# Synthesis of polycyclic compounds with antiviral activity

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# SYNTHESIS OF POLYCYCLIC COMPOUNDS WITH ANTIVIRAL ACTIVITY

Memòria presentada per Eva Torres Costa

Per obtenir el títol de Doctora en Farmàcia.

Director i Tutor:

Dr. Santiago Vázquez Cruz

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**RESULTS**

The current Thesis had led to the publication of several scientific papers and symposium Communications.

**Papers**

1. 'New oxapolycyclic cage amines with NMDA receptor antagonist and trypanocidal activities' Duque, M.D.; Camps, P.; Torres, E.; Valverde, E.; Sureda, F. X.; López-Querol, M.; Camins, A.; Prathalingam, S. R.; Kelly, J.M.; Vázquez, S. *Bioorganic & Medicinal Chemistry* **2010**, *18*, 46-57.
2. 'Polycyclic N-benzamido imides with potent activity against vaccinia virus' Torres, E.; Duque, M.D.; Camps, P.; Naesens, L.; Calvet, T.; Font-Bardia, M.; Vázquez, S. *ChemMedChem* **2010**, *5*, 2072-2078.
3. 'Synthesis and antiviral evaluation of bisnoradamantane sulfites and related compounds' Valverde, E.; Torres, E.; Guardiola, S.; Naesens, L.; Vázquez, S. *Medicinal Chemistry* **2011**, *7*, 135-140.
4. 'Exploring the size limit of templates for inhibitors of the M2 ion chanel of influenza A virus' Duque, M. D.; Ma, C.; Torres, E.; Wang, J.; Naesens, L.; Juárez-Jiménez, J.; Camps, P.; Luque, F. J.; DeGrado, W. F.; Lamb, R. A.; Pinto, L. H.; Vázquez, S. *Journal of Medicinal Chemistry* **2011**, *54,* 2646-2657.
5. 'Synthesis of benzopolycyclic cage amines: NMDA receptor antagonist, trypanocidal and antiviral activities' Torres, E.; Duque, M. D.; López-Querol, M.; Taylor, M. C.; Naesens, L.; Ma, C.; Pinto, L. H.; Sureda, F. X.; Kelly, J. M.; Vázquez, S. *Bioorganic & Medicinal Chemistry* **2012**, *20*, 942-948.
6. 'Synthesis and Anti-Influenza A Virus Activity of 2,2-dialkylamantadines' Torres, E.; Vanderlinden, E.; Fernández, R.; Miquet, S.; Font-Bardia, M.; Naesens, L.; Vázquez, S. *ACS Medicinal Chemistry Letters* **2012**, *20*, 1065-1069.
7. 'Role of the viral hemagglutinin in the anti-influenza virus activity of newly synthesized polycyclic amine compounds' Torres, E.; Duque, M. D.; Vanderlinden, E.; Ma, C.; Pinto, L. H.; Pelayo, C.; Froeyen, M.; Vázquez, S.; Naesens, L. *Antiviral Research* **2013**, *99*, 281-291.
8. 'M2 ion channel of influenza A virus: from wild-type inhibitors to compounds with potent activity against the V27A mutant', Rey-Carrizo, M.; Torres, E.; Ma, C.; Barniol-Xicota, M.; Wang, J.; Wu, Y.; Naesens, L.; DeGrado, W. F.; Lamb, R. A.; Pinto, L. H.; Vázquez, S. *Journal of Medicinal Chemistry* **2013**, submitted.
9. ´Synthesis of enantioenriched tertiary boronic esters by the lithiation/borylation dialkyl substituted benzotes.', Pulis, A. P.; Blair, D. J.; Torres, E.; Aggarwal, V. *Journal of the American Chemical Society* **2013**, submitted.

**Book publications**

1. 'Inhibitors of the M2 channel of influenza A virus'. Duque, M.D.; Torres, E.; Valverde, E.; Barniol, M.; Guardiola, S.; Rey, M.; Vázquez, S. *Recent Advances in Pharmaceutical Sciences,* Transworld research Network, **2011**, 35-64.
2. 'Dipolar 1,3-cycloadditions of nitrile ylides' Torres, E.; Arróniz, C.; Escolano, C.; Vázquez, S. *Organic Reactions* (invited review, in progress).

**Symposium Communications**

1. 'New polycyclic cage amines with NMDA receptor antagonist activity' Torres, E.; López-Querol, M.; Sureda, F. X.; Vázquez, S. (Poster). 16$^{th}$ European Symposium on Organic Chemistry, ESOC, Praga, Czech Republic, **2009**.
2. 'New benzoxapolycyclic amines with NMDA receptor antagonist activity' Vázquez, S:; Camps, P.; Duque, M. D.; Torres, E.; López-Querol, M.; Sureda, F. X. (Poster). 16$^{th}$ European Symposium on Organic Chemistry, ESOC, Praga, Czech Republic, **2009**.
3. 'Síntesi i avaluació farmacològica de noves amines policícliques'Torres, E.; Naesens, L.: Vázquez, S. (Oral communication) Sisena trobada de Joves Investigadors dels Països Catalans, València, Spain, **2010**.
4. 'Polycyclic benzamides with potent activity against orthopoxviruses' Torres, E.; Naesens, L.; Vázquez, S. (Poster) BOSS XII, Namur, Belgium, **2010**.
5. 'New inhibitors of the M2 ion channel of influenza A virus' Vázquez, S.; Duque, M. D.; Ma, C.; Torres, E.; Juárez-Jiménez, J.; Pinto, L. H.; Luque, F. J. (Poster) 12$^{th}$ Tetrahedron Symposium, Sitges, Spain, **2011**.
6. 'New influenza A M2 channel inhibitors featuring tetracyclic scaffolds' Barniol, M.; Rey, M.; Torres, E.; Ma, C.; Pinto, L.H.; Vázquez, S. (Poster) Balticum Organicum Syntheticum, BOS 2012, Tallinn, Estonia, **2012**.
7. 'Targeting the V27A mutant M2 channel of influenza A virus' Rey, M.; Torres, E.; Ma, C.; Pinto, L.H.; Vázquez, S. (Poster), Balticum Organicum Syntheticum, BOS 2012, Tallinn, Estonia, **2012.**
8. 'Ritter reaction in noradamantanes: access to new adamantane derivatives by a Wagner-Meerwein-type rearrangement' Torres, E.; Fernández, R.; Miquet, S.; Naesens, L.; Vázquez, S. (Poster), Balticum Organicum Syntheticum, BOS 2012, Tallinn, Estonia, **2012.**
9. 'Polycyclic amines with activity against amantadine-resistant H1N1 influenza A virus strains' Vázquez, S.; Duque, M.D.; Torres, E.; Vanderlinden, E.; Naesens, L. (Poster), Balticum Organicum Syntheticum, BOS 2012, Tallinn, Estonia, **2012.**

10. ‘Synthesis of polycyclic amines with anti-influenza A virus activity’ Torres, E.; Rey, M.; Barniol, M.; Vázquez, S. (Oral communication), XXIV Reunión Bienal de Química Orgánica, San Sebastián, Spain, **2012.**
11. ‘Synthesis and anti-influenza A activity of novel polycyclic amines’ E. Torres, M. Barniol, S. Vázquez, M. Rey (Oral communication), XXIV Reunión Bienal de Química Orgánica, San Sebastián, Spain, **2012.**
12. ‘New benzopolycyclic cage amines with NMDA receptor antagonist activity’, S. Vázquez, M. D. Duque, E. Torres, E. Valverde, M. Lafuente, F. X. Sureda, (Poster), VI REQOMED, Granada, Spain, **2013**.
13. ‘Novel anti-influenza drugs: inhibitors of the V27A mutant M2 channel’ M. Barniol-Xicota, E. Torres, C. Ma, L. H. Pinto, S. Vázquez (Poster), VI REQOMED, Granada, Spain, **2013**.
14. ‘Rationally-designed inhibitors of the V27A mutant M2 channel of influenza A virus’ M. Rey, E. Torres, M. Frigolé, C. Ma, L. H. Pinto, S. Vázquez (Oral communication), XXXIV Reunión Bienal de la Real Sociedad Española de Química, Santander, Spain, **2013**.
15. ‘Novel polycyclic compounds with potent activity against the amantadine-resistant L26F and V27A mutant M2 channels of influenza A virus’ M. Barniol-Xicota, M. Rey-Carrizo, E. Torres, C. Ma, M. Frigolé, S. Llabrés, J. Juárez-Jiménez, F. J. Luque, L. H. Pinto, S. Vázquez (Póster), XVII Congreso de la Sociedad Española de Química Terapéutica, Madrid, Spain, **2013**.
16. ‘Novel analogues of memantine with potent activity as glutamate N-methyl-D-aspartate receptor antagonists’ S. Vázquez, E. Valverde, E. Torres, F. X. Sureda (Póster), XVII Congreso de la Sociedad Española de Química Terapéutica, Madrid, Spain, **2013**.

**Research stays**

1. ‘Use of thio groups as leaving groups in lithiation-borylation.’ Varinder Aggarwal’s research group. University of Bristol, Bristol, United Kingdom. June 2011-October 2011

**ABBREVIATIONS**

- Ac = acetyl
- $Ac_2O$ = acetic anhydride
- AIDS = acquired immune deficiency syndrome
- brsm = based on recovered starting material
- BSL-2 = biosafety level 2
- BSL-4 = biosafety level 4
- Bu = butyl
- CDC = Centre for Disease Control and Prevention
- CEV = cell-associated enveloped virus
- CPE = cytopathic effect
- DIPEA = diisopropylethylamine
- DMAP = 4-dimethylaminopyridine
- DMD = dimethyldioxirane
- DMF = dimethylformamide
- DMSO = dimethylsulfoxide
- DNA = deoxyribonucleic acid
- $EC_{50}$ = concentration producing 50% antiviral effect
- EEV = extracellular enveloped virus
- EI = electronic impact
- Et = ethyl
- EWG = electron-withdrawing group
- FDA = Food and Drug Administration
- GC = Gas Chromatography
- HA = hemagglutinin
- HEL cells = human erythroleukemia cells
- HHV = herpes virus
- HIV = human immunodeficiency virus
- IEV = intracellular enveloped virus
- IMV = intracellular mature virus
- IR = infrared
- LDA = lithium diisopropylamide
- LiHMDS = lithium bis(trimethylsilyl)amide
- MCC = minimum cytotoxic concentration
- MD = molecular dynamics
- MDCK = Madin Darby canine Kidney cells
- Me = methyl
- MS = mass spectrometry
- NA = neuraminidase
- NEP = nuclear export protein
- NMR = Nuclear Magnetic Resonance
- NRTIs: = nucleoside and nucleotide reverse-transcriptase inhibitors
- NNRTIs = non-nucleoside reverse-transcriptase inhibitors
- NP = nucleoprotein
- PB1 = polymerase basic 1 protein
- PB2 = polymerase basic 2 protein
- PMB = *para*-methoxybenzyl
- RNA = ribonucleic acid
- SAR = structure-activity relationship
- THF = tetrahydrofuran
- vRNP = viral ribonucleoprotein
- WHO = World Health Organization

**INDEX**

# INTRODUCTION

## 1. The virus: biology, disease and treatment.

The first time that an agent smaller than a bacteria was reported in the literature was in 1892, it resulted to be the causative agent of tobacco mosaic disease. The Russian scientist Dimitrii Ivanowsky observed that this agent was not retained by the unglazed porcelain filters used at that time to remove bacteria from extracts and culture media.

Six years later, Martinus Beijerinck hypothesized that the pathogen responsible for tobacco mosaic disease must have been a smaller agent than bacteria, because it was able to pass through the filters supposed to retain particles of the same size than bacteria.

The same year (1898), the German scientists Friedrich Löffler and Paul Frosch, both former students and assistants of Koch, observed that the causative agent of foot-and-mouth disease, a widespread, devastating infection who affects bovids was also filterable. Not only were tobacco mosaic and foot-and-mouth disease pathogens much smaller than any previously recognized microorganism, but they also replicated only in their host organism.

Beijerinck termed the submicroscopic agent responsible for tobacco mosaic disease *contagium vivium fluidum* to emphasize the infectious nature and distinctive reproductive and physical properties of those pathogens. Agents that pass through filters that retain bacteria came to be called ultrafilterable viruses. This term eventually evolved simply to viruses, appropriating the term **virus** from the Latin for "poison".

The first human virus to be identified, in 1901, was that responsible for yellow fever. Other human viruses were identified during the early decades of the 20$^{th}$ century. However, the pace of the discovery was slow, not least because of the dangers and difficulties associated with experimental manipulation of human viruses. A classic case in point is the virus responsible for influenza, a name derived from Italian in the mid-1700s to indicate that the disease resulted from the "influence" of miasma (bad air) and other astrological signs. The human disease is thought to have arisen as a result of the transfer of the virus among humans and other animals following human domestication of animals about 6.000 years ago. Worldwide epidemics, called pandemics, of influenza have been documented in humans for well over 100 years.[1]

The structure of viruses was determined in 1935 when crystals of tobacco mosaic virus were obtained by Wendell Stanley. Obtaining of an infective agent in crystalline form, fact typically associated with inorganic molecules, made to increase the wonder about if viruses were a life form or not.

The same decade, the electron microscope was discovered, this device would completely change the virology. The magnifying power of this microscope overcomes everything known at that time (eventually over 100.000-fold), this property allowed direct

[1] Hughes, S. S. 'The virus: A History of the concept' **1977**, Heinemann Educational Books, London, United Kingdom.

visualization of virus particles for the first time. Images of many different viruses confirmed that these agents are very small and elegant in appearance (Figure 1).

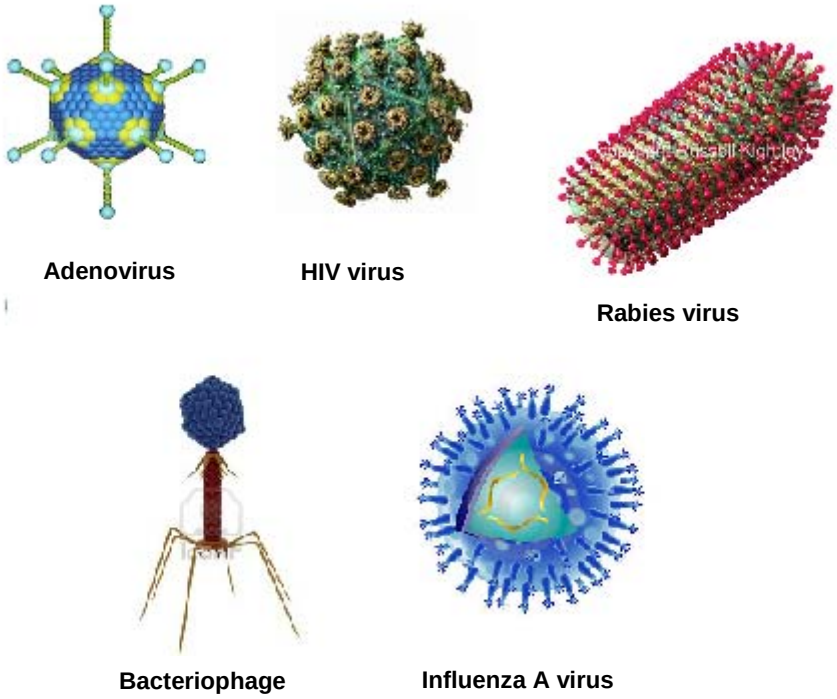



**Figure 1.** Several viruses with different morphologies.

Advances in knowledge of the virus' structure particle and the mechanism by which viruses reproduce in their host cells have been accompanied by increasingly accurate definitions of these unique agents. The definitive properties of viruses are summarized as follows:

- A virus is a very small, infectious, obligate intracellular (molecular) parasite.
- The virus genome comprises either DNA or RNA.
- Within an appropriate host cell, the viral genome is replicated and directs the synthesis, by cellular systems, of other virion components.
- Progeny virions are formed by *de novo* assembly from newly synthesized components within the host cell.

- A new virion assembled during the infectious cycle is the vehicle for transmission of the viral genome to the next host cell or organism, where its disassembly leads to the beginning of the next infectious cycle.[2]

The viruses are far simpler than even the smallest microorganism and lack the complex energy-generating and biosynthetic systems necessary for independent existence.

Virus particles, known as virions, consist of two or three parts: the genetic material made from either DNA or RNA, a protein coat that protects these genes, called capsid, and in some cases, an envelope of lipids that surrounds the protein coat when they are outside a cell.[3]

An enormous variety of genomic structures can be seen among viral species; as a group, they contain more structural genomic diversity than plants, animals, archaea, or bacteria. There are millions of different types of viruses, although only about 5.000 of them have been described in detail.

A virus has either DNA or RNA genes and is called a *DNA virus* or a *RNA virus*, respectively. The vast majority of viruses have RNA genomes. Among RNA viruses and certain DNA viruses, the genome is often divided up into separate parts, in which case it is called *segmented*. For RNA viruses, each segment often codes for only one protein and they are usually found together in one capsid. However, all segments are not required to be in the same virion for the virus to be infectious.

A viral genome, irrespective of nucleic acid type, is either *single-stranded* or *double-stranded*. Single-stranded genomes consist of an unpaired nucleic acid. Double-stranded genomes consist of two complementary paired nucleic acids.

For most viruses with RNA genomes and some with single-stranded DNA genomes, the single strands are said to be either *positive-sense* or *negative-sense* depending on whether or not they are complementary to the viral messenger RNA, mRNA.

Those RNA viruses with continuous (+) strand genomes include the family of *picornaviruses* and *calciviruses*. *Retroviruses* also contain (+) strand RNA genomes, but two copies of genomic RNA are included in each virion, this is a unique feature of this family.

RNA viruses with (-) strand genomes are all enveloped by helical capsids. These genomes could be organized as a single (-) strand RNA molecule, such as *paramyxoviruses*, or could be segmented, such as *Orthomyxoviruses*.[4] Moreover, there are some families that have double-stranded RNA genome, such as *reovirus* (Figure 2).

[2] Flint, S. J.; Enquist, L. W.; Krug, R. M.; Racaniello, V. R.; Skalka, A. M. 'Principles of virology: Molecular Biology, Pathogenesis and Control' **2000**, American Society for Microbiology, Washington, United States of America.

[3] Crick, F. H.; Watson, J. D. *Nature* **1956**, *177*, 473-475.

[4] Baltimore, D. *Bacteriol. Rev.* **1971**, *35*, 235-241.

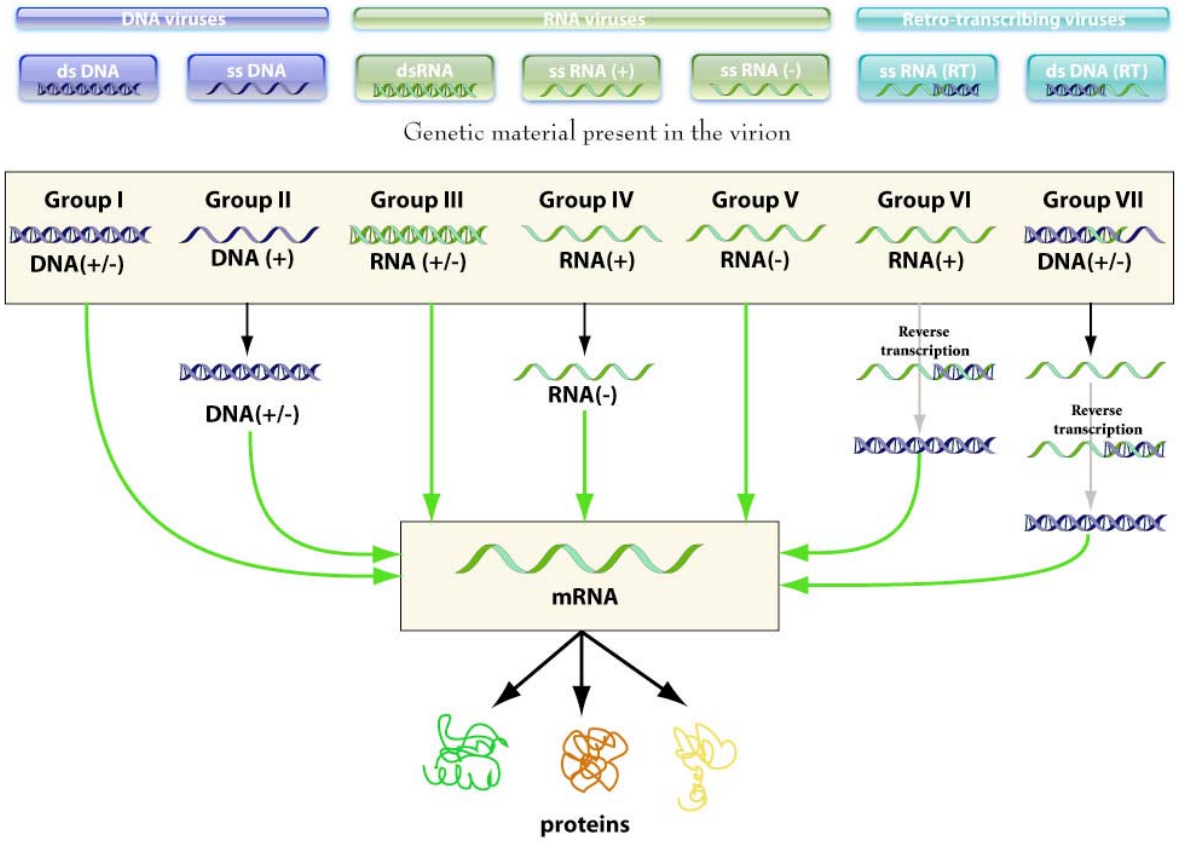



**Figure 2.** Baltimore's classification of viruses.[5]

Viral genomes are encased in protective protein structures called nucleocapsids. Capsids are often formed by the assembly of identical structural units in ways that provide maximal contact between them. There are different nucleocapsids symmetries depending in how the proteins are packaged: helical, icosahedral, prolate and complex particles.[3] These morphological differences permit to differentiate between viruses.

Virus particles are designed for effective transmission of the nucleic acid genome from one host cell to another within a single animal or among host organism. A primary function of the virion is therefore protection of the genome, which can be damaged irreversibly by a break in the nucleic acid or mutation during passage through hostile environments.

[5] Taken from Dr. Jeff Young, Biology Department, Western Washington University: http://biol.wwu.edu/young/321/stuff/collection/baltimore_classification.html (accessed on July, 8th 2013).

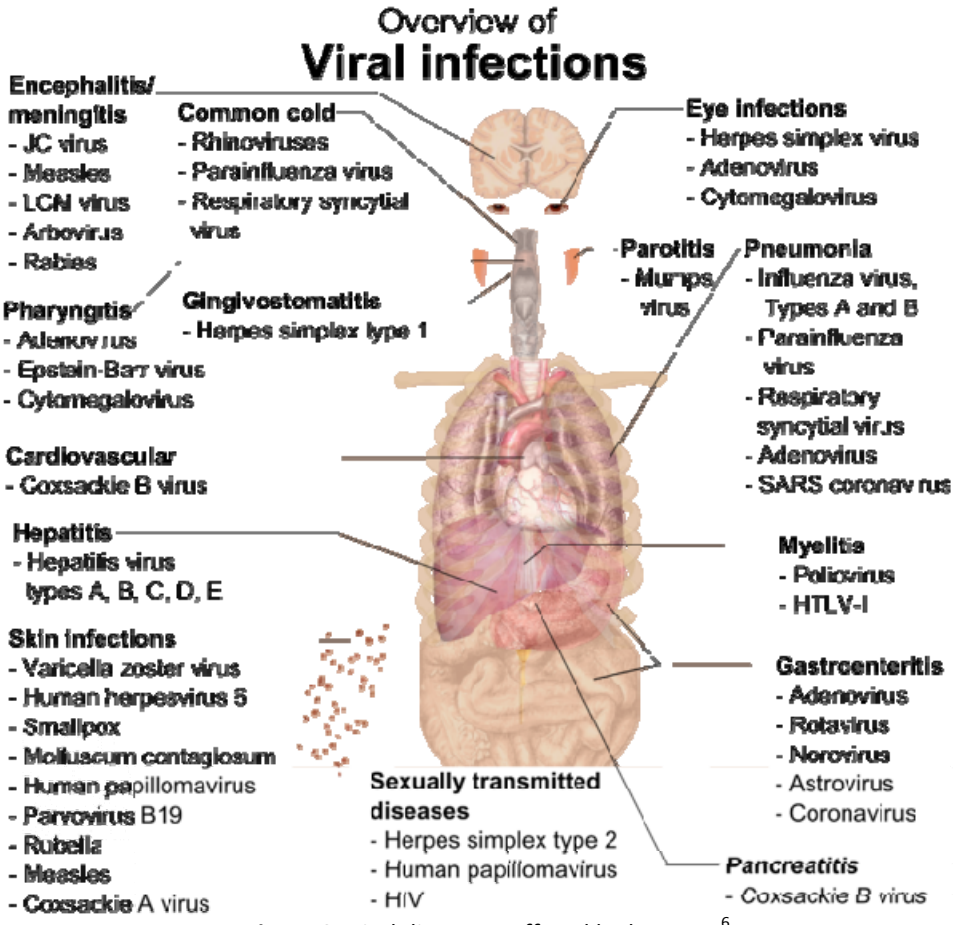



**Figure 3.** Viral diseases suffered by humans.[6]

Examples of common diseases caused by viruses include the common cold, influenza, chickenpox and cold sores. Many serious diseases such as Ebola, AIDS, avian influenza and SARS are caused by viruses (Figure 3).[6] The relative ability of viruses to cause disease is described in terms of virulence.

Viruses have different mechanism to cause a disease in an organism. Mechanisms at the cellular level mainly include cell lysis, which ends up in the death of the cell. In multicellular organism, if enough cells die, the whole organism will start to suffer from the effects. Although viruses cause disruption of healthy homeostasis, resulting in disease, they may exist relatively harmlessly within an organism. An example would include the ability of the herpes simplex virus, which causes cold sores, to remain in a dormant state within the human body. This is called latency and is characteristic of the herpes viruses including Epstein-Barr virus, which causes glandular fever, and varicella zoster virus, which causes chickenpox and shingles. Most people have been infected with at least one of these types of herpes virus.[7]

[6] Taken from: Chapter 33 (Disease summaries), pp 367-392 in: Fisher, B.; Harvey, R.; Champe, P. *Lippincott's Illustrated Reviews: Microbiology* Hagerstown, MD: Lippincott Willims & Wilkins; **2007**. pp 367-392.

[7] Whitley, R. J.; Roizman, B. *Lancet* **2001**, *357*, 1513-1518.

When a viral infection affects a high proportion of individuals in a population are called epidemics. If outbreaks spread worldwide they are called pandemics. Two big pandemics have occurred during the humankind history. The first was the 1918 flu pandemic, which lasted until 1919, was a category 5 influenza pandemic caused by an unusually severe and deadly influenza A virus. And the second was caused by smallpox which was responsible for an estimated 300–500 million deaths during the 20$^{th}$ century.

It is believed that the outbreak of HIV originated in sub-Saharan Africa during the 20$^{th}$ century[8] was a pandemic, with an estimated 38.6 million people now living with the disease worldwide. The World Health Organization estimates that AIDS has killed more than 25 million people since it was first recognised on June, 1981, making it one of the most destructive epidemics in recorded history.[9]

Several highly lethal viral pathogens are members of the Filoviridae. Filoviruses are filament-like viruses that cause viral hemorrhagic fever, and include the ebola and Marburg viruses. The Marburg virus attracted widespread press attention in April 2005 for an outbreak in Angola. Beginning in October 2004 and continuing into 2005, the outbreak was the world's worst epidemic of any kind of viral hemorrhagic fever.

The most effective medical approaches to viral diseases are vaccinations to provide immunity to infection and antiviral drugs that selectively interfere with viral replication. Vaccination is a cheap and effective way of preventing infections by viruses. Their use has resulted in a dramatic decline in morbidity and mortality associated with viral infection such as polio, measles, mumps and rubella. For instance, smallpox infections have been eradicated thanks to vaccination.[10]

Vaccines are very effective on stable viruses, but are of limited use in treating a patient who has already been infected. They are also difficult to successfully deploy against rapidly mutating viruses, such as 'influenza' (the vaccine for which is updated every year) and HIV. Antiviral drugs are particularly useful in these cases.

Over these last three decades several antiviral drugs have been developed to treat human viral diseases. Several strategies have allowed obtaining a broad spectrum of antiviral drugs, these could be classified depending on their structure:

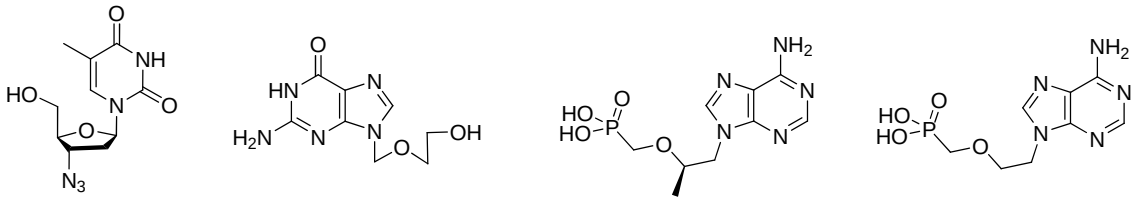
- **Nucleoside and nucleotide analogs reverse-transcriptase inhibitors (NRTIs):**

They inhibit the reverse transcriptase enzyme that retroviruses need to replicate. NRTIs are analogues of the naturally occurring deoxynucleosides or deoxynucleotides needed to synthesize the viral DNA and they compete with the deoxynucleosides or deoxynucleotides for incorporation in the growing DNA chain and thus halting the process.

[8] Gao, F.; Bailes, E.; Robertson, D. L. *Nature* **1999**, *397*, 436-441.

[9] Mawar, N.; Saha, S.; Pandit, A.; Mahajan, U. *Indian J. Med. Res.* **2005**, *122*, 471-484.

[10] Lane, J. M. *Curr. Top. Microbiol. Immunol.* **2006**, *304*, 17-29.



**Zidovudine** (nucleoside) **Aciclovir** (nucleoside) **Tenofovir** (nucleotide) **Adefovir** (nucleotide)

**Figure 4.** Examples of nucleoside and nucleotide analogs reverse-transcriptase inhibitors.

- **Non-nucleoside reverse-transcriptase inhibitors (NNRTIs):**

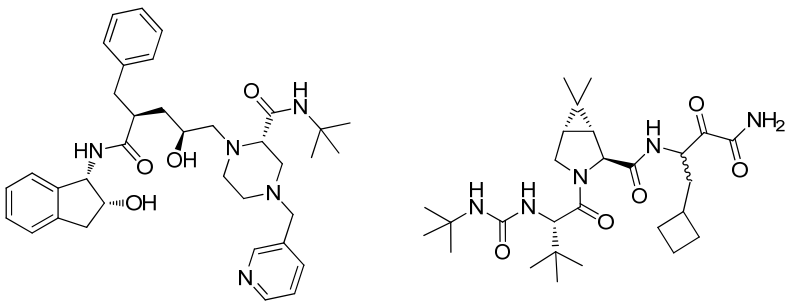
These compounds inhibit the reverse transcriptase by binding at a different site of the enzyme. They are not incorporated in the DNA chain but instead inhibit the movement of protein domains needed to carry out the function of reverse-transcriptase enzyme.

**Efavirenz** **Nevirapine**

**Figure 5.** Examples of non-nucleoside reverse-transcriptase inhibitors.

- **Pseudopeptides (Protease inhibitors):**

They are used to treat hepatitis C and HIV. These drugs prevent viral replication by inhibiting the proteases of these viruses. The function of the proteases is to cleave nascent proteins for final assembly of new virions. These pseudopeptides bind to the enzyme where the protein should be, blocking the proteases for further activity.



**Indinavir** **Boceprevir**

**Figure 6.** Examples of protease inhibitors.

- **Protein and polypeptide antiviral drugs:**

Some examples are: interferons, proteins that enhance the immune response of the host; or enfuvirtide, used for the treatment of HIV. Enfuvirtide is a biomimetic peptide that was designed to mimic components of the HIV-1 fusion machinery and displace them, preventing normal fusion.

- **Sialic acid-like compounds:**

This family is composed, mainly, by the neuraminidase inhibitors, such as: oseltamivir, zanamivir and peramivir. All of them inhibit the neuraminidase of Influenza A virus, an enzyme that cleaves the bond between hemagglutinin and the sialic acids of the cellular membrane to release the new assembled virus particle (Figure 7).

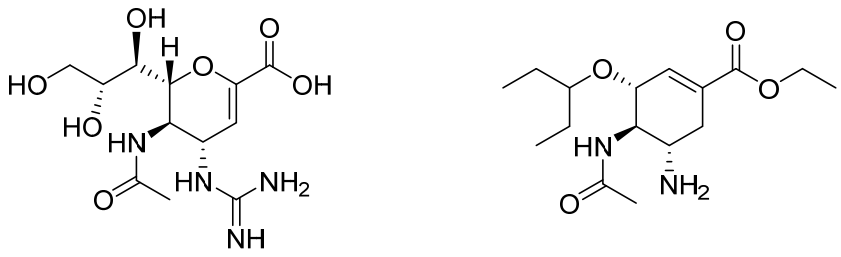
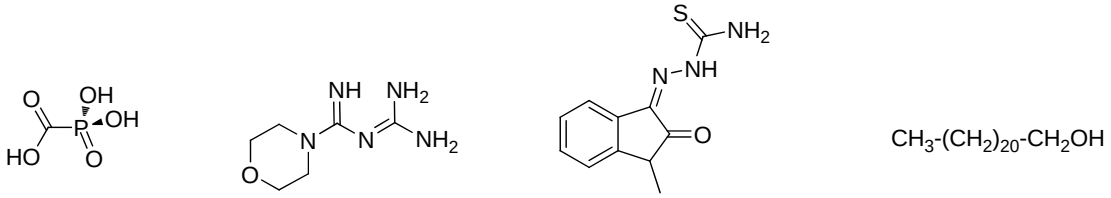



**Figure 7.** Sialic acid-like compounds.

- **Rare structures:**

There are some antiretroviral drugs that are not related to any other, neither the structure nor the mechanism of action. Here, we can include some of the following molecules:



**Foscarnet**
(treatment of AIDS and herpes)

**Moroxydine**
(treatment of the flu)

**Methisazone**
(treatment of smallpox)

**Docosanol**
(treatment of herpes)

**Figure 8.** Some different antiviral drugs.

- **Polycyclic antiviral drugs:**

This kind of compounds possesses a polycyclic ring in their scaffold. Although their mechanism of action is different some resemblance could be found in their structure and so, in the chemistry used to synthesize them.

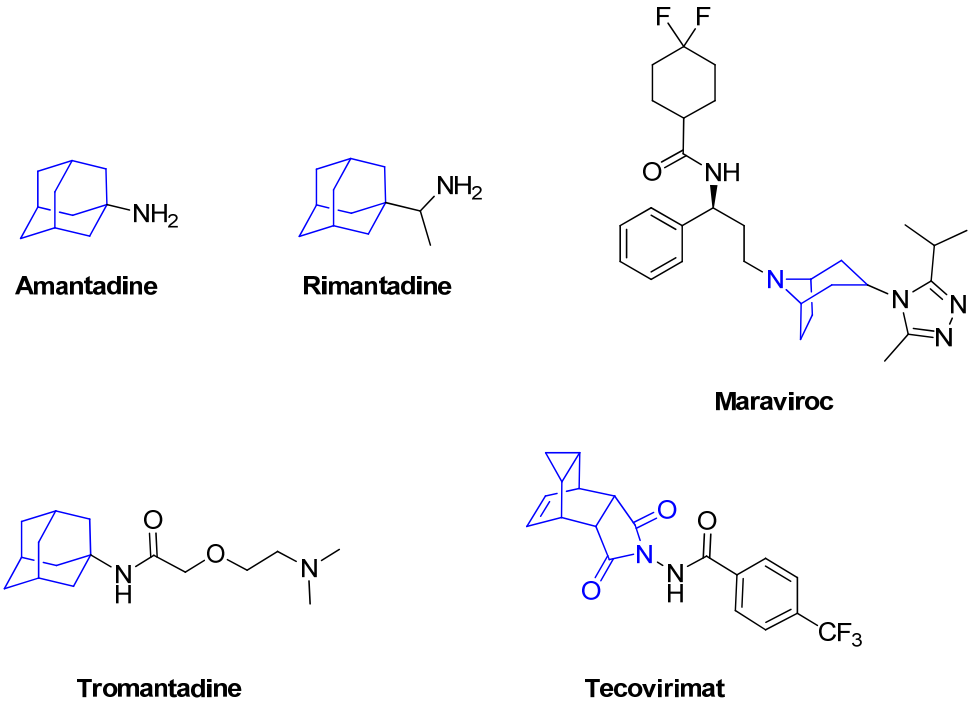
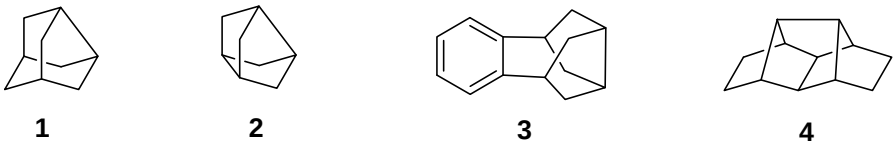



**Figure 9.** Antiviral drugs containing a polycyclic ring in their structure.

## 2. Projects of our research group in polycyclic scaffolds.

For more than twenty years, our laboratory has studied the reactivity and synthesis of several polycyclic compounds, mainly derivatives of the tricyclo[3.3.1.$0^{3,7}$]nonane ("noradamantane") **1**, tricyclo[3.3.0.$0^{3,7}$]octane ("bisnoradamantane") **2**, and related compounds, such as **3** and **4** (Figure 10).[11]

[11] a) Camps, P.; Font-Bardia, M.; Pérez, F.; Solans, X.; Vázquez, S. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 912–914. b) Camps, P.; Fernández, J. A.; Vázquez, S.; Font-Bardia, M.; Solans, X. *Angew. Chem. Int. Ed.* **2003**, *42*, 4049–4051. c) Ayats, C.; Camps, P.; Fernández, J. A.; Vázquez, S. *Chem. Eur. J.* **2007**, *13*, 1522–1532.

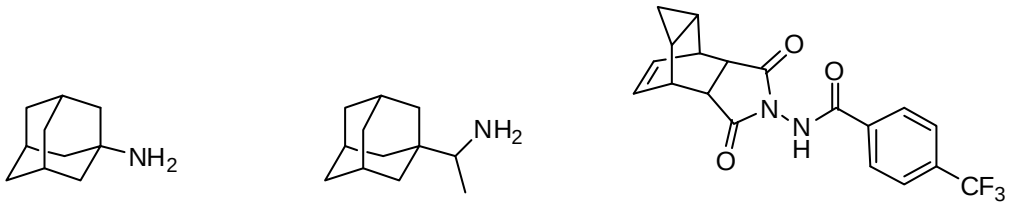


**1** **2** **3** **4**

**Figure 10:** Noradamantane, **1**, bisnoradamantane, **2**, and related compounds **3** and **4**.

It must be pointed out that, for many years, these studies were just carried out from a synthetic point of view, the main aim of our research being the synthesis and the study of the reactivity of these polycyclic scaffolds.

However, taking into account the biological activity of some aminopolycyclic compounds, such as *amantadine* or *memantine*, it seemed logical to us to take advantage of our expertise in the synthesis of such polycyclic compounds in order to develop a new line of research with the goal of obtaining new bioactive compounds. With this aim, eight years ago, our group started, within the Thesis of M. D. Duque, the synthesis and pharmacological evaluation of new derivatives of the polycyclic compounds shown in Figure 10, as potential analogues of the clinically used anti-influenza drugs amantadine and rimantadine; and also as analogues of *tecovirimat*, currently in phase III clinical trials, designed for the treatment of smallpox (Figure 11).




*Amantadine* *Rimantadine* *Tecovirimat*

**Figure 11:** Structures of *amantadine*, *rimantadine* and *tecovirimat*.

Following the work started by Dra. M. D. Duque some analogues of tecovirimat, amantadine and rimantadine are reported within this Thesis. It will be structured in two parts containing the antiviral compounds designed against Vaccinia virus and against Influenza A virus, respectively.

# 1st PART: POLYCYCLIC *N*-BENZAMIDO IMIDES WITH POTENT ACTIVITY AGAINST VACCINIA VIRUS

# PART I: INTRODUCTION

## 1. The relevance of *Orthopoxvirus* and smallpox.

There are close to 100 species of poxviruses found in nature, ranging from those that infect invertebrate hosts (*Entomopoxvirinae* subfamily) to those that infect vertebrate hosts (*Chordopoxvirinae* subfamily).[12] Poxviruses are a double-stranded DNA viruses that replicate entirely in the cytoplasm (Figure 12), being the largest viruses that infect animals.[13]

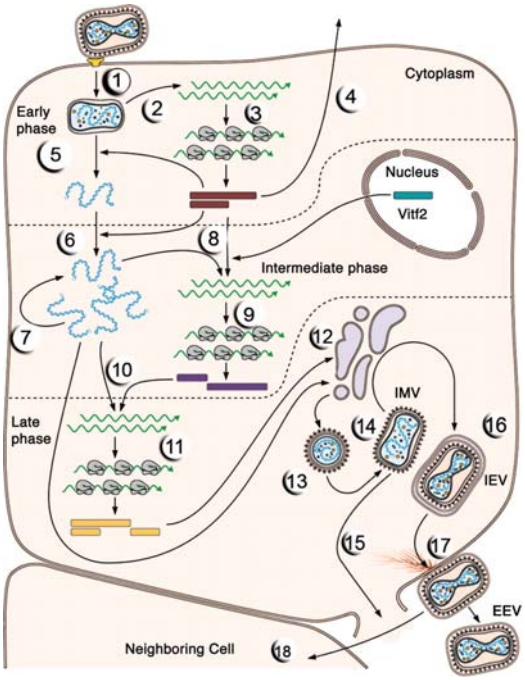



**Figure 12.** Life cycle of *Vaccinia* virus (*Orthopoxvirus*). Taken from Harrison et al. *Proc. Nat. Acad. Sci.* **2004**, *101*, 111778-11192.

The family *Poxviridae* includes eight genera, four of which are of greater importance to humans: *Orthopoxvirus* (variola, vaccinia, cowpox, camelpox and monkeypox viruses), *Parapoxvirus* (orf, Milker's nodules and monkeypox viruses), *Yatapoxvirus* (yatapox and tanapox viruses) and *Molluscipoxvirus* (molluscum contagiosum virus).[14]

The *Orthopoxvirus* is by far the most studied genus, mainly because the variola virus, that has humans as its only host, is the causative agent of smallpox, a devastating and highly contagious disease, which has been responsible for millions of human deaths over centuries.

Their lineal genoma contains 200 genes; those in the central region encode proteins involved in replication of the virion structure. The highly accurate poxviral DNA polymerase has conserved the sequences of these genes among all *Orthopoxviruses.* The flanking regions

[12] Byrd, C. M.; Page, J.; Hruby, D. E.; Jordan, R. *Drugs Future* **2008**, *33*, 875-890.
[13] Moss, B. *Fields Virology 5th ed.* (Ed. D. M. Knipe, P. M. Howley), Lippincott Williams & Wilkins, Philadelphia, **2007**, 2905-2946.
[14] Harrison, S. C.; Alberts, B.; Ehrenfeld, E.; Enquist, L.; Fineberg, H.; McKnight, S. L.; Moss, B.; O'Donnell, M.; Ploegh, H.; Schmid, S. L.; Walter, K. P.; Theriot, J. *Proc. Nat. Acad. Sci.* **2004**, *101*, 11178-11192.

contain genes encoding proteins that modify the intra- and extra-cellular environment in ways that favour viral replication and spread.[15]

Because of the highly transmissibility and dangerousness of variola virus, the research that is currently carried out with variola virus requires biosafety level 4 (BSL-4).[16] For this reason, the life cycle of *Orthopoxvirus* has been extensively studied using vaccinia virus, the prototypic virus because it shares a 97% of its genoma with variola virus, is a biosafety level 2 (BSL-2) agent and ideal for laboratory study. There are four types of infectious poxvirus particles that differ in their lipid membranes location and relative quantity: intracellular mature virus (IMV), intracellular enveloped virus (IEV), cell-associated enveloped virus (CEV), and extracellular enveloped virus (EEV). These particles play distinct roles in pathogenesis of the virus (Figure 13).[14]

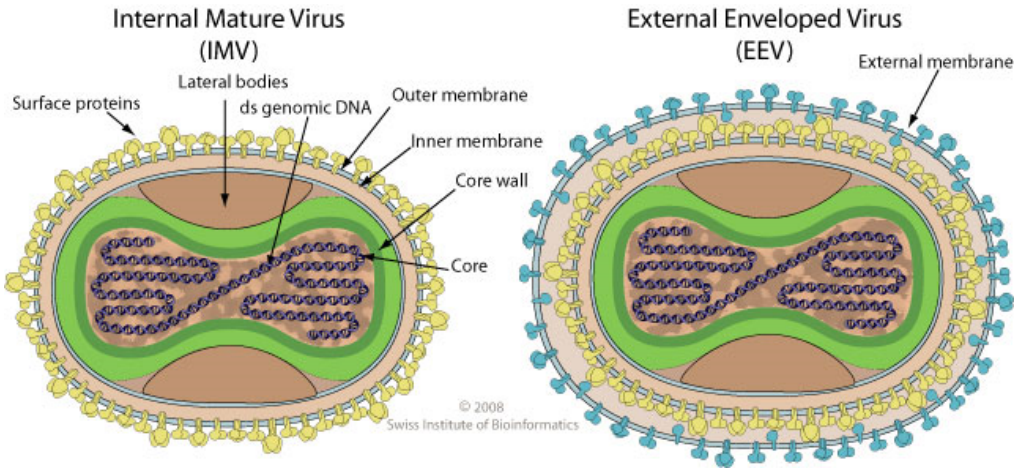



**Figure 13.** IMV and EEV particles of *Vaccinia* virus. Taken from http://viralzone.expasy.org/all_by_species/174.html

The IMV is the most abundant virion, contains a single membrane and remains within the cell until cell lysis. The IMV particle is environmentally stable and is the responsible for the spread of the infection between host organisms. EEV has an additional membrane made of cell membrane and is the responsible for the long-range dissemination of the virus and the systemic disease. Virus maturation and egress are regulated in part by the F13L protein, which encodes a viral late assembly domain. In the absence of F13L activity, vaccinia virulence is significantly reduced by inhibition of EEV formation, revealing F13L as a useful drug target.[12,14,17]

There are at least two forms of smallpox: variola major and variola minor. The first is the most common and severe, with a mortality of up to 30% in unvaccinated patients, compared with 1% for variola minor.

[15] Bray, M.; Buller, M. *Clin. Infect. Dis.* **2004**, *38*, 882-889.
[16] Bolken, T. C.; Hruby, D. E. *Antivir. Res.* **2008**, *77*, 1-5.
[17] Duraffour, S.; Vigne, S.; Vermeire, K.; Garcel, A.; Vanstreels, E.; Daelemans, D.; Yang, G.; Jordan, R.; Hruby, D. E.; Crance, J. M.; Garin, D.; Andrei, G.; Snoeck, R. *Antivir. Ther.* **2008**, *13*, 977-990.

Generally, the virus is transmitted by inhalation, entering the oropharyngeal and respiratory mucosa, and proliferating in the regional lymph nodes, multiplying in particular in the reticulo-endothelial system. However, the cellular entry mechanism is unknown in terms of fusion proteins and cells receptors.[18]

This is followed by systemic spread travelling to the spleen, liver and reticulo-endothelial system where replication in these organs produce a secondary viremia. This is accompanied by clinical latency in which physical symptoms of infection are absent. Clinical latency ends with the rapid onset of severe headache, backache and fever. The virus invades the capillary epithelium of the dermal layer skin, perivascular cells and epidermis where replication results in necrosis and the formation of a rash which can leave permanent scars (Figure 14).[19] Therefore, smallpox is remembered as either being lethal or a disfiguring disease.

Within 1 to 2 days, the rash becomes vesicular and later papular and death, which usually occurs during the second week of illness, most likely results from the toxemia associated with circulating immune complexes and soluble variola antigens. The result of host inflammatory responses is hypotension and coagulopathy which in severe cases ends in vascular dysfunction and multiorgan failure, resembling septic shock.[20]

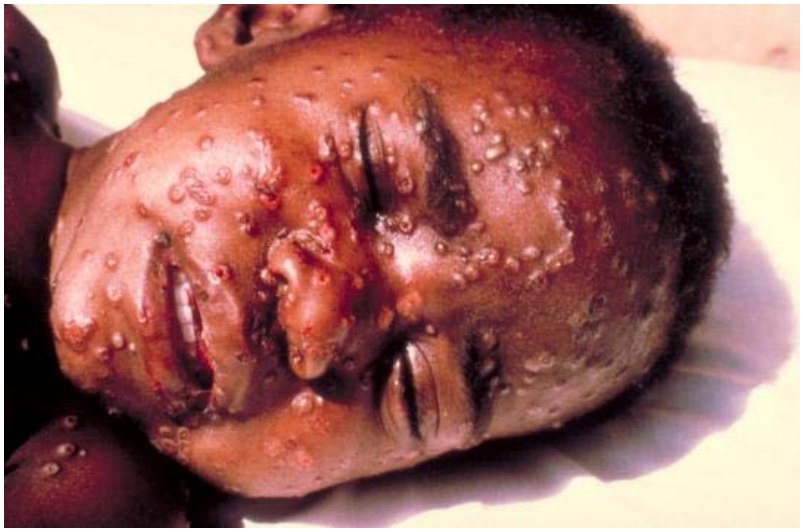


**Figure 14.** Patient of smallpox. Taken from https://www.dshs.state.tx.us/preparedness/factsheet_smallpox_pro.shtm

[18] Sliva, K.; Schnierle, B. *Virology J.* **2007**, 4:8.
[19] Jordan, R.; Hruby, D. *Expert Rev. Anti Infect. Ther*. **2006**, *4*, 277-289.
[20] Henderson, A. D.; Inglesby, T. V.; Bartlett, J. G.; Ascher, M.S.; Eitzen, E.; Jahrling, P. B.; Hauer, J.; Layton, M.; McDade, J.; Osterholm, M. T.; O'Toole, T.; Parker, G.; Russell, P. T.; Tonat, K. *J. Am. Med. Ass.* **1999**, *281*, 2127-2137.

The vaccination against smallpox, first introduced by Edward Jenner more than 200 years ago, was the single most effective public health intervention in human history.[21] Usually, the vaccine was made of live vaccinia virus which was introduced into the skin with a bifurcated needle. Four to five days following the vaccination, a papule appears at the site of viral replication, which becomes pustular a few days later due to the infiltration on inflammatory cells. The pustule heals after 3 weeks leaving a characteristic scar.

Complications from vaccination can result from escape of the virus from the inoculation site and include progressive vaccinia, eczema vaccinatum, generalized vaccinia and postvaccinal encephalitis.[22] Among people who have the highest risk of complications are immunodeficient patients, pregnants, people with cardiopathies and infants less than one year old.[23]

The last natural case of smallpox occurred in 1977 in Somalia. In May 1980, the eradication of smallpox was declared as having been achieved by the World Health Organisation (WHO), following intensive immunisation with the vaccinia virus vaccine.[23]

Three years later from eradication of the disease, variola virus stocks were either supposedly destroyed or submitted to one of the two WHO approved laboratories situated in Atlanta (US) and Novosibirsk (Russia).[24] The controversial destruction of the two variola virus stocks had initially been recommended, but later it was decided to preserve them for research purposes, mainly to allow the further development of vaccines or antiviral drugs.

The eradication of smallpox was followed by cessation of routine vaccination. Thus, the re-introduction of the variola virus would be even more lethal nowadays due to the absence or minimal immunity against smallpox. The herd immunity from prior vaccination is estimated at no more than 18% and is decreasing with time.[23]

The release of the causative agent of anthrax, *Bacillus anthracis*, in the US postal system in 2001, and the allegations of existing undeclared stocks of variola virus, have emphasised the need to be prepared for the use of variola virus as a bioterrorist weapon.[25] Variola virus is considered as an ideal bioterrorism agent because it is highly transmissible and associated with high morbidity. Additionally, it can survive in aerosol form, particularly in cool and dry environments. The US Centers for Disease Control and Prevention has ranked variola virus as a category A, high-threat agent. Moreover, there is also a natural public threat arising from monkeypox virus, a virus that produces a disease in man that closely resembles smallpox,

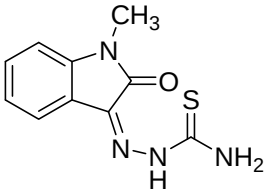
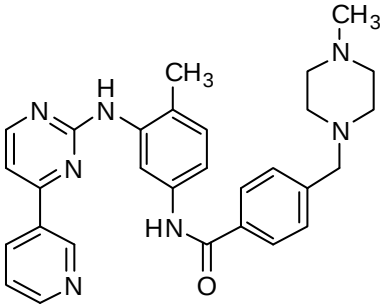
[21] Jacobs, B. L.; Langland, J. O.; Kibler, K. V.; Denzler, K. L.; White, S. D.; Holechek ,S. A.; Wong, S.; Huynh, T.; Baskin, C. R. *Antiviral Res.* **2009**, *84*, 1-13.
[22] a) Lane, J. M.; Ruben, F. L.; Abrutyn, E.; Millar, J. D. *J. Am. Med. Ass.* **1970**, *126*, 160-168; b) Baggs, J.; Chen, R. T.; Damon, I. K.; Rotz, L.; Allen, C.; Fullerton, K. E.; Casey, C.; Nordenberg, D.; Mootrey, G. *Clin. Infect. Dis.* **2005**, *40*, 1133-1140; c) Schwartz, B.; Lebwohl, M. *Int. J. Dermatol.* **2005**, *44*, 289-292; d) Reif, D. M.; McKinney, B. A.; Motsinger, A. A.; Chanock, S. J.; Edwards, K. M.; Rock, M. T.; Moore, J. H.; Crowe, J. E.; *J. Infect. Dis.* **2008**, *198*, 16-22.
[23] Omari, K.; Stammers, D. K. *Expert Opin. Drug Discov.* **2007**, *2*, 1263-1272.
[24] Henderson, D. A. *Smallpox: The Death of a Disease*, Prometheus Books, **2009**.
[25] Whitley, R. J. *Antivir. Res.* **2003**, *57*, 7-12.

though less frequently fatal. Monkeypox exists naturally in Africa and several cases are reported in the US every year.[26]

All previous exposed motives are remarkably reasons for developing safe and specific anti-orthopoxvirus drugs.[27] Recently, several compounds have been identified that inhibit various steps in poxvirus infection, including virion morphogenesis and DNA synthesis.[14]

Table 1 collects several compounds which have been considered for the treatment of a possible breakthrough of smallpox.

**Table 1**. Compounds with activity against orthopoxviruses.

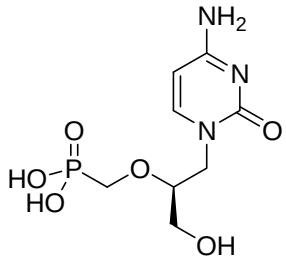
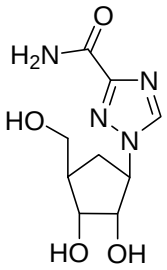
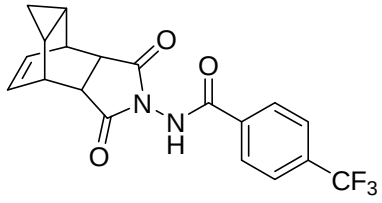
| | |
|---|---|
|  | Thiosemicarbazones were first discovered as antituberculosis agents, later showed to be active against vaccinia virus. *Methisazone* (Morbaran®), was used in the 1960s to treat complications of smallpox vaccination or for prophylaxis against variola infection.[28] It was stated to be protective against smallpox if administered within 3 days after exposure. Side effects of the drug included nausea and vomiting in 10-65% of the drug recipients.<br><br>While the exact mechanism of action is unknown, *in vitro* studies showed that methisazone inhibited the synthesis of a late structural vaccinia virus protein, thereby inhibiting viral assembly. |
|  | *Imatinib*, an anticancer drug, is a tyrosine kinase inhibitor that, administered intravenously, has recently shown to block the release of vaccinia virus from infected cells and to promote survival of vaccinia-infected mice.[29]<br><br>It seems that the approach targeting hosts factors might be very promising as it would be more difficult for the virus to develop resistance, so the virus would have to change its virulence strategy to become resistant. |

[26] a) Stittelaar K. J.; Neyts J.; Naesens L.; Amerongen G.; Lavieren R. F.; Holý A.; De Clercq E.; Niesters H. G.; Fries E.; Maas C.; Mulder P. G.; Zeijst B. A.; Osterhaus A. D. *Nature* **2006**, *439*, 745-748; b) Cowpox infections are being increasingly reported through Eurasia; see, for example: Duraffour, S.; Mertens, B.; Meyer, H.; van der Oord, J. J.; Mitera, T.; Matthys, P.; Snoeck, R.; Andrei, G. *PLoS One* **2013**, *8*(2); e55808.

[27] a) In this regard, it is very interesting to read the following news: "Israel taps Siga Technologies' ST-246 to combat smallpox in simulated bioterror attack", available in www.investar.siga.com/releasedetail.cfm?.releaseID=438564 (accessed on 25-05-2013); b) For recent advances on anti-orthopoxviruses agents see, Kolodziej, M.; Joniec, J.; Bartoszcze, M.; Gryko, R.; Kocik, J.; Knap, J. *Ann. Agric. Environ. Med.* **2013**, *20*, 1-7.

[28] Hamre D.; Brownlee K. A.; Donovick R. *J. Immunol.* **1951**, *67*, 305-312.

[29] Reeves P. M.; Bommarius B.; Lebeis S. *Nature Med*. **2005**, *11*, 731-739.

| | |
|---|---|
|  | *Cidofovir* is a nucleoside analogue whose 5'-diphosphorylated metabolite is recognized by viral DNA polymerases and terminates DNA chain elongation. Cidofovir is licensed for the treatment of cytomegalovirus retinitis in HIV-infected patients. *Cidofovir* was shown to be more effective for the postexposure treatment than postexposure vaccination.<br><br>*Cidofovir* is the only licensed antiviral drug that has been reserved and stockpiled for possible use in therapy and prophylaxis of smallpox and the complications of smallpox vaccination with vaccinia virus.[30] In 2003 *cidofovir* was allowed to its emergency use in the event of an outbreak. Although the therapeutic effectiveness of *cidofovir* is limited by its poor oral bioavailability and nephrotoxicity,[31] its analogue hexadecyloxypropyl-cidofovir shows better oral bioavailability and has shown strong inhibition of poxvirus infections both in vitro and in vivo.[32] |
|  | *Ribavirin* is a nucleoside analogue active against a wide variety of RNA and DNA viruses, including vaccinia virus.<br><br>It is an inhibitor of inosine monophosphate dehydrogenase, a cellular target. The latter enzyme is essential for *the novo* biosynthesis of purine nucleotides. Its selectivity for infected cells is based on the fact that viral RNA and DNA synthesis are predominant in infected cells.<br><br>*Ribavirin* in combination with vaccinia immune globulin has been reported to have successfully treated immunocompromised patients.[33] |
|  | *Tecovirimat* (Arestvyr®) is an orally bioavailable, low-molecular weight compound developed by Siga Technologies that can be given prophylactically, postexposure prophylactically and therapeutically to prevent or treat Orthopoxvirus infection.<br><br>*Tecovirimat* targets vaccinia virus F13L gene, to prevent the production of extracellular enveloped virus, thus preventing egress and spread of the virus.[34] |

[30] De Clercq E. *Med. Res. Rev.* **2008**, *28*, 929-953.
[31] Kern, E. R. in *Antiviral Drug Discovery For Emerging Disease and Bioterrorism Threats,* Torrence, P. F. ed.; Wiley, **2005**, pp 331-351.
[32] a) Clerq, E. *Antiviral Res.* **2002**, *55*, 1-13; b) Smee, D. F.; Sidwell, R.W., Kefauver, D.; Bray, M.; Huggins, J. W. *Antimicrob. Agents Chemother*. **2002**, *46*, 1329-1335; c) Hostetler, K. Y. *Antivir. Res.* **2009**, *82*, A84-A98.
[33] Kesson, A. M.; Ferguson, J. K.; Rawlinson, W. D.; Cunningham, A. L. *Clin. Infect. Dis*. **1997**, *25*, 911-914.

| | |
|---|---|
| | In a phase I clinical trial, *tecovirimat* was found to be readily absorbed after oral administration, well tolerated with no severe adverse events and to provide blood exposure levels predicted to be sufficient for inhibiting Orthopoxvirus disease. It is currently in clinical trial II and has recently been given 'fast-track' status by the FDA. |

These compounds are thought to be a possible treatment in case of a variola outbreak, but up to now, there is no compound that really fits in this context. The most suitable drug is *tecovirimat* because it is specific for orthopoxvirus and seems to be secure, tolerable and is orally bioavailable. However, it is still in clinical phase II and it has not been approved for the clinical use yet. So we consider important to develop new drugs to offer different choices just in case *tecovirimat* failed.

**2. Tecovirimat analogs synthesized by our group.**

In 2004, while Loli Duque was carrying out her doctoral thesis, Siga Technologies, using a high throughput screening of 356000 commercial, low molecular weight compounds, found that several polycyclic imides featuring benzamide rings were endowed with very potent activity against several orthopoxviruses (Figure 15).[35] It was found that these compounds inhibited the formation of the extracellular forms of the viruses, targeting the F13L gene, which encodes a major envelope protein necessary for the formation and egress of extracellular virus particles.[17]

Siga's scientists found that in order to have potent antiviral activity an electron withdrawing group (EWG) should be in the *meta* or *para* positions from the carboxamide function in the aromatic ring. Several compounds showed submicromolar activities and one of these compounds, *tecovirimat*, which has an $IC_{50}$ = 0.04 μM and an oral bioavailability of 31%, is currently in clinical trials.[36]

In 2007, we already knew the outstanding activity of several of our polycyclic amines as anti-influenza agents. Analyzing the structures shown in Figure 15, we realized that Siga Technologies had only tested polycyclic compounds which possessed a tertiary carbon in the two α positions to the imide moiety. We wondered if analogues of *tecovirimat* featuring a polycyclic scaffold with two quaternary carbon atoms in the α positions of the imide would have better activity, and selected two scaffolds synthesized earlier in our laboratory for making such kind of *tecovirimat* analogues.

[34] a) Jordan, R.; Leeds, J. M.; Tyavanagimatt, S.; Hruby, D. E. *Viruses* **2010**, *2*, 2409-2435. b) Grosenbach, D. W.; Jordan, R.; Hruby, D. E. *Future Virol.* **2011**, *6*, 653-671.

[35] a) Jordan R., Bailey T. R., Rippin S. R. (Viropharma Inc.), WO 2008/130348, **2008**; b) Jordan R., Bailey T. R., Rippin S. R., Dai D. (SIGA Technologies, Inc.), WO 2004/112718, **2004**.

[36] a) Bailey, T. R.; Rippin, S. R.; Opsitnick, E.; Burns, C. J.; Pevear, D. C.; Collett, M. S.; Rhodes, G.; Tohan, S.; Huggins, J. W.; Baker, R. O.; Kern, E. R.; Keith, K. A.; Dai, D.; Yang, G.; Hruby, D.; Jordan, R. *J. Med. Chem.* **2007**, *50*, 1442−1444; b) Grosenbach, D. W.; Berhanu, A.; King, D. S.; Mosier, S.; Jones, K. F.; Jordan, R. A.; Bolken, T. C.; Hruby, D. E.; *Proc. Nat. Acad. Sci.* **2010**, *107*, 838−843.

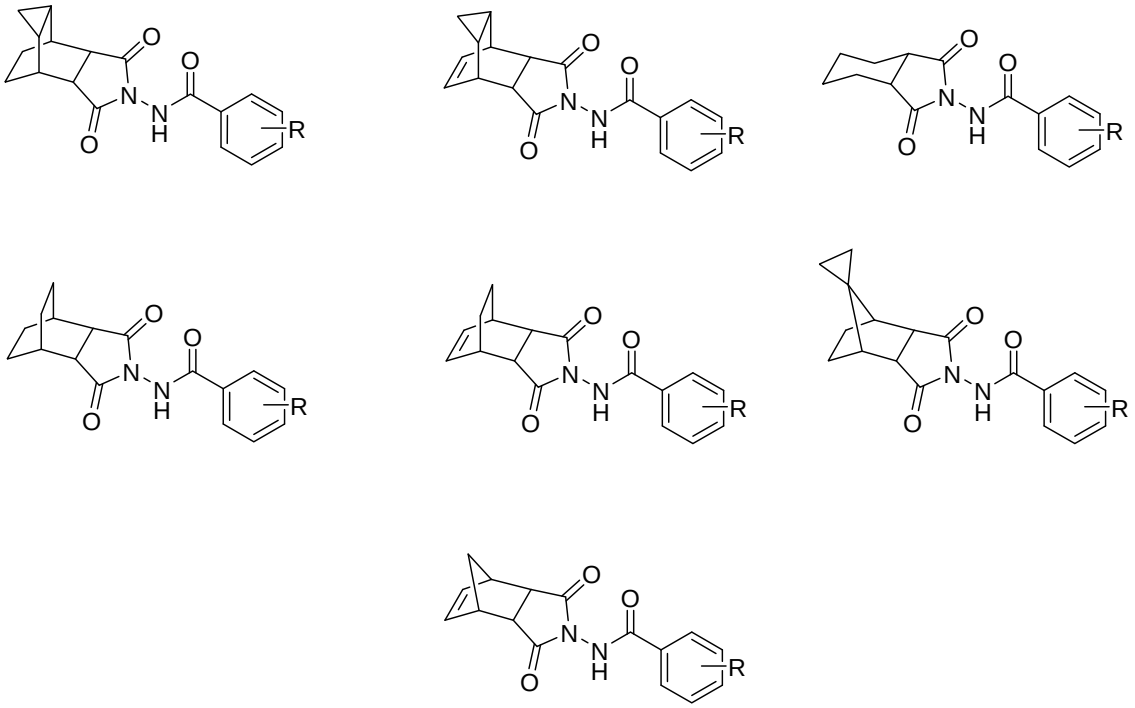
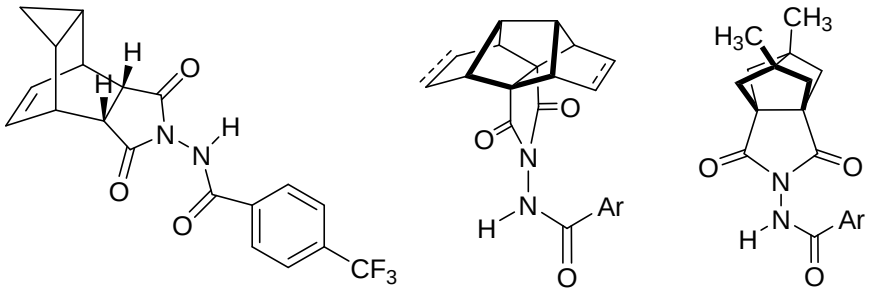


**Figure 15.** Polycyclic scaffolds tested against orthopoxviruses by Siga Technologies.

Thus, Loli Duque, in her doctoral thesis, synthesized new polycyclic benzamides with general structures **I** and **II** (Figure 16). Several aromatic derivatives were synthesized in order to carry out the structure-activity relationship (SAR) needed to rationalize which groups were better for improving the activity of the benzamides against vaccinia virus.

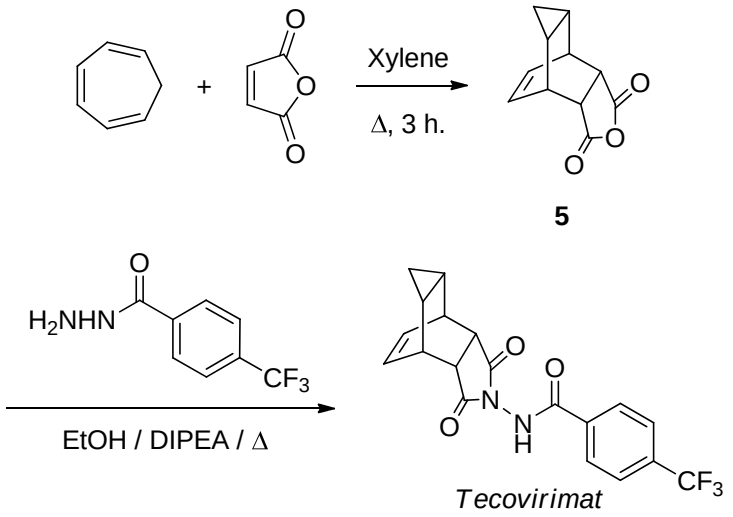


*Tecovirimat* **I** **II**

**Figure 16.**

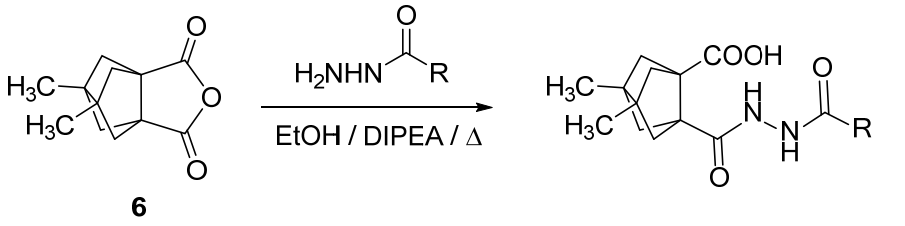
To obtain the benzamides with general structure **II** we followed the methodology described by Siga for synthesizing *tecovirimat*. Following a classic work by K. Alder, the reaction of maleic anhydride with cycloheptatriene led to anhydride **5**.[37] Then, the condensation of this anhydride with 4-(trifluoromethyl)benzohydrazide in absolute ethanol and two drops of diisopropylethylamine furnished *tecovirimat* in very high yield (Scheme 1).

[37] a) Leitich, J.; Sprintschnik, G. *Chem. Ber.* **1986**, *119*, 1640-1660; b) Alder, K.; Jacobs, G. *Chem. Ber.* **1953**, *86*, 1528-1539.




**Scheme 1:** Synthesis of *tecovirimat*.

Following this procedure, we carried out the reaction of anhydride **6**,[11a] previously reported by our group, with a series of benzohydrazides in refluxing ethanol in the presence of a catalytic amount of diisopropylethylamine. In these reactions, we did not obtain the expected imides, but the carboxylic acid derivatives **7-15**, which were fully characterized (Scheme 2).

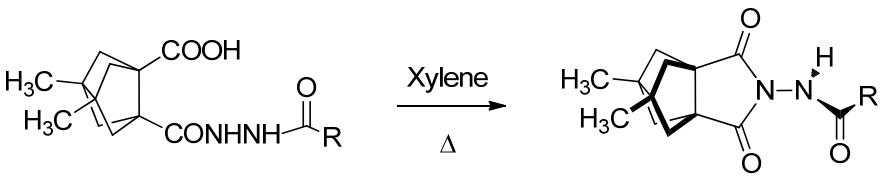



**6**

**7**, R = 4-trifluoromethylphenyl.
**8**, R = 4-bromophenyl.
**9**, R = 3-bromophenyl.
**10**, R = 4-nitrophenyl.
**11**, R = 4-chlorophenyl.
**12**, R = 4-methoxyphenyl.
**13**, R = 4-methylphenyl.
**14**, R = 4-pyridyl.
**15**, R = phenyl.

**Scheme 2.**

Dehydratation of all these compounds in refluxing xylene using a Dean-Stark system gave us the cyclic benzamides in nearly quantitative yields (Scheme 3).




**7**, R = 4-trifluoromethylphenyl.
**8**, R = 4-bromophenyl.
**9**, R = 3-bromophenyl.
**10**, R = 4-nitrophenyl.
**11**, R = 4-chlorophenyl.
**12**, R = 4-methoxyphenyl.
**13**, R = 4-methylphenyl.
**14**, R = 4-pyridyl.
**15**, R = phenyl.

**16**, R = 4-trifluoromethylphenyl.
**17**, R = 4-bromophenyl.
**18**, R = 3-bromophenyl.
**19**, R = 4-nitrophenyl.
**20**, R = 4-chlorophenyl.
**21**, R = 4-methoxyphenyl.
**22**, R = 4-methylphenyl.
**23**, R = 4-pyridyl.
**24**, R = phenyl.

**Scheme 3.**

The preferred conformation of these compounds could be deduced from their NMR spectra and it should be pointed out that is the same than the one obtained by X-ray diffraction of a single crystal of the compound **24**. In agreement with the NMR data, the plane that contains the cyclic imide (CONCO) is perpendicular to the one that contains the benzamide group (Figure 17).

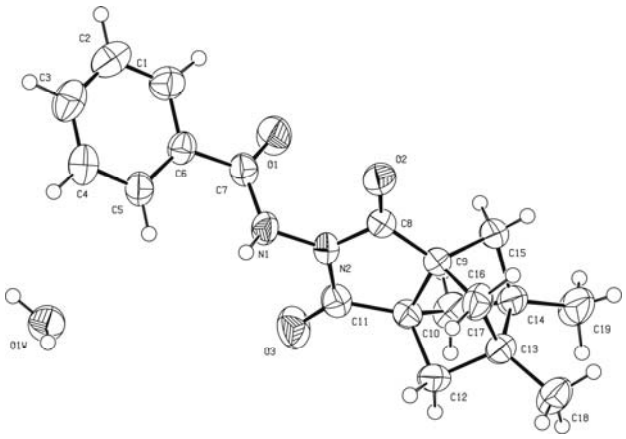
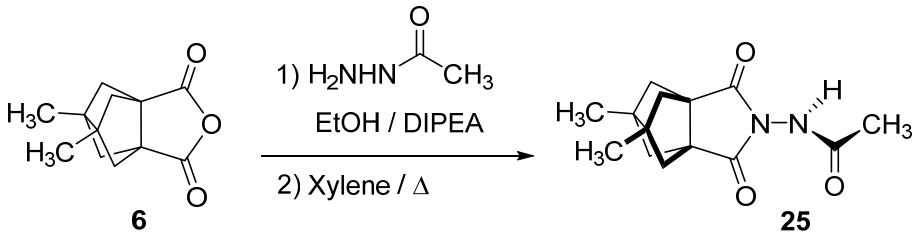


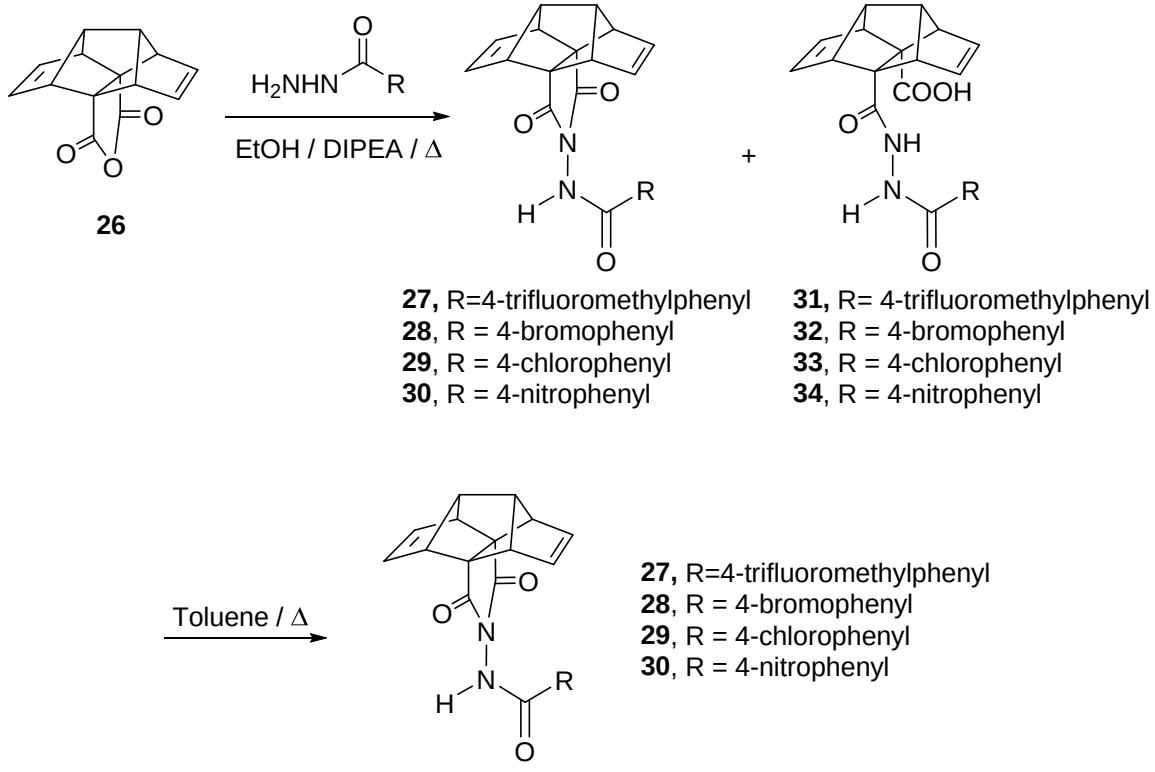

**Figure 17.** X-Ray difraction (ORTEP) structure of **24.**

Finally, the reaction of the anhydride **6** with acetohidrazide under reflux of absolute ethanol and some drops of diisopropylethylamine gave a mixture of the benzamide **25** and its not cyclic precursor. The dehydratation of this mixture in refluxing xylene using a Dean-Stark system yielded the benzamide **25** with 62% overall yield (Scheme 4).




**Scheme 4.**

On the other hand, from the known anhydride **26** Loli Duque synthesized benzamides **27**-**30** (Scheme 5). In this scaffold the reaction gave, initially, a mixture of the expected benzamide **27-30** and the acid **31-34** that we cyclized to the desired compounds using a Dean-Stark system.



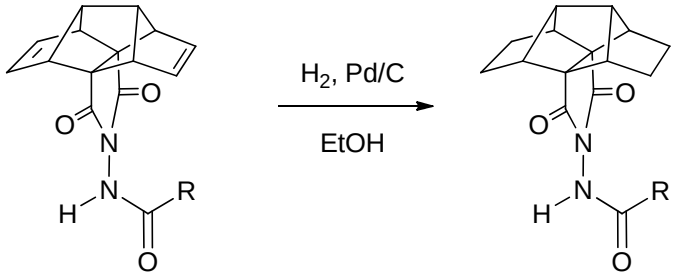

**27,** R=4-trifluoromethylphenyl
**28**, R = 4-bromophenyl
**29**, R = 4-chlorophenyl
**30**, R = 4-nitrophenyl

**31,** R= 4-trifluoromethylphenyl
**32**, R = 4-bromophenyl
**33**, R = 4-chlorophenyl
**34**, R = 4-nitrophenyl

**27,** R=4-trifluoromethylphenyl
**28**, R = 4-bromophenyl
**29**, R = 4-chlorophenyl
**30**, R = 4-nitrophenyl

**Scheme 5.**

From dienes **27** and **29**, Loli Duque also synthesized benzamides **31** and **32**, with the polycyclic scaffold fully saturated (Scheme 6).




**27**, R = 4-trifluoromethylphenyl
**29**, R = 4-chlorophenyl

**31**, R = 4-trifluoromethylphenyl
**32**, R = 4-chlorophenyl

**Scheme 6.**

The pharmacological evaluation of these compounds was conducted in *The Rega Institute for Medical Research* (Leuven, Belgium) by the group of Prof. Erick de Clercq and Lieve Naesens. Worthy of note, Prof. Erick de Clercq is one of the greatest authorities in the antiviral research, being one of the co-inventor of several antiviral drugs currently in clinical use, such as *valaciclovir* (against herpes simplex virus), *brivudine* (against varicella-zoster), *cidofovir* (against citomegalovirus), *adefovir* (against hepatitis B virus) and *tenofovir* (against HIV).[38] We initially started our collaboration with Prof. De Clercq, but when he retired our collaboration with the Rega Institute was followed by Prof. Lieve Naesens.

All these benzamides were tested for antiviral activity against a wide range of DNA and RNA viruses. Moreover, the cytotoxicity of the compounds was also assessed. Overall, the assays carried out at the Rega Institute involved the following viruses:

a) Influenza A and influenza B virus, in MDCK cells.
b) parainfluenza-3 virus, reovirus-1, sindbis virus, coxsackie virus B4, punta toro virus, in Vero cells.
c) herpes simplex virus-1, herpes simplex virus-2, vesicular stomatitis virus, human adenovirus-2, **vaccinia virus**, in HEL cells.
d) stomatitis vesicular virus, coxsackie virus B4 and sincitial respiratory virus, in HeLa cells.
e) herpes virus type 6A and 6B (HHV-6A and HHV-6B) in MOLT-3 and HSB-2 cells.

The compounds showed activity only against vaccinia virus. As previously noted in the introduction, as a consequence of the danger that involves the use of variola virus, the normal way of testing anti-variola virus activity is to assay the potency of the compound first against vaccinia virus. If activity against vaccinia virus is found, assays against other orthopoxvirus, mainly camelpox virus, cowpox virus and monkeypox virus, are carried out. Finally, if all the results are positive it is possible to ask for permissions to test them against variola virus. This

[38] a) De Clercq, E. *Med. Res. Rev.* **2008**, *28*, 929-953; b) De Clercq, E. *Med. Res. Rev.* **2009**, *29*, 571-610; c) De Clercq, E. *Med. Res. Rev.* **2009**, *29*, 611-645; d) De Clercq, E. *Med. Res. Rev.* **2010**, *30*, 667-707; e) De Clercq, E. *Med. Res. Rev.* **2011**, *31*, 118-160.

approach has been validated because all the orthopoxvirus species have similar sensitivity to a given drug. For example, *tecovirimat* has $IC_{50}$ of 65, 10, 10, 20, 50 nM against vaccinia, monkeypox, campelpox, variola-BUT and variola-BSH, respectively.

Table 2 collects the activities of our *tecovirimat* analogues against vaccinia virus in HEL cell cultures. Interestingly, any compound was cytotoxic for the HEL cells at the highest concentration tested (100 μM).

**Table 2.** Anti vaccinia and cytotoxicity activity in HEL cell cultures.

| Compound | Antiviral activity $IC_{50}$ [a] | Toxicity [b] |
|---|---|---|
| **27** | 1.2 (n=3) | >100 |
| **28** | 7.3 (n=3) | >100 |
| **29** | 8.3 (n=3) | >100 |
| **31** | 13.7 (n=3) | >100 |
| **32** | >100 | NE |
| **16** | >100 | >100 |
| **17** | 100 | >100 |
| **18** | >100 | >100 |
| **19** | 45 | >100 |
| **20** | >100 | >100 |
| **21** | >100 | >100 |
| **22** | >100 | >100 |
| **23** | 45 | >100 |
| **24** | >100 | >100 |
| **25** | >100 | >100 |
| ***Tecovirimat*** | 0.1 (n=2) | >200 |
| ***Brivudine*** | 4 (n=3) | >250 |
| ***Cidofovir*** | 4 (n=3) | >250 |
| ***Ganciclovir*** | >100 (n=3) | >100 |

[a] $CE_{50}$: Required concentration (μM) to reduce the cytopatogenicity of the virus up to 50%.
[b] Minimum cytotoxic concentratrion (μM): required concentration to cause a microscopically detectable alteration of normal cell morphology.

From the results shown in Table 1, several conclusions can be drawn:

a) An EWG is essential for the activity of the compound. In the bisnoradamantane series, only **19** (with a $NO_2$ group) and **23** (with a 4-pyridyl group) had some antiviral activity.

b) The bisnoradamantane benzamides are markedly less active than the hexacyclic ones, i. e., compare: **20** vs **29**, **17** vs **28**, **16** vs **27** and **31**.

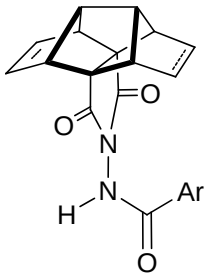
c) For a given diene, a significant reduction of potency is found on going to its corresponding hydrogenated derivative, i. e. **27** vs **31** and **29** vs **32**.

d) Worthy of note, although our most potent compound in this series, **27**, is not as active as *tecovirimat*, is more active than *cidofovir,* the only compound that is approved for emergency use in the event of a smallpox outbreak.

When we received these very promising results, Loli Duque had already finished her thesis, so, at the beginning of this Thesis, we decided to pursue the synthesis of even more potent analogues of **27**.

# PART I: OBJECTIVES

As we have already mentioned in the introduction, our group had synthesized several benzamides with the general structure **I** (Figure 18) that showed very promising anti-vaccinia virus activity.




**I**

**Figure 18**

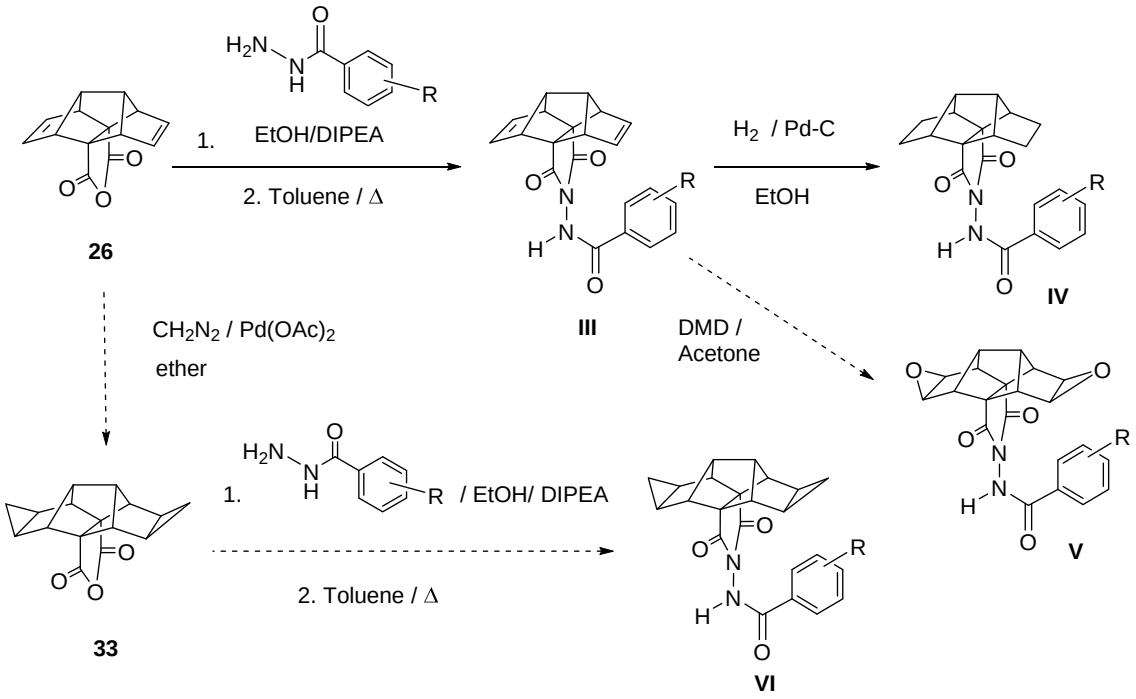
Taking into account these results, at the beginning of this Thesis, we decided to pursue additional synthetic work on this topic, in order to further explore the structure-activity relationship within this family of polycyclic compounds. Our aim was to carry out the synthesis of further dienes with different EWG substituents in the aromatic ring at different positions. On the other hand, we also wanted to synthesize derivatives carrying additional functionality in the polycyclic scaffold.

Thus, for the present work we envisioned two objectives:

a) to synthesize new analogs of general structure **I**, including 4-substituted, 3,4-disubstituted and, finally, 3,4,5-trisubstituted benzamides, in order to know which were the best substitutents in the benzene ring for optimum antiviral activity, and,

b) to modify the policyclic cage in order to further explore the structure-activity relationships. Thus, while Loli Duque made some saturated derivatives in order to increase the lipophilicity of the compounds, we planned to carry out the epoxidation of the two carbon-carbon double bonds in order to increase the polarity, and to attempt the cyclopropanation of the two carbon-carbon double bonds, in order to increase the volume of the scaffold.

In order to fulfil the synthetic goals of this Thesis, we planned to carry out the cyclopropanation of the known anhydride **26** that could led to a new anhydride, **33**. The reaction of anhydride **33** with a series of benzohydrazides following the standard procedure, should furnish a new series of benzamides of general structure **VI**. On the other hand, epoxidation of benzamides of general structure **III** with dimethyldioxirane (DMD) should furnish diepoxides of general structure **V**. Taking into account that the saturated derivatives of general structure **IV** showed to be less potent that their corresponding dienes, no further derivatives of this kind were planned.


1. EtOH/DIPEA
2. Toluene / Δ
H2 / Pd-C
EtOH
26
III
IV
CH2N2 / Pd(OAc)2
ether
DMD /
Acetone
1. / EtOH/ DIPEA
2. Toluene / Δ
33
V
VI

**Scheme 7.**

# PART I: THEORETICAL PART

## 1. Synthesis of new polycyclic analogues of *tecovirimat*.

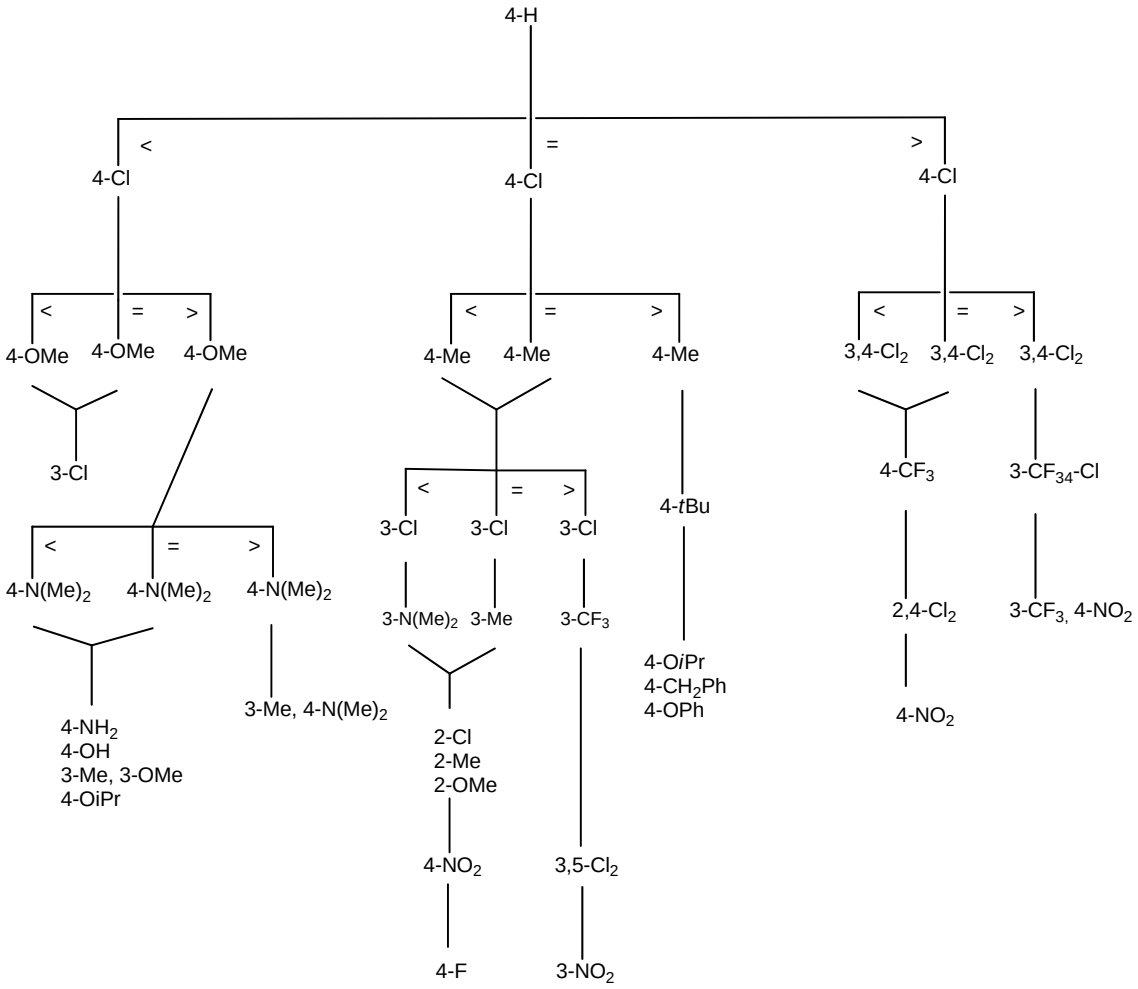
Taking into account the SAR carried out by Siga and our previous work, at the beginning of this Thesis we decided to enlarge the family of benzamides in order to rationalize which structural features were essential to get a very potent anti-vaccinia virus activity.

A typical procedure used in medicinal chemistry to study the SAR on aromatic derivatives is to follow a classical "Topliss diagram", a tool commonly used when a series of compounds are difficult to synthesize and it would be desirable that each new compound leads to a higher potency.[39] Furthermore, it is frequently used when the pharmacological assays are less expensive and less time-consuming than the synthesis. In the context of the foregoing discussion the following Topliss diagram was considered (Scheme 8).[40,41]

For example, if for a given scaffold, the unsubstituted aromatic ring is more potent than the *p*-chloro derivative, the left branch of the scheme must be followed. Then, it is suggested to try with the *p*-methoxy derivative. If, for example, the *p*-methoxy derivative happens to be more active than the *p*-chloro, the synthesis of the *p*-dimethylamino derivative should be done next, and again its activity must be compared with the previous compound. So, if the *p*-dimethylamino derivative is less or equally active than the *p*-methoxy derivative, the *p*-amino, *p*-isopropoxide and *m*-methyl or *m*-methoxy should be synthesized. It has been proved that the Topliss scheme is a reliable tool in medicinal chemistry when applied to benzene derivatives.[42]

Taking into account that both, in the *tecovirimat* analogues studied by Siga technologies and in the compounds synthesized by Loli Duque in her Thesis, the *p*-chloro derivative was more potent than the unsubstituted benzene ring, we started a new series of derivatives following the right branch of the Topliss scheme.

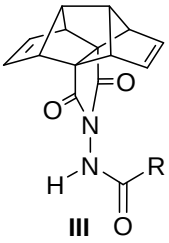
[39] Delgado, A.; Minguillón, C.; Joglar, J. *Introducción a la Química Terapéutica 2ª ed*., **2003**, 167-168.
[40] Topliss, J. G.; *J. Med. Chem*. **1972**, *15*, 1006-1011.
[41] Topliss, J. G.; *J. Med. Chem*. **1977**, *20*, 463-469.
[42] A search in the ISI Web of knowledge (July, 9th 2013) revealed that reference 40 has been cited more than 300 times, while reference 41 has been cited more than 170 times.




**Scheme 8.** Topliss scheme for aromatic substitution.

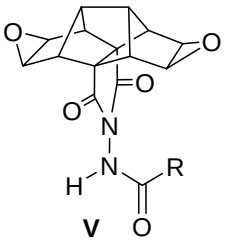
Thus, the 3,4-dichloro compound was selected as the next synthetic target since this would result in both higher lipophilicity and electron withdrawing values. Additionally, we decided to synthesize several compounds that may be as potent as the 3,4-dichloro derivative such as the 3-bromo-4-chloro, 4-bromo-3-chloro, 3-methyl-4-nitro, 4-chloro-3-pyridyl, 4-chloro-3-trifluoromethyl, 3,4-difluoro and 3,4,5-trifluoro derivatives.

We also tried some other EWG in the *para* position like the nitro group and the 4-pyridyl derivative because both substituents worked well in the bisnoradamantane serie (see compounds **19** and **23** in table 1). Finally, we also synthesized related compounds such as the 3,5-bis(trifluoromethyl), 3,5-dichloro and 3-trifluoromethyl derivatives, these compounds being synthesized with the aim of checking the effect of these substituents in the *meta* position (Scheme 9).



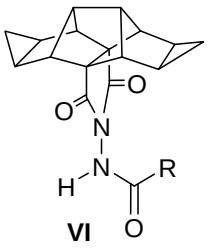
**III**

R = 4-pyridyl
R = 3,5-bis(trifluoromethyl)phenyl
R = 4-nitrophenyl
R = 3,4-dichlorophenyl
R = 3-(trifluoromethyl)phenyl
R = 3,5-dichlorophenyl
R = 4-bromo-3-chlorophenyl
R = 3-bromo-4-chlorophenyl
R = 3-methyl-4-nitrophenyl
R = 6-chloro-3-pyridyl
R = 3,4,5-trifluorophenyl
R = 4-chloro-3-trifluoromethylphenyl
R = 3,4-difluorophenyl



**V**

R = 4-nitrophenyl
R = 3,4-dichlorophenyl
R = 3,5-bis(trifluoromethyl)phenyl
R = 4-trifluoromethylphenyl



**VI**

R = 4-trifluoromethylphenyl
R = 4-bromo-3-chlorophenyl
R = 3-methyl-4-nitrophenyl
R = 3,4-dichlorophenyl
R = 3-bromo-4-chlorophenyl
R = 6-chloro-3-pyridyl

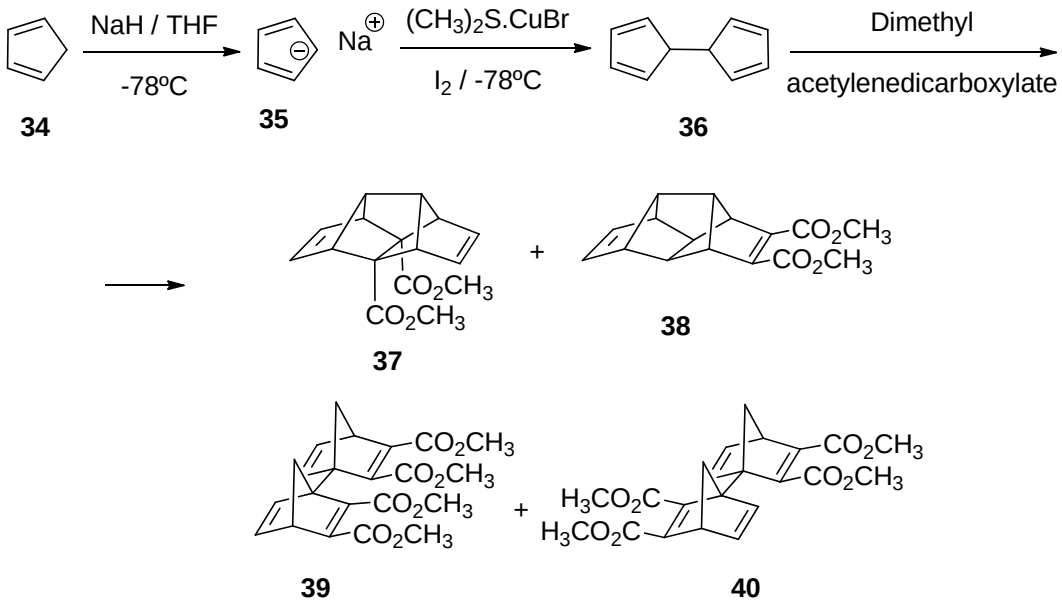
**Scheme 9.** Compounds to be synthesized.

### 1.1. Synthesis of pentacyclo[6.4.0.0$^{2,10}$.0$^{3,7}$.0$^{4,9}$]dodeca-5,11-dien-8,9-dicarboxylic anhydride, 26.

For the synthesis of the new benzamides we started from the known anhydride **26**, previously described by our group.[43] The synthesis of **26** involves the hydrolysis and dehydratation of diester **37**, in turn easily available in multi-gram scale using the tandem reaction shown in Scheme 10.

The synthesis of diester **37** was simultaneously described by Hedaya's and Paquette's research groups in 1974.[44] First, 9,10-dihydrofulvalene, **36**, is generated *in situ* by low-temperature iodine-promoted oxidative coupling of the sodium salt of cyclopentadiene, **35**, in turn obtained from the reaction of cyclopentadiene with sodium hydride. Then, dimethyl acetylenedicarboxylate is added to the cool solution of **36** and a double Diels-Alder reaction occurs,[45] leading to pentacyclic diesters **37** and **38** along with a very complex mixture of side-products, tetraesters **39** and **40** being the major impurities.[46]

[43] Camps, P.; Pujol, X.; Rossi, R. A.; Vázquez, S. *Synthesis* **1999**, 854-858.
[44] a) Paquette, L. A.; Wyvratt, M. J. *J. Am. Chem. Soc.* **1974**, *96*, 4671-4673. b) Neil, D.; Vogt, B. R.; Sudol, J. J.; Theodoropulos, S.; Hedaya, E. *J. Am. Chem. Soc.* **1974**, *96*, 4673-4674.
[45] Review of tandem Diels-Alder reactions: Winkler, J. D. *Chem. Rev.* **1996**, *96*, 167-176.
[46] For a theorical study of this process: Domingo, L. R.; Arnó, M.; Andrés, J. *Tetrahedron Lett.* **1996**, *37*, 7573-7576.

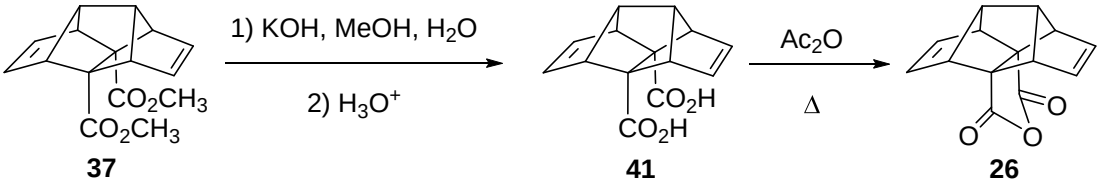



**Scheme 10.**

In the original works by Hedaya and Paquette, the purification of these compounds was very laborious involving a very difficult fractional distillation. Fortunately, Paquette's group later reported an improved method for the purification of diester **37** that allow the synthesis of this product in a 30 g-scale.[47]

According to this improved procedure, the reaction residue which contains the four products is first suspended in diethyl ether. Since tetraesters **39** and **40** are insoluble in diethyl ether they can be easily removed by filtration. Next, the mixture of **37** and **38** are reacted with KOH in methanol at room temperature. Under this mild conditions, **38** is hydrolized to the corresponding diacid, while **37** remains unchanged, probably because of the greater steric hindrance of the ester groups, and a simple acid / base wash allows the purification of the desired diester **37** that is finally isolated with an overall yield of 10-12%.

Under more vigorous conditions, **37** can be hydrolized to the correspoding diacid **41** in high yield (89%). Finally, the reaction of **41** with acetic anhydride under reflux for 1 hour followed by sublimation yields the anhydride **26** in almost quantitative yield (Scheme 11).[43]

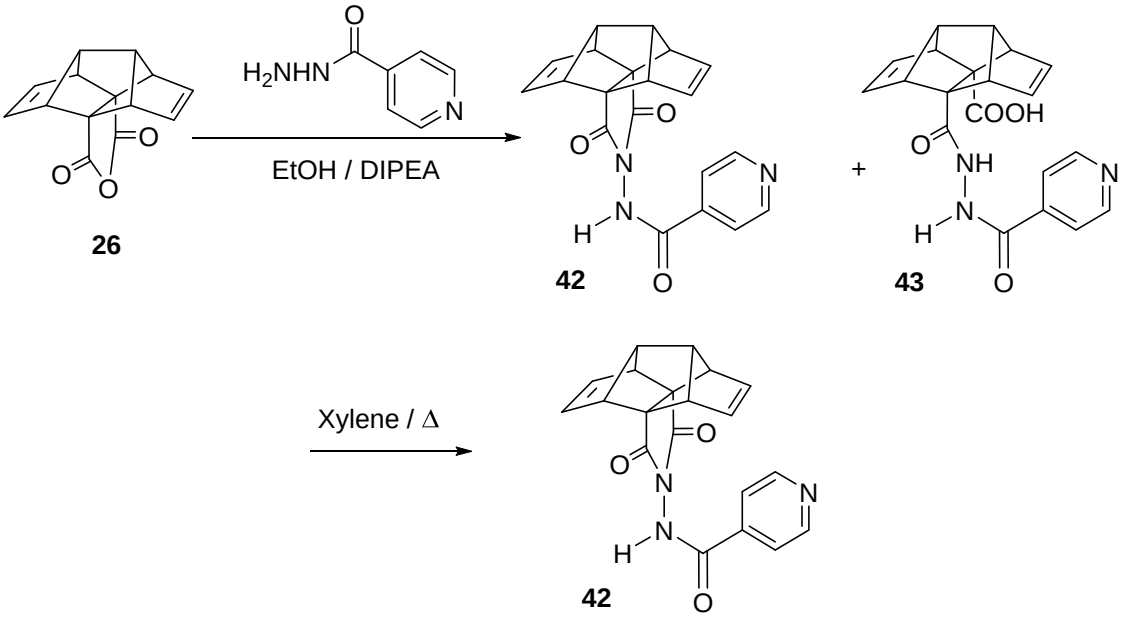



**Scheme 11.**

[47] Taylor, R. J.; Welter, M. W.; Paquette, L. A. *Org. Synth. Coll. VIII* **1993**, 298-302.

**1.2 Synthesis of benzamides containing the diene functionality.**

Once we had the starting material, we proceeded to synthesize the benzamides following the general procedure disclosed by Siga Technologies. Thus, the reaction of anhydride **26** with isoniazide in absolute ethanol containing some drops of diisopropylethylamine (DIPEA) yielded the desired benzamide **42** (56% yield) along with some of its precursor, **43**. No attempts to separate this mixture were carried out, as it was easily converted into pure **42** by refluxing it in xylene overnight using a Dean-Stark system (Scheme 12).




**26** **42** **43** **42**

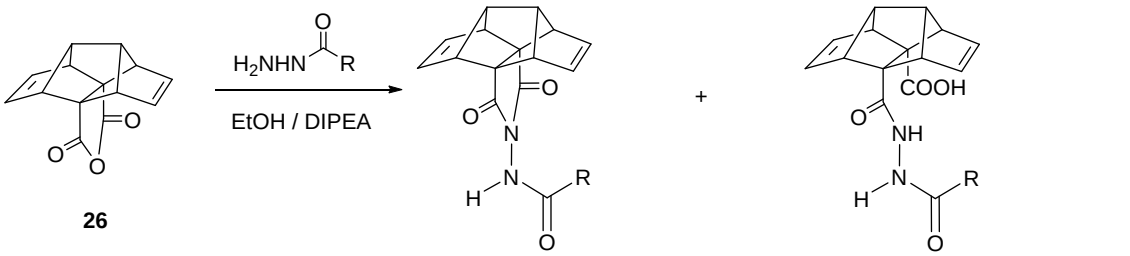
**Scheme 12.**

Similarly, the reaction of anhydride **26** with 3,5-bis(trifluoromethyl)benzohydrazide, 4-nitrobenzohydrazide, 3,4-dichlorobenzohydrazide, 3-(trifluoromethyl)benzohydrazide, 3,5-dichlorobenzohydrazide, 4-bromo-3-chlorobenzohydrazide, 3-bromo-4-chlorobenzohydrazide, 3-methyl-4-nitrobenzohydrazide, 6-chloropyridine-3-carbohydrazide, 3,4,5-trifluorobenzohydrazide, 4-chloro-3-(trifluoromethyl)benzohydrazide and 3,4-difluorobenzohydrazide, followed by a thermally-induced dehydratation led to the expected *tecovirimat* analogues in high yields (Scheme 13).

It should be mentioned that most of the starting benzohydrazides were commercially available products. However, we had to synthesized 3,4,5-trifluorobenzohydrazide, 4-chloro-3-(trifluoromethyl)benzohydrazide and 3,4-difluorobenzohydrazide. These products were synthesized in a general two-step procedure previously described in the literature for related benzohydrazides.[48] First, the corresponding benzoic acid was subjected to Fisher esterification to obtain the expected methyl benzoate. Finally, reaction of the methyl benzoates with

[48] Welch, D. E.; Baron, R. R.; Burton, B. A. *J. Org. Chem.* **1969**, *12*, 299-302.

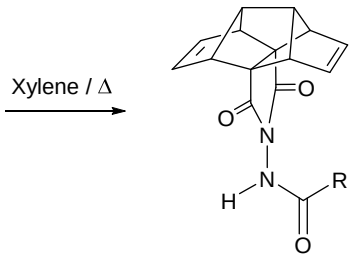
hydrazine monohydrate in ethanol furnished the corresponding benzohydrazides in very high yields (see experimental section for further details).




**26**

**44**, R = 3,5-bis(trifluoromethyl)phenyl
**45**, R = 4-nitrophenyl
**46**, R = 3,4-dichlorophenyl
**47**, R = 3-trifluoromethylphenyl
**48**, R = 3,5-dichlorophenyl
**49**, R = 4-bromo-3-chlorophenyl
**50**, R = 3-bromo-4-chlorophenyl
**51**, R = 3-methyl-4-nitrophenyl
**52**, R = 6-chloro-3-pyridyl
**53**, R = 3,4,5-trifluorophenyl
**54**, R = 4-chloro-3-trifluoromethylphenyl
**55**, R = 3,4-difluorophenyl

**56**, R = 3,5-bis(trifluoromethyl)phenyl
**57**, R = 4-nitrophenyl
**58**, R = 3,4-dichlorophenyl
**59**, R = 3-trifluoromethylphenyl
**60**, R = 3,5-dichlorophenyl
**61**, R = 4-bromo-3-chlorophenyl
**62**, R = 3-bromo-4-chlorophenyl
**63**, R = 3-methyl-4-nitrophenyl
**64**, R = 6-chloro-3-pyridyl
**65**, R = 3,4,5-trifluorophenyl
**66**, R = 4-chloro-3-trifluoromethylphenyl
**67**, R = 3,4-difluorophenyl



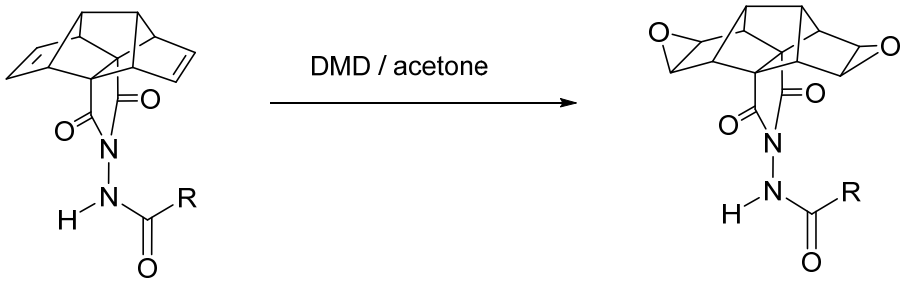

**44**, R = 3,5-bis(trifluoromethyl)phenyl, 48%.
**45**, R = 4-nitrophenyl, 48%.
**46**, R = 3,4-dichlorophenyl, 55%.
**47**, R = 3-trifluoromethylphenyl, 57%.
**48**, R = 3,5-dichlorophenyl, 90%.
**49**, R = 4-bromo-3-chlorophenyl, 70%.
**50**, R = 3-bromo-4-chlorophenyl, 84%.
**51**, R = 3-methyl-4-nitrophenyl, 51%.
**52**, R = 6-chloro-3-pyridyl, 75%.
**53**, R = 3,4,5-trifluorophenyl, 28%.
**54**, R = 4-chloro-3-trifluoromethylphenyl, 74%.
**55**, R = 3,4-difluorophenyl, 96%.

**Scheme 13.** Synthesized products with the corresponding yields.

### 1.3 Synthesis of benzamides without the diene functionality.

After finishing the synthesis of the aforementioned derivatives, we decided to further explore the SAR of this family of derivatives by changing the carbon-carbon double bonds by different functionalities.

From the work carried out in the PhD Thesis of Dolores Duque, we already knew that the hydrogenation of the double bonds led to less potent compounds, so in this Thesis a different set of derivatives were synthesized. Firstly, we increase the polarity of the polycyclic scaffold by epoxidazing the two carbon-carbon double bonds. Thus, four benzamides were epoxidized in almost quantitative yield using a solution of dimethyldioxirane (DMD) in acetone (Scheme 14).

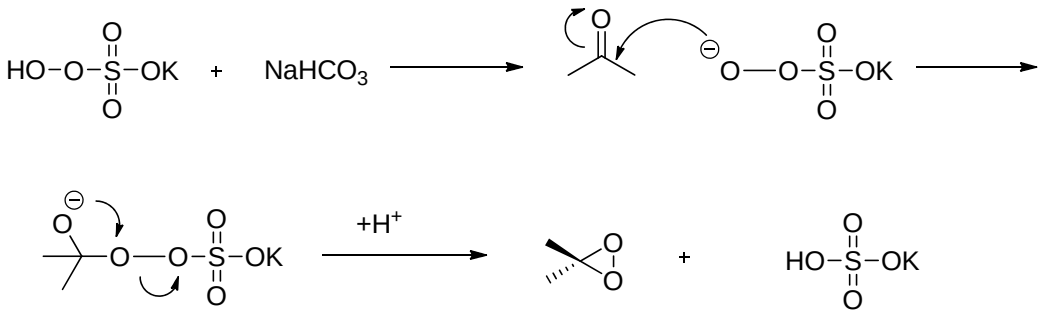



**27**, R = 4-trifluoromethylphenyl
**44**, R = 3,5-bis(trifluoromethyl)phenyl
**45**, R = 4-nitrophenyl
**46**, R = 3,4-dichlorophenyl

**56**, R = 4-trifluoromethylphenyl, 100%.
**57**, R = 3,5-bis(trifluoromethyl)phenyl, 100%.
**58**, R = 4-nitrophenyl, 88%.
**59**, R = 3,4-dichlorophenyl, 99%.

**Scheme 14.**

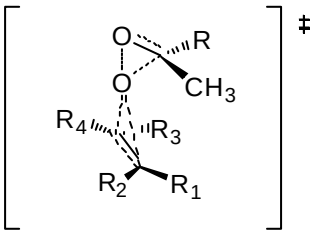
DMD is a gas that is usually prepared and stored at low temperature in acetone solution. In order to generate it, $NaHCO_3$ and Oxone® (potassium peroxomonosulfate) are added to a mixture of acetone/water 1:1. The DMD is thus generated and is co-distilled with acetone giving a solution of DMD in acetone typically of approximately 0.1 M.[49] The mechanism of this reaction occurs as follow (Scheme 15):[50]




**Scheme 15.**

Nowadays, DMD is widely used for olefin epoxidation as the reaction is very clean, fast and very high-yielding. DMD is an electrophilic oxidizing agent that transfers its oxygen through a spiro transition state.[51] The spiro transition state is stabilized by the interaction of the oxygen lone pair electrons with the π* orbital of the alkene (Scheme 16).[52] The reaction is stereospecific and *cis* as both new C-O bonds add on the same face of the alkene.

[49] Baumstark, A. L.; McCloskey, C. J. *Tetrahedron Lett* **1987**, *28*, 3311-3314.
[50] Adam, W.; Curci, R.; Edwards, J. O. *Acc. Chem. Res*. **1989**, *22*, 205-211.
[51] Adam, W.; Smerz, A. K. *J. Org. Chem*. **1996**, *61*, 3506-3510.
[52] Shi, Y. *Acc. Chem. Res*. **2004**, *37*, 488-496.




**Scheme 16.** spiro transition state for the oxygen-atom transfer from DMD to an olefin.

For our dienes, the reaction was fully stereoselective, both epoxides being formed by the *exo* face of the policyclic cage, moving away from the benzamide moiety. This stereoselectivity was proved by X-ray diffraction of a single crystal of the compound **57**, as shown in Figure 19. Interestingly, the plane of the benzamido substituent is essentially orthogonal to the plane of the imide group.

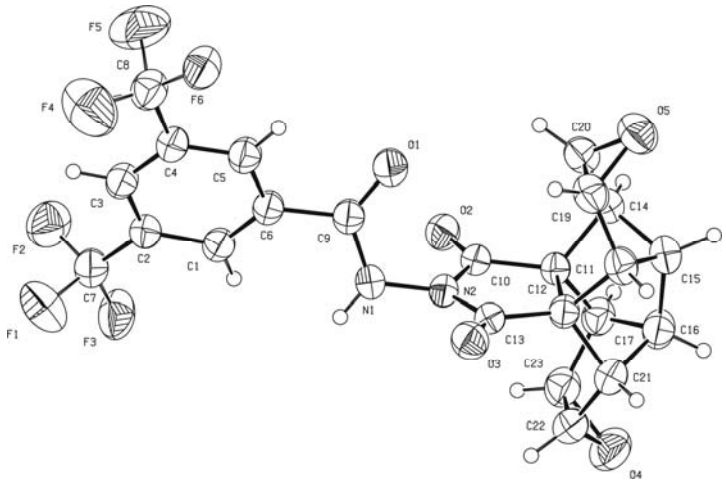
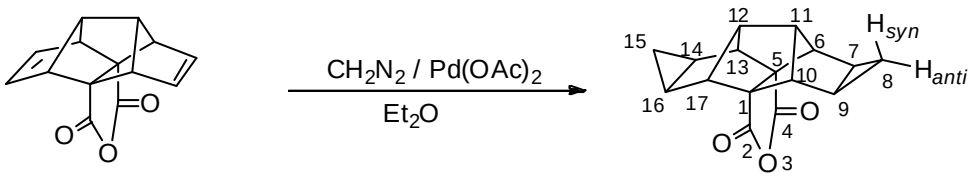



**Figure 19.** X-ray difraction structure (ORTEP) of **57**.

On the other hand, we carried out the cyclopropanation of the double bonds of the polycyclic scaffold in order to increase the volume of the molecule. Thus, the reaction of anhydride **26** with an excess of an ethereal solution of diazomethane using $Pd(OAc)_2$ as a catalyst led to **33** in 98% yield (Scheme 17).

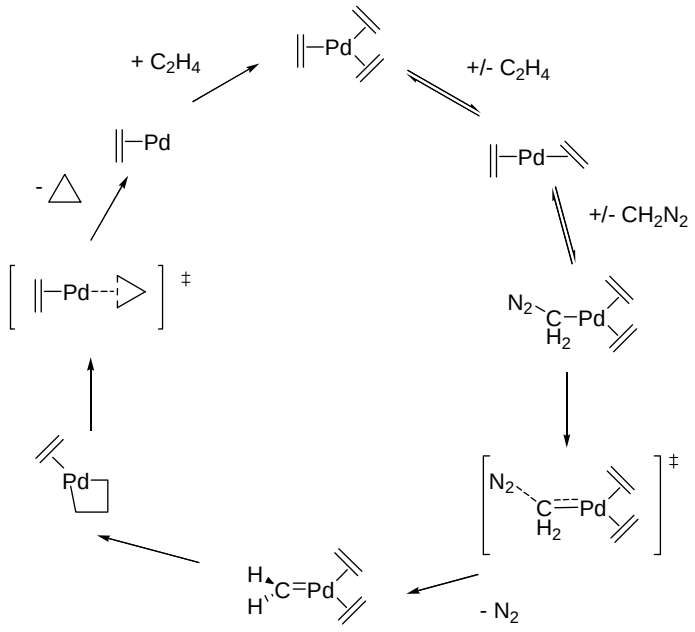



**26** **33**

**Scheme 17.**

The mechanism of this reaction is not clearly understood yet. It is currently assumed that the first step involves the formation of a palladium-carbene complex, resulting from the reaction between palladium (II) and diazomethane.[53] The interaction of the olefin with this complex may lead to the formation of a palladacyclobutane intermediate which by a reductive elimination would give the corresponding cyclopropane.

On the other hand, the ability of palladium to coordinate olefins has led to the postulation of an alternative mechanism in which diazomethane attacks a previously palladium-coordinated olefin. $Pd(OAc)_2$ is thus presumably not the catalyst but the precatalyst of the cyclopropanation reaction. A palladium-coordinated carbene reacts with an olefin coordinated to the same metal via a palladacyclobutane intermediate. This reductively eliminates cyclopropane easily, and subsequent ligand exchange completes the catalytic cycle (Scheme 18).[54]




**Scheme 18.** Catalytic cycle of the Pd species in the cyclopropanation reaction with diazomethane.

As it was the case for the epoxides, the cyclopropanation reaction is fully stereoselective, both cyclopropane rings are in the *exo* face of the polycyclic cage, moving away from the benzamide moiety. 8-$H_{syn}$ / 11-H and 15-$H_{syn}$ / 12-H cross-peaks in the NOE spectrum of **33** strongly support this stereochemistry. Moreover, the stereoselectivity of the reaction was unequivocally established by X-ray diffraction analysis of a single crystal of the compound **33** (Figure 20).

[53] a) Kottwitz, J.; Vorbrüggen, H. *Synthesis* **1975***,* 636-637. b) Illa, O.; Rodríguez-García, C.; Acosta-Silva, C.; Avier, I.; Picurelli, D.; Oliva, A.; Gómez, M.; Branchadell, V.; Ortuño, R. M. *Organometallics* **2007**, *26*, 3306-3314.

[54] Straub, B. F. *J. Am. Chem. Soc*. **2002**, *124*, 14195-14201.

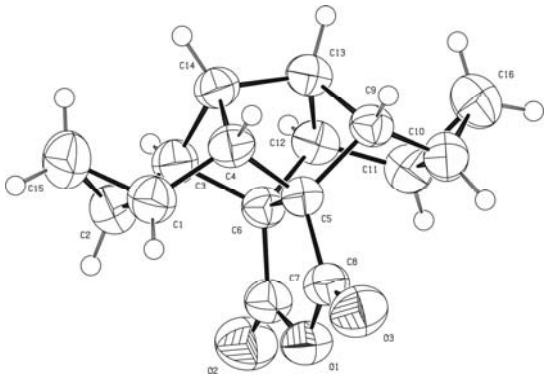
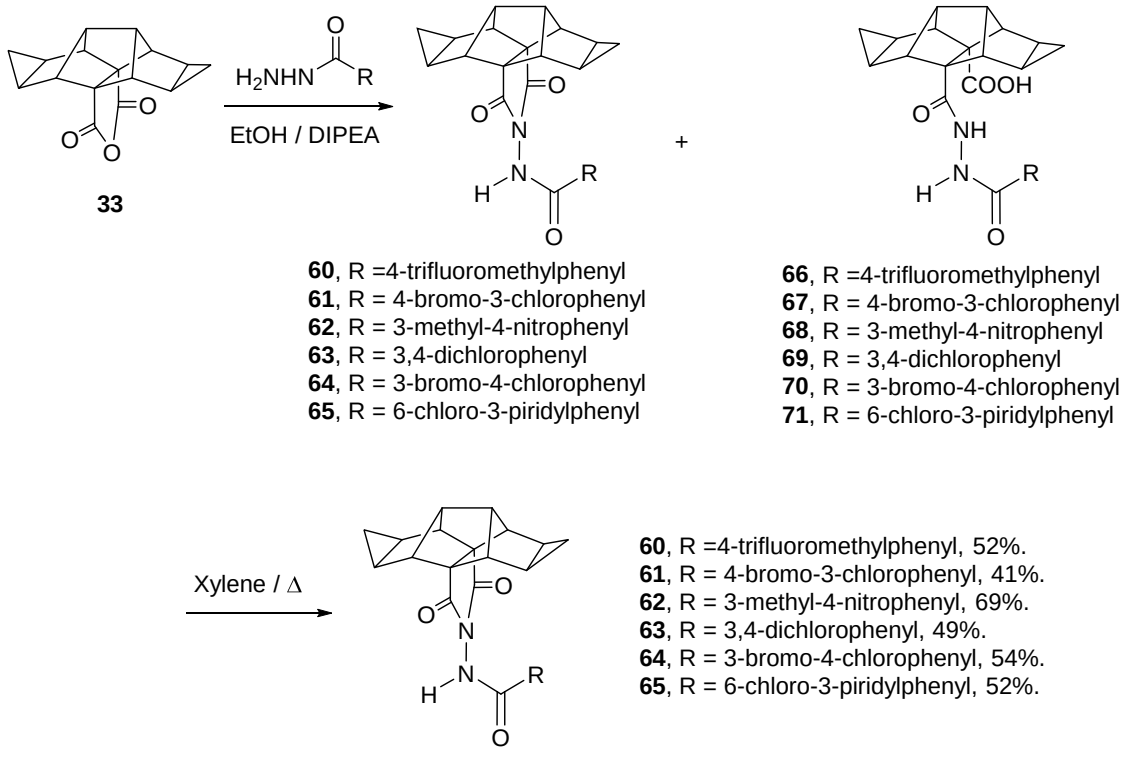



**Figure 20.** X-ray difraction structure (ORTEP) of **33**.

Next, we carried out the reaction of the starting anhydride **33** with a series of benzohydrazides: 4-trifluoromethylbenzohydrazide, 4-bromo-3-chlorobenzohydrazide, 3-methyl-4-nitrobenzohydrazide, 3,4-dichlorobenzohydrazide, 3-bromo-4-chlorobenzohydrazide and 6-chloropyridine-3-carbohydrazide, using the same general procedure previously applied to the diene **26**. As in the previous series, these reactions led to a mixture of two compounds that were treated with xylene under reflux in a Dean-Stark apparatus to give the expected imides in medium overall yields (Scheme 19).




**60**, R =4-trifluoromethylphenyl
**61**, R = 4-bromo-3-chlorophenyl
**62**, R = 3-methyl-4-nitrophenyl
**63**, R = 3,4-dichlorophenyl
**64**, R = 3-bromo-4-chlorophenyl
**65**, R = 6-chloro-3-piridylphenyl

**66**, R =4-trifluoromethylphenyl
**67**, R = 4-bromo-3-chlorophenyl
**68**, R = 3-methyl-4-nitrophenyl
**69**, R = 3,4-dichlorophenyl
**70**, R = 3-bromo-4-chlorophenyl
**71**, R = 6-chloro-3-piridylphenyl

**60**, R =4-trifluoromethylphenyl, 52%.
**61**, R = 4-bromo-3-chlorophenyl, 41%.
**62**, R = 3-methyl-4-nitrophenyl, 69%.
**63**, R = 3,4-dichlorophenyl, 49%.
**64**, R = 3-bromo-4-chlorophenyl, 54%.
**65**, R = 6-chloro-3-piridylphenyl, 52%.

**Scheme 19.**

## 2. Pharmacological evaluation of the polycyclic benzamides.

The antiviral activity and the cytotoxicity of the benzamides were evaluated in the Rega Institute for Medical Research in Leuven (Belgium) by the research group of Lieve Naesens using a cytopathic effect (CPE) reduction assay. All compounds were tested against a wide variety of DNA and RNA viruses, i.e. herpes simplex virus type 1 and type 2, and vaccinia virus [evaluated in HEL cells]; feline coronavirus and feline herpesvirus [in Crandell-Rees Feline Kidney cells]; vesicular stomatitis virus, Coxsackie B4 virus and respiratory syncytium virus [tested in HeLa cells]; para-influenza-3 virus, reovirus-1, Sindbis virus and Punta Toro virus [tested in Vero cells] and influenza virus [in Madin Darby canine kidney cells].[55] Very interestingly, most of the compounds showed no cytotoxicity (as determined by microscopy) at the highest concentration tested in HEL cells (100 μM). Several of the compounds showed potent activity against vaccinia virus (see Table 2) and none of them displayed considerable activity (defined as an antiviral $IC_{50}$ value of 20 μM or less) against any of the other viruses tested.

As previously stated in the introduction, vaccinia virus is the prototypical virus used for testing the activity against *Orthopoxvirus* as working with *variola virus* has very tough limitations. If a compound is found to be active against vaccinia virus is later tested against other members of the family such as *camelpox virus, cowpox virus, monkeypox virus*, and only if the compound has an outstanding activity against several orthopoxviruses, it may be possible to be allowed to test it directly in cells infected with *variola virus*.

From our previous results and the the careful examination of the results shown in Table 2 (page 33) and 3, it must be concluded that:

a) The compounds showed no cytotoxicity at the highest concentration tested (100 μM) in HEL cells.

b) In order to obtain potent antiviral activity it is essential to have an electron withdrawing group on the *para* position of the aromatic ring. These results are in full agreement with the trend found by Siga Technologies and by us in our previous studies.

c) Interestingly, it is even better to introduce a further electron withdrawing group in the *meta* position, e. g., **29** ($IC_{50}$ = 8.3 μM) vs. **46** ($IC_{50}$ = 0.6 μM) and **50** ($IC_{50}$ = 0.4 μM) or **28** ($IC_{50}$ = 7.3 μM) vs. **49** ($IC_{50}$ = 0.7 μM).

[55] For methods: Derudas, M.; Brancale, A.; Naesens, L.; Neyts, J.; Balzarini, J.; McGuigan, C. *Bioorg. Med. Chem.* **2010**, *18*, 2748-2755.

d) The two carbon-carbon double bonds showed to be essential for good antiviral activity. For any given aromatic ring, reduction, epoxidation or cyclopropanation of the double bonds led to less potent compounds.

e) The trisubstitution of the aromatic ring in the two *meta* and in the *para* position of the aromatic ring decreases the antiviral potency, e. g., **55** ($IC_{50}$ = 16 μM) vs **53** ($IC_{50}$ = 32 μM).

**Table 3:** Antiviral activity and cytotoxicity in vaccinia virus-infected HEL cells. Since the epoxide and cyclopropane containing compounds resulted to be inactive, their data is not shown for clarity.

| Substitution of Ar | Compound | Antiviral activity $IC_{50}$[a] | Toxicity[b] |
|---|---|---|---|
| 4-substituted | **27, R = 4-trifluoromethylphenyl** | 1.2 (n=3) | >100 |
| | **42, R = 4-pyridyl** | >100 | >100 |
| | **45, R = 4-nitrophenyl** | 3.0 (n=2) | >100 |
| 3,4-disubstituted | **46, R = 3,4-dichlorophenyl** | 0.6 (n=3) | 100 |
| | **49, R = 4-bromo-3-chlorophenyl** | 0.7 (n=3) | >100 |
| | **50, R = 3-bromo-4-chlorophenyl** | 0.4 (n=3) | >100 |
| | **51, R = 3-methyl-4-nitrophenyl** | 3 (n=2) | >100 |
| | **54, R = 4-chloro-3-trifluoromethylphenyl** | 0.8 (n=2) | >100 |
| | **55, R = 3,4-difluorophenyl** | 16 (n=2) | >100 |
| Others | **44, R = 3,5-bis(trifluoromethyl)phenyl** | >100 | >100 |
| | **47, R = 3-trifluoromethylphenyl** | 16 (n=2) | >100 |
| | **48, R = 3,5-dichlorophenyl** | >20 | >100 |
| | **52, R = 6-chloro-3-pyridyl** | 48 | >100 |
| | **53, R = 3,4,5-trifluorophenyl** | 32 | >100 |
| Reference compounds | ***Tecovirimat*** | 0.065 (n=3) | >200 |
| | ***Brivudine*** | 4 (n=3) | >250 |
| | ***Cidofovir*** | 4 (n=3) | >250 |
| | ***Ganciclovir*** | >100 (n=3) | >100 |

[a] $IC_{50}$: Required concentration (μM) to reduce virus-induced cytopathogenicity by 50%

[b] Minimum cytotoxic concentration (μM): required to cause a mycroscopically detectable alteration of normal cell morphology. The number of independent tests is given in brackets.

Although our compounds did not improve the activity of the reference compound, *tecovirimat*, it is noteworthy that several of the new analogues were up to ten times more potent than *cidofovir*, the other drug currently recommended for short-term prophylaxis, adverse vaccination reactions and emergency treatment against smallpox. Moreover, we have to keep in mind that *tecovirimat* still is in clinical trials, so our derivatives may be considered as interesting back-ups just in case *tecovirimat* had problems during the clinical trials.

# PART I: CONCLUSIONS

1. Starting from anhydride **26** and **33,** we have synthesized and fully characterized a broad family of aromatic benzamides.

2. All the new compounds have been evaluated pharmacologically against a broad DNA and RNA panel of viruses being inactive against all but vaccinia virus.

3. Analyzing all the pharmacological results and the modifications introduced in the aromatic ring as well as in the polycyclic scaffold we can conclude that:

    a. It is essential an EWG in the *para* position in order to have some activity against vaccinia virus.

    b. The introduction of a second EWG in the *meta* position of the aromatic ring lead to more potent compounds.

    c. For any given aromatic ring, neither the epoxydation nor the cyclopropanation of the double bonds of the polycyclic scaffold improves the activity of the compounds.

4. The most active compound is **50** with a $IC_{50}$= 0.4 μM which is less potent than *tecovirimat*, the reference compound, although it is one order of magnitude more potent than *cidofovir*.

# 2nd PART: SYNTHESIS OF ANALOGUES OF AMANTADINE AS ANTIINFLUENZA DRUGS

## PART II: INTRODUCTION

## 1. Highlights of Influenza disease.

Influenza, commonly referred as 'the flu', is a highly contagious viral infection produced by a RNA virus belonging to the *Orthomixoviridae* family, Influenza virus. The flu is a common disease that affects mammals and birds, almost everybody suffers it at least once during its lifetime. The typical symptoms of the illness are fever, myalgia, headache, cough, sore throat, rhinitis and other common cold-like symptoms. The influenza virus infects the respiratory tract of the host destroying the ciliated epithelial cells of the respiratory tract. After an incubation period of one to four days, the appearance of symptoms is abrupt and the illness has an evolution time from 7 to 10 days. Finally, the patient is usually healed up thanks to its immune response. Although it is usually a mild illness, seasonal viruses can also cause severe disease in very young infants, elderly people and those with immunodeficiency, cardiopulmonary disease or other chronic illnesses; in the United States, influenza A virus infections are responsible for nearly 40.000 deaths every year.[56] This fact occurs when the virus is able to pass from the upper respiratory tract to the lower causing a more severe respiratory disease, usually viral or bacterial pneumonia.

The flu is one of the oldest known viral diseases that affects the humankind. The first description of influenza was made by Hippocrates in 412 b. C. and, since 1580 when the first pandemic of flu was described, a large amount of outbreaks have occurred. Without any doubt, the worst pandemic in history was the named 'Spanish flu' caused by the virus strain H1N1 that was responsible during 1917-1918 of 20-50 millions of deaths worldwide, most of them dying few hours later after the first symptoms appearance.[57] Surprisingly, this strain of Influenza A virus targeted the youths, people between 25-35 years mainly, and the elderly were spared, opposite to the usual behaviour of this virus.[58] During the 20$^{th}$ century, two more big pandemics occurred; between 1957-1963 the 'Asian flu', prompted by an H2N2 strain that caused between 2-4 million of deaths and between 1968-1969 the 'Hong Kong flu' prompted by an H3N2 strain causing the death of 1-2 million of people.[59] More recently, a new mutation of the avian flu has generated a new subtype ('Hong Kong flu' H5N1), an unusual aggressive virus that has caused the death of millions of poultry and about 200 of humans since 2003. Fortunately, this new strain is not able to be transmitted human-to-human avoiding a catastrophic pandemic.[60] Finally, in March 2009, the appearance of the H1N1/09 strain, called the 'swine flu', generated a public health alarm due to the possibility of the reassortment

[56] Beigel, J.; Bray, M. *Antiviral Res.* **2008**, *78*, 91-102.

[57] Taubenberger, J. K.; Morens, D. M. *Emerging Infect. Dis.* **2006**, *12*, 15-22. Although it is not known for sure, the Spanish flu is considered to be started in Kansas and the American soldiers sent to fight in the First World War transmitted the virus. Since Spain was one of the few neutral countries, was there where the press informed about the pandemic without censoring, this is why it is called Spanish Flu.

[58] Oxford, J. S.; Lambkin, R.; Elliot, A.; Daniels, R.; Sefton, A.; Gill, D. *Vaccine* **2006**, *24*, 6742-6746.

[59] a) Laver, W. G.; Bischofberger, N.; Webster, R. G. *Sci. Am.* **1999**, *280*, 78-87. b) Reid, A. H. *Microbes Infect*. **2001**, *3*, 81-87. c) Belshe, R. B. *N. Engl. J. Med.* **2005**, *353*, 2209-2211. d) Kilbourne, E. D. *Emerging infect. Dis.* **2006**, *12*, 9-14.

[60] Abdel-Ghafar, A. N; Chotpitayasunondh, T.; Gao, Z.; Hayden, F. G.; Nguyen, D. H.; de Jong, M. D.; Naghdaliyev, A.; Peiris, J. S.; Shindo, N.; Soeroso, S.; Uyeki, T. M. *N. Engl. J. Med.* **2008**, *358*, 261-273.

(exchange of viral RNA segments between viruses) of this strain, mild but easily transmissible, with the avian H5N1, opening the door for a new health disaster.[61]

Influenza virus is transmitted through the airborne route, by coughing or sneezing, creating aerosols containing the virus.[62] It can also be passed through direct contact as it survives some minutes on hands or nasal secretions but it is believed that the airborne route is the most important. [63]

In temperate climates, influenza outbreaks occur especially in the winter season, this corresponds to the months from November to March in the Northern hemisphere and from May to September in the Southern hemisphere. The reason for this seasonal transmission has been determined to reside in conditions of relative humidity and temperature, which are more favourable in winter.[64] Typically, in a normal year of flu infection there are between 3 and 5 million of severe illness cases and up to 500.000 deaths worldwide.[65]

Three or four times each century, however, the virus undergoes a major change, that could arise through antigenic 'drift' from a non-human influenza virus (mainly avian) or antigenic 'shift' through recombination of an avian and human influenza virus, and then the virus is able to cause a pandemia.[66] In this case, the virus will infect a large proportion of the world population killing millions of people due to the higher virulence of the new strain compared to seasonal flu (Figure 21).

Worthy of note, during the writing up of this Thesis two novel influenza avian strains generated public concern. During the spring, the H7N9 avian flu caused the death of dozens of humans in China, while the H7N1 strain killed thousands of poultry in Catalonia in April 2013.[67]

[61] a) Neumann, G.; Noda, T.; Kawaoka, Y. *Nature* **2009**, *459*, 931-939. b) Michaelis, M.; Doerr, H. W., Cinatl, J., Jr. *Curr. Mol. Med.* **2009**, *9*, 131-151.
[62] Brankston, G.; Gitterman, L.; Hirji, Z.; Lemieux, C.; Gardam, M. *Lancet Infect Dis* **2007**, *7*, 257-265.
[63] Weber, T.P.; Stilianakis, N. I. *J. Infect* **2008**, *57*, 361-373.
[64] Lowen, A. C.; Mubareka, S.; Steel J.; Palese P. *PLoS Pathog* **2007**, *3(10)*, e151.
[65] Weinstock, D. M.; Zuccotti, G. *J. Am. Med. Assoc.* **2006**, *295*, 934-936.
[66] Palese, P. *Nature Med.* **2004**, *10*, S82-S87.
[67] a) Lamb, R. A. *Am. J. Respir. Crit. Care Med.* **2013**, *188*, 1-2. b) Morens, D. M.; Taubenberger, J. K.; Fauci, A. S. *MBio* **2013**, *4*, e00445-13. c) Butler, D. *Nature* **2013**, *496*, 145-146. d) Horby, P. *Nature* **2013**, *496*, 399-399.

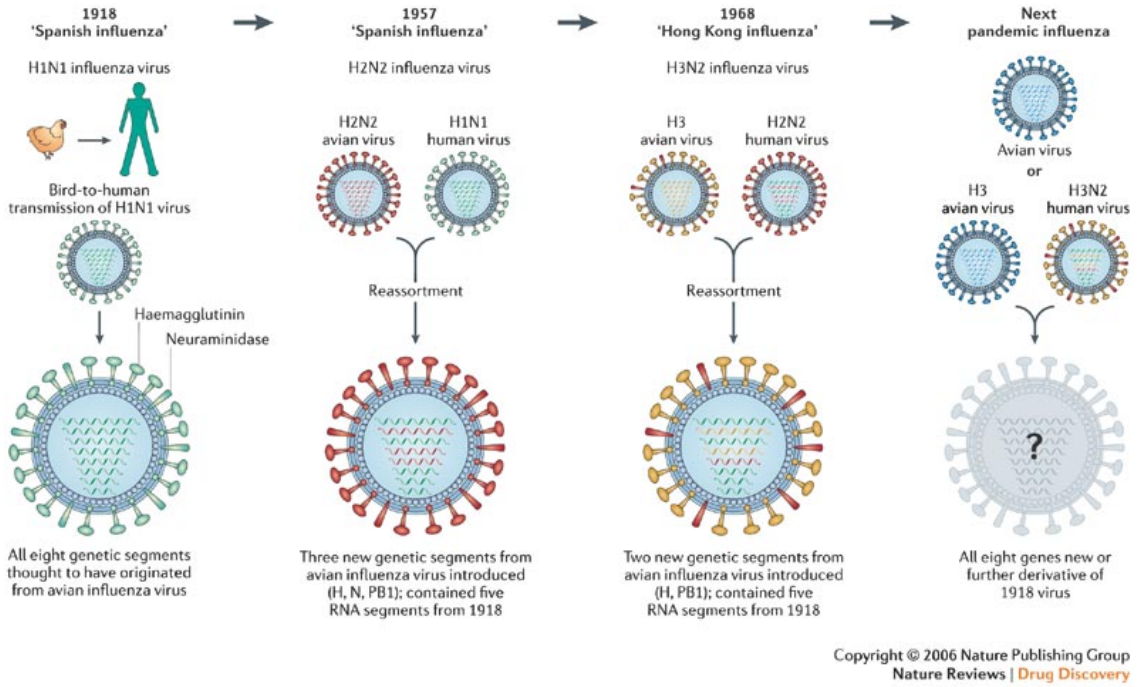



**Figure 21.** How a pandemic could be generated.[68]

Using publically available epidemiology data, analyst from the Centre for Disease Control and Prevention (CDC) have calculated the annual epidemic burden incurred by influenza epidemics in the United States. They determined that every year 3.1 million hospitalized days and 31.4 million outpatient visits can be attributed to this disease and economic burden was pegged at 87.1 billion dollars per year.[69] Workplace absenteeism and associated loss of productivity is a significant factor in driving up the costs incurred by influenza, being between 3.7-5.9 days of work lost by doctor-confirmed case of the disease.

### 2. The influenza A virus.

Influenza viruses have a multipartite, negative-sense, single-stranded RNA genome and a lipid envelope. They are divided into three genera A, B and C within the family *Orthomyxoviridae*, based on the antigenic properties of the viral nucleoprotein. Influenza B and C viruses principally infect humans, usually causing mild illness in children, and undergo only gradual antigenic variation.[70]

Influenza viruses have a standard nomenclature that includes: virus type; species from which it was isolated (if non-human); location at which it was isolated; isolate number; isolate year; and, for Influenza A viruses only, hemagglutinin (HA) and neuraminidase (NA) subtypes. Thus, A/Panama/2007/1999 (H3N2) was the isolate number of 2007 of a human influenza A

[68] De Clercq, E. *Nat. Rev. Drug Discov.* **2006**, *5*, 1015-1025.
[69] Molinari, N. A.; Ortega-Sánchez, I. R.; Messonier, M. L.; Thompson, W. W.; Wortley, P. M.; Weintraub, E.; Bridges, C. B. *Vaccine* **2007**, *25*, 5086-5096.
[70] Wright, P. F.; Webster, R. G. 'Orthomyxoviruses' Knipe, D. M., Howley, P. M. (Eds.), Fields Virology, fourth ed. Lippincott Williams & Wilkins, Philadelphia, **2001**, 1533-1579.

virus taken in Panama in 1999, and it has an HA subtype 3 and a NA subtype 2. While many genetically distinct subtypes (16 for HA and 9 for NA) have been found in circulating influenza A viruses, only three HA (H1, H2 and H3) and two NA (N1 and N2) subtypes have caused human epidemics, as defined by sustained, widespread, person-to-person transmission.[71]

Unusually for a virus, its genome is not a single piece of nucleic acid; instead, it contains eight pieces of segmented negative-sense RNA, each piece of RNA containing either one or two genes, which code for a protein. For example, the influenza A genome contains 11 genes on eight pieces of RNA, encoding for 11 proteins: hemagglutinin, neuraminidase, nucleoprotein (NP), M1, M2, NS1, NS2 (NEP: nuclear export protein), PA, PB1 (polymerase basic 1), PB1-F2 and PB2 (Figure 22).[72]

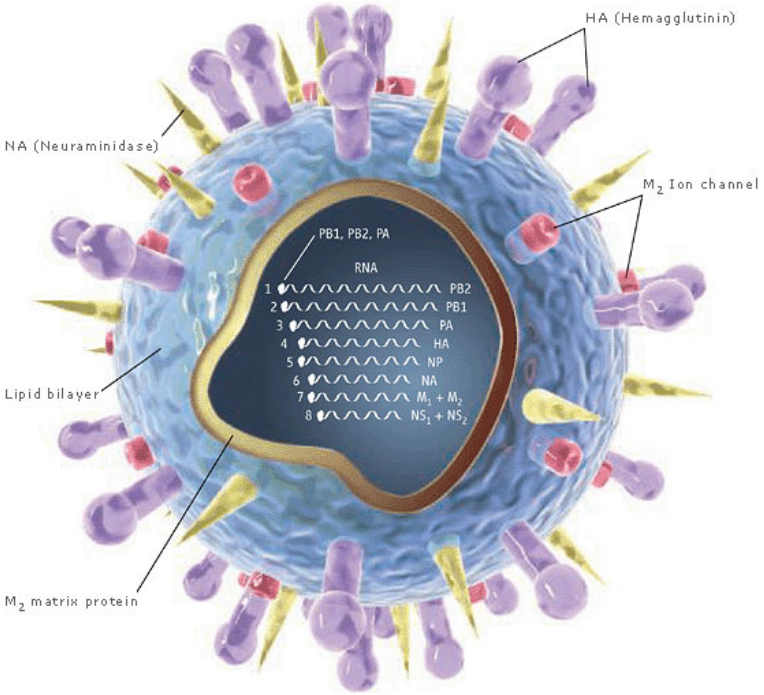
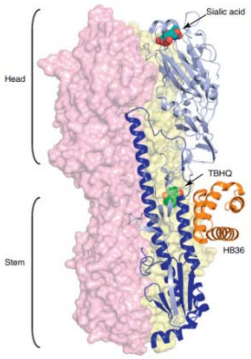
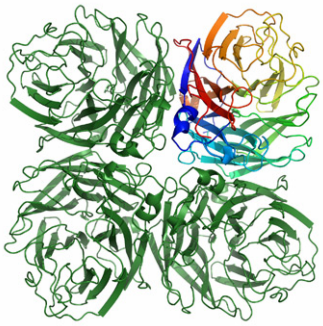
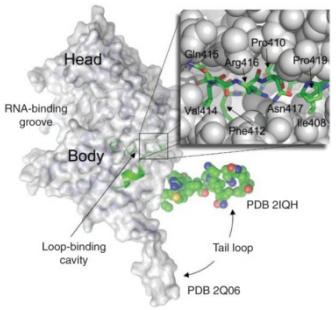
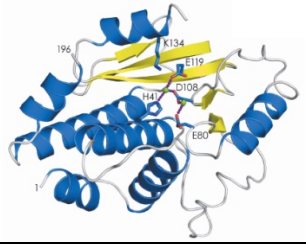



**Figure 22.**Influenza A virus with its genome and proteins.[73]

Each protein has its own function in the influenza replicative cycle and plays an essential role in the infectiveness of the virus (Table 4).[74]

[71] Bouvier, N. M.; Palese, P. *Vaccine* **2008**, *26S*, D49-D53.
[72] Ghedin, E.; Sengamalay, N. A.; Shumway, M.; Zaborsky, J.; Feldblyum, T.; Subbu, V.; Spiro, D. J.; Sitz, J. *Nature* **2005**, *437*, 1162-1166.
[73] Taken from: http://www.ifpma.org/uploads/RTEmagicP_diagram_virus.jpg (accessed on July, 14th 2013).
[74] Du, J.; Cross, T. A.; Zhou, H. *Drug Discovery Today* **2012**, *17*, 1111-1120.

| | |
|---|---|
| HA and NA are the biggest glycoproteins in the outer virus shell. **HA** is a trimer, commonly divided into a head region and a stem region; each chain is synthesized as a precursor polypeptide and then cleaved in two fragments, HA1 (328 amino acids) and HA2 (221 amino acids), linked by a disulphide bond. Its function consists in binding the virus particle to the sialic acids of the cellular surface and to trigger the fusion between the viral and the cellular membrane inside the endosome. |  |
| **NA**, also called sialidase, is a tetramer with relatively independent monomers. Its function consists in cleaving new generated viral particles from the host cell permitting the spread of the infection. |  |
| The **M2 channel** is another surface protein. The 97-residue tetramer has a transmembrane domain (21 residues) and a *C*-terminal cytoplasmatic amphiphilic helix. The *N*-terminal domain consisting in 25 residues is exterior to the virus. This protein facilitates the uncoating of the virus in the interior of the cell. |  |
| The **nucleoprotein** molecules encapsidate the viral single-stranded RNAs and also participate in the nuclear import and export of viral ribonucleoprotein (vRNP) that is incorporated into the virus. A nucleoprotein molecule folds into a crescent shape with a head and a body domain, and the ssRNA binding groove is located on the exterior surface between the two domains of the nucleoprotein. Each nucleoprotein molecule has a tail loop that is inserted into the neighbouring nucleoprotein molecule, resulting in the oligomerization of nucleoprotein, which is required for vRNP formation. |  |
| The **viral polymerase** is an heterotrimer of subunits PA, PB1 and PB2 with a combined mass of ~250k Da. The viral polymerase carries out both mRNA transcription and replication. |  |

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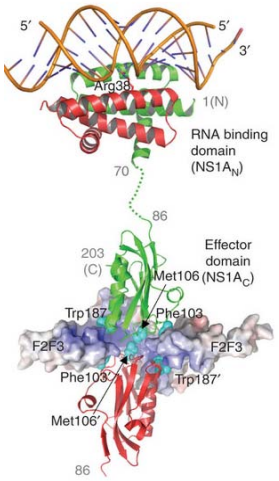
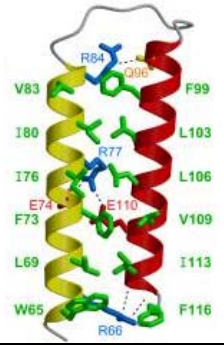
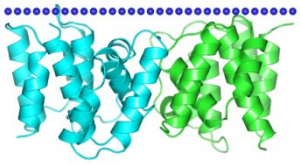
|---|---|
| The nonstrutural protein **NS1A** has two domains. $NS1A_N$ (residues 1-70) exists in homodimeric state that binds double-stranded RNA (dsRNA) in a non-sequence specific manner.<br><br>$NS1A_C$ (residues 86-230/237), also known as the effector domain, binds several cellular proteins in order to stop the cellular mechanism that would otherwise inhibit the replication of the virus. |  |
| The **nuclear export protein** (NEP) or NS2 mediates the nuclear export of new viral RNA and several proteins from the cell nucleus to the cytoplasm. |  |
| The matrix protein **M1** is located below the lipid bilayer. The M1 protein is known to contain hydrophobic domains which interact with lipids to provide structural stability of the virion envelope. On the other hand, the matrix protein interacts with RNP predominantly by electrostatic forces, in this way M1 protein bridges interaction between the viral lipid membrane and the ribonucleoprotein.[75] |  |

**Table 4.** Proteins of the Influenza A virus.[76]

All of these proteins allow the virus to replicate in the host cell, and then developing the illness.

As mentioned, the virus is transmitted by the airborne via. Once the virus has entered in the respiratory tract of the host, the HA recognizes the sialic acid moiety of the epithelial cells (Figure 23). The viruses that infect human possess an HA that has a preferential specificity for sialic acids with α-2,6-linkage which are present in the upper respiratory tract of humans. On the other hand, avian influenza viruses have an HA with an specificity for α-2,3-linked sialic acids that are the majority in the respiratory tract of poultry and in the lower respiratory tract of humans. This fact could explain why avian influenza virus has low infectivity for humans, the

[75] a) Zhirnov, O.P. *Virology* **1990**, *176*, 271-279. b) Cheung, T. K. W.; Poon, L. L. *Ann. N. Y. Acad. Sci.* **2007**, *1102*, 1-25.

[76] Das, K.; Aramini, J.M.; Ma, L.; Krug, R. M.; Arnold, E. *Nat. Struct. Mol. Biol*. **2010**, *17*, 530-538.

human lungs are not as accessible to airborne virus particles as the upper respiratory tract is, but possesses high pathogenicity due to the affectation of lungs.[71]

Following attachment of the influenza virus HA protein to a sialic acid unit of the cellular membrane, the virus is endocytosed. The acidity of the endosomal compartment is crucial to influenza virus uncoating. Hydrogen ions from the endosome are pumped into the virus particle via the M2 ion channel and the interaction between M1 and vRNP is weakened in order the vRNP to be released into the cytoplasm. At the same time, low pH inside the endosomes triggers a conformational change in the HA, exposing a fusion peptide that mediates the merging of the viral envelope with the endosomal membrane, thus opening a pore through which the viral RNPs are released into the host cell cytoplasm.[77]

Once liberated from the virion, RNPs are trafficked to the host cell nucleus. In the nucleus the new viral RNP will be synthesized. Afterwards, the nuclear export of vRNA segments is mediated by viral proteins M1 and NEP.

The envelope proteins HA, NA and M2 channel are synthesized, from mRNA of viral origin, on membrane-bound ribosomes into the endoplasmic reticulum, where they are folded and trafficked to the Golgi apparatus for post-translational modification. Later on, they will be transported to the cell membrane for virion assembly.

Influenza virus budding occurs at the cell membrane, probably initiated by an accumulation of M1 matrix protein at the cytoplasmic side of the lipid bilayer. When budding is complete, HA continues to bind the virions to the sialic acid on the cell surface until virus particles are actively released by the sialidase activity of the NA protein. Then, the new virus particle is now free for infecting new cells and thus, spreading the infection.[78]

[77] Sieczkarski, S. B.; Whittaker, G. R. *Curr. Top. Microbiol. Immunol*. **2005**, *285*, 1-23.
[78] Rossman, J. S.; Lamb, R. A. *Virology* **2011**, *411*, 229-236.

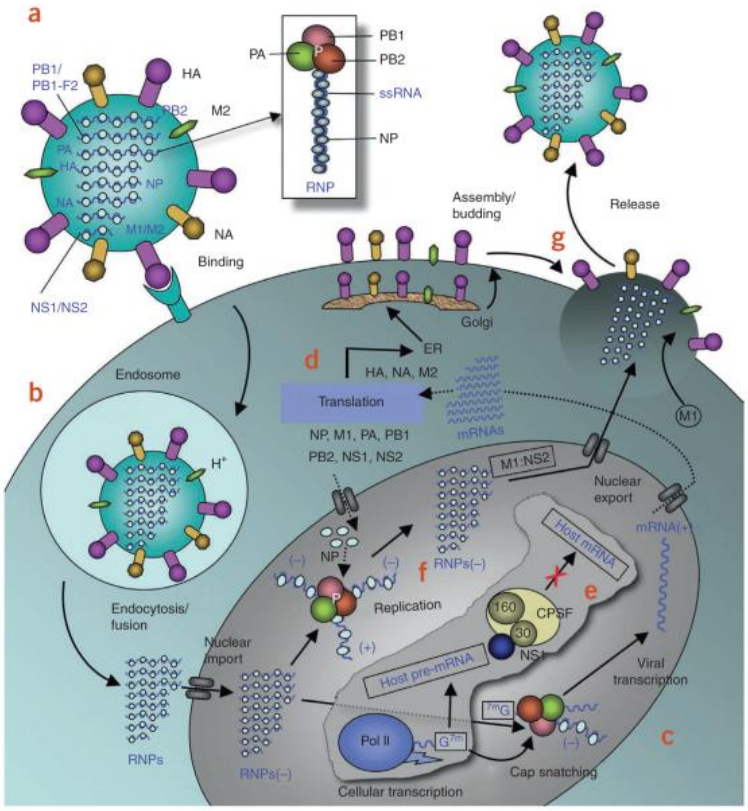



**Figure 23.** Replication cycle of Influenza A virus.[76]

### 3. The M2 channel.

As mentioned before, the M2 channel is a surface protein of the Influenza A virus and the putative target of the compounds synthesized in this Thesis.

It is an homotetramer consisting on disulphide-linked monomers. Each unit of the tetramer has 97 aminoacids, with 24 aminoacids at the *N*-terminal (involved in protein's incorporation into the virion), 19 at the transmembrane domain (involved in tetramerization and proton flux) and 54 aminoacids at the *C*-terminal (involved in budding, scission and interaction with the matrix protein M1).[79] This homotetramer is inserted into the membrane of the virus forming a channel.

The M2 protein act as an ion channel that selectively allows protons to enter inside the virion and then, to trigger the uncoating of the virus genome.[80] Moreover, in the latest steps of

[79] a) Lamb, R. A.; Zebedee, S. L.; Richardson, C.D. *Cell* **1985**, *40*, 627-633. b) Holsinger, L. J.; Lamb, R. A. *Virology* **1991**, *183*, 32-43. c) Sugrue, R. J., Hay, A. *J. Virology* **1991**, *180*, 617-624. d) Park, E. K.; Castrucci, M. R.; Portner, A.; Kawaoka, Y. *J. Virol.* **1998**, *72*, 2449-2455. e) Kochendoerfer, G. G.; Salom, D.; Lear, J. D.; Wilk-Orescan, R.; Kent, S. B.; DeGrado, W. F. *Biochemistry* **1999**, *38*, 11905-11913. f) Rossman, J. S.; Jing, X.; Leser, G. P.; Lamb, R. A.; *Cell* **2010**, *142*, 902-913.

[80] a) Sakaguchi, T.; Leser, G. P.; Lamb, R. A. *J. Cell Bio.* **1996**, *133*, 733-747. b) Pinto, L. H., Holsinger, L. J., Lamb, R. A. *Cell* **1992**, *69*, 517-528.

the virus cycle the M2 channel equilibrates the pH between the acidic trans-Golgi network and the cytoplasm to prevent the early change of hemagglutinin conformation.[81]

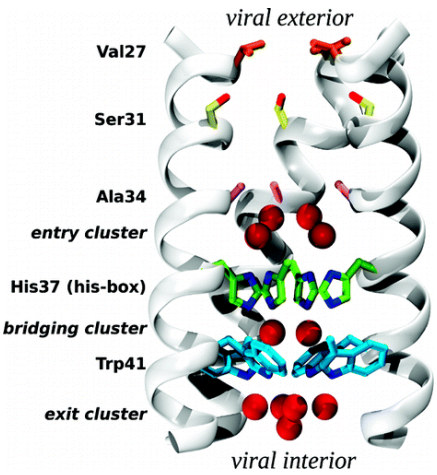



**Figure 24.** Structure of A/M2 channel in the 1.65 Å X-ray structure (PDB: 3LBW). The red spheres are molecules of $H_2O$.

Although the role of M2 protein as a proton channel has been known for decades,[80b,82] the 3-D structure of the protein and detailed mechanism of this transport has been only very recently elucidated, mainly as a consequence of the efforts of Hong's, Cross' and DeGrado's groups. It has been found that the only essential part of the protein for proton transfer to occur is the transmembrane domain, since it was shown that removing the *N*-terminal and the *C*-terminal of the protein do not affect the transport of protons through it.[83] This transmembrane domain includes from 22 to 46 aminoacids where Histidine 37 (His37), Triptophan 41 (Trp41) and Aspartic acid 44 (Asp44) are the important aminoacids for charge storage and proton transfer. Thus, the transmembrane helices are tethered at one end by a pair of conserved *N*-terminal cysteines forming intermolecular disulphides bridges *in vivo* and at the other end by *C*-terminal base, ensuring that destabilization of the four-helix bundle during channel activation does not cause dissociation of the tetramer.[84]

The first gate of the channel is placed in Valine 27, these aminoacids forms a small gate that only allow the pass of protons through it, these aminoacids may contribute to the ion selectivity of this protein.[85] The His37 are the pH sensitive aminoacids of the channel, at physiologic pH (7.7) just two out of four are protonated, stabilizing the closed state of the protein due to the shared hydrogen bonds between the bridging waters placed above the histidines.[86] These waters are hypothesized to delocalize the excess of protons in His37. Furthermore, Trp41 are clustered at high pH closing the internal lumen of the pore, this closed form is stabilized by interaction with Asp44 (Figure 24).

When the virus is endocytosed the external pH becomes more acidic: around 5.7; a proton enters into the channel from the viral exterior and a third imidazole residue is protonated (the $pK_a$s of the third imidazole is 6.3).[87] The activated state is stabilized by a hydrogen bond with a

[81] Hu, J.; Fu, R.; Cross, T. A. *Biophys. J.* **2007**, *93*, 276-283.
[82] Hoffmann, H.; Palese, P.; Shaw, M. L. *Anti. Res.* **2008**, *80*, 124-134.
[83] Ma, C.; Polishchuk, A. L.; Ohigashi, Y.; Stouffer, A. L., Schön, A., Magavern, E.; Jing, X., Lear, J. D.; Freire, E.; Lamb, R. A.; DeGrado, W. F.; Pinto, L. H. *Prod. Nat. Acad. Sci.* **2009**, *106*, 12283-12288.
[84] a) Schnell, J. R.; Chou, J. J. *Nature* **2008**, *451*, 591-596. b) Fiorin, G.; Carnevale, V.; DeGrado, W. F. *Science* **2010**, *330*, 456-458. c) Hu, F.; Luo, W.; Hong, M. *Science* **2010**, *330*, 505-508.
[85] Hu, J.; Asbury, T.; Achuthan, S.; Li, C.; Bertram, R.; Quine, J. R.; Fu, R.; Cross T. A. *Biophys. J.* **2007**, *92*, 4335-4343.
[86] Hong. M.; DeGrado, W. F. *Protein Science* **2012**, *21*, 1620-1633.
[87] Ivanovic, T.; Rozendaal, R.; Floyd, D. L.; Popovic, M.; Van Oijen, A. M.; Harrison, S. C. *PLos One* **2012**, *7*, e31566, 1-9.

molecule of water and a new cation-π interaction with a Trp41.[88] This additional interaction will make the Trp41 to move and open the gate for releasing of the third proton to the interior of the virion. After releasing, the stabilized closed state of the channel is restored, until a new proton gets the third His37 again (Figure 25).[89]

This acidification of the internal of the virion will make the interactions of matrix protein M1 and vRNP to weaken and then the viral RNA can be released in the cellular cytoplasm through the pore opened by the HA.

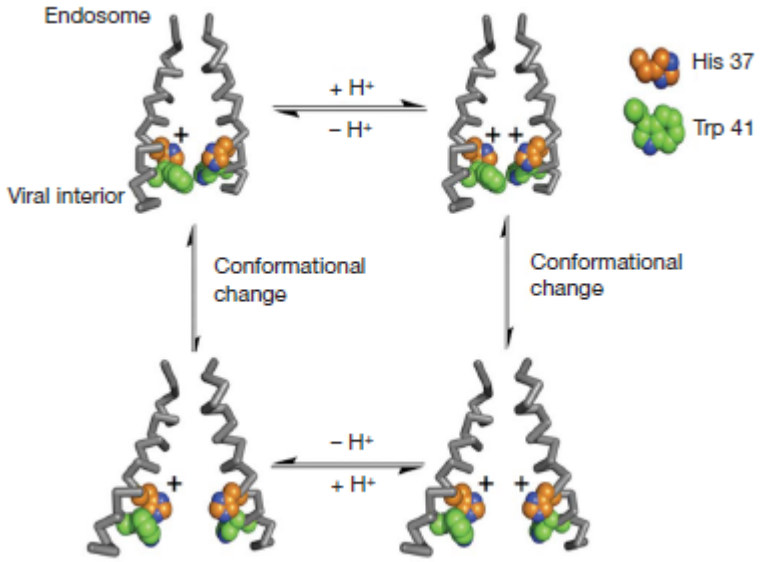



**Figure 25.** Mechanism of low pH activation of the cannel. Two out of four chains are shown for clarification.[84b,90]

## 4. Existing treatments against Influenza A virus.

The first line of effective defence against any influenza strain is vaccination. The influenza vaccine contains three strains of the influenza virus: an influenza A H3N2 strain, an influenza A H1N1 strain and an influenza B strain and it has to be reformulated every year due to the high mutagenicity of the virus. However, in order to develop a vaccine, the strain of the pandemic virus must be first determined; this cannot happen until antigenic shift takes place. As it takes

[88] Okada, A.; Miura, T.; Takeuchi, H. *Biochemistry* **2001**, *40*, 6053-6060.
[89] a) Sharma, M.; Yi, M.; Dong, H.; Qin, H.; Peterson, E.; Busath, D. D.; Zhou, H.; Cross, T. A. *Science* **2010**, *330*, 509-512. b) Carnevale, V.; Fiorin, G.; Levine, B. G.; DeGrado, W. F.; Klein, M. L. *J. Phys. Chem C* **2010**, *114*, 20856-20863. c) Dong, H.; Yi, M.; Cross, T. A.; Zhou, H.-X. *Chem. Sci.* **2013**, *4*, 2776-2787. d) Williams, J. K.; Zhang, Y.; Schmidt-Rohr, K.; Hong, M. *Biophys. J.* **2013**, *104*, 1698-1708.
[90] Stouffer, A. L.; Acharya, R.; Salom, D.; Levine, A. S.; Di Costanzo, L.; Soto, C. S.; Tereshko, V.; Nanda, V.; Stayrook, S.; DeGrado, W. F. *Nature* **2008**, *451*, 596-599.

several months to prepare sufficient quantities of vaccine, none would be available during the early stages of a pandemic.

Thus, it is essential to develop a novel effective anti-influenza drug in order to be able to face a probable pandemic of influenza, since it will be the principal countermeasure to reduce the impact of a new pandemic. This drug must be storable, and it should be able to be used in prophylaxis and treatment, even in the latest stages of the disease.

To date, just six drugs have been licensed to use as the treatment for influenza disease: two adamantanes and four neuraminidase inhibitors. Because their approval was obtained based on studies in healthy adults with uncomplicated seasonal influenza, little is known about how these drugs should be used to treat severe disease.[56]

**a) Neuraminidase inhibitors (NA).**

Neuraminidase has been the most successful influenza drug target. Crystal structures of NA in the early 1980s revealed a conserved sialic acid binding pocket.[91] Subsequently, the structures of the protein and structure-based modelling played a critical role in the development of NA substrate mimics, and these efforts led to the discovery of zanamivir (Relenza®) (Figure 26).[92] Zanamivir was approved for human use as an inhalant drug in 1999.

The replacement of glycerol moiety by a lipophilic group to make it orally biodisponible led to the discovery of oseltamivir (Tamiflu®).[93] More recently, peramivir, a NA inhibitor developed by BioCryst, was approved as an intramolecular injection in Japan (Rapiacta®) and South Corea (PeramiFlu®) for the treatment of patients with severe and complicated influenza.[94] In United States is currently in phase III clinical trials with *fast track* designation.

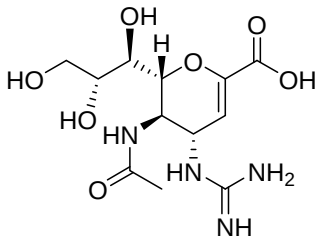
Zanamivir and oseltamivir have been found to be highly potent inhibitors ($IC_{50} \leq 1$ ng $ml^{-1}$) of the influenza neuraminidase, to inhibit influenza A and B virus replication *in vitro* and *in vivo*, to be well tolerated, and to be both prophylactically and therapeutically effective against influenza A and B virus infection in humans.[68, 95]

In order to avoid some emergent resistance against NA inhibitors, some new analogs have been developed. The simple 7-*O*-methyl ether of zanamivir, laninamivir, show similar NA inhibitory potency and slightly improved activity in cell-based assays over zanamivir.[96] To

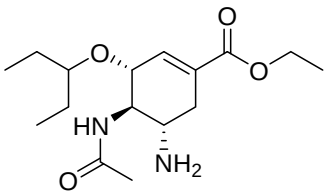
[91] a) Varghese, J. N.; Laver, W. G.; Colman, P. M. *Nature* **1983**, *303*, 35-40. b) Colman, P. M.; Varghese, J. N.; Laver, W. G. *Nature* **1983**, *303*, 41-44.
[92] Von Itzstein, M.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Van Phan, T.; Smythe, M L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. H.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418-423.
[93] Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681-690.
[94] a) Bantia, S.; Arnold, C. S.; Parker, C. D.; Upshaw, R.; Chand, P. *Antiviral Res*. **2006**, *69*, 39-45. b) Shetty, A. K.; Peek, L. A. *Expert Rev. Anti-infect. Ther.* **2012**, *10*, 123-143.
[95] Lagoja, I. M.; De Clercq, E. *Med. Res. Rev.* **2008**, *28*, 1-38.
[96] Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kaneko, S.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921-1924.

improve therapeutic efficacy, Yamashita et al.[97] introduced different acyl groups to esterify the C9 hydroxy of laninamivir and obtain prodrugs. Laninamivir octanoate (CS-8958) was found to be the best compound in terms of its life-prolonging effect ($p>0.0001$ relative to zanamivir in the same infection model). A prolonged survival effect was observed after a single administration of CS-8958, even when it was given 7 days before infection. The drug was approved in Japan in 2010 and marketed by Daiichi Sanky, known as Inavir®.

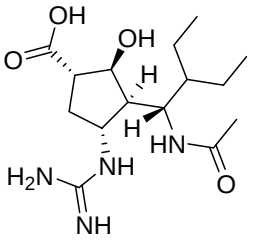
Neuraminidase inhibitors are anticipated to reduce illness duration by 1-3 days, to reduce the risk of virus transmission, to reduce the number and severity of complications, to reduce the use of antibiotics and to prevent seasonal influenza-virus infection. As shown in particular for oseltamivir, the earlier the administration, the shorter the duration of fever, the greater the alleviation of symptoms and the faster the return to baseline activity and health scores.[98,99]



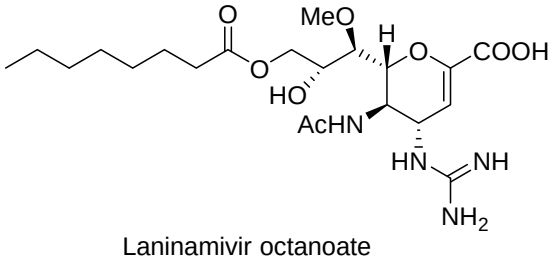
Zanamivir



Oseltamivir



Peramivir



Laninamivir octanoate

**Figure 26.** Structure of Neuraminidase inhibitors.

[97] a) Yamashita, M.; Tomozawa, T.; Kakuta, M.; Tokumitsu, A. Sugaya, N.; Ohashi, Y. *Antimicrob. Agents Chemoter.* **2010**, *54*, 2575-2582. b) Kubo, S. *Antimicrob. Agents Chemother.* **2009**, *53*, 186-192. c) Ikematsu, H.; Kawai, N. *Expert Rev. Anti-infect. Ther.* **2011**, *9*, 851-857.

[98] Aoki, F. Y.; Macleod, M. D.; Paggiaro, P.; Carewicz, O.; El Sawy, A.; Wat, C.; Griffiths, M.; Waalberg, E.; Ward, P. *J. Antimicrob. Chemother.* **2003**, *51*, 123-129.

[99] a) Wang, G. T.; Chen, Y.; Wang, S.; Gentles, R.; Sowin, T.; Kati, W.; Muchmore, S.; Giranda, V.; Stewart, K.; Sham, H.; Kempf, D.; Laver, G. *J. Med. Chem.* **2001**, *44*, 1192-1201. b) Abdel, M. A. F.; Maryanoff, C.A.; Mehrman, S. J. *Curr. Opin. Drug Discover. Develop.* **2001,** *4,* 776-791. c) Hanessian, S.; Bayrakdarian, M.; Luo, X. *J.* Am. Chem. Soc. **2002***, 124,* 4716-4721. d) Maring, C. J.; Stoll, V. S.; Zhao, C.; Sun, M.; Krueger, A. C.; Stewart, K. D.; Madigan, D. L.; Kati, W. M.; Xu, Y.; Carrick, R. J.; Montgomery, D. A.; Kempf-Grote, A.; Marsh, K. C.; Molla, A.; Steffy, K. R.; Sham, H. L.; Laver, W. G.; Gu, Y.; Kempf, D. J.; Kohlbrenner, W. E. *J. Med. Chem.* **2005**, *48*, 3980-3990. e) Momose, T.; Hama, N.; Higashino, C.; Sato, H.; Chida, N. *Tetrahedron Lett.* **2008**, *49*, 1376-1379. f) Liu, Y.; Zhang, J.; Xu, W. *Curr. Med. Chem.* **2007**, *14*, 2872-2891.

### b) Hemagglutinin inhibitors

Hitherto, some hemagglutinin inhibitors have been developed although so far, none of them have reached therapeutical use. Several strategies have been proved to be useful to obtain molecules that affects the hemagglutinin function through all the virus cycle.

For example, Fludase® (DAS 181), a peptide, works as an anti-adhesion agent derived from *Actynomices viscosus* that removes the sialic acid receptors from the airway epitelium. This compound shows potent antiviral and cell-protective efficacies against a broad panel of laboratory strains and clinical isolates of influenza A and influenza B, with virus-replication inhibition $EC_{50}$ values in the range of 0.04-0.9 μM. Fludase® is active against a broad panel of Influenza virus strains including H5N1 and it is currently in phase II clinical trials.[100]

Binding of small molecules such as tert-butylhydroquinone (TBHQ) to HA was found to inhibit membrane fusion. TBHQ binding stabilizes the prefusion trimer conformation and prevents the pH-induced conformational changes of HA1 necessary for membrane fusion (Figure 27).[101]

Based on similarity to these earlier ligands, Tang et al. screened the Roche collection of ~1 million compounds and found that compound **72** was a potent and selective inhibitor to the H1-subtype HAs.[102]

BMY-27709 was identified by Bristol-Myers Squibb as a potent inhibitor of H1 and H2 subtypes of influenza A virus. It was suggested that BMY-27709 interferes with virus infectivity by preventing the low pH-induced conformational rearrangement of hemagglutinin into its fusogenic state, thereby blocking virus and host cell membrane fusion. Its therapeutic interest was lost when it was demonstrated that BMY-27709 is only active against the H1 and H2 types.[103]

Arbidol® was developed by the Russian Research Chemical Pharmaceutical Institute about 20 years ago, and since 1990 ARB has been used as an over-the-counter, broad-spectrum, antiviral drug in Russia, primarily for prophylaxis and treatment of acute respiratory infections

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[100] a) Malakhov, M. P.; Aschenbrenner, L. M.; Smee, D. F.; Wandersee, M. K.; Sidwell, R. W.; Gubareva, L. V.; Mishin, V. P.; Hayden, F. G.; Kim, D. H.; Ing, A.; Campbell, E. R.; Yu, M.; Fang, F. *Antimicrob. Agents Chemother.* **2006**, *50*, 1470-1479. b) Triana-Baltzer, G. B.; Gubareva, L. V.; Klimov, A. I.; Wurtman, D. F.; Moss, R. B.; Hedlund, M.; Larson, J. F.; Belshe, R. B.; Fang, F. *PLOS one* **2009**, *4*, e7838.

[101] a) Bodian, D. L.; Yamasaki, R. D.; Buswell, R. L.; Stearns, J. F.; White, J. M.; Kuntz, I. D.; *Biochemistry* **1993**, *32*, 2967-2978. b) Staschke, K. A.; Hatch, S. D.; Tang, J. C.; Hornback, W. J.; Munroe, J. E.; Colacion, J. M.; Muesing, M. A. *Virology* **1998**, *248*, 264-274. c) Deshpande, M. S.; Wei, J.; Luo, G.; Cianci, C.; Danetz, S.; Torri, A.; Tiley, L.; Krystal, M.; Yu, K.; Huang, S.; Gao, Q.; Meanwell, N. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2393-2396. d) Russell, R. J.; Kerry, P. S.; Stevens, D. J.; Steinhauer, D. A.; Martin, S. R.; Gamblin, S. J.; Skehel, J. J. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17736-17741.

[102] Tang, G.; Qiu, Z.; Lin, X.; Li, W.; Zhu, L.; Li, S.; Li, H.; Wang, L.; Chen, L.; Wu, J. Z.; Yang, W. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3507-3510.

[103] Luo, G.; Torri, A.; Harte, W. E.; Danetz, S.; Cianci, C.; Tiley, L.; Day, S:; Mullaney, D.; Yu, K. L.; Ouellet, C.; Dextraze, P.; Meanwell, N.; Colonno, R.; Krystal, M. *J. Virol.* **1997**, *71*, 4062-4070. b) Cianci, C.; Yu, K. L.; Dischino, D. D.; Harte, W.; Deshapnde, M.; Luo, G.; Colonno, R. J.; Meanwell, N. A.; Krystal, M. *J. Virol.* **1999**, *73*, 1785-1794.

including influenza.[104] ARB demonstrated a broad and potent activity against three antigenic serotypes of human influenza A viruses (H1N1, H2N2 and H3N2), human influenza B and C viruses,[101b] and avian influenza A viruses (H5N1 and H9N2). Arbidol interacts with HA to stabilize it against the low pH transition to its fusogenic state and consequently inhibits HA-mediated membrane fusion during influenza virus infection.[104d] Currently, Arbidol is in clinical trials in USA named as Umiferovir®.

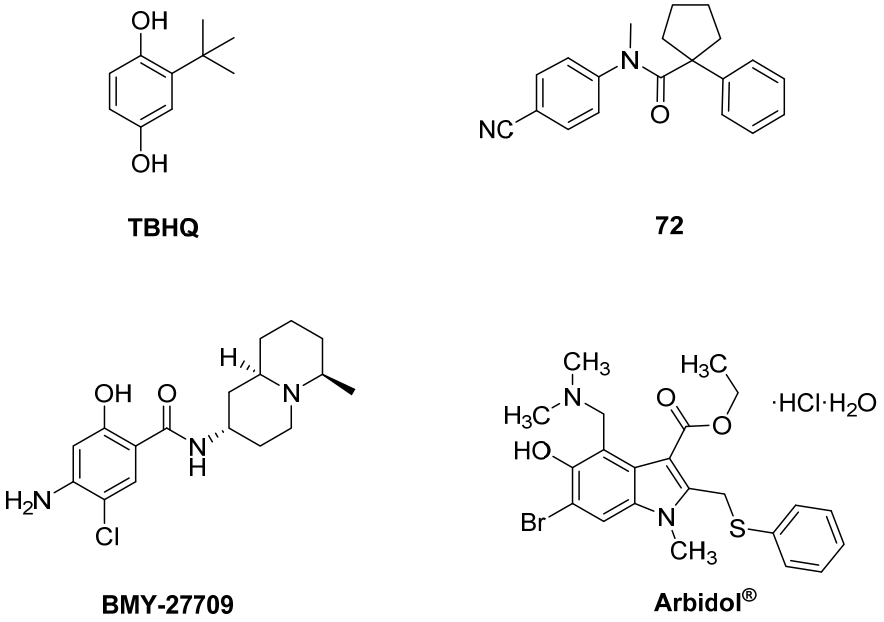


**Figure 27**. Structure of some hemagglutinin inhibitors.

So far, it is clear from the literature that drugs designed to inhibit the hemagglutinin fusion process are effective for only certain serotypes.

### c) M2 channel blockers

The M2 channel is the target of two already approved drugs: amantadine (Symmetrel®, Mantadix®) and rimantadine (Flumadine®) (Figure 28).[105] They exhibit their inhibitory activity at low micromolar concentrations. Rimantadine has a superior intrinsic antiviral activity compared to amantadine, but peak plasma levels of rimantadine are 2-3 fold lower than those achieved with amantadine when given at the same dose.[106]

These two drugs were used for the prophylaxis and treatment of influenza A virus infections. Although initially licensed in 1966 and 1994 respectively, the clinical use of

[104] a) Leneva, I. A.; Fadeeva, N. I.; Fedyakina, I. T. *Antivir. Res.* **1994**, *23*, 187. b) Leneva, I. A.; Fadeeva, N. I.; Fedyakina, I. T.; Guskova, T. A.; Khristova, M. L.; Sokolova, M. V.; Kharitonenkov, I. G.; *Chem. Pharm. J.* **1994**, *9*, 4-15. c) Boriskin, Y. S.; Leneva, I. A.; Pécheur, E. I.; Polyak, S. J. *Curr. Med. Chem.* **2008**, *15*, 997-1005. d) Leneva, I.A.; Russell, R. J.; Bariskin, Y. S.; Hay, A. J. *Antiviral Res.* **2009**, *81*, 132-140.

[105] Davies, W. L.; Grunert, R. R.; Haff, R. F.; McGahen, J. W.; Neumayer, E. M.; Paulshock, M.; Watts, J. C.; Wood, T. R.; Hermann, E. C.; Hoffmann, C. E. *Science* **1964**, *144*, 862-863.

[106] a) Oxford, J.S., Galbraith, A. *Pharmacol. Ther.* **1980**, *11*, 181. b) Belshe, R. B.; Burk, B.; Newman, F.; Cerruti, R. L.; Sim, I. S. *J. Infect. Dis.* **1989**, *159*, 430.

adamantamines has been limited by central nervous system side effects and by the increasingly appearance of resistant virus strains. In fact, earlier observations of the CNS activity of amantadine led to the approval of amantadine as an anti-parkinsonian agent.[107]


**amantadine** **rimantadine**

**Figure 28.** Amantadine and rimantadine.

In order to know how the virus is able to develop resistances against these drugs it is commendatory to learn how adamantylamines bind to the protein. Amantadine binds to the channel with the ratio of one molecule per tetramer,[108] in a lipophilic pocket surrounded by Val27, Ala30, Ser31 and Gly34 (Figure 29).[90,109] Amantadine place its ammonium salt pointing to the His37-box stabilized by forming hydrogen bonds with the carbonyl box formed by the four Ala30. After this first group of waters, there are four more molecules stabilized by hydrogen bond with the Hys37 (Figure 24).[110] The mechanism of blocking is postulated to be by steric hindrance, that is amantadine binds to the channel avoiding the flow of protons through it, by dehydration of the channel and by forming hydrogen bonds with the cluster of water molecules above His37.[111]

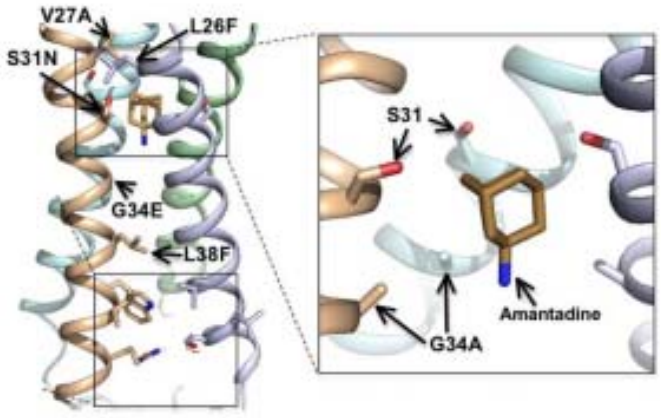


**Figure 29.** Binding site of amantadine in the M2 channel of influenza A.[111]

[107] Hubsher, G.; Haider, M.; Okun, M. S. *Neurology* **2012**, *78*, 1096-1099.
[108] Czabotar, P. E.; Martin, S. R.; Hay, A. J. *Virus Res.* **2004**, *99*, 57-61.
[109] a) Rosenberg, M. R.; Casarotto, M. G. *Proc. Nat. Acad. Sci. USA.* **2009**, *106*, 13866-13871. b) Gkeka, P.; Eleftheratos, S.; Kolocouris, A.; Ournia, Z. *J. Chem. Theory Comput.* **2013**, *9*, 1272-1281. c) Gu, R.-X.; Liu, L. A.; Wang, Y.-H.; Xu, Q.; Wei, D.-Q. *J. Phys. Chem. B* **2013**, *117*, 6042-6051. d) Andreas, L. B.; Barnes, A. B.; Corzilius, B.; Chou, J. J.; Miller, E. A.; Caporini, M.; Rosay, M.; Griffin, R. G. *Biochemistry* **2013**, *52*, 2774-2782.
[110] Wang, J.; Ma, C.; Fiorin, G.; Carnevale, V.; Wang T.; Hu, F.; Lamb, R. A.; Pinto, L. H.; Hong, M.; Klein, M. L.; DeGrado, W. F. *J. Am. Chem. Soc.* **2011**, *133*, 12834-12841.
[111] a) Pielak, R. M.; Schnell, J. R.; Chou, J. J. *Proc. Nat. Acad. Sci.* **2009**, *106*, 7379-7384. b) Acharya, A.; Carnevale, V.; Fiorin, G.; Levine, B. G.; Polishchuk, A.; Balannick, V.; Samish, I.; Lamb, R. A.; Pinto, L. H.; DeGrado, W. F.; Klein, M. L. *Proc. Nat. Acad. Sci. USA.* **2010**, *107*, 15075-15080.

It should be pointed out that these amines are only active against Influenza A virus because the M2 channel of Influenza B virus possesses some polar residues between aminoacids 27-34, this fact prevents the binding of amantadine to the channel due to its high lipophilicity.[112]

Unfortunately, the virus has developed resistance to these drugs mutating in the binding site of amantadine that it is not an essential region for the function of the protein. The most common drug-resistant mutations are S31N (90% of prevalence), V27A and L26F (8-67% of prevalence).[113] The high incidence of drug-resistant mutations in recent years has led the Center for Disease Control and Prevention to recommend against the use of these drugs for the treatment of influenza.[114]

Thereby, there is an urgent need to develop novel orally bioavailable antivirals capable of targeting resistant strains of influenza A viruses.

Recent studies showed that the current predominance of S31N is not the result of drug selection pressure, because S31N was prevalent before the introduction of amantadine and has become widespread in regions where amantadine was never used.[115] Instead, V27A was identified to be the major mutation emerging from drug selection pressure. *While the L26F and S31N mutation causes a 10-20 decrease in the $IC_{50}$s for amantadine inhibition, the corresponding V27A mutation renders the channel entirely resistant to both amantadine and rimantadine*.[116] Overall, the effect of L26F and V27A mutations can be rationalized by observing that the lumen of the pore is widened, on the other hand, the S31N mutation make the lumen of the pore narrower and this mutation places more polar residues in the binding site of amantadine, a very lipophilic molecule.[109] This fact makes amantadine, that blocks the channel by steric hindrance, completely useless against these mutants.

Due to the appearance of mutations, a lot of efforts have been made to obtain new small molecules that inhibit the mutant M2 channel of Influenza A virus. The first derivatives of amantadine and rimantadine were focused in modifying the amine functionality, this fact led to some alkylamino derivatives **73**, hydroxyl derivatives of rimantadine as **74**, aminoalcohols as **75**, and heterocyclic rimantadine analogues **76** (Figure 30). All of them resulted to be equally or less potent than amantadine and rimantadine.[117]

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[112] a) Pinto, L. H.; Lamb, R. A. *J. Biol. Chem.* **2006**, *14*, 8997-9000; b) Zhang, Y.; Shen, H.; Zhang, H.; Li, G.; *J. Phys. Chem. B* **2013**, *117*, 982-988.

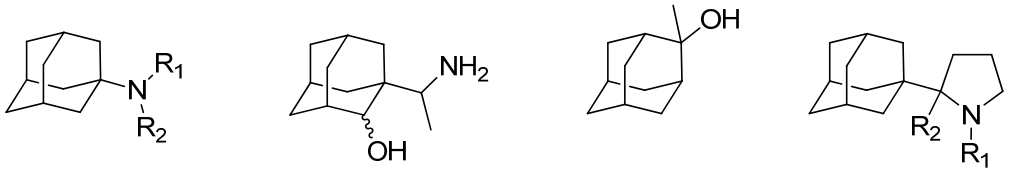
[113] a) Bright, R. A.; Medina, M. J.; Xu, X.; Perez-Oronoz, G.; Wallis, T. R.; Davis, X. M.; Povinelli, L.; Cox, N. J.; Klimov, A. I. *Lancet* **2005**, *366*, 1175-1181. b) Saito, R.; Sakai, T.; Sato, I.; Sano, Y.; Oshitani, H.; Sato, M.; Suzuky, H. *J. Clin. Microbiol.* **2003**, *41*, 2164-2165.

[114] a) Bright, R. A.; Shay, D. K.; Shu, B.; Cox, N. J.; Klimov, A. *J. Am. Med. Assoc.* **2006**, *295*, 891-894. b) Deyde, V. M.; Xu, X. Y.; Bright, R. A.; Shaw, M.; Smith, C. B.; Zhang, Y.; Shu, Y. L.; Gubareva, L. V. Cox, N. J.; Klimov, A. *J. Infect. Dis.* **2007**, *196*, 249-257. c) Bright, R. A. *Morb. Mortal. Weekly Rep.* **2006**, *55*, 44-46.

[115] Furuse, Y.; Suzuki, A.; Oshitani, H. *Antimicrob. Agents Chemoter.* **2009**, *53*, 4457-4463.

[116] Balannik, V.; Wang, J.; Ohigashi, Y.; Jing, X. H.; Magavern, E.; Lamb, R. A.; DeGrado, W. F.; Pinto, L. H. *Biochemistry* **2009**, *48*, 11872-11882.

[117] a) Geluk, H. W.; Schut, J.; Schlatmann, J. L. M. A. *J. Med. Chem.* **1969**, *12*, 712. b) Manchand, P. S.; Cerruti, R. L.; Maritn, J. A.; Hill, C. H., Merrett, J. H.; Keech, E., Belshe, R. B.; Connell, E. V.; Sim, I. S. *J.*

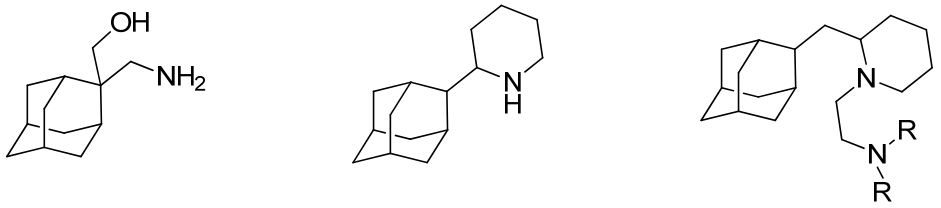



**73** **74** **75** **76**

**Figure 30.** First amantadine and rimantadine derivatives.

Some attempts consisted in adding another nitrogen to rimantadine,[118] the aim of this modification being the incorporation of additional hydrogen bonding interactions with the M2 protein but resulting in no modification on the antiinfluenza activity.

Looking for better compounds Kolocouris' group reported several adamantanaminoalcohols, such as **77**, which had a submicromolar $EC_{50}$ against some H3N2 strain.[119] They also tested the inclusion of the amino group in an heterocyclic ring and the addition of a second amino group far away from the heterocyclic moiety (Figure 31). Among these series they obtained compounds such as **79** showing and $EC_{50}$ between 3 and 7 μM against the X-31 strain, an amantadine-resistant strain, much lower than amantadine and rimantadine.[120]




**77** **78** **79**

**Figure 31.** Substituted 2-adamantanes.

Later on, some azaspiroadamantanes were developed, these unique group of drugs showed to be very active against the Influenza A virus. In fact, one of them, DU34796 that had

*Med. Chem.* **1990**, *33*, 1992-1995. c) Wang, J., Ma, C.; Balannik, V., Pinto, L. H.; Lamb, R. A.; DeGrado, W. F. *ACS Med. Chem. Lett* **2011**, *2*, 307-312. d) Zoidis, G.; Fytas, C.; Papanastasiou, I.; Foscolos, G. B., Fytas, G.; Padalko, E., De Clerq, E.; Naesens, L.; Neyts, J.; Kolocouris, N. *Bioorg. Med. Chem.* **2006**, *14*, 3341.

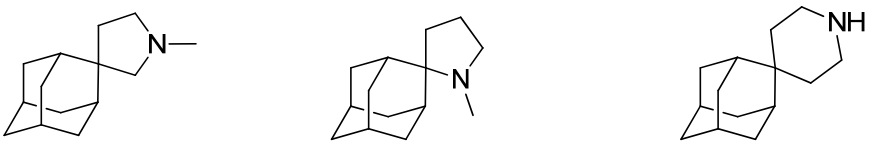
[118] Tataridis, D.; Fytas, G.; Kolocouris, A.; Fytas, C.; Kolocouris, N.; Foscolos, G. B., Padalko, E.; Neyts, J.; De Clercq, E. *Bioorg. Med. Chem.* **2007**, *17*, 692-696.

[119] Zoidis, G.; Kolocouris, N.; Kelly, J. M.; Prathalingam, S. R.; Naesens, L.; DeClerq, E. Eur. *J. Med. Chem.* **2010**, *45*, 5022-5030.

[120] Setaki, D.; Tataridis, D.; Stamatiou, G.; Kolocouris, A.; Foscolos, G. B.; Fytas, G.; Kolocouris, N.; Padalko, E.; Neyts, J.; De Clerq, E. *Bioorg. Chem.* **2006**, *34*, 248-273.

an antiviral spectrum wider than that of amantadine and was more potent than amantadine against mouse influenza, entered clinical trials, although finally the drug was not further developed.[121]

In the nineties, Kolocouris et al. synthesized more analogues of DU34796 obtaining compounds up to 230 times more active than amantadine, such as **80** or **81** (Figure 32).[122] These analogues consisted in changing the position of the nitrogen atom in the heterocycle, adding a methyl group or contracting or expanding the size of the heterocycle. Although being very potent, unfortunately these compounds resulted to be very toxic *in vivo*.




DU34796 **80** **81**

**Figure 32.** Synthesized azaspiroadamantanes.

In 2008, Kolocouris et al. synthesized a series of 1,2-annulated adamantane derivatives. Compounds **82** and **83** showed an $IC_{50}$ of 0.5 and 0.6 μM, respectively, against A/Hong Kong/7/87, an H3N2 strain, being equipotent to rimantadine ($EC_{50}$ = 0.36 μM) (Figure 33).[123]


**82** **83**

**Figure 33.** 1,2-annulated adamantane derivatives.

These results showed that a large lipophilic moiety in the vicinity of the adamantane skeleton is compatible with good-antiviral activity and that moving the amine nitrogen atom away from the 2-adamantyl carbon atom enhances activity.[124]

[121] a) Beare, A. S.; Hall, T. S.; Tyrrel, D. A. *Lancet* **1972**, *299*, 1039. b) Mathur, A.; Beare, A.; Reed, S. E. *Antimicrob. Agents Chemoter.* **1973**, *4*, 421-426. c) Togo, Y. *Antimicrob. Agents Chemoter.* **1973**, *4*, 641-642.

[122] Kolocouris, N.; Foscolos, G. B.; Kolocouris, A.; Marakaos, P.; Pouli, N.; Fytas, G.; Ikeda, S.; De Clerq, E. *J. Med. Chem.* **1994**, *37*, 2896-2902.

[123] a) Zoidis, G.; Tsotinis, A.; Kolocouris, N.; Kelly, J. M.; Prathalingam, S. R.; Kolocouris, N.; Naesens, L.; De Clerq, E. *Org. Biomol. Chem.* **2008**, *6*, 3177-3185. b) Zoidis, G.; Kolocouris, N.; Naesens, L.; De Clerq, E. *Bioorg. Med. Chem.* **2009**, *17*, 1534-1541.

[124] Duque, M. D.; Torres, E.; Valverde, E.; Barniol, M.; Guardiola, S.; Rey, M.; Vázquez, S. *Rec. Adv. Pharm. Sci.* **2011**, *19*, 1655-1663.

## 5. Recent advances in designing M2 channel blockers.

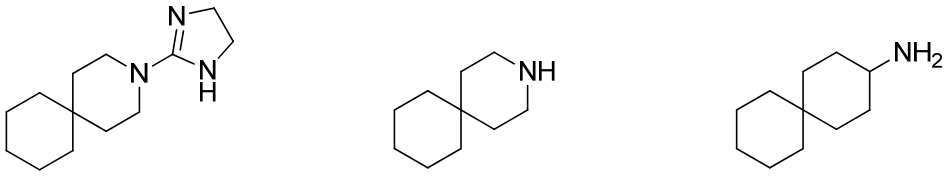
Until 2008, hundreds of M2 channel blockers derived from amantadine and rimantadine had been synthesized and tested against Influenza A virus although little was known about the structure and the mechanism of function of M2 channel. In fact, those compounds were designed and synthesized merely using SAR (Structure-Activity Relationship) studies.

In 2008, a paramount discovery in the field occurred; William F. DeGrado and co-workers disclosed, in Nature, the 3D structures of the transmembrane domain of the protein with and without amantadine which was going to allow studying the interaction of the inhibitor with the protein.[90] Since the publication of the paper several works reporting the mechanism of inhibition of amantadine and the mutations that undergoes the channel to become resistant to this drug have been published.[89a, 111, 125] Moreover, the 3-D structure of the S31N,[111a,126] V27A mutants[127] and A/M2-B/M2 chimeric channels[128] have been published.

All of these events opened the door to the rational design of new inhibitors of the M2 channel, and not surprisingly, years from 2008 to date have been very fruitful for the understanding of the protein and the inhibition of the M2 channel and several good inhibitors have been disclosed including molecules able to inhibit V27A, S31N and L26F resistant strains, most of them reported while this Thesis was in progress. Following, the most relevant examples will be pointed out.

In 1995, Bristol-Myers Squibb's researchers carried out a high-throughput screening based on the ability of inhibitors to reverse the toxicity associate with M2 channels expressed in the yeast *Saccharomyces cerevisae* membranes. They found an azaspiro[5.5]undecane derivative, BL-1743 (Figure 34), able to efficiently inhibit the activity of wt influenza A M2 channels.[129] Taking into account their 3-D structure of the M2 channel of Influenza A virus first reported in 2008, Pinto's and DeGrado's groups started a SAR study of the BL-1743 scaffold with the aim of discovering new inhibitors of amantadine-resistant mutants.[116,130] Interestingly, they found that spiropiperidine **84** had an $EC_{50}$ of 0.9 μM against influenza A wt M2 channel expressed in the *Xenopus* oocytes membrane, which is more than one order of magnitude more potent than amantadine ($EC_{50}$ = 16 μM) and represents a more than 45-fold increase in potency relative to BL-1743 ($EC_{50}$ = 45.3 μM). Moving the nitrogen atom out of the spiro-ring led to amine **85** that has an $EC_{50}$ of 12.6 μM, very similar to amantadine.

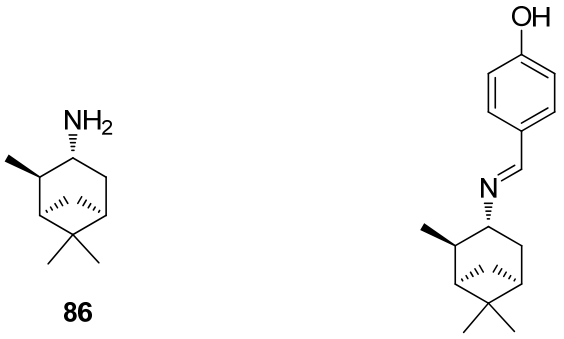
[125] Cady, S. D.; Schmidt-Rohr, K.; Wang, J.; Soto, C. S.; DeGrado, W. F.; Hong, M. *Nature* **2010**, *463*, 689-693.
[126] a) Wang, J.; Wu, Y.; Ma, C.; Fiorin, G.; Wang, J.; Pinto, L. H.; Lamb, R. A.; Klein, M. L.; DeGrado, W. *Proc. Nat. Acad. Sci*. **2013**, *110*, 1315-1320. b) Williams, J. K.; Tietze, D.; Wang, J.; Wu, Y.; DeGrado, W.; Hong, M. *J. Am. Chem. Soc.* **2013**, 135, 9885-9897.
[127] Pielak, R. M.; Chou, J. J. *Biochem. Biophys. Res. Commun.* **2010**, *401*, 58-63.
[128] Pielak, R. M.; Oxenoid, K.; Chou, J. J: *Structure* **2011**, *19*, 1655-1663.
[129] Kurts, S.; Luo, G. X.; Hahnenberger, K. M.; Brooks, C.; Gecha, O.; Ingalls, K.; Numata, K. I.; Krystal, M. *Antimicrob. Agents Chemoter.* **1995**, *39*, 2204.
[130] Wang, J.; Cady, S. D.; Balannik, V.; Pinto, L. H.; DeGrado, W. F.; Hong, M. *J. Am. Chem. Soc.* **2009**, *131*, 8066.




**BL-1743** **84** **85**

**Figure 34.** BL-1743 and its analogues.

In 2010, Chen et al. tested a large number of lineal, aromatic, monocyclic, bicyclic and tricyclic amines against an H1N1 amantadine resistant strain and an H3N2 amantadine sensitive strain, discovering amine **86** as a potent M2 channel inhibitor ($EC_{50}$ = 4.3 μM in the H3N2 strain).[131] After finding the proper polycyclic amine Hu et al. studied the optimum substitution of the amine moiety in order to increase the activity of those compounds doing SAR studies. They found that imine **87** containing a phenol moiety at the end of the molecule possesses an $EC_{50}$ = 0.088 μM against an H3N2 amantadine sensitive strain, nearly 240-fold more potent than amantadine (Figure 35).[132]

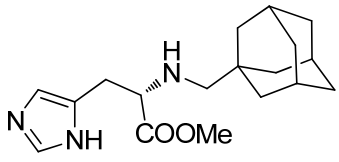



**86** **87**

**Figure 35.** New inhibitors of M2 channel.

In 2010, Guochun et al. explored the effects of adding an heteroaromatic group as a substituent in the amine moiety of adamantane.[133] After synthesizing several molecules possessing different heterocycles, they found that compound **88** that it is endowed with an imidazole group at the end of the molecule (Figure 36) exhibits a more than acceptable $EC_{50}$ against S31N and wild type strains of the Influenza A virus. These compound possesses an $EC_{50}$ = 5.8 μM for wild type, $EC_{50}$ = 10.96 μM for S31N and $EC_{50}$ = 9.77 μM for S31N/L26F strains.

[131] Hu, W.; Zeng, S.; Li, C.; Jie, Y.; Li, Z.; Chen, L. *J. Med. Chem.* **2010**, *53*, 3831-3834.
[132] Zhao, X.; Li, C.; Zeng, S.; Hu, W. *Eur. J. Med. Chem.* **2011**, *46*, 52-57.
[133] Wenjuan, Z.; Jing, X.; Fang, L.; Chufang, L.; Yanling, J.; Shaopeng, C.; Zhiyuan, L.; Jinsong, L.; Ling, C.; Guochun, Z. *Chin. J. Chem.* **2010**, *28*, 1417-1423.




**88**

**Figure 36.** New wt, S31N and L26F inhibitor.

In 2011, DeGrado and co-workers disclosed a new family of M2 inhibitors, organosilanes amines.[134] This approach was based on the fact that the bond between carbon and silicon is a bit larger than the carbon carbon bond. With this idea in mind, they tried to make molecules that occupy more space into the channel lumen. The most active compounds of this series were compounds **89** and **90** (Figure 37Figure ). The first one being more potent than amantadine for wild type ($EC_{50}$ = 2.6 μM) but not showing activity against any of the mutant channels. Amine **90**, with an $EC_{50}$ of 13.7 μM against the wt channel, had also an $EC_{50}$ = 31.3 μM against the V27A mutant channel, being the first compound that showed an acceptable activity against the V27A mutant M2 channel.


**89** **90**

**Figure 37.** New synthesized organosilane amines.

In the same year, DeGrado and co-workers disclosed a different family of compounds; they designed and synthesized compound **91** (Figure 38) which emerged from a superposition of compound **85**, also described by the Grado's group (Figure 34), and amantadine.[110,135] This compound was tested against wt M2 channel of influenza A virus expressed in oocytes of *Xenopus laevis*, and showed an $EC_{50}$ of 18.7 μM, very similar to amantadine. More interestingly, compound **91** revealed to be a submicromolar inhibitor of the clinically important mutant V27A ($EC_{50}$ = 0.31 μM) and also showed to be active against the mutant L26F

[134] Wang, J.; Ma, C.; Wu, Y.; Lamb, R. A.; Pinto, L. H.; DeGrado, W. F. *J. Am. Chem. Soc.* **2011**, *133*, 13844-13847.
[135] DeGrado, W. F.; Wang, J. (University of Pennsylvania), **2011**, WO2011/022191.

($EC_{50}$ = 5.6 µM). To the best of our knowledge, **91** is the most potent compound ever reported against mutant V27A.

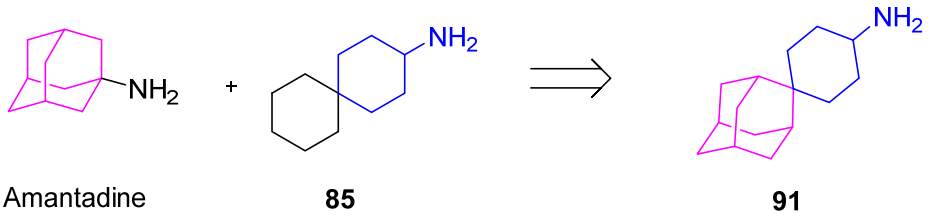



**Figure 38.** The most active compound against V27A strains.

During the writing up of this Thesis, DeGrado and co-workers have reported a new class of M2 inhibitors which acted as dual agent against wild type and S31N strains.[136] These compounds have an adamantane ring and an aromatic ring bonded to the amine moiety. In a first batch of 50 compounds, they pointed out that for obtaining S31N activity was essential to have an aromatic ring and two acceptors of hydrogen bond in the positions 2 and 4 of the aromatic ring. The optimum substitutions resulted to be two hydroxyls. Compound **92a** (Figure 39), the most active of this family, showed and $IC_{50}$ of 59 µM for wild type, one fold less potent than amantadine, and $EC_{50}$ of 35 µM against S31N. Although being less potent than compound **88** this work has shown very important clues for the future synthesis of S31N inhibitors.

In a second batch of compounds,[126] they reported the most active compound against S31N to date, **92b**, with an $EC_{50}$ of 16 µM. This compound possesses an ammonium group that mimics an hydronium cation and it receives additional stabilization interacting with residues Val27 and the side chain of Asn31 (Figure 39b). Unfortunately, **92b** is not active against the wt channel.

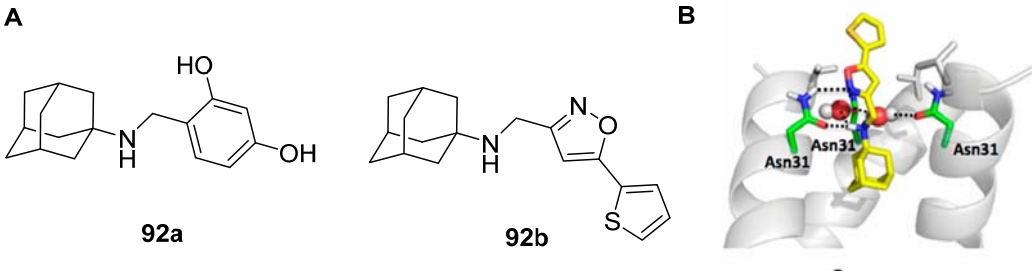


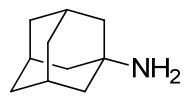

**Figure 39.** a) New class of S31N inhibitors. b) Binding mode of compound **92b**.

[136] Wang, J.; Ma, C.; Wang, J.; Jo, H.; Canturk, B.; Fiorin, G.; Pinto, L. H.; Lamb, R.; Klein, M.; DeGrado, W.F. *J. Med. Chem.* **2013**, *56*, 2804-2812.

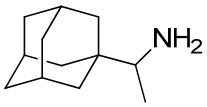
## 6. Previous work of our group in designing M2 inhibitors.

Taking into account the previous work in the field and following the wide expertise of our research group in the synthesis of polycyclic scaffolds some analogs of amantadine and rimantadine were synthesized over the last 8 years. These are some exemples:

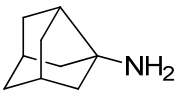
a) noradamantane amines like **93** and **95**, as analogues of *amantadine* and *rimantadine* by ring-contraction, and amine **94** by homologation of **93**.



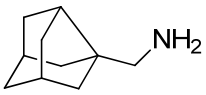

*Amantadine*



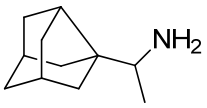

*Rimantadine*




**93**

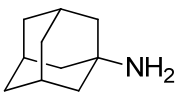



**94**

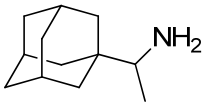



**95**

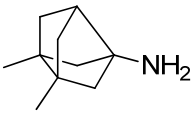
b) bisnoradamantane amines like **96** and **98**, as analogues of *amantine* and *rimantadine* by double ring-contraction, and amine **97** by homologation of **96**.



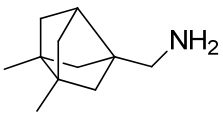

*Amantadine*



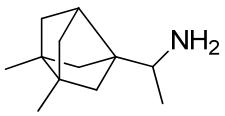

*Rimantadine*




**96**

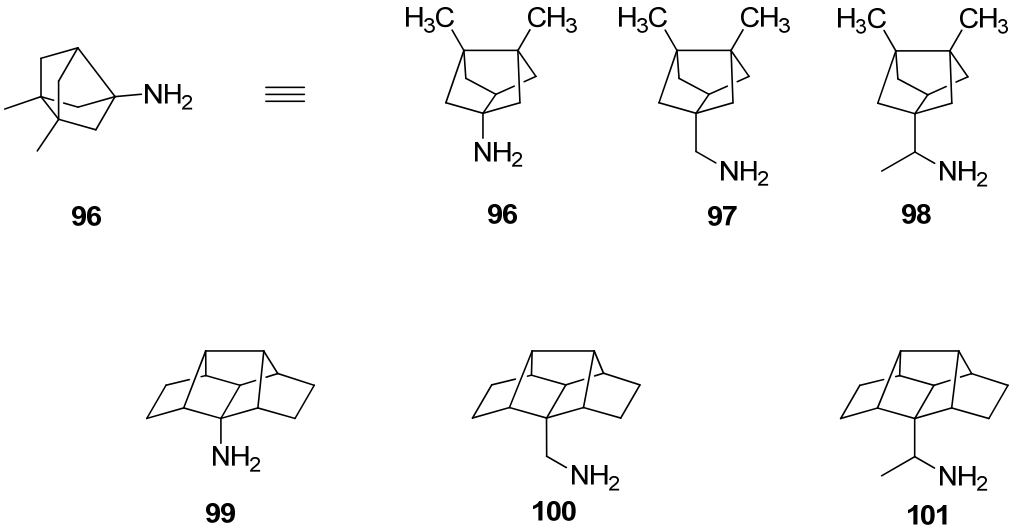



**97**




**98**

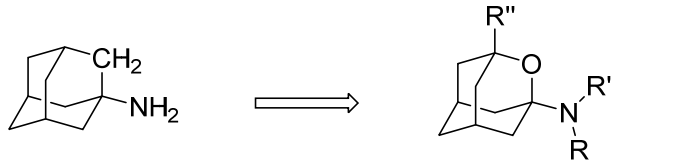
c) pentacyclic amines like **99**, **100**, and **101** as analogues of the previously shown bisnoradamantane amines, by addition of further rings.




**96** **96** **97** **98**

**99** **100** **101**

d) oxadamantane amines of general structure **VII**, as *amantadine* analogues by bioisosterism, replacing one methylene unit by an oxygen atom.




Amantadine **VII** R= H, OH, alkyl

From these starting amines, several derivatives, mainly secondary and tertiary amines were prepared. Also, in several examples, the amino group was replaced by a guanidine or amidine groups, in order to assess the effect of the basicity in the pharmacological activity.

All new compounds from Dra. M. D. Duque Thesis were tested as antivirals by the research group of the Professors Erik de Clercq and Lieve Naesens, from the Rega Institute for Medical Research (Leuven, Belgium). They found that the 2-oxadamantanes had no antiviral activity. Also, the bisnoradamantane and the noradamantane derivatives were either inactive or only slightly active against the influenza A virus. By way of contrast, several of the pentacyclic amines showed to have potent anti-influenza activity against the A/PR/8/34 H1N1, a strain that bears two mutation in the M2 channel associated with resistance to amantadine (S31N and V27A).

Nineteen pentacyclic analogues were synthesized, nine inhibited the influenza virus A/H1N1 replication in infected MDCK cells. As shown in Table 5, the amine **105·HCl** ($IC_{50}$= 2.0 ± 0.0 μM) and acetamidine **106·HCl** ($IC_{50}$= 2.0 ± 0.0 μM), were the most potent compounds against the influenza virus A/H1N1, being 15-fold more potent than rimantadine and 35-fold more potent than amantadine. It is also interesting the activity of compound **103·HCl** with an $IC_{50}$= 8.0 ± 1 μM, which is four fold more active than rimantadine and 9 fold more potent than amantadine. Finally, compounds **102·HCl**, **104·HCl** and **107·HCl** display a similar activity than rimantadine and they are twofold more active than amantadine (Figure 40).

**Table 5**.Anti-influenza activity of the new polycyclic amines.

| Compound | Antiviral activity $IC_{50}$[a] (μM) | | | | | | Toxicity (MCC[b]) |
|---|---|---|---|---|---|---|---|
| | Influenza A/H1N1 | | Influenza A/H3N2 | | Influenza B | | |
| | CPE | MTS | CPE | MTS | CPE | MTS | |
| **93·HCl** | 195±110 | 198±103 | 26±22 | 26±22 | N.A.[c] | N.A. | > 100 |
| **94·HCl** | 36.4±11 | 48.7±7 | 51.5±16 | 65±44 | N.A. | N.A. | > 100 |
| **97·HCl** | N.A. | N.A. | 6.5±3.0 | 5.9±4 | N.A. | N.A. | > 100 |
| **102·HCl** | 27±18 | 23.6±11.3 | N.A. | N.A. | N.A. | N.A. | ≥ 100 |
| **103·HCl** | 8±1 | 8.9±2.3 | N.A. | N.A. | N.A. | N.A. | 100 |
| **104·HCl** | 27±18 | 32.2±21.4 | N.A. | N.A. | N.A. | N.A. | > 100 |
| **105·HCl** | 2.0±0.0 | 3.3±0.3 | N.A. | N.A. | N.A. | N.A. | > 100 |
| **106·HCl** | 2.0±0.0 | 2.2±0.0 | N.A. | N.A. | N.A. | N.A. | > 100 |
| **107·HCl** | 27±18 | 26.6±18.1 | N.A. | N.A. | N.A. | N.A. | > 100 |
| ***Amantadine*** | 72.5±38.9 | 57.5±25.2 | 2.2±0.3 | 1.7±0.8 | N.A. | N.A. | > 100 |
| ***Rimantadine*** | 33.3±11.4 | 24.1±10.2 | 0.25±0.21 | 0.25±0.21 | N.A. | N.A. | > 100 |

[a] $IC_{50}$: Required concentration (μM) to reduce the cytopatogenicity of the virus up to 50% measured by spectrofotometric analysis of the cell viability using an MTS assay. These values are based on the average± error of three experiments

[b] Minimum cytotoxic concentration (μM): needed concentration of the compound to alter the cell morphology mycroscopically.

[c] N.A., no active compound in a range of non cytotoxic concentrations or at the maximum tested concentration.

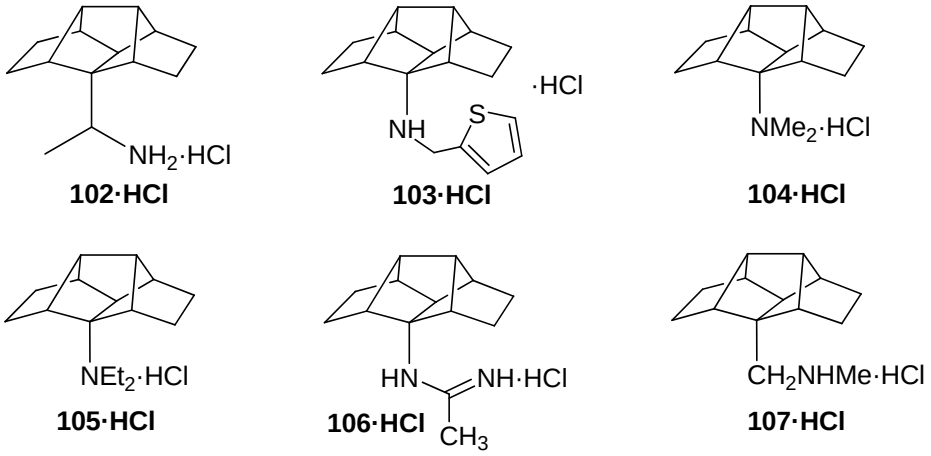
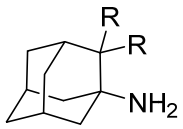



**Figure 40.** Pentacyclic compounds with activity against Influenza A virus.

## PART II: OBJECTIVES

At the beginning of the second part of the Thesis we established as the main objectives the synthesis of the following families of compounds:

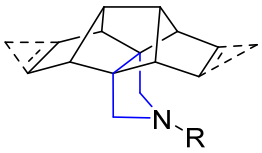
1. Synthesis and pharmacological evaluation of a series of 2,2-dialkylamines with general structure **VIII** and their derivatives. These molecules were designed with the objective of increasing the bulkiness of the adamantane cage to better fill the lumen of the M2 channel protein.




**VIII**

**Figure 41.**

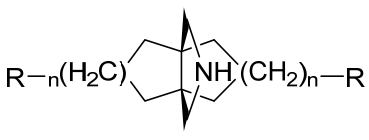
2. Synthesis and pharmacological evaluation of 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-dienes and related compounds as ring rearranged analogues of amantadine and rimantadine. In this case the amine is enclosed in a pirrolidine ring in order to further increase the activity of the previously synthesized (pentacyclo[6.4.0.0$^{2,10}$.0$^{3,7}$.0$^{4,9}$]dodec-8-yl)amines in Dra. M. D. Duque's Thesis.




**IX**

**Figure 42.**

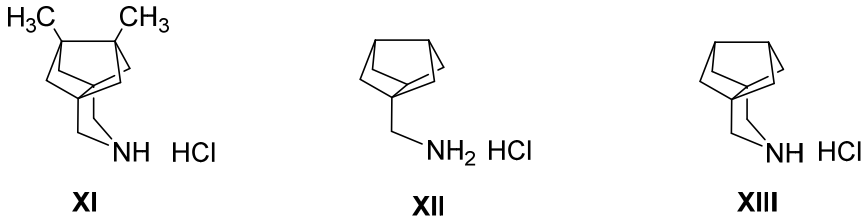
3. Synthesis and pharmacological evaluation of 3-azatricyclo[3.3.3.0]undecanes and related compounds as simplified analogues of 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-dienes.




**X**

**Figure 43.**

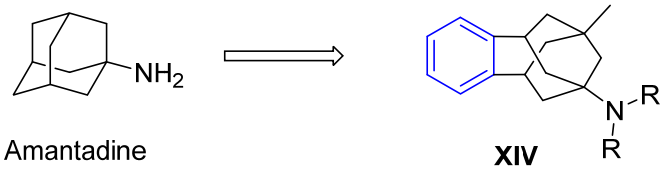
4. Synthesis and pharmacological evaluation of 3-aza-7,8-dimethyltetracyclo[5.2.1.1$^{5,8}$.0$^{1.5}$]undecane, **XI,** (tricyclo[3.3.0.0$^{3,7}$]oct-1-yl)amines, **XII** and 3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1.5}$]undecane, **XIII**, and their derivatives, as ring contracted analogs of amantadine and rimantadine.




**XI** **XII** **XIII**

**Figure 44.**

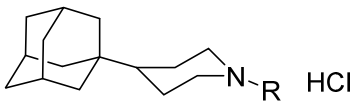
5. Synthesis and pharmacological evaluation 6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5*H*-benzocyclononen-7-amine and its derivatives, of general structure **XIV**, as analogs of amantadine and rimantadine by addition of a ring.




Amantadine **XIV**

**Figure 45.**

6. Synthesis of 4-(1-adamantyl)piperidines and related compounds as larger analogues of amantadine and rimantadine (Figure 46).




**XV**

**Figure 46.**

**PART II: THEORETICAL PART**

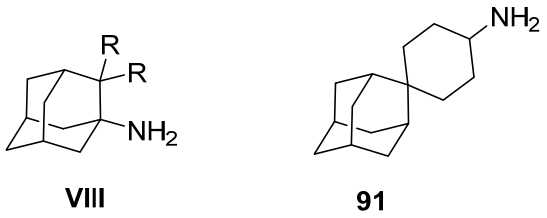
# COMPOUNDS TARGETING HEMAGGLUTININ

# 1. 2,2-Dialkyladamantamines.

## 1.1 Earlier attempts to synthesize 2,2-dimethyl-1-adamantylamine.

Following the work started by Dra. M. D. Duque we decided to further pursue the synthesis of more analogues of amantadine and rimantadine with the aim of obtaining a molecule capable to inhibit several strains of the Influenza A virus.

At the beginning of this work, it was already known that the channel lumen in the V27A mutant was wider than in the wt channel. This fact let us to think that the design and synthesis of larger polycyclic compounds than amantadine may lead to a better inhibitory activity of the V27A mutant channel. Thus, we envisaged the synthesis of new adamantane derivatives of general structure **VIII**, that are endowed with bulky groups in the position 2 of the adamantane cage, with the aim of obtaining a better fulfilment of the M2 channel (Figure 47). In fact, after finishing the synthesis of this family of compounds and while we were waiting for the biological results, at the beginning of 2011, DeGrado's group disclosed in a patent,[135] the spirocompound **91**, which shows an outstanding activity against the V27A mutant as well as against the wild type M2 channel of the Influenza A virus (Figure 47).

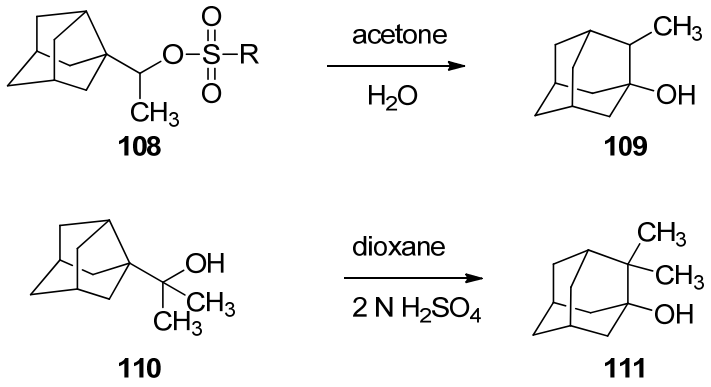



**VIII** **91**

**Figure 47.** New analogs described herein and DeGrado's inhibitor **91**.

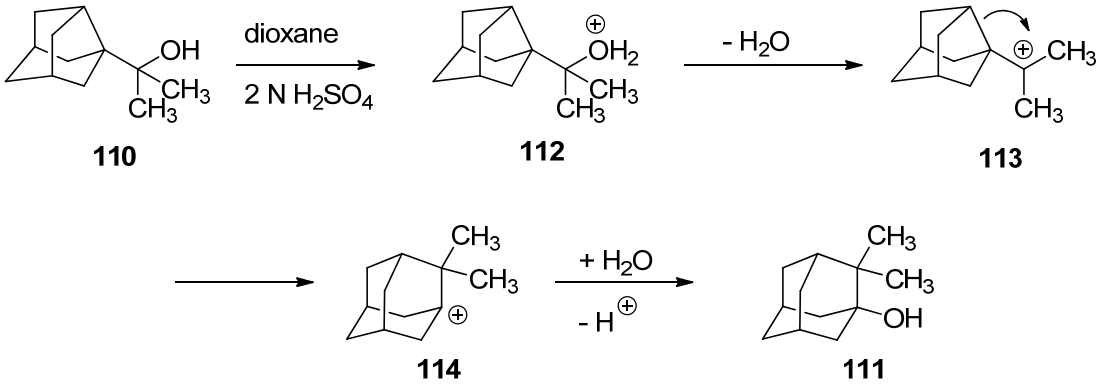
It should be pointed out that although a large amount of amantadine analogs have been described, the 2,2-dialkylated amantadines were unprecedented in the literature.[137] We foresee the synthesis of these new compounds based on the work published in 1993 by Stoelting and Shiner. They reported a detailed study on the solvation reaction of 1-(3-noradamantyl)ethyl sulphonates, showing that this kind of products rearrange in good yields to obtain 2-methyl-1-adamantanol, **109**. On the other hand, they also observed that the reaction of 2-(3-noradamantyl)-2-propanol, **110**, with a mixture of 58:42 of dioxane / 2N $H_2SO_4$ yielded 2,2-dimethyl-1-adamantanol, **111**, with high yields (Scheme 20).[138]

[137] However, some analogs containing some substitution in the position 2 have been described, see for example: Djaidi, D.; Leung, I. S. H.; Bishop, R.; Craig, D. C.; Scudder, M. L. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2037-2042.
[138] Stoelting, D. T.; Shier, V. J. Jr. *J. Am. Chem. Soc.* **1993**, *115*, 1695-1705.




**Scheme 20.** Previous reported rearrangements.

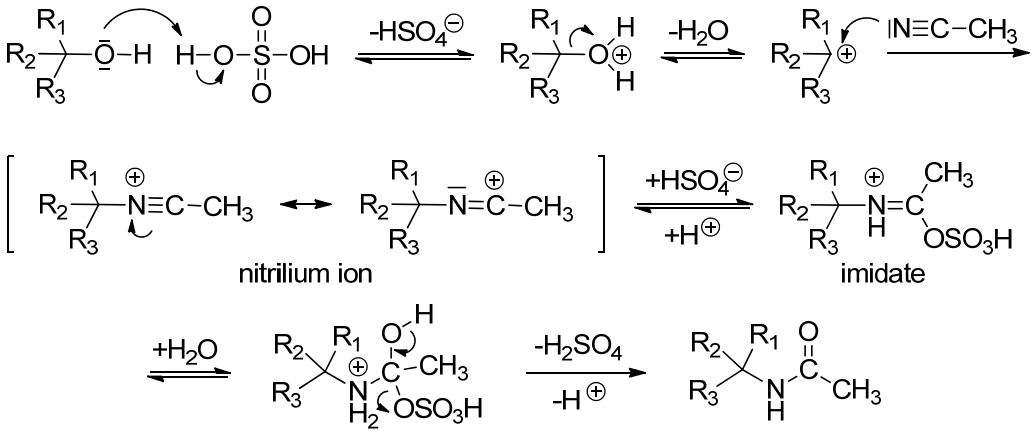
Probably, this transformation involves a Wagner-Meerwein rearrangement of the generated carbocation as it is shown in Scheme 21.




**Scheme 21.** Wagner-Meerwein rearrangement of the noradamantane scaffold.

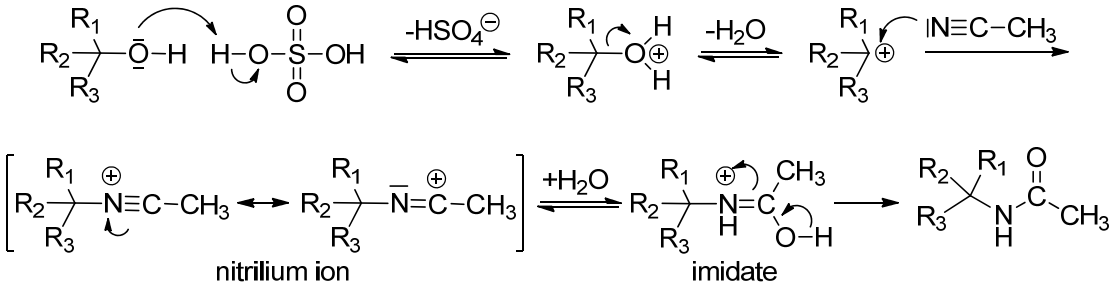
Similarly, the Ritter reaction with alcohols involves, firstly, the protonation of the alcohol and subsequent loss of water generating a carbocation. This carbocation is attacked by the nitrogen of the nitrile, yielding a nitrilium ion that reacts with the conjugated base of the used acid (usually sulphuric acid) obtaining an imidate. Finally, the hydrolysis of the imidate yields the desired *N*-alkyl carboxamide (Scheme 22).[139]

[139] Kürti, L.; Czakó, B. "Strategic Applications of Named Reactions in Organic Sythesis" Elsevier: **2005**, pp 382-383.




**Scheme 22.** Ritter reaction mechanism.

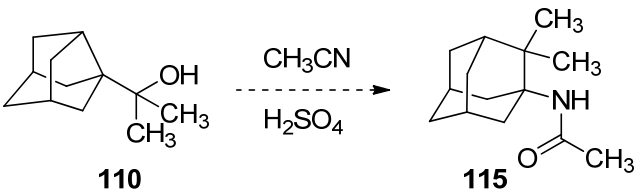
However, sometimes, when the mechanism of the Ritter reaction is drawn, the step where the conjugated base acts is omitted (Scheme 23).[140]




**Scheme 23.** Alternative Ritter reaction mechanism.

Taking into account the examples shown in Scheme 20 and the mechanism of the Ritter reaction, at the beginning of this Thesis we envisaged that the reaction of alcohol **110** under conditions of Ritter reaction may led to the acetamide **115** (Scheme 24) the hydrolysis of the acetamide may lead to a derivative of **VIII**.

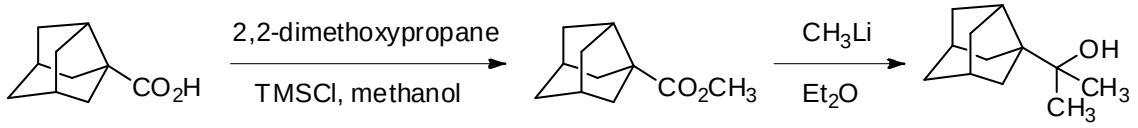
[140] a) Colombo, M. I.; Bohn, M. L.; Rúveda, E. *A. J. Chem. Ed.* **2002**, *79*, 484-485. b) Gerasimova, N. P.; Nozhnin, N. A.; Ermolaeva, V. V.; Ovchinnikova, A. V.; Moskvichev, Y.; Alov, E. M.; Danilova, A. S. *Mendeleev Commun.* **2003**, *13*, 82-83.




**110** **115**

**Scheme 24.**

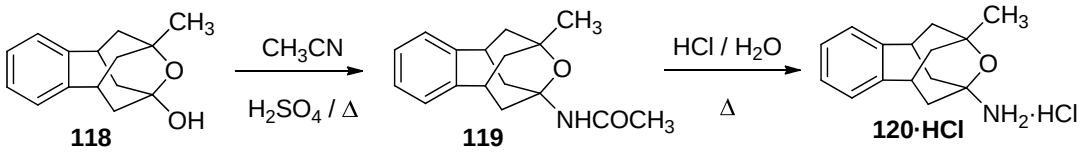
Firstly, we planned the synthesis of enough quantities of the alcohol **110** in order to find the best conditions for such rearrangement. Although the synthesis of the alcohol **110** was already reported by Stoelting and Shiner through addition of an excess of methylmagnesium chloride to 3-acetylnoradamantane, this precursor is not commercially available. So, we envisaged the synthesis of **110** starting from the noradamantane-3-carboxylic acid. Thus, the esterification of the acid **116** in methanol with 2,2-methoxypropane and trimethylsilyl chloride, following a procedure described in the literature, yielded the ester **117** in 84% yield.[141] The reaction of the ester **117** with an excess of methyl lithium in anhydrous diethyl ether gave alcohol **110** in 91% yield (Scheme Scheme 25).




**116** **117** **110**

**Scheme 25.** Synthesis of alcohol **110**.

Having synthesized enough quantities of alcohol **110**, we proceeded to study the best conditions for doing the rearrangement. In our research group, we had previously reported the reaction of alcohol **118** under conditions of Ritter reaction, using acetonitrile and concentrated sulfuric acid, to give acetamide **119** in 81% yield. The hydrolysis of this acetamide in acid media gave the amine **120**, as its hydrochloride salt, in 87% yield (Scheme 26).[142]



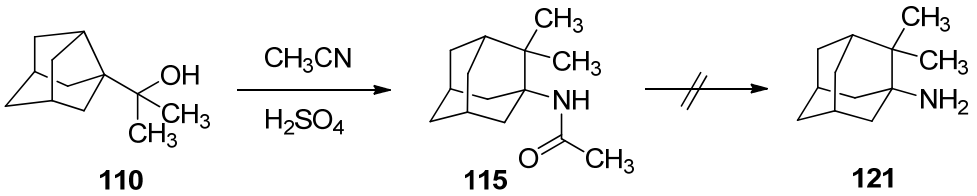

**118** **119** **120·HCl**

**Scheme 26.** Previous hydrolysis of acetamide **119** carried out in our laboratory.

[141] Moss, R. A.; Sauers, R. R.; Sheridan, R. S.; Tian, J.; Zuev, P. S. *J. Am. Chem. Soc.* **2004**, *126*, 10196-10197.

[142] Duque, M. D.; Camps, P.; Torres, E.; Valverde, E.; Sureda, F. X.; López-Querol, M.; Camins, A.; Prathalingam, S. R.; Kelly, J. M.; Vázquez, S. *Bioorg. Chem. Med.* **2010**, *18*, 46-57.

The first attempts to carry out this reaction with the alcohol **110** gave acetamide **115** with good yields but all the attempts to hydrolyze the acetamide **115** to amine **121** led either to the recovering of the starting material or to a complex mixture of decomposition products (Scheme 27).

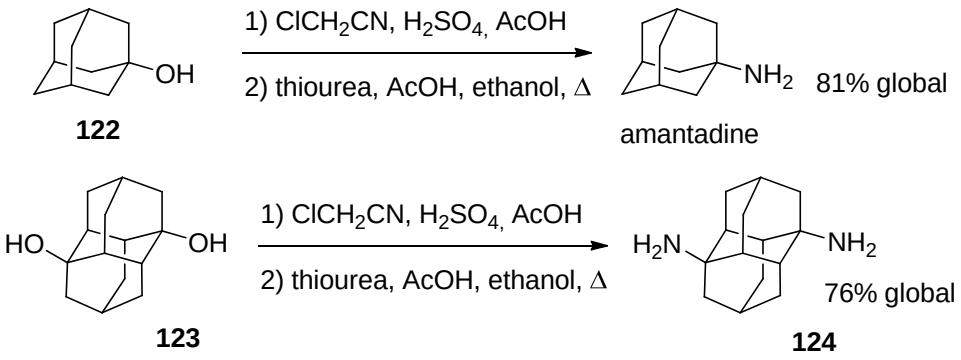



**110** **115** **121**

**Scheme 27.** Failure in the synthesis of amine **121**.

After these negative results we decided to switch the conditions of the Ritter reaction in order to obtain an easily hydrolyzable compound.

In 2000, Jirgensons et al. reported a new effective method for the obtention of *tert*-alkylamines from tertiary alcohols by a Ritter reaction with chloroacetonitrile and subsequent deprotection of the resulting chloroacetamide using thiourea.[143] Recently, Schreiner et al. applied this transformation to several adamantane derivatives.[144] As shown in Scheme 28, this method allows the substitution of a bridgehead alcohol by an amino group in very high overall yields.




**122** amantadine

**123** **124**

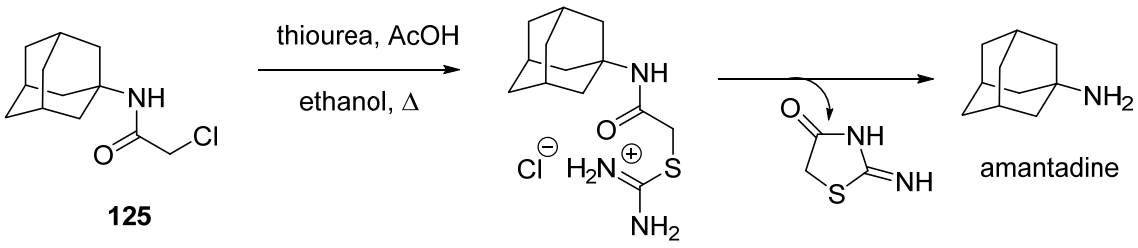
**Scheme 28.** Previously reported synthesis of amines from tertiary alcohols.

The deprotection step involves the nucleophilic substitution of the chlorine atom in the acetamide by the thiourea to give a salt that it can be isolated under certain conditions of

[143] Jirgensons, A.; Kauss, V.; Kalvinsh, I.; Gold, M. R. *Synthesis* **2000**, 1709-1712.

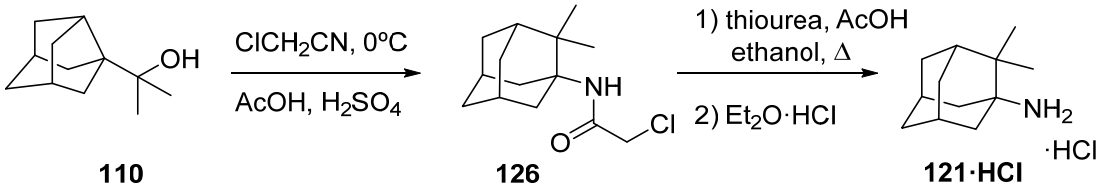
[144] a) Schwertfeger, H.; Würtele, C.; Serafin, M.; Hausmann, H.; Carlson, R. M. K.; Dahl, J. E. P.; Schreiner, P. R. *J. Org. Chem.* **2008**, *73*, 7789-7792. b) Fokin, A. A.; Merz, A.; Fokina, N. A.; Schwertfeger, H.; Liu, S. L.; Dahl, J. E. P.; Carlson, R. M. K.; Schreiner, P. R. *Synthesis* **2009**, 909-912.

reaction. The decomposition of this salt by the intramolecular attack of the nitrogen of the isotiouronium group to the amide carbonyl allowed the release of the amine (Scheme 29).




**Scheme 29.** Mechanism of the deprotection with thiourea.

In our case, the treatment of alcohol **110** with chloroacetonitrile and sulfuric acid in acetic acid gave acetamide **126** with 89% yield. The subsequent transformation of **126** with thiourea gave the desired amine **121**, isolated as its hydrochloride salt in 78% yield (Scheme 30).




**Scheme 30.** Optimized synthesis of amine **121·HCl**.

### 1.2 Synthesis of (2,2-dimethyladamantyl)amine derivatives.

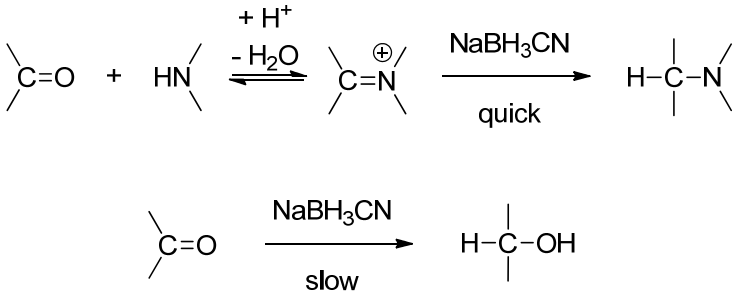
Once we had obtained the desired adamantanyl amine **121**, we planned to face the next goal of this Thesis, thus, the synthesis and pharmacological evaluation of several secondary and tertiary amines derived from the aforementioned amine.

It had been previously reported that substitution in the amine moiety by alkyl or aryl group decreased the antiviral activity of several families of adamantane derivatives.[118, 123a, 145] Generally, the only compatible substitution are small groups with less than 5 or 6 carbon atoms, preferably methyl or ethyl groups. Taking into account this data, we synthesized the secondary and tertiary amines showed in Scheme 33, using reductive alkylation procedures.

Reductive alkylation is the reaction between amines and a carbonyl compound such as aldehydes and ketones, to yield imines that can be reduced by catalytic hydrogenation or several hydrides to the corresponding alkylated amine. One of the most used hydrides is sodium cianoborohydride, as it is a very selective reagent due to the coordination between the boron and the ciano group. Since the ciano group is electronwithdrawing, the reagent

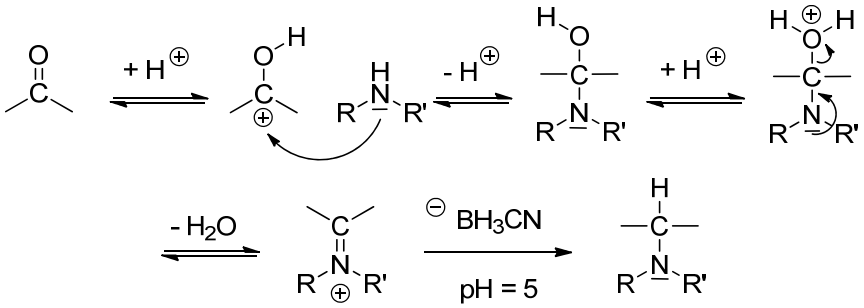
[145] a) Stamatiou, G.; Foscolos, G. B.; Fytas, G.; Kolocouris, A.; Kolocouris, N.; Pannecouque, C.; Witvrouw, M.; Padalko, E.; Neyts, J.; De Clercq, E. *Bioorg. Med. Chem.* **2003**, *11,* 5485-5492. b) Kolocouris , A.; Tataridis, D.; Fytas, G.; Mavromoustakos, T.; Foscolos, G. B.; Kolocouris, N.; De Clercq, E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3465-3470.

becomes milder and more selective than, for example, sodium borohydride. Then, it is possible to carry out the reductive amination of an aldehyde or a ketone by mixing the carbonyl compound and the amine at slightly acid pH in the presence of sodium cianoborohydride in a 'one-pot' procedure. The presence of the carbonyl compound and the hydride in the reaction mixture is not a problem because sodium cianoborohydride reduces far quicker the iminium ion moiety than the carbonyl (Scheme 31).[146]




**Scheme 31.** $NaBH_3CN$ selectivity.

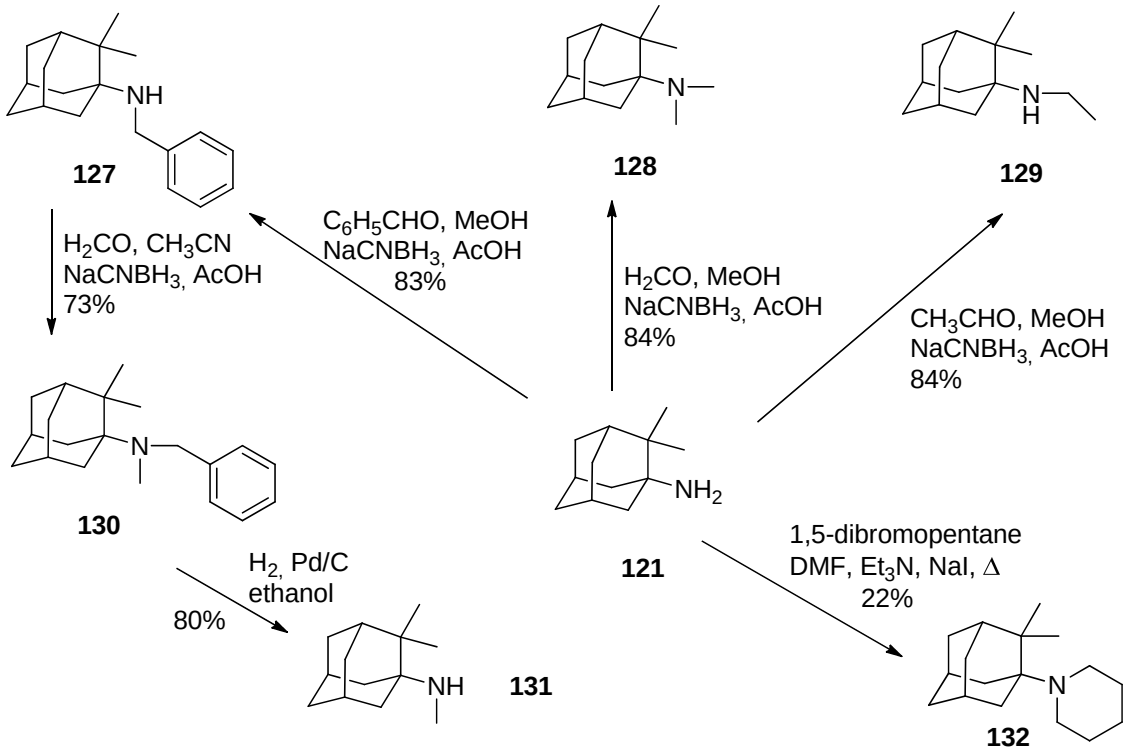
In a usual procedure the carbonyl compound and the amine are in equilibrium with the corresponding iminium ion or the imine in the presence of the reductant.



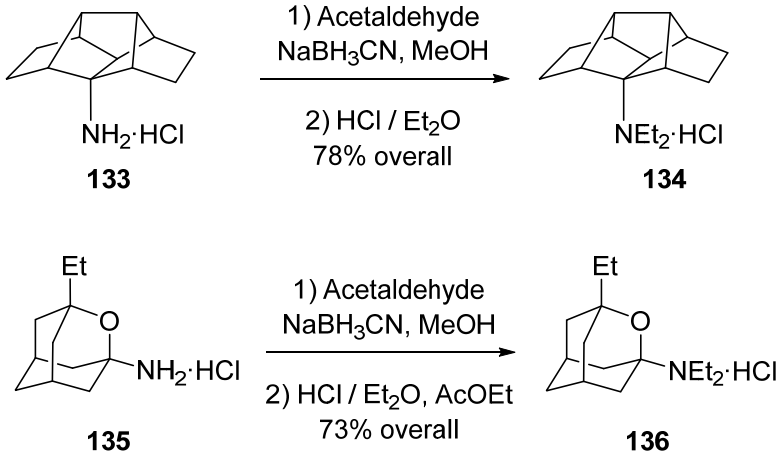

**Scheme 32.** Reductive amination. R, R'= alkyl, aryl, H.

Using this kind of reaction, six new derivatives of the amine **121** were synthesized. Thus, the treatment of **121** with benzaldehyde, $NaCNBH_3$ and acetic acid in methanol yielded the benzylic amine **127** in 83% yield. The treatment of **121** with an excess of aqueous formaldehyde, $NaCNBH_3$ and acetic acid in methanol gave the dimethyl amine **128** with an 84% yield. Surprisingly, the treatment of **121** with an excess of acetaldehyde in the same conditions did not furnish the expected tertiary amine, but the secondary amine **129** in 84% yield (Scheme 33). Attempts to introduce a second ethyl group in **129**, using an excess of acetaldehyde, $NaCNBH_3$ and acetic acid did not give the desired amine but led to the recovery of the starting material. This result was in contrast with some previous results obtained in our laboratory (Scheme 34)[142,147]. We hypothesized that in **129** the geminal dimethyl substitution in the position 2 of the adamantane cage makes enough steric hindrance to avoid the insertion of the second ethyl substituent.

[146] a) Lane, C. F. *Synthesis* **1974**, 135-146. b) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897-2904.
[147] María D. Duque, Doctoral Thesis (Universitat de Barcelona, 2010).




**Scheme 33.** New synthesized derivatives of amine **121·HCl.**

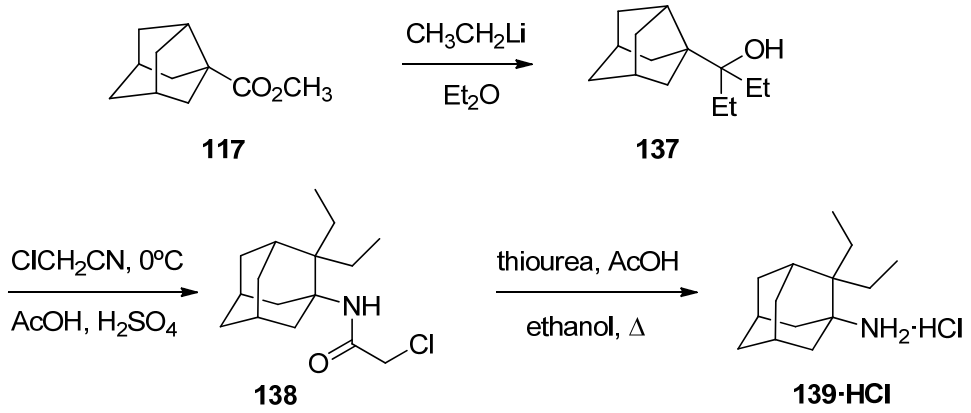



**Scheme 34.** Previous examples of reductive alkylation with acetaldehyde.

The synthesis of **132** also took place in low yield, once again the steric hindrance can be blamed for it. By way of contrast, the reductive amination of **127** with formaldehyde gave the tertiary amine **130** in 73% yield. Finally, the debenzylation reaction of **130** gave **131** with an 80% yield. All compounds were isolated and fully characterized as their hydrochloride salts.

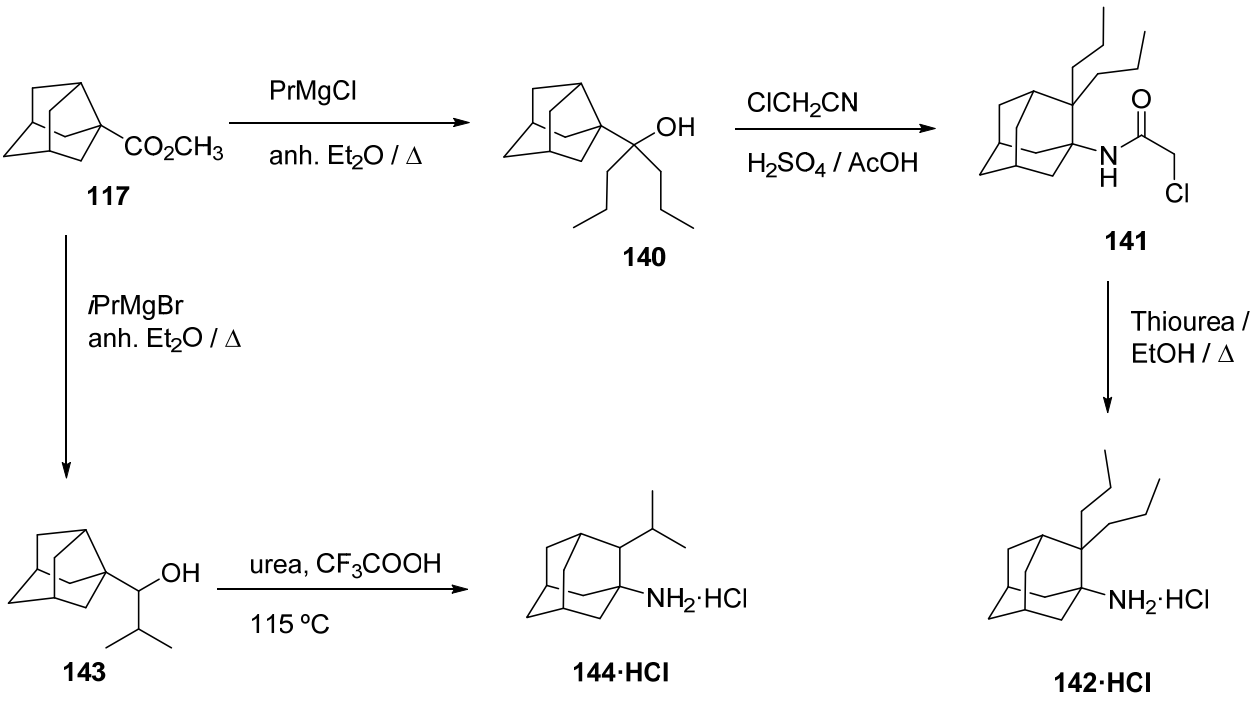
### 1.3 Synthesis of other (2,2-dialkyladamantyl)amines.

As a second objective, we planned to extend the methodology employed for obtaining the amine **121·HCl** to other noradamantyl alcohols analogues to **110**. Firstly, we envisaged the synthesis of the diethyl amine **139·HCl** which synthesis would involve the reaction of the ester **117** with an excess of ethyl lithium, Ritter rearrangement with chloroacetonitrile and treatment of the chloroacetamide with urea. As shown in Scheme 35, the planned synthetic route smoothly led to the amine **139** from the ester **117** in a 32% overall yield (Scheme 35).




**Scheme 35.** Synthesis of amine **139·HCl.**

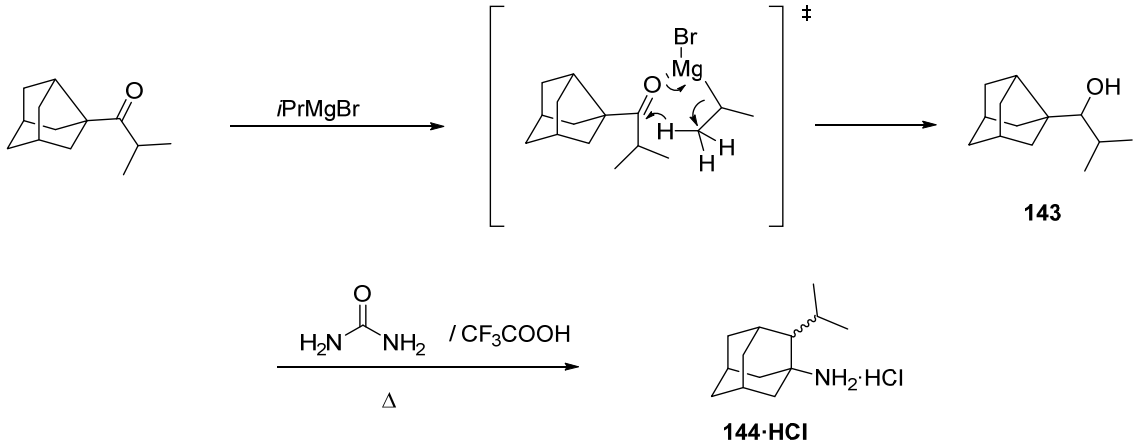
In order to gain further insight in the SAR, more 2,2-dialkylated amines were synthesized. Thereby, amines possessing two propyl groups or an isopropyl group in the position 2 of the polycyclic scaffold were synthesized (Scheme 36).




**Scheme 36.** Synthesis of amines **142·HCl** and **144·HCl.**

Amine **142** was obtained after treatment of the ester **117** with an excess of propyl magnesium chloride in THF to obtain alcohol **140** in a 96% yield. Then the alcohol was allowed to react with chloroacetonitrile and concentrated sulphuric acid in acetic acid to give chloroacetamide **141** in 91% yield. Finally, the reaction of the chloroacetamide **141** with thiourea in ethanol furnished the amine **142·HCl** in 91% yield.

Noteworthy, several attempts to synthesize 1-adamantyl-1,1-diisopropylmethanol from ester **117** using a large excess of *i*PrMgBr were unfruitful, probably as a consequence of too much steric hindrance, the alcohol **143** being obtained, instead. Due to the large excess of the Grignard reagent and the impossibility of a second attack in the carbonyl group, the alcohol is generated by reduction (Scheme 37).[148] After the first nucleophilic attack the ketone would coordinate with another molecule of isopropyl magnesium bromide followed by a hydride transfer from the β carbon atom of the Grignard reagent to the electrophilic carbon of the ketone through a six membered transition state.[149] The aqueous work-up would generate compound **143**.

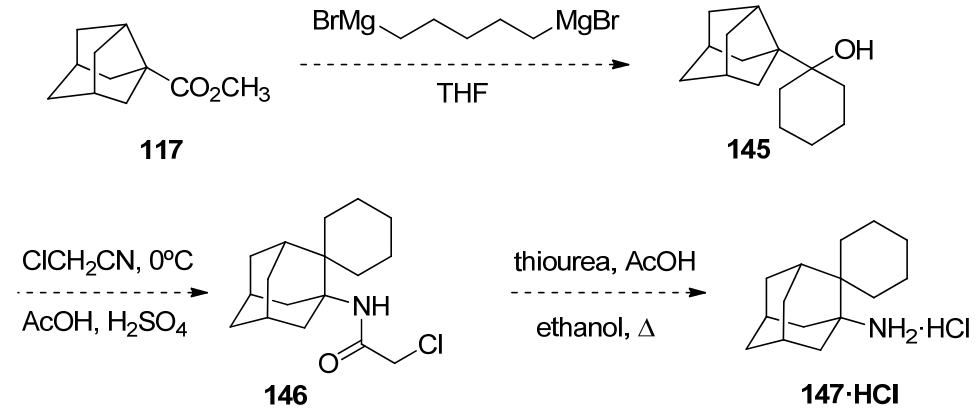



**Scheme 37.** Reduction of the ketone by isopropylmagnesium bromide and synthesis of amine **144·HCl**.

From the noradamantyl alcohol **143**, the amine **144** was obtained using an alternative method that generates the rearranged amine in a "one-pot" procedure by refluxing the alcohol with urea in $CF_3COOH$ media with 43% yield.[150] Although we tried to apply this one-pot procedure to other alcohols, in all the evaluated examples, low yields and complex mixtures were obtained.

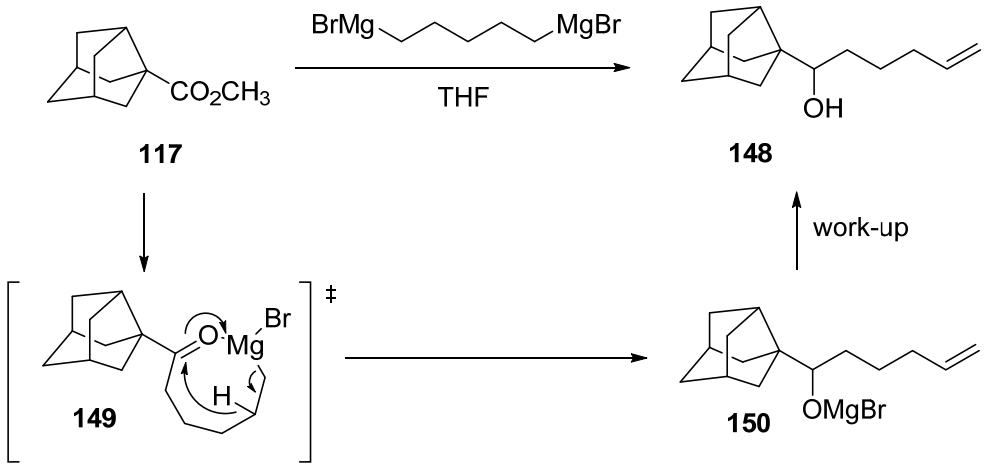
The synthesis of amine **147** from alcohol **145** resulted to be more troublesome than initially expected. Initially, we envisaged that the alcohol **145** would be readily available through the reaction of the ester **117** with pentamethylene magnesium dibromide as shown in Scheme 38. From the alcohol, our usual sequence would lead to the amine **147·HCl**.

[148] The reductive ability of Grignard reagents in sterically encumbered ketones is well precedented: Brucker, R. "Reaction Mechanism. Stereochemistry and Synthesis." Springer-Verlag; Berlin; **2010**, pp 328-330.
[149] Kharasch, M. S.; Weinhouse, S. *J. Org. Chem.* **1936**, *1*, 209-230.
[150] a) Shokova, E.; Mousoulou, T.; Luzikov, Y.; Kovalev, V. *Synthesis* **1997**, *9*, 1034-1040. b) Moiseev, I. K.; Makarova, N. V.; Zemtsova, M. N. *Russ. Chem. Rev.* **1999**, *68*, 1001-1020.




**Scheme 38.** First attempted synthesis of amine **147·HCl.**

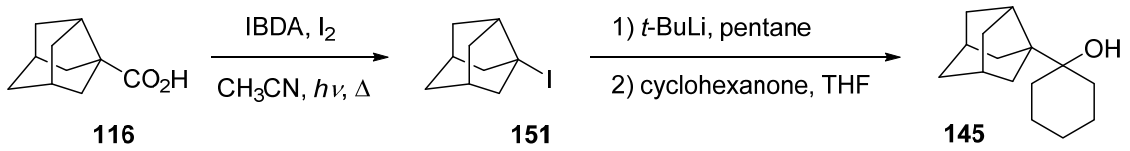
However, the reaction of **117** with the Grignard reagent did not give the desired alcohol but a different product with the same molecular weight. Based on the NMR spectra we concluded that the obtained product was the secondary alcohol **148**. The generation of this product can be explained by the addition of the first nucleophilic carbon atom to the carbonyl of the ester, to generate the intermediate ketone, **149**, which instead of undergoing the second nucleophilic addition it undergoes an intramolecular hydride transfer that finally leads to **148** (Scheme 39).




**Scheme 39.** Mechanism of the reaction to obtain alcohol **148.**

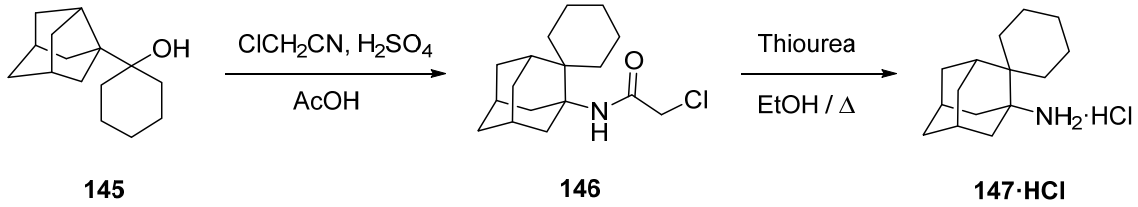
Taking into account this negative result, we envisaged a second, alternative synthetic route that involved the iododecarboxylation reaction of the acid **116** to obtain the iodonoradamantane **151**, a product already known in the literature using another synthetic

procedure.[151] The reaction of **151** with *tert*-butyl lithium followed by the addition of cyclohexanone gave, after column chromatography, the alcohol **145** in 56% yield (Scheme 40).




**Scheme 40.** Synthesis of alcohol **145**.

Alcohol **145** also smoothly underwent the Ritter reaction to give chloroacetamide **146** with 38% yield, which was converted into the desired amine **147·HCl** after the standard treatment with thiourea in ethanol in 62% yield (Scheme 41).




**Scheme 41.** Synthesis of amine **147·HCl.**

The structure of compound **146** was unequivocally assigned by X-Ray crystallography, this fact allowed us to prove the Wagner-Meerwein rearrangement occurred in our molecules since the substitution originally bonded to the 1st carbon in the polycyclic cage ends up in the second position (Figure 48).

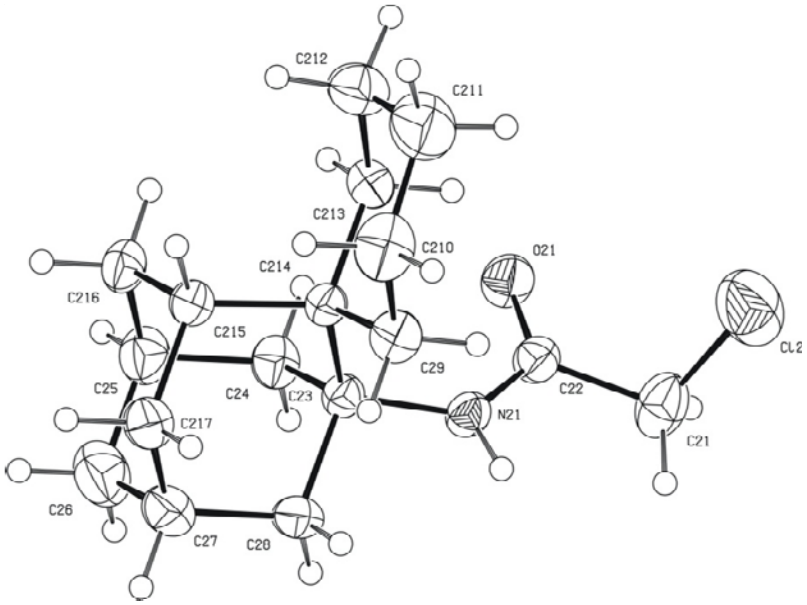
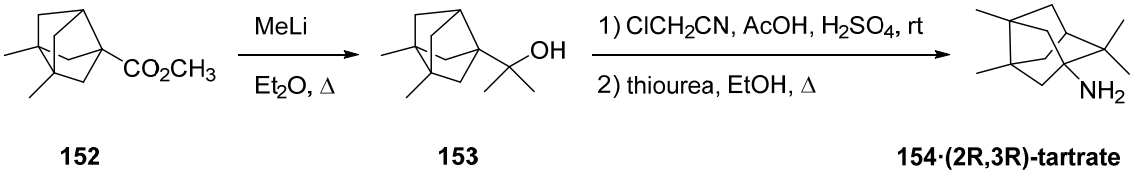



**Figure 48.** X-ray difraction structure (ORTEP) of **146**.

[151] Sosnowski, J. J.; Rheingold, A. L.; Murray, R. K. Jr. *J. Org. Chem.* **1985**, *50*, 3788-3791.

Using the aforementioned methodology, the amine **154** as its corresponding **(2R,3R)-tartrate** was synthesized as a ring-contracted analogue of amantadine. Thus, reaction of known ester **152** with excess of methyllithium followed by Jirgensons' conditions led to the noradamantane derivative **154·(2R,3R)-tartrate** in 52% yield after three synthetic steps (**Scheme** 42).[152]



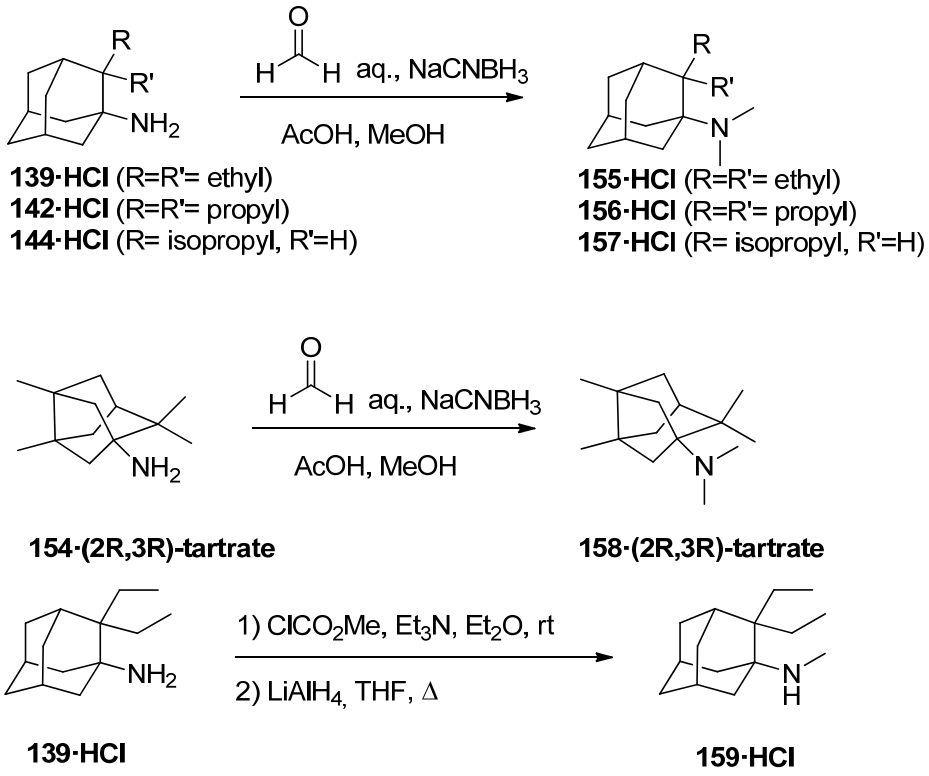

**152** **153** **154·(2R,3R)-tartrate**

MeLi
Et2O, Δ
1) ClCH2CN, AcOH, H2SO4, rt
2) thiourea, EtOH, Δ
CO2CH3
OH
NH2
152
153
154·(2R,3R)-tartrate

**Scheme 42.** Synthesis of amine **154·(2R,3R)-tartrate.**

Afterwards, some *N*-alkylated derivatives of the all aforementioned amines were synthesized in order to gather more information in SAR studies (Scheme 43). The *N*,*N*-dimethyl derivatives of amines **139·HCl**, **142·HCl**, **144·HCl**, **154·(2R,3R)-tartrate** were synthesized reacting them with an excess of formadehyde, $NaCNBH_3$ and acetic acid in methanol in 31%, 83%, 41% and 96% yield, respectively (Scheme 43).

[152] Camps, P.; Lukach, A. E.; Rossi, R. A. *J. Org. Chem.* **2001**, *66*, 5366-5373.




**Scheme 43.** Derivatives of amines **139**, **142**, **144**, and **154**.

Finally, in order to obtain the mono-*N*-methyl derivative of **139·HCl**, this amine was made to react with methylchloroformate and triethylamine in diethyl ether at room temperature to obtain a carbamate that, without further purification, was reduced with lithium aluminium hydride in anhydrous THF to the expected amine **159·HCl** in 62% overall yield.

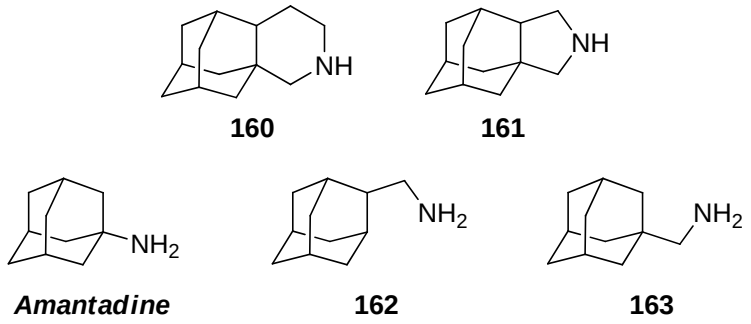
The assignment of the $^{1}H$ and $^{13}C$-NMR spectra was carried out with the support of COSY $^{1}H/^{1}H$ homocorrelation and gHSQC $^{1}H/^{13}C$ heterocorrelation, and with the help of DEPT experiments to differentiate among C, CH, $CH_2$, $CH_3$ and, for some specific cases with NOESY experiments. Using all the data gathered by this set of spectra we could assign all the molecules synthesized in this Thesis without any significant problem. The assignment of all $^{1}H$ and $^{13}C$ spectra, not only from this chapter but from the following ones is described in the experimental section although some significant comments will be done in the theoretical part only if are of particular interest.

# 2. Synthesis of 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene and related compounds.

## 2.1 Previous work on 3-azapentacyclo[6.4.0.0$^{2,10}$.0$^{3,7}$.0$^{4,9}$]dodecanes.

As mentioned before, in Dra. M. D. Duque Thesis some 3-azapentacyclo[6.4.0.0$^{2,10}$.0$^{3,7}$.0$^{4,9}$]dodecanes were described and tested as antivirals, showing a very interesting activity[147]. We considered this polycyclic scaffold as a very promising one because the pharmacological assays showed that several pentacyclic amines possessed a really low $EC_{50}$ against influenza A/H1N1 strains possessing an M2 channel resistant to amantadine. Thereby, we decided to expand this family of compounds looking for a molecule capable to improve the activity of the earlier pentacyclic compounds.

In 2008, Kolocouris' group published a paper where it was shown that nitrogen-containing rings analogues were much more active than their corresponding analogs containing linear amine groups. For example, amines **160** and **161** resulted to be more potent than amines **162** and **163** (Figure 49). This increase in potency was attributed to a better orientation of the amine group inside the M2 channel of the influenza virus.[153]




**160** **161** ***Amantadine*** **162** **163**

**Figure 49.** Kolocouris' amantadine derivatives.

Taking as a source of inspiration this report and that our pentacyclic amines showed significant activity against H1N1 Influenza A virus, being the most potent compounds amine **105·HCl** ($EC_{50}$ = 2.0 μM) and **106·HCl** ($EC_{50}$ = 2.0 μM), we decided to synthesize a new series of compounds with general structure **IX** that include the amino moiety in a pyrrolidine ring conferring rigidity to the molecule. The aim of this approach being to find a better interaction between the compound and the M2 channel (Figure 50).

[153] Kolocouris, A.; Spearpoint, P.; Martin, S. R.; Hay, A. J.; López-Querol, M.; Sureda, F. X.; Padalko, E.; Neyts, J.; De Clercq, E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6156-6160.

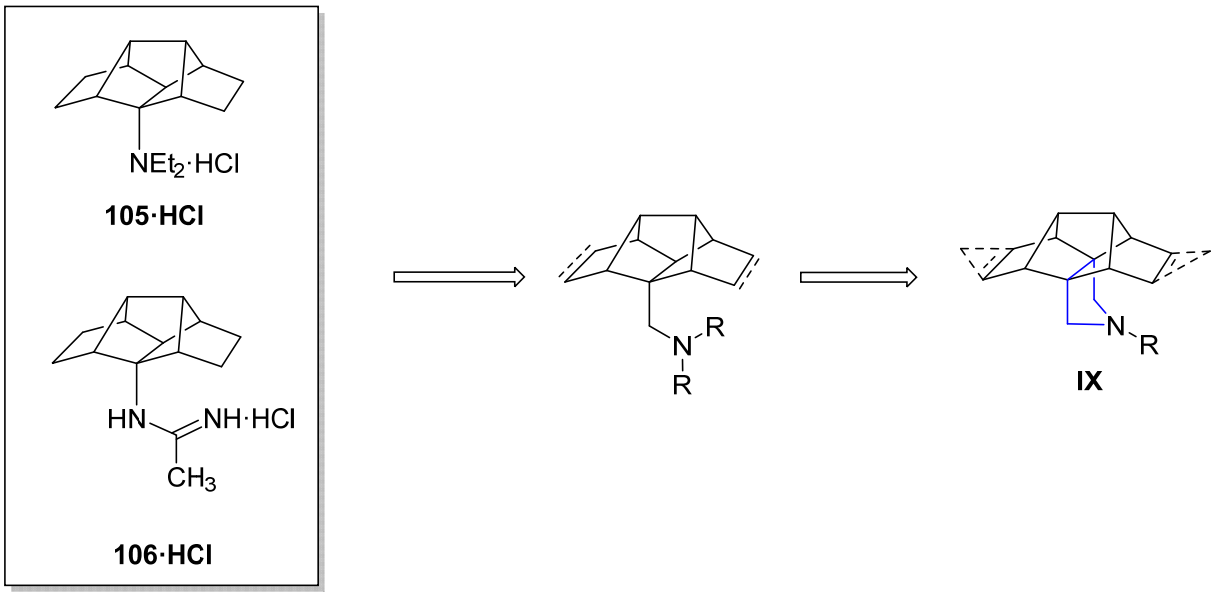
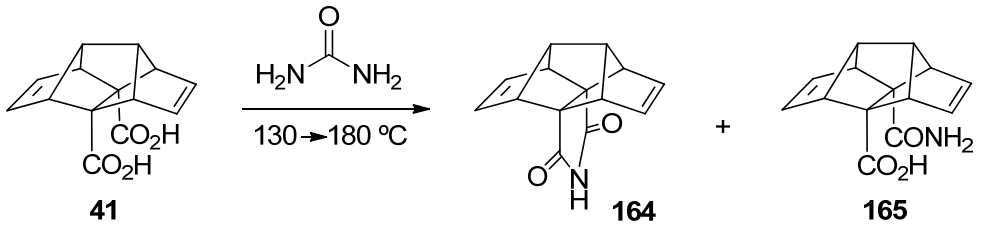



**Figure 50.** General structure of new hexacyclic amines.

**2.2 Synthesis of 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-dienes and related compounds.**

Starting from the known diacid **41,** described in the first part of this Thesis, by reaction with urea at its melting temperature (180 $^{o}$C) over 30 min, the imide **164** was obtained in 79% yield. In this reaction, it is important to keep the internal temperature at 180 $^{o}$C in order to avoid the formation of high amounts of the opened product **165**. This reaction works upon thermal decomposition of urea to ammonia and cyanic acid, being the generated ammonia the actual nucleophile that reacts with both carbonyls to form the imide (Scheme 44.).[154]

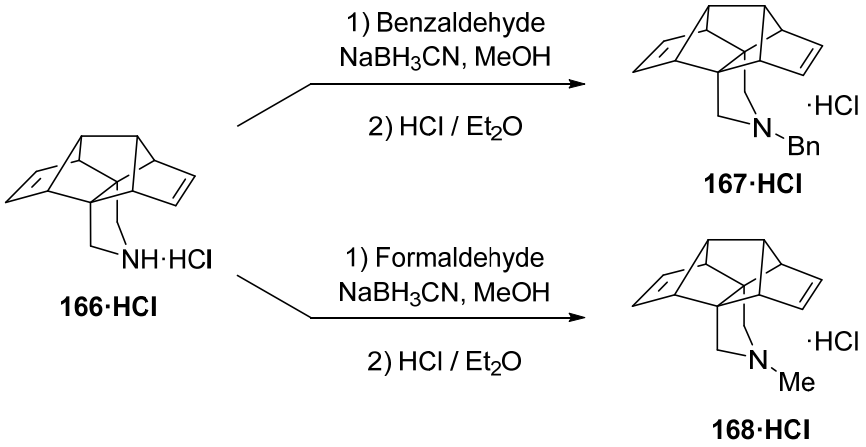



**Scheme 44.**

[154] a) Schaber, P. M.; Colson, J.; Higgins, S.; Thielen, D.; Anspach, B.; Brauer, *J. Therm. Ac.* **2004**, *424*, 131-142. b) Bernhard, A. M.; Peitz, D.; Elsener, M.; Wokaun, A.; Kröcher, O. *App. Cat. B: Env.* **2012**, *115-116*, 129-137.

Then, the hexacyclic amine **166** was obtained by reduction of imide **164** with $LiAlH_4$ in 86% yield. This amine was isolated as its hydrochloride salt after the addition of an excess of HCl / $Et_2O$ solution. This compound is the closed analog of amine **100·HCl** that had been previously synthesized by our group (see page 86).

In order to check if alkyl or aryl derivatives improve the potency of these compounds we synthesized the benzyl derivative **167·HCl** which may form π-stacking interactions with the targeted protein and methyl amine **168·HCl**. Then, reductive alkylation of amine **166·HCl** with benzaldehyde and formaldehyde yielded amines **167·HCl** in 53% yield and **168·HCl** in 91% yield, respectively (Scheme 45).

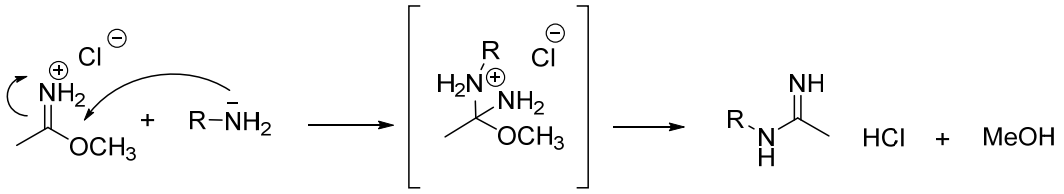



**Scheme 45.** Derivatives of amine **166·HCl.**

At this point, we decided to modify the amine moiety in order to check the importance of the basicity of the nitrogen atom, so we synthesized the corresponding amidine and guanidine. The $pK_a$s of these functionalities goes from $pK_a$=~10 for an amine and a $pK_a$=12.4 for an amidine to a $pK_a$=13.6 for a guanidine, being the latter the more basic one.[155]

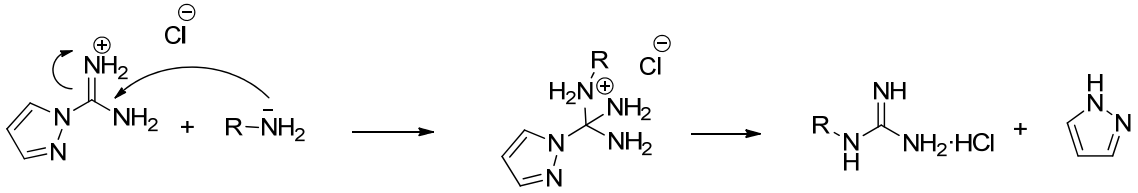
To synthesize the desired amidine, amine **166·HCl** was reacted with methyl imidate hydrochloride and $Et_3N$ in THF for 24 h.[156] This reaction involves the nucleophilic attack of the amine to the electrophilic carbon of the imidate, after an addition/elimination mechanism the amidine is released (Scheme 46).

[155] Clayden, Greeves, Warren, Wothers "Organic Chemistry", 2nd edition, Oxford University Press: Oxford 2012, 174-175.
[156] a) Black, R. M.; Gill, G. B.; *J. Chem. Soc. D, Chem. Commun.* **1970**, 972-973. b) Janjatović, J.; Majerski, Z. *J. Org. Chem.* **1980**, *45*, 4892-4898.



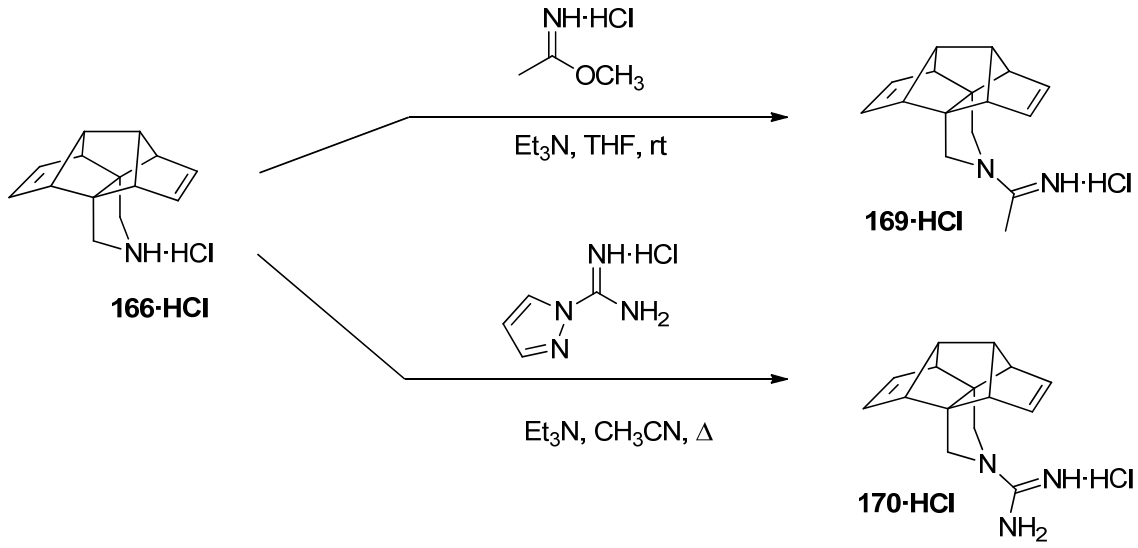
**Scheme 46.** Mechanism of the amidine synthesis.

To synthesize the desired guanidine **170·HCl**, 1*H*-pirazol-1-carboxamidine was used as the reagent to introduce the guanidine moiety. This procedure involves the reaction of a primary or a secondary amine with stoichiometric quantities of 1*H*-pirazol-1-carboxamidine in presence of a tertiary amine, such as $Et_3N$, in mild conditions. These reactions work by the addition/elimination mechanism shown in Scheme 47. It should be pointed out that this procedure does not work with sterically hindered amines.[157]



**Scheme 47.** Mechanism of guanidine synthesis.

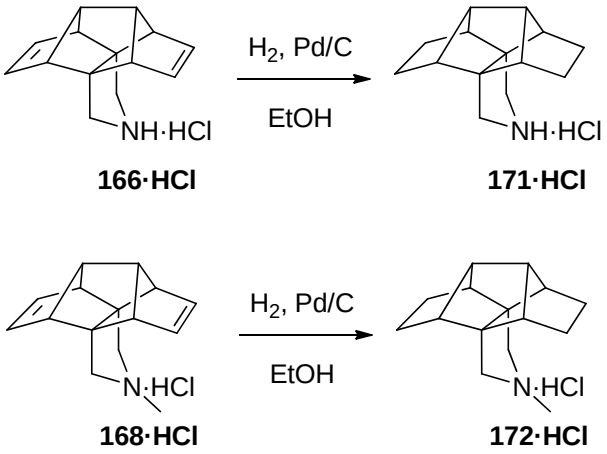
Applying these procedures, amidine **169·HCl** was obtained in 95% yield and guanidine **170·HCl** in 67% yield (Scheme 48).



**Scheme 48.** Derivatives of amine **166·HCl.**

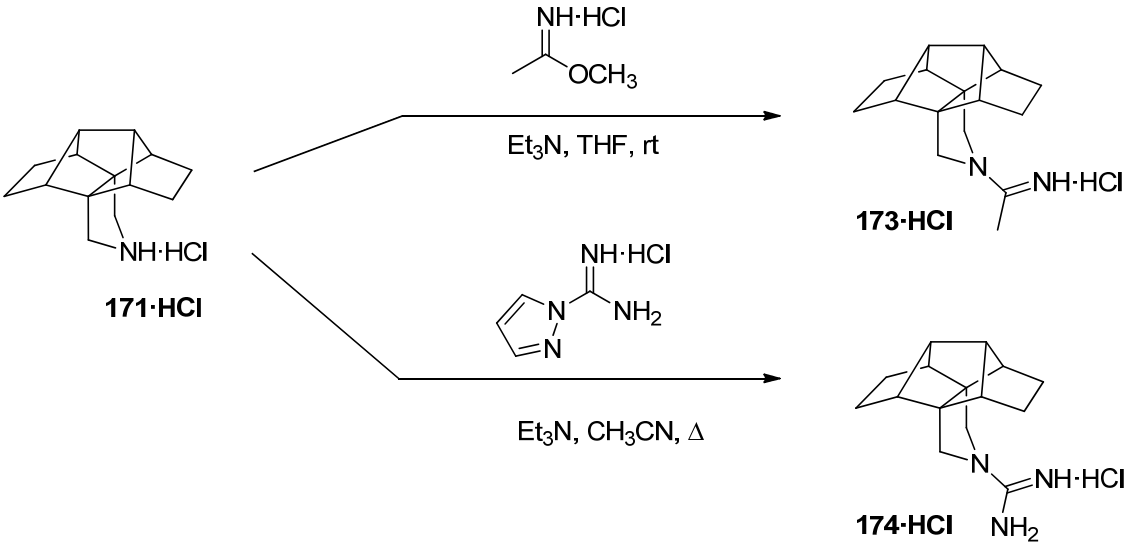
[157] a) Bernatowiez, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497-2502. b) Makovec, F.; Artusi, R.; Zanzola, S.; Rovati, C. Patent US 2005/0049312, **2005**.

In order to gain further insight in the SAR of these molecules, some saturated derivatives were also prepared. This modification will endow our molecules with more bulkiness at the end of the lipophilic polycyclic scaffold. The catalytic hydrogenation of the amine **166·HCl** and its methylated derivative **168·HCl** gave access to saturated amines **171·HCl** and **172·HCl** in quantitative yields (Scheme 49).




**Scheme 49.** Saturated derivatives of amine **166·HCl.**

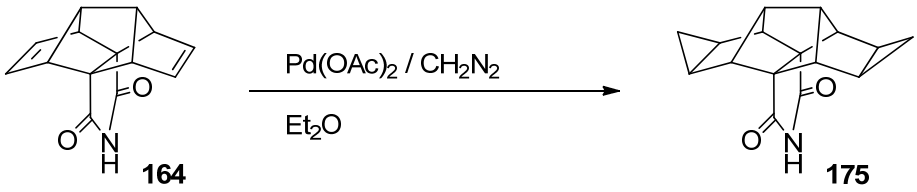
The corresponding amidine and guanidine were prepared by reacting amine **171·HCl** with either methyl imidate hydrochloride or 1*H*-pyrazol-carboxamidine yielding the amidine **173·HCl** in 55% yield and the guanidine **174·HCl** in 77% yield, respectively (Scheme 50).




**Scheme 50.** Derivatives of amine **171·HCl.**

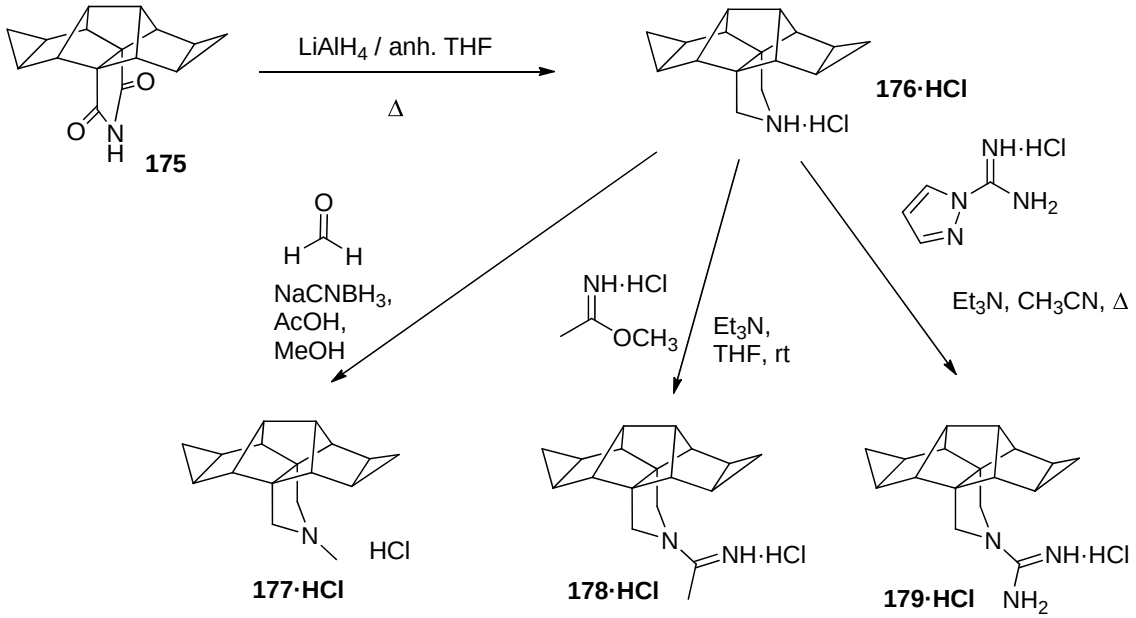
Moreover, we wondered if making bigger the polycyclic cage of the amines would result in a better inhibition of the channel due to a better fulfilment of the channel's lumen.

Following this idea, we decided to add cyclopropane rings to our polycyclic amines. To do so, we used the previous optimized methodology (see page 49, Part I of this Thesis) consisting in reacting the imide **164** with $Pd(OAc)_2$ and $CH_2N_2$ generated *in situ.* In this way, we obtained the desired imide in 95% yield (Scheme 51).




**Scheme 51.** Synthesis of imide **175.**

Once we obtained the targeted imide we proceed to reduce it to amine **176·HCl** using $LiAlH_4$ which gave the amine in 64% yield. Three selected derivatives were also synthesized using the methods stated before (Scheme 52).




**Scheme 52.** Derivatives of amine **176·HCl**.

The methyl derivative **177·HCl** was obtained in 77% yield, the amidine **178·HCl** in 69% and the guanidine **179·HCl** in 67% yield.

# 3. Synthesis of 3-azatricyclo[3.3.3.0$^{1,5}$]undecanes, 8-azatricyclo [4.3.3.0$^{1,6}$]dodecanes and 12-azatricyclo[4.4.3.0$^{1,6}$]tridecanes.

## 3.1. Releasing conformational constraints of the previously synthesized polycyclic amines.

A very usual strategy in medicinal chemistry for designing new drugs is the synthesis of ring opened analogs of a previous bioactive compound.[158] Taking into account this traditional approach and that several of our previously synthesized polycyclic amines displayed a very good activity as antivirals (see later on), we decided to synthesize several analogs with some of the rings opened, in order to gain conformational freedom. If the bond that tethers the hexacycle structure is removed, the remaining rings may be able to accommodate better to the channel's lumen.

We synthesized this kind of compounds because we thought that the rigidity of our previous compounds might be a potential disadvantage for antiviral activity because the putative mechanism of those compounds consists in sterically blocking the channel, thus a higher conformational freedom may lead to a better blocking activity.

In this way, we synthesized 3-azatricyclo[3.3.3.0$^{1,5}$]undecanes, 8-azatricyclo[4.3.3.0$^{1,6}$]dodecanes and 12-azatricyclo[4.4.3.0$^{1,6}$]tridecanes (Figure 50).

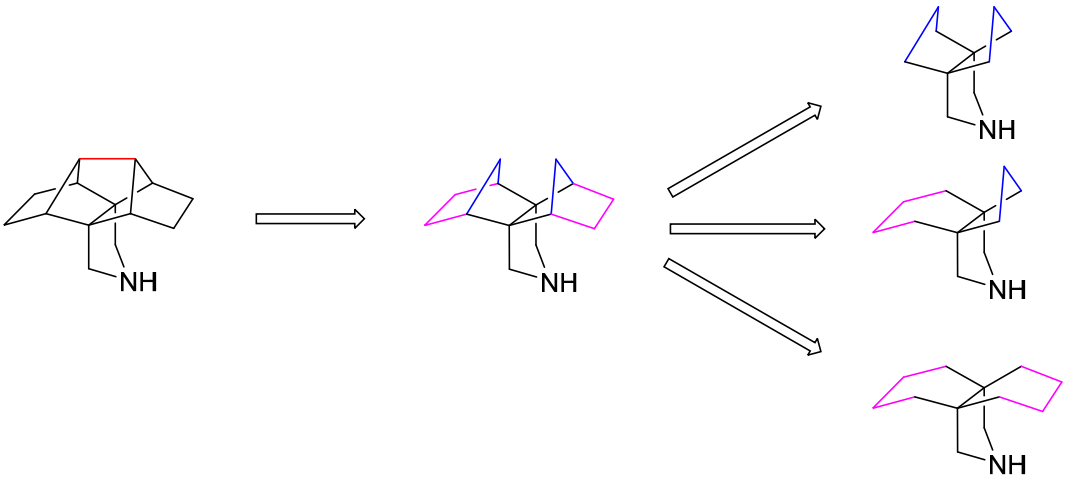



**Figure 51.** General structure of 3-azatricyclo[3.3.3.0$^{1,5}$]undecanes, 8-azatricyclo[4.3.3.0$^{1,6}$]dodecanes and 12-azatricyclo[4.4.3.0$^{1,6}$]tridecanes.

Then, we envisaged the synthesis of the following amines (Figure 52). The exocyclic double bonds and methyls are added to confer more lipophilicity at the end of the molecule.

[158] Delgado, A.; Minguillón, C.; Joglar, J. 'Introducción a la química terapéutica.' Díaz de Santos; Madrid; **2003**, pp 130-140.

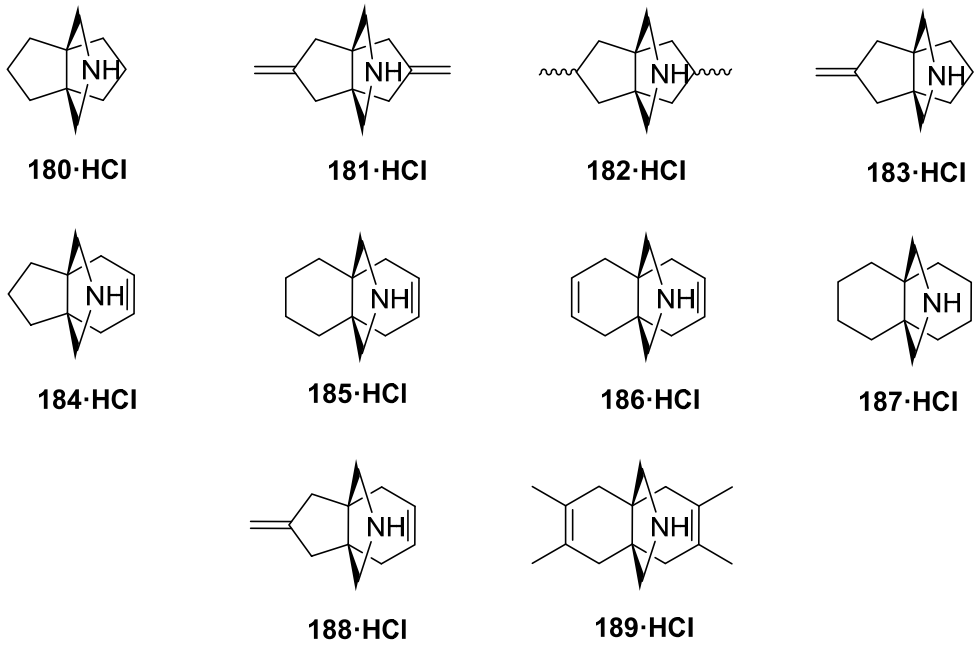



**Figure 52.** New designed molecules.

### 3.2. Synthesis of the tricyclic amines and their derivatives.

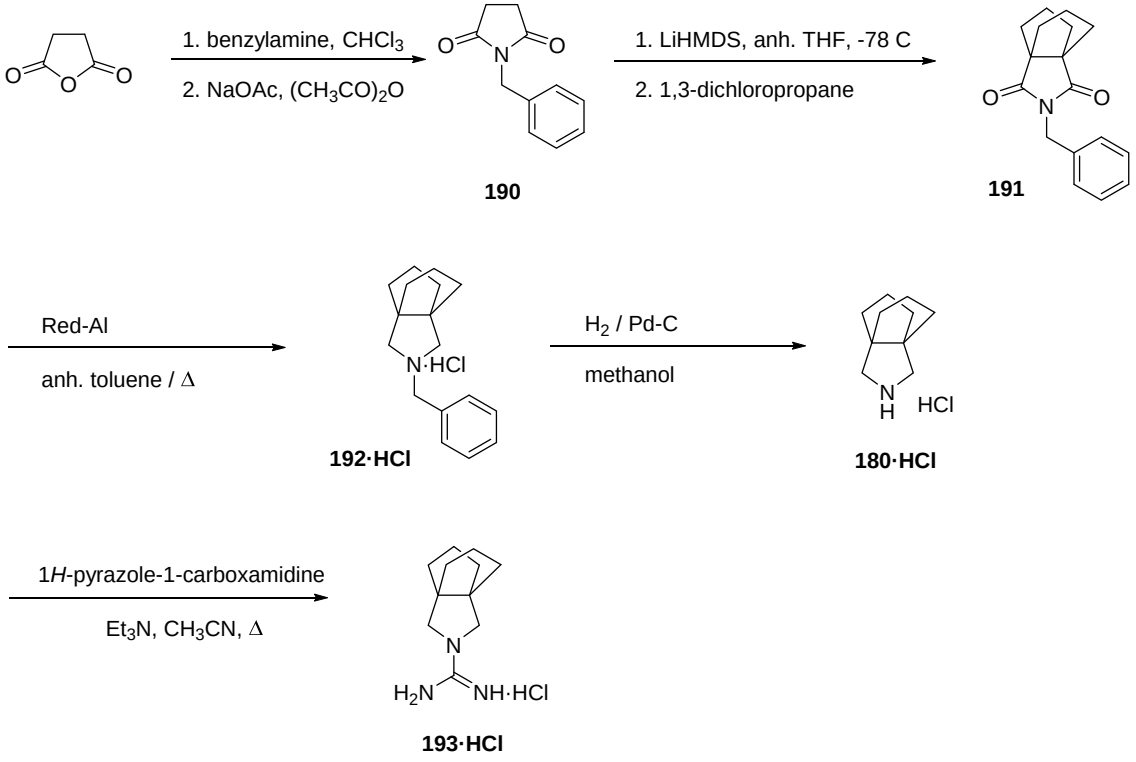
In order to synthesize the amines containing five membered rings, we decided to alkylate a suitable electrophile using as the nucleophile a protected succinimide. Thus, the nucleophilic attack of a putative enolate derived from the succinimide into an alkylating reagent would give us access to the five-membered polycyclic scaffold.

Following this idea, to synthesize amine **180·HCl,** we started from succinic anhydride that was made to react with benzylamine,[159] in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) in glacial acetic acid to get the imide **190** in 85% yield.[160] Imide **190** was dialkylated by a procedure recently developed by our group.[161] Thereby, the imide was reacted with six equivalents of LiHMDS in anhydrous THF at -78 $^{o}$C and after 1 hour of deprotonation 2 equivalents of 1,3-dichloropropane were added to the reaction mixture. This reaction yielded the imide **191** together with a complex mixture of impurities. After a tedious column chromatography the desired product was isolated in very low yield, 8.5%.

The next step of the synthesis was the reduction of the imide's carbonyl group using Red-Al® in anhydrous toluene to obtain amine **192·HCl**.[162] Red-Al® is sodium bis(2-methoxyethoxy)aluminumhydride, its reactivity is comparable to $LiAlH_4$ however, due to the 2-methoxyethoxy radicals it is soluble in organic solvents, property that makes it easier to handle.

[159] Jun, J.; Mundy, B. P. *Bull. Korean Chem. Soc.* **1987**, *8*, 310-313.
[160] Sortino, M.; Garibotto, F.; Cechinel Filho, V.; Gupta, M.; Enriz, R.; Zacchino, S. *Biorg. Med. Chem.* **2011**, *19*, 2823-2834.
[161] Camps, P.; Fernández, J. A.; Rull, J.; Vázquez, S. *Eur. J. Org. Chem.* **2009**, 3081-3087.
[162] Kalo, J.; Ginsburg, D.; Vogel, E. *Tetrahedron* **1977**, *33*, 1177-1182.

Once the carbonyl groups were reduced in 55% yield, the deprotection of the tertiary amine was carried out by a catalytic hydrogenation yielding amine **180·HCl** in quantitative yield (Scheme 53). The guanidine **193·HCl** was synthesized by the previously described procedure.



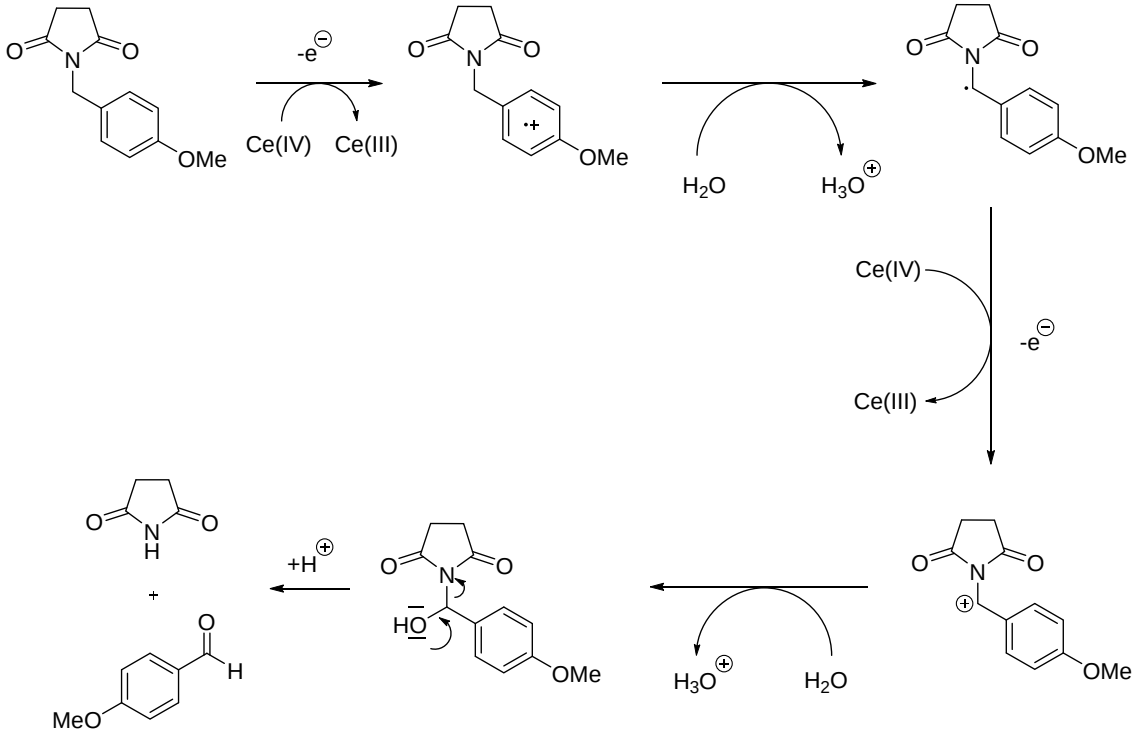

**Scheme 53.** Synthesis of amine **180·HCl** and guanidine **193·HCl**.

Trying to introduce more steric hindrance at the top of these molecules we thought of adding new exocyclic double bonds followed by catalytic hydrogenation to reduce the double bonds with the purpose of occupying more space into the M2 channel lumen. To synthesize amine **181·HCl** and the corresponding guanidine the synthetic route started from succinic anhydride which was reacted with *p*-methoxybenzyl amine.[163] The *p*-methoxybenzyl (PMB) protecting group was selected because it needs oxidative conditions to be cleaved, conditions that would not modify the alkenes. After the obtention of the imide **194** we proceeded to do the bisannulation. To do so, the acidic protons of the imide were removed using LiHMDS as the base and then, 3-chloro-(2-chloromethyl)-1-propene as the electrophile was added to the reaction mixture. This reaction yielded imide **195** in 40% yield.

After obtaining the polycyclic scaffold, imide **195** was deprotected using Cerium (IV) ammonium nitrate in a mixture of 1:1 water/acetonitrile. The mechanism of this reaction is shown in scheme 54. Removing of the PMB group works through a SET-type reaction, where first Cerium removes an electron from the activated aromatic ring to generate a radical-cation species. Then, after the loss of a proton, a radical stabilized in the benzylic position is

[163] Verschueren, W. G., Dierynck, I.; Amssons, K. I. E.; Hu, L.; Boonants, P.; Pille, G.; Daeyaert, F.; Hertogs, K.; Surleraux, D.; Wigerinck, P. *J. Med. Chem* **2005**, *48*, 1930-1940.

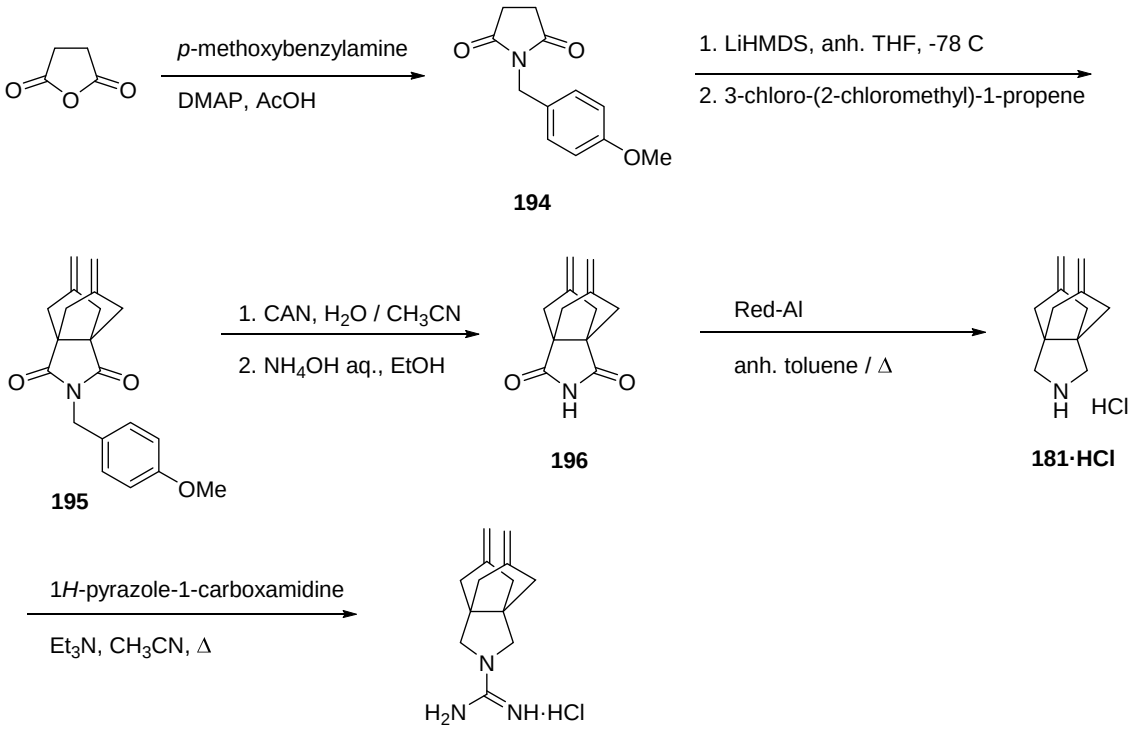
generated which undergoes another radical abstraction to obtain a benzylic carbocation. This carbocation will react with the water of the media generating an hemiaminal that is cleaved in the aqueous work-up to release the imide and *p*-methoxybenzaldehyde (Scheme 54).[164]




**Scheme 54.** Mechanism of PMB deprotection using CAN.

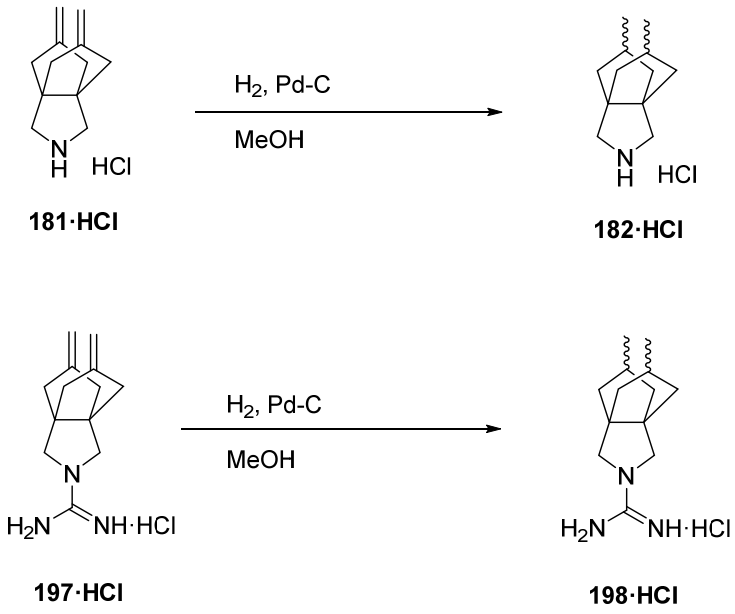
The next step was the reduction of **196** with Red-Al® which yields the desired amine **181·HCl** in 96% yield. Then, the guanidine **197·HCl** was synthesized in 75% yield using the previously stated procedure (Scheme 55).

[164] Ishibashi, H.; Nakaharu, T.; Nishimura, M.; Nishikawa, A. Kameoka, C.; Ikeda, M. *Tetrahedron* **1995**, *51*, 2929-2938.




**Scheme 55**. Synthesis of amine **181·HCl** and guanidine **197·HCl**.

To synthesize the saturated amine **182·HCl** and guanidine **198·HCl**, we carried out the catalytic hydrogenation of amine **181·HCl** and its corresponding guanidine, compound **197·HCl.** These reactions allowed us to get these compounds in quantitative yields as a mixture of stereoisomers (Scheme 56). No attempts to separate them were done.



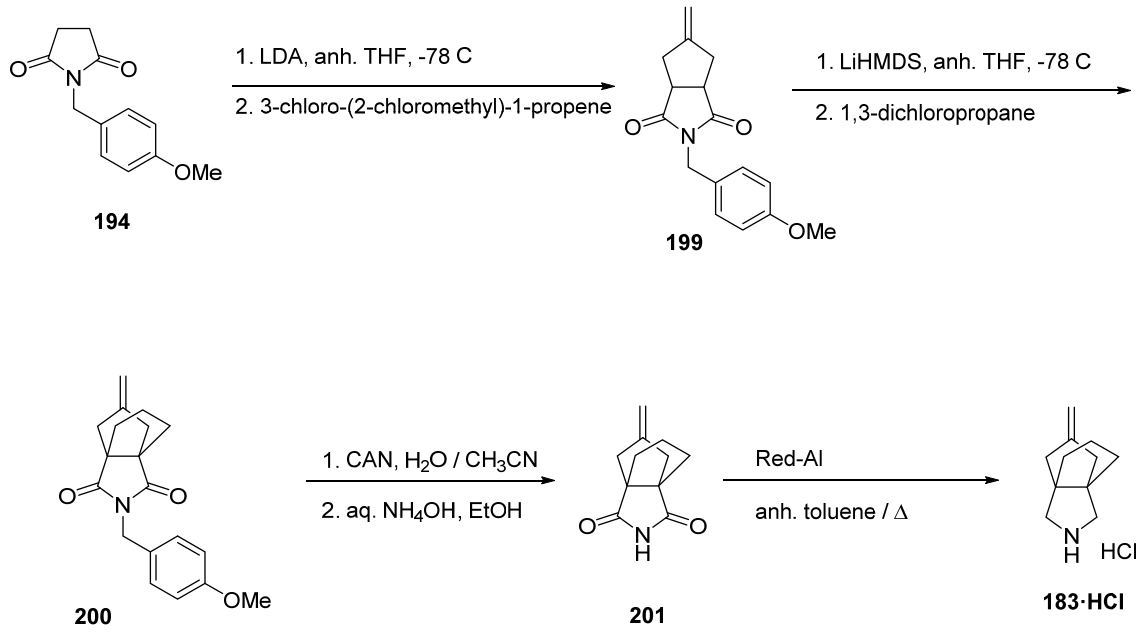

**Scheme 56.** Synthesis of **182·HCl** and **198·HCl.**

Since we wanted to synthesize the unsymmetrical product **183·HCl** the construction of the two rings was carried out in a two-step synthesis. According to previous work of our research group, when imide **194** is treated with LiHMDS and 3-chloro-(2-chloromethyl)-1-propene the

imide undergoes a bisannulation, however when imide **194** is made to react with LDA as the base then a monoannulation selectively occurs (Scheme 57).[161] Taking these previous results into account, unsymmetrical amine **183·HCl** was synthesized starting from imide **194** that upon treatment with LDA and 3-chloro-(2-chloromethyl)-1-propene undergoes monoannulation in 31% yield.

Once the monoannulated product **199** was synthesized we carried out the building of the third ring. Thus, **199** was treated with LiHMDS as a base and 1,3-dichloropropane was used as the electrophile. The desired bisannulated imide **200** was obtained in 46% yield.

After construction of the polycyclic scaffold the deprotection and reduction was done following the usual procedures. Deprotection of the *p*-methoxybenzyl group was carried out upon treatment with CAN and followed by ethanolysis to yield imide **201** in 83%. The reduction was carried out using Red-Al® as the reductive agent. Amine **183·HCl** was obtained in 76% yield (Scheme 57).

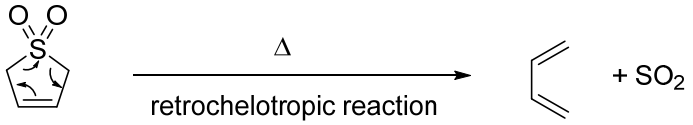



**Scheme 57.** Synthetic route to amine **183·HCl**.

After including some exocyclic groups at the end of the polycyclic scaffolds we decided to modify the size of the rings. So we planned to synthesize molecules containing five- and six-membered rings. We decided to carry out Diels-Alder reactions to achieve the synthesis of the six-membered rings.

The first proposed compound, amine **184·HCl** was synthesized starting from commercially available 1-cyclopentene-1,2-dicarboxylic anhydride that was reacted with 3-sulfolene as a

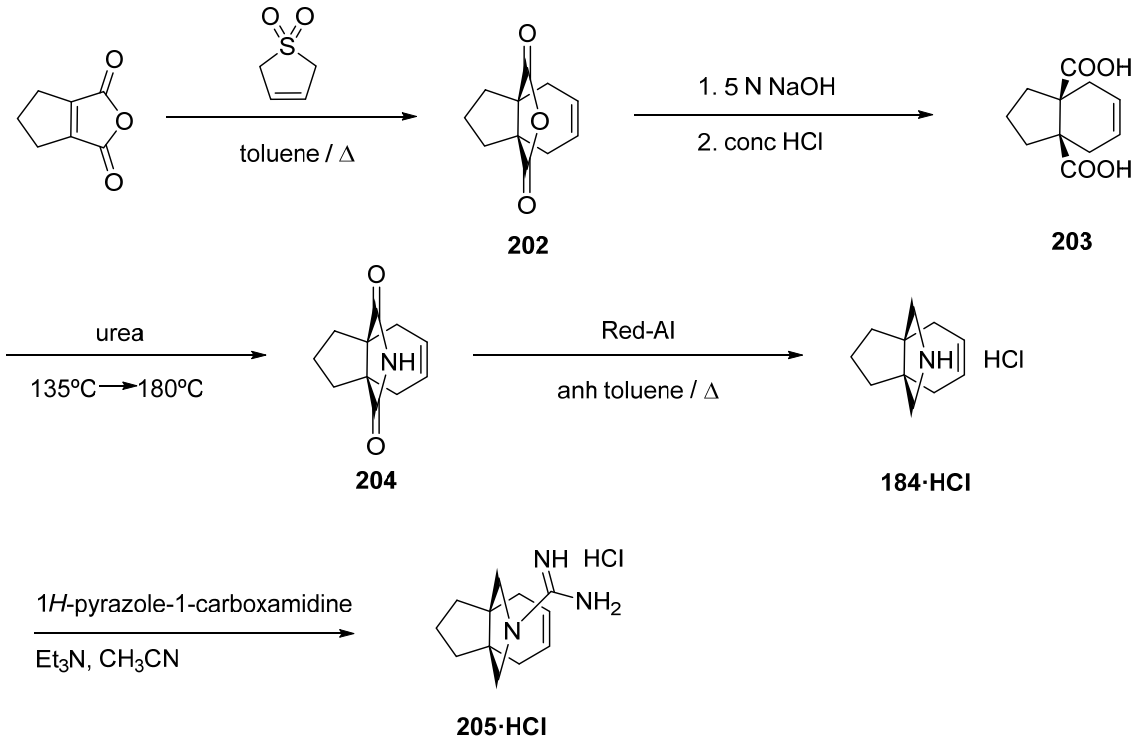
solid source of 1,3-butadiene. 3-sulfolene undergoes a retrochelotropic reaction upon warming (>75 $^{o}$C) releasing sulphur dioxide and 1,3-butadiene (Scheme 58).[165]




**Scheme 58.** 3-sulfolene as a source of 1,3-butadiene**.**

The reaction between cyclopentene-1,2-dicarboxylic anhydride and 3-sulfolene was carried out in a sealed tube using toluene as the solvent. The high pressures generated in a sealed tube upon warming helps to push the equilibrium of the Diels-Alder reaction to the products' side. The Diels-Alder product **202** was obtained in 77 % yield after sublimation at 75 $^{o}$C / 0.5 Torr to purify it.

The basic hydrolysis of compound **202** yielded diacid **203** quantitatively. This reaction was followed by the formation of the imide **204** in 56% yield using the same procedure stated before. Finally, imide **204** was reduced to the amine using Red-Al®, **184·HCl** was obtained in 82% yield (Scheme 59). The corresponding guanidine **205·HCl** was synthesized as usual in 50% yield.




**Scheme 59.** Synthesis of amine **184·HCl** and guanidine **205·HCl.**

[165] Sample, T. E.; Hatch, L. F. *J. Chem. Educ.* **1968**, *45*, 55-56.

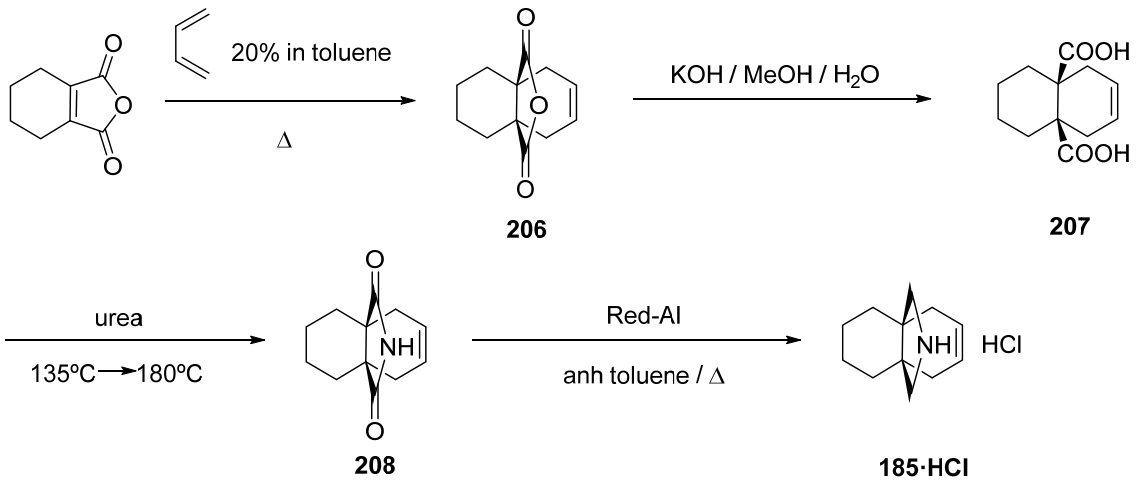
We envisaged the synthesis of amine **185·HCl** in an analogous way than the previous synthesis.[166] However, several attempts to afford compound **206** using 3-sulfolene as the source of 1,3-butadiene were unsuccessful. Presumably, because 3,4,5,6-tetrahydrophthalic anhydride is more stable than cyclopentene-1,2-dicarboxylic anhydride, so more harsh conditions would be required to carry out this reaction successfully.

Firstly, we changed the source of 1,3-butadiene, we used a 20% solution of 1,3-butadiene in toluene instead of 3-sulfolene. We hypothesized that the equilibrium of 3-sulfolene to 1,3-butadiene and sulphur dioxide was competing with the equilibrium of the Diels-Alder reaction considering that the reaction was done in a sealed tube and the sulphur dioxide is not removed from the reaction media.

Finally, the reaction worked properly using a large excess of the solution of 20% of 1,3-butadiene in toluene and heating at 180 $^{o}$C for 3 days. The desired anhydride **206** was obtained in 95% of yield.

The possible higher stability of 3,4,5,6-tetrahydrophthalic anhydride versus cyclopentene-1,2-dicarboxylic anhydride was demonstrated doing molecular mechanic calculations which show that while going from cyclopentene-1,2-dicarboxylic anhydride to compound **202**, 25 Kcal/mol are released, in going from 3,4,5,6-tetrahydrophthalic anhydride to compound **206** only 15 Kcal/mol are released. This fact shows up that the former reaction is possibly favoured because a relatively unstable product is converted into a more stable one.

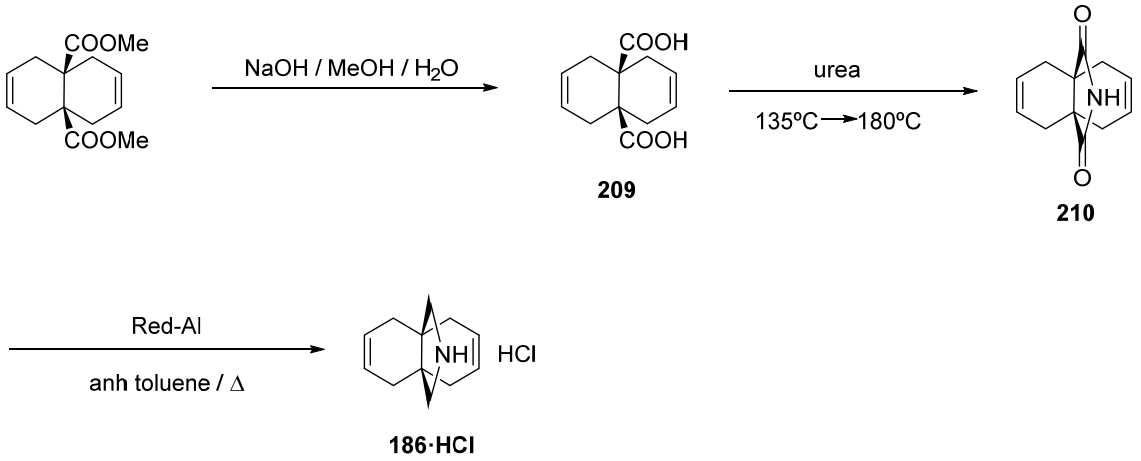
Basic hydrolysis of **206** yielded **207** in 79% yield. Next, the reaction with urea yielded compound **208** in 61% and after reduction with Red-Al®, amine **185·HCl** was obtained in 43% yield (Scheme 60).




**Scheme 60.** Synthesis of amine **185·HCl.**

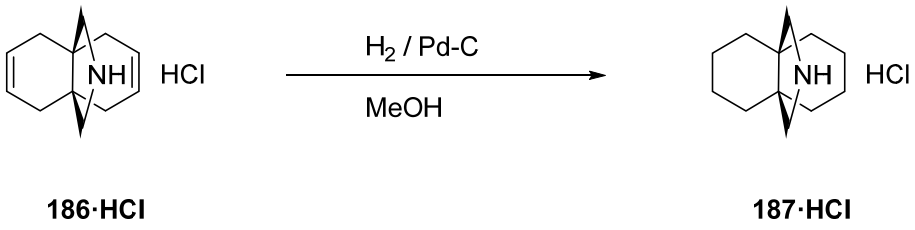
[166] Amith, C.; Kalo, J.; North, B. E.; Ginsburg, D. *Tetrahedron* **1974**, *30*, 479-481.

To face the challenge of synthesizing compound **186·HCl**, we envisage the synthetic route starting from the commercial dimethyl 1,4,5,8-tetrahydro-4a,8a-naphthalenedicarboxylate. After its basic hydrolysis in a mixture of 1:1 methanol:water, the corresponding acid was treated with urea at its melting temperature (180 $^{o}$C internal temperature) to give imide **210** in 81% yield. Then, the reduction of this imide allowed us to obtain the known amine **186·HCl** in 47% yield (Scheme 61).[162]



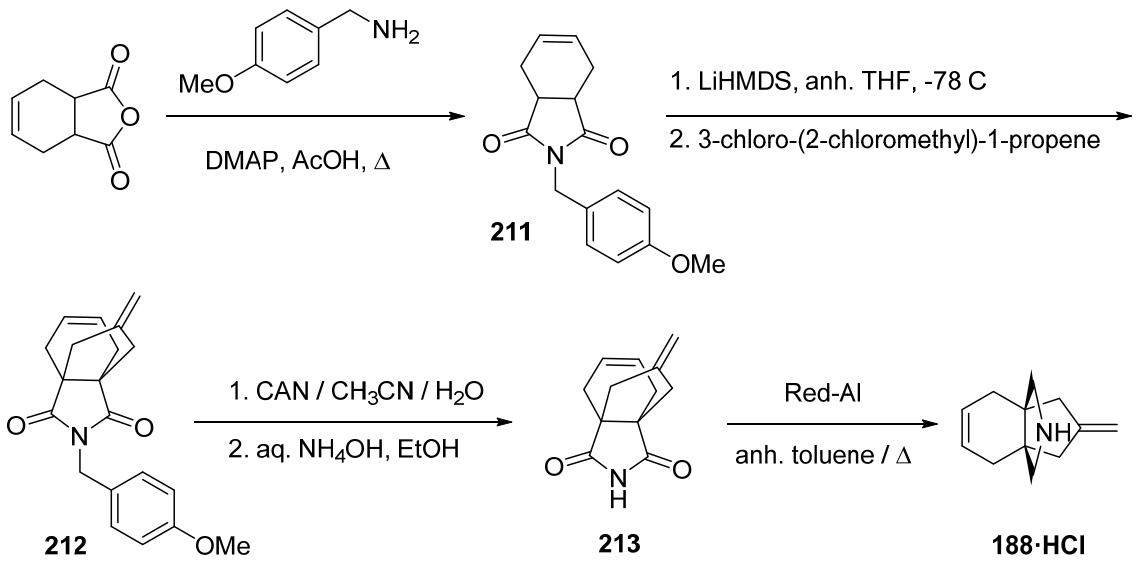

**Scheme 61.** Obtention of amine **186·HCl**.

Amine **187·HCl** was obtained in quantitative yield by catalytic hydrogenation of amine **186·HCl**, this reaction worked quantitatively (Scheme 62).




**Scheme 62.** Catalytic hydrogenation of **186·HCl.**

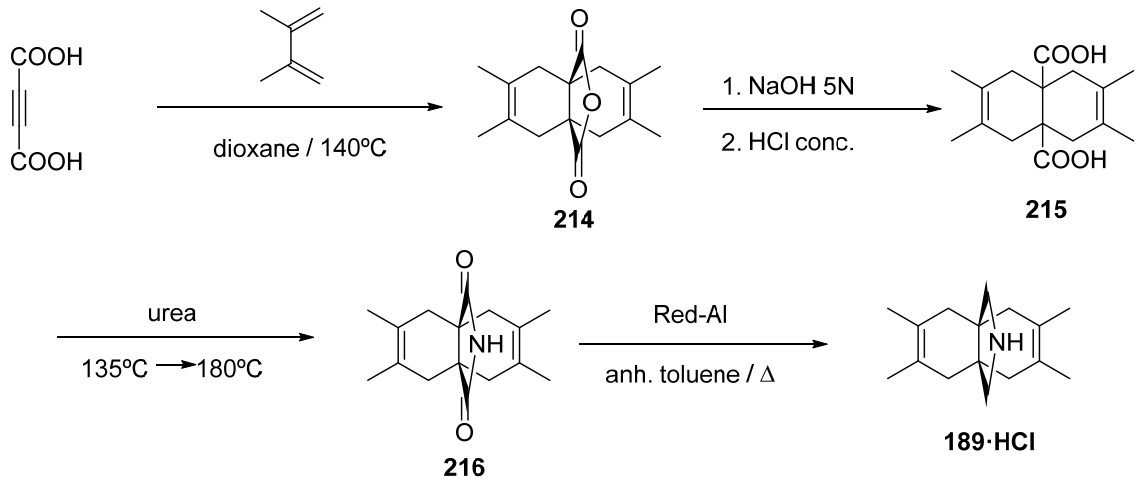
The synthesis of unsymmetrical amine **188·HCl** started from imide **211**. This compound was synthesized from the commercially available (3aR,7aS)-3a,4,7,7a-tetrahydro-2-benzofuran-1,3-dione, *p*-methoxybenzylamine and a catalytic amount of DMAP in glacial acetic acid that worked with 51% yield. Then, the alkylation of imide **211** was carried out using LiHMDS as base and 3-chloro-(2-chloromethyl)-1-propene as the electrophile to give imide **212** in 70% yield. Deprotection of imide **212** (67% yield) and reduction with Red-Al® (61% yield) completed the synthesis of amine **188·HCl** (Scheme 63).




**Scheme 63.** Synthesis of amine **188·HCl.**

Finally, we wanted to add more steric hindrance at the end of these molecules so we thought of placing four methyl groups in the double bonds of amine **186·HCl**, so we synthesized amine **189·HCl**. This synthesis started with the Diels-Alder reaction between acetylenedicarboxylic acid and 2,3-dimethyl-1,3-butadiene in dioxane at 140 $^{o}$C. This reaction yielded directly the double Diels-Alder adduct, following these steps: first, a Diels-Alder reaction, then, thermally induced dehydratation of the diacid to form the anhydride and finally another Diels-Alder reaction.

Hydrolisis of anhydride **214** in aqueous solution of 5 N sodium hydroxide yielded the desired diacid in 81% yield. Then, formation of imide **216** and then reduction yielded amine **189·HCl,** in 40% of yield.




**Scheme 64.** Obtention of amine **189·HCl**.

# 4. Pharmacological evaluation of the 2,2-dialkyladamantamines, the 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-dienes and 3-azatricyclo[3.3.3.0$^{1,5}$]undecanes and related compounds.

After finishing the synthesis and characterization of these 3 families of compounds, we proceed to evaluate their antiviral activity. This work was carried out by the research group of Prof. Lieve Naesens at the *Rega Institute for Medical Research* (KU Leuven, Belgium).

Taking into account the structural similarity of all our derivatives with amantadine, we expected our compounds to have anti-influenza activity. However, since the *Rega Institute* is used to test compounds against a very broad panel of viruses by plaque reduction assays, they also tested our compounds against the following viruses, in order to check if they were endowed with other antiviral activities. These are the performed assays:

a) antiviral activity and citotoxicity assays in MDCK (Madin Darby canine kidney cells) culture: Influenza A virus and Influenza B virus.
b) antiviral activity and citotoxicity assays in Vero cells: parainfluenza-3 virus, reovirus-1, sindbis virus, coxsackie virus B4, punta toro virus.
c) antiviral activity and citotoxicity assays in HEL cell culture: herpes simplex virus-1, herpes simplex virus-2, stomatitis vesicular virus, human adenovirus-2 and vaccinia virus.
d) antiviral activity and citotoxicity assays in HeLa cell culture: stomatitis vesicular virus, coxsackie virus B4 and respiratory syncytial virus.
e) antiviral activity and citotoxicity assays in MOLT-3 and HSB-2 cell culture: herpes virus 6A and 6B.

Thereby, the antiviral activity of the synthesized compounds was tested in different cell cultures using a broad panel of RNA and DNA viruses. As expected, these compounds were only active against the influenza A virus.

Table 6, 7 and 8 gather all the antiviral data of the synthesized polycyclic amines and guanidines, including two strains of Influenza A virus [A/Puerto Rico/8/34 (H1N1) and A/Hong Kong/7/87 (H3N2)] and a strains of Influenza B virus (B/Hong Kong/5/72), using as reference compounds: amantadine and rimantadine. Worthy of note, while the A/HK/7/87 strain, that carries a wt M2 channel, was known to be sensitive to amantadine, neither the A/PR/8/34 strain, that has a mutant, amantadine-resistant, M2 channel, nor the Influenza B strains were sensitive to amantadine.

**Table 6.** Antiviral activity of 2,2-dialkylamantadines.

| Compound | Antiviral activity $EC_{50}$[a] (μM) | | | | | | Toxicity (MCC[b]) |
|---|---|---|---|---|---|---|---|
| | Influenza A/H1N1 A/PR/8/34 | | Influenza A/H3N2 A/HK/7/87 | | Influenza B B/HK/5/72 | | |
| | CPE | MTS | CPE | MTS | CPE | MTS | |
| **121·HCl** | 15[c] | 10[d] | > 100 | > 100 | > 100 | > 100 | > 100 |
| **127·HCl** | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 | 4 |
| **128·HCl** | 4 | 5 | > 100 | > 100 | > 100 | > 100 | 200 |
| **129·HCl** | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 | 200 |
| **130·HCl** | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 | 4 |
| **131·HCl** | 6 | 10 | > 100 | > 100 | > 100 | > 100 | 200 |
| **132·HCl** | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 | 4 |
| **139·HCl** | 2.0 | 1.7 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **142·HCl** | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 | 8.5 |
| **144·HCl** | 3.1 | 0.9 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **147·HCl** | 1.1 | 0.9 | > 100 | > 100 | > 100 | > 100 | 11 |
| **154·(2R, 3R)-** | 4.6 | 4.4 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **155·HCl** | 4.0 | 9.6 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **156·HCl** | 6.0 | 6.7 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **157·HCl** | 5.1 | 6.0 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **158·(2R, 3R)-tartrate** | 5.9 | 5.7 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **159·HCl** | 4.0 | 8.8 | > 100 | > 100 | > 100 | > 100 | > 100 |
| ***Amantadine*** | 72.5±38.9 | 57.5±25.2 | 2.2±0.3 | 1.7±0.8 | > 100 | > 100 | > 500 |
| ***Rimantadine*** | 33.3±11.4 | 24.1±10.2 | 0.25±0.21 | 0.25±0.21 | > 100 | > 100 | > 500 |

[a] $EC_{50}$: concentration producing 50% antiviral effect, as determined by microscopy of the virus-induced CPE.
[b] MCC: minimum cytotoxic concentration, i.e. concentrations causing minimal changes in cell morphology.
[c] Average value after three experiments.
[d] Average value after two experiments.

The inhibitory activity of the viral replication and the cytotoxicity was determined by microscopic examination of the CPE (cytopatic effect: degenerative changes induced by the virus) and by spectophotometry of the cellular viability using an MTS assay. As anticipated, all compounds proved to be inactive against influenza B virus, which is known to be insensitive to amantadine and rimantadine.

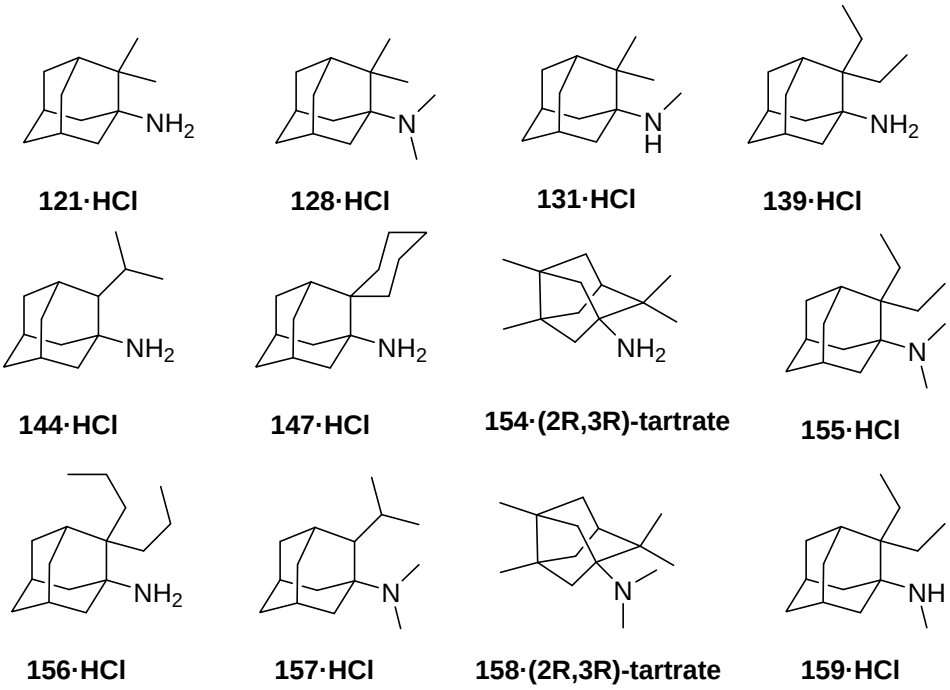



**Figure 53.** Active compounds in the series of 2,2-dialkylamantadines.

Although we did expect to find anti-influenza activity, when we first received the results of the influenza virus CPE assays, shown in Table 6, we were quite shocked. Surprisingly, *while several compounds displayed low micromolar activity against the influenza A/PR/8/34 (H1N1) subtype, which carries an amantadine-resistant M2 channel, no compound was active against the influenza A/HK/7/87 (H3N2) subtype, an influenza strain known to be amantadine-sensitive* (Table 6). The antiviral data obtained by microscopy were confirmed by a colorimetric cell viability assay.

From the analysis of the data from Table 6 some trends are evident. First, with the single exception of the dipropyl derivative **142·HCl**, all the primary amines were more potent than amantadine and rimantadine against the A/PR/8/34 strain of the influenza A/H1N1. Although the more potent compound was the spiroderivative **147·HCl** ($EC_{50}$ = 1.1 μM), the highest selectivity was noted with compound **139·HCl** ($EC_{50}$ = 2.0 μM). Secondly, while the introduction of one or two methyl groups into the primary amine was beneficial (e.g. **142·HCl** vs **156·HCl**) or had an almost neutral effect (e.g. **139·HCl** vs **155·HCl** or **159·HCl**; **154·(2R, 3R)-tartrate** vs **158·(2R, 3R)-tartrate**; and **144·HCl** vs **157·HCl**) in the antiviral activity, the introduction of larger groups was really deleterious for the activity, leading largely to inactive, cytotoxic compounds (Figure 53).

The same pharmacological assays were carried out with 3-azahexacyclo[7.6.0.$0^{1,5}$.$0^{5,12}$.$0^{6,10}$.$0^{11,15}$]pentadecanes, revealing again very interesting activities against the tested H1N1 Influenza A strain (Table 6).

**Table 7.** Antiviral activity of 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-dienes and related compounds.

| Compound | Antiviral activity $EC_{50}$[a] (µM) | | | | | | Toxicity (MCC[b]) |
|---|---|---|---|---|---|---|---|
| | Influenza A/H1N1 A/PR/8/34 | | Influenza A/H3N2 A/HK/7/87 | | Influenza B B/HK/5/72 | | |
| | CPE | MTS | CPE | MTS | CPE | MTS | |
| **166·HCl** | 16[c] | 34[d] | 14 | 16 | > 100 | > 100 | > 100 |
| **167·HCl** | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **168·HCl** | 0.4 | 1.3 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **169·HCl** | 4.9 | < 0.8 | > 100 | > 100 | > 100 | > 100 | 100 |
| **170·HCl** | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 | ≥ 20 |
| **171·HCl** | 2.0 | 1.9 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **172·HCl** | 2.9 | 4.5 | > 100 | > 100 | > 100 | > 100 | 27 |
| **173·HCl** | ≤ 2.3 | ≤ 2.0 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **174·HCl** | 1.3 | 1.2 | > 100 | > 100 | > 100 | > 100 | ≥ 20 |
| **176·HCl** | 0.5 | 1.2 | 57 | 60 | > 100 | > 100 | 22 |
| **177·HCl** | 4.6 | 6.0 | > 100 | > 100 | > 100 | > 100 | 31 |
| **178·HCl** | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 | 56 |
| **179·HCl** | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 | 69 |
| ***Amantadine*** | 72.5±38.9 | 57.5±25.2 | 2.2±0.3 | 1.7±0.8 | > 100 | > 100 | > 500 |
| ***Rimantadine*** | 33.3±11.4 | 24.1±10.2 | 0.25±0.21 | 0.25±0.21 | > 100 | > 100 | > 500 |

[a] $EC_{50}$: concentration producing 50% antiviral effect, as determined by microscopy of the virus-induced CPE.
[b] MCC: minimum cytotoxic concentration, i.e. concentrations causing minimal changes in cell morphology.
[c] Average value after three experiments.
[d] Average value after two experiments.

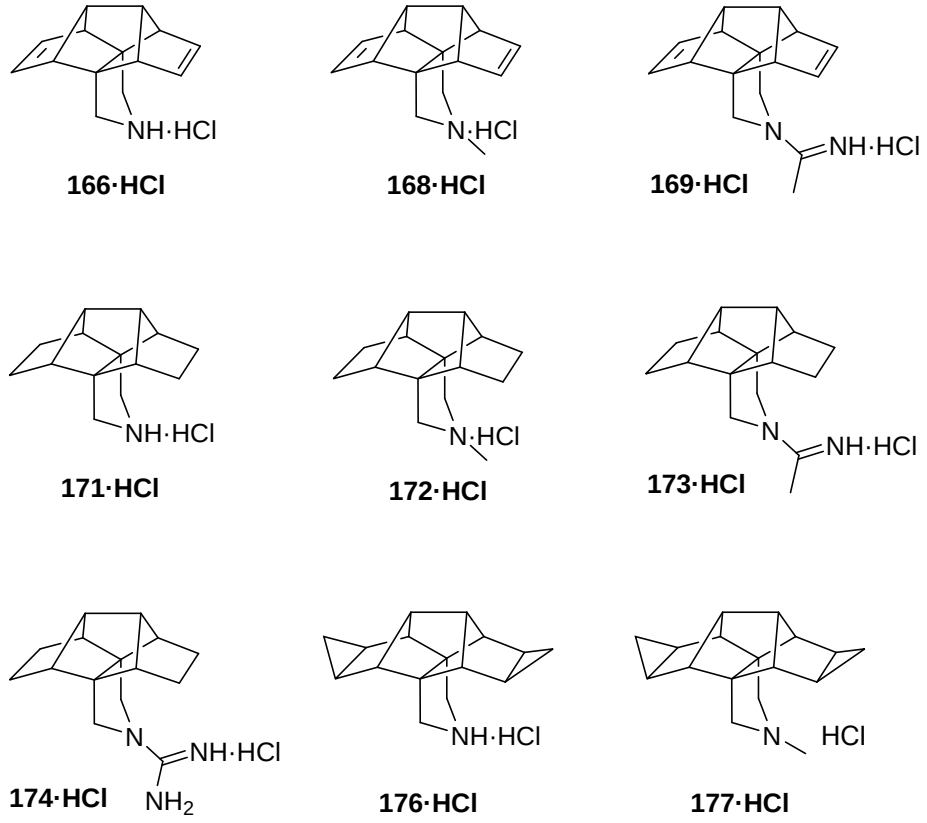



**Figure 54.** Active compounds in 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene and related compounds.

As shown in Table 7, only one compound, secondary amine **166·HCl** displayed activity against both the A/H1N1 and the A/H3N2 virus (antiviral $EC_{50}$ values of 14 and 16 μM, respectively). Apart from this, several compounds showed a nice activity against A/H1N1, being in most cases up to 10 times more active than amantadine (the reference compound), the most distinguished compounds being **168·HCl**, **171·HCl**, **172·HCl**, **174·HCl** and **176·HCl**. Although the latter compound had quite pronounced cytotoxicity for the MDCK cells in the three-day CPE reduction assay (MCC: 22 μM and $CC_{50}$: 44 μM), and this may have masked its potential inhibitory effect towards the A/HK/7/87 virus; the rest of the active compounds produced no cytotoxicity at 100 μM (the highest concentration tested), yielding a favorable selectivity index (defined as the ratio between the cytotoxic and the antiviral concentration for the A/H1N1 virus).

In striking contrast to the overall outstanding activity against the A/H1N1 virus, all of our polycyclic amines displayed no activity against the influenza A/H3N2 virus. As anticipated, all compounds proved to be inactive against influenza B virus, which is known to be insensitive to Amt and rimantadine.

Although a clear SAR was not fully evident in this series, the following insights could be found:

a) Regarding the secondary amines **166·HCl**, **171·HCl** and **176·HCl**, the corresponding methylated compounds (**168·HCl**, **172·HCl** and **177·HCl**, respectively) displayed similar antiviral activity with $EC_{50}$ values in the low micromolar range.

b) The amidine and guanidine substitution, in general does not involve a significant decrease in potency, so as in **171·HCl** ($EC_{50}$ = 2 μM), **173·HCl** ($EC_{50}$ = ≤ 2.3 μM) and **174·HCl** ($EC_{50}$ = 1.3 μM) but in the particular case of the cyclopropanated compounds the introduction of these functionalities lead to a complete loss of potency: **176·HCl** ($EC_{50}$ = 1 μM), **178·HCl** ($EC_{50}$ = >100 μM) and **179·HCl** ($EC_{50}$ = >100 μM).

c) The effect of the double bond is quite puzzling, while in going from **166** to **171** an increase in activity is found ($EC_{50}$ = 16 μM vs $EC_{50}$ = 12 μM), the opposite effect is observed for the pair **168** / **172**, and no significant variation is observed when comparing **169** vs **173**.

Similarly, the antiviral activity of 3-azatricyclo[3.3.3.$0^{1,5}$]undecanes, 8-azatricyclo[4.3.3.$0^{1,6}$]dodecanes and 12-azatricyclo[4.4.3.$0^{1,6}$]tridecanes was determined by microscopic examination of the CPE and by spectrophotometry of the cellular viability using an MTS assay along with all other assays against a broad panel of viruses. From 9 compounds tested, only three of them showed to be active against the H1N1 strain, and none was active agaist the H3N2 strain. Once again, all compounds proved to be inactive against influenza B virus.

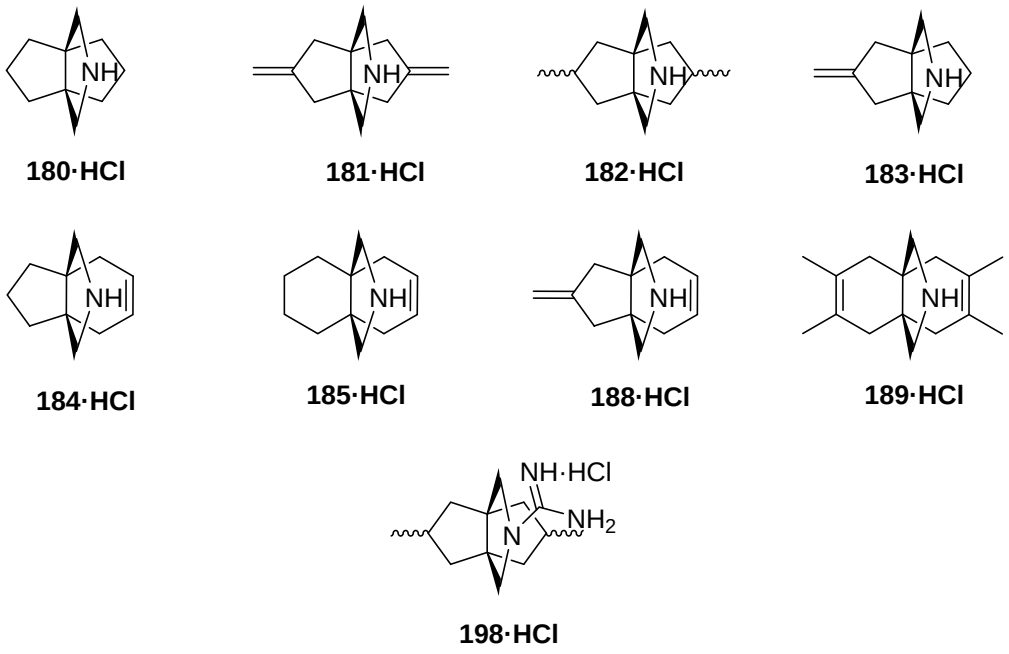



**Figure 55.** Active compounds belonging to the 3-azatricyclo[3.3.3.$0^{1,5}$]undecane, 8-azatricyclo[4.3.3.$0^{1,6}$]dodecane and 8-azatricyclo[4.4.3.$0^{1,6}$]tridecane series.

Analyzing the data showed in Table 8, it seems that the larger structures (**181**, **189**, **198**) displayed anti-H1N1 activity, with the smaller compounds, such as **180** or **183**, being fully inactive. We can also conclude that reducing the exocyclic double bond decreases the antiviral activity, **181·HCl** ($EC_{50}$ = <0.80 μM), and **182·HCl** ($EC_{50}$ = 24 μM). While introducing the guanidine moiety results in an increase of the potency: **182·HCl** ($EC_{50}$ = 24 μM) and **198·HCl** ($EC_{50}$ = 2.0 μM). Remarkably, this series includes the most potent compound synthesized in this Thesis against the tested A/H1N1 strain, being **182·HCl** the lead compound of this family with an $EC_{50}$ = <0.80 μM, two orders of magnitude more potent than the reference compounds, amantadine and rimantadine (Table 8 and Figure 55).

**Table 8.** Antiviral activity of selected 3-azatricyclo[3.3.3.$0^{1,5}$]undecanes, 8-azatricyclo[4.3.3.$0^{1,6}$]dodecanes and 12-azatricyclo[4.4.3.$0^{1,6}$]tridecanes.

| Compound | Antiviral activity $EC_{50}$[a] (μM) | | | | | | Toxicity (MCC[b]) |
|---|---|---|---|---|---|---|---|
| | Influenza A/H1N1 A/PR/8/34 | | Influenza A/H3N2 A/HK/7/87 | | Influenza B B/HK/5/72 | | |
| | CPE | MTS | CPE | MTS | CPE | MTS | |
| **180·HCl** | >100 | >100 | >100 | >100 | > 100 | > 100 | > 100 |
| **181·HCl** | <0.80[c] | <0.80[d] | >100 | >100 | > 100 | > 100 | > 100 |
| **182·HCl** | 24 | 20 | > 100 | > 100 | > 100 | > 100 | > 100 |
| **183·HCl** | >100 | >100 | >100 | >100 | > 100 | > 100 | > 100 |
| **184·HCl** | 70 | 56 | >100 | >100 | > 100 | > 100 | > 100 |
| **185·HCl** | 30 | 14 | >100 | >100 | > 100 | > 100 | > 100 |
| **188·HCl** | 45 | 43 | >100 | >100 | > 100 | > 100 | > 100 |
| **189·HCl** | 7.0 | 5.6 | >100 | >100 | > 100 | > 100 | > 100 |
| **198·HCl** | 2.0 | 1.6 | > 100 | > 100 | >100 | 65 | 100 |
| ***Amantadine*** | 72.5±38.9 | 57.5±25.2 | 2.2±0.3 | 1.7±0.8 | > 100 | > 100 | > 500 |
| ***Rimantadine*** | 33.3±11.4 | 24.1±10.2 | 0.25±0.21 | 0.25±0.21 | > 100 | > 100 | > 500 |

[a] $EC_{50}$: concentration producing 50% antiviral effect, as determined by microscopy of the virus-induced CPE.
[b] MCC: minimum cytotoxic concentration, i.e. concentrations causing minimal changes in cell morphology.
[c] Average value after three experiments.
[d] Average value after two experiments.

From the results collected in tables 6, 7 and 8, it seems that these three families work through the same mechanism, because they possess an outstanding activity against the A/H1N1 strain but they are completely inactive against the A/H3N2. At this point, more studies were required in order to clarify the true mechanism of our compounds.

As we have previously mentioned, it is well-known that the target of amantadine and rimantadine is the influenza A virus M2 channel protein and that a single S31N mutation in M2 renders the virus resistant to both drugs.[112-115] Since most of the currently circulating subtypes of influenza A virus, A/H3N2 and A/H1N1, carry the S31N mutation in M2, there is an urgent need for the development of novel anti-influenza drugs that are effective against the most common amantadine-resistant mutants.[112] The influenza A/PuertoRico/8/34 strain used for testing the activity of our new amines has an M2 channel carrying two substitutions associated with amantadine resistance (i.e. S31N and V27T).

Since these compounds were initially designed as amantadine and rimantadine analogs, we *initially* assumed that their target would be the M2 channel of the Influenza A virus. In order to demonstrate their mechanism of action the most active compounds in this series were tested by the group of Lawrence H. Pinto (Northwestern University, Evanston, Illinois, USA). Prof. Pinto's group performs an assay that consists in measuring the inhibition of a compound directly in the M2 channel expressed in *Oocytes of Xenopus laevis.*

These assays consist in measuring the activity of the channel after the addition of a putative inhibitor and comparing the recorded data with the basal activity of the protein. The gold standard of measuring compound activity on an ion channel is the patch clamp technique. Even though it is low throughput, the high quality data generated from this assay offers insights in guiding drug design. In the patch clamp assay, a constant electric potential (voltage clamp) is applied across an electrically isolated area (patch) of membrane containing the protein of interest, and the current is recorded directly in real time under different conditions, e.g. in the presence or absence of inhibitor.

The assayed protein can either be expressed in cell membrane or reconstituted in artificially formed bilayer-like vesicles. Both single channel and whole cell current recording are possible, but in the case of A/M2, due to the extremely small conductance per channel, whole cell recording are applied for drug screening purpose. In a typical screening assay experiment, Oocyte expressing A/M2 channel is clamped at -20 mV. *Xenopus laevis* (an African clawed frog) was chosen for this purpose since their oocytes are large cells with exceeding 1 mm in diameter and are therefore easy to manipulate, and can be induced to express transport proteins of interest by intracytoplasmatic injection of mRNA encoding the desired protein. Initially oocyte is bathing at pH 8.5 buffer, where A/M2 channel is closed; then A/M2 channel is activated by lowering the buffer solution to pH 5.5. When the inward A/M2 current reaches maximum, a pH 5.5 buffered solution of 100 μM of the inhibitor was applied for 2 min. The remaining current after 2 min application was compared to the maximum current before the application of the inhibitor. The potency of the inhibitors was expressed as percentage inhibition of A/M2 current by 2 min application of 100 μM inhibitiors. Typical trace of the current change is shown in Figure 56.[80b]

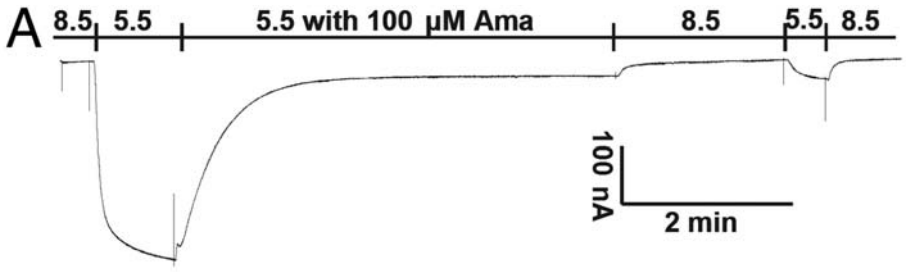



**Figure 56.** Current trace of A/M2 channel upon acidic activation and compound inhibition.

Several selected compounds from Figures 53, 54 and 55 were tested using this assay, the main aim of this work was to demonstrate if, as assumed, the M2 protein of the virus was the target of our compounds. The following tables gather all the information obtained from these studies.

**Table 9.** Inhibition of the wt, V27A and S31N M2 channels by (2,2-dialkyl)amantadines and related compounds.

| Compound | WT A/M2 channel | | V27A A/M2 channel | | S31N A/M2 channel | |
|---|---|---|---|---|---|---|
| | % inh. | $IC_{50}$ (µM) | % inh. | $IC_{50}$ (µM) | % inh. | $IC_{50}$ (µM) |
| **121·HCl** | 58.8±2.3 | ND | 31.6±0.1 | ND | 0 | ND |
| **139·HCl** | 41.5±1.6 | ND | 1.3±0.6 | ND | 7.2±1.1 | ND |
| **144·HCl** | 83.2±1.2 | 37.0 | 41.4±1.0 | 113.1 | 0 | ND |
| **147·HCl** | 4.8±0.8 | ND | 0 | ND | ND | ND |
| **157·HCl** | 4.7±4.7 | ND | 0 | ND | 0 | ND |
| **158·(2R,3R)-tartrate** | 30.4±0.9 | ND | 57.9±1.3 | 51.4 | 0 | ND |
| **159·HCl** | 14.9±2.6 | ND | 10.9±1.2 | ND | 2±0.7 | ND |
| **Amantadine** | 91.0±2.1 | 16.0±1.2 | 10.8±2.0 | ND | 35.6±1.5 | 199.9±13.5 |

% inh.: inhibition by 100 µM of the compound for 2 min (%). ND: not determined.

Values shown are the mean ±SEM of 2-3 determinations.

Table 9 collects the results obtained by Pinto's group regarding the series of 2,2-dialkylamantadines. Although **144** weakly inhibited the wt and the V27A mutant M2 channels, this activity does not match with the antiviral activities obtained in the CPE assays by L. Naesens. For example, one of the most active compound, **147·HCl** that resulted to have an $EC_{50}$=1 µM in CPE assays, only inhibit a 4.8% of the activity of the wt M2 channel, and is fully inactive against the mutant V27A and S31N channels. Moreover, **139·HCl** that exhibited an $EC_{50}$=2.0 µM in the CPE assays, inhibited only the 41.5% of the activity of the channel after the addition of 100 µM of the compound. *All these data strongly suggest that these compounds do not act in this protein but in another one*.

Regarding the series of 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadecanes and related compounds we drew the same conclusion stated before. The data shown in table 10 strongly suggest that the M2 channel is not the target of this family of compounds.

**Table 10.** Inhibition of the wt, V27A and S31N M2 channels by hexacyclic and octacyclic amines.

| Compound | WT A/M2 channel | | V27A A/M2 channel | | S31N A/M2 channel | |
|---|---|---|---|---|---|---|
| | % inh. | $IC_{50}$(μM) | % inh. | $IC_{50}$ (μM) | % inh. | $IC_{50}$ (μM) |
| **166·HCl** | 82±1 | 34±2 | 9.2±1 | ND | 0 | ND |
| **171·HCl** | 40±1 | ND | 14.8±0.5 | ND | 0 | ND |
| **172·HCl** | 4.1±1.1 | ND | 2.8±0.4 | ND | 1.2±0.6 | ND |
| **176·HCl** | 86±1 | 24±2 | 17.7±0.5 | ND | 0 | ND |
| **177·HCl** | 27±1 | ND | 1.9±0.2 | ND | 0 | ND |
| **178·HCl** | 20±2 | ND | 2.2±10.3 | ND | 1.4±1.4 | ND |
| **Amantadine** | 91.0±2.1 | 16.0±1.2 | 10.8±2.0 | ND | 35.6±1.5 | 199.9±13.5 |

% inh.: inhibition by 100 μM of the compound for 2 min (%). ND: not determined.

Values shown are the mean ±SEM of 2-3 determinations.

As shown, for compound **166·HCl** and amantadine, a nice correlation was seen between their cell culture activities for the A/HK/7/87 virus and their $IC_{50}$ values against A/M2 wt proton channel function (Table 7 and 10). This was, however, not the case for the bis-cyclopropanated derivative of **166·HCl**, the octacyclo compound **176·HCl**. Compound **176·HCl** showed quite pronounced cytotoxicity for the MDCK cells in the CPE reduction assay, and this may have masked its potential inhibitory effect towards the A/HK/7/87 virus. Referring to the other compounds, the $EC_{50}$ determined by patch clamp assays do not match with the ones detected in plaque assays. It seemed that we were again considering an erroneous target.

Since the 3-azatricyclo[3.3.3.0$^{1,5}$]undecanes and the related polycyclic compounds showed a similar profile in the CPE assays than the previous two families we hypothesized that they would not block the M2 channel. In the spring of 2012, Prof. Lawrence H. Pinto retired and this stopped our collaboration with him. However, before his retirement, he put in contact our group with that of Prof. Anna Moroni and Sabrina Gazzarrini, former post-docts of him that are currently working in the Department of Biology of the Università degli Studi di Milano (Milan, Italy), in order to secure the testing of the compounds. In this way, the Italian group has recently tested some selected 3-azatricyclo[3.3.3.0$^{1,5}$]undecane, 8-azatricyclo[4.3.3.0$^{1,6}$]dodecanes and 12-azatricyclo[3.3.3.0$^{1,6}$]tridecanes.

As shown in Table 11, our hypothesis about the similar behaviour of these compounds with the two previous families of polycyclic compounds was confirmed after receiving the results from Prof. Moroni's group.

**Table 11.** Inhibition of the wt, V27A and S31N M2 channels by selected 3-azatricyclo[3.3.3.0$^{1,5}$]undecanes, 8-azatricyclo[4.3.3.0$^{1,6}$]dodecanes and 12-azatricyclo[4.4.3.0$^{1,6}$]tridecanes.

| Compound | WT A/M2 channel | | V27A A/M2 channel | | S31N A/M2 channel | |
|---|---|---|---|---|---|---|
| | % inh. | $IC_{50}$(μM) | % inh. | $IC_{50}$ (μM) | % inh. | $IC_{50}$ (μM) |
| **180·HCl** | 47.1±2.3 | ND | 6.7±0.3 | ND | 0 | ND |
| **181·HCl** | 29.3±1.6 | ND | 0.3±0.3 | ND | 0 | ND |
| **182·HCl** | 58.7±2.9 | ND | 5.6±2.2 | ND | 0 | ND |
| **183·HCl** | 29.9±1.1 | ND | 0 | ND | 0 | ND |
| **185·HCl** | 19.1±1.8 | ND | ND | ND | ND | ND |
| **188·HCl** | 14.9±3.9 | ND | 0 | ND | ND | ND |
| **198·HCl** | 8.8±2.2 | ND | ND | ND | ND | ND |
| **Amantadine** | 91.0±2.1 | 16.0±1.2 | 10.8±2.0 | ND | 35.6±1.5 | 199.9±13.5 |

% inh.: inhibition by 100 μM of the compound for 2 min (%). ND: not determined.

Values shown are the mean ±SEM of 3-4 determinations.

Thereby, after receiving the shocking results from Prof. Larry Pinto shown in Table 9 and 10, which stated that our amines were not acting on the M2 channel, Prof. Lieve Naesens decided to find out the mechanism of these drugs. Firstly, they analysed if the inactivity of them against the tested H3N2 strain was specific for this subtype. Selected compounds with activity against the A/PR/8/34 (A/H1N1) strain but not the A/HK/7/87 (A/H3N2) strain were further evaluated against the A/Victoria/3/75 (A/H3N2) strain. Both these A/H3N2 strains carry a wt A/M2 protein. The selected compounds (**166·HCl**, **171·HCl**, **172·HCl** and **177·HCl**) were also inactive against the A/Victoria/3/75 strain ($EC_{50}$ >100 μM, the highest concentration tested; data not shown). These data argue against the possibility that the inactivity of these compounds against A/H3N2 virus may have been specific for the A/HK/7/87 strain.

In order to do further investigations on these molecules the research group of Prof. Lieve Naesens tested them against a broader panel of Influenza A virus strains and afterwards they collected some mutants obtained under the pressure of some of our compounds, in order to discover the targeted protein. Taking into account its high potency and low cytotoxicity five compounds were selected: a primary amine, **139·HCl**, a secondary amine **171·HCl** and three tertiary amines, **155·HCl**, **158·(2R, 3R)-tartrate** and **168·HCl**. Chronologically, the compounds shown in Tables 8 and 11 were the last set of compounds to be synthesized, so by the time of starting this study we had not synthesized them, logically were not included in it.

This panel contained:

a) Two Amt-sensitive A/H1N1 strains: A/Ned/378/05 and A/FM/1/47, carrying a wt M2 channel.

b) The Amt-resistant A/H1N1 strain: A/PR/8/34, carrying a V27T / S31N M2 channel.

c) The Amt-resistant A/H1N1 2009 pandemic strain, carrying a S31N M2 channel.

d) Two Amt-sensitive H3N2 strains: A/HK/7/87 and A/Ishikawa/7/82

e) A chimeric strain, A/X-31, carrying the H3 and N2 protein of the Amt-sensitive A/Aichi/2/68 strain and the other proteins (including M2) from the Amt-resistant A/PR/8/34 strain.

All seven influenza A virus strains used were characterized by A/M2 gene sequencing to detect Amt-resistant mutations, and by determining their hemolysis pH (Table 12). The latter was done in the context of our finding that resistance to our polycyclic amines was associated with mutations in the HA protein, leading to an increased hemolysis pH (see below).

Table 12 shows that our compounds are completely inactive against any strain of H3N2 *regardless of carrying a wt, amantadine sensitive, or a mutated, amantadine resistant, M2 protein*. Moreover, this table shows that our amines display activity against any tested H1N1 strain, with the strain A/PR/8/34 being the more sensitive and A/FM/1/47 the less sensitive strain. Additionally, it should be pointed out that some of these compounds, especially **171·HCl,** showed an interesting activity against the strain of the pandemic of 2009, one order of magnitude lower than that of amantadine or rimantadine and with the same $EC_{50}$ than the approved antiviral rivabirin.

Taking into account the results obtained by Pinto's group (Tables 9 and 10) and the results collected in table 12, we concluded that, *although the activity of the amines clearly differs for each H1N1 strain, the antiviral activity of the newly synthesized amines depends on the type of hemagglutinin (H1 or H3) that the virus strains carries*.

In order to check if our hypothesis was true, Naesens' team isolated mutant resistant to our compounds, obtained after passing two of our selected compounds several times in cell cultures. The aim of these experiments was to sequence the genome of the resistant virus and to observe where the mutations had occurred. If the mutated protein was hemagglutinin, this would mean that our compounds were acting on it.

The strict activity of our structurally diverse polycyclic amines against A/H1N1 viruses, whether containing a wt or Amt-resistant M2 protein, pointed to the viral hemagglutinin as the likely antiviral target. It has been known since many years that M2 inhibition by amantadine occurs at lower (micromolar) compound concentrations, while at higher concentrations (100 µM or more), amantadine increases the endosomal pH, thereby interfering with the low pH-induced and HA-mediated membrane fusion.[167]

[167] Daniels, R. S., Downie, J. C., Hay, A. J., Knossow, M., Skehel, J. J., Wang, M. L., Wiley, D. C., *Cell* **1985**, *40*, 431-439.

**Table 12.** Activity against a broader panel of influenza A/H1N1 and A/H3N2 viruses.

| Compound | Antiviral activity $EC_{50}$[a] (μM) | | | | | | | Toxicity (MCC[b]) |
|---|---|---|---|---|---|---|---|---|
| | A/H1N1 subtype | | | | A/H3N2 subtype | | | |
| | A/PR/8/34 | A/Ned/378/05 | A/FM/1/47 | A/2009pan* | A/HK/7/87 | A/Ishikawa/7/82 | A/X-31 | |
| | Amantadine resistant mutations on M2[c] | | | | | | | |
| | V27T/S31N | wt | wt | S31N | wt | wt | V27T/S31N | |
| | Virus pH hemolysis[d] | | | | | | | |
| | 5.0 | 5.1 | 5.2 | 5.2* | 5.0 | 5.2 | 5.3 | |
| **139·HCl** | 1.2 ± 0.5 | 4.7 ± 1.3 | 7.0 ± 0.0 | ND | >100 | >100 | >100 | 100 |
| **155·HCl** | 0.70 ± 0.39 | 23 ± 3 | 43 ± 12 | 28±8 | >100 | >100 | >100 | >100 |
| **158·(2R, 3R)-tartrate** | 5.0 ± 2.5 | 8.7 ± 0.3 | 9.3 ±0.3 | ND | >100 | >100 | >100 | 100 |
| **168·HCl** | 8.0 ± 2.2 | 50 ± 0 | 12±3 | 75 ± 12 | >100 | >100 | >100 | 100 |
| **171·HCl** | 0.40 ± 0.0 | 7.0 ± 0.0 | 10±3 | 11 ± 6 | >100 | >100 | >100 | >100 |
| ***Amantadine*** | 53 ± 11 | 2.0 ± 0.0 | 5.1±2.4 | 137±10 | 3.4 ± 1.7 | 11 ± 8 | 61 ± 12 | 500 |
| ***Rimantadine*** | 63 ± 18 | 0.50 ± 0.22 | 1.9±1.1 | ≥158 | 0.17 ± 0.08 | 0.45 ± 0.15 | 7.0 ± 1.7 | >100 |
| **Oseltamivir carboxylate** | 21 ± 9 | 1.5 ± 0.8 | 16±2 | ND | 29 ± 5 | 3.0 ± 1.0 | 0.11 ± 0.05 | >100 |
| **Zanamivir** | 6.0 ± 1.3 | 1.7 ± 0.5 | 5.0±1.6 | ND | 1.2 ± 0.9 | 8.7 ± 3.8 | 0.16 ± 0.05 | >200 |
| **Ribavirin** | 8.7 ± 0.7 | 7.6 ± 1.0 | 10±1 | 11 ± 1 | 8.6 ± 0.4 | 8.8 ± 0.3 | 8.8 ± 0.2 | >100 |

[a]The $EC_{50}$ represents the compound concentration producing 50% inhibition of virus replication, as determined by microscopic scoring of the CPE.

[b]MCC: minimum cytotoxic concentration, or compound concentration producing minimal alterations in cell morphology. Values shown are the mean ± SEM of 2-5 determinations.

[c]For each virus strain, the entire M2 coding region was sequenced, but only the residues associated with the Amt resistance (i.e. located between M2 residues 26-34) are mentioned here.

[d]For each virus strain, the hemolysis pH was determined (defined as the pH at which 50% hemolysis occurs). ND = not determined.

*A/H1N1 2009 pandemic virus (strain: A/Virginia/ATCC3/2009).

This explains why viruses that have been selected under amantadine in cell culture regularly contain mutations in HA that increases the fusion pH of the virus. These mutant HAs adopt their fusogenic conformation at less acidic pH, thus escaping the pH-increasing effect of amantadine. In this study, we serially passed the influenza A/PR/8/34 virus in the presence of the secondary hexacyclic amine **168·HCl** or the (2,2-diethyl)-1-adamantyl tertiary amine **155·HCl**, which were chosen because of their superior selectivity which enabled to apply compound concentrations as high as 150 µM and because they are representative of each series being a secondary amine and a tertiary one, respectively. A control experiment in which the virus was passed in the absence of test compound was included, to identify which HA mutations appear as the virus, produced in eggs, is adapted to cell culture.

**Table 13.** Amino acid substitutions in the viral HA after passaging with **168·HCl** or **155·HCl**, and their impact on the pH of hemolysis.

| Virus[a] | Amino acid residue[b] | | | | | Hemolysis pH[d] |
|---|---|---|---|---|---|---|
| | $HA_1$-13 | $HA_1$-186 | $HA_1$-324 | $HA_2$-3 | $HA_2$-10 | |
| Published[c] | Ala | Ala | Ala | Ala | Ala | |
| Parent allantois | Ala | Pro | Ile | Phe | Ile | 4.9 ± 0.0 |
| No cpd #1 | Ala | Pro/Ser | Ile | Phe | Ile | |
| No cpd #2 | Ala | Pro/Ser | Ile | Phe | Ile/Val | |
| No cpd cl 1-2-3 | Ala | Pro | Ile | Phe | Val | 5.2 ± 0.0 |
| **168·HCl** #1 | Ala | Ser | Ile/Thr | Phe | Ile | |
| **168·HCl** #2 | Ala | Ser | Thr | Phe | Ile | |
| **168·HCl** #8 cl 1-2-3 | Thr | Ser | Thr | Phe | Ile | 5.7 ± 0.1 |
| **155·HCl** #1 | Ala | Pro | Ile | Phe | Val | |
| **155·HCl** #2 | Ala | Pro | Ile | Leu | Val | |
| **155·HCl** #8 cl 1-2-3 | Ala | Pro | Ile | Leu | Val | 5.5 ± 0.0 |

[a]Influenza virus (A/H1N1; strain A/PR/8/34) was passed in MDCK cells in the presence of **168·HCl**, **155·HCl** or no compound, and passages #1; #2 and #8 were subjected to HA sequence analysis. For passage #8, individual virus clones (cl1, cl2 and cl3) were first isolated by plaque purification.
[b]Amino acid residues at the indicated positions in the $HA_1$ or $HA_2$ polypeptides of the HA protein. HA numbering as by Gamblin et al.[168]
[c]Published hemagglutinin sequences for the A/PR/8/34 strain (GI accession numbers 89779321, 392340000 and 323696012).[169]
[d]To prepare concentrated stocks for determining the hemolysis pH, the isolated virus clones were expanded by one passage in eggs.[170] Data shown are the mean ± SEM (n=2).

[168] Gamblin, S. J.; Haire, L. F.; Russell, R. J.; Stevens, D. J.; Xiao, B.; Ha, Y.; Vasisht, N.; Steinhauer, D. A.; Daniels, R. S.; Elliot, A.; Wiley, D. C.; Skehel, J. J. *Science* **2004**, *303*, 1838-1842.

As summarized in Table 13, five residue changes were detected in the HA1 or HA2 polypeptide parts of the HA protein. Sequence analysis on our parent A/PR/8/34 allantoic stock showed that, at this stage, the virus was still homogeneous, since no double peaks were observed. A wide survey of published sequences of the A/PR/8/34 HA[169] demonstrated that for two of the five changes, the observed variation has been reported (i.e. $HA_1$-P186S and $HA_1$-I324T), suggesting that these changes naturally occur without selective pressure. In the virus passed in the absence of compound, an $HA_2$-I10V substitution became apparent after two passages, and this mutant was the only one remaining after eight passages, indicating that it results from cell culture adaptation of the A/PR/8/34 virus. The two remaining changes were clearly selected by our polycyclic amines: $HA_1$-A13T, which was present in the #8 passage selected under compound **168·HCl**, and $HA_2$-F3L, which was already apparent after two passages with **155·HCl**. The virus selected under **168·HCl** also contained the $HA_1$-P186S and $HA_1$-I324T changes, which appeared early on and of which the relevance is not clear since, as explained above, these may well be polymorphic changes.

Our sequencing data strongly argued for a role of the HA protein in the antiviral mode of action of **168·HCl** and **155·HCl**, and, most likely, the other polycyclic amines studied here. A parallel can be seen with the HA changes obtained under Amt, which result in an increased fusion pH, thus rendering the virus resistant to the pH-increasing effect of Amt.[167] We therefore determined the hemolysis pH, defined as the pH at which 50% hemolysis occurs when virus attached to erythrocytes is exposed to different acidic buffers.[170] The hemolysis pH of our parent allantoic A/PR/8/34 virus was quite low (Table 12). This value was increased (to 5.2) in the cell culture-adapted virus containing the $HA_2$-I10V change. The mutant viruses obtained under **168·HCl** or **155·HCl** had a considerably higher hemolysis pH of 5.7 and 5.5, respectively, indicating that acquisition of a less stable HA that is able of fusing at higher pH, confers resistance to the polycyclic amines.

Antiviral evaluation of the #8 passages, with the allantoic stock tested in parallel, demonstrated that the fusion mutants obtained under **168·HCl** and **155·HCl** were fully resistant to both polycyclic amines, and cross-resistant to Amt and rimantadine, whereas their sensitivity to ribavirin was the same as that of the allantoic virus (Table 14). The #8 virus obtained in the absence of compound, which had an intermediate hemolysis pH of 5.2, was less sensitive to all four Amt derivatives, the factor increase in antiviral $EC_{50}$ (compared to the allantoic virus) being 4 (compound **168·HCl**); 11 (compound **155·HCl** and Amt) and >27 (rimantadine). Thus, a correlation was seen between the viral hemolysis pH and the antiviral sensitivity to each of the four amines.

[169] a) Bao, Y.; Bolotov, P.; Dernovoy, D.; Kiryutin, B.; Zaslavsky, L.; Tatusova, T.; Ostell, J.; Lipman, D. *J. Virol.* **2008**, *82*, 596-601. b) http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html

[170] Vanderlinden E.; Göktas F.; Cesur Z.; Froeyen M.; Reed M. L.; Russell, C. J.; Cesur, N.; Naesens L. *J. Virol.* **2010**, *84*, 4277-4288.

**Table 14.** Antiviral resistance profile of the influenza virus clones obtained after selection with **168·HCl** or **155·HCl**.

| Virus[a] | Hemolysis pH[b] | Antiviral activity ($EC_{50}$ in µM)[c] | | | | |
|---|---|---|---|---|---|---|
| | | **168·HCl** | **155·HCl** | Amantadine | Rimantadine | Ribavirin |
| Parent allantois[d] | 4.9 ± 0.0 | 1.6 ± 0.6 | 1.0 ± 0.3 | 67 ± 2.8 | 3.7 ± 2.7 | 11 ± 5 |
| No cpd #8 cl3 | 5.2 ± 0.0 | 18 ± 8 | 4.5 ± 1.9 | 70 ± 30 | >100 | 15 ± 5 |
| **168·HCl** #8 cl3 | 5.7 ± 0.1 | >100 | >100 | >250 | >100 | 26 ± 4 |
| **155·HCl** #8 cl3 | 5.5 ± 0.0 | >100 | >100 | >250 | >100 | 24 ± 3 |

[a]The $EC_{50}$ represents the compound concentration producing 50% inhibition of virus replication, as determined by microscopic scoring of the CPE.
[b]MCC: minimum cytotoxic concentration, or compound concentration producing minimal alterations in cell morphology. Values shown are the mean ± SEM of 2-5 determinations.
[c]$EC_{50}$: compound concentration producing 50% inhibition of virus-induced CPE.
[d]Parent A/PR/8/34 allantois stock, used for selection of the virus clones in the presence of **168·HCl** or **155·HCl,** or in the absence of compound.
Data shown are the mean ± SEM (n=2).

After endocytic uptake of the influenza virus into the host cell, the viral genome segments must be released into the cytoplasm and transported to the nucleus to initiate RNA transcription and replication. This endosomal escape of the virus critically depends on the activity of two viral proteins which are both activated at the low endosomal pH: the M2 proton channel, required for uncoating of the viral ribonucleoproteins, and the HA protein, which upon acidification adopts a fusogenic conformation to cause fusion of the endosomal and viral membranes and formation of a fusion pore.[171] Therefore, dually acting polycyclic amines which combine blockade of the M2 channel with an inhibitory effect on HA refolding, appear highly attractive. This dual approach could also, at least in theory, increase the barrier for selecting adamantane resistance.[172] Optimized adamantane derivatives might be able to exert this dual pharmacological effect, provided that both the M2 and HA inhibition occur at similar and relevant compound concentrations. For comparison, the M2 blocking effect of Amt is achieved at micromolar concentrations, whereas its endosomal pH increasing effect requires much higher concentrations that may not be achieved *in vivo*.

We hypothesized that the potent activity of our polycyclic amines against the Amt-resistant A/PR/8/34 virus most likely results from interference with HA-mediated fusion, alike seen with high concentrations of Amt. A/PR/8/34 virus mutants obtained after serial passaging in the presence of the secondary amine **168·HCl** or the tertiary amine **155·HCl** indeed contained mutations in the HA protein that considerably increased the pH of hemolysis, meaning that these mutant HAs adopt their fusogenic conformation at higher pH.

[171] Cross, K. J.; Burleigh, L. M.; Steinhauer, D. A. *Exp. Rev. Mol. Med.* **2001**, *3*, 1-18.
[172] Scholtissek, C.; Quack, G.; Klenk, H. D.; Webster, R. G. *Antiviral Res.* **1998**, *37*, 83-95.

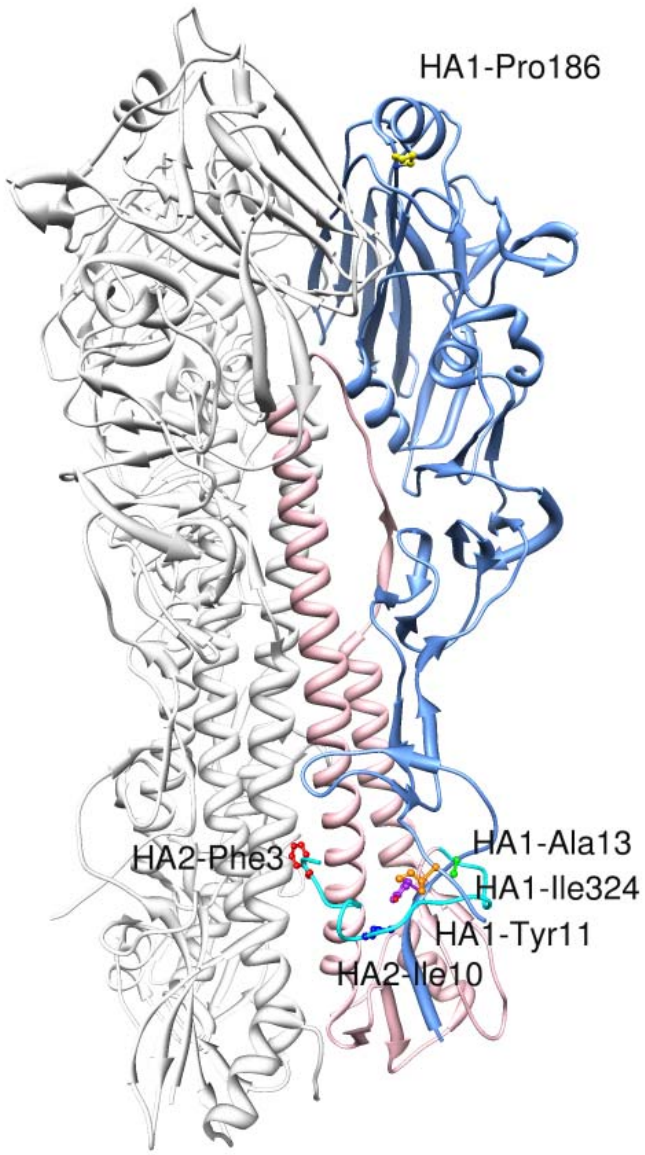



**Figure 57**. HA1 in blue; HA2 in red. Marked residues: 13A green; 186P pink; 324I orange; 503F grey; 510I yellow.

In Fig. 57, the five residues that were subjected to mutation in our passaged viruses were located in the published crystal structure of the A/PR/8/34 HA.[168] Three of these changes [$HA_1$-P186S, located in the globular head, and $HA_1$-I324T and $HA_2$-I10V, both located in the HA stem near the fusion peptide] seemed to be polymorphic and/or relate to cell culture adaptation and, hence, were considered irrelevant in the context of our polycyclic amines. The $HA_2$-F3L residue change selected under **155·HCl** was also identified by Plotch et al. who selected a virus for resistance to the small molecule fusion inhibitor CL-61917, starting from an Amt-resistant A/FM/47 virus.[173] The increased fusion pH of the $HA_2$-F3L mutant virus is not unexpected, since this residue lies in the hydrophobic fusion peptide of HA. Others have

[173] Plotch, S. J.; O'Hara, B.; Morin, J.; Palant, O.; LaRocque, J.; Bloom, J. D.; Lang, S. A., Jr.; DiGrandi, M. J.; Bradley, M.; Nilakantan, R.; Gluzman, Y. *J Virol.* **1999**, *73*, 140-151.

reported that introduction of less hydrophobic residues into the fusion peptide results in an increased fusion pH. Regarding the $HA_1$-A13T substitution selected under compound **168·HCl**, the impact of this residue change is less obvious. In the neutral pH structure of the A/PR/8/34 HA protein, this Ala-13 residue in HA1 lies adjacent to Tyr-11, which is reported to directly interact with the fusion peptide *via* formation of two hydrogen bonds. The corresponding residue in H3 HA is His-17, which requires a water molecule to interact with the fusion peptide and, in contrast to tyrosine, is protonated at fusion pH. The critical role of this residue in triggering membrane fusion explains why Tyr-11 is conserved in all group-1 HAs (including H1 HA), whereas all group-2 HAs (including H3 HA) contain His-17 at the corresponding position. The importance of this HA region is consistent with our observation that the $HA_1$-A13T substitution drastically reduces the stability of the HA, resulting in a hemolysis pH as high as 5.7. One explanation may be that a hydroxyl-containing Thr residue at position 13 may disturb the hydrogen bonding between Tyr-11 and the fusion peptide.

The fact that the polycyclic amines select for A/H1N1 escape mutants with increased fusion pH indicates that these compounds interfere with the HA-mediated fusion process. One potential mode of action, i.e. direct binding of the polycyclic amine compound to the prefusogenic HA protein, thereby preventing its refolding at low pH, was excluded in a hemolysis inhibition experiment with the A/PR/8/34 virus. We previously used the hemolysis assay to identify a small molecule fusion inhibitor of A/H3N2 viruses that acts by binding to a hydrophobic pocket in the prefusogenic HA protein.[170] Neither of the two polycyclic amines tested (**168·HCl** and **155·HCl**) inhibited the low pH-induced hemolysis by A/PR/8/34 at 100 µM (data not shown). In addition, the compounds did not produce any effect in a hemagglutination inhibition assay. These data argue against a direct binding interaction between the polycyclic amines and any of the HA regions involved in receptor binding or membrane fusion. Thus, in analogy to Amt, it is probable that the polycyclic amines act by causing a slight increase in the endosomal pH. Whereas this pH effect of Amt is only seen at ~100 µM concentrations, our compounds act at ~50-fold lower concentrations. This mode of action explains why, within the panel of A/H1N1 strains tested, a correlation was seen between the antiviral $EC_{50}$ values and the viral hemolysis pH (Table 14). Also, the markedly increased hemolysis pH of the A/PR/8/34 mutants resistant to the polycyclic amines means that these mutant HAs are able to fuse at conditions of higher endosomal pH, induced by the amine compounds. However, it is clear that the antiviral sensitivity to the polycyclic amines is not merely determined by the viral fusion pH, since the HA subtype (i.e. H1 or H3) acts as the real discriminating factor. The most convincing evidence comes from our result that the A/PR/8/34 (A/H1N1) and A/X-31 (A/H3N2) strains display opposite sensitivity to our polycyclic amines, despite having a similarly low hemolysis pH of 5.0. It thus appears that the A/PR/8/34 HA has some feature which makes it less stable and more susceptible to a slight increase in the endosomal pH. Some parallel may be seen with the reports that the A/PR/8/34 virus is particularly sensitive to inactivation at low pH demonstrated that the fusion activity of A/PR/8/34 virus was rapidly lost when the virus was preincubated at pH 5.4.[174] For A/X-31 virus, this inactivation required a longer preincubation time and a lower pH of 5.0. These authors further showed that, at pH 5.4, the A/PR/8/34 HA displays a higher exposure of hydrophobic residues compared to A/X-31 HA, which is relevant for the initiation of membrane

[174] Korte, T.; Ludwig, K.; Booy, F. P.; Blumenthal, R.; Herrmann, A. *J Virol.* **1999**, *73*, 4567-4574.

fusion. The fast inactivation of A/PR/8/34 HA at low pH was proposed to be related to two characteristic Glu residues that are lying close to each other in the globular head of the HA protein, causing electrostatic repulsion and a weaker trimeric stability.[175] An alternative explanation for the H1-specific antiviral activity of our polycyclic amines may be that some H1 HAs (such as that of the 1918 virus or the A/PR/8/34 strain) possess a second cluster of basic (particularly histidine) residues adjacent to the vestigial esterase domain.[176] A second basic patch was also found in the 2009 pandemic H1 HA,[177] but not in other HAs, such as H3 HA.[176] It was suggested that this second basic patch creates extra electrostatic repulsions when the HA is exposed to acidic pH. Since a second cluster of basic residues is also present in the HA of some avian influenza A/H5N1 viruses,[178] it would be relevant to investigate whether these viruses are sensitive to our polycyclic amines.[178b]

At the time of writing this Thesis a similar study with compounds shown in Figure 55 had not been done yet, but probably, in the near future we will have the results of these studies to demonstrate the true mechanism of action of these compounds.

[175] Rachakonda, P. S.; Veit, M.; Korte, T.; Ludwig, K.; Böttcher, C.; Huang, Q.; Schmidt, M. F.; Herrmann, A. *FASEB J.* **2007**, *21*, 995-1002.
[176] Stevens, J.; Corper, A. L.; Basler, C. F.; Taubenberger, J. K.; Palese, P.; Wilson, I. A. *Science* **2004**, *303*, 1866-1870.
[177] Zhang, W.; Qi, J.; Shi, Y.; Li, Q.; Gao, F.; Sun, Y.; Lu, X.; Lu, Q.; Vavricka, C. J.; Liu, D.; Yan, J.; Gao, G. F.; *Protein Cell*. **2010**, *1*, 459-467.
[178] a) Stevens, J.; Blixt, O.; Tumpey, T. M.; Taubenberger, J. K.; Paulson J. C.; Wilson, I. A. *Science* **2006**, *312*, 404-410. b) Biosafety regulations do not allow Prof. Naesens' group to work with the Influenza A/H5N1 viruses.

# COMPOUNDS TARGETING M2 CHANNEL

# 5. Design of new inhibitors for the S31N mutant M2 channel.

## 5.1 (Tricyclo[3.3.0.0$^{3,7}$]oct-1-yl)amines, 3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecanes and 7,8-dimethyl-3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecanes.

In 2010, Jiang and coworkers published that molecular dinamics (MD) simulations predicted a narrower pore for the S31N mutant channel than that of the wt channel (Figure 58).[179] More recent MD simulations published this year are in agreement with this earlier disclosure.[109b,c]

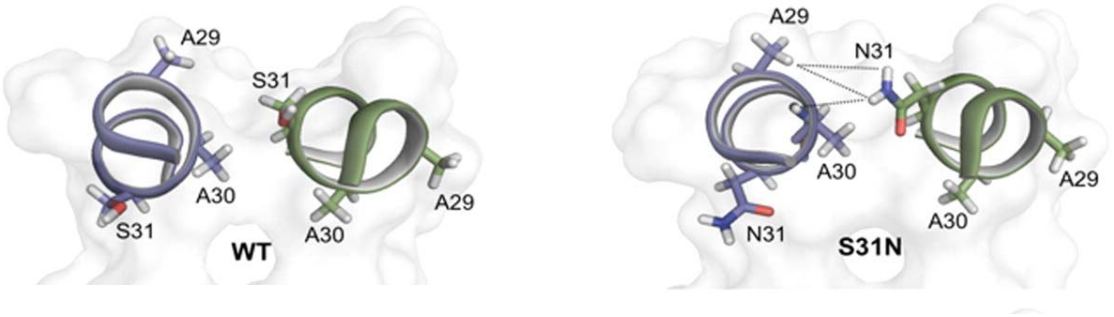



**Figure 58.** Narrowing of the channel lumen in going from serine to asparagine: left: wt channel, right: S31N mutant channel.[179]

We hypothesized that a smaller polycyclic scaffold than amantadine would result in a better inhibition of the S31N mutant channel. For this reason, during the PhD Thesis of M. D. Duque, our group prepared two series of bisnoradamantane derivatives, **XVI** and **XVII**, as ring-contracted analogues of amantadine. Unfortunately, although several of these derivatives displayed interesting anti-influenza activity against amantadine-sensitive strains and they were able to effectively blocking the wt M2 channel of influenza A,[180] neither anti-viral activity against amantadine-resistant strains nor blocking activity of the mutant M2 channels was found for any of the new derivatives. For example, in CPE assays, primary amine **XVII** (R=R'=H) displayed low micromolar inhibition of the amantadine-sensitive A/HK/7/87 strain ($EC_{50}$ = 2.5 μM), most probably by M2 channel inhibition, as Pinto's group found that this compound had an $IC_{50}$ = 7.2 μM, twofold more potent than amantadine, for the blocking of the wt M2 channel. However, this compound was unable to inhibit the S31N mutant M2 channel.

Taking into account these results, as the fourth objective of this Thesis, we wanted to synthesize new bisnoradamantanes derivatives of general structures **XI**, **XII** and **XIII** (Figure 59).

[179] Qin, J.; Yu, K.; Shi, T.; Luo, C.; Li, G.; Zhu, W.; Jiang, H. *J. Phys. Chem. B* **2010**, *114*, 8487-8493.
[180] Duque, M. D.; Ma, C.; Torres, E.; Wang, J.; Naesens, L.; Juárez-Jiménez, J.; Camps, P.; Luque, F. J.; DeGrado, W. F.; Lamb, R. A.; Pinto, L. H.; Vázquez S. *J. Med. Chem.* **2011**, *54*, 2646-2657.

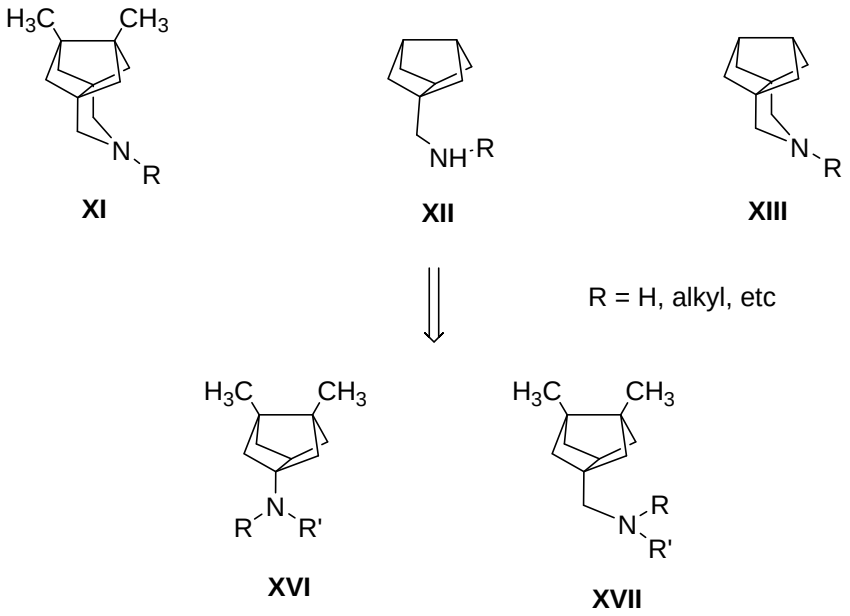
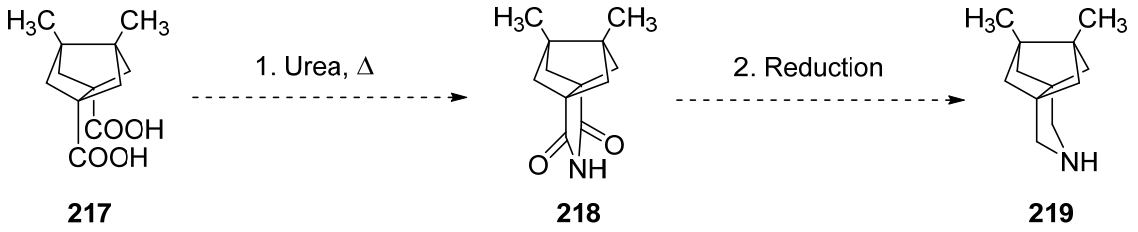



**Figure 59.** Bisnoradamantane amines.

### 5.2. Synthesis of 7,8-dimethyl-3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecanes.

As we have previously mentioned, our group had synthesized several amines of general structures **XVI** and **XVII** for which anti-influenza activity was found (Figure 59).[180] We have already seen in Chapter 2 that the inclusion of the nitrogen atom in a pyrrolidine ring led, in some examples, to an enhancement of the antiviral activity. With these precedents in mind, we first decided to synthesize compounds of general structure **XI** (Figure 59).

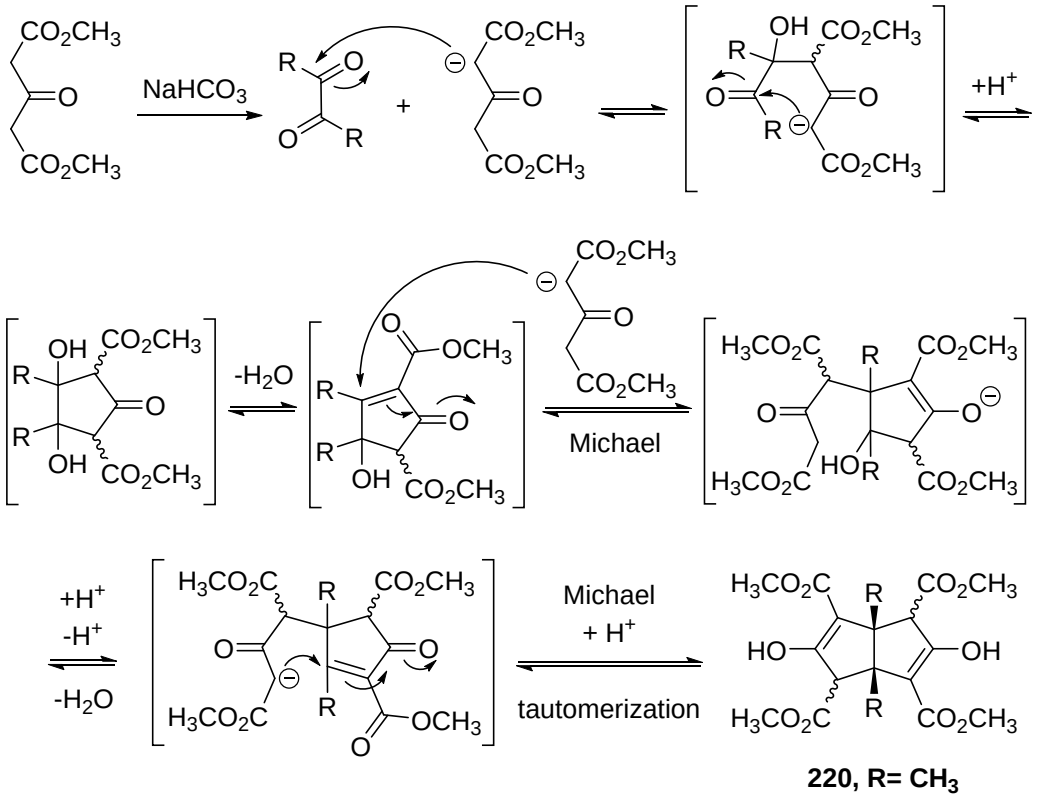
For the synthesis of 7,8-dimethyl-3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecane, **219**, we thought of dicarboxylic acid **217** as a suitable starting material. This acid had been reported by our group several years ago.[11a,181] The reaction of **217** with urea followed by reduction of the obtained imide should led to **219** (Scheme 65).




**Scheme 65.** Envisioned synthesis of amine **219**.

[181] Camps, P.; Iglesias, C.; Rodríguez, M. J.; Grancha, M. D.; Gregori, M. E.; Lozano, R.; Miranda, M. A.; Figueredo, M.; Linares, A. *Chem. Ber.* **1988**, *121*, 647-654.

The synthesis of **219** started from diketone **221** (Scheme 67). This compound is readily available in a multigram scale through the Weiss reaction,[182] which involves the condensation of an α-dicarbonylic compound, in this case 2,3-butanedione, with two equivalents of dimethyl 1,3-acetonedicarboxylate in basic media. After a series of aldolic condensations, dehydrations and Michael reactions, the dienol-tetraester **220** is obtained. Its hydrolysis and decarboxylation in acid media furnished diketone **221** in 84% overall yield (Scheme 66).[183]

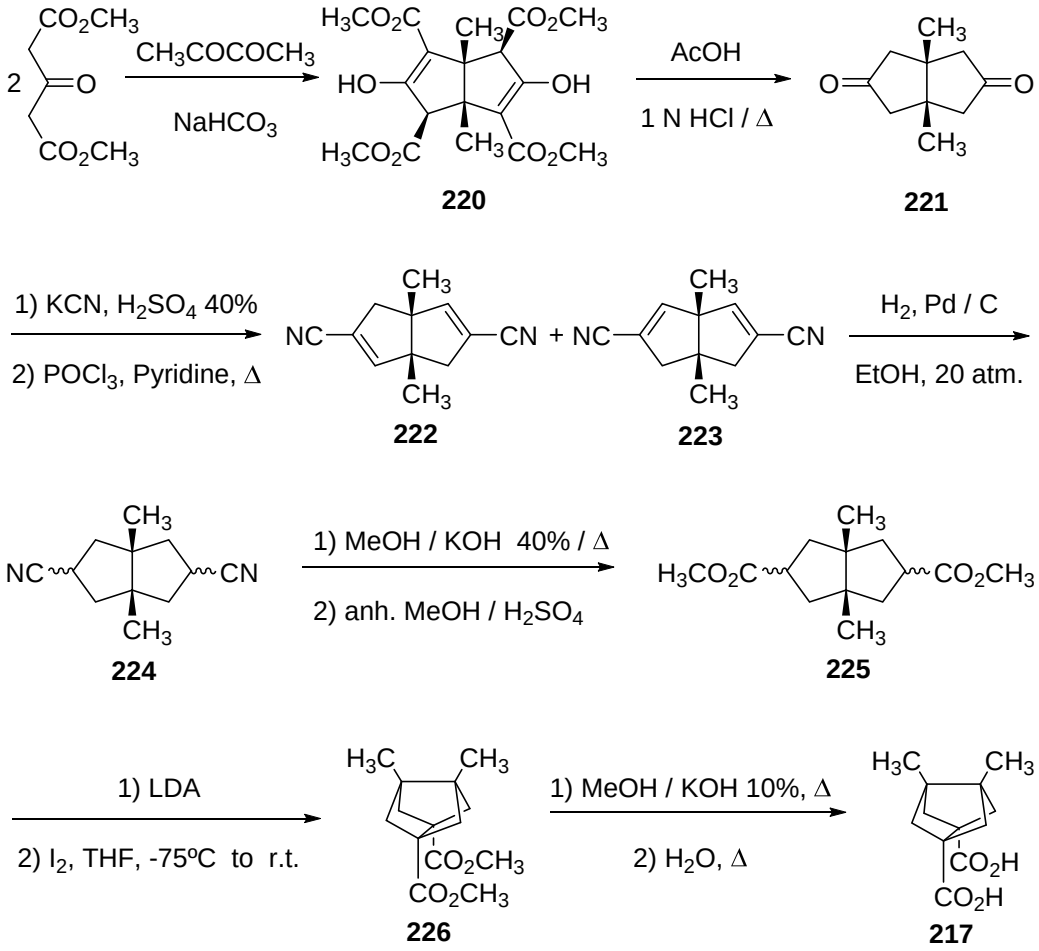



**Scheme 66.** Mechanism of the Weiss reaction.

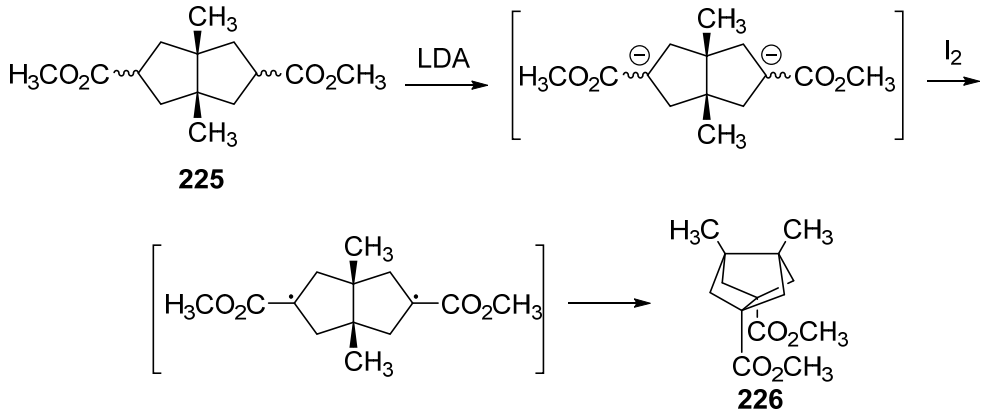
The reaction of diketone **221** with potassium cyanide in aqueous media keeping the neutral pH by adding dropwise a 40% solution of sulphuric acid gave a mixture of *bis*-cyanohydrins which were dehydrated with $POCl_3$ in pyridine at reflux. This reaction gave the stereoisomeric mixture of dinitriles **222** and **223** with 55% overall yield. The catalytic hydrogenation of these dinitriles using 10 % of Pd/C as catalyst, ethanol as solvent and at 20 atm of pressure furnished an stereoisomeric mixture of the three possible reduced products in quantitative yield. This mixture was hydrolysed with KOH in a methanol/water media and after esterification using Fisher's conditions, the stereoisomeric mixture of diesters **225** was obtained with an overall yield of 62%.

[182] a) Fu, X.; Cook, J. M. *Aldrichimica Acta* **1992**, *25*, 43-54. b) Gupta, A. K.; Fu, X.; Snyder, J. P.; Cook, J. M. *Tetrahedron* **1991**, *47*, 3665-3710.
[183] Bertz, S. H.; Cook, J. M.; Gawish, A.; Weiss, U. *Org. Synth. Coll. Vol. VII*, Wiley: New York, **1990**, pp 50-56.

The key step of this synthetic route was the cyclization of the diesters to construct the bisnoradamantane scaffold. This reaction was carried out by the oxidative coupling mediated by iodine of the corresponding lithium *bis*-enolates to obtain the diester **226** in 54% yield (Scheme 68). Finally, the hydrolysis of this diester in basic media gave the diacid **217** in 95% yield (Scheme 67).

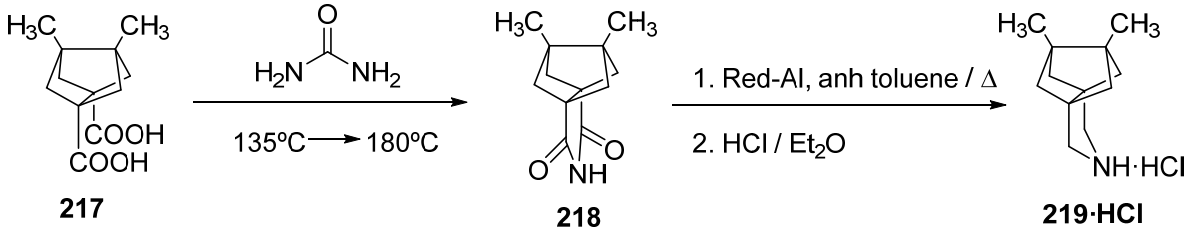



**Scheme 67.** Obtention of diacid **217**.



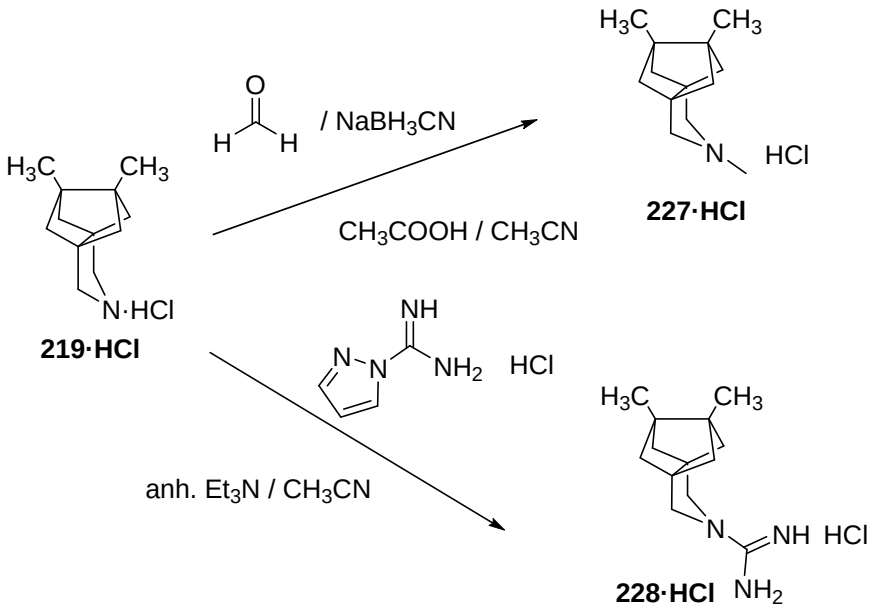

**Scheme 68.** Oxidative coupling of ester **225** mediated by iodine.

As expected, the treatment of diacid **217** with urea at 180 $^{o}$C (internal temperature) gave the imide **218** in 75% yield as a yellowish solid . The reduction of this imide with Red-Al® in anhydrous toluene yielded amine **219·HCl** in 99% yield (Scheme 69).




**Scheme 69.** Synthesis of of amine **219·HCl**.

With enough amount of the secondary amine **219·HCl** in our hands, we next proceeded to synthesize its *N*-methyl derivative, **227·HCl**, and the guanidine **228·HCl**. Both reactions led smoothly to the expected derivatives in 73 and 61% yield, respectively (Scheme 70).



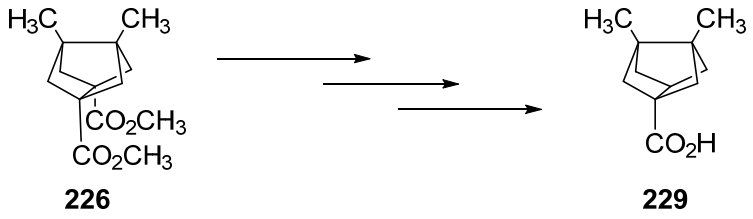

**Scheme 70.** Synthesis of derivatives of amine **219·HCl**.

### 5.3. Preparation of (tricyclo[3.3.0.0$^{3,7}$]oct-1-yl)amines.

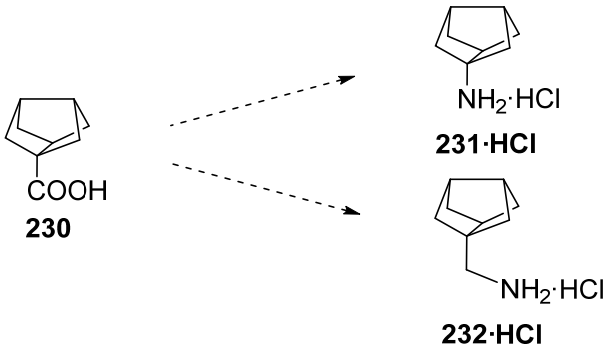
As we have already mentioned, the aim of synthesizing compounds of general structures **XVI** and **XVII** was to test the blocking activity of ring-contracted analogs of amantadine in the S31N mutant M2 channel of influenza A. However, as previously stated, although several derivatives of **XVI** and **XVII** were active against the wt M2 channel, they failed to successfully block the activity of the S31N mutant channel. Keeping in mind these results, we reasoned that by removing the two methyl groups present in **XVI**, **XVII** and **XI** we would obtain even smaller analogues of amantadine.

Several years ago, our group synthesized the carboxylic acid **229** from diester **226** (Scheme 71).[43,152,184] Taking into account that our group had also reported the synthesis of diester **239** (Scheme 73),[181] when we planned to synthesize amines **231·HCl** and **232·HCl** we thought that a logic common precursor for these amines would be the carboxylic acid **230**, that may be available from diester **239** using the same sequence previously developed by our group for the synthesis of **229** from **226**. From **230**, classical transformations would lead to the requested amines. Thus, **231** may be accessed through a Schmidt rearrangement of **230**, while **232** may be synthesized by reduction of an amide derived from **230** (Scheme 72).

[184] Xavier Pujol, Tesis Doctoral, Universidad de Barcelona, **2001**.

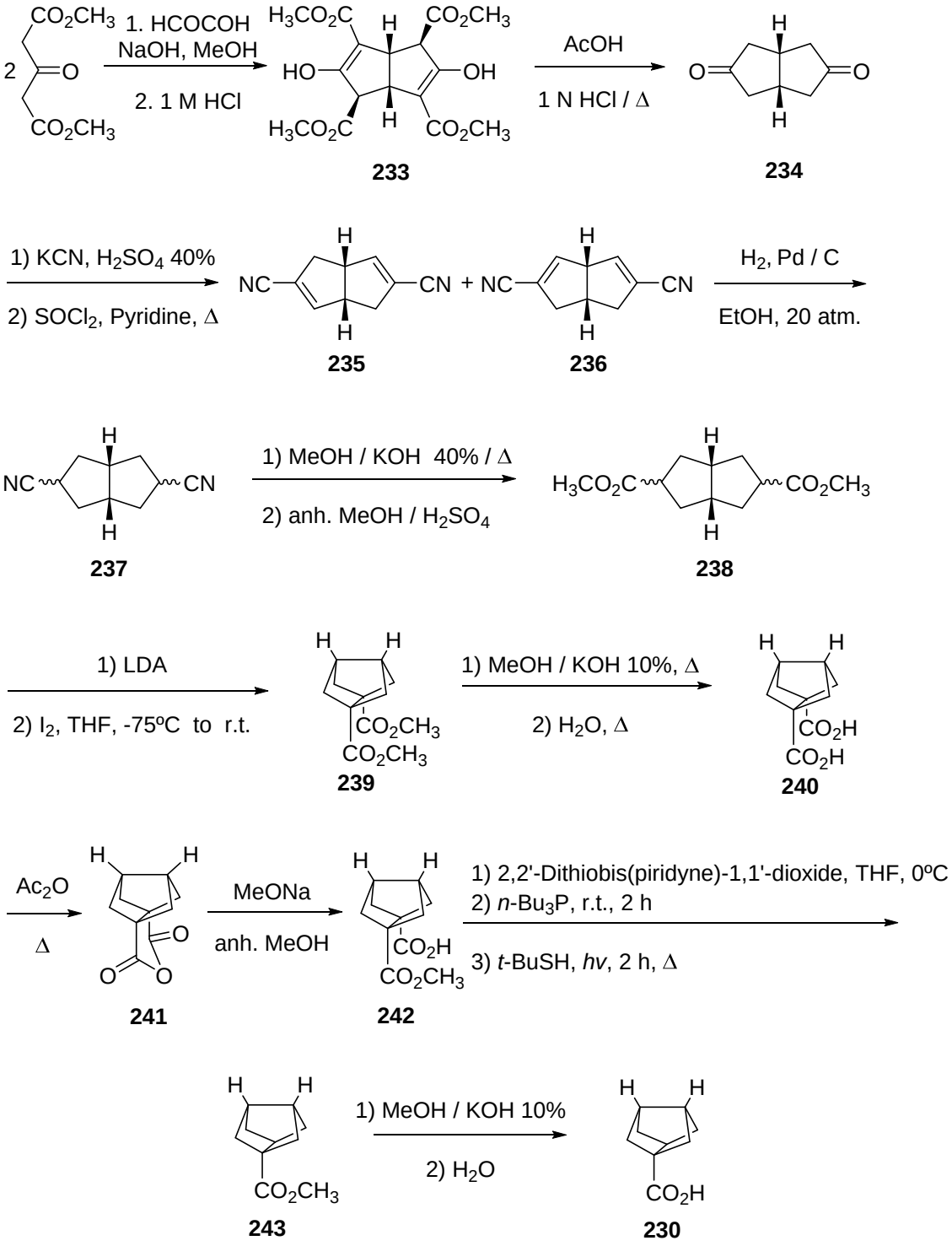



**Scheme 71.** Diester **226** as a synthetic precursor of monoacid **229.**




**Scheme 72.** Planned synthesis of amines **231·HCl** and **232·HCl.**

In order to achieve these aims the diester **239** was first synthesized. Similarly to the synthesis of **221** (Schemes 66 and 67), diketone **234** is readily available using the Weiss reaction, just changing the 2,3-butanedione used for **221** by glyoxal in a 40% wt solution.[182] This reaction was followed by a hydrolysis in acid media and a decarboxylation which gave diketone **234** in a 33% overall yield.[183] The treatment of diketone **234** with potassium cyanide in aqueous media, keeping a neutral pH by a slow addition of a 40% $H_2SO_4$ solution, gave a stereoisomeric mixture of *bis*-cyanohydrins which after dehydration with $SOCl_2$ in refluxing pyridine yielded the regioisomeric mixture of dinitriles **235** and **236** in a 55% overall yield.




**Scheme 73.** Obtention of the monoacid **230**.

The catalytic hydrogenation of the dinitrile mixture at 20 atm of pressure, using Pd/C (10% mol) as the catalyst in ethanol, gave a stereoisomeric mixture of the three possible products in quantitative yield. The hydrolysis of the aforementioned mixture with KOH/water followed by a Fischer esterification reaction gave the stereoisomeric mixture of diesters **238** with an overall yield of 33%.

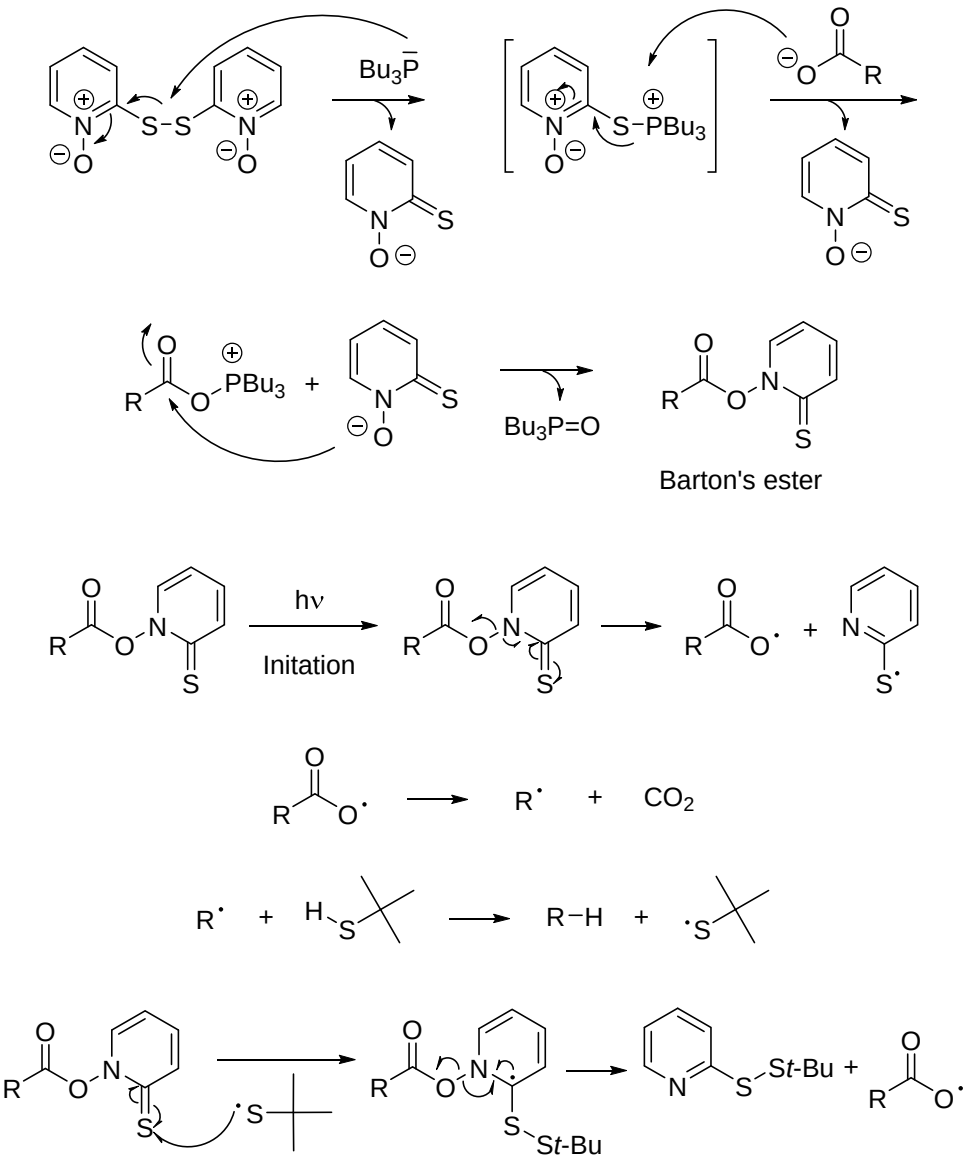
The construction of the tricyclic scaffold was carried out starting from the stereoisomeric mixture of diesters by oxidative coupling with iodine of the corresponding lithium *bis*-enolates. After a column chromatography the diester **239** was obtained in 43% yield. As previously reported by our group, the basic hydrolysis of this diester gave diacid **240** in 87% yield.[185]

In order to remove one of the carboxylic groups of **240**, firstly they had to be differentiated. To do so, **240** was treated with acetic anhydride at reflux for one hour and the obtained anhydride **241** was subsequently treated with sodium methoxide in anhydrous methanol to give the hemiester **242** as a white solid. The conversion of hemiester **242** into monoester **243** revolves around the synthesis of an intermediate "Barton's ester" or thiohydroxamic ester followed by its thermally- or photochemically-induced homolysis in the presence of a suitable hydrogen atom donor such as *t*-butylthiol or tributyltin hydride (Scheme 74).[186]

In our hands, the synthesis of the Barton's ester was carried out by reaction of **242** with 2,2'-dithiobis(pyridine)-1,1'-dioxide and *n*-tributylphosphine in anhydrous THF, at low temperature and in a round-bottomed flask protected from the light. The putative mechanism of this reaction is showed in Scheme 74. Without isolation, the lemon-yellow Barton's ester was reacted with *t*-butylthiol in a radical reaction triggered by irradiation with two 100 W wolfram lamps. After the work-up, the crude monoester **243** was isolated and, without any purification because of its volatility, its hydrolysis using the usual conditions was carried out. This reaction allowed us to obtain the monoacid **230** with a 36% yield after two synthetic steps.

After obtaining enough quantities of the common precursor of amines **231·HCl** and **232·HCl**, we carried on with the synthesis of them.

[185] Camps, P.; Luque, F. J.; Orozco, M.; Pérez, F.; Vázquez, S. *Tetrahedron Lett.* **1996**, *37*, 8605-8608.
[186] a) Barton, D. H. R.; Crich, D.; Motherwell, W. B. *Tetrahedron* **1985**, *41*, 3901-3924. b) Barton, D. H. R.; Samadi, M. *Tetrahedron* **1992**, *48*, 7083-7090.

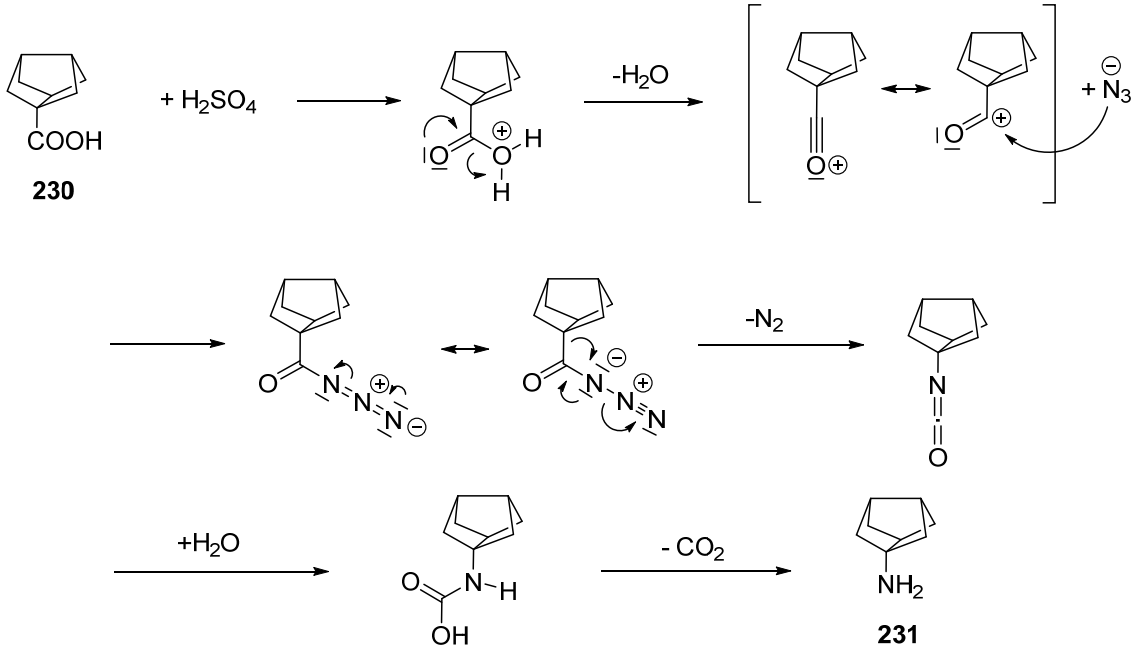



**Scheme 74.** Mechanism of the Barton's ester formation and decarboxylation.

To do the conversion of the acid **230** in the amine **231·HCl** we decided to apply the Schmidt rearrangement, based on the favourable results that our research group obtained previously in related compounds. This reaction allows, in only one step, to access to the amine functionality from a carboxylic acid with the loss of a carbon atom. This transformation consists in, firstly, the acid-base reaction of the carboxylic acid and concentrated sulphuric acid to protonate the OH moiety of the carboxylic acid, fact that triggers the loss of water to form an acylium ion. This cation reacts with the azide to generate an acyl azide that undergoes the rearrangement of the carbon atom bonded to the carbonyl and subsequent loss of nitrogen gas and generation of an isocyanate. The isocyanate will be attacked by water to give a carbamate which after hydrolysis will release the amine (Scheme 75).[187] We carried on this

[187] Kürti, L.; Czakó, B. "Strategic Applications of Named Reactions in Organic Sythesis" Elsevier: 2005, pp 396-397.

reaction using the classical conditions of the Schimdt rearrangement, that is, the addition of $NaN_3$ onto a solution of the acid in chloroform and sulphuric acid at reflux.[188]

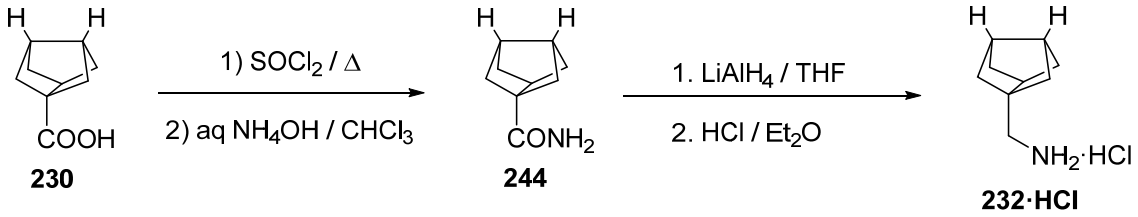



**Scheme 75.** Mechanism of the Schmidt's rearrangement.

This reaction gave amine **231·HCl** in 47 % yield, the low yield is associated with the fact that amine **231** is highly volatile, property that makes it difficult to isolate. This bisnoradamantane analogue corresponds to a double ring-contracted amantadine analogue with the nitrogen directly bonded to the polycyclic scaffold.

To synthesize the analogue that possesses the amine moiety separated from the polycyclic cage by a methylene bridge, firstly, we converted the monoacid **230** in the corresponding amide through a reaction with thionyl chloride followed by the reaction with an excess of ammonium hydroxide in chloroform (Scheme 76). The amide **244** was obtained in 99% yield as an easy to handle, white solid. Then, reduction of the amide with $LiAlH_4$ in anhydrous THF gave amine **232·HCl** in 76% yield. This amine was isolated directly as its hydrochloride salt due to its volatility. This compound was synthesized with the aim of enlarge the structure of the bisnoradamantane cage trying to place the amine deeper in the M2 channel to establish new interactions with the surrounding aminoacids or water molecules of the channel.

[188] Jawdosiuk, M.; Kovacic, P. *Synth. Commun.* **1983**, *13*, 53-62.




**Scheme 76**. Synthesis of amide **232·HCl**.

Unfortunately, we did not synthesize any derivative of **231·HCl** and **232·HCl** because we arrived at the end of the synthetic route with a very low amount of each compound. In case that these amines displayed activity against the S31N mutant channel, more derivatives would be synthesized in the future.

### 5.4 Synthesis of 3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecanes.

We have previously seen in chapter 2 that the inclusion of the nitrogen atom in a pyrrolidine ring led, in some examples, to an enhacement of the antiviral activity. With these precedents in mind, we decided to synthesize 3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecanes of general structure **XIII** (Figure 60).

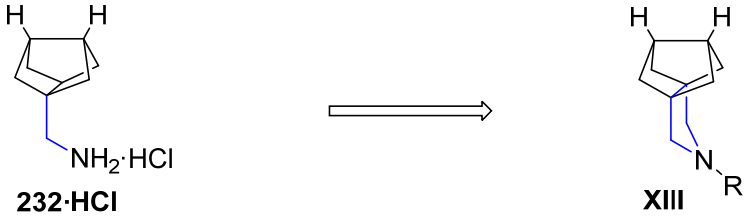
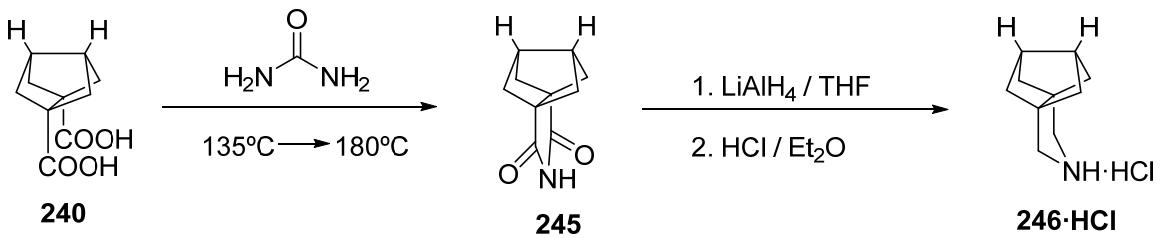


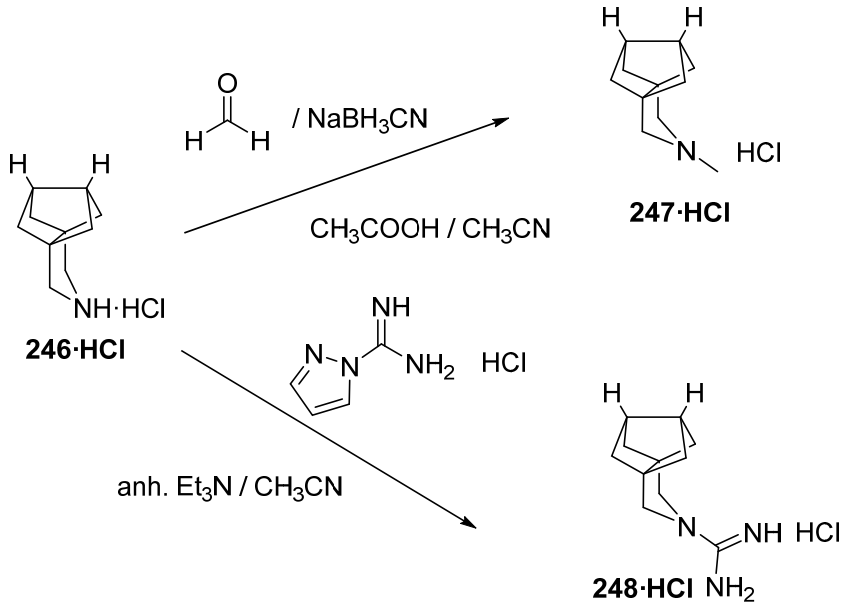

**Figure 60.** New bisnoradamantane analogues.

The synthesis of the parent 3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecane started from the diacid **240** (Scheme 77). The reaction of **240** with neat urea at its melting temperature (180 °C) for 30 min furnished the imide **245** in 73% yield. The subsequent reduction of imide **236** with $LiAlH_4$ in anhydrous THF heated at reflux for 3 days gave amine **246** in 75% yield.




**Scheme 77.** Preparation of amine **246·HCl** from **240**.

When we got amine **246·HCl** we planned to prepare some derivatives with the purpose of modulating its biological activity. We synthesized the methyl derivative **247·HCl** in 70% yield by reductive alkylation with aqueous formaldehyde and $NaBH_3CN$ as the reductive agent. Finally, as in previous guanidines described in this Thesis, we synthesized **248·HCl** in 96% yield using 1*H*-pyrazole-1-carboxamidine, $Et_3N$ and acetonitrile (Scheme 78).




**Scheme 78.** Synthesized derivatives of amine **246·HCl**.

**5.5 Pharmacological evaluation.**

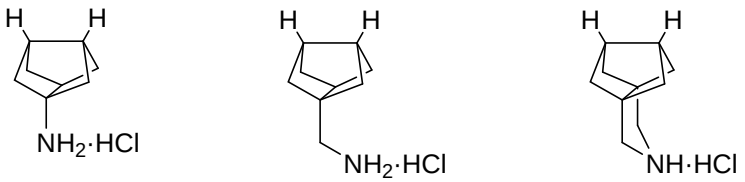
Once we have finished the synthesis and characterization of all these compounds, we sent them to the *Rega Institute for Medical Research* to be tested as antinfluenza drugs, and to the Northwestern University to be tested as M2 channel blockers.

At the *Rega Institute*, they were tested against a broad panel of viruses, but as expected, they resulted to be inactive against all of them but Influenza A virus. Table 15 gather all the data collected in these assays including two different strains of Influenza A virus: A/PR/8/34 and A/HK/7/87 and an Influenza B strain: B/HK/5/72. As shown in Table 15, several of our bisnoradamantane amines were endowed with an antiviral activity similar to that of amantadine against the H3N2 influenza A strain, the more potent compound being the primary amine **232·HCl**. Within this family, the inclusion of the nitrogen atom in a pyrrolidine ring did not increase the antiviral activity (compare **232·HCl** vs **246·HCl**). Also, in going from a secondary to a tertiary amine or from the amine to the guanidine, did not increase the activity. Taking into account the activity of **232·HCl** ($EC_{50}$= 2.7 μM) and that of its dimethyl analog ($EC_{50}$= 6.5 μM) and the lack of antiviral activity for the dimethyl derivatives **219, 227** and **228**, it seems that the presence of the methyl groups in the bisnoradamantane scaffold is negative. In fact, memantine, a 3,5-dimethylderivative of amantadine, clinically approved for Alzheimer's disease, is inactive as antiviral.

**Table 15.** Antiinfluenza activity of the bisnoradamantane amines in MCDK cells.

| Compound | Antiviral activity $EC_{50}$[a] (μM) | | | | | | Toxicity ($MCC^b$) |
|---|---|---|---|---|---|---|---|
| | Influenza A/H1N1 | | Influenza A/H3N2 | | Influenza B | | |
| | CPE | MTS | CPE | MTS | CPE | MTS | |
| **219·HCl** | >100 | >100 | >100 | >100 | > 100 | > 100 | > 100 |
| **227·HCl** | >100 | >100 | >100 | >100 | > 100 | > 100 | ≥20 |
| **228·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 4 |
| **231·HCl** | >100 | 37 | 52 | 22 | >100 | >100 | >100 |
| **232·HCl** | 63 | 52 | 2.7 | 2.2 | >100 | >100 | >100 |
| **246·HCl** | >100 | >100 | 7.9 | 7.2 | >100 | >100 | 100 |
| **247·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **248·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 8.5 |
| ***Amantadine*** | 72.5±38.9 | 57.5±25.2 | 2.2±0.3 | 1.7±0.8 | > 100 | > 100 | > 500 |
| ***Rimantadine*** | 33.3±11.4 | 24.1±10.2 | 0.25±0.21 | 0.25±0.21 | > 100 | > 100 | > 500 |

[a] $EC_{50}$: concentration producing 50% antiviral effect, as determined by microscopy of the virus-induced CPE.
[b] MCC: minimum cytotoxic concentration, i.e. concentrations causing minimal changes in cell morphology.




**231·HCl** **232·HCl** **246·HCl**

**Figure 61.** Active compounds against the tested A/H3N2 strain.

Since these compounds were initially designed as M2 inhibitors, Prof. Larry Pinto's group tested them directly on the M2 channel. Table 16 collects the results of these studies. We can clearly see that, with the single exception of **231·HCl**, all the compounds are blockers of the wt M2 channel, being the guanidine **248·HCl** the most active one ($IC_{50}$= 1.05 μM). The lack of antiviral activity of this guanidine in the CPE experiments may reflect its cytotoxicity for the MDCK cells (see Table 15).

Although our aim in designing these compounds was to target the S31N mutant M2 channel, all the compounds were inactive against it. It seems clear that although the S31N mutant M2 channel is narrower than the wt, in order to effectively block it, a reduction of the size of the putative blocker is not enough.

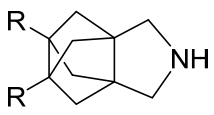
**Table 16.** Activity of bisnoradamantane amine in M2 channel expressed in Oocytes of *Xenopus laevis*.

| Compound | WT A/M2 channel | | V27A A/M2 channel | | S31N A/M2 channel | |
|---|---|---|---|---|---|---|
| | % inh. | $IC_{50}$ (μM) | % inh. | $IC_{50}$ (μM) | % inh. | $IC_{50}$(μM) |
| **219·HCl** | 93.8±0.8 | 9.7 | 26.0±0.3 | ND | 0 | ND |
| **227·HCl** | 87.9±0.6 | 16.3 | 12.3±1.6 | ND | 0 | ND |
| **228·HCl** | 92.4±0.6 | 6.1 | 84.2±1.0 | 11.4 | 1.0±1.0 | ND |
| **231·HCl** | 55.8±2.3 | ND | 0 | ND | 0 | ND |
| **232·HCl** | 92.0±0.8 | 3.0 | 0 | ND | 0 | ND |
| **246·HCl** | 93.9±0.2 | 11.7 | 5.6±1.5 | ND | 6.2±0.7 | ND |
| **247·HCl** | 88.3±0.2 | 25.2 | 0 | ND | 0 | ND |
| **248·HCl** | 95.7±1.5 | 1.1 | 0 | ND | 0 | ND |
| **Amantadine** | 91.0±2.1 | 16.0±1.2 | 10.8±2.0 | ND | 35.6±1.5 | 199.9±13.5 |

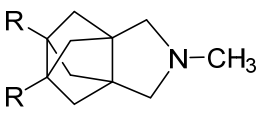
% inh.: inhibition by 100 μM of the compound for 2 min (%). ND: not determined.

Values shown are the mean ±SEM of 2-3 determinations.

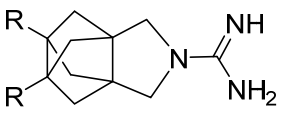
Taking into account that the bisnoradamantane derivatives were initially conceived against the S31N mutant M2 channel, we were really disappointed when we first saw the results from Pinto's laboratory. However, we were surprised for the dual action of compound **228·HCl** which inhibited both the wt and the V27A mutant M2 channels with a very good activity in both cases (wt, $EC_{50}$= 6.10 μM; V27A, $EC_{50}$= 11.4 μM). In fact, **228·HCl** is more active against both channel than amantadine is against the wt M2 channel. It must be pointed out that these results are noteworthy for two reasons. First, when we received this data, **228·HCl** was the more potent compound ever found against the V27A mutant M2 channel. More interestingly, we found a plausible trend to follow when designing newer V27A mutant M2 channel inhibitors, that is, if we compare **246**, **247** and **248** with their corresponding dimethyl analogues, **219**, **227** and **228**, without exception, the dimethyl derivatives were more potent against the V27A channel (Figure 62). Taking into account that these molecules are located along the M2 channel, with the nitrogen atom pointing towards the histidines, it seems that the longer the molecule the better the activity.




R = H, **246**; %inh = 6%
R = $CH_3$, **219**; %inh = 26%



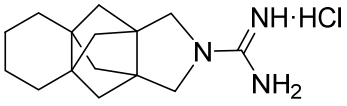

R = H, **247**; %inh = 0%
R = $CH_3$, **227**; %inh = 12%




R = H, **248**; %inh = 0%
R = $CH_3$, **228**; %inh = 84%

**Figure 62.** Structures and activities (% inh. 100 μM) of several pyrrolidines against the V27A mutant M2 channel.

With this idea in mind, during the writing of this Thesis, Matías Rey-Carrizo, working in our group, has recently synthesized even larger compounds, and one of them, guanidine **249·HCl**, has shown to be a submicromolar inhibitor of the V27A ($EC_{50}$= 0.29 μM) channel, while keeping low micromolar activity against the wt M2 channel ($EC_{50}$= 3.0 μM) (Figure 63).[189]




**249·HCl**

**Figure 63.** Compound synthesized by Matías Rey.

[189] In July, 2011, DeGrado and coworkers published the first submicrolar inhibitor of the V27A mutant M2 channel ($EC_{50}$= 0.30 μM). However, their compound, **91**, is less potent against the wt channel ($EC_{50}$= 18.7 μM). See Figure 38 and references 110 and 135.

# 6. Design of new inhibitors for the V27A mutant M2 channel.

## 6.1. Synthesis of benzopolycyclic cage amines.

As observed in the previous chapter, the best way to target the V27A mutant M2 channel seems to be the synthesis of larger molecules than Amt. In order to make larger compounds, the first idea we came up with was to introduce a benzene ring at the "top" of the polycyclic cage (Figure 64). Following this idea, we prepared a small series of compounds having the general structure **XIV**.

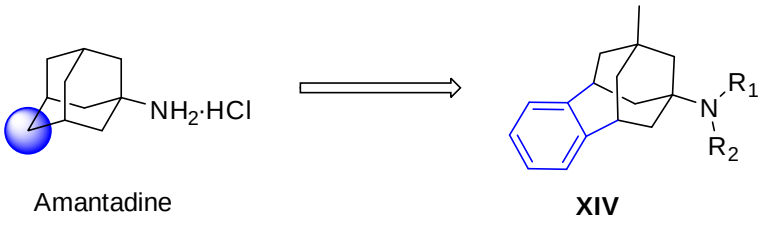



**Figure 64.** New benzopolycyclic amines.

The idea of making larger molecules match with the fact that the V27A mutant M2 channel of Influenza A virus have a considerable wider pore at the gate of the channel, so a bigger molecule should fit better in the binding site (Figure 65).[109b,109c,110]

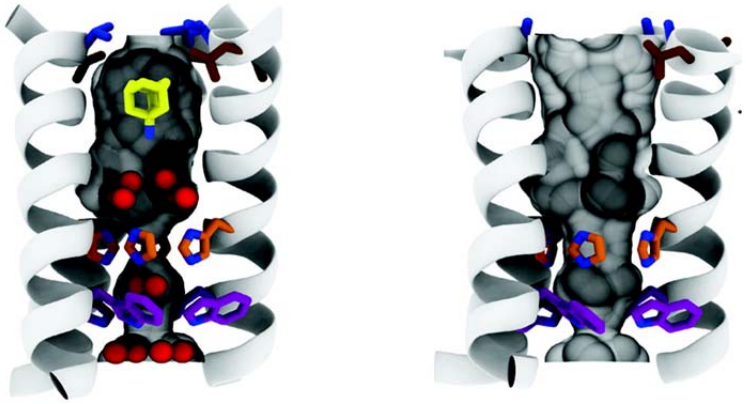


**Figure 65.** On the left, wt M2 channel with amantadine in its binding site. On the right, V27A mutant M2 channel.

At first sight the idea of adding an aromatic ring may be a bit risky but, we must take into account that recently several aromatic inhibitors of M2 channel have been published (see Figures 36 and 39, compounds **88**, **92a** and **92b**).[126,133,136]

In order to synthesize the amines of general structure **XIV** we decided to take advantage of our expertise in the Ritter Reaction (see Chapter 1, Part II). Diene **252** was first described by Bishop et al. by a double Wittig reaction from diketone **250**,[190] in turn easily available from the Weiss reaction of *o*-phthaldialdehyde with two equivalents of dimethyl-1,3-acetonedicarboxylate.[191]

Worthy of note, the Weiss reaction between *o*-phthaldialdehyde and two equivalents of dimethyl-1,3-acetonedicarboxylate led, after the hydrolysis and decarboxylation of the intermediate tetraester, to a mixture of diketone **250** along with its hydrate **251**, in 62% overall yield (Scheme 79). To obtain the pure diketone **250**, the crude of this reaction was sublimated at 160 $^{o}$C / 0.5 Torr to remove the water from the hydrate and release pure **250**.[192]

Although the synthesis of **252** from **250** was already described in the literature,[190] there were no much details about the experimental process and, in fact, the synthesis of **252** was the most troublesome reaction of this synthetic route which needed some optimization to get good yields and good selectivity of the diene **252** over the enone **255** (Scheme 80).

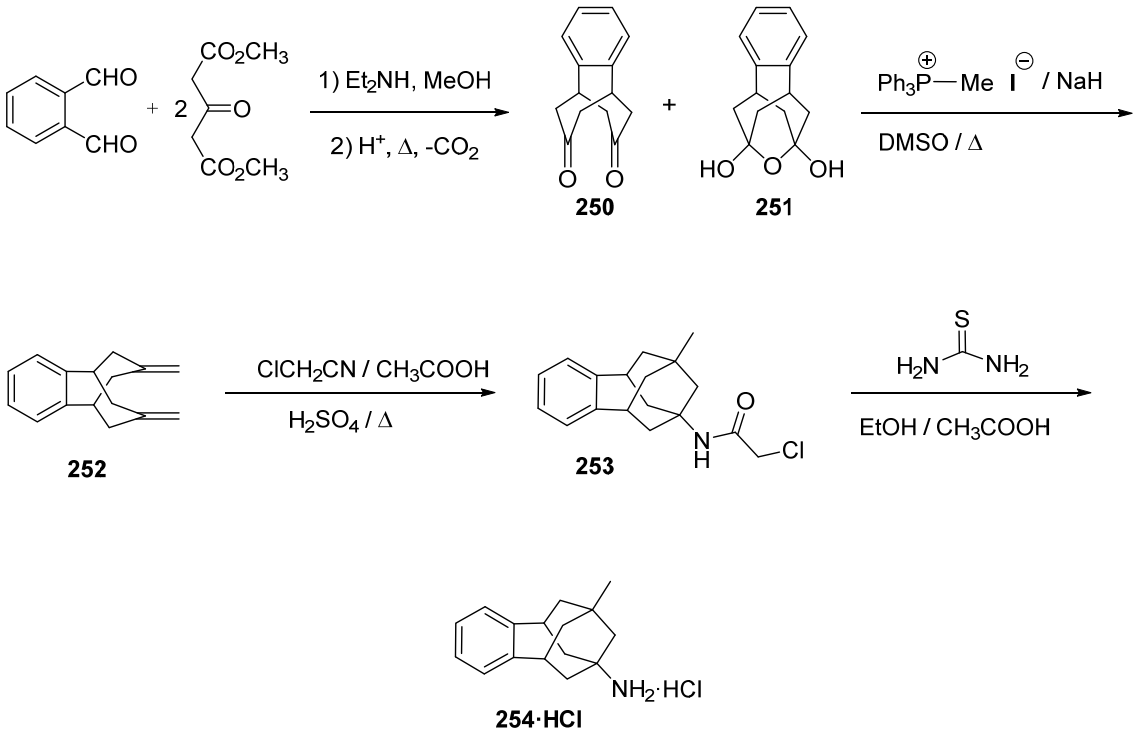
The Wittig reaction was carried out preparing a suspension of NaH in anhydrous DMSO and heating 45 min at 70 $^{o}$C to form the conjugated base of the DMSO.[193] A solution of methyltriphenylphosphonium iodide and diketone **250** in anhydrous DMSO was added to the mixture and the reaction was heated at 90 $^{o}$C. We needed to modify the equivalents of the sodium hydride and methyltriphenylphosphonium iodide and optimize the reaction time and the dilution in order to get good yields of the desired diene.

[190] a) Bishop, R.; Landers, A. E. *Aust. J. Chem.* **1979**, *32*, 2675–2679. b) Amini; Bishop, R. *Aust. J. Chem.* **1983**, *36*, 2465–2472. c) Amini; Bishop, R.; Burgess, G.; Craig, D. C.; Dance, I. G.; Scudder, M. L. *Aust. J. Chem.* **1989**, *42*, 1919-1928.

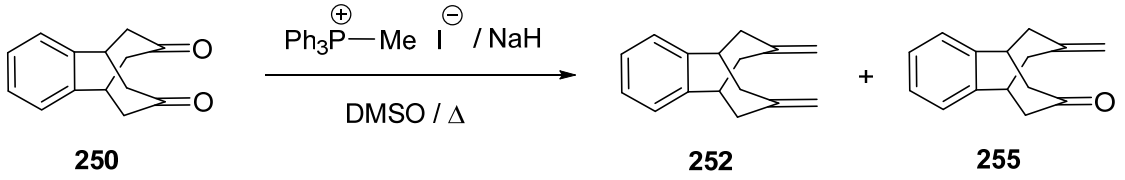
[191] a) Föhlisch, B.; Widmann, E.; Schupp, E. *Tetrahedron Lett.* **1969**, 2355-2358. b) Föhlisch, B.; Dukek, U.; Graeble, I.; Novotny, B.; Schupp, E.; Schwaiger, G.; Widmann, E. *Liebigs Ann. Chem.* **1973**, 1839-1850.

[192] Alternatively, refluxing the mixture in toluene overnight in a Dean-Stark system also led to pure **250**.

[193] a) Greenwald R.; Chaykovsky M.; Corey E. J. *J. Org. Chem*. **1963**, *28*, 1128-1129. b) Dehmlow E. V.; Gröning C. *J. Chem. Res.*, **1992**, 108-109.




**Scheme 79**. Synthetic route for obtaining amine **254·HCl.**



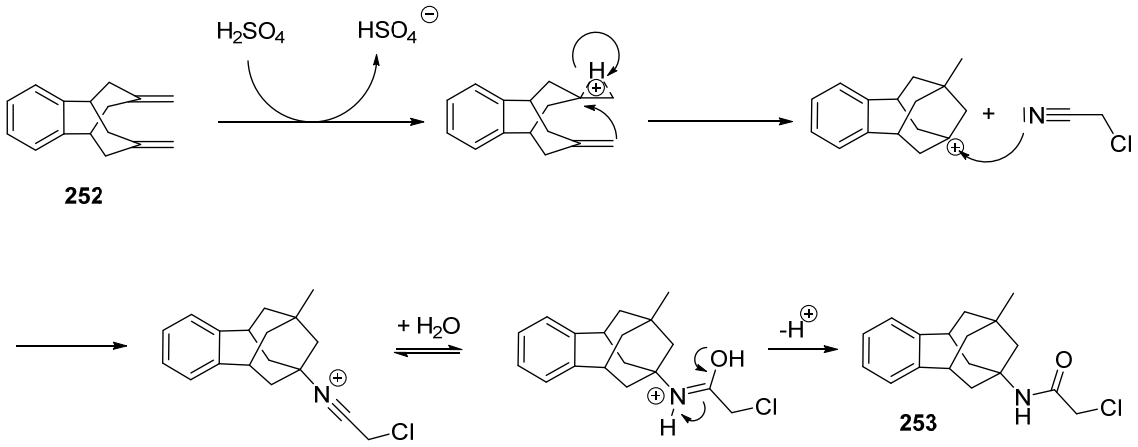

**Scheme 80.** Wittig reaction on compound **250**.

Table 17 shows all the attempts to get compound **252**, the optimal conditions resulted to be the use of 8 equivalents of NaH and methyltriphenylphosphonium iodide in a dilution of 1:40 and heating the reaction at 90 $^{o}$C overnight, to obtain pure diene **252** in 72% yield.

**Table 17.** Optimization of the Wittig reaction to obtain compound **252**.

| Entry | 250 (eq.) | NaH (eq.) | $Ph_3MeP^+ I^-$ (eq.) | Dilution | Reaction time (h) | 255:252 |
|---|---|---|---|---|---|---|
| **1** | 1 | 2.8 | 2.8 | 1:150 | 6 | No prod |
| **2** | 1 | 2.4 | 2.2 | 1:70 | 18 | No prod |
| **3** | 1 | 2.7 | 2.7 | 1:50 | 5 | 100:0 |
| **4** | 1 | 2.7 | 2.7 | 1:50 | 18 | 100:0 |
| **5** | 1 | 2.7 | 2.7 | 1:22 | 2 | 88:12 |
| **6** | 1 | 10.7 | 2.7 | 1:38 | 18 | 85:15 |
| **7** | 1 | 8 | 8 | 1:40 | 18 | 0:100 |

Once we have enough quantities of compound **252**, we planned to do a transannular Ritter reaction in order to close the polycyclic structure. This kind of reaction is possible thanks to the transannular interaction through the space between the two π orbitals of the double bounds.[194] Thus, the Ritter reaction may start with the interaction of a proton with one of the double bonds making it electrophile, then, the other will attack to generate a more stable tertiary carbocation. The carbocation will react with chloroacetonitrile to form the nitrilium ion which, after the addition of water, will be converted into chloroacetamide **253** (Scheme 81).[195]




**Scheme 81.** Mechanism of the transannular Ritter reaction.

As in our previous work with the 2,2,-dialkylamantadines (see Chapter 1 part II), the deprotection of the chloroacetamide **253** was carried out with thiourea, a catalytic amount of acetic acid and ethanol as solvent to obtain amine **254·HCl** in 44% yield after crystallization with 2-propanol.

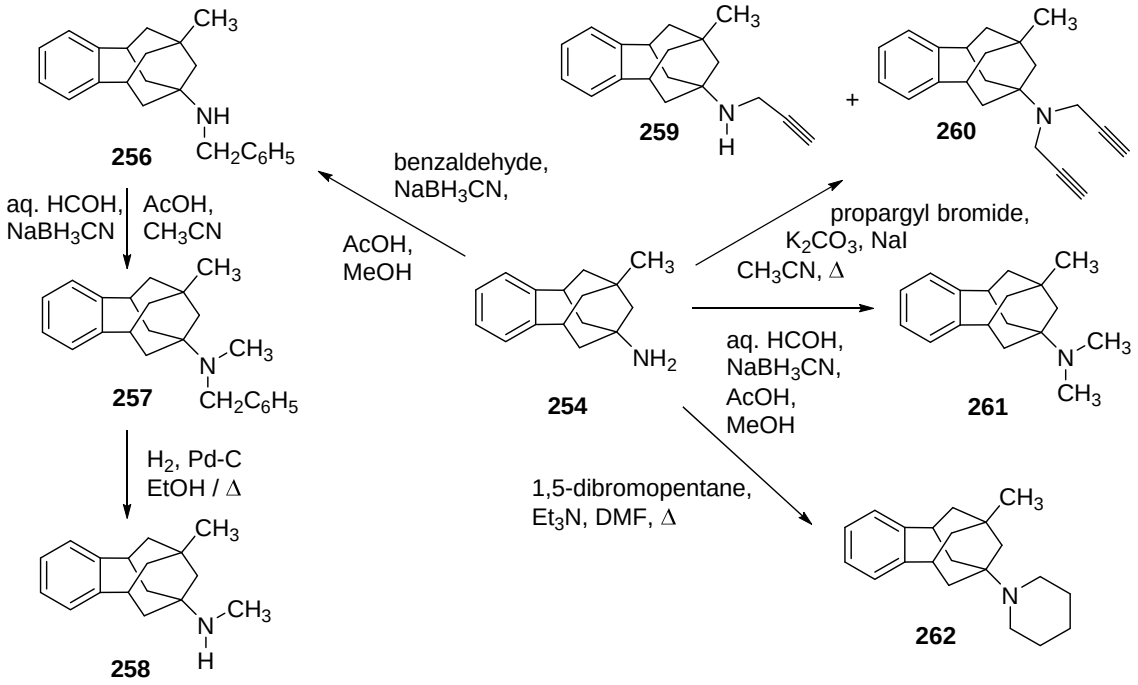
Then, some derivatives of amine **254·HCl** were prepared using classical methods in amine chemistry, mainly reductive alkylations (Scheme 82). Reductive alkylation of amine **254·HCl** with benzaldehyde gave amine **256·HCl** in 68% yield (brsm). Then, reaction with aqueous formaldehyde yielded compound **257·HCl** in 55% yield. Hydrogenolysis of **257·HCl** gave **258·HCl** in quantitative yield. Analogously, the reductive alkylation of amine **254·HCl** with an excess of aqueous formaldehyde gave amine **261·HCl** in 75% yield.

On the other hand, alkylation of amine **254·HCl** with propargyl bromide, sodium iodide and potassium carbonate in acetonitrile at reflux yielded a mixture of monoalkylated and dialkylated amines which were separated by column chromatography. Amine **259·HCl** was

[194] a) Ishiyama, J.; Senda, Y.; Imaizumi, S. *Chem. Lett.* **1983**, 771-774. b) Chow, T. J.; Li, L. *Tetrahedron* **1999**, *55*, 6067-6074. c) Bishop, R. *Aust. J. Chem.* **1984**, *37*, 319-325. d) Olah, G. A.; Krishnamurti, R.; Prakash S. *Synthesis* **1990**, 646-648.

[195] The Ritter reaction of diene **252** with acetonitrile had been described in reference 190c. However, in our hands, the hydrolysis of this acetamide with aq. HCl gave amine **254** in low yield. Only under very drastic basic conditions (NaOH, diethyleneglycol at 210 °C for 24 hours) **254** was obtained in high yield (89%).

obtained in 17% and amine **260·HCl** in 48% yield. Finally, amine **254·HCl** was reacted with 1,5-dibromopentane and $Et_3N$, in DMF at 60 $^oC$ to obtain piperidine **262·HCl** in 35% yield.




**Scheme 82.** Synthesized derivatives of amine **254·HCl.**

### 6.2. Pharmacological evaluation of 6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5*H*-benzocyclononen-7-amine, 254·HCl and its derivatives.

These compounds were evaluated for antiviral activity by the group of Prof. Lieve Naesens in the *Rega Institute for Medical Research*. They carried out CPE assays in MCDK cells infected with different strains of Inluenza A and B. Table 18 collects all the information drawn from these studies.

Unfortunately, all the compounds proved to be inactive against the A/H3N2 subtype and influenza B virus and only one compound, the benzyl derivative **256·HCl**, displayed a reasonable activity against the A/H1N1 subtype (antiviral $EC_{50}$ = 10 μM). Taking into account these results it seems obvious that the inclusion of an aromatic ring directly fused to the adamantane nucleous is very deleterious for the antiviral activity.

Despite the discouraging results obtained in the CPE assays, we wanted to test selected derivatives of this family of compounds in the patch clamp assay to see if our hypothesis of introducing the benzene ring in the polycyclic scaffold resulted in an improvement of the inhibition of the V27A mutant M2 channel (Table 19). However, we were able to test only three compounds, due to their poor solubility in the solvent system employed to carry out this assay. No compound resulted active against the M2 channel of Influenza A virus, independently that if it was wt of mutant.

**Table 18.** Antiinfluenza activity of the benzopolycyclic amines in MCDK cells.

| Compound | Antiviral activity $EC_{50}$[a] (µM) | | | | | | Toxicity (MCC[b]) |
|---|---|---|---|---|---|---|---|
| | Influenza A/H1N1 | | Influenza A/H3N2 | | Influenza B | | |
| | CPE | MTS | CPE | MTS | CPE | MTS | |
| **254·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 100 |
| **256·HCl** | 10 | 12 | >100 | >100 | >100 | >100 | 40 |
| **257·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 150 |
| **258·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 4 |
| **259·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 20 |
| **260·HCl** | 66 | 61 | >100 | >100 | >100 | >100 | 150 |
| **261·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 150 |
| **262·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 20 |
| ***Amantadine*** | 72.5±38.9 | 57.5±25.2 | 2.2±0.3 | 1.7±0.8 | > 100 | > 100 | > 500 |
| ***Rimantadine*** | 33.3±11.4 | 24.1±10.2 | 0.25±0.21 | 0.25±0.21 | > 100 | > 100 | > 500 |

[a] $EC_{50}$: concentration producing 50% antiviral effect, as determined by microscopy of the virus-induced CPE.
[b] MCC: minimum cytotoxic concentration, i.e. concentrations causing minimal changes in cell morphology.

**Table 19.** Activity of selected benzopolycyclic amine in M2 channel expressed in Oocytes of *Xenopus laevis*.

| Compound | WT A/M2 channel | | V27A A/M2 channel | | S31N A/M2 channel | |
|---|---|---|---|---|---|---|
| | % inh. | $IC_{50}$ (µM) | % inh. | $IC_{50}$ (µM) | % inh. | $IC_{50}$(µM) |
| **254·HCl** | 13.2 ± 0.8 | ND | 0 | ND | 0 | ND |
| **256·HCl** | 0.7 ± 0.7 | ND | 0 | ND | 0 | ND |
| **257·HCl** | 5.5 ± 2.1 | ND | 1.7 ± 0.5 | ND | 0 | ND |
| **Amantadine** | 91.0±2.1 | 16.0±1.2 | 10.8±2.0 | ND | 35.6±1.5 | 199.9±13.5 |

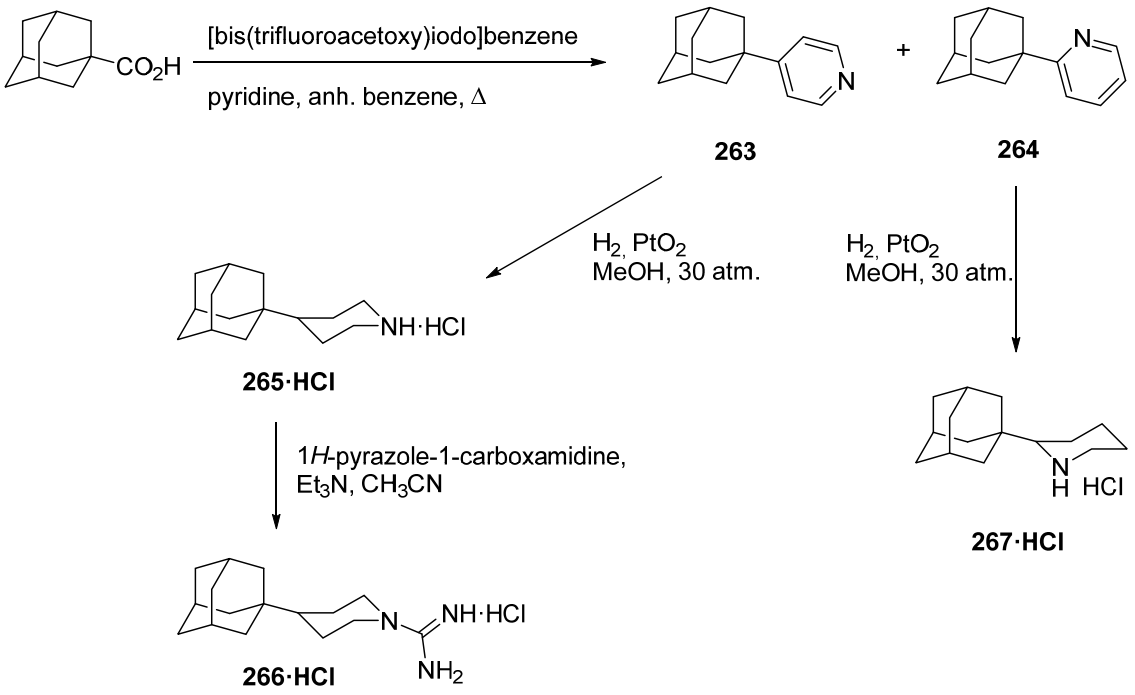
% inh.: inhibition by 100 µM of the compound for 2 min (%). ND: not determined.

Values shown are the mean ±SEM of 2-3 determinations.

### 6.3 Synthesis of (1-adamantyl)piperidines.

Although our first attempt to inhibit the V27A mutant M2 channel by making larger molecules failed, we did believe that this strategy may be the correct one if a suitable scaffold was chosen. We thought that an obvious way to proceed was to start from adamantane, as we indeed know that binds to the channel, and add an additional structure to enlarge the molecule.

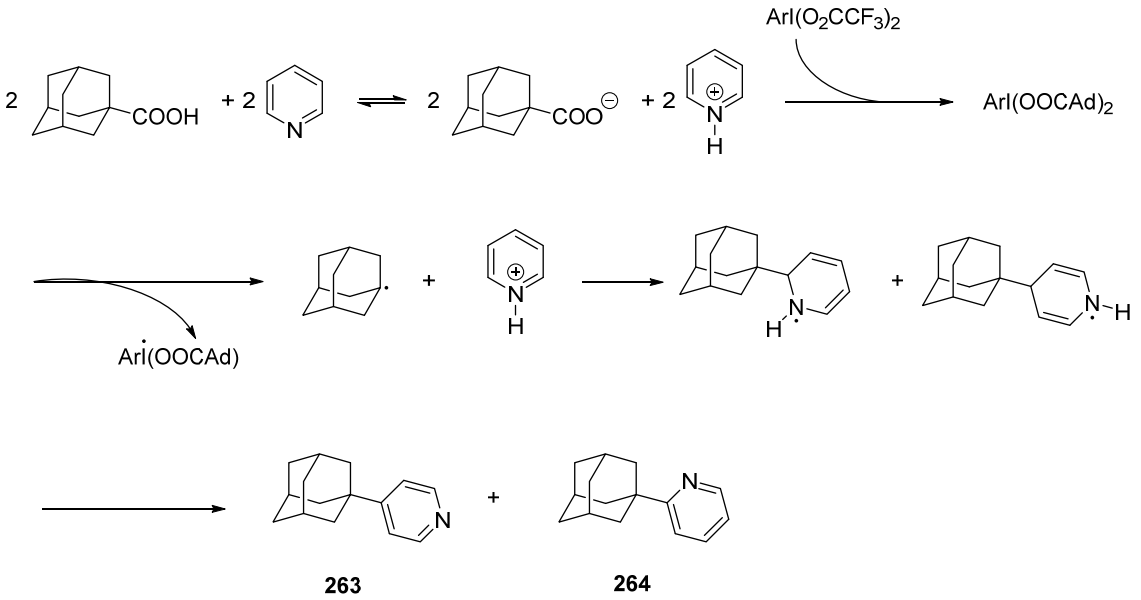
So we thought of synthesizing 4-(1-adamantyl)piperidine, **265·HCl**, and some related compounds. The synthesis of these molecules was carried out as shown in Scheme 83.




**Scheme 83.** Synthesis of 4-(1-adamantyl)piperidine **265·HCl** and related compounds **266·HCl** and **267·HCl**.

Togo and coworkers had reported the synthesis of a mixture of **263** and **264** by radical decarboxylation of 1-adamantane carboxylic acid with [bis(trifluoroacetoxy)iodo]benzene in the presence of pyridine.[196] The first step in this reaction is the acid-base exchange between 1-adamantane carboxylic acid and pyridine. Then, the [bis(trifluoroacetoxy)iodo]benzene changes anions, coordinating with the 1-adamantane carboxylate and displacing the trifluoroacetate moieties, in order to generate [bis(1-adamantanecarboxyl)iodo]benzene. This species undergoes radical decarboxylation to generate an adamantly radical. This radical adds to the pyridinium cation that finally, rearomatizes to yield the desired addition products (Scheme 84).

[196] Togo, H.; Aoki, M.; Kuramochi, M.; Yokoyama, M. *J. Chem. Soc. Perkin Trans. 1* **1993**, 2417-2427.




**Scheme 84.** Mechansim of the radical decarboxylation-addition reaction to prepare compounds **263** and **264.**

In our hands, compounds **263** and **264** were obtained in a 39% yield in a 0.8:2 ratio in favour of the *ortho* product. These two isomers were easily separated by column chromatography. Then compound **263** was hydrogenated using $PtO_2$ as catalyst during 48 hours at 30 atmospheres of pressure, which gave amine **265·HCl** in 97% yield. Amine **265·HCl** was converted into the corresponding guanidine **266·HCl** in 76% yield. Of note, this guanidine is the largest compound we synthesized in this Thesis, the length of this molecule was measured doing *ab initio* calculations, and it resulted to be 8.5 Å. Their spatial conformation was confirmed by X-Ray diffraction which shows their extended conformation (Figure 66).

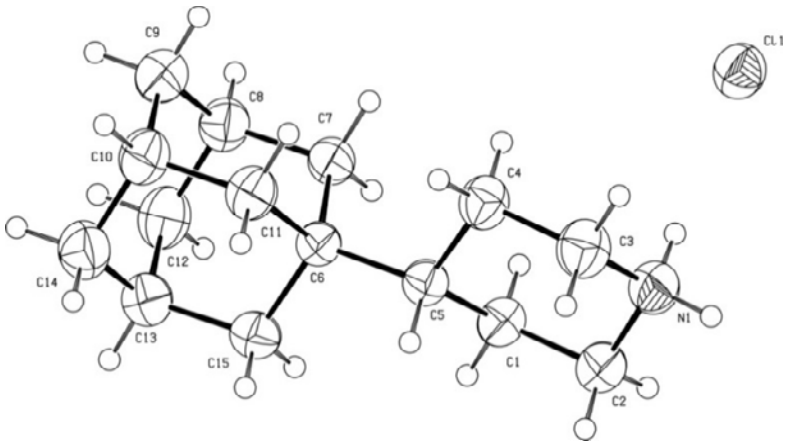



**Figure 66.** Structure (ORTEP) of compound **265·HCl** by X-Ray diffraction.

Concurrently, we hydrogenated compound **264** to obtain amine **267·HCl** in quantitative yield. However, several attempts to prepare the corresponding guanidine were unsuccessful, presumably because the 2-(1-adamantyl)piperidine is too sterically hindered to do the nucleophilic attack to 1*H*-pyrazole-1-carboxamidine.

### 6.4. Pharmacological evaluation of (1-adamantyl)piperidines.

These compounds were evaluated as antivirals by the research group of Prof. Lieve Naesens in the Rega Institute for Medical Research. As shown in Table 20, the three compounds were inactive against all the tested strains of Influenza. This result may reflect the high cytotoxicity of the compounds against MCDK cells, as their cytotoxicity may mask their true antiviral activity. In the next future, it is scheduled to do an influenza virus yield reduction assay, an experiment that allows to measure the activity of the compounds regardless of their cell toxicity.

**Table 20.** Antiinfluenza activity of the **265·HCl**, **266·HCl** and **267·HCl** in MCDK cells.

| Compound | Antiviral activity $EC_{50}$[a] (μM) | | | | | | Toxicity (MCC[b]) |
|---|---|---|---|---|---|---|---|
| | Influenza A/H1N1 | | Influenza A/H3N2 | | Influenza B | | |
| | CPE | MTS | CPE | MTS | CPE | MTS | |
| **265·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 4 |
| **266·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | ≥20 |
| **267·HCl** | >100 | >100 | >100 | >100 | >100 | >100 | 4 |
| ***Amantadine*** | 72.5±38.9 | 57.5±25.2 | 2.2±0.3 | 1.7±0.8 | > 100 | > 100 | > 500 |
| ***Rimantadine*** | 33.3±11.4 | 24.1±10.2 | 0.25±0.21 | 0.25±0.21 | > 100 | > 100 | > 500 |

[a] $EC_{50}$: concentration producing 50% antiviral effect, as determined by microscopy of the virus-induced CPE.
[b] MCC: minimum cytotoxic concentration, i.e. concentrations causing minimal changes in cell morphology.

Although these compounds showed to be inactive in the CPE assays, since they were designed to block the V27A mutant M2 channel, we wanted to check if our hypothesis was true and larger molecules would inhibit the V27A mutant M2 channel. Table 21 shows the results of the patch clamp assays carried out by Pinto's group. Compound **267·HCl** was not tested due to problems of solubility.

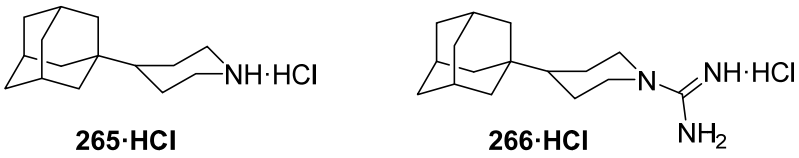
These compounds, as expected, were inactive against the S31N mutant M2 channel. However, they resulted to be dual agents, being able to block the wt M2 channel and the V27A mutant. Thus compound **265·HCl** inhibits a 91.7% when 100 μM of compound was tested, comparable to the activity of amantadine on wt channel. Compound **265·HCl** also inhibits an 87% of the activity of the V27A mutant channel when 100 μM of the compound was tested, overcoming by far the activity of amantadine.

**Table 21.** Activity of **265·HCl** and **266·HCl** in M2 channel expressed in Oocytes of *Xenopus laevis*.

| Compound | WT A/M2 channel | | V27A A/M2 channel | | S31N A/M2 channel | |
|---|---|---|---|---|---|---|
| | % inh. | $IC_{50}$ (μM) | % inh. | $IC_{50}$ (μM) | % inh. | $IC_{50}$(μM) |
| **265·HCl** | 91.7 ± 0.7 | ND | 87.3 ± 1.1 | ND | 4.6 ± 1.1 | ND |
| **266·HCl** | 94.7 ± 2.1 | 2.9 | 92.1 ± 0.5 | 4.2 | 0 | ND |
| **Amantadine** | 91.0±2.1 | 16.0±1.2 | 10.8±2.0 | ND | 35.6±1.5 | 199.9±13.5 |

% inh.: inhibition by 100 μM of the compound for 2 min (%). ND: not determined.
Values shown are the mean ±SEM of 2-3 determinations.

However, it was more impressive the activities of compound **266·HCl** which resulted to be a superior inhibitor of wt and V27A channels than **265·HCl**. While its wt activity is one of the best we have ever obtained, with a percentage of inhibition of the 94.7% and with an $IC_{50}$= 2.9 μM, an order of magnitude better than amantadine, it is highly remarkable its activity against the V27A mutant channel ($EC_{50}$= 4.2 μM).




**265·HCl** **266·HCl**

**Figure 67.** Active compounds against wt and V27A M2 channel.

Overall, compound **266·HCl** is the best M2 channel inhibitor we have synthesized during the current Thesis. This compound is endowed with an outstanding activity against wt and V27A M2 channels.

These results also allowed us to prove that our hypothesis of synthesizing larger molecules to target V27A channel was correct so, in the near future we will continue following this hypothesis to find even better inhibitors.

# PART II: CONCLUSIONS

1. In this second part of the Thesis, 120 new compounds have been synthesized and fully characterized, with its NMR data and elemental analysis. Among them, 88 have been tested as antivirals. Specifically, it has been synthesized:

a) A series of 2,2-dialkylamantadines as analogs of amantadine with more steric hindrance in the position 2 of the polycyclic cage.

b) A series of 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene and related compounds as ring rearranged analogues of amantadine, featuring the nitrogen atom in a pyrrolidine ring.

c) A series of 3-azatricyclo[3.3.3.0$^{1,5}$]undecanes, 8-azatricyclo[4.3.3.0$^{1,6}$]dodecanes and 12-azatricyclo[4.4.3.0$^{1,6}$]tridecanes, as analogues of 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-dienes with more conformational freedom.

d) A series of (tricyclo[3.3.0.0$^{3,7}$]oct-1-yl)amines, 3-azatetracyclo[5.2.1.1$^{1,5}$.0$^{5,8}$]undecanes and 7,8-dimethyl-3-azatetracyclo[5.2.1.1$^{1,5}$.0$^{5,8}$]undecanes as ring-contracted analogues of amantadine.

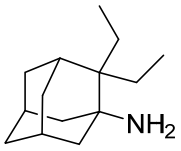
e) A series of 6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5*H*-benzocyclononen-7-amines as enlarged amantadine analogues.

f) A series of (1-adamantyl)piperidines as enlarged amantadine analogues.

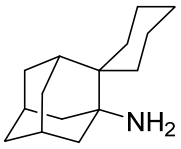
2. Regarding anti-Influenza activity some conclusions can be drawn:

a) It is possible to synthesize polycyclic amines able to target the hemagglutinin of several H1N1 influenza A strains. These compounds do not inhibit the M2 channel of influenza A virus. Three families of compounds support this statement.

   i. In the series of (2,2-dialkyl)amantadines, twelve compounds showed to be active in the CPE assays against A/PR/8/34 strain, being the best, compounds **139·HCl** and **147·HCl** with $EC_{50}$= 2.0 and 1.1 respectively.
   In the patch clamp assays did not show any significant activity against M2 channel, independently if they were Amt-sensitive or resistant.




**139·HCl**




**147·HCl**

**Figure 68.** Most active compounds in the series of (2,2-dialkyl)amantadines.

   ii. In the series of 3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-dienes, several compounds showed interesting activity against A/PR/8/34.
   The most distinguished compounds were **168·HCl**, **171·HCl**, **172·HCl**, **174·HCl** and **176·HCl** with $EC_{50}$ of 0.4, 2.0, 2.9, 1.3 and 0.5 µM respectively.

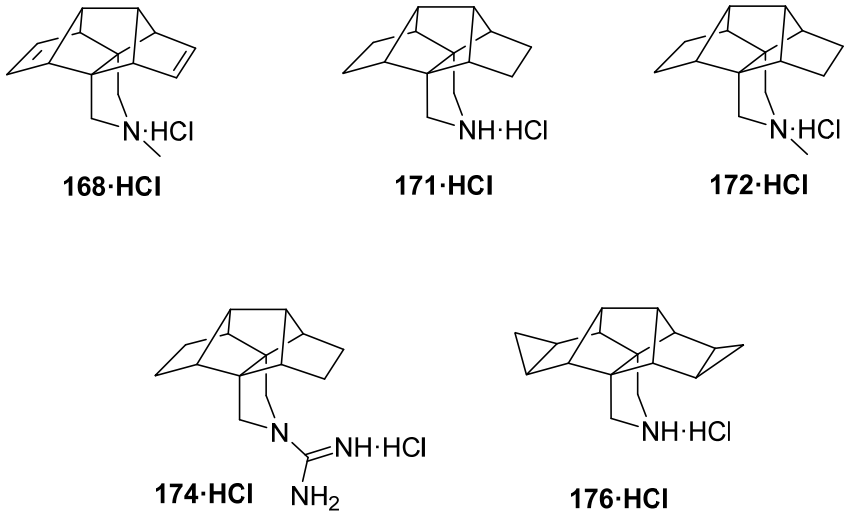



**Figure 69.** Most active compounds in (hexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadec-3-yl)amines series.

None of these compounds showed a remarkably activity in the patch clamp assay.

iii. In the series of 3-azatricyclo[3.3.3.0$^{1,5}$]undecanes, 8-azatricyclo[4.3.3.0$^{1,6}$]dodecanes and 12-azatricyclo[4.4.3.0$^{1,6}$]tridecanes, two compounds showed a nice activity against A/PR/8/34 strain, compounds **181·HCl** and **198·HCl** with $EC_{50}$= <0.80 and 2.0.

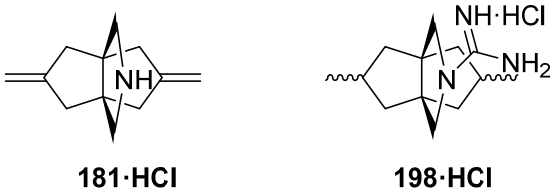


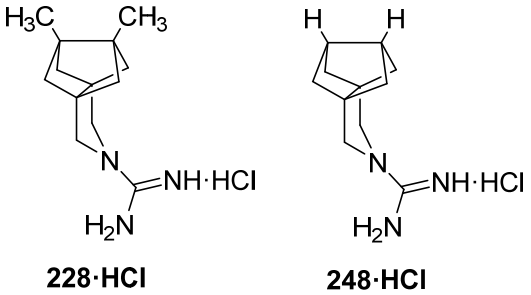

**Figure 70.** Active compounds among 3-azatricyclo[3.3.3.0$^{1,5}$]undecanes, 8-azatricyclo[4.3.3.0$^{1,6}$]dodecanes and 12-azatricyclo[4.4.3.0$^{1,6}$]tridecanes.

None of them resulted to be active in the patch clamp assay.

iv. The most active compounds among these three families were compounds **168·HCl** and **181·HCl**, with an $EC_{50}$= 0.4 μM and <0.8 μM, respectively, anaginst A/PR/8/34.

v. The research group of Prof. Lieve Naesens carried out some studies to determine the target of these compounds. This revealed that likely, they were interfering with the HA-mediated fusion process, only acting in strains carrying HA of the type H1 but not against the strains carrying the HA of the type H3.

b) Bisnoradamantane compounds, initially designed to target S31N M2 channel, did not show the expected results but revealed to possess some interesting activity against the wt and V27A M2 channel. Thus, compound **248·HCl** resulted to inhibit a 95.7% the activity of the channel when 100 μM of compound was tested and have an $EC_{50}$= 1.05 μM. The activity of compound **228·HCl** was more interesting because it resulted to be a dual agent, acting on the wt and V27A channel. Compound **228·HCl** inhibit a 92.4% the wt M2 channel with an $EC_{50}$= 6.10 μM and 84.2% the V27A channel with an $EC_{50}$= 11.4 μM.



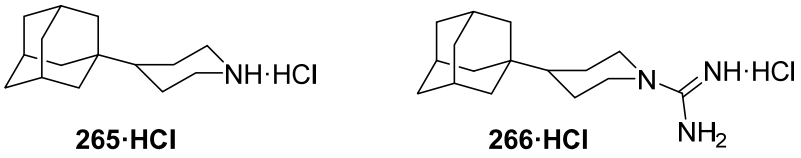

**228·HCl** **248·HCl**

**Figure 71.** Active compounds in the bisnoradamantane series.

c) Among the families of compounds designed to target V27A channel, these are the conclusions that can be drawn:

i. In the series of benzopolycyclic amines which were endowed with a phenyl ring to enlarge the molecule, none of them showed a significant activity neither in the CPE assays nor in the patch clamp assays.

ii. In the series of (1-adamantyl)piperidines we observed that they inhibit both wt and V27A M2 channels. For example, amine **265·HCl** inhibit a 91.7% the wt channel and a 87.3% the V27A channel.
Moreover, compound **266·HCl** inhibit a 94.7% the wt channel with an $EC_{50}$= 2.9 μM and a 92.1% the V27A channel with an $EC_{50}$= 4.2 μM.




**265·HCl** **266·HCl**

**Figure 72.** 4-(1-adamantyl)piperidines.

3. Taking into account that when Amantadine binds the M2 channel, the amino moiety points down towards the Histidine-37, as a general conclusion of this Thesis it must be stated that, when we synthesized polycyclic amines introducing the bulkiness in the horizontal axis of the molecule of amantadine (Figure 73, left), these compounds will target Influenza A hemagglutinin and lose their M2 channel blocking activity.
On the other hand, if we synthesize longer molecules than amantadine in the vertical axis, we will obtain molecules targeting Influenza A M2 channel (Figure 73, right), with

the largest compounds targeting the V27 mutant. Compound **266·HCl**, with an $EC_{50}$ = 4.2 µM for V27A M2 channel and an $EC_{50}$ = 2.9 µM for wt channel, is the best example.

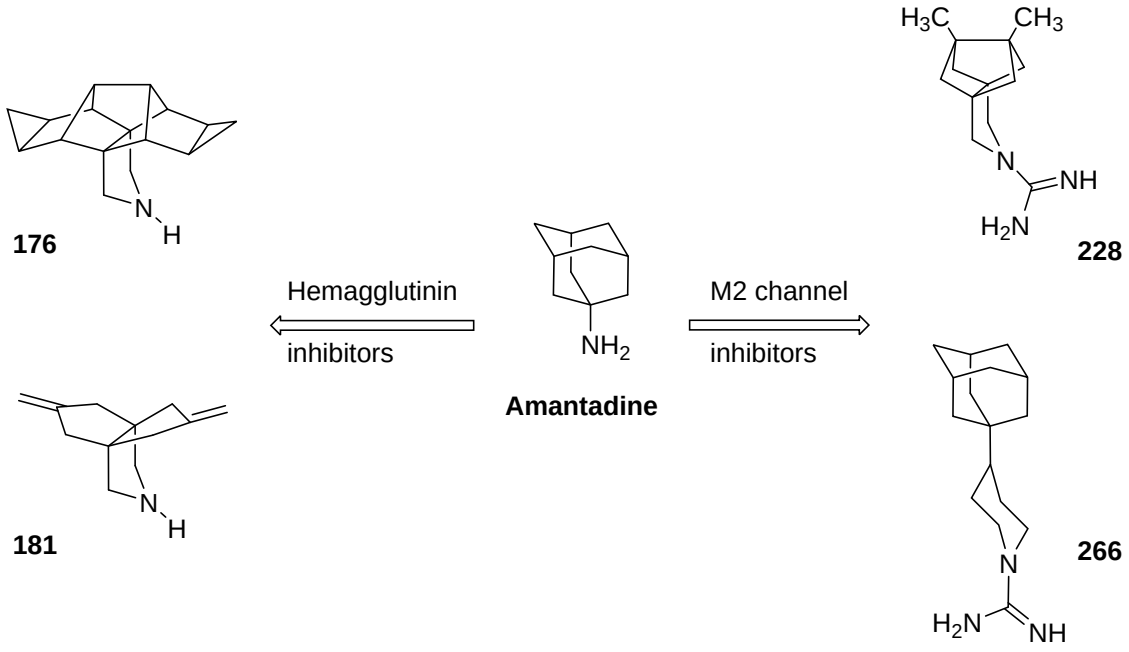



**Figure 73.** Polycyclic amines with different target.

# EXPERIMENTAL PART

## General data

Melting points were determined in open capillary tubes with a MFB 595010M Gallenkamp or a Büchi B-540 melting point apparatus.

300 MHz $^{1}$H/75.4 MHz $^{13}$C NMR spectra, 400 MHz $^{1}$H/100.6 MHz $^{13}$C NMR spectra, and 500 MHz $^{1}$H/125.7 MHz $^{13}$C NMR spectra were recorded on Varian Gemini 300, Varian Mercury 400, and Varian Inova 500 spectrometers, respectively. The chemical shifts are reported in ppm ($\delta$ scale) relative to internal tetramethylsilane, and coupling constants are reported in Hertz (Hz). Assignments given for the NMR spectra of the new compounds have been carried out on the basis of DEPT, COSY $^{1}$H/$^{1}$H (standard procedures), and COSY $^{1}$H/$^{13}$C (gHSQC and gHMBC sequences) experiments. The used abbreviations were: s, singlet; d, doublet; t, triplet; q, quadruplet; quint, quintuplet; hept, septet; m, multiplet; or combinations thereof.

IR spectra were run on FTIR Perkin-Elmer Spectrum RX I spectrophotometer using *Attenuated Total Reflectance* (ATR) Technique. Absorption values are expressed as wave-numbers ($cm^{-1}$); only significant absorption bands are given.

The GC/MS analysis was carried out in an inert Agilent Technologies 5975 gas chromatograph equipped with a DB-5MS (30 m × 25 mm) capillary column with a stationary phase of phenylmethylsilicon (5% diphenyl – 95% dimethylpolysiloxane), using the following conditions: initial temperature of 50 ºC (1 min), with a gradient of 15 ºC / min up to 300 ºC, and a temperature in the source of 230 ºC. *Solvent Delay* (SD) of 3 minutes and a pressure of 7,5 psi.

The accurate mass analysis was carried out at Unitat d'Espectrometria de Masses dels Centres Científics i Tecnològics de la Universitat de Barcelona (CCiTUB), Chemistry Faculty, using a LC/MSD-TOF spectrophotometer.

The elemental analysis was carried out in a Flash 1112 series Thermofinnigan elemental microanalyzator (A5) to determine C, H and N, and in a titroprocessor Methrom 808 to determine I and F, in Servei de Microanàlisi of IIQAB (CSIC) of Barcelona.

The X ray analysis was carried out in Serveis Científico-Tècnics of Universitat de Barcelona, using MNAR345 difractometer.

To concentrate solvents in vacuo a Büchi GKR-50 rotavapor was used.

Column chromatography was performed on silica gel 60 Å (35–70 mesh, SDS, ref 2000027).

Thin-layer chromatography was performed with aluminium-backed sheets with silica gel 60 $F_{254}$ (Merck, ref 1.05554), and spots were visualized with UV light and 1% aqueous solution of $KMnO_4$.

The analytical samples of all of the new compounds which were subjected to pharmacological evaluation possessed a purity ≥95% as evidenced by their elemental analyses.

Solvent purification was carried out following the procedures described in: Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 4th Edition, Butterworth-Heinemann: Oxford, 1996.

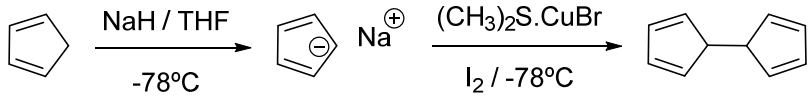
The NMR data and the synthetic procedure of all the compounds synthesized for the first time in our laboratory were included in the current Thesis. Regarding the compounds described previously in the literature, the synthetic procedure was described along with the literature reference where they were first described.

A complete characterization of all the new compounds synthesized in this Thesis was carried out including $^{1}H$ and $^{13}C$, IR, elemental analysis i GC/MS, all NMR signals of $^{1}H$ and $^{13}C$ thanks to homocorrelation experiments (COSY $^{1}H/^{1}H$ and NOESY) and heterocorrelation $^{1}H/^{13}C$ (HSQC).

# 1. SYNTHESIS OF POLYCYCLIC *N*-BENZAMIDO IMIDES

**Synthesis of dimethyl pentacyclo[6.4.0.0$^{2,10}$.0$^{3,7}$.0$^{4,9}$]dodeca-5,11-diene-8,9-dicarboxylate, 37.**[47]

**a)** Synthesis of 9,10-dihydrofulvalene, **36**:

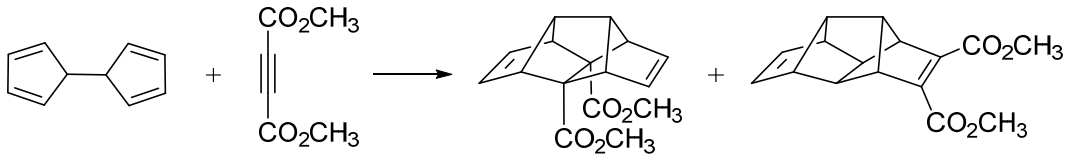



**34** **35** **36**

In a 2 L reactor equipped with a thermometer, argon atmosphere, mechanic stirring, and two pressure-equalizing dropping funnels, a suspension of sodium hydride (50.0 g, 2.08 mol) in anhydrous THF (1 L) was prepared. The suspension was cooled to 0 ºC with an ice bath and freshly distilled cyclopentadiene (137 g, 2.08 mol) was added dropwise over 30-40 minutes avoiding excess foaming. After the addition is complete, the reaction was allowed to reach room temperature and was stirred for an hour.

Then, complex cuprous bromide-dimethyl sulfide complex (0.75 g) was added and the solution was cooled at −78 ºC with a $CO_2$/acetone bath. A solution of iodine (265 g, 1.04 mol) in anhydrous THF (250 mL) was added dropwise over 90 minutes. At the end, the suspension was kept at –78 ºC for 15 minutes.

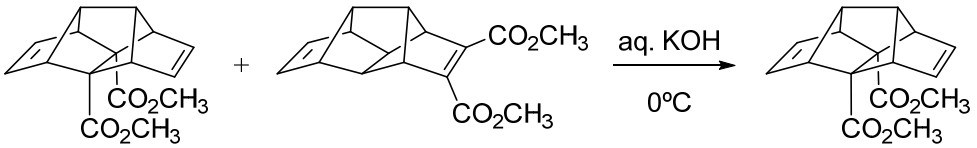
**b)** Reaction of **36** with dimethyl acetylenedicarboxylate:




**36** **37** **38**

Dimethyl acetylenedicarboxylate (165 g, 1.16 mol) was added dropwise for 15 min to the previous slurry. The solution was kept at −78 ºC for 30 minutes, after that the reaction was allowed to reach room temperature and stirred for 4 hours. During this period of time, a softly exothermic reaction was observed and a yellow solid appeared corresponding to sodium iodide. The suspension was filtered through Celite® and washed with THF (750 mL). The combined filtrates were concentrated under vacuo without exceeding 30 ºC, obtaining a red oil which was dissolved in diethyl ether (750 mL). The solution was stirred for 15 minutes, filtered through Celite® and concentrated under reduced pressure without exceeding 30 ºC. A mixture of diesters **37** and **38** was obtained as a red oil, which was stored at 0 ºC overnight.

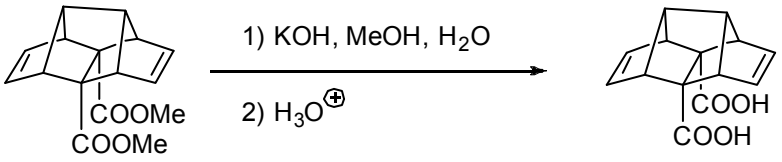
**c)** Isolation of **37**:




**37** **38** **37**

In a 2 L round-bottomed flask equipped with a pressure-equalizing dropping funnel, gas outlet, mechanic stirring and a thermometer, the previous crude was dissolved in MeOH (1 L), the solution was cooled at −5 ºC with a salt/ice bath. A precooled (0 ºC) solution of KOH (110 g, 85% purity, 1.67 mol) in water (200 mL) was added dropwise at such a rate as to keep the reaction temperature below 10 ºC. When the addition was finished, the stirring was kept at 0 ºC for 2 hours and then, one hour at room temperature prior to the addition of glacial AcOH (50 mL). Solid sodium carbonate was added to bring the pH to 8 and the solution was filtered through Celite®. Concentration off the filtrate at 35 ºC and reduced pressure affords about 500 mL of a dark liquid. The residue was diluted with 1 L of water and extracted with petroleum ether (6x300 mL). The combined extracts were washed with aqueous (10%) sodium thiosulphate (2x200 mL), dried over anh. $Na_2SO_4$ and concentrated under vacuo without exceeding 30 ºC, obtaining the diester **37** (35.7 g, 13% overall yield), whose spectroscopic data coincide which the ones described in the bibliography.[47]

**Synthesis of pentacyclo[6.4.0.0$^{2,10}$.0$^{3,7}$.0$^{4,9}$]dodeca-5,11-diene-8,9-dicarboxylic acid, 41.**

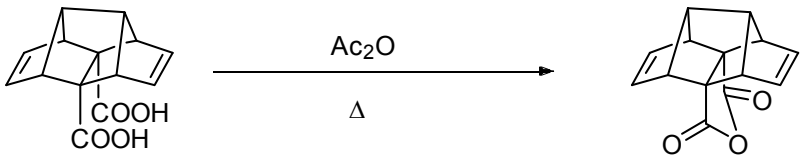



**37** **41**

In a 500 mL round-bottom flask equipped with a magnetic stirrer, thermometer, addition funnel, and a condenser, a solution of diester **37** (35.7 g, 0.13 mol) in methanol (75 mL) was prepared. Then, KOH (20 g in 75 mL water) was added dropwise, the mixture was heated under reflux for 1 h, giving a black solution. The methanol was evaporated under vaccuo, water (145 mL) was added and the obtained mixture was heated under reflux for 5 hours. After that, the solution was cooled at room temperature and activated charcoal (6 g) was added. The suspension was stirred at room temperature overnight, and filtered through Celite® obtaining a brown solution which was cooled at 0 ºC with an ice bath. When the solution reached 0ºC was acidified with concentrated HCl to pH=1. The suspension was filtered under vacuo, and the solid was re-dissolved in ethyl acetate (1 L) and the organic layer was dried over sodium sulphate, filtered and concentrated under vacuo to give pure acid **41** as a white solid. In order to increase the yield, the aqueous layer was extracted with ethyl acetate

(3 x 50 mL) obtaining a further amount of diacid **41**. A total amount of 25.7 g (82% yield) of diacid were obtained, whose spectroscopic data coincide with the described in the bibliography.[47]

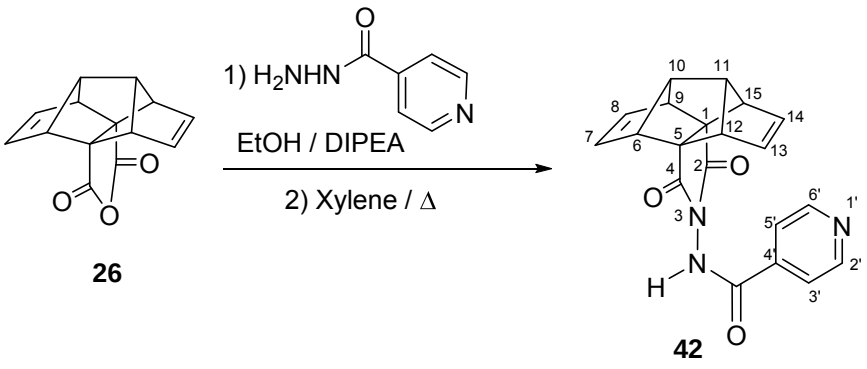
**Synthesis of pentacyclo[6.4.0.0$^{2,10}$.0$^{3,7}$.0$^{4,9}$]dodeca-5,11-diene-8,9-dicarboxylic anhydride, 26.**




**41** **26**

In a round-bottomed flask of 250 mL equipped with a magnetic stirrer and a condenser a suspension of the diacid **41** (4 g, 16.4 mmol) in acetic anhydride (103 ml) was prepared. The suspension was heated under reflux for 90 min. The solution was concentrated under vaccuo to give a yellow solid (4.16 g). The product was purified by sublimation at 100 $^{o}$C / 0.7 Torr to obtain pure anhydride **26** (3.16 g, 14 mmol, 85% yield) whose spectroscopic data coincide which the ones described in the bibliography.[43]

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(4-pyridylcarbonyl)amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-2,4-dione, 42.**




**26** **42**

In a 5 mL round-bottomed flask with a condenser and magnetic stirrer, a mixture of anhydride **26** (100 mg, 0.44 mmol), isoniazid (60 mg, 0.44 mmol), absolute EtOH (2 mL), and a drop of diisopropylethylamine was prepared. The suspension was heated at 90 $^{o}$C for 6 hours. The suspension was stored at 0 $^{o}$C overnight and the precipitate was collected by filtration and washed with cold ethanol (1mL). The white solid was suspended in xylene (10 mL) and heated under reflux for 24 hours in a Dean-Stark system. The solution was concentrated under vacuo to give pure (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(4-pyridylcarbonyl)amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-2,4-dione as a white solid (85 mg, 56% yield).

Analytic and spectroscopic data of **42**.

Melting point: 241 °C decompose ($CH_2Cl_2$).

IR (KBr) *v*: 3189, 3068, 2968, 2361, 1784, 1732, 1703, 1604, 1557, 1525, 1493, 1412, 1380, 1341, 1282, 1240, 1185, 1155, 1127, 1062, 1022, 908, 819, 773, 713, 696, 577 $cm^{-1}$.

$^1H$ NMR (500 MHz, DMSO-$d_6$) δ: 2.86 (broad s, 2 H) (10-H and 11-H), 3.57 (broad s, 4 H) [6(9)-H and 12(15)-H], 6.06 (s, 2 H) and 6.11 (s, 2 H) [7(8)-H and 13(14)-H], 7.77 [d, *J* = 6.8 Hz, 2 H, pyr-3(5)-H], 8.78 [d, *J* = 6.8 Hz, 2 H, pyr-2(6)-H], 11.41 (s, 1 H, NH) ppm.

$^{13}C$-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.86 (CH) and 61.93 (CH) [C6(9) and C12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 65.0 [C, C1(5)], 121.3 [CH, pyr-C3(5)], 132.0 (CH) and 132.4 (CH) [C7(8) and C13(14)], 137.9 (C, pyr-C4), 150.6 [CH, pyr-C2(6)], 162.5 (C, CONH), 171.8 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 345 (2) $[M]^{\cdot+}$, 281 (7), 280 (100) $[M-C_5H_5]^{\cdot+}$, 153 (20), 152 (15), 106 (25) $[C_5H_4N\text{-}CO]^+$.

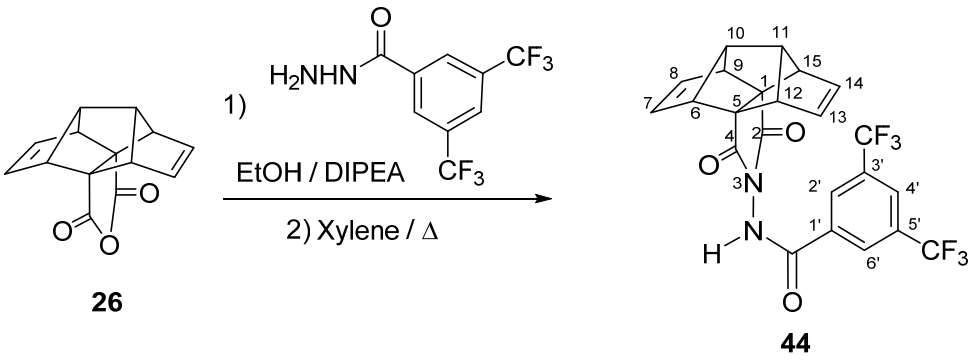
Elemental analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{20}H_{15}N_3O_3$: | C 69.56% | H 4.38% | N 12.17% |
| Found: | C 69.47% | H 4.63% | N 11.98% |

Accurate mass:

| | |
|---|---|
| Calculated for $[C_{20}H_{15}N_3O_3+H]^+$: | 346.1186 |
| Found: | 346.1191 |

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[3,5-bis(trifluoromethyl)benzoylamino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$] pentadeca-7,13-diene-2,4-dione, 44.**




This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (100 mg, 0.44 mmol) and 3,5-bis(trifluoromethyl)benzoic acid hydrazide (120 mg, 0.44 mmol), benzamide **44** was isolated as a white solid (101 mg, 48% yield). An analytical sample of **44** was obtained by crystallization from $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **44**.

Melting point of: 248-249 °C ($C_{10}H_8$).

IR (KBr) *v*: 3289, 3106, 2999, 2359, 1791, 1720, 1703, 1625, 1507, 1463, 1380, 1342, 1286, 11272, 1246, 1223, 1195, 1173, 1157, 1130, 1033, 1022, 906, 821, 714 cm$^{-1}$.

$^1$H-RMN (400 MHz, DMSO-$d_6$) δ: 2.88 (broad s, 2 H) (10-H and 11-H), 3.59 (broad s, 4 H) [6(9)-H and 12(15)-H], 6.07 (s, 2 H) and 6.13 (s, 2 H) [7(8)-H and 13(14)-H], 8.44 (broad s, 1 H, Ar-4-H), 8.53 [broad s, 2 H, Ar-2(6)-H], 11.61 (s, 1 H, NH) ppm.

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.9 [CH, C6(9) and C12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.7 [C, C1(5)], 123.9 (C, q, $J_{C\text{-}F}$ = 272.4 Hz, $CF_3$), 126.3 [CH, m, Ar-C4), 128.4 [CH, m, Ar-C2(6)], 130.9 (C, q, $J_{C\text{-}F}$ = 33.4 Hz, Ar-C3(5)], 132.0 (CH) and 132.3 (CH) [C7(8) and C13(14)], 132.9 (C, Ar-C1), 161.1 (C, CONH), 171.7 [C, C2(4)] ppm.

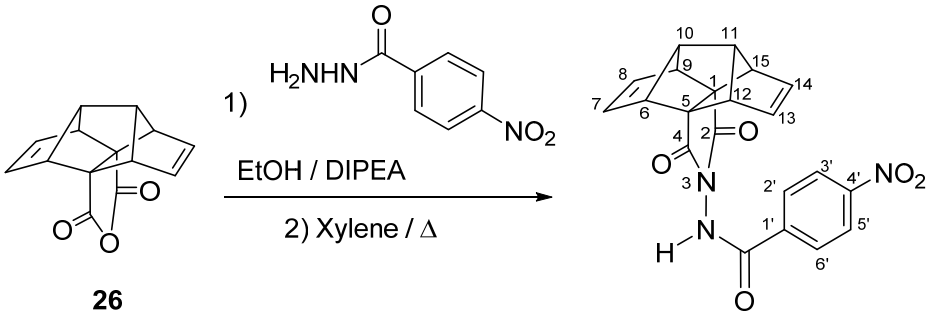
MS (EI), m/e (%); main ions: 480 (<1) $[M]^{\cdot+}$, 461 (<1) $[M-F]^+$, 416 (22), 415 (100) $[M-C_5H_5]^{\cdot+}$, 241 (46) $[(CF_3)_2C_6H_3\text{-}CO]^+$, 213 (22) $[(CF_3)_2C_6H_3]^+$, 153 (29), 152 (24).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{23}H_{14}F_6N_2O_3$: | C 57.51% | H 2.94% | N 5.83% | F 23.73% |
| Calculated for $C_{23}H_{14}F_6N_2O_3 \cdot 0.1\ C_8H_{10}$: | C 58.22% | H 3.08% | N 5.71% | F 23.22% |
| Found: | C 57.94% | H 3.42% | N 5.73% | F 23.34% |

Accurate mass:
Calculated for $[C_{23}H_{14}F_6N_2O_3+H]^+$: 481.0981
Found: 481.0986

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(4-nitrobenzoyl)amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-2,4-dione), 45.**




This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (100 mg, 0.44 mmol) and 4-nitrobenzoic acid hydrazide (80 mg, 0.44 mmol), benzamide **45** was isolated as a white solid (83 mg, 48% yield). An analytical sample of **45** was obtained by crystallization from $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **45**.

Melting point: 225 °C decompose ($CH_2Cl_2$).

IR (KBr) *v*: 3284, 3062, 2989, 1786, 1720, 1705, 1603, 1559, 1522, 1477, 1399, 1343, 1269, 1243, 1188, 1159, 1133, 1097, 1021, 821, 779, 710 cm$^{-1}$.

$^1$H-RMN (500 MHz, DMSO-$d_6$) δ: 2.86 (broad s, 2 H, 10-H and 11-H), 3.57 [broad s, 4 H, 6(9)-H and 12(15)-H], 6.06 (broad s, 2 H) and 6.10 (broad s, 2 H) [7(8)-H and 13(14)-H], 8.09 [d, *J* = 8.0 Hz, 2 H, Ar-2(6)-H], 8.34 [d, *J* = 8.0 Hz, 2 H, Ar-3(5)-H], 11.42 (s, 1 H, NH) ppm.

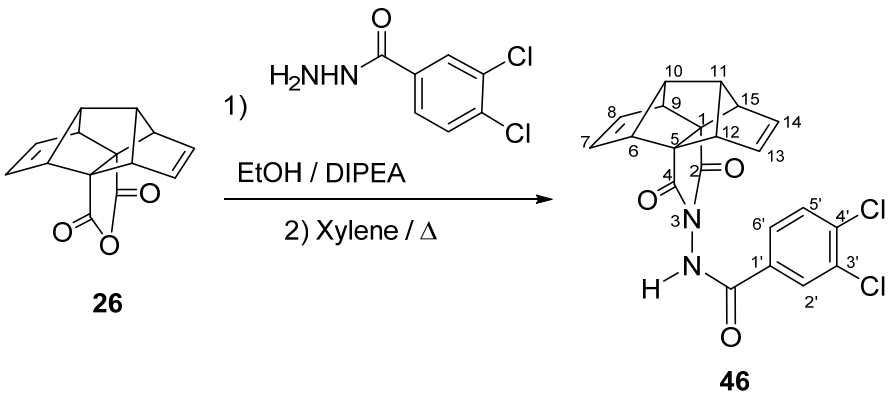
$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 62.1 [CH, C6(9) and 12(15)], 63.7 (CH) and 64.2 (CH) (C10 and C11), 64.9 [C, C1(5)], 124.0 [CH, Ar-C3(5)], 129.4 [CH, Ar-C2(6)], 132.2 (CH) and 132.5 (CH) [C7(8) and C13(14)], 136.7 (C, Ar-C1), 149.9 (C, Ar-C4), 162.6 (C, CONH), 172.0 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 389 (1) $[M]^{\cdot+}$, 325 (18), 324 (100) $[M-C_5H_5]^+$, 153 (29), 152 (21), 150 (34) $[O_2N\text{-}C_6H_4CO]^+$, 120 (17), 104 (18).

Elemental analysis
Calculated for $C_{21}H_{15}N_3O_5$: C 64.78% H 3.88% N 10.79%
Calculated for $C_{21}H_{15}N_3O_5 \cdot 0.1\ C_7H_8$: C 65.19% H 4.15% N 10.18%
Found: C 65.28% H 4.15% N 10.56%

Accurate mass
Calculated for $[C_{21}H_{15}N_3O_5+H]^+$: 390.1084
Found: 390.1079

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(3,4-Dichlorobenzoyl)amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-2,4-dione, 46.**




This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (100 mg, 0.44 mmol) and 3,4-dichlorobenzoic acid hydrazide (90 mg, 0.44 mmol), benzamide **46** was isolated as a white solid (101 mg, 55% yield). An analytical sample of **46** was obtained by crystallization from $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **46**.

Melting point: 244-245 °C ($CH_2Cl_2$).

IR (KBr) *v*: 3296, 3062, 2982, 1790, 1723, 1700, 1586, 1461, 1400, 1338, 1275, 1242, 1188, 1159, 1132, 1101, 1029, 922, 821, 710 cm$^{-1}$.

$^1$H-RMN (500 MHz, DMSO-$d_6$) δ: 2.86 (broad s, 2 H, 10-H and 11-H), 3.57 [broad s, 4 H, 6(9)-H and 12(15)-H], 6.05 (broad s, 2 H) and 6.10 (broad s, 2 H) [7(8)-H and 13(14)-H], 7.82 (d, *J* = 8.4 Hz, 1 H, Ar-5-H), 7.85 (dd, *J* = 8.4 Hz, *J'* = 1.9 Hz, 1 H, Ar-6-H), 8.11 [d, *J* = 1.9 Hz, 1 H, Ar-2-H), 11.26 (s, 1 H, NH) ppm.

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.86 (CH) and 61.90 (CH) [C6(9) and 12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.6 [C, C1(5)], 127.8 (CH, Ar-C5), 129.5 (CH, Ar-C2), 131.1 (CH, Ar-C6), 131.2 (C), 131.7 (C) (Ar-C1 and Ar-C3), 132.0 (CH) and 132.3 (CH) [C7(8) and C13(14)], 135.4 (C, Ar-C4), 161.7 (C, CONH), 171.8 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 414 (2) [M, $^{37}Cl$ and $^{35}Cl]^{\cdot+}$, 412 (2) [M, 2 $^{35}Cl]^{\cdot+}$, 349 (58) [M−$C_5H_5$, $^{37}Cl$ and $^{35}Cl]^+$, 348 (17), 347 (87) [M−$C_5H_5$, 2 $^{35}Cl]^+$, 177 (11) [$C_6H_3Cl_2CO$, 2 $^{37}Cl]^+$, 175 (61) [$C_6H_3Cl_2CO$, $^{37}Cl$ and $^{35}Cl]^+$, 173 (100) [$C_6H_3Cl_2CO$, 2 $^{35}Cl]^+$, 153 (32), 152 (24), 147 (13) [$C_6H_3Cl_2$, $^{37}Cl$ and $^{35}Cl]^+$, 145 (20) [$C_6H_3Cl_2$, 2 $^{35}Cl]^+$.

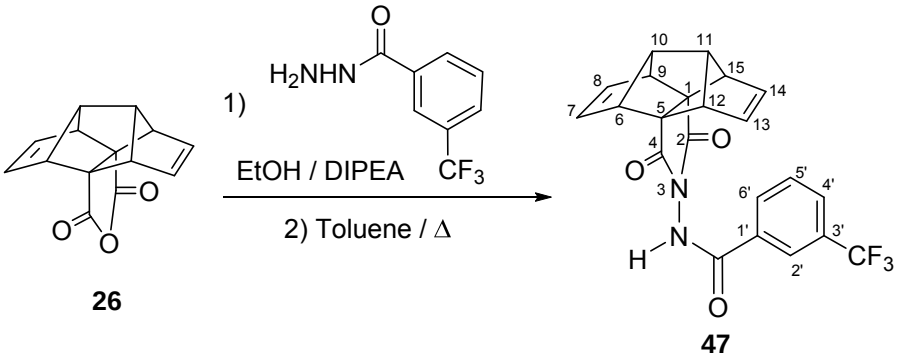
Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{21}H_{14}Cl_2N_2O_3$: | C 61.03% | H 3.41% | N 6.78% | Cl 17.16% |
| Calculated for $C_{21}H_{14}Cl_2N_2O_3 \cdot 0.1\ C_8H_{10}$: | C 61.77% | H 3.57% | N 6.61% | Cl 16.73% |
| Found: | C 61.66% | H 3.63% | N 6.35% | Cl 16.24% |

Accurate mass

| | |
|---|---|
| Calculated for $[C_{21}H_{14}Cl_2N_2O_3+H]^+$: | 413.0454 |
| Found: | 413.0454 |

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-{[3-(trifluoromethyl)benzoyl]amino}-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$] pentadeca-7,13-diene-2,4-dione, 47.**




**26** **47**

This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (100 mg, 0.44 mmol) and 3-(trifluoromethyl)benzoic acid hydrazide (90 mg, 0.44 mmol), benzamide **47** was isolated as a white solid (103 mg, 57% yield). An analytical sample of **47** was obtained by crystallization from $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **47**.

Melting point: 257-258 °C ($CH_2Cl_2$).

IR (KBr) *v*: 3268, 3073, 2980, 2360, 1783, 1713, 1702, 1617, 1529, 1484, 1438, 1398, 1338, 1259, 1185, 1156, 1125, 1074, 1018, 916, 820, 714 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, DMSO-$d_6$) δ: 2.87 (broad s, 2 H, 10-H and 11-H), 3.58 (m, 4 H) [6(9)-H and 12(15)-H], 6.07 (broad s, 2 H) and 6.11 (broad s, 2 H) [7(8)-H and 13(14)-H], 7.79 (t, *J* = 7.8 Hz, 1 H, Ar-5-H), 8.01 (d, *J* = 7.8 Hz, 1 H, Ar-4-H), 8.18 (d, *J* = 7.8 Hz, 1 H, Ar-6-H), 8.23 (broad s, 1 H, Ar-2-H), 11.34 (s, 1 H, NH) ppm.

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.8 (CH) and 61.9 (CH) [C6(9) and C12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.7 [C, C1(5)], 123.8 (C, q, $J_{C\text{-}F}$ = 273.0 Hz, $CF_3$), 124.2 (CH, q, $J_{C\text{-}F}$ = 4.0 Hz, Ar-C2), 129.1 (CH, q, $J_{C\text{-}F}$ = 3.1 Hz, Ar-C4), 129.4 (C, q, $J_{C\text{-}F}$ = 32.7 Hz, Ar-C3), 130.1 (CH, Ar-C5), 131.77 (CH, Ar-C6), 131.80 (C, Ar-C1), 132.0 (CH) and 132.3 (CH) [C7(8) and C13(14)], 162.4 (C, CONH), 171.9 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 412 (1) $[M]^{\cdot+}$, 348 (19) 347 (100) $[M-C_5H_5]^+$, 173 (81) $[CF_3\text{-}C_6H_4\text{-}CO]^+$, 153 (24), 152 (20), 145 (32) $[CF_3\text{-}C_6H_4]^+$.

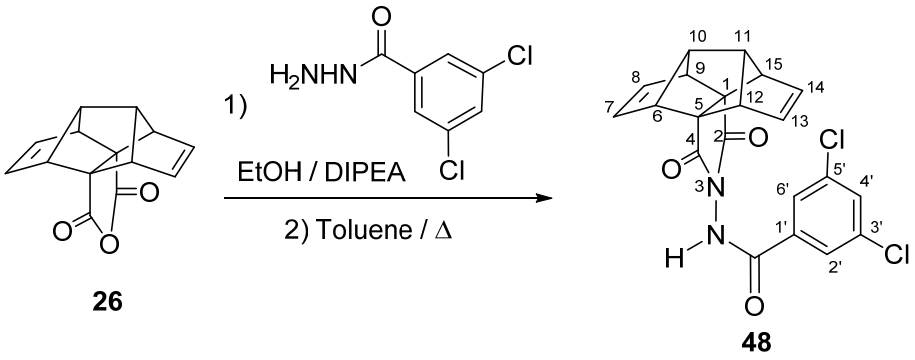
Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{22}H_{15}F_3N_2O_3$: | C 64.08% | H 3.67% | N 6.79% | F 13.82% |
| Calculated for $C_{22}H_{15}F_3N_2O_3 \cdot 0.25\ H_2O$: | C 63.39% | H 3.75% | N 6.72% | F 13.67% |
| Found: | C 63.68% | H 3.97% | N 6.47% | F 13.55% |

Accurate mass

| | |
|---|---|
| Calculated for $[C_{22}H_{15}F_3N_2O_3+H]^+$: | 413.1108 |
| Found: | 413.1107 |

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(3,5-dichlorobenzoyl)amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-2,4-dione, 48.**




This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (100 mg, 0.44 mmol) and 3,5-dichlorobenzoic acid hydrazide (90 mg, 0.44 mmol), benzamide **48** was isolated as a white solid (163 mg, 90% yield). An analytical sample of **48** was obtained by crystallization from $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **48**.

Melting point: 278-279 ºC ($CH_2Cl_2$).

IR (KBr) *v*: 3267, 3063, 2984, 1791, 1716, 1693, 1569, 1520, 1403, 1339, 1301, 1267, 1243, 1188, 1159, 1141, 1102, 1020, 908, 821, 704 $cm^{-1}$.

$^1$H-RMN (500 MHz, DMSO-$d_6$) δ: 2.87 (broad s, 2 H) (10-H and 11-H), 3.57 [m, 4 H, 6(9)-H and 12(15)-H], 6.05 (s, 2 H) and 6.11 (s, 2 H) [7(8)-H and 13(14)-H], 7.89 [d, *J* = 1.8 Hz, 2 H, Ar-2(6)-H], 7.93 (t, *J* = 1.8 Hz, 1 H, Ar-4-H), 11.31 (s, 1 H, NH) ppm.

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.9 [CH, C6(9) and C12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.7 [C, C1(5)], 126.4 [CH, ArC2(6)], 132.0 (C, Ar-C4), 132.0 (CH) and 132.3 (CH) [C7(8) and C13(14)], 134.0 (C, Ar-C1), 134.7 [CH, Ar-C3(5)], 161.3 (C, CONH), 171.7 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 414 (1) [M, $^{37}$Cl and $^{35}$Cl]$^{\cdot+}$, 412 (1) [M, 2 $^{35}$Cl]$^{\cdot+}$, 349 (66) [M−$C_5H_5$, $^{37}$Cl and $^{35}$Cl]$^+$, 348 (18), 347 (100) [M−$C_5H_5$, 2 $^{35}$Cl]$^+$, 175 (47) [$C_6H_3Cl_2CO$, $^{37}$Cl and $^{35}$Cl]$^+$, 173 (78) [$C_6H_3Cl_2CO$, 2 $^{35}$Cl]$^+$, 154 (20), 153 (48), 152 (36), 147 (15) [$C_6H_3Cl_2$, $^{37}$Cl and $^{35}$Cl]$^+$, 145 (24) [$C_6H_3Cl_2$, 2 $^{35}$Cl]$^+$.

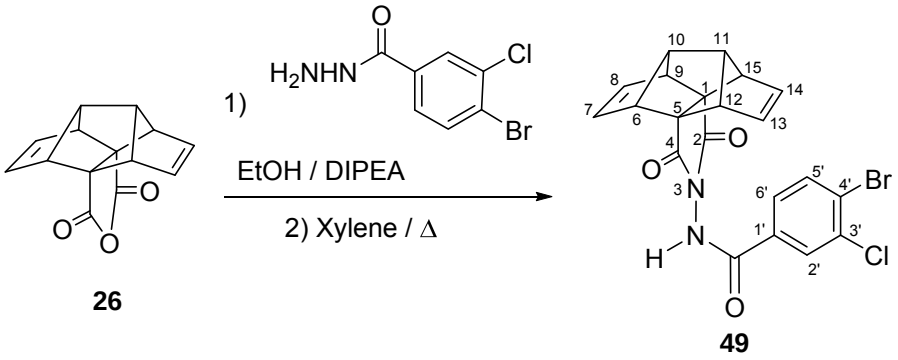
Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{21}H_{14}Cl_2N_2O_3$: | C 61.03% | H 3.41% | N 6.78% | Cl 17.16% |
| Found: | C 60.93% | H 3.41% | N 6.59% | Cl 16.90% |

Accurate mass

| | |
|---|---|
| Calculated for $[C_{21}H_{14}Cl_2N_2O_3+H]^+$: | 413.0454 |
| Found: | 413.0448 |

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(4-bromo-3-chlorobenzoyl) amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-2,4-dione, 49.**




This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (100 mg, 0.44 mmol) and 4-bromo-3-chlorobenzoic acid hydrazide (110 mg, 0.44 mmol), benzamide **49** was isolated as a white solid (141 mg, 70% yield). An analytical sample of **49** was obtained by crystallization from $CH_2Cl_2$.

Analytic and spectroscopic data **49**.

Melting point: 260-262 °C ($CH_2Cl_2$).

IR (KBr) *v*: 3486, 3271, 3067, 2980, 2539, 2357, 1789, 1721, 1714, 1673, 1586, 1552, 1519, 1506, 1460, 1384, 1339, 1320, 1284, 1270, 1245, 1235, 1185, 1156, 1135, 1099, 1087, 1037, 1018, 908, 820, 706 cm$^{-1}$.

$^1$H-RMN (400 MHz, DMSO-$d_6$) δ: 2.86 (broad s, 2H, 10-H and 11-H), 3.57 (m, 4H) [6(9)-H and 12(15)-H], 6.05 (broad s, 2H) and 6.10 (broad s, 2H) [7(8)-H and 13(14)-H], 7.75 (dd, *J* = 8.4 Hz, *J'* = 2.0 Hz, 1H, Ar-6-H), 7.96 (d, *J* = 8.4 Hz, 1H, Ar-5-H), 8.08 (d, *J* = 2.0 Hz, 1H, Ar-2-H), 11.24 (s, 1 H, NH) ppm.

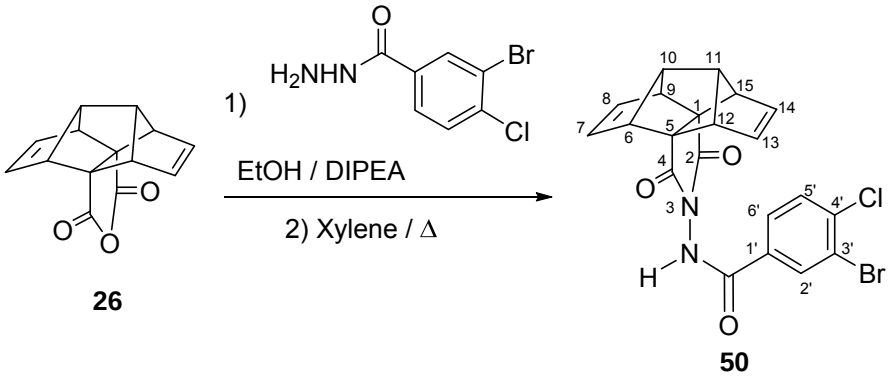
$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.86 (CH) and 61.90 (CH) [C6(9) and C12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.6 [C, C1(5)], 126.3 (C, Ar-C4), 127.8 (CH, Ar-C6), 129.3 (CH, Ar-C2), 131.8 (C, Ar-C3), 132.0 (CH) and 132.3 (CH) [C7(8) and C13(14)], 133.7 (C, Ar-C1), 134.4 (CH, Ar-C5), 161.9 (C, CONH), 171.8 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 460 (1) [M, $^{81}Br^{37}Cl]^{\cdot+}$, 458 (3) [M, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^{\cdot+}$, 456 (2) [M, $^{79}Br^{35}Cl]^{\cdot+}$, 395 (23) [M−$C_5H_5$, $^{81}Br^{37}Cl]^+$, 394 (17), 393 (88) [M−$C_5H_5$, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^+$, 392 (12), 391 (56) [M−$C_5H_5$, $^{79}Br^{35}Cl]^+$, 221 (27) [$C_6H_3BrCl$-CO, $^{81}Br^{37}Cl]^+$, 219 (100) [$C_6H_3BrCl$-CO, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^+$, 217 (82) [$C_6H_3BrCl$-CO, $^{79}Br^{35}Cl]^+$, 191 (29) [$C_6H_3BrCl$, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^+$, 189 (26) [$C_6H_3BrCl$, $^{79}Br^{35}Cl]^+$, 154 (31), 153 (70), 152 (64).

Elemental analysis
Calculated for $C_{21}H_{14}ClN_2O_3Br$: C 55.11% H 3.08% N 6.12% Br 17.46% Cl 7.75%
Found: C 55.46% H 3.15% N 5.93% Br 17.81% Cl 6.88%

Accurate mass
Calculated for $[C_{21}H_{14}ClN_2O_3Br+H]^+$: 456.9949
Found: 456.9949

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(3-bromo-4-chlorobenzoyl) amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-2,4-dione, 50.**




**26** **50**

This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (100 mg, 0.44 mmol) and 3-bromo-4-chlorobenzoic acid hydrazide (110 mg, 0.44 mmol), benzamide **50** was isolated as a white solid (170 mg, 84% yield). An analytical sample of **50** was obtained by crystallization from $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **50**.

Melting point: 266-267 ºC ($CH_2Cl_2$).

IR (KBr) *v*: 3311, 2994, 1786, 1715, 1704, 1588, 1504, 1461, 1392, 1371, 1337, 1259, 1187, 1153, 1131, 1090, 1023, 915, 821, 706 cm$^{-1}$.

$^1$H-RMN (400 MHz, DMSO-$d_6$) δ: 2.86 (broad s, 2H, 10-H and 11-H), 3.57 (m, 4H) [6(9)-H and 12(15)-H], 6.05 (broad s, 2H) and 6.11 (broad s, 2H) [7(8)-H and 13(14)-H], 7.80 (d, *J* = 8.4 Hz, 1 H, Ar-5-H), 7.88 (dd, *J* = 8.4 Hz, *J'* = 2.0 Hz, 1 H, Ar-6-H), 8.24 (d, *J* = 2.0 Hz, 1 H, Ar-2-H), 11.25 (s, 1 H, NH) ppm.

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.85 (CH) and 61.89 (CH) [C6(9) and C12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.6 [C, C1(5)], 121.9 (C, Ar-C3), 128.4 (CH, Ar-C6), 130.9 (CH,

Ar-C5), 131.2 (C, Ar-C1), 132.0 (CH) and 132.3 (CH) [C7(8) and C13(14)], 132.7 (CH, Ar-C2), 137.4, (C, Ar-C4), 161.7 (C, CONH), 171.8 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 458 (3) [M, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^{\cdot+}$, 456 (2) [M, $^{79}Br^{35}Cl]^{\cdot+}$, 395 (25) [M−$C_5H_5$, $^{81}Br^{37}Cl]^+$, 394 (16), 393 (96) [M−$C_5H_5$, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^+$, 392 (14), 391 (77) [M−$C_5H_5$, $^{79}Br^{35}Cl]^+$, 221 (27) [$C_6H_3BrCl$-CO, $^{81}Br^{37}Cl]^+$, 219 (100) [$C_6H_3BrCl$-CO, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^+$, 217 (83) [$C_6H_3BrCl$-CO, $^{79}Br^{35}Cl]^+$, 207 (18), 191 (29) [$C_6H_3BrCl$, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^+$, 189 (23) [$C_6H_3BrCl$, $^{79}Br^{35}Cl]^+$, 160 (19), 154 (38), 153 (83), 152 (66).

Elemental analysis

Calculated for $C_{21}H_{14}ClN_2O_3Br$: C 55.11% H 3.08% N 6.12% Br 17.46% Cl 7.75%

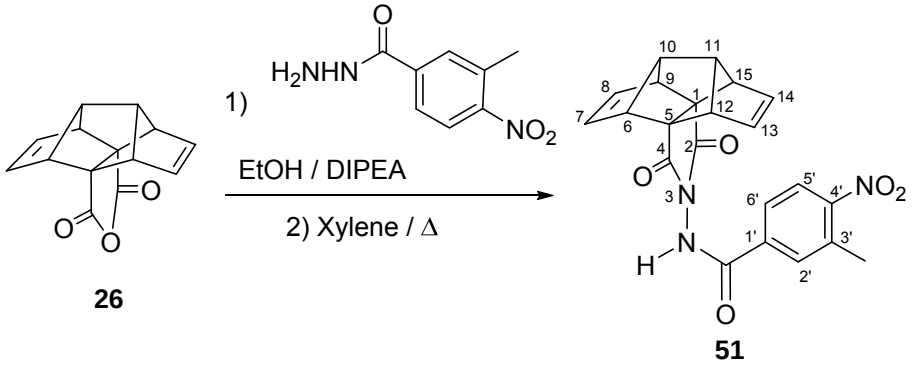
Found: C 55.04% H 3.14% N 5.86%

Accurate mass:

Calculated for $C_{21}H_{14}ClN_2O_3Br$: 456.9949

Found: 456.9947

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(3-methyl-4-nitrobenzoyl) amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-2,4-dione, 51.**




**26** **51**

This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (200 mg, 0.88 mmol) and 3-methyl-4-nitrobenzoic acid hydrazide (164 mg, 0.88 mmol), benzamide **51** was isolated as a white solid (180 mg, 51% yield). An analytical sample of **51** was obtained by crystallization from $CH_2Cl_2$.

Analytic and spectroscopic data of **51**.

Melting point: 237 °C decompose ($CH_2Cl_2$).

IR (KBr) *v*: 3297, 3067, 2987, 1785, 1720, 1702, 1609, 1587, 1524, 1475, 1403, 1357, 1340, 1314, 1276, 1243, 1198, 1159, 1134, 1105, 1022, 908, 822, 711 cm$^{-1}$.

$^{1}$H-RMN (500 MHz, DMSO-$d_6$) δ: 2.54 (s, 3 H, Ar-$CH_3$), 2.87 (broad s, 2 H, 10-H and 11-H), 3.58 [broad s, 4 H, 6(9)-H and 12(15)-H], 6.07 (broad s, 2 H) and 6.11 (broad s, 2 H) [7(8)-H and 13(14)-H], 7.89 (d, *J* = 8.4 Hz, 1 H, Ar-6-H), 7.98 (s, 1 H, Ar-2-H), 8.09 (d, *J* = 8.4 Hz, 1 H, Ar-5-H), 11.32 (s, 1 H, NH) ppm.

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 19.2 ($CH_3$, Ar-$CH_3$), 61.8 (CH) and 61.9 (CH) [C6(9) and 12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.7 [C, C1(5)], 124.7 (CH, Ar-C5), 126.5 (CH, Ar-C6), 132.0 (CH) and 132.3 (CH) [C7(8) and C13(14)], 132.1 (CH, Ar-C2), 133.0 (C, Ar-C3), 134.7 (C, Ar-C1), 151.1 (C, Ar-C4), 162.4 (C, CONH), 171.8 [C, C2(4)] ppm..

MS (EI), m/e (%); main ions: 403 (2) $[M]^{\cdot+}$, 339 (23), 338 (100) $[M-C_5H_5]^+$, 164 (39) $[(CH_3)(NO_2)C_6H_3\text{-}CO]^+$, 153 (18), 152 (13), 118 (12).

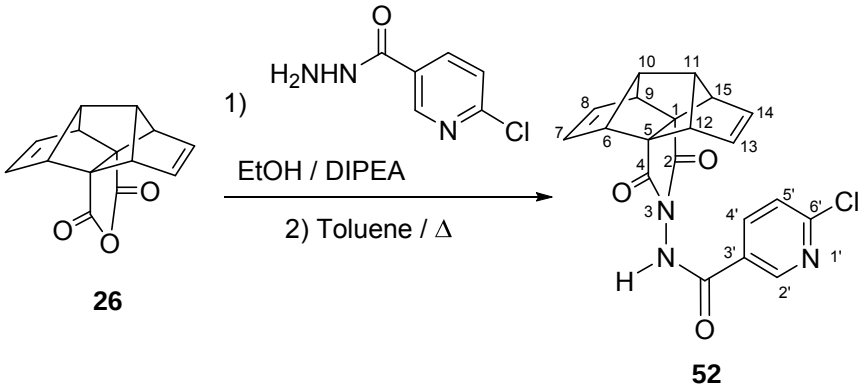
Elemental analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{22}H_{17}N_3O_5$: | C 65.50% | H 4.25% | N 10.42% |
| Found: | C 65.76% | H 4.35% | N 10.41% |

Accurate mass

| | |
|---|---|
| Calculated for $C_{22}H_{17}N_3O_5$: | 404.1241 |
| Found: | 404.1246 |

**Synthtesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-{[(6-chloro-3-pyridyl)carbonyl]amino}-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$] pentadeca-7,13-diene-2,4-dione, 52.**




**26** **52**

This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (200 mg, 0.88 mmol) and 6-chloropyridine-3-carboxylic acid hydrazide (151 mg, 0.88 mmol), benzamide **52** was isolated as a white solid (252 mg, 75% yield). An analytical sample of **52** was obtained by crystallization from $CH_2Cl_2$.

Analytic and spectroscopic data of **52**.

Melting point:268-269 $^{o}$C ($CH_2Cl_2$).

IR (KBr) *v*: 3501, 3269, 3064, 2985, 1789, 1736, 1677, 1587, 1558, 1523, 1461, 1397, 1364, 1341, 1315, 1301, 1279, 1244, 1189, 1157, 1129, 1107, 1036, 1021, 1006, 937, 905, 819, 715, 589 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, DMSO-$d_6$) δ: 2.86 (broad s, 2 H, 10-H and 11-H), 3.57 [broad s, 4 H, 6(9)-H and 12(15)-H], 6.06 (broad s, 2 H) and 6.11 (broad s, 2 H) [7(8)-H and 13(14)-H], 7.71 (dm, *J* = 8.4 Hz, 1 H, Pyr-5-H), 8.26 (dd, *J* = 8.4 Hz, *J'* = 2.7 Hz 1 H, Pyr-4-H), 8.60 (dd, *J* = 2.7 Hz, *J'* = 1.5 Hz 1 H, Pyr-2-H), 11.40 (s, 1 H, NH) ppm.

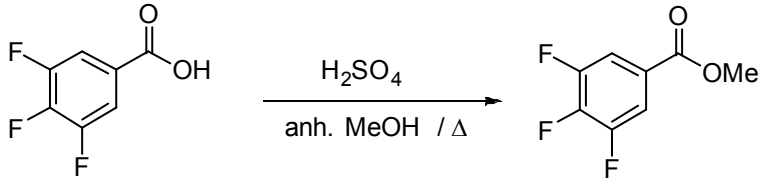
$^{13}$C-RMN (125.7 MHz, DMSO-$d_6$) δ: 61.89 (CH) and 61.95 (CH) [C6(9) and 12(15)], 63.5 (CH) and 64.1 (CH) (C10 and C11), 64.7 [C, C1(5)], 124.6 (CH, Pyr-C5), 126.1 (C, Pyr-C3), 132.0 (CH) and 132.4 (CH) [C7(8) and C13(14)], 138.9 (CH, Pyr-C4), 149.3 (CH, Pyr-C2), 153.8 (C, Pyr-C6), 161.6 (C, CONH), 171.9 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 381 (1) [M, $^{37}Cl]^{\cdot+}$, 379 (4) [M, $^{35}Cl]^{\cdot+}$, 316 (30) [M−$C_5H_5$, $^{37}Cl]^{+}$, 315 (125), 314 (88) [M−$C_5H_5$, $^{35}Cl]^{\cdot+}$, 154 (20), 153 (50), 152 (40), 142 (33) [$C_5H_3$Cl-CO]$^{+}$, $^{37}Cl]^{+}$, 140 (100) [$C_5H_3$Cl-CO]$^{+}$, $^{35}Cl]^{+}$, 112 (31).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{20}H_{14}ClN_3O_3$: | C 63.25% | H 3.72% | Cl 9.33% | N 11.06% |
| Found: | C 63.01% | H 3.55% | Cl 9.41% | N 11.00% |

**Synthesis of methyl 3,4,5-trifluorobenzoate, 268.**

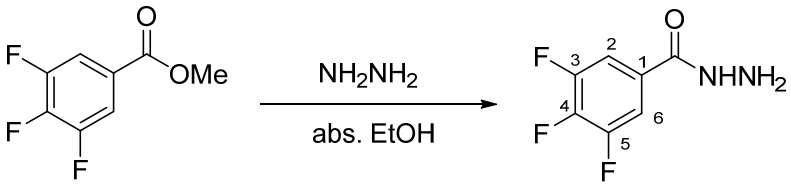



**268**

In a round-bottomed flask of 50 mL equipped with a condenser, argon atmosphere and a magnetic stirrer, a solution of 3,4,5-trifluorobenzoic acid (500 mg, 2.84 mmol), anhydrous methanol (27 mL) and concentrated sulfuric acid (1.4 mL) was prepared. The solution was heated under reflux overnight. The solution was concentrated under vacuo and the residue was disolved in $CH_2Cl_2$ (50 mL) and $H_2O$ (30 mL). The layers were separated and the aqueous one was further extracted with $CH_2Cl_2$ (3 x 30 mL). The combined extracts were dried over $Na_2SO_4$,

filtered and concentrated under vacuo to give pure carboxylate **268** as an oil (285 mg, 53% yield), whose spectroscopic data coincide with the ones described in the bibliography.[197]

**Synthesis of 3,4,5-trifluorobenzohydrazide, 269.**

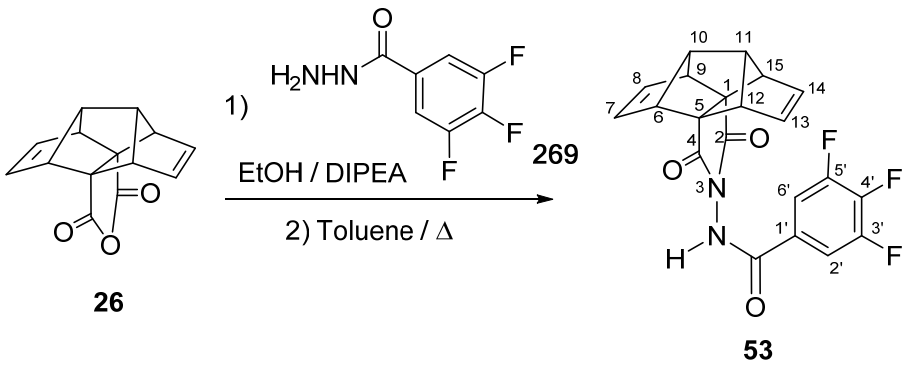



**268** **269**

In a round-bottomed flask of 25 mL with a gas outlet and a magnetic stirrer a solution of methyl 3,4,5-trifluorobenzoate (285 mg, 1.50 mmol), absolute ethanol (2 mL) and hydrazine monohydrate (0.09 mL, 1.80 mmol) was prepared. The solution was stirred at room temperature for 48 hours. The yellow solution was concentrated under vacuo to give the benzohydrazide **269** as a yellow solid (276 mg, 97% yield).

$^1$H-RMN (300 MHz, DMSO-$d_6$) δ: 4.57 (bs, 2H, $NH_2$), 7.85 [dt, *J* = 15.9 Hz, *J'* = 4.2 Hz, 2 H, Ar-2(6)-H], 9.95 (bs, 1 H, CONH).

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(3,4,5-trifluorobenzoyl)amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca -7,13-diene-2,4-dione, 53.**




**26** **53**

This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (275 mg, 1.22 mmol) and 3,4,5-trifluorobenzoic acid hydrazide **269** (231 mg, 1.22 mmol), benzamide **53** was isolated as a white solid (137 mg, 28% yield). An analytical sample of **53** was obtained by crystallization from $CH_2Cl_2$/pentane.

[197] Hachiya, S.; Oku, M.; Mukai, H.; Shin, T.; Matsuura, K.; Seo, R.; Kamikubo, T.; Terada, Y.; Sanagi, M.; Yoshihara, K.; Takahashi, T. (Astellas Pharma Inc.), WO2006/123725, **2006**.

Analytic and spectroscopic data of **53**.

Melting point: 236-237 ºC decompose ($CH_2Cl_2$).

IR (KBr) *v*: 3509, 3210, 3078, 2985, 1791, 1737, 1674, 1626, 1600, 1543, 1515, 1438, 1359, 1275, 1242, 1183, 1156, 1131, 1051, 1020, 983, 821, 777, 711 $cm^{-1}$.

$^1$H-RMN (400 MHz, DMSO-$d_6$) δ: 2.87 (broad s, 2 H) (10-H and 11-H), 3.57 [m, 4 H, 6(9)-H and 12(15)-H], 6.08 (m, 4 H, 7(8)-H and 13(14)-H], 7.83 [m, 2 H, Ar-2(6)-H], 11.29 (s, 1 H, NH) ppm.

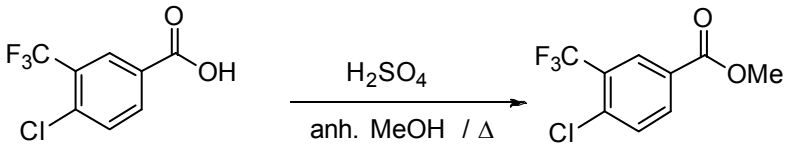
$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.87 (CH) and 61.92 (CH) [C6(9) and C12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.7 [C, C1(5)], 112.9 [CH, dd, *J* = 16.6 Hz, *J'* = 5.4 Hz, Ar-C2(6)], 127.1 (C, m, Ar-C1), 132.0 (CH) and 132.4 (CH) [C7(8) and C13(14)], 141.6 (C, dt, *J* = 255.9 Hz, *J'* = 15.2 Hz, Ar-C4), 150.2 [C, *J* = 249.3 Hz, *J'* = 10.1 Hz, *J"* = 3.6 Hz, Ar-C3(5)], 160.8 (C, CONH), 171.8 [C, C2(4)] ppm.

MS (IE), m/e (%); main ions: 398 (1), 334 (19), 333 (100) $[M-C_5H_5]^+$, 159 (83) $[C_6H_2F_3CO]^+$, 153 (39), 152 (28), 131 (22) $[C_6H_2F_3]^+$.

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{21}H_{13}F_3N_2O_3$: | C 63.32% | H 3.29% | N 7.03% | F 14.31% |
| Calculated for $C_{21}H_{13}F_3N_2O_3 \cdot 0.4\ H_2O$: | C 62.20% | H 3.43% | N 6.91% | F 14.05% |
| Found: | C 62.26% | H 3.72% | N 6.91% | |

**Syntehsis of methyl 4-chloro-3-trifluoromethylbenzoate, 270.**

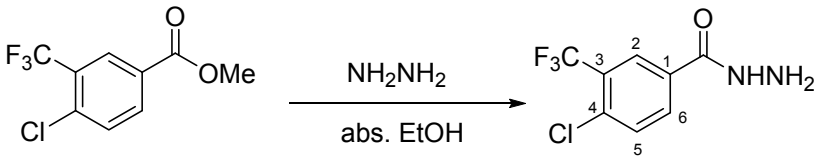



**270**

In a round-bottomed flask of 50 mL equipped with a condenser, argon atmosphere and a magnetic stirrer, a solution of 4-chloro-3-trifluoromethylbenzoic acid (225 mg, 1 mmol), anhydrous methanol (9.5 mL) and concentrated sulfuric acid (0.5 mL), was prepared. The solution was heated under reflux overnight. The solution was concentrated under vacuo and the residue was dissolved in $CH_2Cl_2$ (20 mL) and $H_2O$ (15 mL). The layers were separated and the aqueous one was further extracted with $CH_2Cl_2$ (3 x 15 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under vacuo to give pure carboxylate **270** as a colourless oil (227 mg, 99% yield), whose spectroscopic data coincide with the ones described in the bibliography.[198]

[198] Tanabe, Y.; Matsuo, N.; Ohno, N. *J. Org. Chem* **1988**, *53*, 4582-4585.

**Synthesis of 4-chloro-3-trifluoromethylbenzohydrazide, 271.**

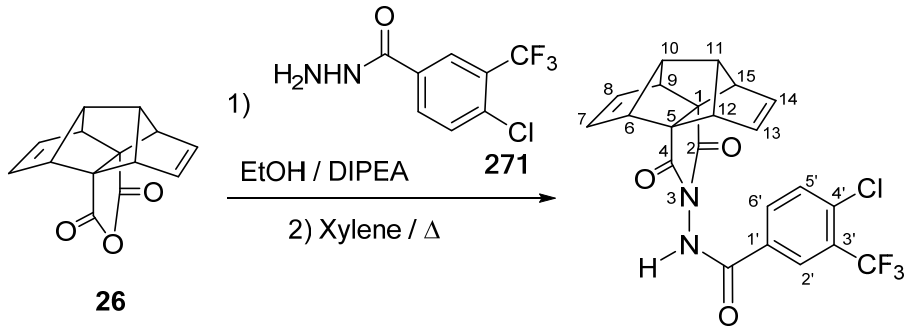



**270** **271**

In a round-bottomed flask of 25 mL with a gas outlet and a magnetic stirrer a solution of methyl 4-chloro-3-trifluoromethylbenzoate (355 mg, 1.49 mmol), absolute ethanol (9 mL) and hydrazine monohydrate (0.09 mL, 1.80 mmol), was prepared, the solution was stirred at room temperature for 48 hours. The yellow solution was concentrated under vauo to give benzohydrazide **271** as a beige solid (303 mg, 85% yield).

$^1$H-RMN (300 MHz, DMSO-$d_6$) δ: 5.33 (bs, 2H, $NH_2$), 7.85 (d, *J* = 8.4 Hz, 1 H, Ar-5-H), 8.12 (dd, *J* = 8.1 Hz, *J'* = 2.1 Hz, 1 H, Ar-6-H), 8.26 (d, *J* = 1.8 Hz, 1 H, Ar-2-H), 10.19 (bs, 1 H, CONH).

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-{[4-chloro-3-(trifluoromethyl)benzoyl]amino}-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$] pentadeca-7,13-diene-2,4-dione, 54.**




**26** **54**

This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (150 mg, 0.66 mmol) and 4-chloro-3-(trifluoromethyl)benzoic acid hydrazide **271** (157 mg, 0.66 mmol), benzamide **54** was isolated as a white solid (217 mg, 74% yield). An analytical sample of **54** was obtained by crystallization from $CH_2Cl_2$.

Analytic and spectroscopic data of **54**.

Melting point: 246-247 ºC ($CH_2Cl_2$).

IR (KBr) *v*: 3328, 3076, 2994, 2977, 1792, 1718, 1701, 1607, 1575, 1508, 1473, 1405, 1338, 1317, 1279, 1246, 1187, 1166, 1148, 1129, 1035, 1021, 900, 821, 706 $cm^{-1}$.

$^1$H-RMN (400 MHz, DMSO-$d_6$) δ: 2.87 (broad s, 2H, 10-H and 11-H), 3.57 (m, 4H) [6(9)-H and 12(15)-H], 6.06 (broad s, 2H) and 6.11 (broad s, 2H) [7(8)-H and 13(14)-H], 7.92 (d, *J* = 8.0 Hz, 1 H, Ar-5-H), 8.17 (dd, *J* = 8.0 Hz, *J'* = 1.6 Hz, 1 H, Ar-6-H), 8.33 (d, *J* = 1.6 Hz, 1 H, Ar-2H), 11.42 (s, 1 H, NH) ppm.

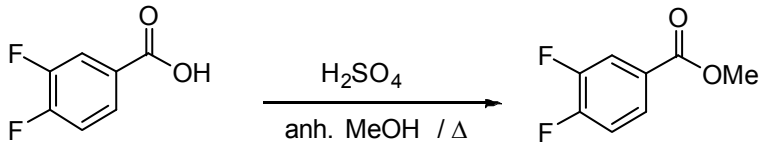
$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.86 (CH) and 61.90 (CH) [C6(9) and C12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.7 [C, C1(5)], 122.4 (C, q, $J_{C\text{-}F}$ = 273.0 Hz, $CF_3$), 126.9 (CH, q, $J_{C\text{-}F}$ = 5.4 Hz, Ar-C2), 127.2 (C, q, $J_{C\text{-}F}$ = 30.4 Hz, Ar-C3), 130.1 (C, Ar-C1), 132.0 (CH) and 132.3 (CH) [C7(8) and C13(14)], 132.4 (CH, Ar-C5), 133.3, (CH, Ar-C6), 135.0, (C, m, Ar-C4), 161.6 (C, CONH), 171.8 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 446 (1) [M, $^{35}Cl]^{\cdot+}$, 383 (24) [M−$C_5H_5$, $^{37}Cl]^+$, 382 (14), 381 (72) [M−$C_5H_5$, $^{35}Cl]^+$, 209 (33) [$CF_3$-$C_6H_3$Cl-CO, $^{37}Cl]^+$, 207 (100) [$CF_3$-$C_6H_3$Cl-CO, $^{35}Cl]^+$, 181 (21) [$CF_3$-$C_6H_3$Cl, $^{37}Cl]^+$, 179 (32) [$CF_3$-$C_6H_3$Cl, $^{35}Cl]^+$, 154 (17), 153 (41), 152 (30).

Elemental analysis

| | | | | | |
|---|---|---|---|---|---|
| Calculated for $C_{22}H_{14}ClF_3N_2O_3$: | C 59.14% | H 3.16% | N 6.27% | Cl 7.93% | F 12.76% |
| Calculated for $C_{22}H_{14}ClF_3N_2O_3 \cdot 0.1\ C_5H_{12}$: | C 59.52% | H 3.37% | N 6.17% | Cl 7.81% | F 12.55% |
| Found: | C 59.56% | H 3.29% | N 6.11% | | |

**Synthesis of methyl 3,4-difluorobenzoate, 272.**

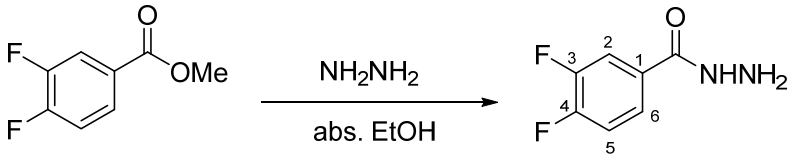



**272**

In a round-bottomed flask of 50 mL equipped with a condenser, argon atmosphere and a magnetic stirrer, a solution of 3,4-difluorobenzoic acid (500 mg, 3.16 mmol), anhydrous methanol (27 mL) and concentrated sulfuric acid (1.4 mL), was prepared. The solution was heated under reflux overnight. The solution was concentrated under vacuo and the residue was dissolved in $CH_2Cl_2$ (50 mL) and $H_2O$ (30 mL). The layers were separated and the aqueous one was extracted with $CH_2Cl_2$ (3 x 30 mL). The combined extracts were dried over $Na_2SO_4$,

filtered and concentrated under vacuo to give pure the carboxylate **272** (390 mg, 72% yield), whose spectroscopic data coincide with the ones described in the bibliography.[199]

**Synthesis of 3,4,-difluorobenzohydrazide, 273.**

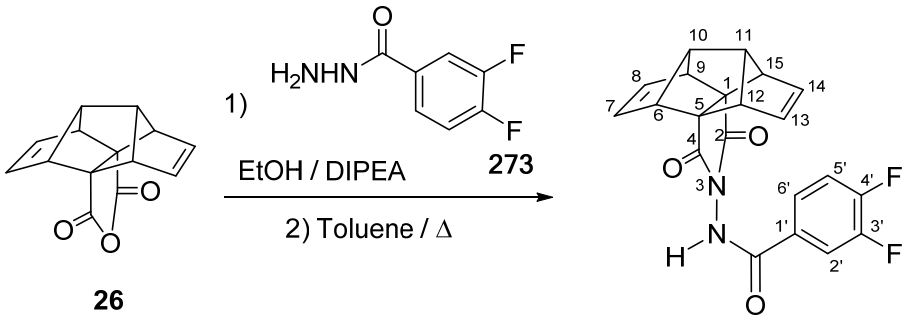



**272** **273**

In a round-bottomed flask of 25 mL with a gas outlet and a magnetic stirrer a solution of methyl 3,4-difluorobenzoate (390 mg, 2.27 mmol), absolute ethanol (2.5 mL) and hydrazine monohydrate (0.2 mL, 2.7 mmol), was prepared, the solution was stirred at room temperature for 48 hours. The colourless solution was concentrated under vacuo to give the benzohydrazide **273** as a white solid (276 mg, 87% yield).

$^1$H-RMN (300 MHz, DMSO-$d_6$) δ: 4.74 (bs, 2H, $NH_2$), 7.54 (dt, *J* = 10.5 Hz, *J'* = 8.4 Hz, 1 H, Ar-5-H), 7.71 (m, *J* = 1.2 Hz, 1 H, Ar-6-H), 8.26 (ddd, *J* = 11.8 Hz, *J'* = 7.8 Hz, *J''* = 2.1 Hz, 1 H, Ar-2-H), 10.19 (bs, 1 H, CONH).

**Synthesis of (1*r*,5*s*,6*R*,9*S*,10*s*,11*r*,12*S*,15*R*)-3-[(3,4-difluorobenzoyl)amino]-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-2,4-dione, 55.**




**26** **55**

This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **26** (200 mg, 0.88 mmol) and 3,4-difluorobenzoic acid hydrazide **273** (152 mg, 0.88 mmol), benzamide **55** was isolated as a white solid (322 mg, 96% yield). An analytical sample of **55** was obtained by crystallization from $CH_2Cl_2$/pentane.

[199] Rando, D. G.; Avery, M. A.; Tekwani, B. L.; Khan, S. I.; Ferreira, E. I. *Bioorg Med. Chem.* **2008**, *16*, 6724-6731.

Analytic and spectroscopic data of **55**.

Melting point: 243-244 ºC ($CH_2Cl_2$).

IR (KBr) *v*: 3198, 3064, 2988, 1789, 1736, 1670, 1617,1606, 1539, 1508, 1432, 1397, 1341, 1320, 1295, 1244, 1202, 1183, 1155, 1132, 1116, 1021, 961, 821, 782, 714 $cm^{-1}$.

$^1$H-RMN (400 MHz, DMSO-$d_6$) δ: 2.85 (broad s, 2 H, 10-H and 11-H), 3.57 [broad s, 4 H, 6(9)-H and 12(15)-H], 6.05 (broad s, 2 H) and 6.11 (broad s, 2 H) [7(8)-H and 13(14)-H], 7.61 (dt, *J* = 10.2 Hz, *J'* = 8.4 Hz, 1 H, Ar-5-H), 7.79 (m, 1 H, Ar-6-H), 7.92 (ddd, *J* = 11.2 Hz, *J'* = 8.0 Hz, *J"* = 1.8 Hz, 1 H, Ar-2-H), 11.17 (s, 1 H, NH) ppm.

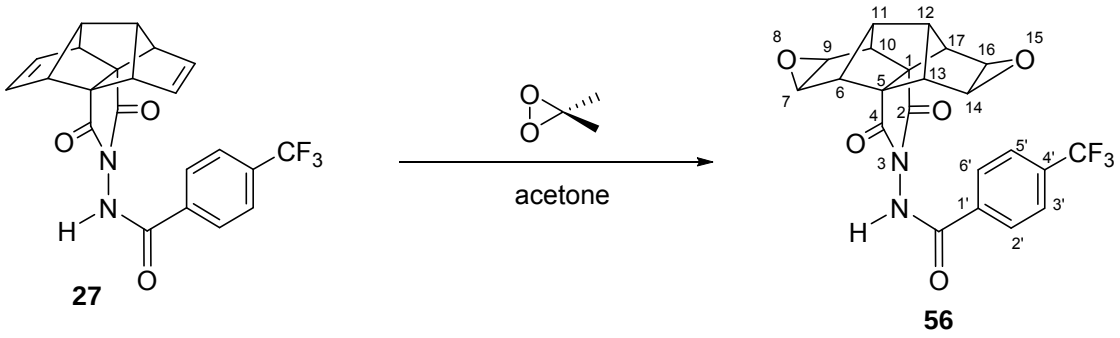
$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 61.86 (CH) and 61.91 (CH) [C6(9) and 12(15)], 63.5 (CH) and 64.0 (CH) (C10 and C11), 64.6 [C, C1(5)], 117.2 (CH, d, $J_{C\text{-}F}$ = 18.6 Hz, Ar-C2), 118.1 (CH, d, $J_{C\text{-}F}$ = 17.9 Hz, Ar-C6), 125.3 (CH, m, Ar-C5), 128.3 (C, broad s, Ar-C1), 132.0 (CH) and 132.3 (CH) [C7(8) and C13(14)], 149.3 (C, dd, $J_{C\text{-}F}$ = 248.8 Hz, $J'_{C\text{-}F}$ = 13.2 Hz, Ar-C3), 152.1 (C, dd, $J_{C\text{-}F}$ = 252.1 Hz, $J'_{C\text{-}F}$ = 12.5 Hz, Ar-C4), 161.7 (C, CONH), 171.9 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 380 (1) $[M]^{\cdot+}$, 316 (14), 315 (77) $[M\text{-}C_5H_5]^+$, 153 (23), 152 (18), 141 (100) $[C_6H_3F_2\text{-}CO]^+$, 113 (23) $[C_6H_3F_2]^+$.

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{21}H_{14}F_2N_2O_3$: | C 66.31% | H 3.71% | N 7.37% | F 9.99% |
| Found: | C 66.23% | H 3.70% | N 7.29% | F 9.89% |

**Synthesis of (1*r*,5*s*,6*R*,7*R*,9*S*,10*S*,11*s*,12*r*,13*S*,14*S*,16*R*,17*R*)-3-[4-(trifluoromethyl)benzoylamino]-3-aza-8,15-dioxaoctacyclo [8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]heptadecane-2,4-dione, 56.**




In a 50 mL round-bottomed flask equipped with a magnetic stirrer and a gas outlet the benzamide **27** (70 mg, 0.17 mmol) and a solution of dimethyldioxirane in acetone (25 mL) were added. The solution was stirred at room temperature overnight. After this, the solution was concentrated under vacuo to give pure (1*r*,5*s*,6*R*,7*R*,9*S*,10*S*,11*s*,12*r*,13*S*,14*S*,16*R*,17*R*)-3-[4-(Trifluoromethyl)benzoylamino]-3-aza-8,15-dioxaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]

heptadecane-2,4-dione **56** as a white solid. (75 mg, quantitative yield). An analytical sample of **56** was obtained by crystallization from a mixture $CH_2Cl_2$/hexane.

Analytic and spectroscopic data of **56**.

Melting point: 290 $^oC$ decompose (acetone).

IR (KBr) *v*: 3248, 3047, 3018, 1792, 1741, 1703, 1582, 1521, 1497, 1407, 1398, 1326, 1305, 1268, 1247, 1228, 1164, 1133, 1115, 1100, 1065, 1012, 978, 845, 695 $cm^{-1}$.

$^1$H-RMN (500 MHz, DMSO-$d_6$) δ: 2.14 (broad s, 2 H, 11-H and 12-H), 3.31 (broad s, 4 H, 6(10)-H and 13(17)-H], 3.36 (broad s, 2 H) and 3.43 (broad s, 2 H) [7(9)-H and 14(16)-H], 7.95 [d, *J* = 8.3 Hz, 2 H, Ar-3(5)-H], 8.11 [d, *J* = 8.3 Hz, 2 H, Ar-2(6)-H], 11.62 (s, 1 H, NH) ppm.

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 38.4 (CH) and 39.0 (CH) (C11 and C12), 47.3 (CH) and 47.7 (CH) [C7(9) and C14(16)], 54.8 (CH) and 55.1 (CH) [C6(10) and C13(17)], 59.2 [C, C1(5)], 123.7 (C, q, $J_{C\text{-}F}$ = 272.8 Hz, $CF_3$), 125.9 [CH, q, $J_{C\text{-}F}$ = 3.8 Hz, Ar-C3(5)], 128.8 [CH, Ar-C2(6)], 132.5 (C, q, $J_{C\text{-}F}$ = 31.8 Hz, Ar-C4), 134.3 (C, Ar-C1), 163.4 (C, CONH), 171.0 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: (4) $[M]^{\cdot+}$, 393 (4) $[M-F]^+$, 173 (100) $[CF_3\text{-}C_6H_4\text{-}CO]^+$, 145 (29) $[CF_3\text{-}C_6H_4]^+$, 81 (20).

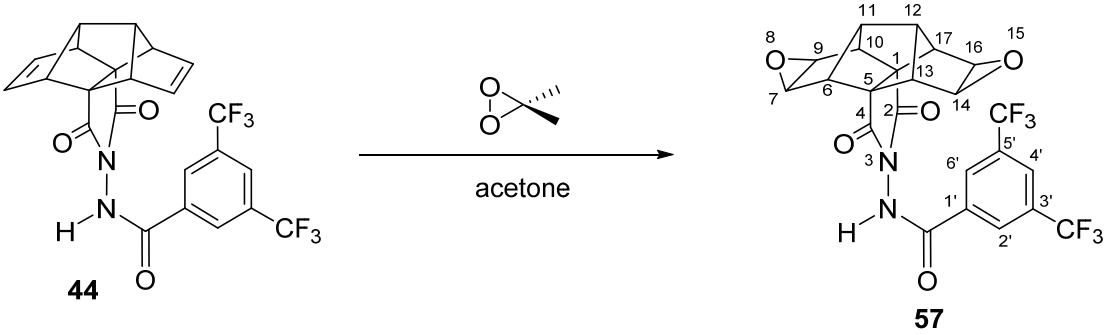
Elemental analysis

| | | | | | | |
|---|---|---|---|---|---|---|
| Calculated for $C_{22}H_{15}F_3N_2O_5$: | C | 59.46% | H | 3.40% | N | 6.30% | F | 12.83% |
| Calculated for $C_{22}H_{15}F_3N_2O_5 \cdot 0.25\ C_3H_6O$: | C | 59.55% | H | 3.62% | N | 6.10% | F | 12.42% |
| Found: | C | 59.38% | H | 3.82% | N | 5.84% | F | 12.15% |

Accurate mass

| | |
|---|---|
| Calculated for $C_{22}H_{15}F_3N_2O_5$: | 445.1006 |
| Found: | 445.1007 |

**Synthesis of (1*r*,5*s*,6*R*,7*R*,9*S*,10*S*,11*s*,12*r*,13*S*,14*S*,16*R*,17*R*)-3-[(3,5-bis(trifluoromethyl)benzoyl)amino]-3-aza-8,15-dioxaoctacyclo [8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]heptadecane-2,4-dione, 57.**




This compound was obtained as described before for **56**. Starting from benzamide **44** (80 mg, 0.17 mmol), compound **57** was isolated as a white solid (85 mg, quantitative yield). An analytical sample of **57** was obtained by crystallization from a mixture $CH_2Cl_2$/hexane.

Analytic and spectroscopic data of **57**.

Melting point: 265-266 ºC ($CH_2Cl_2$).

IR (KBr) *v*: 3272, 2924, 2855, 1791, 1734, 1623, 1526, 1458, 1382, 1280, 1184, 1136, 1016, 995, 852, 824, 681 cm$^{-1}$.

$^1$H-RMN (500 MHz, DMSO-$d_6$) δ: 2.14 (broad s, 2 H, 11-H and 12-H), 3.33 (broad s, 4 H, 6(10)-H and 13(17)-H], 3.35 (broad s, 2 H) and 3.45 (broad s, 2 H) [7(9)-H and 14(16)-H], 8.48 (broad s, 1 H, Ar-4-H), 8.56 [broad s, 2 H, Ar-2(6)-H], 11.93 (s, 1 H, NH) ppm.

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 38.4 (CH) and 39.0 (CH) (C11 and C12), 47.2 (CH) and 47.6 (CH) [C7(9) and C14(16)], 54.8 (CH) and 55.1 (CH) [C6(10) and C13(17)], 59.3 [C, C1(5)], 122.9 (C, q, $J_{C\text{-}F}$ = 272.8 Hz, $CF_3$), 126.6 [CH, m, Ar-C4), 128.5 [CH, m, ArC2(6)], 131.0 (C, q, $J_{C\text{-}F}$ = 33.5 Hz, Ar-C3(5)], 132.6 (C, Ar-C1), 161.8 (C, CONH), 170.8 [C, C2(4)] ppm.

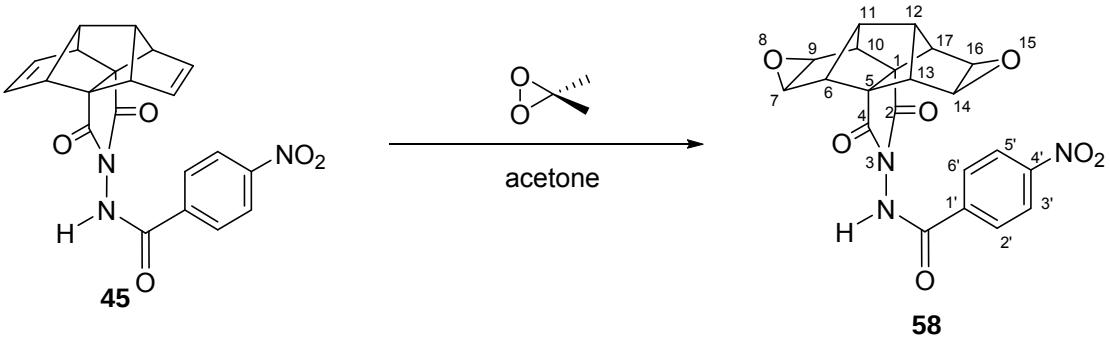
MS (EI), m/e (%); main ions: 512 (2) $[M]^{\cdot+}$, 241 (100) $[(CF_3)_2C_6H_3\text{-}CO]^+$, 213 (13) $[(CF_3)_2C_6H_3]^+$, 81 (85).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{23}H_{14}F_6N_2O_5$: | C 53.92% | H 2.75% | N 5.47% | F 22.25% |
| Found: | C 53.57% | H 3.22% | N 5.21% | |

Accurate mass
Calculated for $C_{23}H_{14}F_6N_2O_5$: 513.088
Found: 513.0879

**Synthesis of (1*r*,5*s*,6*R*,7*R*,9*S*,10*S*,11*s*,12*r*,13*S*,14*S*,16*R*,17*R*)-3-[(4-nitrobenzoyl)amino]-3-aza-8,15-dioxaoctacyclo [8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]heptadecane-2,4-dione, 58.**




This compound was obtained as described before for **56**. Starting from benzamide **45** (165 mg, 0.17 mmol), compound **58** was isolated as a yellow solid (157 mg, 87.9% yield).

Analytic and spectroscopic data of **58**.

Melting point: 255-256 °C decompose (acetone).

IR (KBr) *v*: 3258, 3039, 2992, 1793, 1741, 1699, 1604, 1560, 1525, 1478, 1396, 1348, 1320, 1266, 1187, 1165, 1138, 1097, 1012, 929, 851, 719 cm$^{-1}$.

$^{1}$H-RMN (500 MHz, DMSO-d$_6$) δ: 2.14 (s, 2H, 11-H y 12-H), 3.31 (s, 4 H) [6(10)-H y 13(17)-H], 3.36 (s, 2H) y 3.43 (s, 2H) [7(9)-H y 14(16)-H], 8.14 [d, *J* = 8.6 Hz, 2 H, Ar-2'(6')-H], 8.39 [d, *J* = 8.6 Hz, 2 H, Ar-3'(5')-H], 11.74 (s, 1 H, NH).

$^{13}$C-RMN (100.6 MHz, DMSO-d$_6$) δ: 38.4 (CH) y 38.9 (CH) (C11 y C12), 47.2 (s,CH) y 47.7 (s, CH) [C7(9) y C14(16)], 54.8 (s, CH) y 55.1 (s, CH) [C6(10) y 14(16)], 59.3 [C, C1(5)], 124.0 [CH, Ar-C3'(5')], 129.3 [CH, Ar-C2'(6')], 136.0 (C, Ar-C1'), 149.9 (C, Ar-C4'), 163.0 (C, CONH), 170.9 [C, C2(4)].

MS (EI), m/e (%); main ions: 421 (2) [M$^{\cdot+}$], 150 (100) [NO$_2$-C$_6$H$_4$CO$^+$], 120 (16), 104 (28) [C$_7$H$_5$O$^{\cdot}$], 92 (15), 81 (55) [C$_5$H$_5$O$^{\cdot}$], 76 (15) [C$_6$H$_5^{\cdot}$].

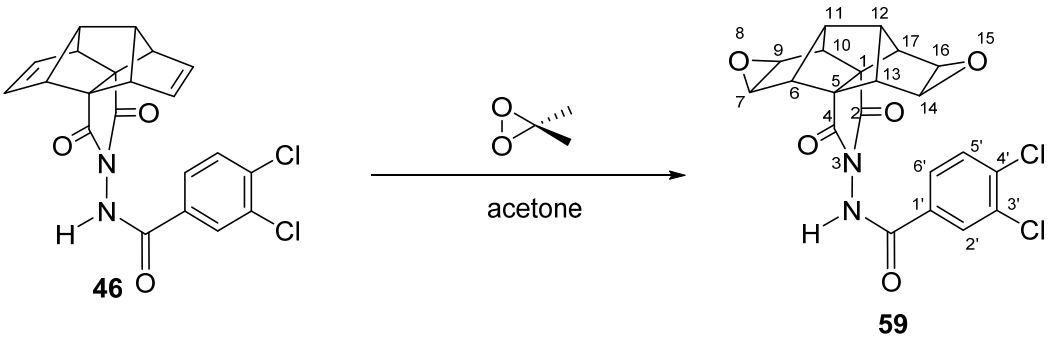
Elemental analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{21}H_{25}N_3O_7$: | C 59.86% | H 3.59% | N 9.97% |
| Calculated for $C_{21}H_{25}N_3O_7 \cdot 0.25\ C_4H_6O_3$: | C 59.13% | H 3.72% | N 9.40% |
| Found: | C 58.91% | H 3.80% | N 9.08% |

Accurate mass

| | |
|---|---|
| Calculated for $C_{21}H_{25}N_3O_7$: | 422.0983 |
| Found: | 422.0982 |

**Synthesis of (1*r*,5*s*,6*R*,7*R*,9*S*,10*S*,11*s*,12*r*,13*S*,14*S*,16*R*,17*R*)-3-[(3,4-dichlorobenzoyl)amino]-3-aza-8,15-dioxaoctacyclo [8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]heptadecane-2,4-dione, 59.**




This compound was obtained as described before for **56**. Starting from benzamide **46** (141 mg, 0.34 mmol), compound **59** was isolated as a white solid (150 mg, 99% yield). An analytical sample of **59** was obtained by crystallization from a mixture $CH_2Cl_2$/hexane.

Analytic and spectroscopic data of **59**.

Melting point: 275 °C decompose ($CH_2Cl_2$).

IR (KBr) *v*: 3286, 3020, 1792, 1739, 1720, 1699, 1686, 1589, 1557, 1500, 1460, 1398, 1288, 1239, 1205, 1186, 1165, 1132, 1109, 1064, 1031, 1012, 930, 852, 747 cm$^{-1}$.

$^1$H-RMN (500 MHz, DMSO-$d_6$) δ: 2.12 (broad s, 2 H, 11-H and 12-H), 3.29 (broad s, 4 H, 6(10)-H and 13(17)-H], 3.32 (broad s, 2 H) and 3.41 (broad s, 2 H) [7(9)-H and 14(16)-H], 7.84 (d, *J* = 8.4 Hz, 1 H, Ar-5-H), 7.88 (dd, *J* = 8.4 Hz, *J'* = 1.9 Hz, 1 H, Ar-6-H), 8.13 [d, *J* = 1.9 Hz, 1 H, Ar-2-H), 11.59 (s, 1 H, NH) ppm.

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 38.4 (CH) and 39.0 (CH) (C11 and C12), 47.2 (CH) and 47.7 (CH) [C7(9) and C14(16)], 54.8 (CH) and 55.1 (CH) [C6(10) and C13(17)], 59.2 [C, C1(5)], 127.9 (CH, Ar-C5), 129.6 (CH, Ar-C2), 131.3 (CH, Ar-C6), 130.8 (C), 131.8 (C) (Ar-C1 and Ar-C3), 135.8 (C, Ar-C4), 162.4 (C, CONH), 170.9 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 446 (4) [M, $^{37}Cl$ and $^{35}Cl]^{\cdot+}$, 444 (6) [M, 2 $^{35}Cl]^{\cdot+}$, 177 (10) [$C_6H_3Cl_2CO$, 2 $^{37}Cl]^+$, 175 (64) [$C_6H_3Cl_2CO$, $^{37}Cl$ and $^{35}Cl]^+$, 173 (100) [$C_6H_3Cl_2CO$, 2 $^{35}Cl]^+$, 147 (12) [$C_6H_3Cl_2$, $^{37}Cl$ and $^{35}Cl]^+$, 145 (19) [$C_6H_3Cl_2$, 2 $^{35}Cl]^+$.

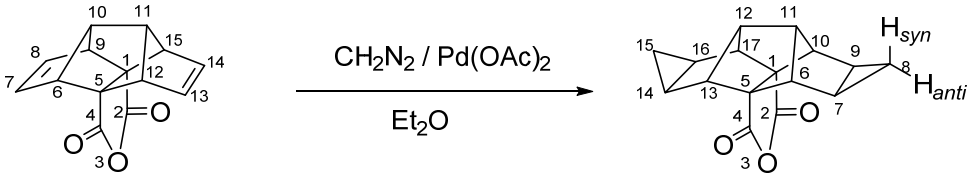
Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for: $C_{21}H_{14}Cl_2N_2O_5$: | C 56.65% | H 3.17% | N 6.29% | Cl 15.92% |
| Calculated for: $C_{21}H_{14}Cl_2N_2O_5 \cdot 0.5\ H_2O$: | C 54.98% | H 3.41% | N 6.11% | Cl 15.46% |
| Found: | C 54.88% | H 3.21% | N 5.86% | |

Accurate mass

| | |
|---|---|
| Calculated for: $C_{21}H_{14}ClN_2O_5$: | 445.0353 |
| Found: | 445.035 |

**Synthesis of the anhydride of (1*r*,5*s*,6*R*,7*S*,9*R*,10*S*,11*s*,12*r*,13*S*,14*R*,16*S*,17*R*)-3-oxaoctacyclo [8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]heptadecane-2,4-dione, 33.**




**26** **33**

Excess of an ethereal solution of diazomethane (100 mL) was added to a mixture of anhydride **26** (200 mg, 0.88 mmol) and $Pd(OAc)_2$ (5 mg, 0.02 mmol) and the suspension was stirred overnight at room temperature. The mixture was filtered and the filtrate was dried with anhydrous $Na_2SO_4$ and concentrated in vacuo to give a yellow solid, that was subjected to silica gel column chromatography (hexane/EtOAc mixtures) On elution with hexane/EtOAc 95:5, anhydride **33** (220 mg, 98% yield) was obtained as a white solid. An analytical sample of **33** was obtained by crystallization from a mixture $CH_2Cl_2$/pentane.

Analytic and spectroscopic data **33**.

Melting point: 180 $^oC$ ($CH_2Cl_2$).

IR (KBr) *v*: 3434, 3024, 2965, 1839, 1778, 1457, 1335, 1265, 1203, 1068, 1030, 911, 849, 816, 755, 679, 555, 526 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, DMSO-$d_6$) δ: 0.21 [dt, *J* = 5.6 Hz, *J'* = 7.3 Hz, 2 H, 8(15)-$H_{anti}$], 0.30 [dt, *J* = 5.6 Hz, *J'* = 3.3 Hz, 2 H, 8(15)-$H_{syn}$], 1.19 [dd, *J* = 7.3 Hz, *J'* = 3.3 Hz, 4 H, 7(9,14,16)-H], 2.00 [m, 2 H, 11(12)-H], 2.92 (dd, *J* = 1.8 Hz, *J'* = 0.8 Hz, 4 H, 6(10,13,17)-H] ppm.

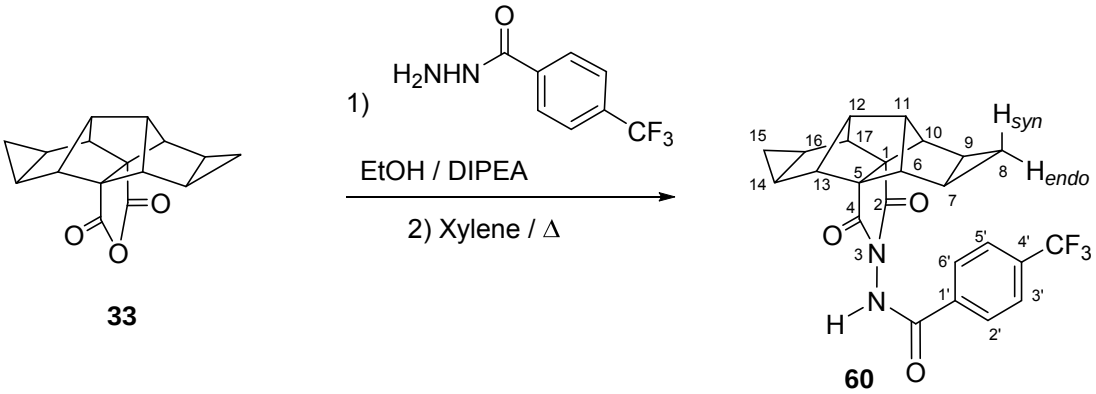
$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 2.1 [$CH_2$, C8(15)], 9.6 [CH, C7(9,14,16)], 39.7 [CH, C11(12)], 56.6 [CH, C6(10,13,17)], 66.3 [C, C1(5)], 170.7 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 254 (16) $[M]^{\cdot+}$, 210 (54) $[M\text{-}CO_2]^{\cdot+}$, 182 (29) $[M\text{-}C_2O_3]^{\cdot+}$, 167 (100), 166 (42), 165 (80), 153 (36), 152 (43), 141 (42), 128 (52), 115 (46), 104 (21), 103 (23), 91 (31) 79 (60).

Elemental analysis

| | | |
|---|---|---|
| Calculated for $C_{16}H_{14}O_3$: | C 75.57% | H 5.55% |
| Found: | C 75.49% | H 5.56% |

**Synthesis of (1*r*,5*s*,6*R*,7*S*,9*R*,10*S*,11*s*,12*r*,13*S*,14*R*,16*S*,17*R*)-3-[4-(trifluoromethyl)benzoylamino]-3-azaoctacyclo [8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]heptadecane-2,4-dione, 60.**




This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **33** (200 mg, 0.79 mmol) and 4-(trifluoromethyl)benzoic acid hydrazide (162 mg, 0.79 mmol), compound **60** was isolated as a white solid (182 mg, 52% yield). An analytical sample of **60** was obtained by crystallization from a mixture $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **60**.

Melting point: 258-259 °C ($CH_2Cl_2$).

IR (KBr) *v*: 3351, 3079, 3012, 2965, 1780, 1718, 1694, 1520, 1473, 1406, 1332, 1291, 1265, 1205, 1167, 1143, 1128, 1116, 1096, 1067, 1033, 1016, 930, 854, 817, 677 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, DMSO-$d_6$) δ: 0.16 (m, 2 H, 8-$H_{anti}$ and 15-$H_{anti}$), 0.35 (m, 2 H, 8-$H_{syn}$ and 15-$H_{syn}$), 0.96 (dd, *J* = 7.1 Hz, *J'* = 3.0 Hz, 2 H) and 1.04 (dd, *J* = 7.1 Hz, *J'* = 3.2 Hz, 2 H) [7(9)-H and 14(16)-H], 2.04 (m, 2 H, 11-H and 12-H), 2.86 (broad s, 4 H, 6(10)-H and 13(17)-H], 7.94 [d, *J* = 8.2 Hz, 2 H, Ar-3(5)-H], 8.12 [d, *J* = 8.2 Hz, 2 H, Ar-2(6)-H], 11.43 (s, 1 H, NH) ppm.

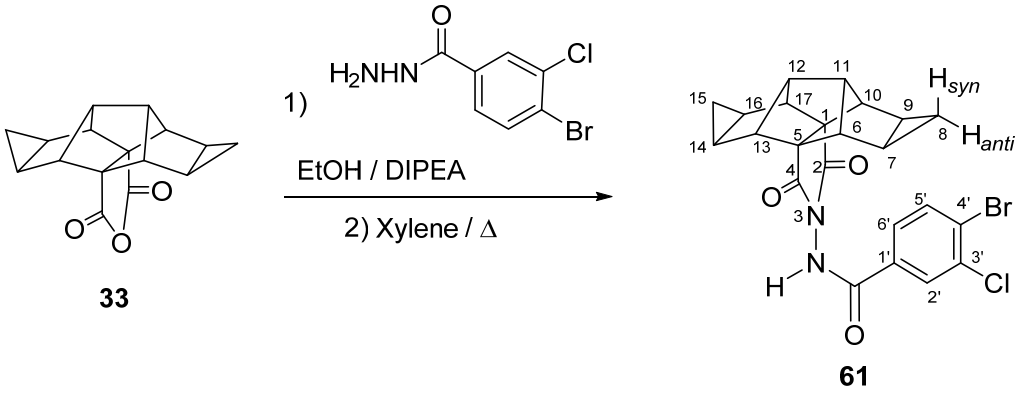
$^{13}$C-RMN (125.7 MHz, DMSO-$d_6$) δ: 1.8 ($CH_2$) and 2.0 ($CH_2$) (C8 and C15), 9.5 (CH) and 9.6 (CH) [C7(9) and C14(16)], 38.3 (CH) and 38.6 (CH) (C11 and C12), 54.4 (CH) and 54.7 (CH) [C6(10) and C13(17)], 62.5 [C, C1(5)], 123.7 (C, q, $J_{C\text{-}F}$ = 272.8 Hz, $CF_3$), 125.8 [CH, q, $J_{C\text{-}F}$ = 3.9 Hz, Ar-C3(5)], 128.6 [CH, Ar-C2(6)], 132.2 (C, q, $J_{C\text{-}F}$ = 32.3 Hz, Ar-C4), 134.7 (C, Ar-C1), 163.2 (C, CONH), 172.9 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 440 (4) $[M]^{\cdot+}$, 421 (2) $[M-F]^{+}$, 267 (4) $[M-(CF_3\text{-}C_6H_4\text{-}CO)]^{+}$, 173 (100) $[CF_3\text{-}C_6H_4\text{-}CO]^{+}$, 145 (22) $[CF_3\text{-}C_6H_4]^{+}$.

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{24}H_{19}F_3N_2O_5$: | C 65.45% | H 4.35% | N 6.36% | F 12.94% |
| Found: | C 65.55% | H 4.59% | N 6.21% | F 12.76% |

**Synthesis of (1*r*,5*s*,6*R*,7*S*,9*R*,10*S*,11*s*,12*r*,13*S*,14*R*,16*S*,17*R*)-3-[(4-bromo-3-chlorobenzoyl)amino]-3-azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$] heptadecane-2,4-dione, 61.**




This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **33** (200 mg, 0.79 mmol) and 4-bromo-3-chlorobenzoic acid hydrazide (197 mg, 0.79 mmol), compound **61** was isolated as a white solid (159 mg, 41% yield). An analytical sample of **61** was obtained by crystallization from a mixture $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **61**.

Melting point: 294-295 °C ($CH_2Cl_2$).

IR (KBr) *v*: 3328, 3074, 3007, 2961, 2935, 1780, 1716, 1587, 1558, 1488, 1457, 1401, 1337, 1290, 1261, 1233, 1203, 1173, 1144, 1096, 1054, 1020, 928, 890, 816, 745, 676 $cm^{-1}$.

$^1$H-RMN (500 MHz, DMSO-$d_6$) δ: 0.15 (m, 2 H, 8-$H_{anti}$ and 15-$H_{anti}$), 0.33 (m, 2 H, 8-$H_{syn}$ and 15-$H_{syn}$), 0.95 (dd, *J* = 6.9 Hz, *J'* = 2.8 Hz, 2 H) and 1.02 (dd, *J* = 6.9 Hz, *J'* = 3.1 Hz, 2 H) [7(9)-H and 14(16)-H], 2.03 (m, 2 H, 11-H and 12-H), 2.85 (broad s, 4 H, 6(10)-H and 13(17)-H], 7.79 (dd, *J* = 8.4 Hz, *J'* = 2.0 Hz, 1 H, Ar-6-H), 7.99 (d, *J* = 8.4 Hz, 2 H, Ar-5-H), 8.12 [d, *J* = 2.0 Hz, 1 H, Ar-2-H), 11.39 (s, 1 H, NH) ppm.

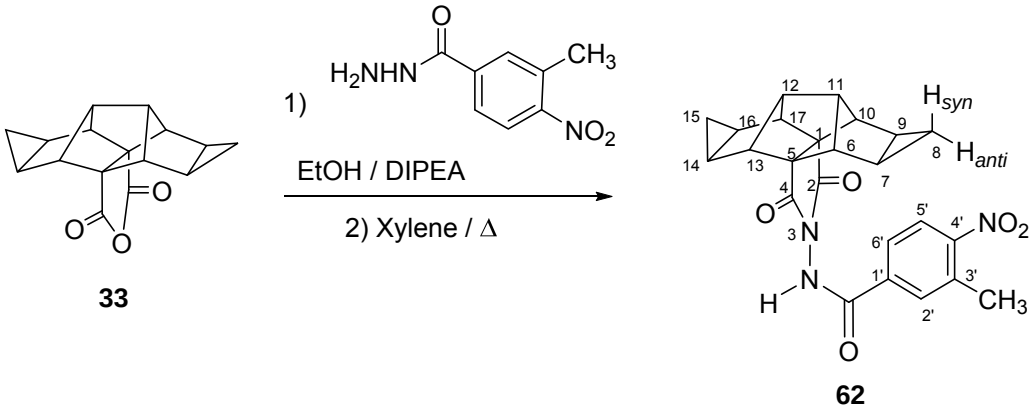
$^{13}$C-RMN (125.7 MHz, DMSO-$d_6$) δ: 1.8 ($CH_2$) and 2.0 ($CH_2$) (C8 and C15), 9.5 (CH) and 9.6 (CH) [C7(9) and C14(16)], 38.3 (CH) and 38.6 (CH) (C11 and C12), 54.4 (CH) and 54.7 (CH) [C6(10) and C13(17)], 62.5 [C, C1(5)], 126.4 (C, Ar-C4), 127.8 (CH, Ar-C6), 129.3 (CH, Ar-C2), 131.8 (C, Ar-C3), 133.8 (C, Ar-C1), 134.5 (CH, Ar-C5), 162.3 (C, CONH), 172.9 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 488 (2) [M, $^{81}Br^{37}Cl]^{\cdot+}$, 486 (5) [M, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^{\cdot+}$, 484 (4) [M, $^{79}Br^{35}Cl]^{\cdot+}$, 267 (8) [M−$C_6H_3BrCl$-CO]$^+$, 221 (22) [$C_6H_3BrCl$-CO, $^{81}Br^{37}Cl]^+$, 219 (100) [$C_6H_3BrCl$-CO, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^+$, 217 (78) [$C_6H_3BrCl$-CO, $^{79}Br^{35}Cl]^+$, 191 (13) [$C_6H_3BrCl$, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^+$, 189 (12) [$C_6H_3BrCl$, $^{79}Br^{35}Cl]^+$, 165 (13).

Elemental analysis

| | | | | | |
|---|---|---|---|---|---|
| Calculated for $C_{23}H_{18}BrClN_2O_3$: | C 56.87% | H 3.73% | N 5.77% | Br 16.45% | Cl 7.30% |
| Found: | C 56.76% | H 3.77% | N 5.58% | Br 17.03% | Cl 7.01% |

**Synthesis of (1*r*,5*s*,6*R*,7*S*,9*R*,10*S*,11*s*,12*r*,13*S*,14*R*,16*S*,17*R*)-3-[(3-methyl-4-nitrobenzoyl)amino]-3-azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$] heptadecane-2,4-dione, 62.**




**33** **62**

This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **33** (200 mg, 0.79 mmol) and 3-methyl-4-nitrobenzoic acid hydrazide (154 mg, 0.79 mmol), compound **62** was isolated as a white solid (236 mg, 69% yield). An analytical sample of **62** was obtained by crystallization from a mixture $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **62**.

Melting point:>300 °C ($CH_2Cl_2$).

IR (KBr) *v*: 3323, 3079, 3011, 2963, 1779, 1716, 1698, 1609, 1588, 1518, 1471, 1401, 1385, 1354, 1340, 1315, 1289, 1276, 1256, 1224, 1203, 1186, 1171, 1143, 1113, 1095, 1076, 1055, 1029, 928, 819, 731, 675 $cm^{-1}$.

$^1$H-RMN (500 MHz, DMSO-$d_6$) δ: 0.16 (m, 2 H, 8-$H_{anti}$ and 15-$H_{anti}$), 0.34 (m, 2 H, 8-$H_{syn}$ and 15-$H_{syn}$), 0.96 (m, 2 H) and 1.04 (m, 2 H) [7(9)-H and 14(16)-H], 2.04 (broad s, 2 H, 11-H and 12-H), 2.56 (s, 3 H, Ar-$CH_3$), 2.86 (broad s, 4 H, 6(10)-H and 13(17)-H], 7.94 (d, *J* = 8.4 Hz, 1 H, Ar-6-H), 8.03 (s, 1 H, Ar-2H), 8.12 (d, *J* = 8.4 Hz, 1 H, Ar-5-H), 11.44 (s, 1 H, NH) ppm.

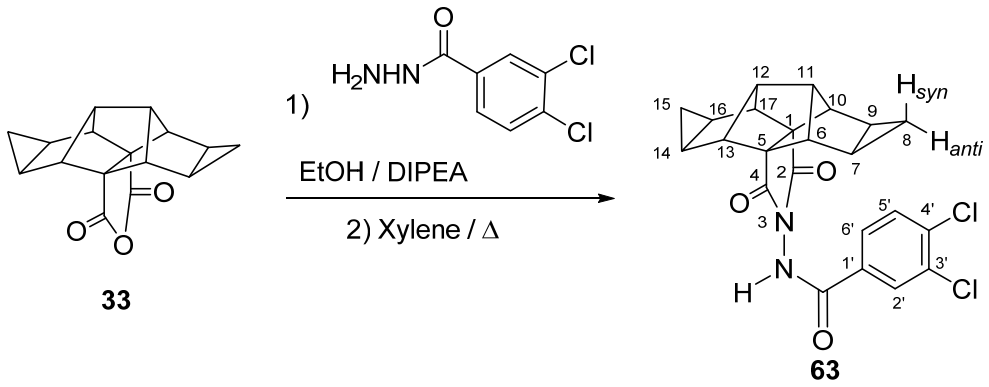
$^{13}$C-RMN (125.7 MHz, DMSO-$d_6$) δ: 1.8 ($CH_2$) and 2.0 ($CH_2$) (C8 and C15), 9.5 (CH) and 9.6 (CH) [C7(9) and C14(16)], 19.2 ($CH_3$, Ar-$CH_3$), 38.3 (CH) and 38.6 (CH) (C11 and C12), 54.4 (CH) and 54.7 (CH) [C6(10) and C13(17)], 62.5 [C, C1(5)], 124.7 (CH, Ar-C5), 126.4 (CH, Ar-C6), 132.2 (CH, Ar-C2), 133.0 (C, Ar-C3), 134.6 (C, Ar-C1), 151.1 (C, Ar-C4), 162.8 (C, CONH), 172.8 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions: 431 (7) $[M]^{\cdot+}$, 267 (5) $[M-(CH_3)(NO_2)C_6H_3\text{-}CO]^+$, 207 (9), 165 (21), 164 (100) $[CH_3\text{-}C_6H_3\text{-}CO]^+$, 134 (40), $[CH_3\text{-}C_6H_3\text{-}CONH_2]^+$, 118 (20) $[CH_3\text{-}C_6H_3\text{-}CO]^+$.

Elemental analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{24}H_{21}N_3O_5$: | C 66.81% | H 4.91% | N 9.74% |
| Calculated for $C_{24}H_{21}N_3O_5 \cdot 0.3\ H_2O$: | C 65.99% | H 4.98% | N 9.62% |
| Found: | C 65.98% | H 4.98% | N 9.54% |

**Synthesis of (1*r*,5*s*,6*R*,7*S*,9*R*,10*S*,11*s*,12*r*,13*S*,14*R*,16*S*,17*R*)-3-[(3,4-Dichlorobenzoyl)amino]-3-azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$] heptadecane-2,4-dione, 63.**




This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **33** (200 mg, 0.79 mmol) and 3,4-dichlorobenzoic acid hydrazide (162 mg, 0.79 mmol), compound **63** was isolated as a white solid (173 mg, 49% yield). An analytical sample of **63** was obtained by crystallization from a mixture $CH_2Cl_2$/pentane.

Analytic and spectroscopic of **63**.

Melting point: 272-273 ºC ($CH_2Cl_2$).

IR (KBr) *v*: 3486, 3307, 3070, 3007, 2986, 1780, 1715, 1696, 1589, 1558, 1488, 1461, 1400, 1337, 1290, 1274, 1249, 1239, 1203, 1172, 1140, 1106, 1093, 1054, 1028, 998, 890, 816, 675 cm$^{-1}$.

$^1$H-RMN (500 MHz, DMSO-d$_6$) δ: 0.16 (m, 2 H, 8-H$_{anti}$ and 15-H$_{anti}$), 0.34 (m, 2 H, 8-H$_{syn}$ and 15-H$_{syn}$), 0.95 (dd, *J* = 6.8 Hz, *J'* = 2.6 Hz, 2 H) and 1.02 (dd, *J* = 6.8 Hz, *J'* = 3.0 Hz, 2 H) [7(9)-H and 14(16)-H], 2.03 (m, 2 H, 11-H and 12-H), 2.85 (broad s, 4 H, 6(10)-H and 13(17)-H], 7.85 (d, *J* = 8.4 Hz, 2 H, Ar-5-H), 7.90 (dd, *J* = 8.4 Hz, *J'* = 2.0 Hz, 1 H, Ar-6-H), 8.15 [d, *J* = 2.0 Hz, 1 H, Ar-2-H), 11.37 (s, 1 H, NH) ppm.

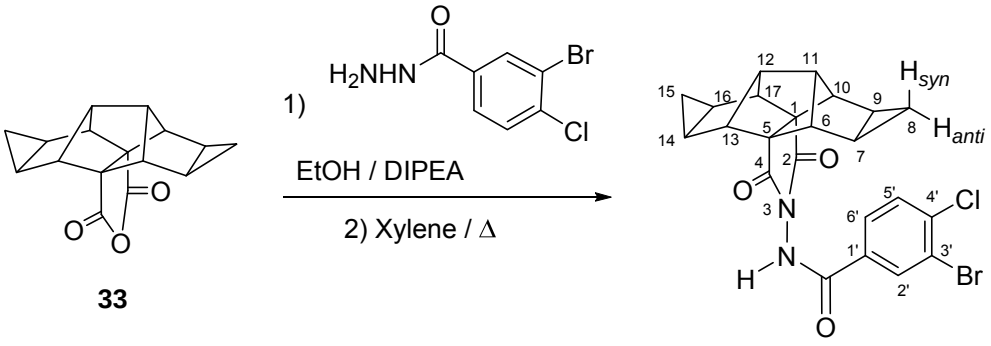
$^{13}$C-RMN (125.7 MHz, DMSO-d$_6$) δ: 1.8 ($CH_2$) and 2.0 ($CH_2$) (C8 and C15), 9.5 (CH) and 9.6 (CH) [C7(9) and C14(16)], 38.3 (CH) and 38.6 (CH) (C11 and C12), 54.4 (CH) and 54.7 (CH) [C6(10) and C13(17)], 62.5 [C, C1(5)], 127.8 (CH, Ar-C5), 129.6 (CH, Ar-C2), 131.2 (CH, Ar-C6), 131.18 (C) and 131.7 (C) (Ar-C1 and Ar-C3), 135.5 (C, Ar-C4), 162.2 (C, CONH), 172.8 [C, C2(4)] ppm.

MS (EI), m/e (%); main ions:. 442 (2) [M, $^{37}$Cl and $^{35}$Cl]$^{\cdot+}$, 440 (4) [M, 2 $^{35}$Cl]$^{\cdot+}$, 267 (4) [M–$C_6H_3Cl_2CO$]$^+$, 177 (11) [$C_6H_3Cl_2CO$, 2 $^{37}$Cl]$^+$, 175 (64) [$C_6H_3Cl_2CO$, $^{37}$Cl and $^{35}$Cl]$^+$, 173 (100) [$C_6H_3Cl_2CO$, 2 $^{35}$Cl]$^+$, 145 (13) [$C_6H_3Cl_2$, 2 $^{35}$Cl]$^+$.

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{23}H_{18}Cl_2N_2O_3$: | C 62.60% | H 4.11% | N 6.35% | Cl 16.07% |
| Calculated for $C_{23}H_{18}Cl_2N_2O_3 \cdot 0.05\ CH_2Cl_2$: | C 62.14% | H 4.09% | N 6.29% | Cl 16.71% |
| Found: | C 62.21% | H 4.21% | N 6.06% | Cl 16.68% |

**Synthesis of (1*r*,5*s*,6*R*,7*S*,9*R*,10*S*,11*s*,12*r*,13*S*,14*R*,16*S*,17*R*)-3-[(3-Bromo-4-chlorobenzoyl)amino]-3-azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$] heptadecane-2,4-dione, 64.**




**33** **64**

This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **33** (240 mg, 0.94 mmol) and 3-bromo-4-chlorobenzoic acid hydrazide (235 mg, 0.94 mmol), compound **64** was isolated as a white solid (258 mg, 54% yield). An analytical sample of **64** was obtained by crystallization from a mixture $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **64**.

Melting point: 263-264 ºC ($CH_2Cl_2$).

IR (KBr) *v*: 3308, 3072, 3009, 2960, 1780, 1715, 1694, 1589, 1494, 1458, 1400, 1336, 1286, 1249, 1233, 1171, 1140, 1110, 1094, 1053, 1036, 1023, 890, 816, 748, 676 cm$^{-1}$.

$^1$H-RMN (500 MHz, DMSO-d$_6$) δ: 0.15 (m, 2 H, 8-H$_{anti}$ and 15-H$_{anti}$), 0.33 (m, 2 H, 8-H$_{syn}$ and 15-H$_{syn}$), 0.95 (m, 2 H) and 1.02 (m, 2 H) [7(9)-H and 14(16)-H], 2.04 (broad s, 2 H, 11-H and 12-H), 2.85 (broad s, 4 H, 6(10)-H and 13(17)-H], 7.83 (d, *J* = 8.4 Hz, 2 H, Ar-5-H), 7.93 (dd, *J* = 8.4 Hz, *J'* = 1.8 Hz, 1 H, Ar-6-H), 8.28 [d, *J* = 1.8 Hz, 1 H, Ar-2-H), 11.38 (s, 1 H, NH) ppm.

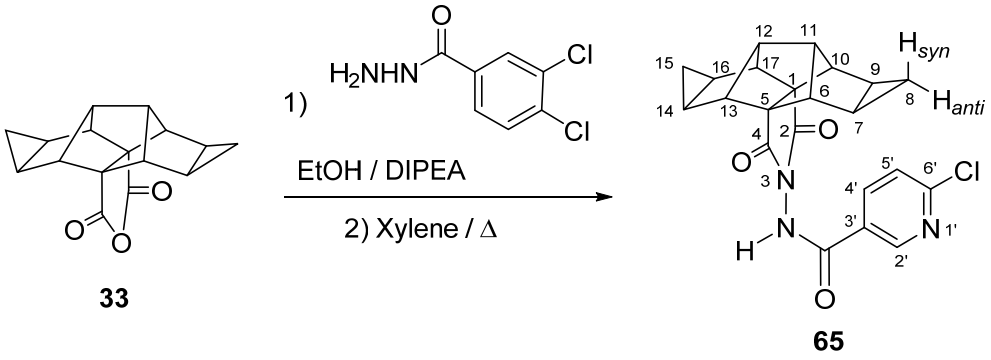
$^{13}$C-RMN (125.7 MHz, DMSO-d$_6$) δ: 1.8 ($CH_2$) and 2.0 ($CH_2$) (C8 and C15), 9.5 (CH) and 9.6 (CH) [C7(9) and C14(16)], 38.3 (CH) and 38.6 (CH) (C11 and C12), 54.4 (CH) and 54.7 (CH) [C6(10) and C13(17)], 62.5 [C, C1(5)], 121.9 (C, Ar-C3), 128.4 (CH, Ar-C6), 131.0 (CH, Ar-C5), 131.1 (C, Ar-C1), 132.7 (CH, Ar-C2), 137.5, (C, Ar-C4), 162.3 (C, CONH), 172.8 [C, C2(4)] ppm.

MS (IE), m/e (%); main ions: 488 (1) [M, $^{81}Br^{37}Cl]^{\cdot+}$, 486 (5) [M, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^{\cdot+}$, 484 (4) [M, $^{79}Br^{35}Cl]^{\cdot+}$, 267 (5) [M−$C_6H_3BrCl$-CO]$^+$, 221 (25) [$C_6H_3BrCl$-CO, $^{81}Br^{37}Cl]^+$, 219 (100) [$C_6H_3BrCl$-CO, $^{81}Br^{35}Cl$ and $^{79}Br^{37}Cl]^+$, 217 (77) [$C_6H_3BrCl$-CO, $^{79}Br^{35}Cl]^+$, 191 (15) [$C_6H_3BrCl$, $^{81}Br^{35}Cl$ and $^{79}Br^{35}C]^+$ 189 (12) [$C_6H_3BrCl$, $^{79}Br^{35}Cl]^+$, 165 (14).

Elemental analysis

| | | | | | |
|---|---|---|---|---|---|
| Calculated for $C_{23}H_{18}BrClN_2O_3$: | C 56.87% | H 3.73% | N 5.77% | Cl 7.30% | Br 16.45% |
| Found: | C 56.85% | H 3.66% | N 5.42% | Cl 6.95% | Br 16.83% |

**Synthesis of (1*r*,5*s*,6*R*,7*S*,9*R*,10*S*,11*s*,12*r*,13*S*,14*R*,16*S*,17*R*)-3-[(6-Chloro-3-pyridylcarbonyl)amino]-3-azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$] heptadecane-2,4-dione, 65.**




This compound was obtained in a similar manner to that described before for compound **42**. Starting from anhydride **33** (200 mg, 0.79 mmol) and 6-chloronicotinic acid hydrazide (136 mg, 0.79 mmol), compound **65** was isolated as a white solid (169 mg, 52% yield). An analytical sample of **65** was obtained by crystallization from a mixture $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of **65**.

Melting point: >300 °C ($CH_2Cl_2$).

IR (KBr) *v*: 3489, 3141, 3093, 3011, 2957, 1778, 1731, 1697, 1592, 1568, 1560, 1527, 1458, 1395, 1365, 1336, 1327, 1311, 1289, 1279, 1251, 1221, 1202, 1168, 1138, 1122, 1088, 1030, 995, 817, 677 cm$^{-1}$.

$^1$H-RMN (500 MHz, DMSO-$d_6$) δ: 0.18 (m, 2 H, 8-$H_{anti}$ and 15-$H_{anti}$), 0.36 (m, 2 H, 8-$H_{syn}$ and 15-$H_{syn}$), 0.98 (dd, *J* = 6.9 Hz, *J*' = 2.7 Hz, 2 H) and 1.05 (dd, *J* = 6.9 Hz, *J*' = 3.0 Hz, 2 H) [7(9)-H and 14(16)-H], 2.06 (broad s, 2 H, 11-H and 12-H), 2.88 (broad s, 4 H, 6(10)-H and 13(17)-H], 7.89

(dm, *J* = 8.4 Hz, 1 H, Pyr-5-H), 8.46 (dd, *J* = 8.4 Hz, *J'* = 2.5 Hz 1 H, Pyr-4-H), 9.06 (dd, *J* = 2.5 Hz, 1 H, Pyr-2-H), 11.52 (s, 1 H, NH) ppm.

$^{13}$C-RMN (125.7 MHz, DMSO-$d_6$) δ: 1.8 ($CH_2$) and 2.0 ($CH_2$) (C8 and C15), 9.5 (CH) and 9.6 (CH) [C7(9) and C14(16)], 38.3 (CH) and 38.6 (CH) (C11 and C12), 54.4 (CH) and 54.7 (CH) [C6(10) and C13(17)], 62.5 [C, C1(5)], 124.6 (CH, Pyr-C5), 126.1 (C, Pyr-C3), 138.9 (CH, Pyr-C4), 149.3 (CH, Pyr-C2), 153.8 (C, Pyr-C6), 162.0 (C, CONH), 172.8 [C, C2(4)] ppm.

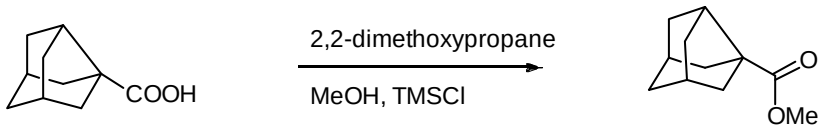
MS (EI), m/e (%); main ions: 409 (2) [M, $^{37}Cl]^{\cdot+}$, 407 (6) [M, $^{35}Cl]^{\cdot+}$, 267 (6) $[M-C_5H_3NClCO]^+$, 165 (12), 142 (34) $[C_5H_3NCl\text{-}CO]^+$, $^{37}Cl]^+$, 141 (12), 140 (100) $[C_5H_3NCl\text{-}CO]^+$, $^{35}Cl]^+$, 112 (18) $[C_5H_3Cl$, $^{35}Cl]^+$.

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{22}H_{18}ClN_3O_3$: | C 64.79% | H 4.45% | N 10.30% | Cl 10.30% |
| Found: | C 64.47% | H 4.46% | N 10.31% | Cl 8.87% |

# 2. SYNTHESIS OF 2,2-DIALKYLATED ADAMANTYLAMINES

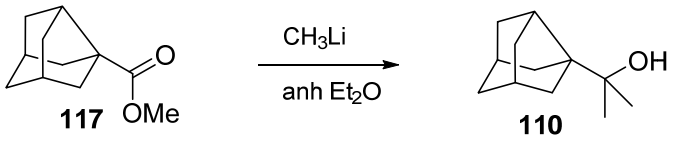
**Synthesis of methyl 3-noradamantanecarboxylate, 117.**




**116** **117**

In a 100 mL round-bottomed flask equipped with a gas outlet and magnetic stirring, a solution of 3-noradamantane carboxylic acid **116** (4 g, 24 mmol), 2,2-dimethoxypropane (40 mL), methanol (16 mL) and TMSCl (0.30 mL, d = 0.86 g $ml^{-1}$, 2.37 mmol) was stirred at room temperature for 24 h, then, the solvent was concentrated in vacuo obtaining a dark yellow oil (4.47 g). The residue was dissolved in methanol and activated carbon (1 g) was added, the suspension was stirred 2 h at room temperature. Afterwards, the suspension was filtered off obtaining ester **117** (3.63 g, 84% yield) as a light yellow oil, whose spectroscopical data concede with the reported.[141]

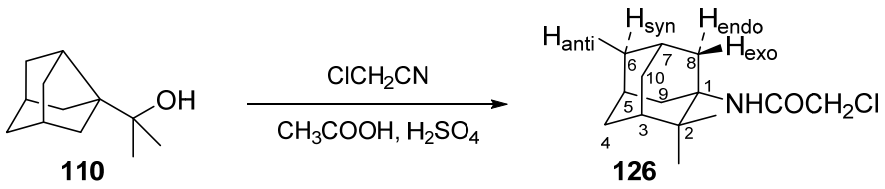
**Synthesis of 2-(3-noradamantyl)-2-propanol, 110.**




**117** **110**

To a stirred solution of **117** (3.63 g, 20 mmol) in anhydrous $Et_2O$ (80 mL) at 0 $^oC$, methyllithium (100 mL, 1.6 M in $Et_2O$, 160 mmol) was added dropwise. The reaction was heated under reflux overnight, cooled to 0 $^oC$, carefully quenched with $H_2O$ (14 mL) and stirred at 0 $^oC$ for one hour. The layers were separated and the aqueous one was extracted with $Et_2O$ (3x40 mL). The combined organic layers were dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to dryness to give the known alcohol **110** as a pale yellow oil (3.3 g, 91% yield).[138]

**Synthesis of *N*-(2,2-Dimethyladamant-1-yl)-2-chloroacetamide, 126.**




**110** **126**

A solution of alcohol **110** (3.16 g, 17.5 mmol) and chloroacetonitrile (5 mL, 79.2 mmol) in acetic acid (6.3 mL) was cooled to 0 $^{o}$C. Concentrated $H_2SO_4$ (6.3 mL) was added dropwise without exceeding 10 $^{o}$C. The reaction was stirred at room temperature overnight. Afterwards, the crude of the reaction was poured into ice (90 g) and the residue was filtered under vacuo. The filtrate was dissolved in $CH_2Cl_2$ (100 mL) and the organic layer was dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain chloroacetamide **126** as a white solid (3.98 g, 89% yield). The analytical sample was obtained by crystallization from EtOAc / Hexane.

Analytical and spectroscopical data **126**.

Melting point: 136-137 $^{o}$C

IR (KBr) *v*: 3311, 3220, 3089, 3014, 2993, 2957, 2916, 2900, 2880, 2677, 2361, 1682, 1661, 1560, 1487, 1464, 1450, 1391, 1365, 1347, 1323, 1272, 1252, 1219, 1165, 1146, 1112, 1091, 978, 969, 950, 926, 901, 849, 821, 795, 717, 651, 621, 539, 460 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CDCl_3$) δ: 1.11 (s, 6H, $2CH_3$), 1.49 [dm, *J* = 12.5 Hz, 2H, 4(10)-$H_{endo}$], 1.51 (bs, 1H, 3-H), 1.62 (dm, *J* = 12.5 Hz, 1H, 6-$H_{anti}$), 1.68 (dtt, *J* = 12.5 Hz, *J'* = 5.5 Hz, *J''* = 2.5 Hz, 1H, 6-$H_{syn}$), 2.04 [m, 2H, 5(7)-H], 2.06 [dm, *J* = 12.5 Hz, 2H, 4(10)-$H_{exo}$], 2.19 [dd, *J* = 13 Hz, *J'* = 1.5 Hz, 2H, 8(9)-$H_{exo}$], 2.31 [d, *J* = 13 Hz, 2H, 8(9)-$H_{endo}$], 3.96 (s, 2H, $C\underline{H}_2Cl$), 6.42 (bs, 1H, NH).

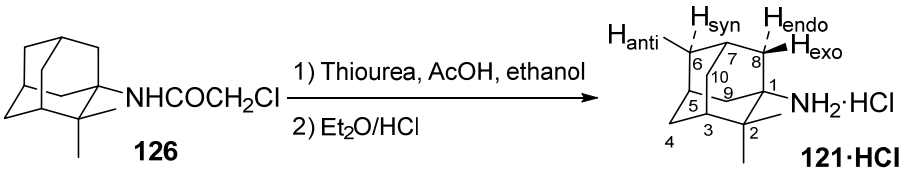
$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 23.7 ($CH_3$, $\underline{C}H_3$-C2), 29.6 [CH, C5(7)], 32.5 [$CH_2$, C4(10)], 36.4 [$CH_2$, C8(9)], 37.7 ($CH_2$, C6), 39.7 (C, C2), 40.6 (CH, C3), 43.4 ($CH_2$, $\underline{C}H_2Cl$), 57.5 (C, C1), 164.5 (C, CO).

GC/MS (EI), m/e (%); Main ions: 257 ($M^{\cdot+}$, 15), 255 (44), 221 (13), 220 (81), 212 (13), 172 (34), 171 (11), 170 (100), 163 [$(C_{12}H_{19})^{+}$, 25], 162 (44), 122 (11), 119 (10), 105 (11), 94 (16), 93 (10), 91 (14), 79 (22), 77 (14).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{14}H_{22}NClO$: | C 65.74% | H 8.67% | N 5.48% | Cl 13.86% |
| Calculated for $C_{14}H_{22}NClO{\cdot}0.15H_2O$: | C 65.05% | H 8.70% | N 5.42% | Cl 13.71% |
| Found: | C 65.05% | H 8.70% | N 5.40% | Cl 13.68% |

**Synthesis of (2,2-Dimethyladamant-1-yl)amine hydrocloride, 121·HCl.**




A solution of chloroacetamide **126** (3.98 g, 15.6 mmol), thiourea (1.43 g, 18.8 mmol), glacial acetic acid (6.2 mL) in absolute EtOH (30 mL) was heated under reflux overnight. The resulting suspension was cooled to room temperature and water (160 mL) was added. A solid was separated by filtration and the filtrate was basified with an aqueous solution of 5 N NaOH til pH = 14. The aqueous layer was extracted with EtOAc (3x150 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired amine **121** as a pale orange solid (2.65 g). The residue was dissolved in the minimum amount of EtOAc, treated with an excess of an ethereal solution of HCl and allowed to stand at 0 $^oC$ for 24 h. Filtration of the precipitate gave **121·HCl** as a white solid (2.63 g, 78% yield). An analytical sample was obtained by crystallization from EtOAc.

Analytical and spectroscopical data **121·HCl**.

Melting point: > 300 $^oC$ (decompose)

IR (KBr) $\nu$: 3418, 3205, 3003, 2930, 2868, 2764, 2614, 2590, 2518, 2467, 2358, 2049, 1614, 1519, 1487, 1465, 1454, 1395, 1376, 1358, 1344, 1311, 1298, 1161, 1082, 1001, 975, 921, 848, 818, 761, 554, 485 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 1.17 (s, 6H, $2CH_3$), 1.58 (bs, 1H, 3-H), 1.59 [dd, *J* = 12 Hz, *J'* = 2.5 Hz, 2H, 4(10)-$H_{endo}$], 1.65-1.69 [complex signal, 3 H, 6-$H_{syn}$ + 8(9)-$H_{endo}$], 1.76 (dm, *J* = 12.5 Hz, 1H, 6-$H_{anti}$), 2.10 [dm, *J* = 12 Hz, 2H, 4(10)-$H_{exo}$], 2.12 [m, 2H, 5(7)-H], 2.28 [dm, *J* = 12 Hz, 2H, 8(9)-$H_{exo}$], 4.89 (bs, $NH_3$).

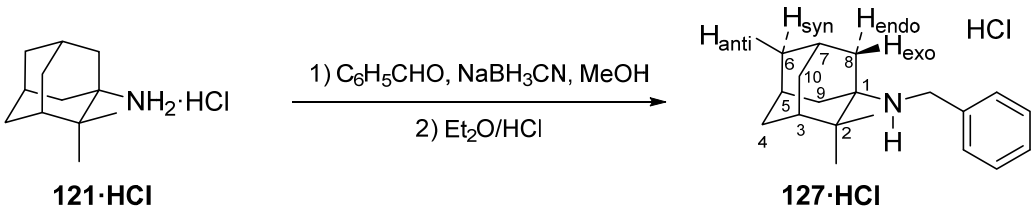
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 23.5 ($CH_3$, $\underline{C}H_3$-C2), 30.8 [CH, C5(7)], 32.9 [$CH_2$, C4(10)], 38.2 [$CH_2$, C8(9)], 38.3 ($CH_2$, C6), 39.4 (C, C2), 42.1 (CH, C3), 58.0 (C, C1).

GC/MS (EI), m/e (%); main ions: 179 ($M^{\cdot+}$, 23), 95 (10), 94 (100), 57 (10).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{12}H_{21}N$·HCl: | C 66.80% | H 10.28% | N 6.49% | Cl 16.43% |
| Found: | C 67.03% | H 10.39% | N 6.69% | Cl 16.32% |

**Synthesis of *N*-Benzyl(2,2-dimethyladamant-1-yl)amine hydrochloride, 127·HCl.**




**121·HCl** **127·HCl**

To a solution of **121·HCl** (0.393 g, 1.82 mmol) in methanol (5.3 mL), $NaBH_3CN$ (95% content, 0.243 g, 3.53 mmol), AcOH (0.16 mL) and benzaldehyde (0.26 mL, 2.56 mmol) were added and the mixture was magnetically stirred at room temperature for 2 h. Then, more $NaBH_3CN$ (95% content, 0.119 g, 1.75 mmol) and benzaldehyde (0.15 mL, 1.48 mmol) were added and stirring at room temperature was continued for 16 h more. The mixture was concentrated in vacuo, the residue was taken in water (11 mL), the solution was basified with an aqueous solution of 1N NaOH and extracted with EtOAc (3×6 mL). The combined organic extracts were washed with brine (2×6 mL), dried (anhydrous $Na_2SO_4$) and concentrated in vacuo. The residue was dissolved in the minimum amount of EtOAc, treated with an excess of an ethereal solution of HCl and allowed to stand at 0 $^{o}$C for 24 h. Filtration of the precipitate gave **127·HCl** (0.46 g, 83% yield) as a pale yellow solid. The analytical sample was obtained by crystallization from MeOH/$Et_2O$.

Analyticial and spectroscopical **127·HCl**.

Melting point: 239-240 $^{o}$C

IR (KBr) ν: 3418, 3054, 3042, 3011, 2991, 2921, 2888, 2782, 2703, 2535, 2441, 2333, 2145, 1966, 1903, 1579, 1565, 1499, 1455, 1397, 1377, 1358, 1342, 1308, 1294, 1276, 1241, 1216, 1172, 1146, 1110, 1097, 1088, 1058, 1033, 995, 977, 947, 925, 890, 844, 770, 755, 737, 698, 665, 618, 579, 541, 511, 470 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 1.32 (s, 6H, $2CH_3$), 1.57-1.63 [complex signal, 3H, 3-H + 4(10)-$H_{endo}$], 1.71 (dm, *J* = 12.5 Hz, 1H, 6-$H_{syn}$), 1.77 (dtt, *J* = 12.5 Hz, *J'* = 3 Hz, *J''* = 1.5 Hz, 1H, 6-$H_{anti}$), 1.95 [d, *J* = 11 Hz, 2H, 8(9)-$H_{endo}$], 2.12 [dd, *J* = 12.5 Hz, *J'* = 1.5 Hz, 2H, 4(10)-$H_{exo}$], 2.20 [bs, 2H, 5(7)-H], 2.36 [dm, *J* = 13 Hz, 2H, 8(9)-$H_{exo}$], 4.28 (s, 2H, $C\underline{H}_2$-$C_6H_5$), 4.86 (bs, $NH_2$), 7.42-7.48 (complex signal, 3H, $2H_{meta}$ + $1H_{para}$), 7.56 (dd, *J* = 8 Hz, *J'* = 2 Hz, 2H, $H_{ortho}$).

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 24.7 ($CH_3$, $\underline{C}H_3$-C2), 31.1 [CH, C5(7)], 32.8 [$CH_2$, C4(10)], 35.5 [$CH_2$, C8(9)], 38.1 ($CH_2$, C6), 40.6 (C, C2), 44.4 (CH, C3), 47.1 ($CH_2$, $\underline{C}H_2$-$C_6H_5$), 65.9 (C, C1), 130.1 (CH, $C_{meta}$), 130.5 (CH, $C_{para}$), 131.3 (CH, $C_{ortho}$), 133.2 (C, $C_{ipso}$).

MS (EI), m/e (%); main ions: 270 [$(M+H)^{\cdot+}$, 11], 269 ($M^{\cdot+}$, 50), 226 (17), 185 (18), 184 (100), 147 (22), 106 (18), 91 [$(C_6H_5CH_2)^+$, 99].

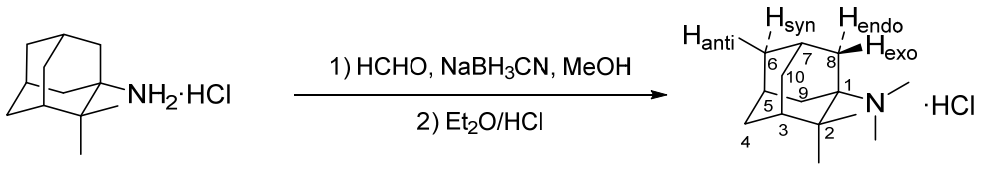
Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{19}H_{27}N{\cdot}HCl$: | C 74.60% | H 9.23% | N 4.58% | Cl 11.59% |
| Calculated for $C_{19}H_{27}N{\cdot}1.08HCl$: | C 73.89% | H 9.16% | N 4.54% | Cl 12.41% |
| Found: | C 73.51% | H 9.24% | N 4.65% | Cl 12.04% |

Accurate mass:

| | |
|---|---|
| Calculated for $[C_{19}H_{28}N]^+$ | 270.2216 |
| Found | 270.2218 |

**Synthesis of *N,N*-Dimethyl(2,2-dimethyladamant-1-yl)amine hydrochloride, 128·HCl.**




**121·HCl** **128·HCl**

To a solution of **121·HCl** (200 mg, 0.93 mmol) in MeOH (7 mL), $NaBH_3CN$ (95% content, 180 mg, 2.66 mmol), AcOH (0.2 mL) and formaldehyde (37% aqueous solution 0.21 mL, 2.84 mmol) were added and the mixture was magnetically stirred at room temperature for 6 h.

Then, more $NaBH_3CN$ (95% content, 180 mg, 2.66 mmol) and formaldehyde (37% aqueous solution 0.21 mL, 2.84 mmol) were added and stirring at room temperature was continued for 18 h more. The mixture was concentrated in vacuo, the residue was taken in water (7 mL) and the solution was made basic with aqueous solution of 2N NaOH and extracted with EtOAc (4×7 mL). The combined organic extracts were washed with brine (2×10 mL), dried (anhydrous $Na_2SO_4$) and concentrated in vacuo. The orange residue (0.20 g) was dissolved in $CH_2Cl_2$ (10 mL), treated with an excess of an ethereal solution of HCl and kept at 0 $^oC$ for 24 h. Filtration of the precipitate gave **128·HCl** (190 mg, 84% yield) as a white solid. An analytical sample was obtained by crystallization from $MeOH/Et_2O$.

Analytical and spectroscopical data **128·HCl**.

Melting point: 228-229 $^oC$

IR (KBr) ν: 3435, 2911, 2894, 2867, 2746, 2623, 2596, 2517, 2475, 2427, 1638, 1499, 1478, 1464, 1450, 1412, 1402, 1380, 1353, 1281, 1202, 1177, 1139, 1115, 1100, 1062, 1035, 1010, 962, 916, 873, 862, 836, 753, 573 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 1.35 (s, 6H, C2-C$\underline{H}_3$), 1.56 (bs, 1H, 3-H), 1.57 [dd, *J* = 13 Hz, *J'* = 1.5 Hz, 2H, 4(10)-$H_{endo}$], 1.69 (dm, *J* = 12.5 Hz, 1H, 6-$H_{syn}$), 1.74 [dm, *J* = 12.5 Hz, 1H, 6-$H_{anti}$), 1.87 [d, *J* = 11.5 Hz, 2H, 8(9)-$H_{endo}$], 2.10 [d, *J* = 13 Hz, 2H, 4(10)-$H_{exo}$], 2.23 [bs, 2H, 5(7)-H], 2.44 [dm, *J* = 11.5 Hz, 2H, 8(9)-$H_{exo}$], 2.87 [s, 6H, N(C$\underline{H}_3)_2$], 4.85 (bs, NH).

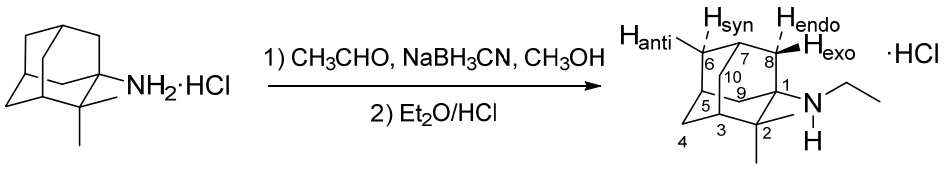
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 25.9 ($CH_3$, C2-$\underline{C}H_3$), 31.7 [CH, C5(7)], 32.7 [$CH_2$, C4(10)], 34.9 [$CH_2$, C8(9)], 37.8 ($CH_2$, C6), 41.6 [$CH_3$, N($\underline{C}H_3)_2$], 41.6 (C, C2), 46.5 (CH, C3), 71.6 (C, C1).

MS (EI), m/e (%); main ions: 207 ($M^{\cdot+}$, 17), 164 (19), 123 (13), 122 (100), 85 (16).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{14}H_{26}NCl$: | C 68.97% | H 10.75% | N 5.74% | Cl 14.54% |
| Calculated for $C_{14}H_{26}NCl·0.4H_2O$: | C 66.99% | H 10.76% | N 5.58% | Cl 14.12% |
| Found: | C 66.82% | H 10.59% | N 5.64% | Cl 14.61% |

**Synthesis of *N*-Ethyl(2,2-dimethyladamant-1-yl)amine hydrochloride, 129·HCl.**




**121·HCl** **129·HCl**

To a solution of **121·HCl** (200 mg, 0.93 mmol) in MeOH (7.5 mL), $NaBH_3CN$ (95% content, 123 mg, 1.86 mmol), AcOH (0.3 mL) and acetaldehyde (0.16 mL, 2.8 mmol) were added and the mixture was magnetically stirred at room temperature for 4 h. Then, more $NaBH_3CN$ (95% content, 62 mg, 0.93 mmol) and acetaldehyde (0.1 mL, 1.75 mmol) were added and stirring at room temperature was continued for 18 h more. The mixture was concentrated in vacuo, the residue was taken in water (11 mL) and the solution was made basic with aqueous solution of 2N NaOH and extracted with EtOAc (4×7.5 mL). The combined organic extracts were washed with brine (2×18 mL), dried (anhydrous $Na_2SO_4$) and filtered. The filtrate was treated with an excess of an ethereal solution of HCl and concentrated in vacuo to give pure **129·HCl** as a white solid (0.19 g, 84% yield). The residue was crystallized from MeOH/$Et_2O$.

Analytical and spectroscopical data **129·HCl**.

Melting point: >255 $^oC$ (decompose)

IR (KBr) ν: 3420, 2993, 2900, 2885, 2860, 2770, 2676, 2459, 2389, 1576, 1470, 1455, 1407, 1377, 1355, 1341, 1305, 1230, 1204, 1153, 1121, 1098, 1062, 1033, 1015, 981, 964, 926, 891, 847, 822, 786, 754, 664, 624, 579, 477 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 1.25 (s, 6H, $2CH_3$), 1.36 (t, *J* = 7.5 Hz, 1H, 2'-H), 1.56-1.62 [complex signal, 3H, 3-H + 4(10)-$H_{endo}$], 1.68 (dm, *J* = 12.5 Hz, 1H, 6-$H_{syn}$), 1.75 (dm, *J* = 12.5 Hz, 1H, 6-$H_{anti}$), 1.88 (d, *J* = 11.5 Hz, 2H, 8(9)-$H_{endo}$), 2.10 (broad d, *J* = 14 Hz, 2H, 4(10)-$H_{exo}$), 2.17 (bs, 2H, 5(7)-H), 2.20 (dm, *J* = 12.5 Hz, 2H, 8(9)-$H_{exo}$), 3.00 (q, *J* = 7.5 Hz, 2H,1'-H), 4.86 (broad s, NH).

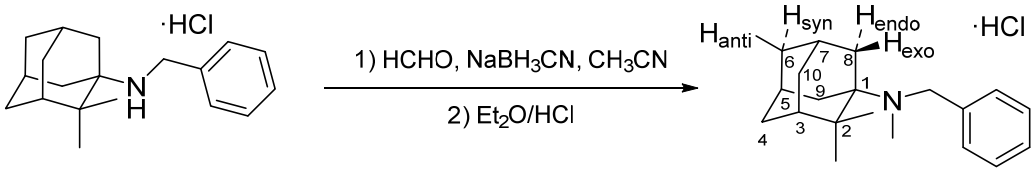
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 11.9 (C, N-$CH_2\underline{C}H_3$), 24.1 ($CH_3$, $\underline{C}H_3$-C2), 31.0 [CH, C5(7)], 32.7 [$CH_2$, C4(10)], 34.8 [$CH_2$, C8(9)], 38.0 ($CH_2$, N-$\underline{C}H_2CH_3$), 38.2 ($CH_2$, C6), 40.4 (C, C2), 43.6 (CH, C3), 64.5 (C, C1).

MS (EI), m/e (%); main ions: 207 ($M^{\cdot+}$, 17), 164 (10), 123 (12), 122 (100), 85 (16).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{14}H_{26}NCl$: | C 68.97% | H 10.75% | N 5.74% | Cl 14.54% |
| Calculated for $C_{14}H_{26}NCl{\cdot}015H_2O$: | C 68.21% | H 10.75% | N 5.68% | Cl 14.38% |
| Found: | C 67.95% | H 10.50% | N 5.60% | Cl 14.78% |

**Synthesis of *N*-Benzyl-*N*-methyl(2,2-dimethyladamant-1-yl)amine hydrochloride, 130·HCl.**




**127·HCl** **130·HCl**

To a suspension of **127·HCl** (0.51 g, 1.67 mmol) in acetonitrile (11 mL), $NaBH_3CN$ (95% content, 314 mg, 4.74 mmol) and formaldehyde (37% aqueous solution 1.23 mL, 16.6 mmol) were added and the mixture was magnetically stirred at room temperature for 30 min. AcOH (0.33 mL) was added until pH = 5 and the mixture was vigorously stirred at room temperature for 2 h. Then, more $NaBH_3CN$ (95% content, 314 mg, 4.74 mmol) was added and stirring at room temperature was continued for 2 h more. The mixture was concentrated in vacuo, the residue was treated with aqueous solution of 2N NaOH (22 mL) and extracted with $CH_2Cl_2$ (3×20 mL). The combined organic extracts were washed with water (2×20 mL), dried (anhydrous $Na_2SO_4$) and concentrated in vacuo to give a yellow oil (0.39 g). The residue was dissolved in EtOAc (15 mL), treated with an excess of an ethereal solution of HCl and allowed to stand at 0 $^oC$ for 24 h. Filtration of the precipitate gave **130·HCl** (390 mg, 73% yield) as a white solid. The analytical sample was obtained by crystallization from 2-propanol.

Analytical and spectroscopical data **130·HCl**.

Melting point: 215-216 $^oC$

IR (KBr) $\nu$: 3419, 3037, 2919, 2890, 2859, 2747, 2655, 2550, 1965, 1911, 1825, 1622, 1497, 1478, 1457, 1422, 1401, 1379, 1355, 1342, 1312, 1285, 1253, 1227, 1211, 1189, 1165, 1145, 1124, 1103, 1066, 1029, 963, 939, 929, 881, 870, 858, 758, 742, 701, 633, 518, 490 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 1.48 (s, 3H) and 1.53 (s, 3H) [C2-($C\underline{H}_3$)$_2$ diastereotopic], 1.56 (m, 1H, 3-H), 1.60 [dm, *J* = 13.5 Hz, 3H, 4(10)-H$_{endo}$], 1.73 (dm, *J* = 12.5 Hz, 1H, 6-H$_{syn}$), 1.79 (dm, *J* = 12.5 Hz, 1H, 6-H$_{anti}$), 1.91 [t, *J* = 15 Hz, 2H, 8(9)-H$_{endo}$], 2.15 [m, 2H, 4(10)-H$_{exo}$], 2.27 (s, 1 H) y 2.30 (bs, 1H) (5-H and 7-H), 2.66 (dm, *J* = 12.5 Hz, 1H) y 2.82 (dm, J = 12.5 Hz, 1H) (8-H$_{exo}$ and 9-H$_{exo}$), 2.83 (bs, 3H, N-$C\underline{H}_3$), 4.05 (d, *J* = 12.5 Hz, 1H) and 4.92 (d, *J* = 12.5 Hz, 1H) ($C\underline{H}_2$-$C_6H_5$ diastereotopic), 7.48-7.56 (complex signal, 5H, Ar-H).

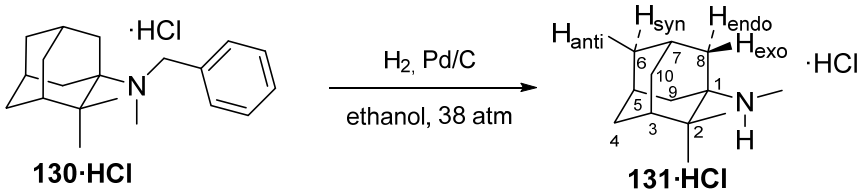
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 27.0 and 27.5 (2 $CH_3$ diastereotopic, $\underline{C}H_3$-C2), 31.7 and 31.8 (2 CH diastereotopic, C5 and C7), 32.7 and 32.9 (2 $CH_2$ diastereotopic, C4 and C10), 35.2 and 36.8 (2 $CH_2$ diastereotopic, C8 and C9), 36.9 ($CH_3$, N-$\underline{C}H_3$), 37.6 ($CH_2$, C6), 42.0 (C, C2), 48.2 (CH, C3), 58.2 ($CH_2$, $\underline{C}H_2$-$C_6H_5$), 73.8 (C, C1), 130.3 (CH, C$_{meta}$), 131.1 (CH, C$_{para}$), 132.1 (C, C$_{ipso}$), 132.6 (CH, C$_{ortho}$).

MS (EI), m/e (%); main ions 283 ($M^{\cdot+}$, 51), 240 (31), 199 (19), 198 (100), 161 (19), 160 (13), 120 [$(C_6H_5CH_2NCH_3)^+$, 15], 91 [$(C_6H_5CH_2)^+$, 77], 284 (11).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{20}H_{30}NCl$: | C 75.09% | H 9.45% | N 4.38% | Cl 11.08% |
| Calculated for $C_{20}H_{30}NCl\cdot0.2H_2O$: | C 74.25% | H 9.47% | N 4.33% | Cl 10.96% |
| Found: | C 74.25% | H 9.45% | N 4.29% | Cl 11.20% |

**Synthesis of *N*-Methyl(2,2-dimethyladamant-1-yl)amine hydrochloride, 131·HCl.**




A suspension of **130·HCl** (175 mg, 0.55 mmol) and 10% Pd/C (50% in water, 0.1 g) in absolute EtOH (25 mL) was hydrogenated at 100 $^{o}$C and 38 atm for 24 h. The suspension was filtered, the solid was washed with absolute EtOH and the combined filtrate and washings were concentrated in vacuo to give **131·HCl** (100 mg, 80% yield). The analytical sample was obtained as a white solid by crystallization from MeOH/$Et_2O$.

Analytical and spectroscopical data of **131·HCl**

Melting point: >200 $^{o}$C (decompose)

IR (KBr) ν: 3422, 2990, 2957, 2924, 2886, 2861, 2751, 2422, 1583, 1508, 1457, 1419, 1397, 1367, 1340 ,1313, 1240, 1202, 1161, 1140, 1129, 1098, 1065,1038, 1010, 978, 927, 905, 885, 854, 548 cm$^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 1.22 (s, 6H, $2CH_3$), 1.58-1.64 [complex signal, 3H, 3-H + 4(10)-$H_{endo}$], 1.68 (dtt, *J* = 13 Hz, *J'* = 3 Hz, *J''* = 2.5 Hz, 1H, 6-$H_{syn}$), 1.75 [dtt, *J* = 13 Hz, *J'* = 3 Hz, *J''* = 2 Hz, 1H, 6-$H_{anti}$), 1.88 (dm, *J* = 12.5 Hz, 2H, 8(9)-$H_{endo}$], 2.07-2.13 [complex signal, 4H, 4(10)-$H_{exo}$ + 8(9)-$H_{exo}$], 2.18 (bs, 2H, 5(7)-H), 2.60 (s, 3H, $NC\underline{H}_3$).

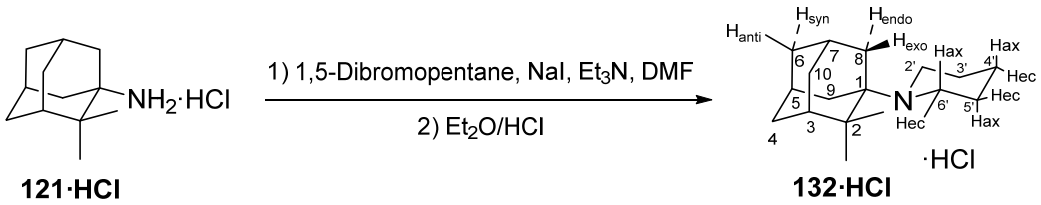
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 23.7 ($CH_3$, $\underline{C}H_3$-C2), 26.8 ($CH_3$, N-$\underline{C}H_3$), 30.9 [CH, C5(7)], 32.8 [$CH_2$, C4(10)], 34.0 [$CH_2$, C8(9)], 38.2 ($CH_2$, C6), 40.4 (C, C2), 43.1 (CH, C3), 63.1 (C, C1).

MS (EI), m/e (%); main ions: 193 ($M^{\cdot+}$, 20), 150 (10), 109 (11), 108 (100), 71 (15).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{13}H_{24}NCl$: | C 67.95% | H 10.53% | N 6.10% | Cl 15.43% |
| Calculated for $C_{13}H_{24}NCl·0.2H_2O$: | C 66.91% | H 10.54% | N 6.00% | Cl 15.19% |
| Found: | C 66.70% | H 10.51% | N 6.03% | Cl 15.55% |

**Synthesis of *N*-(2,2-Dimethyladamant-1-yl)piperidine hydrochloride, 132·HCl.**




**121·HCl** **132·HCl**

To a solution of **121·HCl** (175 mg, 0.81 mmol) in anhydrous DMF (4 mL), anhydrous $Et_3N$ (0.4 mL, 2.74 mmol) was added and the mixture was stirred at room temperature for 2 h. Then, 1,5-dibromopentane (0.16 mL, 1.12 mmol) and NaI (0.34 g, 2.25 mmol) were added and the mixture was heated at 60 $^oC$ for 26 h. The mixture was allowed to cool to room temperature, water (13 mL) was added and the mixture was washed with EtOAc (3×10 mL). The organic layer was dried (anhydrous $Na_2SO_4$) and concentrated in vacuo to give a red residue (370 mg) that was taken into EtOAc (25 mL). Aqueous solution of 2N NaOH (10 mL) was added and the layers were separated. The organic one was washed with aqueous solution of 2N NaOH (2x10 mL), dried (anhydrous $Na_2SO_4$) and concentrated in vacuo. The residue was dissolved in the minimum amount of $Et_2O$ and treated with an excess of an ethereal solution of HCl. Filtration of the precipitate gave **132·HCl** (50 mg, 22% yield) as a solid. The analytical sample was obtained by crystallization from MeOH/$Et_2O$.

Analytical and spectroscopical data of **132·HCl**.

Melting point: 249-250 $^oC$

IR (KBr) ν: 3422, 3022, 2989, 2949, 2919, 2893, 2872, 2854, 2759, 2738, 2676, 2601, 2568, 2527, 2508, 2394, 1633, 1495, 1477, 1458, 1444, 1399, 1376, 1354, 1345, 1313, 1283, 1260, 1233, 1193, 1167, 1144, 1109, 1104, 1078, 1053, 1019, 977, 956, 926, 900, 883, 872, 765, 652, 497, 475 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 1.39 (s, 6H, $2CH_3$), 1.48 (s, 1H, 3-H), 1.54 [dm, *J* = 13 Hz , 3H, 4(10)-$H_{endo}$], 1.56 (dtt, *J* = 13 Hz, *J'* = 12.5 Hz, *J''* = 4.5 Hz, 1H, 4'-$H_{ax}$), 1.70 (dtt, *J* = 12.5 Hz, *J'* = 3 Hz, *J''* = 2 Hz, 1H, 6-$H_{syn}$), 1.74 (dtt, *J* = 12.5 Hz, *J'* = 3 Hz, *J''* = 1.5 Hz, 1H, 6-$H_{anti}$), 1.80 (m, 1H, 4'-$H_{eq}$), 1.83 [dm, *J* = 12 Hz, 3H, 8(9)-$H_{endo}$], 1.86-1.99 (complex signal, 4H, 3'(5')-$H_{ax}$ y 3'(5')-$H_{eq}$), 2.08 [broad d, *J* = 13 Hz, 2H, 4(10)-$H_{exo}$], 2.22 [bs, 2H, 5(7)-H], 2.62 [dm, *J* = 12 Hz, 2H, 8(9)-$H_{exo}$], 3.10 [dt, *J* = 2 Hz, *J'* = 12 Hz, 2H, 2'(6')-$H_{ax}$], 3.80 [d, *J* = 12 Hz, 2H,2'(6')-$H_{eq}$], 4.85 (bs, NH).

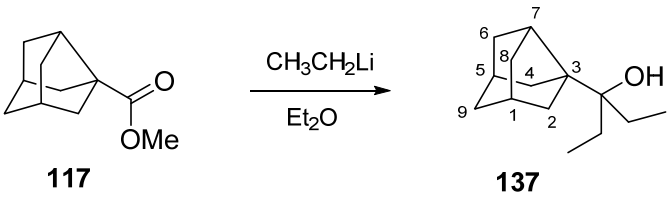
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 23.2 ($CH_2$, C4'), 25.5 [$CH_2$, C3'(5')], 27.2 ($CH_3$, $\underline{C}H_3$-C2), 31.6 [CH, C5(7)], 32.8 [$CH_2$, C4(10)], 35.6 [$CH_2$, C8(9)], 37.6 ($CH_2$, C6), 41.7 (C, C2), 48.4 (CH, C3), 51.9 [$CH_2$, C2'(6')], 73.8 (C, C1).

MS (EI), m/e (%); main ions: 247 ($M^{\cdot+}$, 21), 204 (26), 163 [$(C_{12}H_{19})^+$, 17], 162 (100), 125 (15).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{17}H_{30}NCl$: | C 71.93% | H 10.65% | N 4.93% | Cl 12.49% |
| Calculated for $C_{17}H_{30}NCl{\cdot}0.2H_2O$: | C 71.02% | H 10.66% | N 4.87% | Cl 12.33% |
| Found: | C 71.02% | H 10.61% | N 4.71% | Cl 12.50% |

**Synthesis of 3-(3-Noradamantil)-3-pentanol, 137.**




**117** **137**

To a stirred solution of **117** (4.85 g, 27 mmol) in anhydrous $Et_2O$ (110 mL) at 0 $^oC$, ethyllithium (127 mL, 1.7 M in *n*-$Bu_2O$, 216 mmol) was added dropwise. The reaction was heated under reflux overnight, cooled to 0 $^oC$, carefully quenched with $H_2O$ (135 mL) and stirred at 0 $^oC$ for one hour. The layers were separated and the aqueous one was extracted with $Et_2O$ (3x50 mL). The combined organic layers were dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to dryness to give 4.8 g of a mixture of **137** and the intermediate ketone (NMR, IR). This oil was dissolved in anhydrous $Et_2O$ (110 mL), cooled at 0 $^oC$, treated with more ethyllithium (60 mL, 1.7 M in *n*-$Bu_2O$, 102 mmol) and heated under reflux overnight. Following the aforementioned work-up a yellow-orange oil was obtained that was purified by microdestillation (140 $^oC$, 30 Torr). Pure alcohol **137** was obtained as a colorless oil (3.1 g, 56% yield).

Analytical and spectroscopical data of **137**.

IR (NaCl) ν: 3489, 2920, 2864, 2740, 2640, 2551, 1458, 1380, 1322, 1303, 1251, 1184, 1119, 1086, 1063, 1034, 1001, 950, 918, 865, 844, 777, 624 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 0.92 (t, *J* = 7.5 Hz, 6H, 2 $CH_2C\underline{H}_3$), 1.25 (s, 1H, 7-H), 1.51 (q, *J* = 7.5 Hz, 2H) and 1.66 (q, *J* = 7.5 Hz, 2 H) (2 $C\underline{H}_2CH_3$ diastereotopic), 1.52-1.65 (complex signal, 8-H, 9-$H_{anti,syn}$ + 6(8)-$H_2$ + 2(4)-$H_{exo}$], 1.84 [dm, *J* = 10.5 Hz, 2H, 2(4)-$H_{endo}$], 2.23 [bs, 2H,1(5)-H], 2.36 (t, *J* = 6.5 Hz, 1H, 7-H).

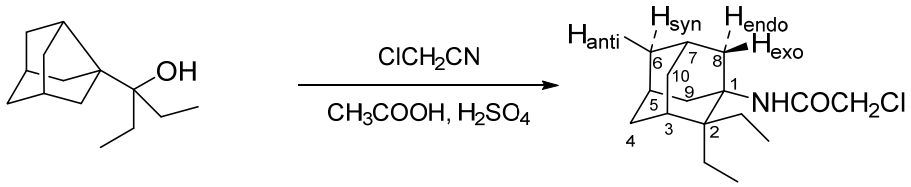
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 8.7 [$CH_3$, C1'(5')], 28.2 [$CH_2$, C2'(4')], 35.7 ($CH_2$, C9), 37.5 [CH, C1(5)], 40.6 (CH, C7), 44.0 [$CH_2$, C6(8)], 45.4 [$CH_2$, C2(4)], 58.5 (C, C3), 76.5 (C, C3').

GC/MS (EI), m/e (%); main ions: 208 ($M^{\cdot+}$, 1), 190 [$(M\text{-}H_2O)^{\cdot+}$, 4], 180 (13), 179 (100), 161 [$(C_{12}H_{17})^{+}$, 10], 86 (14), 80 (11), 79 (14), 57 (19).

Elemental analysis

| | | |
|---|---|---|
| Calculated for $C_{14}H_{24}O$: | C 80.71% | H 11.61% |
| Found: | C 80.85% | H 11.58% |

**Synthesis of *N*-(2,2-Diethyladamant-1-yl)-2-cloroacetamide, 138.**




**137** **138**

A solution of alcohol **137** (2.45 g, 11.8 mmol) and chloroacetonitrile (3 mL, 47.5 mmol) in acetic acid (3.8 mL) was cooled to 0 ºC. Concentrated $H_2SO_4$ (3.8 mL) was added dropwise without exceeding 10 ºC. The reaction was stirred at room temperature overnight. The crude of the reaction was poured into ice (54 g) and the residue was filtered under vacuo. The filtrate was dissolved in $CH_2Cl_2$ (200 mL) and the organic layer was dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain chloroacetamide **138** as a brownish solid (2.99 g, 89.5% yield). The analytical sample was obtained by crystallization from EtOAc.

Analytical and spectroscopical data of **138**.

Melting point: 65-66 $^{o}$C

IR (KBr) ν: 3328, 3073, 2977, 2968, 2927, 2904, 2870, 2676, 2367, 1681, 1660, 1550, 1487, 1464, 1443, 1376, 1346, 1326, 1282, 1270, 1250, 1213, 1167, 1156, 1131, 1111, 1090, 1049, 1020, 984, 947, 910, 879, 855, 810, 793, 774, 721, 705, 648, 611, 549, 474 cm$^{-1}$.

$^{1}$H-RMN (500 MHz, $CDCl_3$) δ: 0.90 (t, *J* = 7.5 Hz, 6H, $2CH_2C\underline{H}_3$), 1.44 [dm, *J* = 13 Hz, 2H, 4(10)-$H_{endo}$], 1.59 (dtt, *J* = 12.5 Hz, *J'* = 3 Hz, *J''* = 1.5 Hz, 1H, 6-$H_{anti}$), 1.62-1.72 (complex signal, 4H, 1 $C\underline{H}_2CH_3$ diastereotopic + 3-H + 6-$H_{syn}$), 1.81 (m, 2H, $1C\underline{H}_2CH_3$ diastereotopic), 1.95 [dm, *J* = 13.5 Hz, 2H, 4(10)-$H_{exo}$], 2.02 [bs, 2H, 5(7)-H], 2.30 [dm, *J* = 13 Hz, 2H, 8(9)-$H_{exo}$], 2.34 [dm, *J* = 13 Hz, 2H, 8(9)-$H_{endo}$], 3.93 (s, $CH_2$, $C\underline{H}_2Cl$), 6.49 (s, 1H, NH).

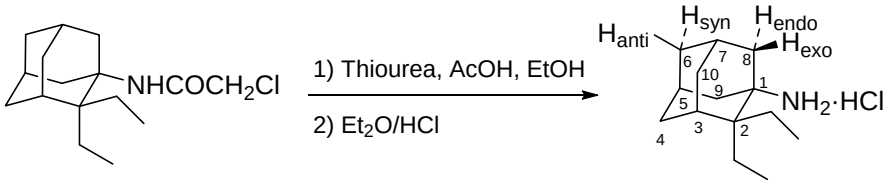
$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 8.7 ($CH_3$, $\underline{C}H_3CH_2$-C2), 23.4 ($CH_2$, $CH_3\underline{C}H_2$-C2), 29.1 [CH, C5(7)], 31.9 [$CH_2$, C4(10)], 34.8 (CH,C3), 36.7 [$CH_2$, C8(9)], 37.9 ($CH_2$, C6), 43.2 ($CH_2$, $\underline{C}H_2Cl$), 43.4 (C, C2), 59.3 (C, C1), 164.2 (C, CO).

GC/MS (EI), m/e (%); main ions: 283 ($M^{\cdot+}$, 26), 254 (18), 249 (13), 248 (72), 212 (12), 191 [$(C_{14}H_{23})^{+}$, 18], 190 (89), 172 (37), 171 (12), 170 (100), 161 (19), 136 (11), 119 (12), 105 (10), 94 (17), 93 (11), 79 (19), 77 (15), 55 (10).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{16}H_{26}ClNO$: | C 67.70% | H 9.23% | N 4.93% | Cl 12.49% |
| Found: | C 67.86% | H 9.40% | N 5.23% | Cl 12.15% |

**Synthesis of (2,2-Diethyladamant-1-yl)amine hydrochloride, 139·HCl.**




**138** **139·HCl**

A solution of chloroacetamide **138** (2.6 g, 9.2 mmol), thiourea (0.83 g, 10.9 mmol), glacial acetic acid (3.6 mL) in absolute EtOH (18 mL) was heated under reflux overnight. The resulting suspension was cooled to room temperature and water (90 mL) was added. A solid was separated by filtration and the filtrate was basified with an aqueous solution of NaOH 5 N til pH = 14. The aqueous layer was extracted with EtOAc (3x150 mL). The combined organic

extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired amine **139** as a yellow solid (1.58 g). The residue was dissolved in the minimum amount of EtOAc, treated with an excess of an ethereal solution of HCl and allowed to stand at 0 $^oC$ for 24 h. Filtration of the precipitate gave **139·HCl** as a white solid (1.43 g, 64% yield). An analytical sample was obtained by crystallization from MeOH/$Et_2O$.

Analytical and spectroscopical data of **139·HCl**.

Melting point: 293-294 $^oC$

IR (KBr) ν: 3422, 2985, 2963, 2926, 2871, 2827, 2766, 2637, 2569, 2469, 2439, 2017, 1599, 1576, 1505, 1473, 1463, 1442, 1382, 1362, 1295, 1246, 1219, 1169, 1140, 1114, 1104, 1075, 1012, 980, 966, 932, 917, 904, 878, 807, 790, 767, 732, 548, 513 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 0.94 (t, *J* = 7.5 Hz, 6H, $2CH_2C\underline{H}_3$), 1.56 [dd, *J* = 13 Hz, *J'* = 1.5 Hz, 2H, 4(10)-$H_{endo}$], 1.64-1.70 [complex signal, 3H, 8(9)-$H_{endo}$ + 6-$H_{syn}$], 1.72 (m, 1H, 6-$H_{anti}$), 1.77 (q, 4H, *J* = 7.5 Hz, $2C\underline{H}_2CH_3$), 1.86 (m, 1H, 3-H), 1.96 [d, *J* = 13 Hz, 2H, 4(10)-$H_{exo}$], 2.11 [bs, 2H, 5(7)-H], 2.42 [dm, *J* = 12.5 Hz, *J'* = 1.5 Hz, 2H, 8(9)-$H_{exo}$], 4.85 (broad signal, $NH_2$).

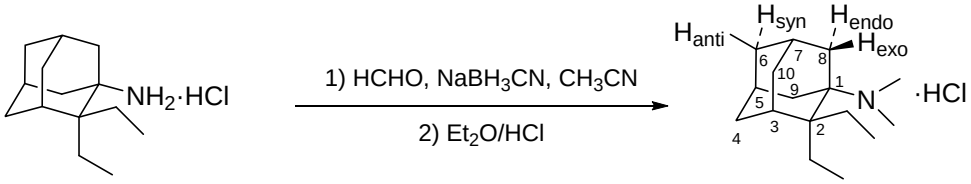
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 9.0 ($CH_3$, $\underline{C}H_3CH_2$-C2), 24.7 ($CH_2$, $CH_3\underline{C}H_2$-C2), 30.3 [CH, C5(7)], 32.3 [$CH_2$, C4(10)], 35.4 (CH,C3), 38.4 ($CH_2$, C6), 38.7 [$CH_2$, C8(9)], 43.3 (C, C2), 59.7 (C, C1).

MS (EI), m/e (%); main ions: 207 ($M^{\cdot+}$, 15), 95 (10), 94 (100).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{14}H_{26}NCl$: | C 68.97% | H 10.75% | N 5.74% | Cl 14.54% |
| Found: | C 68.96% | H 10.97% | N 5.76% | Cl 14.62% |

**Synthesis of *N,N*-Dimethyl(2,2-diethyladamant-1-yl)amine hydrochloride, 155·HCl.**




**139·HCl** **155·HCl**

To a solution of **139·HCl** (350 mg, 1.43 mmol) in MeOH (10 mL), $NaBH_3CN$ (95% content, 265 mg, 4.0 mmol), AcOH (0.3 mL) and formaldehyde (37% aqueous solution, 0.32 mL, 4.3 mmol) were added and the mixture was magnetically stirred at room temperature for 6 h. Then, more $NaBH_3CN$ (95% content, 265 mg, 4.0 mmol) and formaldehyde (37% aqueous solution, 0.32 mL, 4.3 mmol) were added and stirring at room temperature was continued for 18 h more. The mixture was concentrated in vacuo, the residue was taken in water (10 mL) and the solution was made basic with aqueous solution of 2N NaOH and extracted with EtOAc (4×10 mL). The combined organic extracts were washed with brine (2×10 mL), dried (anhydrous $Na_2SO_4$) and concentrated in vacuo. The orange residue (0.13 g) was dissolved in EtOAc, treated with an excess of an ethereal solution of HCl and allowed to stand at 0 $^oC$ for 24 h. Filtration of the precipitate gave **155·HCl** (120 mg, 31% yield) as a white solid. An analytical sample was obtained by crystallization from MeOH/$Et_2O$.

Analytical and spectroscopical data of **155·HCl**.

Melting point: 235-236 $^oC$

IR (KBr) ν: 3428, 3033, 2978, 2962, 2921, 2903, 2858, 2778, 2721, 2461, 1634, 1484, 1463, 1399, 1369, 1332, 1305, 1276, 1227, 1180, 1158, 1122, 1104, 1039, 1027, 991, 974, 963, 891, 867, 857, 796, 722, 664, 576 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 1.03 (t, *J* = 7.5 Hz, 6H, $2CH_2C\underline{H}_3$), 1.57 [d, *J* = 12.5 Hz, 2H, 4(10)-$H_{endo}$], 1.71 (m, 2H, 6-$H_2$), 1.83 (m, 2H, 2 $C\underline{H}_2CH_3$ diastereotopic), 1.89 [d, *J* = 12.5 Hz, 8(9)-$H_{endo}$], 1.94 [d, *J* = 12.5 Hz, 2H, 4(10)-$H_{exo}$], 1.97-2.05 (complex signal, 3H, 3-H + 2 $C\underline{H}_2CH_3$ diastereotopic), 2.23 [bs, 2H, 5(7)-H], 2.54 [d, *J* = 12.5 Hz, 2H, 8(9)-$H_{exo}$], 2.84 [s, 6H, $N(CH_3)_2$], 4.84 (bs, NH).

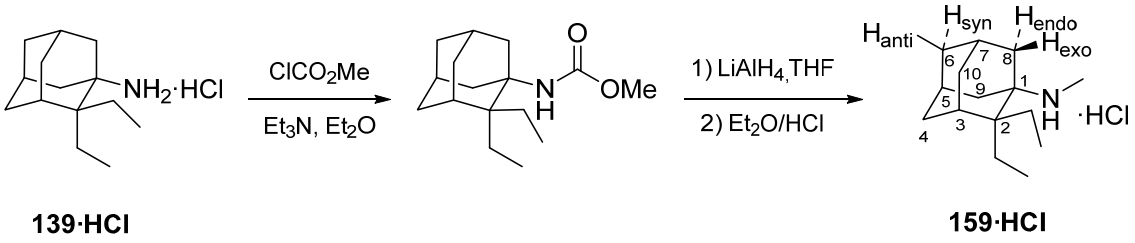
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 9.5 ($CH_3$, $\underline{C}H_3CH_2$-C2), 26.9 ($CH_2$, $CH_3\underline{C}H_2$-C2), 31.4 [CH, C5(7)], 32.2 [$CH_2$, C4(10)], 35.4 [$CH_2$, C8(9)], 35.9 (CH,C3), 37.8 ($CH_2$, C6), 42.1 [$CH_3$, $N(CH_3)_2$], 46.2 (C, C2), 73.8 (C, C1).

MS (EI), m/e (%); main ions: 235 ($M^{\cdot+}$, 14), 164 (21), 123 (13), 122 (100), 85 (17).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{16}H_{29}N\cdot HCl$: | C 70.69% | H 11.12% | N 5.15% | Cl 13.04% |
| Calculated for $C_{16}H_{29}N\cdot 1.1HCl\cdot 0.5H_2O$: | C 67.54% | H 11.02% | N 4.92% | Cl 13.71% |
| Found: | C 67.30% | H 10.71% | N 5.27% | Cl 14.06% |

**Synthesis of *N*-methyl(2,2-diethyladamant-1-yl)amine hydrochloride, 159·HCl.**




**139·HCl** **159·HCl**

To a solution of **139·HCl** (400 mg, 1.64 mmol) and anh. $Et_3N$ (0.76 mL, 5.4 mmol) in anhydrous $Et_2O$ (15 mL) at 0 $^oC$ methyl chloroformiate (0.6 mL, 7.76 mmol) was added dropwise. When the addition was over the mixture was allowed to reach room temperature and was stirred at this temperature overnight. Then, water (25 mL) was added, the layers were separated and the aqueous one was washed with EtOAc (3 x 15 mL). The combined organic extracts were washed with water (1 x 15 mL), aqueous solution 2N HCl (2 x 15 mL), dried (anhydrous $Na_2SO_4$) and concentrated in vacuo to give the carbamate as an orange oil (300 g). To this oil in anhydrous THF (10 mL) was added a suspension of $LiAlH_4$ (430 mg, 11.3 mmol) in anhydrous THF (10 mL). The resulting suspension was stirred at reflux for 20 h and cooled to room temperature. The suspension was carefully made basic through the dropwise addition of aqueous solution of 5N NaOH (4 mL) and stirred at room temperature for 2 h. A solid was removed by filtration through Celite® and washed with $Et_2O$ (3x15 mL). The filtrate and washings were dried (anhydrous $Na_2SO_4$) and concentrated in vacuo to give a yellow oil (270 mg). This residue was dissolved in $Et_2O$, treated with an excess of an ethereal solution of HCl and kept at 0 $^oC$ for 24 h. Filtration of the precipitate gave **159·HCl** (264 mg, 62% yield overall) as a white crystalline solid.

Analytical and spectroscopical data of **159·HCl**.

Melting point: 259-260 ºC

IR (KBr) ν: 3429, 2962, 2925, 2859, 2748, 2419, 2023, 1583, 1462, 1424, 1412, 1392, 1365, 1298, 1280, 1236, 1216, 1152, 1140, 1119, 1097, 1079, 1063, 1002, 977, 970, 942, 905, 878, 856, 815, 790, 727, 661, 554 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 0.99 (t, *J* = 7.5 Hz, 6H, $2CH_2C\underline{H}_3$), 1.58 [d, *J* = 12 Hz, 2H, 4(10)-$H_{endo}$], 1.66-1.73 (complex signal, 2H, 6-$H_2$), 1.74-1.85 (complex signal, 4H, $2C\underline{H}_2CH_3$), 1.88 [d, *J* = 13 Hz, 3H, 8(9)-$H_{endo}$], 1.94 (bs, 1H, 3-H), 1.96 [d, *J* = 12 Hz, 2H, 4(10)-$H_{exo}$], 2.16 [bs, 2H, 5(7)-H], 2.21 [d, *J* = 13 Hz, 2H, 8(9)-$H_{exo}$], 2.58 (s, 3H, $NCH_3$), 4.84 (broad s, NH).

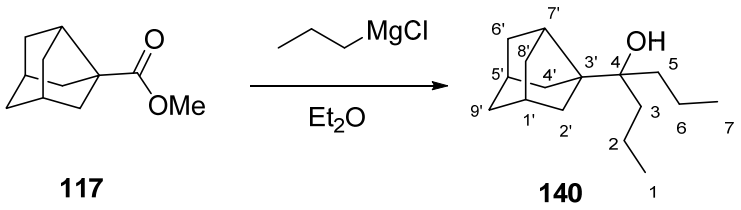
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 9.3 ($CH_3$, $\underline{C}H_3CH_2$-C2), 25.3 ($CH_2$, $CH_3\underline{C}H_2$-C2), 27.2 ($CH_3$, $NCH_3$), 30.3 [CH, C5(7)], 32.3 [$CH_2$, C4(10)], 34.2 [$CH_2$, C8(9)], 35.1 (CH,C3), 38.3 ($CH_2$, C6), 44.3 (C, C2), 65.1 (C, C1).

MS (EI), m/e (%); main ions: 221 ($M^{\cdot+}$, 14), 150 (12), 109 (11), 108 (100), 71 (15).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{15}H_{28}NCl$: | C 69.87% | H 10.94% | N 5.43% | Cl 13.75% |
| Found: | C 69.74% | H 10.99% | N 5.62% | Cl 13.66%. |

**Synthesis of 4-(3-Noradamantyl)-4-heptanol, 140.**




**117** **140**

To a stirred solution of ester **117** (0.54 g, 3.0 mmol) in anhydrous $Et_2O$ (14 mL) at 0 ºC, propylmagnesium chloride (12 mL, 2 M in THF, 24 mmol) was added dropwise. The reaction was heated under reflux overnight. The reaction was cooled to 0 ºC and quenched with $H_2O$ (5 mL) and the mixture was stirred at 0 ºC for one hour. More water (5 mL) was added and the reaction was allowed to reach room temperature. The layers were separated and the aqueous one was extracted with $Et_2O$ (3x10 mL). The combined organic layers were dried with

anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to dryness to give the alcohol **140** as a colorless oil (0.63 g, 89% yield).

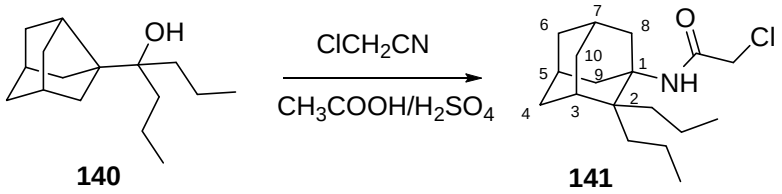
Analytic and spectroscopic data of the alcohol **140**.

IR (KBr) ν: 3492, 2955, 2929, 2870, 1458, 1377, 1338, 1323, 1302, 1235, 1186, 1135, 1122, 1090, 987, 907, 825, 747 $cm^{-1}$.

$^1$H NMR (500 MHz, $CDCl_3$) δ: 0.91 (t, *J* = 7.2 Hz, 6 H, 2 $CH_2CH_2C\underline{H}_3$), 1.17 (bs, 1 H, OH), 1.35-1.47 (complex signal, 6 H) and 1.54-1.69 (complex signal, 10 H) [2 $C\underline{H}_2C\underline{H}_2CH_3$ + 9'-$H_{anti,syn}$ + 6'(8')-$H_2$ + 2'(4')-$H_{exo}$], 1.83 [dm, *J* = 10.8 Hz, 2 H, 2'(4')-$H_{endo}$], 2.23 [bs, 2 H, 1'(5')-H], 2.36 (t, *J* = 6.6 Hz, 1 H, 7'-H).
$^{13}$C NMR (100.6 MHz, $CDCl_3$) δ: 15.1 [$CH_3$, C1(7)], 17.5 [$CH_2$, C2(6)], 35.7 ($CH_2$, C9'), 37.4 [CH, C1'(5')], 39.2 [$CH_2$, C3(5)], 40.5 (CH, C7'), 44.0 [$CH_2$, C6'(8')], 45.3 [$CH_2$, C2'(4')], 58.7 (C, C3'), 76.4 (C, C4).
GC/MS (EI), m/e (%); main ions: 194 (16), 193 (100), 175 (13), 115 (16), 114 (15), 79 (14), 71 (22).
Accurate mass

| | |
|---|---|
| Calculated for $[C_{16}H_{28}O–H_2O+NH_4]^+$ | 236.2372 |
| Found | 236.2370 |

**Synthesis of *N*-(2,2-di-*n*-propyladamant-1-yl)-2-chloroacetamide, 141.**

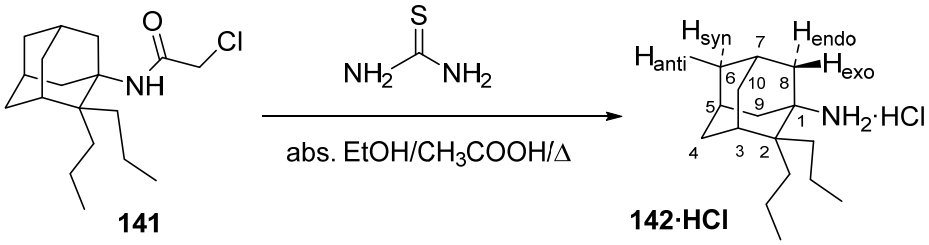



**140** **141**

A solution of alcohol **140** (0.6 g, 2.9 mmol) and chloroacetonitrile (0.7 mL, 11.5 mmol) in acetic acid (6 mL) was cooled to 0 $^oC$. Concentrated sulfuric acid (0.9 mL, 17.2 mmol) was added dropwise without exceeding 10 $^oC$. The reaction was stirred at room temperature overnight. The crude of the reaction was poured into ice (15 g) and the residue was filtered under vacuo. The filtrate was dissolved in $CH_2Cl_2$ (10 mL) and the organic layer was dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired chloroacetamide **141** as an orange solid (0.8 g, 91% yield).

The crude of the reaction was used in the next synthetic step without further purification.

**Synthesis of (2,2-Di-*n*-propyladamant-1-yl)amine hydrochloride, 142·HCl.**




A solution of chloroacetamide **141** (0.8 g, 2.6 mmol), thiourea (0.2 g, 3.1 mmol), glacial acetic acid (0.9 mL) in absolute EtOH (4.5 mL) was heated under reflux overnight. The reaction was tempered and the suspension was dissolved in water (30 mL) and basified with a solution of 10 N NaOH (10 mL). The aqueous layer was extracted with AcOEt (3x40 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired amine **142** as a yellowish solid (0.6 g). The residue was dissolved in AcOEt and HCl/$Et_2O$ was added. The obtained solid was filtered under vacuo and washed with cold $Et_2O$ to obtain the amine **142·HCl** as a white solid (0.6 g, 91% yield). The analytical sample was obtained by crystallization with MeOH/$Et_2O$.

Analytic and spectroscopic data of the amine **142·HCl**.

Melting point: 245-246 ºC (MeOH).

IR (KBr) ν: 3495, 3303, 2954, 2930, 2902, 2870, 2612, 2582, 2554, 2064, 1607, 1530, 1505, 1476, 1462, 1448, 1367, 1350, 1309, 1301, 1151, 1113, 1079, 1002, 952, 747, 669 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 0.93 (t, *J* = 7.3 Hz, 3 H) and 0.94 (t, *J* = 7.3 Hz, 3 H) (2 $CH_2CH_2C\underline{H}_3$), 1.16-1.24 (complex signal, 4 H, 2 $CH_2C\underline{H}_2CH_3$), 1.46-1.53 [complex signal, 6 H, 2 $C\underline{H}_2CH_2CH_3$, 4(10)-$H_{endo}$], 1.63 [d, *J* = 12.3 Hz, 2 H, C8(9)-$H_{endo}$], 1.78 [bs, 2 H, 5(7)-H], 1.81 (bs, 2 H, 6-$H_2$), 2.11-2.13 [complex signal, 3 H, 3-H + 4(10)-$H_{exo}$], 2.22 [d, *J* = 12.0 Hz, 2 H, C8(9)-$H_{exo}$].

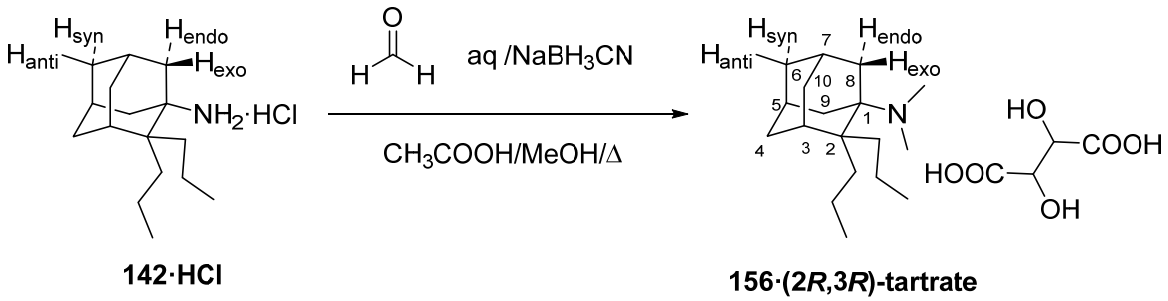
$^{13}$C-RMN (125.7 MHz, $CD_3OD$) δ: 15.3 ($CH_3$, 2 $CH_2CH_2\underline{C}H_3$), 16.4 and 16.7 ($CH_2$, 2 $CH_2\underline{C}H_2CH_3$), 30.0 (CH, C3), 31.8 [$CH_2$, C4(10)], 35.9 and 36.5 ($CH_2$, 2 $\underline{C}H_2CH_2CH_3$), 36.0 [CH, C5(7)], 36.8 [$CH_2$, (EI)C8(9)], 39.4 (C, C2), 43.4 ($CH_2$, C6), 53.1 (C, C1).

MS (EI), m/e (%); main ions: 236 (17), 235 ($M^{\cdot+}$, 28), 193 (16), 192 (24), 164 (12), 96 (18), 95 (52), 94 (100), 83 (16).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{16}H_{30}NCl$: | C 70.69% | H 11.12% | N 5.15% | Cl 13.04% |
| Calculated for $C_{16}H_{30}NCl \cdot 0.25\ Et_2O$: | C 70.31% | H 11.28% | N 4.82% | Cl 12.21% |
| Found: | C 70.01% | H 11.26% | N 4.88% | Cl 11.85% |

***N,N*-Dimethyl-(2,2-di-*n*-propyladamant-1-yl)amine (2*R*,3*R*)-tartrate, 156·(2*R*,3*R*)-tartrate.**




**142·HCl** **156·(2*R*,3*R*)-tartrate**

A mixture of amine HCl **142·HCl** (0.64 g, 2.4 mmol), $NaBH_3CN$ (0.45 g, 6.8 mmol), formaldehyde (0.60 mL, 37% in aqueous solution, 7.2 mmol), acetic acid (0.4 mL) in MeOH (25 mL) with a $CaCl_2$ tube was stirred at room temperature for 8 h. More $NaBH_3CN$ (0.48 g, 6.8 mmol) and formaldehyde (0.60 mL, 37% in aqueous solution, 7.2 mmol) were added and the reaction was stirred at room temperature overnight. The reaction was concentrated under vacuo. The residue was dissolved in $H_2O$ (30 mL) and the aqueous layer was basified with a solution of 10 N NaOH (3 mL). The aqueous layer was extracted with AcOEt (4x30 mL) and the combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired amine **156** as a yellow oil (0.52 g, 83% yield). The amine (217 mg, 0.83 mmol) was dissolved in MeOH (5 mL) and tartaric acid (151 mg, 0.83 mmol) was added to obtain the analytical sample.

Analytic and spectroscopic data of the amine **156·(2*R*,3*R*)-tartrate**.

Melting point: 51-52 $^oC$ (MeOH).

IR (KBr) ν: 3488, 3320, 3273, 2964, 2925, 2869, 1890, 1735, 1604, 1458, 1400, 1375, 1338, 1305, 1264, 1213, 1135, 1103, 1076, 1068, 988, 903, 886, 789, 680, 484 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 0.99 (t, *J* = 7.2 Hz, 6 H, 2 $CH_2CH_2C\underline{H}_3$), 1.42 (complex signal, 4 H, 2 $CH_2C\underline{H}_2CH_3$), 1.54 [dd, *J* = 12.7 Hz, *J'* = 1.6 Hz, 2 H, 4(10)-$H_{endo}$], 1.66-1.68 [m, 2 H, 8(9)-$H_{endo}$], 1.71 (bs, 2 H, 6-$H_2$), 1.88 (complex signal, 5 H, 3-H + 2 $C\underline{H}_2CH_2CH_3$), 1.95 [m, 2 H, 4(10)-$H_{exo}$], 2.22 [bs, 2 H, 5(7)-H], 2.56 [dm, *J* = 13.0 Hz, 2 H, 8(9)-$H_{exo}$], 2.83 [s, 6 H, N-($C\underline{H}_3$)$_2$], 4.38 [s, 2 H, $C\underline{H}(OH)CO_2H$].

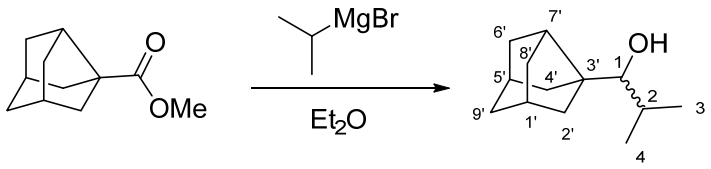
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 15.3 ($CH_3$, 2 $CH_2CH_2\underline{C}H_3$), 18.7 ($CH_2$, 2 $CH_2\underline{C}H_2CH_3$), 31.4 [CH, C5(7)], 32.3 [$CH_2$, C4(10)], 35.3 [$CH_2$, C8(9)], 37.4 ($CH_2$, 2 $\underline{C}H_2CH_2CH_3$), 37.5 (CH, C3), 37.9 ($CH_2$, C6), 42.0 ($CH_3$, N-$\underline{C}H_3$), 46.5 (C, C2), 73.1 (C, C1), 74.2 (CH, $\underline{C}H(OH)COOH$), 177.0 (C, C=O).

MS (EI), m/e (%); main ions: 264 (11), 263 ($M^{+}$,100), 234 (14), 220 (16),165 (12), 164 (73), 136 (12), 123(34), 122 (100), 85 (41), 79 (11), 77 (12), 70 (11).

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_{22}H_{39}NO_6$: | C 63.89% | H 9.51% | N 3.39% |
| Calculated for $C_{22}H_{39}NO_6 \cdot 0.75\ H_2O$: | C 61.87% | H 9.56% | N 3.28% |
| Found: | C 61.90% | H 9.60% | N 3.26% |

**Synthesis of 2-Methyl-1-(3-noradamantyl)-1-propanol, 143.**




**117** **143**

To a stirred solution of ester **117** (0.4 g, 2.4 mmol) in anhydrous $Et_2O$ (12 mL) at 0 ºC, isopropylmagnesium bromide (4 mL, 3 M in methyl THF, 12 mmol) was added dropwise. The reaction was heated under reflux overnight. The reaction was cooled to 0 ºC and quenched with $H_2O$ (5 mL) and the mixture was stirred at 0 ºC for one hour. More water (5 mL) was added and the reaction was tempered. The layer were separated and the aqueous was extracted with $Et_2O$ (3x5 mL). The combined organic layers were dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to dryness to give a white solid (0.5 g). The crude was purified by coloumn chromatography in silica (hexane/AcOEt 98:2) to give alcohol **143** as a white solid (0.4 g, 75% yield).

Analytic and spectroscopic data of the alcohol **143**.

Melting point: 65-66 $^{o}$C.

IR (KBr) ν: 3372, 2924, 2910, 2867, 1456, 1409, 1327, 1296, 1277, 1171, 1135, 1106, 997, 984, 968, 920, 864, 794, 738, 622, 594 cm$^{-1}$.

$^{1}$H NMR (400 MHz, $CDCl_3$) δ: 0.91 (d, *J* = 6.8 Hz, 3 H) and (d, *J* = 7.2 Hz, 3 H) (3-C<u>H</u>$_3$ and 4-C<u>H</u>$_3$), 1.36 (bs, 1 H, OH), 1.58-1.68 (complex signal, 8 H) (9'-$H_2$ + 6'-$H_2$ + 8'-$H_2$ + 2'-$H_{exo}$ + 4'-$H_{exo}$), 1.78-1.82 (complex signal, 2 H, 2'-$H_{endo}$ + 4'-$H_{endo}$), 1.86 (septuplet, *J* = 6.8 Hz, *J'* = 2.5 Hz, 1 H, 2-H), 2.21 (bs, 1 H, 7'-H), 2.25 (complex signal, 2 H, 1'-H + 5'-H), 3.42 (d, *J* = 2.5 Hz, 1 H, 1-H).

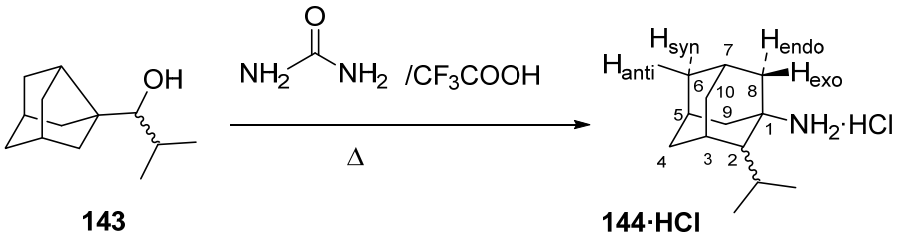
$^{13}$C NMR (100.6 MHz, $CDCl_3$) δ: 16.6 ($CH_3$) and 22.1 ($CH_3$) (C3 and C4), 30.5 (CH, C2), 35.8 ($CH_2$, C9'), 37.4 (CH) and 37.8 (CH) (C1' and C5'), 43.1 (CH, C7'), 43.6 ($CH_2$), 43.7 ($CH_2$), 43.9 ($CH_2$) and 47.1 ($CH_2$) (C2', C4', C6' and C8'), 54.2 (C, C3'), 80.8 (CH, C1).

GC/MS (EI), m/e (%); main ions: 181 (12), 180 ($C_{12}H_{20}O^+$, 100), 149 (18), 148 (31), 121 ($C_9H_{13}^{\cdot}$, 34), 120 (24), 101 (25), 93 (24), 91 (31), 81 (74), 80 (40), 70 (81), 78 (20), 77 (35).

Accurate mass

| | |
|---|---|
| Calculated for $[C_{13}H_{21}+H]^+$ | 177.1638 |
| Found | 177.1639 |

**<u>Synthesis of (2-Isopropyladamant-1-yl)amine hydrochloride, 144·HCl.</u>**




**143** **144·HCl**

A mixture of alcohol **143** (0.5 g, 2.6 mmol), urea (0.33 g, 5.2 mmol) in trifluoroacetic acid (1.1 mL, 13 mmol) was heated at 115 $^{o}$C overnight. The reaction was cooled to 0 $^{o}$C and $H_2O$ (5 mL) was added. The aqueous layer was basified by adding a solution of NaOH 10 N to pH 14. The aqueous layer was extracted with $Et_2O$ (4x10 mL). The combined organic extracts

were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired amine **144·HCl** as a beige oil (0.5 g). The residue was dissolved in AcOEt and $HCl/Et_2O$ was added to yield the amine hydrochloride as a white solid (0.23 g, 99% yield). The analytical sample was obtained by crystallization from MeOH / $Et_2O$.

Analytic and spectroscopic data of the amine **144·HCl**.

Melting point: >300 $^oC$.

IR (KBr) ν: 3487, 2917, 2858, 2687, 2538, 2059, 1605, 1509, 1474, 1457, 1367, 1314, 1117, 1087, 1003, 982, 471 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 0.92 (d, *J* = 6.6 Hz, 3 H) and 0.94 (d, *J* = 6.6 Hz, 3 H) ($C\underline{H}_3CHC\underline{H}_3$), 1.15 (t, *J* = 9.0 Hz, 1 H, 2-H), 1.47 (d, *J* = 13.0 Hz, 1 H, 4-$H_{endo}$ or 10-$H_{endo}$), 1.64 (d, *J* = 12.2 Hz, 1 H, 8-$H_{endo}$ or 9-$H_{endo}$), 1.71-1.98 (complex signal, 10 H, 2 $CH_3C\underline{H}CH_3$ + 6-$H_2$ + 9-$H_{endo}$ or 8-$H_{endo}$ + 10-$H_{endo}$ or 4-$H_{endo}$ + 4-$H_{exo}$ + 8-$H_{exo}$ + 9-$H_{exo}$ + 10-$H_{exo}$), 2.16 (m, 1 H, 3-H), 2.21 (s, 1H) and 2.25 (s, 1 H) (5-H + 7-H).

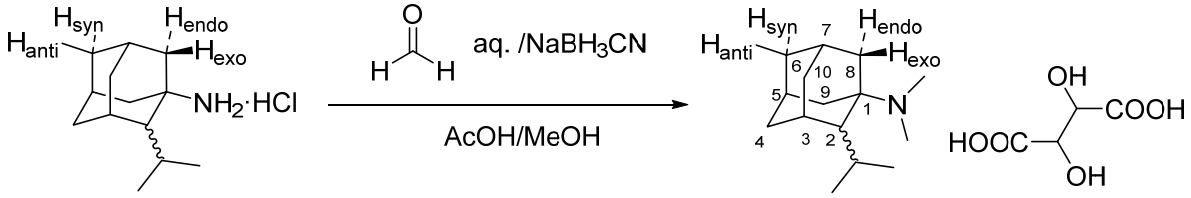
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 21.0 ($CH_3$) and 21.2 ($CH_3$) ($\underline{C}H_3CH\underline{C}H_3$), 28.0 (CH) and 28.2 (CH) ($CH_3\underline{C}HCH_3$), 30.0 and 30.2 (CH, C3), 30.7 ($CH_2$), 36.0 ($CH_2$), 38.1 ($CH_2$), 42.0 ($CH_2$), 42.1 ($CH_2$) and 42.9 ($CH_2$) (C4, C6, C8, C9, C10), 31.5 (CH) and 31.7 (CH) (C5 and C7), 51.0 (CH) and 51.4 (CH) (C2), 52.8 (C, C1).

MS (EI), m/e (%); main ions: 194 (15), 193 ($M^{\cdot+}$, 25), 137 (12), 136 (27), 96 (11), 95 (51), 94 (100), 93 (11).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{13}H_{24}NCl$: | C 67.95% | H 10.53% | N 6.10% | Cl 15.43% |
| Calculated for $C_{13}H_{24}NCl \cdot 0.25\ H_2O$: | C 66.64% | H 10.54% | N 5.98% | Cl 15.13% |
| Found: | C 66.26% | H 10.23% | N 5.83% | Cl 14.83% |

**Synthesis of *N,N*-dimethyl-(2-isopropyladamant-1-yl)amine (2*R*, 3*R*)-tartrate, 157·(2*R*,3*R*)-tartrate.**




**144·HCl** **157·(2R,3R)-tartrate**

A mixture of amine HCl **144** (0.23 g, 1 mmol), $NaBH_3CN$ (0.18 g, 2.9 mmol), formaldehyde (0.25 mL, 37% in aqueous solution, 3.0 mmol), acetic acid (0.15 mL) in MeOH (5 mL) with a $CaCl_2$ tube was stirred at room temperature for 8 h. More $NaBH_3CN$ (0.18 g, 2.9 mmol) and formaldehyde (0.25 mL, 37% in aqueous solution, 3.0 mmol) were added and the reaction was stirred at room temperature overnight. The reaction was concentrated under vacuo and the residue was dissolved in $H_2O$ (10 mL), the aqueous layer was basified with a solution of NaOH 10N (3 mL). The aqueous layer was extracted with AcOEt (4x10 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired amine **157** as an orange oil (0.3 g). The amine (89 mg, 0.40 mmol) was dissolved in MeOH (5 mL) and L-(+)-tartaric acid (60 mg, 0.40 mmol) was added to obtain the analytical sample.

Analytic and spectroscopic data of the amine **157·(2*R*,3*R*)-tartrate**.

Melting point: 63-64 $^oC$ (MeOH).

IR (KBr) ν: 3336, 3057, 2919, 2906, 2732, 2531, 1735, 1676, 1467, 1451, 1420, 1354, 1306, 1264, 1213, 1141, 1111, 1086, 997, 900, 842, 790, 679, 604, 481 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.10 (d, *J* = 6.4 Hz, 3 H) and 1.14 (d, *J* = 6.8 Hz, 3 H) ($C\underline{H}_3CHC\underline{H}_3$), 1.57 (d, *J* = 13.2 Hz, 1 H, 2-H), 1.68-1.84 (complex signal, 4 H, 4-$H_{endo}$ + 6-$H_2$ + 10-$H_{endo}$), 1.91-1.94 (complex signal, 2 H, 8-$H_{endo}$ + 9-$H_{endo}$), 2.01-2.14 (complex signal, 5 H, $CH_3C\underline{H}CH_3$ + 4-$H_{exo}$ + 8-$H_{exo}$ + 9-$H_{exo}$ + 10-$H_{exo}$), 2.23-2.26 (complex signal, 2 H, 5-H + 7-H), 2.32 (bs, 1 H, 3-H), 2.82 [s, 6 H, N-($C\underline{H}_3$)$_2$], 4.41 [s, 2 H, C$\underline{H}$(OH)$CO_2H$].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 21.6 ($CH_3$) and 25.0 ($CH_3$) ($\underline{C}H_3CH\underline{C}H_3$), 27.1 (CH, $CH_3\underline{C}HCH_3$), 30.7 (CH, C3), 31.1 (CH) and 31.5 (CH) (C5 and C7), 32.4 [$CH_2$, C4(10)], 34.6 ($CH_2$) and 37. 4

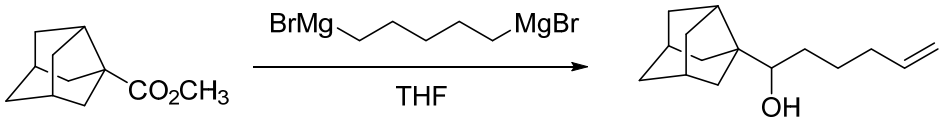
($CH_2$) (C8 and C9), 36.2 ($CH_2$, C6), 37.7 [$CH_3$, N-($\underline{C}H_3)_2$], 40.0 (CH, C2), 68.1 (C, C1), 74.1 [CH, $CH(OH)CO_2H$], 176.6 (C, C=O).

GC/MS (EI), m/e (%); main ions: 222 (9), 221 ($M^{\cdot+}$, 50), 178 (23), 164 (21), 136 (15), 123 (35), 122 (100), 121 (14), 91 (10), 85 (37), 79 (11).

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_{19}H_{33}NO_6$: | C 61.43% | H 8.95% | N 3.77% |
| Calculated for $C_{19}H_{33}NO_6 \cdot 1\ H_2O$: | C 58.59% | H 9.06% | N 3.60% |
| Found: | C 58.32% | H 8.85% | N 3.40% |

**Synthesis of 1-(3-Noradamantyl)-5-hexen-1-ol, 148.**




**117** **148**

In a 25 mL three-necked round-bottomed flask equipped with a condenser, inert atmosphere and magnetic stirring, a solution of ester **117** (220 mg, 1.22 mmol) in anh. THF (5 mL) was cooled to 0 $^{o}$C with an ice-bath. To this solution, dimagnesiumpentamethylene dibromide (3.66 mL, 0.5 M en THF, 1.83 mmol) was added dropwise and the reaction was heated under reflux for 24h. To the reaction, water (1 mL) was added and stirred at room temperature for 1h. Afterwards, more water (3 mL) was added and the layers were separated. The aqueous layer was extracted with pentane (3x40 mL) and the combined extracts were dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to obtain alcohol **148** as a colourless oil (249 mg, 93% yield).

Analytical and spectroscopical data of **148**.

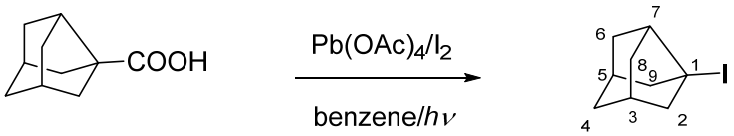
IR (KBr) $\nu$: 3399, 3076, 2922, 2861, 1639, 1458, 1436, 1304, 1075, 1050, 991, 962, 908 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CDCl_3$) δ: 1.30-1.74 (complex signal, 15 H, 2-$H_2$, 3-$H_2$, 2'-$H_2$, 4'-$H_2$, 6'-$H_2$, 8'-$H_2$, 9'-$H_2$ and OH), 2.10 (m, 2 H, 4-$H_2$), 2.17 (t, *J* = 6.8 Hz, 1 H, 7'-H), 2.21 [broad s, 2 H, 1(5)-H], 3.50 (dd, *J* = 10.4 Hz, *J'* = 1.6 Hz, 1H, 1-H), 4.95 (ddt, *J* = 10 Hz, *J'* = 2 Hz, *J"* = 1.5 Hz, 1 H, 6-$H_E$), 5.02 (ddt, *J* = 17 Hz, *J'* = 2 Hz, *J"* = 1.6 Hz, 1 H, 6-$H_Z$), 5.82 (ddt, *J* = 17 Hz, *J'* = 10 Hz, *J"* = 6.4 Hz, 1 H, 5-H).

$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 25.9 ($CH_2$, C3), 32.8 ($CH_2$, C2), 33.7 ($CH_2$, C4), 35.7 ($CH_2$, C9), 37.40 (CH) and 37.41 (CH) (C1' y C5'), 41.2 (CH, C7), 43.5 ($CH_2$) and 44.0 ($CH_2$) (C6' and C8'), 43.7 ($CH_2$) and 45.8 ($CH_2$) (C2' and C4'), 54.3 (C, C3'), 76.7 (CH, C1), 114.4 ($CH_2$, C6), 138.9 (CH, C5).

GC/MS (EI), m/e (%); main ions: 220 ($M^{\cdot+}$, 1), 177 (51), 164 (18), 152 (12), 151 (100), 149 (36), 133 (40), 121 [$(C_9H_{13})^+$, 23], 107 (19), 105 (22), 95 (13), 92 (11), 91 (71), 81 (30), 80 (19), 79 (54), 77 (21), 67 (22), 55 (21).

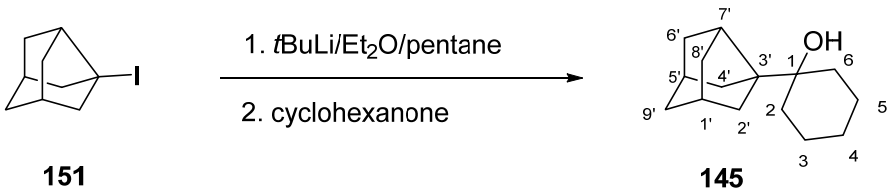
**Synthesis of 1-iodonoradamantane, 151.**




**116** **151**

A solution of acid **116** (1 g, 6 mmol), iodine (7.6 g, 30 mmol) and $Pb(OAc)_4$ (3.5 g, 7.4 mmol) in benzene (100 mL) in argon atmosphere was stirred at room temperature for 5 min. The reaction was irradiated with two tungsten lamps over 2.5 h. The reaction was tempered and the organic layer was washed with sodium tiosulphate solution (3x100 mL) and a saturated solution of $NaHCO_3$ (1x100 mL). The organic layer was dried with anhyd $Na_2SO_4$, filtered and evaporated in vacuo to give the compound **151** as a pale yellow oil (1.4 g, 99% yield).[151]

**Synthesis of 1-(3-Noradamantyl)cyclohexan-1-ol, 145.**




A solution of 1-iodonoradamantane **151** (2.5 g, 10.1 mmol) in anh. $Et_2O$ (83 mL) and anh pentane (42 mL) in argon atmosphere was cooled to -78 ºC, and *t*-BuLi 1.4 M (12 mL, 20.2 mmol) was added dropwise. The reaction was stirred at -78 ºC for 2 h. This solution was added dropwise into a solution of cyclohexanone (1.6 mL, 15.1 mmol) in anhydrous $Et_2O$ (68 mL) and anh pentane (34 mL) at -78 ºC. The reaction was stirred at -78 ºC for 2 h, at 0 ºC for an hour and at room temperature overnight. Water (15 mL) was added dropwise, the layers were separated and the aqueous extracted with $Et_2O$ (2x20 mL). The combined organic layers were dried with anhydrous $Na_2SO_4$, filtered and evaporated in vacuo to obtain a colourless oil (2.20 g). The crude was purified by coloumn chromatography in silica (hexane/AcOEt 99:1) to obtain the desired alcohol **145** as a colourless oil (1.25 g, 56% yield).

Analytic and spectroscopic data of alcohol **145**.

IR (KBr) *v*: 3459, 2930, 2859, 1710, 1447, 1349, 1313, 1252, 1210, 1166, 1122, 1067, 1033, 960, 897, 828, 757, 700 $cm^{-1}$.

$^1H$ NMR (400 MHz, $CDCl_3$) δ: 1.14 (bs, 1 H, OH), 1.40-1.67 [complex signal, 18 H, 2(6)-$H_{eq}$ + 3(5)-$H_2$ + 4-$H_2$ + 2'(4',6',8')-$H_2$ + 9'-$H_2$], 1.84 [dm, *J* = 10.4 Hz, 2 H, 2(6)-$H_{ax}$], 2.25 [bs, 2 H, 1'(5')-H], 2.32 (t, *J* = 6.4 Hz, 7'-H).

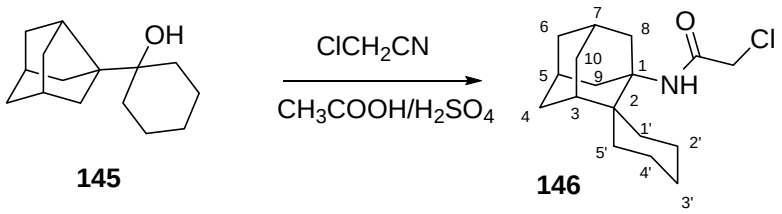
$^{13}C$ NMR (100.6 MHz, $CDCl_3$) δ: 21.7 [$CH_2$, C3(5)], 26.0 ($CH_2$, C4), 32.8 [$CH_2$, C2(6)], 35.6 ($CH_2$, C9'), 37.4 [CH, C1'(5')], 38.9 (CH, C7'), 44.2 ($CH_2$) and 44.4 ($CH_2$) [C2'(4') and C6'(8')], 58.0 (C, C3'), 73.6 (C, C1).

GC/MS (EI), m/e (%): 220 ($M^{+}$, 21), 202 (23), 178 (15), 177 (100), 164 (39), 149 (88), 121 (57), 99 (24), 98 (38), 93 (32), 91 (33), 81 (44), 80 (28), 79 (64), 77 (31), 67 (22), 55 (30).

Elemental analysis

| | | |
|---|---|---|
| Calculated for $C_{15}H_{24}O$: | C 81.76% | H 10.98% |
| Found | C 81.69% | H 11.19% |

**Synthesis of *N*-(spiro[adamantane-2,1'-cyclohexan]-1-yl)-2-chloroacetamide, 146.**




**145** **146**

A solution of alcohol **145** (1.3 g, 5.7 mmol) and chloroacetonitrile (1.7 mL, 25.5 mmol) in acetic acid (2 mL) was cooled to 0 $^{o}$C. Concentrated sulfuric acid (2 mL) was added dropwise without exceeding 10 $^{o}$C. The reaction was stirred at room temperature overnight. The crude of the reaction was poured into ice (15 g) and the residue was filtered under vacuo. The filtrate was dissolved in $CH_2Cl_2$ (10 mL) and the organic layer were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired chloroacetamide **146** as an violet solid (0.65 g, 38% yield).

Analytic and spectroscopic data of the chloroacetamide **146**.

Melting point: 136-137 $^{o}$C (decompose).

IR (KBr) $\nu$: 3386, 3303, 3093, 2921, 2900, 2863, 1662, 1560, 1458, 1446, 1342, 1254, 1221, 1153, 1109, 1089, 981, 898, 797, 712, 620, 535 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CDCl_3$) $\delta$: 1.11-1.18 [complex signal, 3 H, 3'-$H_{eq}$ + 2'(6')-$H_{eq}$], 1.36-1.55 [complex signal, 6 H, 4(10)-$H_{endo}$ + 3'(5')-H], 1.62 (dm, *J* = 12.3 Hz, 1 H, 4'-$H_{ax}$), 1.68 (dm, *J* = 12.1 Hz, 2 H, 6-$H_2$), 1.94 [d, *J* = 1.6 Hz, 2 H, 4(10)-$H_{exo}$], 2.02 [bs, 2 H, 5(7)-H], 2.13 [dm, *J* = 9.9 Hz, 8(9)-$H_{endo}$ + 2'(6')-$H_{ax}$], 2.21 (t, *J* = 2.9 Hz, 1 H, 3-H), 2.33 [dd, *J* = 13.3 Hz, *J*' = 1.5 Hz, 8(9)-$H_{exo}$], 3.96 (s, 2 H, C(O)C$\underline{H}_2$Cl), 6.39 (bs, 1 H, N$\underline{H}$).

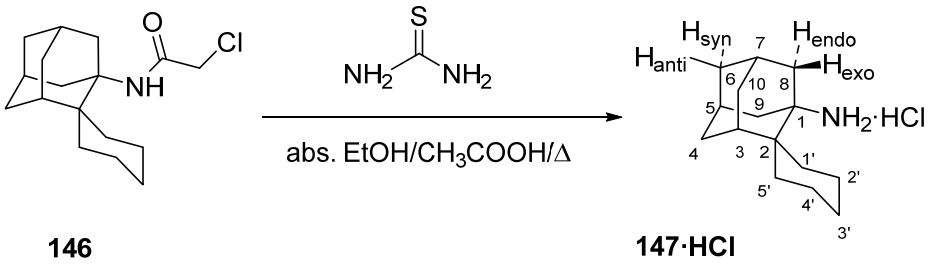
$^{13}$C-RMN (125.7 MHz, $CDCl_3$) $\delta$: 20.5 [$CH_2$, C3'(5')], 26.2 ($CH_2$, C4'), 28.7 [$CH_2$, C2'(6')], 29.1 [CH, C5(7)], 29.4 (CH, C3), 31.2 [$CH_2$, C4(10)], 36.0 [$CH_2$, C8(9)], 38.0 ($CH_2$, C6), 42.1 (C, C2), 43.6 [$CH_2$, C(O)$\underline{C}H_2$Cl], 58.1 (C,C1), 164.5 (C, C=O).

GC/MS (EI), m/e (%); main ions: 295 ($M^{\cdot+}$, 9), 260 (23), 203 (20), 202 (100), 172 (15), 170 (38), 159 (13), 94 (17), 91 (16), 79 (16), 77 (11).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{17}H_{26}ClNO$: | C 69.02% | H 8.86% | N 4.73% | Cl 11.98% |
| Found: | C 69.12% | H 9.15% | N 4.71% | Cl 11.76% |

**Synthesis of spiro[adamantane-2,1'-cyclohexan]-1-amine, 147·HCl.**




**146** **147·HCl**

A solution of chloroacetamide **146** (0.65 g, 2.2 mmol), thiourea (0.18 g, 2.6 mmol), glacial acetic acid (1 mL) in absolute EtOH (4.5 mL) was heated under reflux overnight. The reaction was tempered and the suspension was dissolved in water (20 mL) and basified with a solution of 10 N NaOH (10 mL). The aqueous layer was extracted with AcOEt (3x20 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired amine **147** as a yellowish oil. The residue was dissolved in AcOEt and $HCl/Et_2O$ was added. The solid was filtered under vacuo and washed with cold $Et_2O$ to obtain the amine **147·HCl** as a white solid (0.35 g, 62% yield). The analytical sample was obtained by crystallization with $MeOH/Et_2O$.

Analytic and spectroscopic data of the amine **147·HCl**.

Melting point: >300 $^oC$ (MeOH).

IR (KBr) ν: 3037, 2970, 2927, 2906, 2865, 2037, 1612, 1597, 1517, 1463, 1449, 1361, 1344, 1316, 1296, 1216, 1125, 1086, 1003, 983, 899, 542 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 1.15.1.26 [complex signal, 3 H, 2'(6')-$H_{eq}$ + 4'-$H_b$], 1.46-1.64 [complex signal, 8 H, 8(9)-$H_{endo}$ + 4(10)-$H_{endo}$ + 3'(5')-H], 1.65-1.78 (complex signal, 3 H, 6-$H_2$ + 4'-$H_a$), 2.00 [d, *J* = 10.5 Hz, 2 H, 4(10)-$H_{exo}$], 2.12 [bs, 2 H, 5(7)-H], 2.17 [d, *J* = 12.0 Hz, 2 H, 2'(6')-$H_{ax}$], 2.33 [broad d, 2 H, 8(9)-$H_{exo}$], 2.34 (bs, 1 H, 3-H).

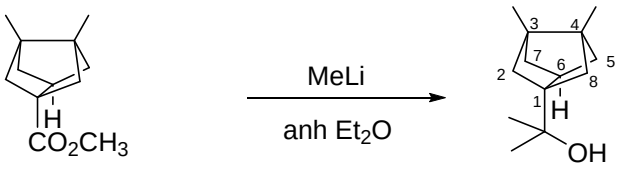
$^{13}$C-RMN (125.7 MHz, $CD_3OD$) δ: 21.3 [$CH_2$, C3'(5')], 27.0 ($CH_2$, C4'), 29.2 [$CH_2$, C2'(6')], 30.4 [CH, C5(7)], 30.6 (CH, C3), 31.6 [$CH_2$, C4(10)], 37.5 [$CH_2$, C8(9)], 38.4 ($CH_2$, C6), 41.5 (C, C2), 58.7 (C, C1).

MS (EI), m/e (%); main ions: 220 (22), 219 ($M^{\cdot+}$, 13), 203 (12), 202 (18), 97 (13), 96 (52), 95 (100), 94 (68), 91 (11), 79 (13), 77 (11).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{15}H_{26}ClN$: | C 70.42% | H 10.24% | N 5.48% | Cl 13.86% |
| Found: | C 70.44% | H 10.24% | N 5.48% | Cl 13.86% |

**Synthesis of 2-(3,7-dimethyl)tricyclo[3.3.0.0$^{3,7}$]oct-1-yl)propan-2-ol, 153.**




**152** **153**

To a stirred solution of ester **152** (1.7 g, 8.7 mmol) in anhydrous $Et_2O$ (40 mL) at 0 $^oC$, methyl lithium (44 mL, 1.6 M, 70.0 mmol) was added dropwise. The reaction was heated under reflux overnight. The reaction was cooled to 0 $^oC$ and quenched with $H_2O$ (4 mL) and the mixture was stirred at 0 $^oC$ for one hour. More water (4 mL) was added and the reaction was tempered. The layers were separated and the aqueous was extracted with $Et_2O$ (3x20 mL). The combined organic layers were dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to dryness to give a yellowish oil (1.26 g). The crude was purified by microdistillation (160 $^oC$, 160 mmHg) to obtain the alcohol **153** as a yellowish oil (0.85 g, 51% yield).

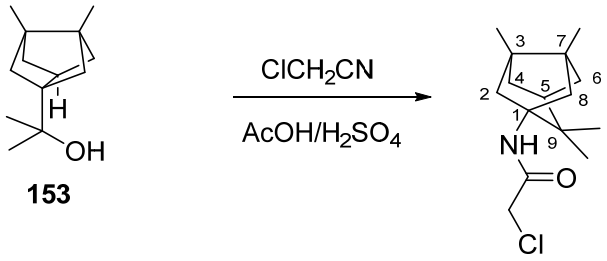
Analytic and spectroscopic data of the alcohol **153**.

IR (KBr) *v*: 3467, 2950, 2880, 2865, 1478, 1458, 1446, 1379, 1366, 1303, 1181, 1150, 1126, 1102, 942, 922, 904, 818 $cm^{-1}$.

$^1$H NMR (400 MHz, $CDCl_3$) δ: 1.14 [s, 6 H, ($C\underline{H}_3$)-C3'(7')], 1.22 [s, 6 H, $C\underline{H}_3$-C1(3)], 1.33 [complex signal, 4 H, 2'(8')-H], 1.41 [complex signal, 3 H, OH + 4'(6')-$H_{exo}$], 1.49 [dd, *J* = 8.0 Hz, *J'*= 2.8 Hz, 4'(6')-$H_{endo}$], 2.25 (t, *J* = 2.8 Hz, 5'-H).

$^{13}$C NMR (100.6 MHz, $CDCl_3$) δ: 16.9 [$CH_3$, $\underline{C}H_3$-C3'(7')], 26.9 [$CH_3$, C1(3)], 40.1 (CH, C5'), 47.4 [C, C3'(7')], 54.0 ($CH_2$) and 54.4 ($CH_2$) [C2'(8') and C4'(6')], 60.2 (C, C1'), 71.9 (C, C2).

**Synthesis of *N*-[(3',7',9',9'-Tetramethyl)tricyclo[3.3.1.0$^{3,7}$]non-1'-yl]-2-chloroacetamide.**




**153**

A solution of alcohol **153** (0.85 g, 4.4 mmol) and chloroacetonitrile (1.3 mL, 20.0 mmol) in acetic acid (1.6 mL) was cooled to 0 $^{o}$C. Concentrated sulfuric acid (1.6 mL) was added dropwise without exceeding 10 $^{o}$C. The reaction was stirred at room temperature overnight. The crude of the reaction was poured into ice (23 g) and the residue was filtered under vacuo. The filtrate was dissolved in $CH_2Cl_2$ (10 mL) and the organic layer were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired chloroacetamide as an violet solid (1.10 g, 93% yield).

Analytic and spectroscopic data of the chloroacetamide.

Melting point: 150-151 $^{o}$C.

IR (KBr) *v*: 3281, 3093, 2985, 2945, 2865, 1660, 1572, 1463, 1444, 1334, 1247, 1234, 1183, 1145, 1070, 947, 799, 759, 741, 631, 547, 489 cm$^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 1.00 [s, 6 H, C$\underline{H}_3$-3'(7')], 1.04 [s, 6 H, C$\underline{H}_3$-C1(3)], 1.30 [dt, *J* = 10.5 Hz, *J'*= 2.8 Hz, 2 H, 4'(6')-$H_{endo}$], 1.68 (t, *J* = 3.5 Hz, 1 H, 5'-H), 1.77 [dd, *J* = 11.0 Hz, *J'*= 1.1 Hz, 4'(6')-$H_{exo}$], 1.87 [dd, *J* = 10.8 Hz, *J'*= 2.7 Hz, 2'(8')-$H_{endo}$], 2.10 [dd, *J* = 13.3 Hz, *J'*= 2.4 Hz, 2'(8')-$H_{exo}$], 3.95 (bs, 2 H, 2-H), 6.44 (bs, 1 H, NH).

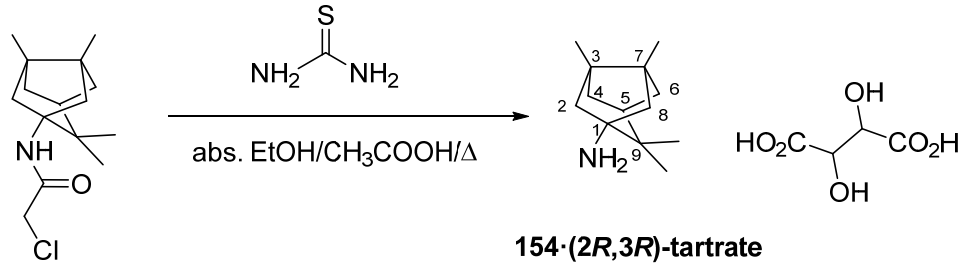
$^{13}$C-RMN (125.7 MHz, $CD_3OD$) δ: 21.6 [$CH_3$, $\underline{C}H_3$-C3'(7')], 23.7 [$CH_3$, ($\underline{C}H_3$)$_2$-C9'], 39.2 (C, C9'), 43.2 ($CH_2$, C2), 43.7 [C, C3'(7')], 46.1 [$CH_2$, C4'(6')], 46.4 (CH, C5'), 50.2 [$CH_2$, C2'(8')], 63.3 (C, C1'), 164.6 (C, C=O).

GC/MS (EI), m/e (%); main ions: 269 ($M^{\cdot+}$, 1), 198 (11), 187 (33), 186 (12), 185 (100).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{15}H_{24}ClNO$: | C 66.77% | H 8.97% | N 5.19% | Cl 13.14% |
| Found: | C 66.61% | H 9.15% | N 5.15% | Cl 13.02% |

**Synthesis of [(3,7,9,9-Tetramethyl)tricyclo[3.3.1.0$^{3,7}$]non-1-yl]amine (2*R*,3*R*)-tartrate, 154·(2*R*,3*R*)-tartrate**




**154·(2*R*,3*R*)-tartrate**

A solution of chloroacetamide (1.1 g, 4.1 mmol), thiourea (0.37 g, 4.9 mmol), glacial acetic acid (1.7 mL) in absolute EtOH (10 mL) was heated under reflux overnight. The reaction was tempered and the suspension was dissolved in water (25 mL) and basified with a solution of 10 N NaOH (10 mL). The aqueous layer was extracted with AcOEt (3x25 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired amine **154** as a yellowish oil (560 mg, 74% yield). The residue was dissolved in MeOH and L-(+)-tartaric acid (0.43, 3 mmol) was added. The suspension was filtered off to obtain the amine **154** as a tartrate salt (0.74 g). The analytical sample was obtained by crystallization with MeOH/$Et_2O$.

Analytic and spectroscopic data of the amine **154·(2*R*,3*R*)-tartrate**.

Melting point: 232-233 $^oC$ (MeOH).

IR (KBr) ν: 3411, 3140, 2970, 2951, 2941, 2867, 1718, 1623, 1558, 1537, 1467, 1326, 1137, 1074, 906, 838, 680, 567, 476 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 1.06 [s, 6 H, C$\underline{H}_3$-3(7)], 1.14 [s, 6 H, (C$\underline{H}_3$)$_2$-9], 1.40 [dt, *J* = 11.0 Hz, *J'*= 3.0 Hz, 2 H, 4(6)-$H_{endo}$], 1.64 [dd, *J* = 10.0 Hz, *J'* = 2.5 Hz,2 H, 2(8)-$H_{endo}$], 1.77 (t, *J* = 3.0 Hz, 5-H), 1.84 [d, *J* = 10.5 Hz, 2 H, 4(6)-$H_{exo}$], 1.98 [dd, *J* = 13.0 Hz, *J'*= 2.0 Hz, 2(8)-$H_{exo}$], 4.35 [s, 2 H, C$\underline{H}$(OH)$CO_2H$].

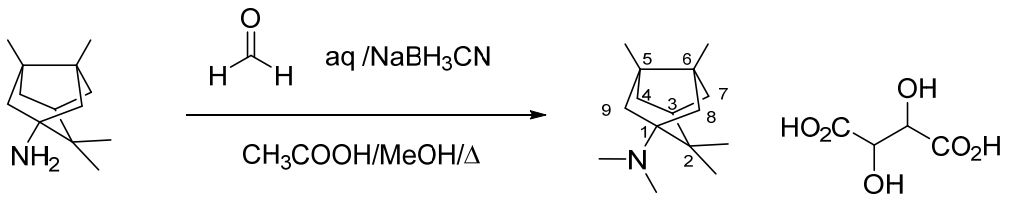
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 21.7 [$CH_3$, C$\underline{H}_3$-C3(7)], 23.3 [$CH_3$, (C$\underline{H}_3$)$_2$-C9], 38.0 [C, C3(7)], 45.6 [$CH_2$, C4(6)], 46.7 (CH, C5), 48.3 (C, C9), 50.4 [$CH_2$, C2(8)], 63.1 (C, C1), 74.2 [CH, C$\underline{H}$(OH)$CO_2H$], 177.0 (C, C=O).

MS (EI), m/e (%); main ions: 178 (5), 122 (17), 110 (10), 109 (100), 108 (20).

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_{17}H_{29}NO_6$: | C 59.46% | H 8.51% | N 4.08% |
| Found: | C 59.21% | H 8.69% | N 4.03% |

**Synthesis of *N*,*N*-Dimethyl-(3,7,9,9-tetramethyltricyclo[3.3.1.0$^{3,7}$]non-1-yl)amine (2*R*,3*R*)-tartrate, 158·(2*R*,3*R*)-tartrate.**




**154** **158·(2*R*,3*R*)-tartrate**

A mixture of amine **154** (0.15 g, 0.8 mmol), $NaBH_3CN$ (0.15 g, 2.3 mmol), formaldehyde (0.20 mL, 37% in aqueous solution, 2.4 mmol), acetic acid (0.1 mL) in MeOH (6 mL) with a $CaCl_2$ tube was stirred at room temperature for 8 h. More $NaBH_3CN$ (0.15 g, 2.3 mmol) and formaldehyde (0.20 mL, 37% in aqueous solution, 2.4 mmol) were added and the reaction was stirred at room temperature overnight. The reaction was concentrated under vacuo. The residue was dissolved in $H_2O$ (7 mL) and the aqueous layer was basified with a solution of NaOH 10N (3 mL). The aqueous layer was extracted with AcOEt (3x10 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain the desired amine **158** as an yellow oil (0.17 g, 96% yield). The amine (167 mg, 0.76 mmol) was dissolved in MeOH (5 mL) and L-(+)-tartaric acid (114 mg, 0.76 mmol) was added to obtain the tartrate amine **158**.

Analytic and spectroscopic data of the amine **158·(2*R*,3*R*)-tartrate**.

Melting point: 155-156 $^{o}$C (MeOH).

IR (KBr) ν: 3425, 3318, 2925, 2866, 1749, 1624, 1600, 1517, 1465, 1445, 1406, 1345, 1321, 1290, 1229, 1132, 1118, 1110, 1078, 1006, 989, 940, 901, 684, 611, 567, 524 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.09 [s, 6 H, C$\underline{H_3}$-3(7)], 1.29 [s, 6 H, (C$\underline{H_3}$)$_2$-9], 1.39 [dt, *J* = 11.2 Hz, *J'*= 3.0 Hz, 2 H, 4(6)-H$_{endo}$], 1.77 (t, *J* = 3.0 Hz, 1 H, 5-H), 1.85 [dd, *J* = 11.2 Hz, *J'* = 1.2 Hz, 2 H,

4(6)-$H_{exo}$], 1.93 [dd, *J* = 10.4 Hz, *J'*= 2.0 Hz, 2(8)-$H_{endo}$], 2.09 [dd, *J* = 13.2 Hz, *J'*= 2.4 Hz, 2(8)-$H_{exo}$], 2.85 [s, 6 H, N-(C<u>H</u>$_3$)$_2$], 4.40 [s, 2 H, C<u>H</u>(OH)$CO_2H$].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 21.7 [$CH_3$, <u>C</u>$H_3$-C3(7)], 24.1 [$CH_3$, (<u>C</u>$H_3$)$_2$-C9], 40.1 [C, C3(7)], 43.2 [$CH_3$, N-(<u>C</u>$H_3$)$_2$], 44.9 (C, C9), 46.3 [$CH_2$, C4(6)], 48.2 [$CH_2$, C2(8)], 50.9 (CH, C5), 74.1 [CH, <u>C</u>H(OH)$CO_2H$], 76.0 (C, C1), 176.8 (C, C=O).

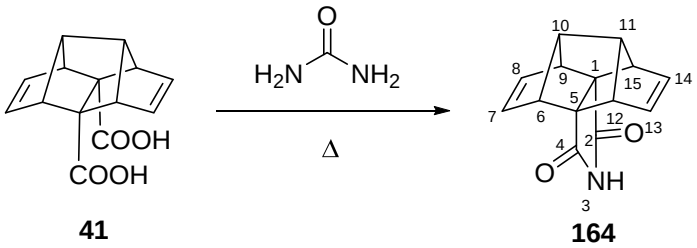
MS (EI), m/e (%); main ions: 222 (2), 221 ($M^{\cdot+}$, 9), 206 (12), 138 (13), 137 (100), 122 (12).

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_{19}H_{33}NO_6$: | C 61.43% | H 8.95% | N 3.77% |
| Calculated for $C_{19}H_{33}NO_6 \cdot 0.5\ H_2O$: | C 59.98% | H 9.01% | N 3.68% |
| Found: | C 60.28% | H 8.85% | N 3.43% |

# 3. SYNTHESIS OF 3-AZAHEXACYCLO[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]PENTADECA-7,13-DIENE AND RELATED COMPOUNDS

**Synthesis of 3-Azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene-1,2-dione, 164.**




**41** **164**

A mixture of diacid **41** (5 g, 20.5 mmol) and urea (6.20 g, 97% purity, 102.5 mmol) was heated slowly to 135 $^{o}$C. When the mixture melted it was heated to 180 $^{o}$C for 30 min and then, it was cooled to room temperature. Water (100 mL) was added and the suspension was extracted with $CH_2Cl_2$ (6×60 mL). The combined organic extracts were washed with brine (1×90 mL), dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to dryness to give imide **164** as a white solid (3.66 g, 79% yield). An analytical sample of **164** was obtained by crystallization from $CH_2Cl_2$.

Analytic and spectroscopic data of the imide **164**.

Melting point: 244-245 $^{o}$C (decompose)

IR (KBr) $\nu$ : 3187, 3075, 2992, 2982, 2756, 1751, 1718, 1387, 1346, 1323, 1277, 1247, 1069, 1015, 869, 819, 788, 769, 724, 705, 620, 604 cm$^{-1}$.

$^{1}$H NMR (500 MHz, $CDCl_3$) δ: 2.87 [m, 2 H, 10(11)-H], 3.46 [m, 4 H, 6(9, 12, 15)-H], 6.07 [t, *J* = 2.0 Hz, 4 H, 7(8,13,14)-H], 7.96 (broad s, 1 H, NH).

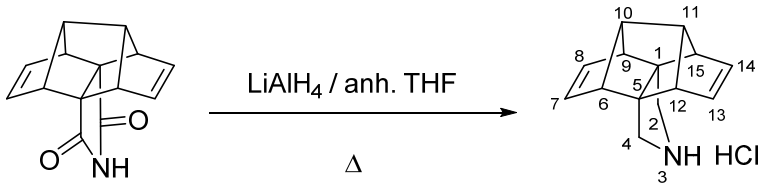
$^{13}$C NMR (100.6 MHz, $CDCl_3$) δ: 62.4 [CH, C6(9,12,15)], 64.4 [CH, C10(11)], 68.3 [C, C1(5)], 132.2 [CH, C7(8,13,14)], 175.5 (C, C=O).

MS (EI), m/e (%); main ions: 225 ($M^{\cdot+}$, 4), 160 (100), 153 (31), 152 (18).

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_{14}H_{14}NO_2$: | C 74.65% | H 4.92% | N 6.22% |
| Found | C 74.29% | H 4.97% | N 6.31% |

**Synthesis of 3-Azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene hydrochloride, 166·HCl.**




**164** **166·HCl**

To a stirred solution of imide **164** (1.45 g, 6.42 mmol) in anhydrous THF (50 mL) at 0 $^{o}$C, $LiAlH_4$ (2.44 g, 64.2 mmol) was carefully added. When the addition was finished, the suspension was heated under reflux for 72 h. The mixture was cooled to 0 $^{o}$C (ice-water bath), treated with 10 N NaOH until basic pH and stirred at room temperature for 1 h. The obtained solid was filtered in vacuo through Celite® and washed with $CH_2Cl_2$ (3×25 mL). The filtrate was dried with anhydrous $Na_2SO_4$, filtered and evaporated in vacuo. The obtained solid residue was taken in $Et_2O$ (20 mL) and treated with excess of HCl in $Et_2O$ to give the hydrochloride of **166**. An analytical sample of **166·HCl** was obtained as a white solid by crystallization from MeOH/$Et_2O$.

Analytic and spectroscopic data of the amine **166·HCl**.

Melting point: 239-240 $^{o}$C (decompose).

IR (KBr) *v*: 3160, 3060, 2974, 2949, 2883, 2836, 2736, 2631, 2551, 2509, 2478, 1566, 1458, 1445, 1409, 1339, 1283, 1269, 1238, 1225, 1203, 1185, 1056, 946, 913, 871, 762, 731 cm$^{-1}$.

$^{1}$H NMR (500 MHz, DMSO-$d_6$) δ: 2.77 (m, 2 H, 10(11)-H), 2.97 [s, 4 H, 2(4)-$H_2$], 3.11 [pseudo q, *J* = 2.5 Hz, 4 H, 6(9,12,15)-H], 4.86 (s, $NH_2$), 6.19 [t, *J* = 2.0 Hz, 4 H, 7(8,13,14)-H].

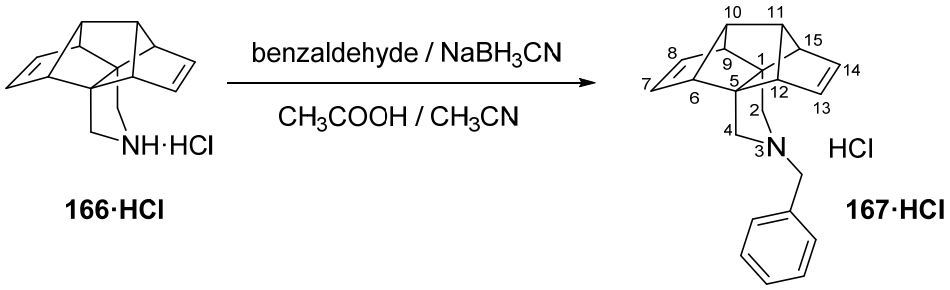
$^{13}$C NMR (100.6 MHz, DMSO-$d_6$) δ: 36.3 [$CH_2$, C2(4)], 53.3 [CH, C6(9,12,15)], 55.3 [CH, C10(11)], 63.3 [C, C1(5)], 125.4 [CH, C7(8,13,14)].

MS (EI), m/e (%); main ions: 197 ($M^{\cdot+}$, 55), 196 (35), 168 (24), 167 (26), 165 (27), 156 (30), 153 (25), 152 (30), 132 (64), 131 (91), 130 (100), 118 (48), 117 (38), 115 (51).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{14}H_{15}N$·HCl: | C 71.94% | H 6.90% | N 5.99% | Cl 15.17% |
| Found: | C 71.80% | H 7.08% | N 5.91% | Cl 15.47% |

**Synthesis of 3-Benzyl-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene hydrochloride, 167·HCl.**




In a 25 ml round bottomed-flask equipped with a condenser a suspension of amine **166·HCl** (450 mg, 1.9 mmol), benzaldehyde (0.3 mL, 2.7 mmol) and $NaBH_3CN$ (256 mg, 3.9 mmol) in methanol was stirred at room temperature for 30 min. Then, acetic acid (0.3 mL) was added and the reaction was stirred for 6 hours. Then, more $NaBH_3CN$ (128 mg, 1.9 mmol) was added and the reaction stirred at room temperature overnight. The reaction was concentrated in vacuo and the residue dissolved in 2N NaOH (40 mL), the aqueous layer was extracted with $CH_2Cl_2$ (3 x 50 mL). The combined organic layers were dryed over $Na_2SO_4$, filtered and concentrated in vacuo. The residue was dissolved in AcOEt and HCl / $Et_2O$ was added dropwise to obtain the amine **167·HCl** as a beige solid (330 mg, 53% yield).

Analytic and spectroscopic data of the amine **167·HCl**.

IR (KBr) ν: 3600-2800 (max. at 3383, 3064, 2951, 2672, 2580, 2487, 2288), 1621, 1568, 1495, 1457, 1444, 1413, 1391, 1341, 1211, 1151, 1105, 1075, 1032, 967, 950, 939, 874, 769, 752, 741, 700, 675, 606 cm$^{-1}$.

$^1$H NMR (500 MHz, $CD_3OD$) δ: 2.67 [m, 1 H, 10-H or 11-H], 2.77 [d, *J* = 13.0 Hz, 2 H, 2(4)-H$_{cis}$], 2.78 (m, 1 H, 11-H or 10-H), 3.07 [m, 2 H, 6(9)-H or 12(15)-H], 3.11 [m, 2 H, 12(15)-H or 6(9)-H], 3.42 [d, *J* = 13.0 Hz, 2 H, 2(4)-H$_{trans}$], 4.23 (s, 2 H, C$\underline{H_2}$C$_6$H$_5$), 4.86 (s, mobile H), 6.10 [t, *J* = 2.0 Hz, 2 H, 7(8)-H or 13(14)-H], 6.23 [t, *J* = 2.0 Hz, 2 H, 13(14)-H or 7(8)-H], 7.44-7.51 (m, 5 H, CH$_2$C$_6$H$\underline{_5}$).

$^{13}$C NMR (125.7 MHz, $CD_3OD$) δ: 54.7 [$CH_2$, C2(4)], 60.1 ($CH_2$, $\underline{C}H_2C_6H_5$), 62.7 [CH, C6(9) or C12(15)], 63.0 [CH, C12(15) or C6(9)], 63.6 (CH, C10 or C11), 65.2 (CH, C11 or C10), 71.6 [C, C1(5)], 130.3 8CH, Ar-C$_{meta}$), 130.8 (C, Ar-C$_{ipso}$), 131.2 (CH, Ar-C$_{para}$), 132.3 (CH, Ar-C$_{orto}$), 135.1 [CH, C7(8) or C13(14)], 135.2 [CH, C13(14) or C7(8)].

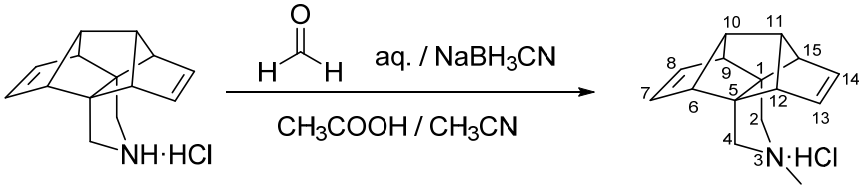
MS (EI), m/e (%); main ions: 288 ([M+H$^{\cdot+}$], 13), 287 (M$^{\cdot+}$, 60), 286 (22), 246 (10), 222 (14), 221 (35), 220 (13), 208 (22), 196 (12), 167 (11), 165 (12), 152 (12), 130 (22), 91 (100), 65 (11).

Accurate mass:

Calculated for $[C_{21}H_{22}N+H]^+$ 288.1747

Found: 288.1749

**Synthesis of 3-Methyl-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene hydrochloride, 168·HCl.**




**166·HCl** **168·HCl**

In a 25 ml round bottomed-flask equipped with a condenser a suspension of amine **166·HCl** (690 mg, 3.0 mmol), aqueous formaldehyde 37% (1.6 mL, 19.5 mmol) and $NaBH_3CN$ (397 mg, 6 mmol) in $CH_3CN$ was stirred at room temperature for 30 min. Then, acetic acid (1.8 mL) was added and the reaction was stirred for 2 hours. Then, more $NaBH_3CN$ (397 mg, 6 mmol) was added and the reaction stirred at room temperature overnight. The reaction was concentrated in vacuo and the residue dissolved in 2 N NaOH (40 mL), the aqueous layer was extracted with $CH_2Cl_2$ (3 x 50 mL). The combined organic layers were dryed over $Na_2SO_4$, filtered and concentrated in vacuo. The residue was dissolved in AcOEt and $HCl/Et_2O$ was added dropwise to obtain the amine **168·HCl** as a beige solid (520 mg, 70% yield).

Analytic and spectroscopic data of the amine **168·HCl**.

IR (KBr) ν: 3600-2400 (max. at 3400, 3062, 2968, 2801, 2716), 1625, 1456, 1404, 1340, 1138, 978, 746, 731, 670 cm$^{-1}$.

$^1$H NMR (500 MHz, $CD_3OD$) δ: 2.59 [d, *J* = 13.0 Hz, 2H, 2(4)-H$_{cis}$], 2.69 (m, 1H, 10-H or 11-H), 2.74 (s, 3H, N-C$\underline{H_3}$), 2.82 (m, 1H, 11-H or 10-H), 3.09 [m, 2H, 6(9)-H or 12(15)-H], 3.13 [m, 2H, 12(15)-H or 6(9)-H], 3.60 [d, *J* = 13.0 Hz, 2H, 2(4)-H$_{trans}$], 4.86 (s, mobile H), 6.20 [t, *J* = 2.0 Hz, 2H, 7(8)-H or 13(14)-H], 6.24 [t, *J* = 2.0 Hz, 2H, 13(14)-H or 7(8)-H].

$^{13}$C NMR (125.7 MHz, $CD_3OD$) δ: 42.9 ($CH_3$, $NCH_3$), 57.1 [$CH_2$, C2(4)], 62.4 [CH, C6(9) or C12(15)], 63.0 [CH, C12(15) or C6(9)], 63.4 (CH, C10 or C11), 65.4 (CH, C11 or C10), 72.2 [C, C1(5)], 135.1 [CH, C7(8) or C13(14)], 135.2 [CH, C13(14) or C7(8)].

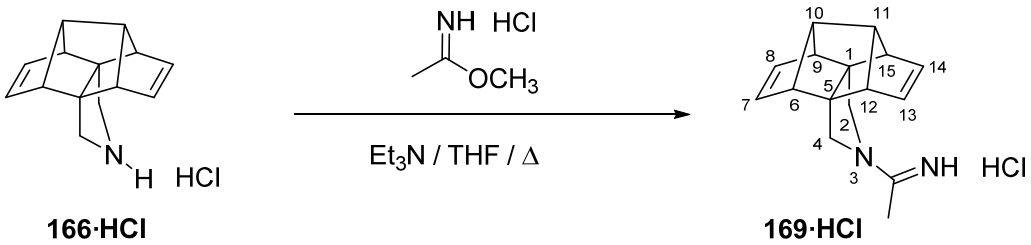
MS (EI), m/e (%); main ions: 211 ($M^{+}$, 91), 210 (57), 146 (51), 145 (100), 144 (95), 132 (88), 115 (45), 94 (45).

Accurate mass:

Calculated for $[C_{15}H_{18}N+H]^+$ 212.1434

Found: 212.1428

**Synthesis of 3-Acetimidoyl-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene hydrochloride, 169·HCl**




**166·HCl** **169·HCl**

In a 10 mL round-bottomed flask a suspension of amine **166·HCl** (250 mg, 1.1 mmol), $Et_3N$ (0.5 mL, 3.2 mmol) and dimethyl acetimidate (235 mg, 2.1 mmol) in THF (7 mL) was stirred at room temperature for 24 hours. The suspension was filtrated in vacuo to obtain the desired acetamidine **169·HCl** as a beige solid (280 mg, 95% yield). The analytical sample was obtained by crystallization from MeOH / $Et_2O$.

Analytic and spectroscopic data of the acetamidine **169·HCl**.

Melting point: 236-237 $^{o}$C (MeOH).

IR (KBr) ν: 3401, 3125, 3059, 2961, 1693, 1634, 1503, 1452, 1365, 1340, 1207, 1169, 758, 734, 683 cm$^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 2.20 (s, 3H, NH=C-C*H*$_3$), 2.74 [broad s, 2H, 10(11)-H], 3.12 [broad s, 4H, 6(9,12,15)-H], 3.16 (s, 2H) and 3.41 (s, 2H) [2(4)-$H_2$], 6.12 [ddd, *J* = 6.0 Hz, *J'* = 3.0 Hz, *J''* = 1.0 Hz, 2H) and 6.16 [ddd, *J* = 6.0 Hz, *J'* = 3.0 Hz, *J''* = 1.0 Hz, 2H) [7(14)-H and 8(13)-H].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 18.9 ($CH_3$, HN=$\underline{C}H_3$), 49.3 ($CH_2$) and 50.6 ($CH_2$) (C2 and C4), 62.8 [CH, C10(11)], 63.1 (CH) and 64.1 (CH) [C6(15) and C9(12)], 71.9 (C) and 72.1 (C) (C1 and C5), 134.5 (CH) and 135.1 (CH) [C7(14) and C8(13)], 163.8 (C, C=N).

MS (EI), m/e (%); main ions: 238 ($M^{\cdot+}$, 95), 196 ($C_{14}H_{14}N^+$, 46), 182 ($C_{14}H_{14}^+$, 31), 180 (66), 179 (82), 173 (80), 165 (100), 153 (38), 152 (45), 132 (42), 131 (56), 130 (81), 128 (31), 118 (35), 117 (41), 115 (54), 77 (35).

Elemental analysis:

Calculated for $C_{16}H_{19}ClN_2$: C 69.93% H 6.97% N 10.19%
Cl 12.90%

Calculated for $C_{16}H_{19}ClN_2 \cdot 1.1\ H_2O \cdot 0.1$ HCl: C 64.43% H 7.20% N 9.39%
Cl 13.08%

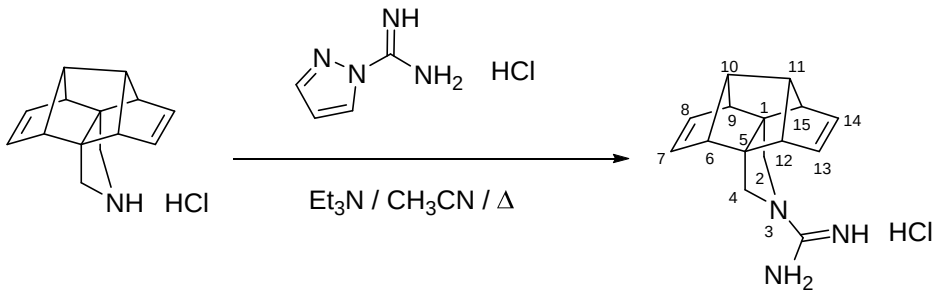
Found: C 64.80% H 7.30% N 8.76%
Cl 12.66%

Accurate mass:

Calculated for $[C_{16}H_{19}ClN_2+H]^+$: 239.1543

Found: 239.1544

**Synthesis of 3-Amidino-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadeca-7,13-diene hydrochloride, 170·HCl.**




**166·HCl** **170·HCl**

In a 25 mL round-bottomed flask equipped with a condenser, a suspension of amine **166·HCl** (250 mg, 1.1 mmol), $Et_3N$ (0.3 mL, 1.9 mmol), 1*H*-pirazol-carboximidine (188 mg, 1.3 mmol) in $CH_3CN$ (5 mL) was heated at 70 $^oC$ over 6 hours. Then, the suspension was cooled at 4 $^oC$ overnight and filtered in vacuo to obtain the guanidine **170·HCl** as a beige solid (319 mg, 67% yield). The analytical sample was obtained by crystallization from MeOH / $Et_2O$.

Analytic and spectroscopic data of the guanidine **170·HCl**.

Melting point: 289-290 $^{o}$C (decompose).

IR (KBr) ν: 3286, 3217, 3125, 2962, 2873, 1654, 1640, 1609, 1467, 1453, 1367, 1210, 1056, 990, 747, 731, 679, 614 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 2.73 [broad t, 2H, 10(11)-H], 3.10 [dt, *J* = 2.8 Hz, *J'* = 1.8 Hz, 4H, 6(9,12,15)-H], 3.13 [s, 4H, 2(4)-$H_2$], 6.13 [t, *J* = 1.8 Hz, 7(8,13,14)-H].

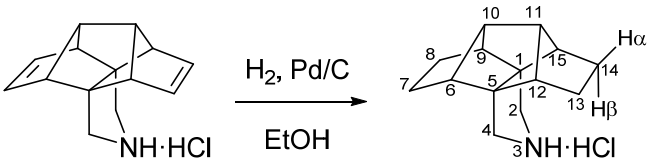
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 48.1 [$CH_2$, C2(4)], 63.1 [CH, C6(9,12,15)], 64.2 [CH, C10(11)], 72.2 [C, C1(5)], 134.7 [CH, C7(8,13,14)], 155.6 (C, C=NH).

MS (EI), m/e (%); main ions: 240 (M, 30), 239 ($M^{\cdot+}$, 76), 197($C_{14}H_{14}N^{\cdot}$, 55), 196 (44), 182 ($C_{14}H_{14}^{+}$, 33), 180 (50), 179 (69), 174 (42), 167 (39), 166 (29), 165 (100), 154 (28), 153 (44), 152 (58), 147 (41), 132 (41), 131 (51), 130 (85), 128 (36), 118 (47), 117 (42), 115 (58), 91 (34), 77 (48), 72 (32).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{15}H_{18}ClN_3$: | C 65.33% | H 6.58% | N | 15.24% |
| Cl 12.86% | | | | |
| Calculated for $C_{15}H_{18}ClN_3$·0.33 $H_2O$·0.1 HCl: | C 63.13% | H 6.63% | N | 14.38% |
| Cl 13.67% | | | | |
| Found: | C 62.92% | H 6.51% | N | 14.50% |
| Cl 13.54% | | | | |

**Synthesis of 3-Azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadecane hydrochloride, 171·HCl.**




**166·HCl** **171·HCl**

A suspension of **166·HCl** (150 mg, 0.64 mmol) and 5% Pd/C (50% in water, 10 mg) in absolute EtOH (20 mL) was hydrogenated at 1 atm for 48 h. The suspension was filtered, the residue was washed with EtOH and the filtrate was concentrated in vacuo to give **171·HCl** as a white solid (150 mg, 99% yield). An analytical sample of **171·HCl** was obtained by crystallization from MeOH/$Et_2O$.

Analytical and spectroscopic data of the amine **171·HCl**.

Melting point: >240 $^oC$ (decompose).

IR (KBr) ν: 2938, 2871, 2793, 2765, 2692, 2663, 1567, 1465, 1414, 1305, 1288, 1200, 1020, 966, 919 $cm^{-1}$.

$^1H$ NMR (500 MHz, $CD_3OD$) δ: 1.62-1.68 [complex signal, 8 H, 7(8, 13, 14)-$H_2$ and 7(8, 13, 14)-$H_2$], 2.24 [m, 4 H, 6(9, 12, 15)-H], 2.55 (broad s, 2 H, 10(11)-H), 3.11 [s, 4 H, 2(4)-$H_2$], 4.86 (s, $NH_2$).

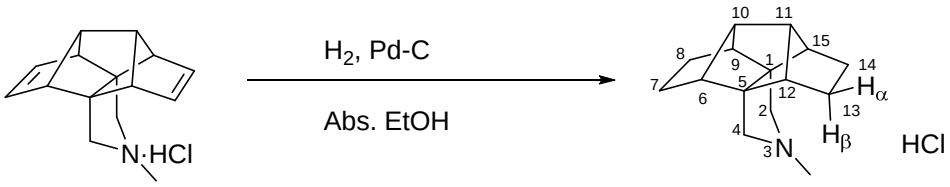
$^{13}C$ NMR (125.7 MHz, $CD_3OD$) δ: 22.3 [$CH_2$, C7(8,13,14)], 41.5 [$CH_2$, C2(4)], 51.7 [CH, C10(11)], 56.1 [CH, C6(9, 12, 15)], 62.2 [C, C1(5)].

MS (EI), m/e (%): 201 ($M^{+}$, 100), 186 (23), 184 (36), 169 (50), 156 (27), 155 (27), 143 (32), 129 (45), 106 (70), 91 (50).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{14}H_{19}N·HCl$: | C 70.72% | H 8.48% | N 5.89% | Cl 14.91% |
| Calculated for $C_{14}H_{19}N·HCl·0.4H_2O$: | C 68.64% | H 8.56% | N 5.72% | Cl 14.47% |
| Found: | C 68.59% | H 8.56% | N 5.56% | Cl 14.41% |

**Synthesis of 3-Methyl-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadecane hydrochloride, 172·HCl**




**168·HCl** **172·HCl**

Amine **168·HCl** (520 mg, 2.1 mmol) dissolved in absolute ethanol (60 mL) was hydrogenated at one atmosphere of pressure overnight. The crude of the reaction was filtrated and concentrated in vacuo to obtain the desired amine **172·HCl** as a white solid (500 mg, 96% yield).

Analytic and spectroscopic data of the amine **172·HCl**.

Melting point: 256-257 $^{o}$C.

IR (KBr) ν: 3389, 2942, 2871, 2603, 2478, 1639, 1457, 1398, 1299, 1276, 1243, 1200, 1152, 1085, 1059, 946, 922, 729, 526, 482 cm$^{-1}$.

$^{1}$H-RMN (500 MHz, $CDCl_3$) δ: 1.44–1.49 (m, 1H), 1.64–1.69 (m, 1H) [7(8)-$H_2$ or 13(14)-$H_2$], 1.59–1.64 [m, 1H, 13(14)-H or 7(8)-H], 2.15–2.17 [m, 6H, 13(14)-H or 7(8)-H, 6(9)-H and 12(15)-H], 2.45 (m, 1H, 10-H or 11-H), 2.52–2.54 (m, 1H, 11-H or 10-H), 2.52–2.56 (overlapped dd, *J* = 12.7 Hz, *J'* = 8.5 Hz, 2H, 2(4)-H], 2.89 (d, *J* = 4.8 Hz, 3H, NH-C*H*$_3$), 3.63 [dd, *J* = 12.7 Hz, *J'* = 6.0 Hz, 2H, 2(4)-H], 11.94 (broad s, 1H, N<u>H</u>-$CH_3$).

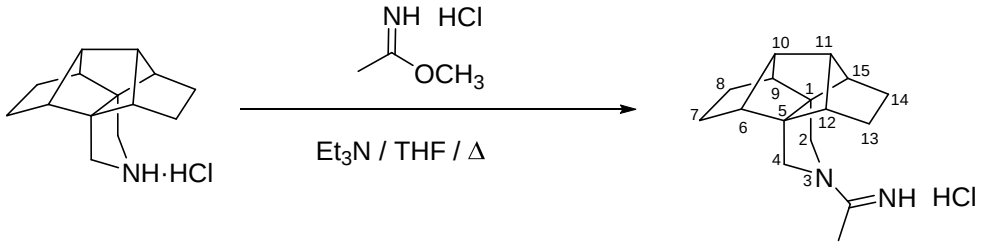
$^{13}$C-RMN (125.7 MHz, $CDCl_3$) δ: 21.3 ($CH_2$) and 21.9 ($CH_2$) [C7(8) and C13(14)], 44.2 ($CH_3$, NH*C*H$_3$), 49.1 (CH) and 51.1 (CH) (C10 and C11), 51.0 [$CH_2$, C2(4)], 54.2 (CH) and 55.7 (CH) [C6(9) and C12(15)], 61.2 [C, C1(5)].

MS (EI), m/e (%); main ions: 215 ($M^{+\cdot}$, 100), 214 (34), 200 (13), 185 (11), 184 (38), 172 (11), 170 (11), 169 (41), 156 (17), 155 (19), 144 (12), 143 (24), 142 (13), 141 (16), 134 ($C_{10}H_{14}^{+}$, 19), 132 (13), 131 (12), 130 (14), 129 (24), 128 (19), 119 (14), 118 (19), 117 (18), 115 (18), 106 (14), 105 (14), 94 (11), 93 (13), 92 (12), 91 (35), 79 (15), 77 (18), 67 (12), 65 (10), 58 (34), 57 (11).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{15}H_{22}ClN$: | C 71.55% | H 8.81% | N 5.56% | Cl 14.08% |
| Calculated for $C_{15}H_{22}ClN \cdot 0.9\ H_2O$: | C 67.22% | H 8.95% | N 5.23% | Cl 13.23% |
| Found: | C 67.15% | H 8.78% | N 4.94% | |

**Synthesis of 3-Acetimidoyl-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadecane hydrochloride, 173·HCl.**




**171·HCl** **173·HCl**

In a 10 mL round-bottomed flask a suspension of amine **171·HCl** (235 mg, 1.0 mmol), $Et_3N$ (0.4 mL, 3.0 mmol) and dimethyl acetimidate (217 mg, 2.0 mmol) in THF (7 mL) was stirred at room temperature for 24 hours. The suspension was filtered in vacuo to obtain the desired acetamidine **173·HCl** as a beige solid (150 mg, 55% yield). The analytical sample was obtained by crystallization of MeOH / $Et_2O$.

Analytic and spectroscopic data of the acetamidine **173·HCl**.

Melting point: 297-298 $^oC$ (MeOH).

IR (KBr) $\nu$: 3407, 3131, 2986, 2950, 2866, 2267, 2205, 1685, 1674, 1627, 1477, 1463, 1450, 1363, 1342, 1285, 1205, 1100, 782, 587 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) $\delta$ 1.51–1.56 (complex signal, 2H) and 1.58–1.66 (complex signal, 6H) [7(8,13,14)-$H_2$], 2.23 [broad s, 4H, 6(9,12,15)-H], 2.33–2.35 (complex signal, 3H, N=C-C$\underline{H_3}$), 2.51–2.55 (complex signal, 2H, 10(11)-H), 3.30 (broad s, 2H) and 3.54 (broad s, 2H) [2-$H_2$ and 4-$H_2$], 4.85 (mobile H).

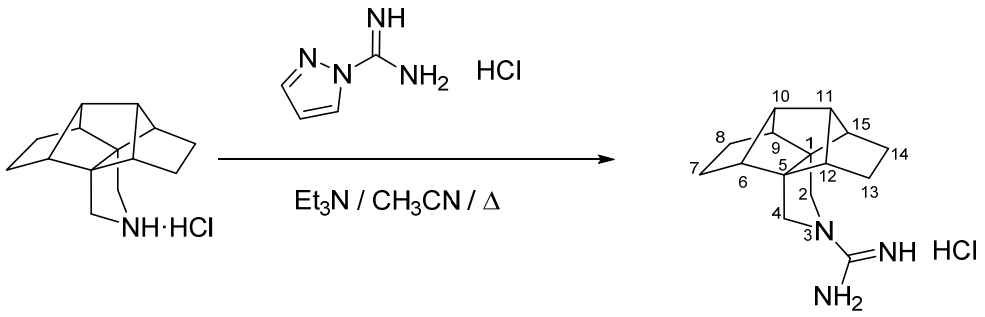
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) $\delta$: 19.2 ($CH_3$, N=C-*C*$H_3$), 22.4 [$CH_2$, C7(8,13,14)], 44.6 ($CH_2$) and 46.1 ($CH_2$) (C2 and C4), 50.9 [CH, C6(9,12,15)], 56.27 (CH) and 56.30 (CH) (C10 and C11), 60.3 (C) and 60.6 (C) (C1 and C5), 164.7 (C, C=N).

MS (EI), m/e (%); main ions: 244 (22), 243 (70), 242 ($M^{\cdot+}$,100), 227 (25), 226 (21), 200 ($C_{14}H_{18}N^{\cdot+}$, 65), 200 (26), 134 ($C_{10}H_{14}^{+}$, 100), 184 ($C_{14}H_{16}^{+}$, 57), 169 (57), 156 (39), 155 (41), 144 (22), 143 (40), 142 (26), 140 (43), 132 (21), 131 (21), 130 (34), 129 (58), 128 (50), 120 (22), 118 (40), 117 (58), 116 (22), 115 (49), 106 (65), 105 (37), 104 (37), 93 (34), 92 (33), 91 (79), 80 (22), 79 (35), 78 (20), 77 (45), 67 (35), 65 (24), 59 (22).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{16}H_{23}NCl$: | C 68.92% | H 8.31% | N 10.05% | Cl 12.72% |
| Calculated for $C_{16}H_{23}N_2Cl \cdot 0.5\ H_2O$: | C 66.78% | H 8.35% | N 9.74% | Cl 12.35% |
| Found: | C 66.39% | H 8.19% | N 9.60% | Cl 12.04% |

**Synthesis of 3-Amidino-3-azahexacyclo[7.6.0.0$^{1,5}$.0$^{5,12}$.0$^{6,10}$.0$^{11,15}$]pentadecane hydrochloride, 174·HCl.**




**171·HCl** **174·HCl**

In a 10 mL round-bottomed flask equipped with a condenser, a suspension of amine **171·HCl** (235 mg, 1.0 mmol), $Et_3N$ (0.3 mL, 1.8 mmol), 1*H*-pirazol-carboximidine (173 mg, 1.2 mmol) in $CH_3CN$ (5 mL) was heated at 70 $^oC$ over 6 hours. Then, the suspension was cooled at 4 $^oC$ overnight and filtered in vacuo to obtain the guanidine **174·HCl** as a white solid (223 mg, 77% yield). The analytical sample was obtained by crystallization of MeOH / $Et_2O$.

Analytic and spectroscopic data of the guanidine **174·HCl**.

Melting point: >300 $^oC$ (MeOH).

IR (KBr) ν: 3214, 3139, 2989, 2950, 2900, 2865, 2473, 2367, 2312, 1646, 1616, 1604, 1557, 1481, 1470, 1453, 1365, 1344, 1205, 1182, 1103, 983, 889, 724, 607 cm$^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.48–1.62 [complex signal, 8H, 7(8, 13, 14)-$H_2$], 2.16 [broad d, *J* = 1.9 Hz, 4H, 6(9, 12, 15)-H], 2.47 [broad s, 2H, 10(11)-H], 3.24 [broad s, 4H, 2(4)-$H_2$], 4.81 (mobile H).

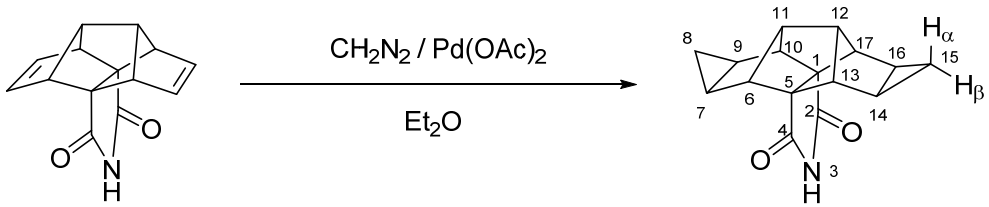
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 22.4 [$CH_2$, C7(8, 13, 14)], 43.4 [$CH_2$, C2(4)], 50.9 [CH, C10(11)], 56.2 [CH, C6(9, 12, 15)], 60.4 [C, C1(5)], 156.4 (C, C=NH).

MS (EI), m/e (%); main ions: 245 (100), 244 ($M^{+}$, 78), 243 (26), 184 (26), 169 (22), 156 (18), 155 (19), 143 (14), 142 (12), 141 (19), 129 (24), 128 (24), 117 (26), 115 (23), 105 (18), 104 (19), 79 (15), 77 (21), 67 (15), 65 (11), 62 (15), 45 (16).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{15}H_{22}NCl_3$: | C 64.39% | H 7.92% | N 15.02% | Cl 12.67% |
| Calculated for $C_{12}H_{20}NCl \cdot 0.33\ H_2O$: | C 63.05% | H 7.99% | N 14.70% | Cl 12.41% |
| Found: | C 62.91% | H 8.06% | N 14.50% | |

**Synthesis of 3-Azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$0$^{12,17}$0$^{14,16}$]heptadecane-2,4-dione, 175.**




**164** **175**

To a suspension of imide **164** (2.50 g, 11.1 mmol) and $Pd(OAc)_2$ (62.8 mg, 0.28 mmol) in $CH_2Cl_2$ (5 mL) an ethereal solution of diazomethane (freshly prepared from *N*-methyl-*N*-nitrosourea, 10 g, 9.7 mmol) was added and the mixture was stirred at room temperature. Two further additions of ethereal solution of diazomethane were performed to achieve total conversion ($^1$H NMR control). The suspension was filtered, $NaHCO_3$ (saturated aq. solution, 100 mL) was added and the mixture was stirred at room temperature overnight. The organic layer was separated, dried with anhydrous $Na_2SO_4$, filtered and evaporated in vacuo to give the imide **175** as a pale yellow solid (2.66 g, 95% yield). An analytical sample of **175** was obtained by crystallization from $CH_2Cl_2$/pentane.

Analytic and spectroscopic data of imide **175**.

Melting point: 277-278 $^{o}$C (decompose).

IR (KBr) $\nu$: 3225, 3008, 2986, 2956, 1751, 1721, 1698, 1454, 1376, 1345, 1326, 1294, 1278, 1247, 1095, 1072, 1035, 791, 777, 680, 493 cm$^{-1}$.

$^{1}$H NMR (500 MHz, $CDCl_3$) δ: 0.13 [dt, 2 H, *J* = 5.5 Hz, *J'* = 7.5 Hz, 8(15)-$H_{endo}$], 0.26 [dt, 2 H, *J* = 5.5 Hz, *J'* = 3.3 Hz, 8(15)-$H_{exo}$], 1.11 [dd, 4 H, *J* = 7.5 Hz, *J'* = 3.3 Hz, 7(9,14,16)-H], 1.93 [m, 2 H, 11(12)-H], 2.81 [dd, 4 H, *J* = 2.0 Hz, *J'* = 0.5 Hz, 6(10,13,17)-H], 8.28 (s, 1 H, NH).

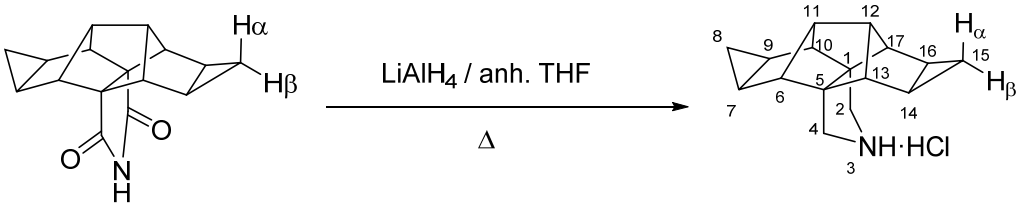
$^{13}$C NMR (125.7 MHz, $CDCl_3$) δ: 2.1 [$CH_2$, C8(15)], 9.6 [CH, C7(9,14,16)], 39.1 [CH, C11(12)], 55.2 [CH, C6(10,13,17)], 66.3 [C, C1(5)], 176.6 [C, C2(4)].

MS (EI), m/e (%): 253 ($M^{+}$, 21), 187 (24), 182 (20), 175 (31), 174 (26), 167 (32), 166 (23), 165 (48), 152 (27), 148 (30), 128 (28), 115 (38), 104 (25), 91 (38), 79 (100), 77 (61).

Elemental Analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{16}H_{15}NO_2$: | C 75.87% | H 5.97% | N 5.53% |
| Found: | C 75.64% | H 5.77% | N 5.49% |

**Synthesis of 3-Azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$0$^{12,17}$0$^{14,16}$]heptadecane hydrochloride, 176·HCl.**




**175** **176·HCl**

To a stirred solution of imide **175** (763 mg, 3.01 mmol) in anhydrous THF (28 mL) at 0 $^{o}$C, $LiAlH_4$ (1.14 g, 30.1 mmol) was carefully added. When the addition was finished, the suspension was heated under reflux for 72 h. The mixture was cooled to 0 $^{o}$C (ice-water bath), treated with 10 N NaOH til basic pH and stirred at room temperature for 1 h. The obtained solid was filtered in vacuo through Celite and was washed with $CH_2Cl_2$ (3×25 mL). The filtrate was dried with anhydrous $Na_2SO_4$, filtered and evaporated in vacuo. The obtained solid residue was taken in EtOAc/$Et_2O$ and treated with excess of HCl in $Et_2O$ to give the hydrochloride of **176** as a solid (506 mg, 64% yield). An analytical sample of **176·HCl** was obtained by crystallization from MeOH/$Et_2O$.

Analytic and spectroscopic data of amine **176·HCl**.

Melting point: 242-243 $^{o}$C (decompose).

IR (KBr) ν: 3446, 2996, 2960, 2921, 2798, 2767, 2663, 2560, 2497, 1581, 1463, 1454, 1423, 1338, 1281, 1247, 1209, 1104, 1037, 1021, 987, 864, 842, 829, 620 $cm^{-1}$.

$^{1}H$ NMR (500 MHz, $CD_3OD$) δ: 0.19 [dt, 2 H, *J* = 5.0 Hz, *J'* = 7.2 Hz, 8(15)-$H_{endo}$], 0.43 [dt, 2 H, *J* = 5.0 Hz, *J'* = 3.0 Hz, 8(15)-$H_{exo}$], 1.13 [dd, 4 H, *J* = 7.2 Hz, *J'* = 3.1 Hz, 7(9, 14, 16)-H], 1.89 [m, 2 H, 11(12)-H], 2.37 [broad s, 4 H, 6(10, 13, 17)-H], 3.29 [s, 4 H, 2(4)-$H_2$].

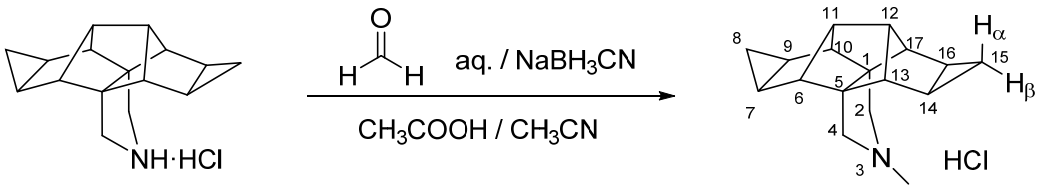
$^{13}C$ NMR (125.7 MHz, $CD_3OD$) δ: 3.2 [$CH_2$, C8(15)], 10.3 [CH, C7(9, 14, 16)], 39.7 [CH, C11(12)], 41.9 [$CH_2$, C2(4)], 54.8 [CH, C6(10, 13, 17)], 66.5 [C, C1(5)].

MS (EI), m/e (%): 226 (19), 225 ($M^{+}$, 100), 224 (74), 170 (20), 165 (21), 146 (27), 145 (31), 144 (70), 132 (22), 130 (30), 129 (25), 128 (26), 118 (38), 117 (45), 115 (41), 91 (38), 80 (41), 79 (44), 77 (39).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{16}H_{19}N{\cdot}HCl$: | C 73.41% | H 7.70% | N 5.35% | Cl 13.54% |
| Calculated for $C_{16}H_{19}N{\cdot}HCl{\cdot}1.25H_2O$: | C 67.59% | H 7.98% | N 4.93% | Cl 12.47% |
| Found | C 67.42% | H 7.75% | N 4.87% | Cl 12.80% |

**Synthesis of 3-Methyl-3-azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]heptadecane hydrochloride, 177·HCl.**




**176·HCl** **177·HCl**

In a 25 ml round-bottomed flask equipped with a condenser a suspension of amine **176·HCl** (250 mg, 1.0 mmol), aqueous formaldehyde 37% (0.5 mL, 6.2 mmol) and $NaBH_3CN$ (126 mg, 1.9 mmol) in $CH_3CN$ was stirred at room temperature for 30 min. Then, acetic acid (0.9 mL) was added and the reaction was stirred for 2 hours. Then more $NaBH_3CN$ (126 mg, 1.9 mmol) was added and the reaction stirred at room temperature overnight. The reaction was concentrated in vacuo and the residue dissolved in a solution of 2 N NaOH (20 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (3 x 30 mL). The combined organic layers were dried

over $Na_2SO_4$, filtered and concentrated in vacuo. The residue was dissolved in AcOEt and $HCl/Et_2O$ was added dropwise to obtain the amine **177·HCl** as a beige solid (200 mg, 77% yield).

Analytic and spectroscopic data of the amine **177·HCl**.

Melting point: 269-270 ºC (decompose).

IR (KBr) ν: 3409, 3063, 2999, 2984, 2949, 2933, 2297, 1643, 1463, 1334, 1247, 1211, 1173, 1111, 1080, 1027, 1015, 975, 826, 754, 628 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CD_3OD$) δ: 0.25 (q, *J* = 6.5 Hz, 2H, 8-$H_\beta$ and 15-$H_\beta$), 0.42–0.44 (m, 1H) and 0.49–0.51 (m, 1H) (8-$H_\alpha$ and 15-$H_\alpha$), 1.20 (dd, *J* = 7.3 Hz, *J'* = 3.1 Hz, 2H) and 1.24 (dd, *J* = 7.3 Hz, *J'* = 3.1 Hz, 2H) [7(9)-H and 14(16)-H], 1.87 (q, *J* = 3.5 Hz, 1H) and 1.95 [q, *J* = 3.5 Hz, 1H) (11-H and 12-H), 2.36 (broad s, 2H) and 2.43 (d, *J* = 3.0 Hz, 2H) [6(10)-H and 13(17)-H], 3.00 (s, 3H, N-C$H_3$), 3.07 (d, *J* = 12.7 Hz, 2 H) and 3.83 (d, *J* = 12.7 Hz, 2 H) [2(4)-$H_{syn}$ and 2(4)-$H_{anti}$].

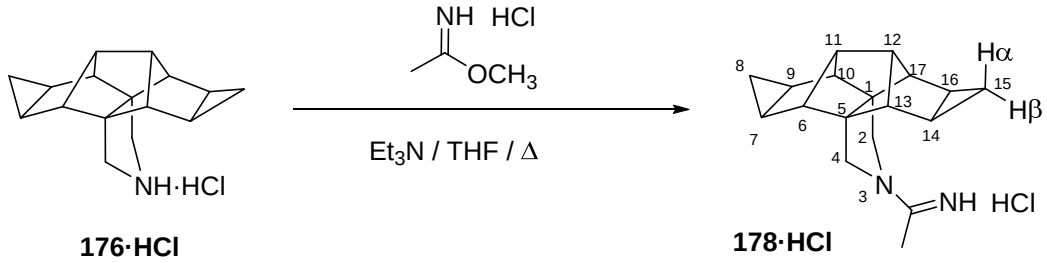
$^{13}$C-RMN (125.6 MHz, $CD_3OD$) δ: 3.25 ($CH_2$) and 3.28 ($CH_2$) (C8 and C15), 10.3 (CH) and 10.5 (CH) [C7(9) and C14(16)], 37.8 (CH) and 40.6 (CH) (C11 and 12), 43.2 ($CH_3$, N-*C*$H_3$), 53.4 [$CH_2$, C2(4)], 54.0 (CH) and 55.4 (CH) [C6(10) and C13(17)], 66.2 [C, C1(5)].

MS (EI), m/e (%); main ions: 240 ($M^{\cdot+}$, 15), 239 (88), 238 (100), 158 (19), 132 ($C_{10}H_{12}^+$, 13), 115 (11), 94 (12), 91 (12), 79 (14), 77 (12).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{17}H_{22}NCl$: | C 74.03% | H 8.04% | N 5.08% | Cl 12.85% |
| Calculated for $C_{17}H_{22}NCl \cdot 0.5\ H_2O$: | C 71.69% | H 8.14% | N 4.92% | Cl 12.45% |
| Found: | C 71.52% | H 8.00% | N 5.29% | |

**Synthesis of 3-Acetimidoyl-3-azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]heptadecane hydrochloride, 178·HCl.**




**176·HCl** **178·HCl**

In a 10 mL round-bottomed flask a suspension of amine **176·HCl** (250 mg, 1.0 mmol), $Et_3N$ (0.4 mL, 2.9 mmol) and dimethyl acetimidate (208 mg, 1.9 mmol) in THF (7 mL) was stirred at room temperature for 24 hours. The suspension was filtered in vacuo to obtain the desired acetamidine **178·HCl** as a beige solid (222 mg, 69% yield). The analytical sample was obtained by crystallization of MeOH / $Et_2O$.

Analytic and spectroscopic data of the acetamidine **178·HCl**.

Melting point: 282-283 $^oC$ (MeOH).

IR (KBr) $\nu$: 3069, 2998, 2948, 2343, 2262, 1669, 1617, 1482, 1449, 1372, 1340, 1265, 1251, 1209, 1173, 1123, 1095, 1053, 1037, 1021, 855, 825, 737, 695 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 0.14 [dt, *J* = 5.2 Hz, *J'* = 7.2 Hz, 2H, 8(15)-$H_\beta$], 0.38 [dt, *J* = 5.1 Hz, *J'* = 3.2 Hz, 2H, 8(15)-$H_\alpha$], 1.01–1.05 (m, 2H) and 1.09–1.13 (m, 2H) [7(14)-H and 9(16)-H], 1.86 [broad s, 2H, 11(12)-H], 2.34 (s, $CH_3$, N=C-C$\underline{H_3}$), 2.36 [m, 4H, 6(14)-H and 13(17)-H], 3.48 (s, 2H) and 3.73 (s, 2H) (2-$H_2$ and 4-$H_2$).

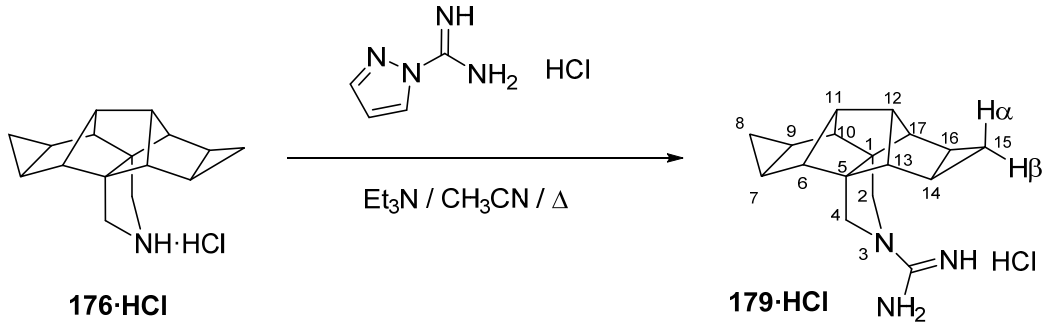
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 2.8 [$CH_2$, C8(15)], 9.9 (CH) and 10.0 (CH) [C7(14) and C9(16)], 19.1 ($CH_3$, N=C-$\underline{C}H_3$), 39.2 [CH, C11(12)], 44.6 ($CH_2$) and 46.1 ($CH_2$) (C2 and C4), 55.2 [CH, C6(13) and C10(17)], 64.9 (C) and 65.3 (C) (C1 and C5), 164.0 (C, C=N).

MS (EI), m/e (%); main ions: 268 (17), 267 (53), 266 ($M^{\cdot+}$, 100), 265 (47), 193 (16), 167 (13), 165 (18), 162 (20), 161 (14), 148 (12), 147 (14), 146 (10), 145 (11), 144 (14), 141 (12), 132 (10), 131 (14), 130 (21), 129 (33), 128 (24), 118 (14), 117 (23), 115 (28), 91 (27), 79 (21), 77 (26).

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_{18}H_{23}N_2Cl$: Cl 12.06% | C 71.39% | H 7.66% | N 9.25% |
| Calculated for $C_{18}H_{23}N_2Cl{\cdot}0{,}5\ H_2O{\cdot}0{,}03$ HCl: Cl 11.67% | C 69.08% | H 7.74% | N 8.95% |
| Found: Cl 12.06% | C 69.29% | H 7.55% | N 8.62% |

**Synthesis of 3-Amidino-3-azaoctacyclo[8.7.0.0$^{1,5}$.0$^{5,13}$.0$^{6,11}$.0$^{7,9}$.0$^{12,17}$.0$^{14,16}$]heptadecane hydrochloride, 179·HCl**




In a 25 mL round-bottomed flask equipped with a condenser, a suspension of amine **176·HCl** (250 mg, 1.0 mmol), $Et_3N$ (0.2 mL, 1.7 mmol), 1*H*-pirazol-carboximidine (170 mg, 1.2 mmol) in $CH_3CN$ (5 mL) was heated at 70 $^oC$ over 6 hours. Then, the suspension was cooled at 4 $^oC$ overnight and filtered in vacuo to obtain the guanidine **179·HCl** as a white solid (203 mg, 67% yield). The analytical sample was obtained by crystallization of MeOH / $Et_2O$.

Analytic and spectroscopic data of the guanidine **179·HCl**.

Melting point: >300 $^oC$ (MeOH).

IR (KBr) ν: 3293, 3142, 3026, 2996, 2969, 2933, 2876, 1641, 1608, 1509, 1474, 1460, 1365, 1334, 1238, 1217, 1189, 1103, 1046, 1026, 856, 816, 746, 605, 499 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 0.14 [dt, *J* = 5.1 Hz, *J'* = 7.2 Hz, 2H, 8(15)-$H_\beta$], 0.37 [dt, *J* = 5.0 Hz, *J'* = 3.2 Hz, 2H, 8(15)-$H_\alpha$], 1.04 [dd, *J* = 7.2 Hz, *J'* = 3.2 Hz, 4H, 7(9, 14, 16)-H], 1.85 [broad s, 2H, 11(12)-H], 2.33 [d, *J* = 1.3 Hz, 4H, 6(10, 13, 17)-H], 3.45 [s, 4H, 2(4)-$H_2$].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 2.8 [$CH_2$, C8(15)], 9.9 [CH, C7(9, 14, 16)], 39.2 [CH, C11(12)], 43.3 [$CH_2$, C2(4)], 55.2 [CH, C6(10, 13, 17)], 65.1 [C, C1(5)], 156.0 (C, C=N).

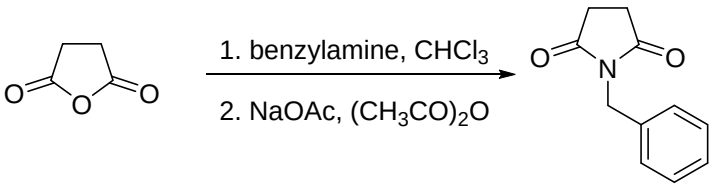
MS (EI), m/e (%); main ions: 270 (37), 269 (95), 268 (100), 267 ($M^{\cdot+}$, 47), 193 (27), 179 (24), 178 (24), 167 (23), 165 (46), 164 (25), 152 (24), 151 (21), 150 (21), 147 (28), 131 (22), 130 (75), 129 (58), 128 (40), 118 (24), 117 (37), 115 (48), 91 (43), 79 (34), 77 (46).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{17}H_{22}N_3Cl$: | C 67.20% | H 7.30% | N 13.83% | Cl 11.67% |
| Found: | C 67.04% | H 7.51% | N 13.70% | Cl 11.31% |

# 4. SYNTHESIS OF 3-AZATRICYCLO[3.3.3.$0^{1,5}$]UNDECANES, 8-AZATRICYCLO[4.3.3.$0^{1,6}$]DODECANES, 12-AZATRICYCLO[4.4.3.$0^{1,6}$]TRIDECANES.

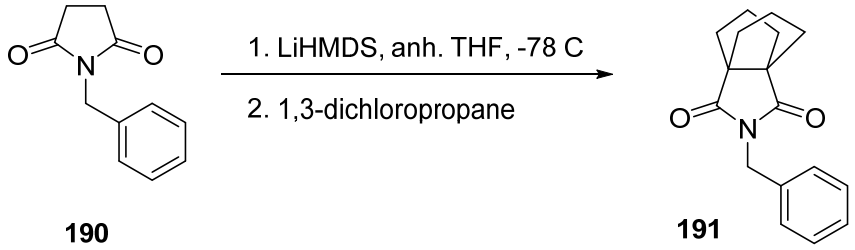
**Synthesis of *N*-benzylsuccinimide, 190.**




**190**

In a 250 mL round-bottomed flask equipped with a gas outlet and magnetic stirring, a suspension of succinic anhydride (5.00 g, 50.0 mmol), benzylamine (5.5 mL, 50.0 mmol) in chloroform (150 mL) was prepared and stirred at room temperature for an hour. The suspension was filtered off to obtain a bright white solid. This solid was dissolved in acetic anhydride (100 mL) followed by the addition of sodium acetate (2 g, 24.4 mmol). The suspension was heated at reflux for 2 hours, cooled down and concentrated in vacuo. The residue was dissolved in water (50 mL) and extracted with $Et_2O$ (3 x 50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the succinimide **190** (8.01 g, 85% yield), which spectroscopic data coincide with the reported in the literature.[163]

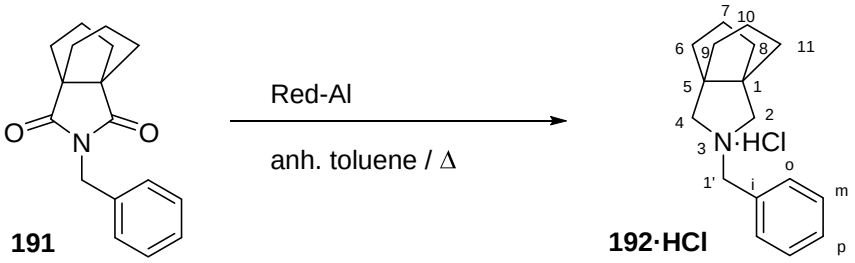
**Synthesis of *N*-benzyl-3-azatricyclo[3.3.3.0$^{1,5}$]undeca-2,4-dione, 191.**




**190** **191**

In a 250 mL 3-necked round-bottomed flask equipped with a pressure equalizing addition funnel, argon atmosphere, gas outlet and a magnetic stirring, a solution of hexamethyldisilazane (55.5 mL, 265.0 mmol) in anhydrous THF (100 mL) was cooled to -78 $^{o}$C. *n*BuLi (106 mL, 2.5 M in hexanes, 265 mmol) was added dropwise and the reaction was stirred 1 hour at -78 $^{o}$C. Then, a solution of *N*-benzylsuccinimide (10 g, 53 mmol) in anhydrous THF (140 mL) was added dropwise to the reaction. The solution was stirred 15 min at -78 $^{o}$C and 1 hour at room temperature. The reaction was cooled again to -78 $^{o}$C and 1,3-dichloropropane (12 mL, 1.19 g/mL, 126 mmol) was added dropwise, followed by stirring at room temperature overnight. The reaction was quenched with a 1N solution of HCl (300 mL). The layers were separated and the aqueous was extracted with $Et_2O$ (3 x 150 mL). The combined organic extracts were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo. The crude was purified by silica column chromatography (hexane:EtOAc 99:1) obtaining succinimide **191** (1.20 g, 8.4% yield), which spectroscopic data coincide with the one reported in the literature.[159]

**Synthesis of *N*-benzyl-3-azatricyclo[3.3.3.0$^{1,5}$]undecane hydrochloride, 192·HCl.**




In a 3-necked 25 mL round-bottomed flask equipped with a condenser, argon atmosphere and magnetic stirring, the imide **191** (130 mg, 0.48 mmol) was dissolved in anhydrous toluene (6 mL). Red-Al® (0.73 mL, 65% in toluene, 2.4 mmol) was added dropwise and the reaction was heated under reflux overnight. The reaction was cooled down and quenched with a solution of KOH 30% in water (10 mL). The layers were separated and the aqueous extracted with $CH_2Cl_2$ (2x10 mL). The combined organic extracts were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo to obtain a beige oil. The residue was dissolved in MeOH (5 mL) and an excess of a solution of HCl/$Et_2O$ was added. The suspension was filtered off obtaining the amine **192·HCl** (100 mg, 75% yield) as a white solid.

Analytical and spectroscopical data of **192·HCl**.

Melting point: 234-235 ºC (methanol).

IR (KBr) *v*: 2932, 2861, 1451, 993, 756, 699, 629, 572, 552 cm$^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.34 (t, *J* = 13.2 Hz, 1 H) and 1.36 (t, *J* = 13.2 Hz, 1 H) (7-$H_a$ and 10-$H_a$), 1.53-1.85 [complex signal, 8 H, 6(8)-$H_2$ and 9(11)-$H_2$], 1.89 (d, *J* = 13.2 Hz, 1 H) and 1.91 (d, *J* = 13.2 Hz, 1 H) (7-$H_b$ and 10-$H_b$), 2.91 [d, *J* = 12.2 Hz, 2 H, 2(4)-$H_a$], 3.54 [d, *J* = 12.2 Hz, 2 H, 2(4)-$H_b$], 4.40 (s, 2 H, C$\underline{H}_2$C$_6$H$_5$), 7.44-7.50 [complex signal, 3 H, Ar-$H_{ortho}$ and Ar-$H_{para}$], 7.58-7.62 [complex signal, 2 H, Ar-$H_{meta}$].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 25.6 and 25.7 [broad signals, $CH_2$, C7 and C10], 39.3 and 39.7 [broad signals $CH_2$, C6(8) and C9(11)], 58.9 ($CH_2$, $\underline{C}H_2C_6H_5$), 61.7 [C, C1(5)], 65.6 [$CH_2$, C2(4)], 130.3 (CH, $C_{ortho}$), 131.1 (CH, $C_{para}$), 131.2 (C, $C_{ipso}$), 132.2 (CH, $C_{meta}$).

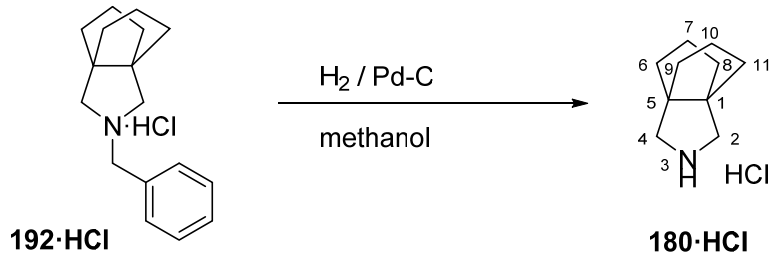
MS (EI), m/e (%); main ions: 241 ($M^{+}$, 58), 240 (100), 164 [$(M-C_6H_5)^+$, 29], 150 [$(M-CH_2C_6H_5)^+$, 49], 133 (12), 132 (14), 91 [$(C_7H_7)^+$, 62].

Accurate mass:

Calculated for $[C_{17}H_{24}N+H]^+$ 242.1903

Found 242.1911

**Synthesis of 3-azatricyclo[3.3.3.0$^{1,5}$]undecane hydrochloride, 180·HCl.**




192·HCl 180·HCl

To a solution of amine **192·HCl** (180 mg, 0.65 mol) in methanol (20 mL) Pd on charcoal (20 mg, ca. 10% Pd) was added and the resulting suspension was hydrogenated at room temperature and atmospheric pressure until no more hydrogen absorption was observed. The resulting suspension was filtered and the residue was washed with methanol. The solvent was removed under vacuo to obtain amine **180·HCl** (110 mg, 90% yield) as a white solid.

Analytical and spectroscopical data of **180·HCl**.

Melting point: 244-245 ºC (chloroform).

IR (KBr) *v*: 3356, 2941, 2860, 2764, 1661, 1605, 1448, 1406, 1384, 1264, 1226, 1189, 1038, 994, 954, 925, 704, 670, 614 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.60-1.71 [complex signal, 12 H, 6(8,9,11)-$H_2$ and 7(10)-$H_2$], 3.12 [s, 4 H, 2(4)-$H_2$].

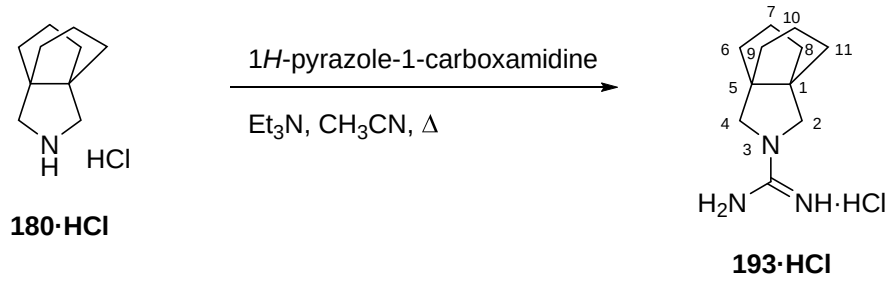
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 25.7 [$CH_2$, C7(10)], 39.5 [$CH_2$, C6(8, 9, 11)], 57.7 [$CH_2$, C2(4)], 62.2 [C, C1(5)].

ME (EI), m/e (%); main ions: 151 ($M^{\cdot+}$, 79), 150 (100), 122 (21), 110 (10), 108 (15), 94 (17), 93 (17), 91 (23), 81 (10), 80 (23), 79 (36), 77 (18), 67 (16), 53 (11).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{10}H_{17}N$·HCl: | C 63.99% | H 9.67% | N 7.46% | Cl 18.89% |
| Calculated for $C_{10}H_{17}N$·1.1HCl·1.2$H_2O$: | C 56.40% | H 9.70% | N 6.58% | Cl 18.31% |
| Found: | C 56.46% | H 9.59% | N 6.42% | Cl 17.99%. |

**Synthesis of 3-amidino-3-azatricyclo[3.3.3.0$^{1,5}$]undecane hydrochloride, 193·HCl.**




**180·HCl** **193·HCl**

In a 10 mL round-bottomed flask equipped with a condenser and a magnetic stirrer, a suspension of amine **180·HCl** (134 mg, 0.7 mmol), 1*H*-pyrazole-1-carboxamidine hydrochloride (426 mg, 0.86 mmol), anhydrous triethylamine (0.15 mL) in acetonitrile (5 mL) was prepared. The reaction was heated at 70 $^{o}$C for 6 hours. The reaction was tempered and cooled at 4ºC overnight. The resulting suspension was filtered out and the filtrate washed with cold acetonitrile obtaining the guanidine **193·HCl** as a white solid (30 mg, 18% yield).

Analytical and spectroscopical data of **193·HCl**.

Melting point: 268-269 $^{o}$C ($CH_3CN$).

IR (KBr) *v*: 3120, 2948, 2864, 1620, 1470, 1368, 1207, 1146, 658 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.62-1.70 [complex signal, 12 H, 6(7, 8, 9, 10, 11)-$H_2$], 3.42 [s, 4 H, 2(4)-$H_2$].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 26.5 [$CH_2$, C7(10)], 40.7 [$CH_2$, C6(8, 9, 11)], 60.9 [$CH_2$, C2(4)], 62.7 [C, C1(5)], 156.7 (C, C=NH).

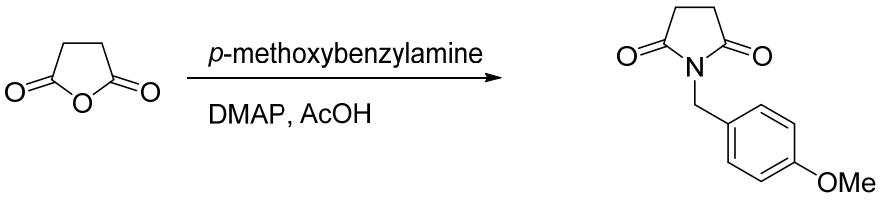
MS (DI), m/e (%); main ions: 193 ($M^{+}$, 42), 192 (15), 152 (19), 151 (14), 150 (52), 108 (11), 91 (17), 86 (10), 85 (100), 80 (10), 79 (19), 77 (10), 72 (14).

Accurate mass:

Calculated for $[C_{11}H_{19}N_3+H]^{+}$ 194.1647

Found 194.1647

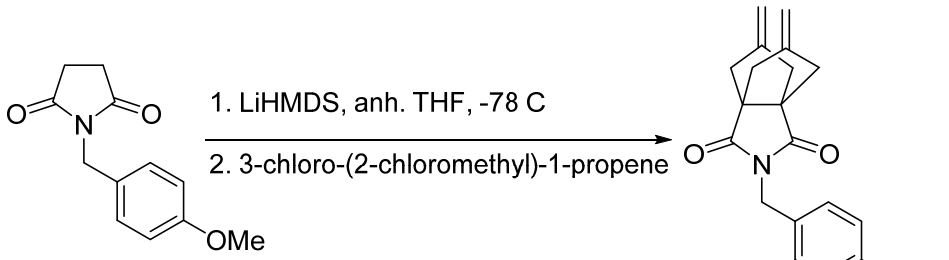
**Synthesis of *N*-(*p*-methoxybenzyl)succinimide, 194.**




**194**

In a 500 mL round-bottomed flask equipped with a condenser and magnetic stirring, a solution of succinic anhydride (10.0 g, 99.9 mmol), DMAP (1 g, 8.19 mmol) and p-methoxybenzylamine (13 mL, 100 mmol) in glacial acetic acid (250 mL) was heated under reflux for 4 days. The reaction was cooled down and concentrated in vacuo. The solid was dissolved in $CH_2Cl_2$ (300 mL) and washed with a saturated solution of $NaHCO_3$ (3 x 100 mL) and a 1N solution of HCl (3 x 100 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the imide **194** (20.5 g, 93% yield), which spectroscopic data coincide with the bibliographic ones.[200]

**Synthesis of *N*-(*p*-methoxybenzyl)-7,10-dimethylene-3-azatricyclo[3.3.3.0$^{1,5}$]undeca-2,4-dione, 195.**



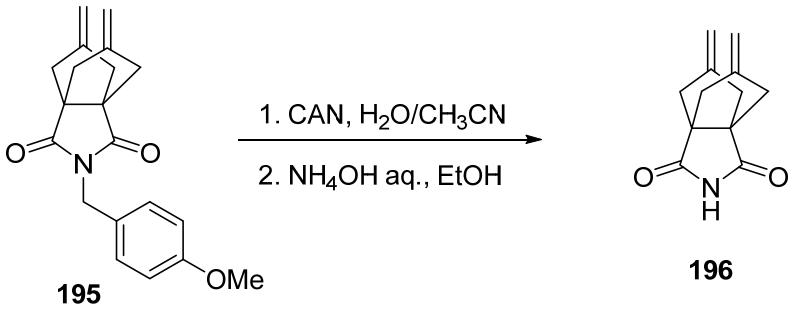

**194** **195**

In a 250 mL, 3-necked round-bottomed flask equipped with a pressure equalizing addition funnel, argon atmosphere, gas outlet and magnetic stirring, a solution of hexamethyldisilazane (23.0 mL, 110.0 mmol) in anhydrous THF (100 mL) was cooled to -78 $^{o}$C. *n*-BuLi (44 mL, 2.5 M in hexanes, 110.0 mmol) was added dropwise and the reaction was stirred 1 hour at -78 $^{o}$C. A solution of imide **194** (4 g, 18.2 mmol) in anhydrous THF (50 mL) was added dropwise to the reaction. The solution was stirred 15 min at -78 $^{o}$C and 1 hour at room temperature. The reaction was cooled again to -78 $^{o}$C and 3-chloro-(2-chloromethyl)-1-propene (8.40 mL, 72.6 mmol) was added dropwise and the reaction was stirred at room temperature for 4 days. The reaction was quenched with a 1 N solution of HCl (150 mL). The layers were separated and the aqueous one was extracted with $Et_2O$ (3 x 200 mL). The combined organic layers were dried

[200] Verschueren, W.; Dierynck, I.; Amssoms, K.; Hu, L.; Boonants, P.; Pille, G.; Daeyaert, F.; Hertogs, K.; Surleraux, D.; Wigerinck, P. *J. Med. Chem.* **2005**, *48*, 1930-1940.

over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo. The crude was purified by column chromatography (silica gel, hexane: EtOAc 8:2) obtaining the imide **195** (2.08 g, 44% yield) as a yellowish solid, which spectroscopical data coincide with the bibliographic ones.[164]

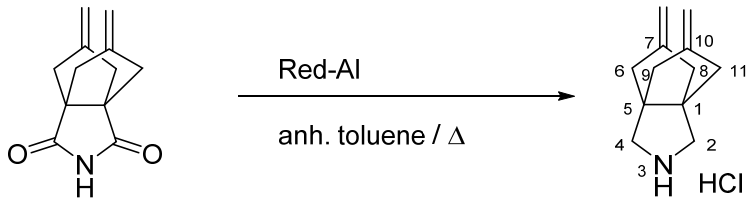
**Synthesis of 7,10-dimethylene-3-azatricyclo[3.3.3.0$^{1,5}$]undeca-2,4-dione, 196.**




**195** **196**

In a 250 mL round-bottomed flask equipped with magnetic stirring, a solution of CAN (19.73 g, 36 mmol) in $H_2O$ (45 mL) was added dropwise to a solution of imide **195** (2.91 g, 9.0 mmol) in $CH_3CN$ (45 mL). The reaction was stirred at room temperature for 2 h and water (100 mL) was added. The aqueous layer was extracted with EtOAc (3x150 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was dissolved in absolute EtOH (40 mL) and $NH_4OH$ 50% v/v (34 mL) was added. The resulting solution was heated at 100 $^oC$ for 2 h in a sealed tube and after cooling down the solvent was concentrated in vacuo. The residue was extracted with hot EtOAc (2 x 150 mL) and the solvent concentrated in vacuo. The crude was purified by column chromatography (silica gel, hexane:EtOAc, 9:1) to obtain imide **196** (1 g, 55% yield). The spectroscopical data of imide **196** coincide with the reported ones.[164,201]

**Synthesis of 7,10-dimethylene-3-azatricyclo[3.3.3.0$^{1,5}$]undecane hydrochloride, 181·HCl.**




**196** **181·HCl**

In a 3-necked, 25 mL round-bottomed flask equipped with a condenser, argon atmosphere and magnetic stirring, the imide **196** (1 g, 4.9 mmol) was dissolved in anhydrous toluene (22 mL). Red-Al® (7.5 mL, 65% in toluene, 25 mmol) was added dropwise and the reaction was heated under reflux for 3 days. The reaction was cooled down and quenched with a 30% aqueous solution of KOH (35 mL). The layers were separated and the aqueous one was

[201] Thesis of Dr. Jordi Rull, Universitat de Barcelona, **2010**.

extracted with $CH_2Cl_2$ (3 x 40 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo to obtain amine **181** as a beige oil. The residue was dissolved in MeOH (5 mL) and an excess of a solution of HCl/$Et_2O$ was added. The resulting suspension was filtered obtaining the amine **181·HCl** (830 mg, 80% yield) as a white solid. An analytical sample of **181·HCl** was obtained by recrystallization from MeOH/$Et_2O$.

Analytical and spectroscopical data of **181·HCl**.

Melting point: 141-142 ºC (methanol).

IR (KBr) *v*: 3390, 3071, 2981, 2941, 2897, 2736, 2703, 2568, 2471, 2159, 2087, 2042, 1663, 1594, 1442, 1430, 1394, 1301, 1250, 1218, 893, 880, 854, 678, 514 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 2.31 [d, *J* = 14.8 Hz, 4 H, 6(8,9,11)-$H_a$] and 2.46 [d, *J* = 14.8 Hz, 4 H, 6(8,9,11)-$H_b$], 3.17 [s, 4 H, 2(4)-$H_2$], 4.92 (m, 4 H, =C$\underline{H}_2$).

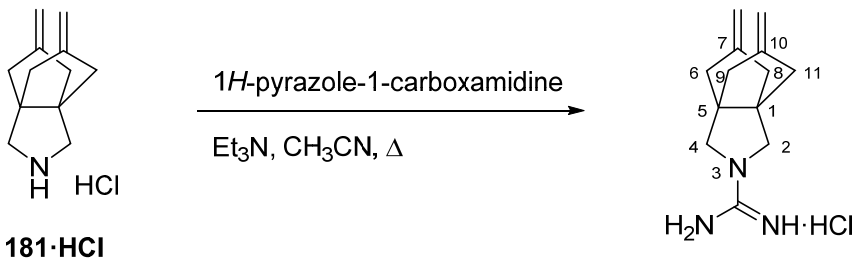
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 45.7 [$CH_2$, C6(8, 9, 11)], 57.3 [$CH_2$, C2(4)], 59.4 [C, C1(5)], 109.5 ($CH_2$, =$\underline{C}H_2$), 149.5 [C, C7(10)].

MS (EI), m/e (%); main ions: 175 ($M^{+}$, 76), 174 (15), 132 (26), 131 (34), 120 (100), 118 (19), 117 (19), 91 (30).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{12}H_{17}N$·HCl: | C 68.07% | H 8.57% | N 6.62% | Cl 16.74% |
| Found: | C 67.72% | H 8.95% | N 6.54% | Cl 16.45% |

**Synthesis of 3-amidino-7,10-dimethylene-3-azatricyclo[3.3.3.0$^{1,5}$]undecane hydrochloride, 197·HCl.**




**181·HCl** **197·HCl**

In a 10 mL round-bottomed flask equipped with a condenser and a magnetic stirrer, a suspension of amine **181·HCl** (0.21 g, 1.0 mmol), 1*H*-pyrazole-1-carboxamidine hydrochloride (0.18 g, 1.2 mmol), anh. triethylamine (0.24 mL) in acetonitrile (5 mL) was prepared. The reaction was heated at 70 ºC for 6 hours. The reaction was tempered and cooled at 4 ºC overnight. The suspension was filtered out and the filtrate washed with cold acetonitrile

obtaining the guanidine **197·HCl** as a beige solid (0.19 g, 75% yield). An analytical sample was obtained by crystallization from MeOH/$Et_2O$.

Analytical and spectroscopical data of **197·HCl**.

Melting point: 191-192 ºC (methanol).

IR (KBr) *v*: 3307, 3122, 2976, 2930, 2827, 2673, 2490, 1624, 1469, 1458, 1435, 1364, 1292, 1201, 1170, 1118, 1076, 1036, 899, 828, 739, 708, 625.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 2.49 [m, 8 H, 6(8,9,11)-$H_2$], 3.45 [s, 4 H, 2(4)-$H_2$], 4.95 [m, 4 H, 7(10)=C$\underline{H}_2$].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 44.1 [$CH_2$, C6(8,9,11)], 58.2 [$CH_2$, C2(4)], 60.6 [C, C1(5)], 109.2 [$CH_2$, C7(10)=$\underline{C}H_2$], 150.8 [C, C7(10)], 156.2 (C, C=NH).

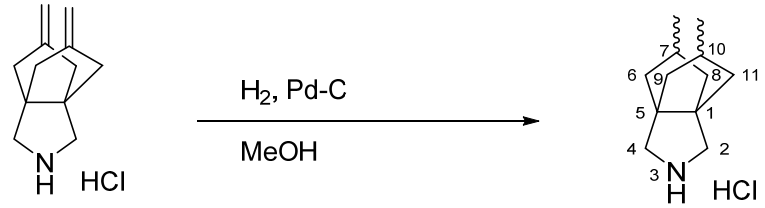
MS (EI), m/e (%); main ions: 217 ($M^{+}$, 100), 162 (67), 145 (13), 143 (13), 120 (39), 118 (13), 117 (13), 91 (25), 85 (19), 72 (33).

Accurate mass:

Calculated for $[C_{13}H_{19}N_3+H]^+$ 218.1652

Found 218.1646

**Synthesis of 7,10-dimethyl-3-azatricyclo[3.3.3.0$^{1,5}$]undecane hydrochloride (mixture of stereoisomers), 182·HCl.**




**181·HCl** **182·HCl**

A solution of **181·HCl** (300 mg, 1.42 mol) and Pd on charcoal (60 mg, ca. 10% Pd) in MeOH (35 mL) was hydrogenated at room temperature and atmospheric pressure until no more hydrogen absorption was observed. The resulting suspension was filtered and the residue washed with methanol. The solvent was removed under vacuo to obtain **182·HCl** (267 mg, 87% yield) as a white solid.

Analytical and spectroscopical data of **182·HCl**.

Melting point: 217-218 ºC (methanol).

IR (KBr) *v*: 2948, 2927, 2865, 2740, 2659, 2638, 2588, 2559, 2432, 2050, 1868, 1587, 1465, 1455, 1414, 1372, 1297, 1018, 994, 839, 649 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ (major stereoisomer): 1.02 [d, *J* = 6.4 Hz, 6 H, C7(10)-C$H_3$], 1.37 [dd, *J* = 12.4 Hz, *J'* = 10.8 Hz, 4 H, 6(8,9,11)-$H_a$], 2.00 [dd, *J* = 13.6 Hz, *J'* = 6.8 Hz, 4 H, 6(8,9,11)-$H_b$], 2.30 [m, 2 H, 7(10)-H], 3.25 [s, 4 H, 2(4)-$H_2$].

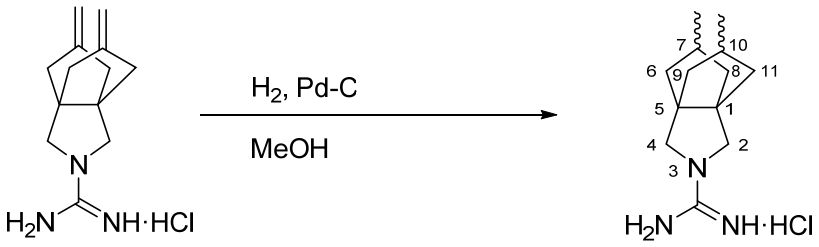
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ (major stereoisomer): 19.6 [$CH_3$, C7(10)-C$H_3$], 38.4 [CH, C7(10)], 50.0 [$CH_2$, C6(8,9,11)], 60.5 [$CH_2$, C2(4)], 64.6 [C, C1(5)].

ME (EI), m/e (%); main ions: 179 ($M^{\cdot+}$, 49), 178 (59), 164 (64), 162 (18), 137 (15), 136 (100), 108 (15), 107 (15), 93 (37), 91 (23), 79 (19), 77 (18).

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_{12}H_{21}N{\cdot}HCl$: | C 66.80% | H 10.28% | N 6.49% |
| Calculated for $C_{12}H_{21}N{\cdot}HCl{\cdot}0.4\ H_2O$: | C 64.64% | H 10.31% | N 6.28% |
| Found: | C 64.60% | H 10.11% | N 5.93% |

**Synthesis of 3-amidino-7,10-dimethyl-3-azatricyclo[3.3.3.0$^{1,5}$]undecane hydrochloride, 198·HCl.**




**197·HCl** **198·HCl**

A solution of **197·HCl** (600 mg, 2.36 mol) and Pd on charcoal (60 mg, ca. 10% Pd) in MeOH (50 mL) was hydrogenated at room temperature and atmospheric pressure until no more hydrogen absorption was observed. The resulting suspension was filtered and the residue washed with methanol. The solvent was removed under vacuo to obtain **198·HCl** (580 mg, 95% yield) as a white solid.

Analytical and spectroscopical data of **198·HCl**.

Melting point: 294-295 ºC (methanol).

IR (KBr) *v*: 3306, 3256, 3185, 3135, 2928, 2869, 2417, 1635, 1624, 1475, 1455, 1373, 1284, 1211, 1153, 1087, 603 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ (major stereoisomer): 1.02 [d, *J* = 6.4 Hz, 6 H, C7(10)-C$\underline{H_3}$], 1.40 [dd, *J* = 13.2 Hz, *J'* = 11.0 Hz, 4 H, 6(8,9,11)-$H_a$], 2.00 [dd, *J* = 13.2 Hz, *J'* = 6.8 Hz, 4 H, 6(8,9,11)-$H_b$], 2.30 [m, 2 H, 7(10)-H], 3.50 [s, 4 H, 2(4)-$H_2$].

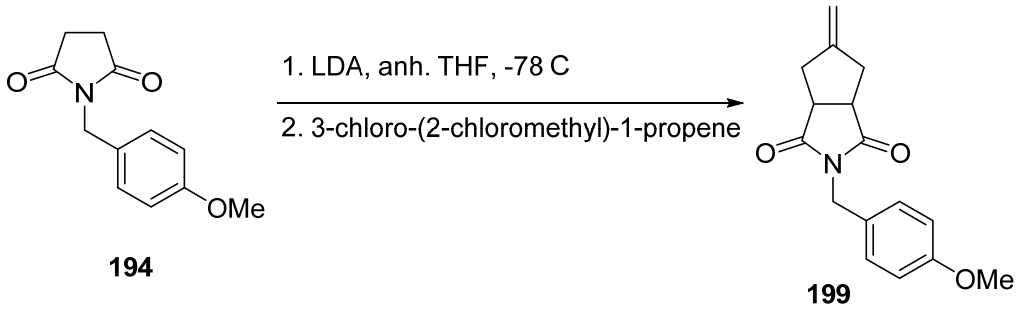
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ (major stereoisomer): 20.4 [$CH_3$, C7(10)-$\underline{C}H_3$], 38.0 [CH, C7(10)], 50.1 [$CH_2$, C6(8,9,11)], 62.2 [$CH_2$, C2(4)], 64.0 [C, C1(5)], 156.2 (C, C=NH).

MS (EI), m/e (%); main ions: 221 ($M^{+}$, 52), 220 (37), 206 (82), 178 (34), 166 (28), 164 (17), 122 (12), 105 (11), 93 (14), 91 (15), 85 (100), 72 (17), 55 (10).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{13}H_{23}N_3$·HCl: | C 60.57% | H 9.38% | N 16.30% | Cl 13.75% |
| Found: | C 60.80% | H 9.65% | N 16.25% | Cl 13.50% |

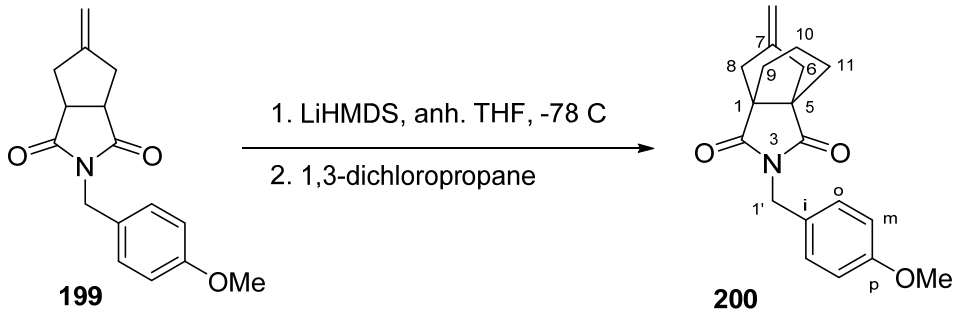
**Synthesis of *N*-(*p*-methoxybenzyl)-4-methylenecyclopentane-1,2-dicarboximide, 199.**




**194** **199**

In a 250 mL 3-necked round-bottomed flask equipped with a pressure equalizing addition funnel, argon atmosphere, gas outlet and magnetic stirring, a solution of diisopropylamine (7.8 mL, 56 mmol) in anhydrous THF (100 mL) was cooled to -40 $^{o}$C. *n*-BuLi (22.0 mL, 2.5 M in hexanes, 55 mmol) was added dropwise and the reaction was stirred 1 hour at -40 $^{o}$C. A solution of imide **194** (4 g, 18.2 mmol) in anhydrous THF (50 mL) was added dropwise to the reaction. The solution was stirred 15 min at -40 $^{o}$C and 1 hour at room temperature. The reaction was cooled again to -78 $^{o}$C and 3-chloro-(2-chloromethyl)-1-propene (4.2 mL, 36.6 mmol) was added dropwise and the reaction was stirred at room temperature for 4 days. The reaction was quenched with a 1 N aqueous solution of HCl (150 mL). The layers were separated and the aqueous one was extracted with $Et_2O$ (3 x 100 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo. The residue was purified by column chromatography (silica gel, hexane: EtOAc, 7:3) obtaining the imide **199** (2.06 g, 42% yield) as a yellowish solid, which spectroscopical data coincide with the bibliographic ones.[161]

**Synthesis of *N*-(*p*-methoxybenzyl)-7-methylene-3-azatricyclo[3.3.3.0$^{1,5}$]undeca-2,4-dione, 200.**




In a 250 mL 3-necked round-bottomed flask equipped with a pressure equalizing addition funnel, argon atmosphere, gas outlet and magnetic stirring, a solution of HMDS (1.6 mL, 7.8 mmol) in anhydrous THF (20 mL) was cooled to -78 $^{o}$C. *n*-BuLi (3.1 mL, 2.5 M in hexanes, 7.8 mmol) was added dropwise and the reaction was stirred 1 hour at -78 $^{o}$C. A solution of imide **199** (700 mg, 2.6 mmol) in anhydrous THF (10 mL) was added dropwise to the reaction. The solution was stirred 15 min at -78 $^{o}$C and 1 hour at room temperature. The reaction was cooled again to -78 $^{o}$C and 1,3-dichloropropane (0.5 mL, 5.2 mmol) was added dropwise and the reaction was stirred at room temperature overnight. The reaction was quenched with a 1 N solution of HCl (20 mL). The layers were separated and the aqueous layer was extracted with $Et_2O$ (3 x 20 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo. The crude was purified by column chromatography (silica gel, hexane: EtOAc, 9:1) obtaining the imide **200** (580 mg, 72% yield) as a yellowish oil.

Analytical and spectroscopical data of the imide **200**.

IR (KBr) *v*: 3453, 3070, 2955, 2866, 1768, 1698, 1613, 1512, 1432, 1389, 1346, 1300, 1248, 1177, 1143, 1108, 1033, 969, 899, 819 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.39 (m, 1 H, 10-$H_a$), 1.66 [m, 2 H, 9(11)-$H_a$], 1.77 (m, 1 H, 10-$H_b$), 2.22 [ddd, *J* = 13.2 Hz, *J'* = 6.4 Hz, *J''* = 4.0 Hz, 2 H, 9(11)-$H_b$], 2.40 [d, *J* = 15.8 Hz, 2 H, 6(8)-$H_a$], 2.69 [d, *J* = 15.8 Hz, 2 H, 6(8)-$H_b$], 3.77 (s, 3 H, O$\underline{C}H_3$), 4.54 (s, 2 H, 1'-$H_2$), 4.81 (t, *J* = 1.6 Hz, 7C=C$\underline{H}_2$), 6.80 [m, 2 H, $H_{meta}$], 7.23 [m, 2 H, $H_{ortho}$].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 27.0 ($CH_2$, C10), 36.7 [$CH_2$, C9(11)], 41.9 ($CH_2$, C1'), 42.4 [$CH_2$, C6(8)], 55.2 ($CH_3$, O$\underline{C}H_3$), 61.8 [C, C1(5)], 109.3 ($CH_2$, C7=$\underline{C}H_2$), 113.9 (CH, $C_{meta}$), 128.3 (C, $C_{ipso}$), 129.5 (CH, $C_{ortho}$), 148.1 (C, C7), 159.1 (C, $C_{para}$), 181.3 (C, C=O).

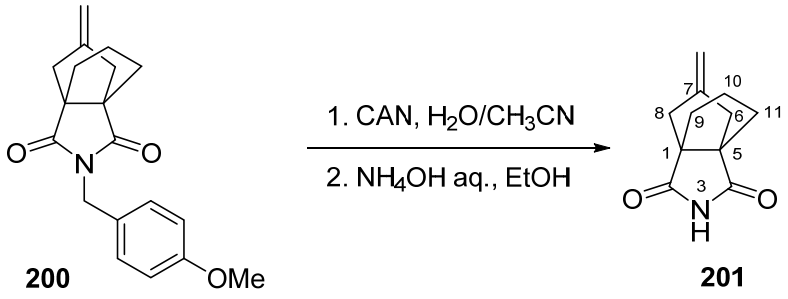
MS (EI), m/e (%); main ions: 311 ($M^{\cdot+}$, 100), 228 (27), 162 (12), 121 (79), 120 (12), 119 (13), 91 (21), 77 (11).

Accurate mass:

Calculated for $[C_{19}H_{21}NO_3+H]^+$ 312.1594

Found 312.1604

**Synthesis of 7-methylene-3-azatricyclo[3.3.3.0$^{1,5}$]undeca-2,4-dione, 201.**




In a 250 mL round-bottomed flask equipped with magnetic stirring, a solution of CAN (6.30 g, 11.6 mmol) in $H_2O$ (15 mL) was added dropwise to a solution of imide **200** (900 mg, 2.9 mmol) in $CH_3CN$ (15 mL). The reaction was stirred at room temperature for 2 h and. Water (40 mL) was added and the aqueous layer was extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was dissolved in absolute EtOH (15 mL) and $NH_4OH$ 50% v/v (15 mL) in a sealed tube. The solution was heated at 100 $^oC$ for 2 h and after cooling down the solvent was concentrated in vacuo. The residue was extracted with hot EtOAc (2 x 70 mL), filtered and the solvent concentrated in vacuo. The crude was purified by column chromatography (silica gel, hexane:EtOAc, 8:2) to obtain imide **201** (460 mg, 83% yield).

Analytic and spectroscopic data of the imide **201**.

Melting point: 155-156 $^oC$ (ethyl acetate).

IR (KBr) *v*: 3069, 2922, 1755, 1703, 1429, 1401, 1351, 1327, 1173, 1139, 1096, 894, 845, 704, 634, 554 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.60 (m, 1 H, 10-$H_a$), 1.68 [m, 2 H, 9(11)-$H_a$], 1.85 (m, 1 H, 10-$H_b$), 2.23 [m, 2 H, 9(11)-$H_b$], 2.40 [d, *J* = 15.6 Hz, 2 H, 6(8)-$H_a$], 2.76 [dd, *J* = 15.6 Hz, *J'* = 1.6 Hz, 2 H, 6(8)-$H_b$], 4.87 (t, *J* = 1.6 Hz, 2 H, 7=C$\underline{H}_2$), 8.59 (bs, 1 H, NH).

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 27.1 ($CH_2$, C10), 36.6 [$CH_2$, C9(11)], 42.4 [$CH_2$, C6(8)], 63.2 [C, C1(5)], 109.5 ($CH_2$, C7=$\underline{C}H_2$), 147.9 (C, C7), 182.1 (C, C=O).

MS (EI), m/e (%); main ions: 191 ($M^{+}$, 100), 151 (16), 148 (44), 120 (29), 119 (30), 105 (27), 92 (42), 91 (53), 79 (13), 77 (14).

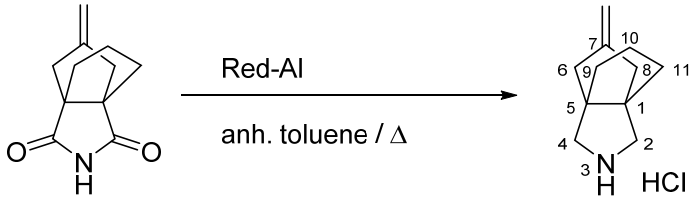
Accurate mass:

Calculated for $[C_{11}H_{13}NO_2+H]^+$ 192.1019 Found 192.1012

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_{11}H_{13}NO_2$: | C 69.09% | H 6.85% | N 7.32% |
| Found: | C 69.12% | H 6.88% | N 7.01% |

**Synthesis of 7,9-dimethylene-3-azatricyclo[3.3.3.0$^{1,5}$]undecane hydrochloride, 183·HCl.**




**201** **183·HCl**

In a 3-necked 25 mL round-bottomed flask equipped with a condenser, argon atmosphere and magnetic stirring, the imide **201** (410 mg, 2.1 mmol) was dissolved in anhydrous toluene (20 mL). Red-Al® (3.2 mL, 65% in toluene, 10.7 mmol) was added dropwise. The reaction was heated under reflux overnight. The reaction was cooled down and quenched with a 30% aqueous solution of KOH (22 mL). The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (2 x 10 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$ and filtered. An excess of HCl/MeOH was added to the organic phase and they were concentrated under vacuo to obtain **183·HCl** (400 mg, 93% yield) as a white solid. An analytical sample was obtained by crystallization from MeOH/$Et_2O$.

Analytical and spectroscopical data of **183·HCl**.

Melting point: 150-151 ºC (methanol/diethyl ether).

IR (KBr) *v*: 3445, 3380, 2937, 2762, 2606, 1664, 1609, 1444, 1416, 1388, 1365, 1272, 1222, 1180, 879, 704, 641, 605 cm$^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.68-1.72 [complex signal, 6 H, 9(11)-$H_2$ and 10-$H_2$], 2.30 [dt, *J* = 14.8 Hz, *J'* = 2.0 Hz, 2 H, 6(8)-$H_a$], 2.38 [dt, *J* = 14.8 Hz, *J'* = 1.6 Hz, 2 H, 6(8)-$H_b$], 3.04 [d, *J* = 11.6 Hz, 2H, 2(4)-$H_a$], 3.22 [d, *J* = 11.6 Hz, 2 H, 2(4)-$H_b$], 4.89 (m, 2 H, 7=C$\underline{H}_2$).

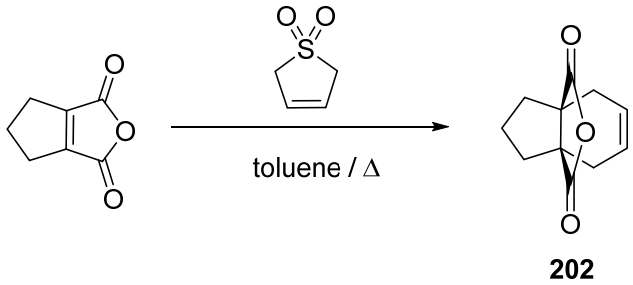
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 25.7 ($CH_2$, C10), 39.6 [$CH_2$, C9(11)], 46.1 [$CH_2$, C6(8)], 57.6 [$CH_2$, C2(4)], 60.7 [C, C1(5)], 109.2 ($CH_2$, C7=$\underline{C}H_2$), 149.8 (C, C7).

MS (EI), m/e (%); main ions: 163 ($M^{\cdot+}$, 41), 134 (24), 122 (28), 120 (13), 119 (14), 109 (41), 108 (100), 105 (19), 91 (35), 79 (14), 77 (16).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{11}H_{17}N{\cdot}HCl$: | C 66.15% | H 9.08% | N 7.01% | Cl 17.75% |
| Calculated for $C_{11}H_{17}N{\cdot}HCl{\cdot}H_2O$: | C 60.68% | H 9.26% | N 6.43% | Cl 16.28% |
| Found: | C 60.73% | H 9.17% | N 6.71% | Cl 16.28% |

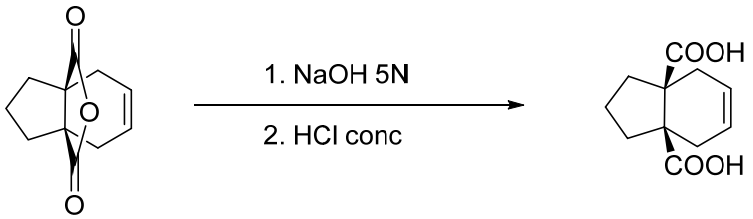
**Synthesis of 8-oxatricyclo[4.3.3.0$^{1,6}$]dodec-3-en-7,9-dione, 202.**




**202**

In a 100 mL sealed tube, a solution of 1-cyclopentene-1,2-dicarboxylic anhydride (750 mg, 5.4 mmol) and 3-sulfolene (1.9 g, 16.3 mmol) in toluene (1.5 mL) was heated at reflux for 3 days. The mixture was concentrated in vacuo and the crude was purified by sublimation at 75 $^{o}$C / 0.5 Torr to obtain pure the anhydride **202** (804 mg, 77% yield), which spectroscopic data coincide with the reported.[202]

**Synthesis of bicyclo[4.3.0]non-3-en-1,6-dicarboxilic acid, 203.**



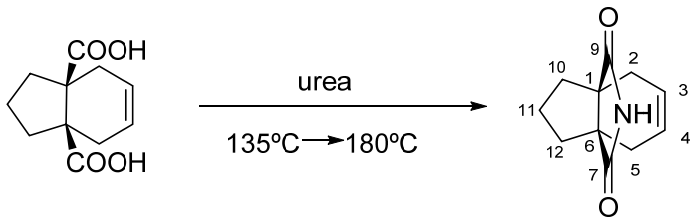

**202** **203**

In a 25 mL round-bottomed flask equipped with a condenser and magnetic stirring, a solution of anhydride **202** (250 mg, 1.3 mmol) in a 5N aqueous solution of NaOH (5 mL) was heated at reflux for 6 h. The reaction was cooled down and the aqueous layer was washed with $Et_2O$ (4 x 15 mL). The aqueous layer was acidified with concentrated HCl until pH=1, and the organic layer was extracted with $CH_2Cl_2$ (3 x 15 mL). The combined organic extracts were

[202] Altman, J.; Babad, E.; Itzchaki, J.; Ginsburg, D. *Tetrahedron*, **1966**, *22*, Supp. 8, 279-304.

dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain diacid **203** (224 mg, 82% yield) as a white solid. The analytical data coincide with the reported.[203]

**Synthesis of 8-azatricyclo[4.3.3.0$^{1,6}$]dodec-3-en-7,9-dione, 204.**




**203** **204**

In a 50 mL round-bottomed flask equipped with a condenser and a gas outlet, a mixture of diacid **203** (224 mg, 1.1 mmol) and urea (335 mg, 95% purity, 5.3 mmol) was stirred and heated at 220 $^{o}$C for 30 min. The reaction was cooled down and the residue was dissolved in water (10 mL) and extracted with $CH_2Cl_2$ (6 x 10 mL). The combined organic extracts were dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuo obtaining imide **204** as a yellowish solid (163 mg, 80% yield). An analytical sample of **204** was obtained by crystallization from $CH_2Cl_2$/pentane.[204]

Analytical and spectroscopical data of the imide **204**.

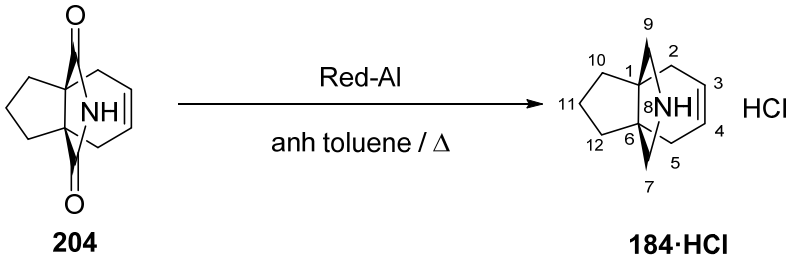
$^{1}$H-RMN (400 MHz, $CDCl_3$) δ: 1.44 (m, 1 H, C11-$H_a$), 1.64 [m, 2 H, C10(12)-$H_a$], 1.75 (m, 1 H, C11-$H_b$), 2.03 (dd, *J* = 16.0 Hz, *J'* = 1.6 Hz, 2 H, C10(12)-$H_b$], 2.27 [ddd, *J* = 12.0 Hz, *J'* = 4.0 Hz, *J''* = 2.0 Hz, 2 H, C2(5)-$H_a$], 2.67 [ddd, *J* = 12.0 Hz, *J'* = 2.0 Hz, *J''* = 1.2 Hz, 2 H, C2(5)-$H_b$], 5.93 [m, 2 H, 3(4)-H], 8.69 (bs, NH).

$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 24.4 ($CH_2$, C11), 30.9 [$CH_2$, C2(5)], 38.0 [$CH_2$, C10(12)], 57.2 [C, C1(6)], 128.5 [CH, C3(4)], 183.0 (C, C=O).

MS (EI), m/e (%); main ions: 191 ($M^{+}$, 100), 149 (13), 148 (51), 147 (15), 130 (21), 120 (59), 119 (36), 105 (24), 92 (30), 91 (67), 77 (11).

[203] Camps, C.; Figueredo, M. *Can. J. Chem.* **1984**, *62*, 1184-1193.
[204] Wagner, E.; Rudzick, A. D. *J. Med. Chem.* **1967**, *10*, 607-611.

**Synthesis of 8-azatricyclo[4.3.3.0$^{1,6}$]dodec-3-ene hydrochloride, 184·HCl.**




**204** **184·HCl**

In a 3-necked 50 mL round-bottomed flask equipped with a condenser, argon atmosphere and magnetic stirring, the imide **204** (470 mg, 2.5 mmol) was dissolved in anhydrous toluene (20 mL). Red-Al® (3.7 mL, 65% in toluene, 12.3 mmol) was added dropwise. The reaction was heated under reflux overnight. The reaction was cooled down and quenched with a solution of KOH 30% in water (25 mL).The layers were separated and the aqueous extracted with $CH_2Cl_2$ (2 x 10 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered. An excess of HCl/MeOH was added to the mother liquors and they were concentrated under vacuo to obtain **184·HCl** (400 mg, 82% yield) as a white solid. The product was recrystallized of MeOH/$Et_2O$ to obtain the analytical sample.

Analytical and spectroscopical data of **184·HCl**.

Melting point: 158-159 $^oC$ (methanol/diethyl ether).

IR (KBr) *v*: 3170, 3050, 2952, 2922, 2850, 1770, 1698, 1442, 1393, 1378, 1358, 1332, 1322, 1175, 1140, 1063, 1038, 969, 876, 853, 778, 716, 660, 651 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.67-1.81 [complex signal, 6 H, 10(12)-$H_2$ and 11-$H_2$], 2.09 [dd, *J* = 15.2 Hz, *J'* = 2.0 Hz, 2 H, 2(5)-$H_a$], 2.19 [dd, *J* = 15.2 Hz, *J'* = 2.4 Hz, 2 H, 2(5)-$H_b$], 3.05 [d, *J* = 12.0 Hz, 2 H, C7(9)-$H_a$], 3.20 [d, *J* = 12.0 Hz, 2 H, 7(9)-$H_b$], 5.90 [m, 2 H, 3(4)-H].

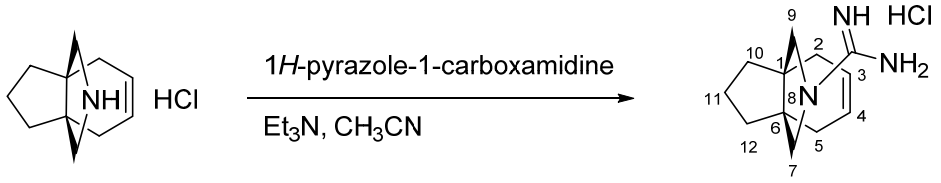
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 24.4 ($CH_2$, C11), 33.8 [$CH_2$, C2(5)], 41.0 [$CH_2$, C10(12)], 55.0 [C, C1(6)], 58.9 [$CH_2$, C7(9)], 129.7 [CH, C3(4)].

MS (EI), m/e (%); main ions: 163 ($M^{+}$, 52), 162 (12), 135 (27), 134 (100), 122 (20), 120 (29), 117 (19), 108 (26), 91 (56), 80 (22), 77 (22), 65 (13).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{11}H_{17}N$·HCl: | C 66.15% | H 9.08% | N 7.01% | Cl 17.75% |
| Found: | C 66.05% | H 9.09% | N 6.80% | Cl 17.71%. |

**Synthesis 8-amidino-8-azatricyclo[4.3.3.0$^{1,6}$]dodec-3-ene hydrochloride, 205·HCl.**




**184·HCl** **205·HCl**

In a 10 mL round-bottomed flask equipped with a condenser and a magnetic stirrer, a suspension of amine **184·HCl** (0.17 g, 0.8 mmol), 1*H*-pyrazole-1-carboxamidine hydrochloride (0.15 g, 1.0 mmol), anh. triethylamine (0.21 mL, 1.5 mmol) in acetonitrile (3.5 mL) was prepared. The reaction was heated at 70 $^{o}$C for 6 hours. The reaction was cooled down and cooled at 4 $^{o}$C overnight. The suspension was filtered out and the filtrate was washed with cold acetonitrile obtaining the guanidine **205·HCl** as a beige solid (0.10 g, 49% yield). The analytical sample was obtained by crystallization from MeOH/$Et_2O$.

Analytic and spectroscopic data of **205·HCl**.

Melting point: 176-177 $^{o}$C (dec., methanol/diethyl ether).

IR (KBr) v: 3311, 3183, 3136, 2946, 2875, 1643, 1613, 1470, 1426, 1365, 1183, 1086, 830, 652 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.67-1.93 [complex signal, 6 H, 10(12)-$H_2$ and 11-$H_2$], 2.05-2.20 [complex signal, 4 H, 2(5)-$H_2$], 3.28 [d, *J* = 10.4 Hz, 2 H, 7(9)-$H_a$], 3.44 [d, *J* = 10.4 Hz, 2 H, 7(9)-$H_b$], 5.66 [t, *J* = 2.0 Hz, 3(4)-H].

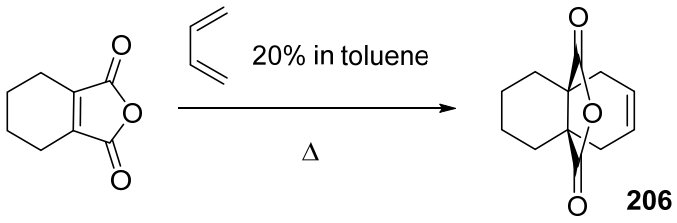
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 20.9 ($CH_2$, C11), 31.6 [$CH_2$, C2(5)], 36.6 [$CH_2$, C10(12)], 50.3 [C, C1(6)], 58.5 [$CH_2$, C7(9)], 125.2 [CH, C3(4)], 157.0 (C, C=NH).

MS (EI), m/e (%); main ions: 205 ($M^{+}$, 100), 204 (29), 163 (22), 162 (51), 151 (98), 150 (14), 135 (19), 134 (31), 120 (25), 118 (15), 117 (20), 108 (31), 105 (15), 91 (58), 85 (18), 80 (20), 79 (19), 77 (23), 72 (31).

Elemental analysis:

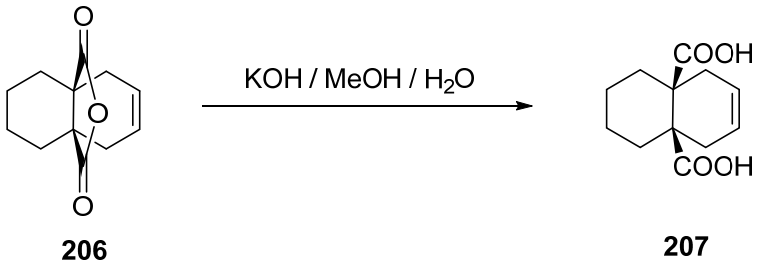
| | | | | |
|---|---|---|---|---|
| Calculated for $C_{12}H_{19}N_3$·HCl: | C 59.62% | H 8.34% | N 17.38% | Cl 14.66% |
| Calculated for $C_{12}H_{19}N_3$·HCl·0.8 $H_2O$: | C 56.26% | H 8.50% | N 16.40% | Cl 13.84% |
| Found: | C 55.86% | H 8.13% | N 16.30% | Cl 14.09% |

**Synthesis of 12-oxatricyclo[4.4.3.0$^{1,6}$]tridec-3-en-11,13-dione, 206.**




In a 100 mL sealed tube, a solution of 1-cyclohexene-1,2-dicarboxylic anhydride (1 g, 6.6 mmol) and 1,3-butadiene (15 mL, 20% solution in toluene, 9.9 mmol) was heated at reflux for 4 days. The mixture was concentrated in vacuo to obtain pure the anhydride **206** (1.43 g, 95% yield). The analytical data coincide with the reported.[161, 205]

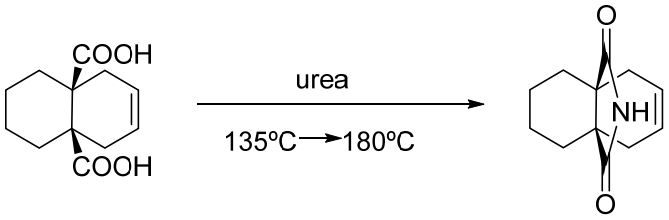
**Synthesis of *cis*-bicyclo[4.4.0]dec-3-en-1,6-dicarboxylic acid, 207.**




In a 25 mL round-bottomed flask equipped with a condenser and magnetic stirring, a solution of anhydride **206** (760 mg, 3.7 mmol) in a 5 N aqueous solution of NaOH (15 mL) was heated at reflux for 6 h. The reaction was cooled down and the aqueous layer was washed with $Et_2O$ (4 x 45 mL). The aqueous layer was acidified with concentrated HCl until pH=1 and extracted with $CH_2Cl_2$ (3 x 45 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain diacid **207** (650 mg, 79% yield) as a white solid. The analytical data coincide with the reported.[204]

[205] Alder, K. *Ber. Dtsch. Chem. Ges.* **1938**, *71B*, 2199-2209.

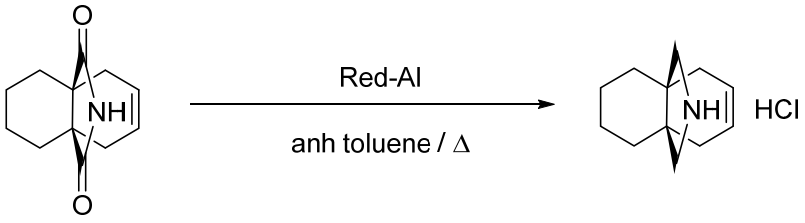
**Synthesis of 12-azatricyclo[4.4.3.0$^{1,6}$]tridec-3-en-11,13-dione, 208.**




**207** **208**

In a 100 mL round-bottomed flask equipped with a condenser and a gas outlet, a mixture of diacid **207** (650 mg, 2.9 mmol) and urea (871 mg, 95% purity, 14.5 mmol) was stirred and heated at 220 $^{o}$C for 30 min. The reaction was cooled down and the residue was dissolved in water (30 mL) and extracted with $CH_2Cl_2$ (6 x 30 mL). The combined organic extracts were dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuo obtaining imide **208** as a yellowish solid (450 mg, 76% yield). The analytical sample was obtained by crystallization from $CH_2Cl_2$/pentane. The spectroscopic data of compound **208** were identical to the reported.[162]

**Synthesis of 12-azatricyclo[4.4.3.0$^{1,6}$]tridec-3-ene hydrochloride, 185·HCl.**

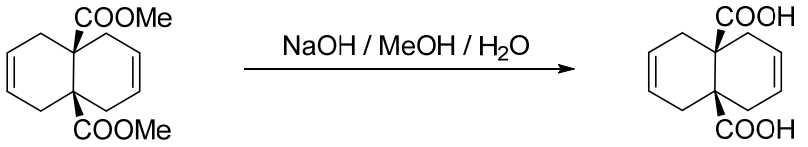



**208** **185·HCl**

In a 3-necked 50 mL round-bottomed flask equipped with a condenser, argon atmosphere and magnetic stirring, the imide **208** (210 mg, 1.0 mmol) was dissolved in anhydrous toluene (10 mL). Red-Al® (1.6 mL, 65% in toluene, 5.1 mmol) was added dropwise. The reaction was heated under reflux overnight. The reaction was cooled down and quenched with a solution of KOH 30% in water (10 mL).The layers were separated and the aqueous extracted with $CH_2Cl_2$ (2 x 10 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered. An excess of HCl/MeOH was added to the mother liquors and they were concentrated under vacuo to obtain amine **185·HCl** (153 mg, 71% yield) as a white solid. The product was recrystallized of MeOH/$Et_2O$ to obtain the analytical sample, that showed identical analytical data than the reported in the literature.[206]

[206] Wagner, E.; Davisson, J. *J. Med. Chem.* **1968**, *11*, 805-807.

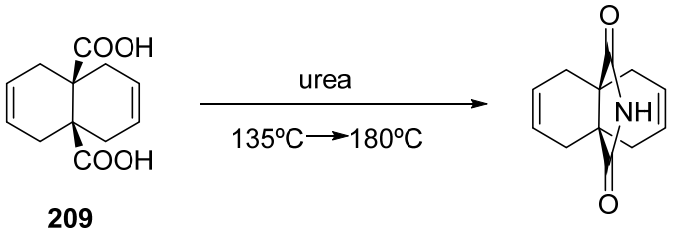
**Synthesis of *cis*-bicyclo[4.4.0$^{1,6}$]decan-2,6-diene-1,6-dicarboxylic acid, 209.**




**209**

In a 50 mL round-bottomed flask equipped with a condenser and magnetic stirring, a solution of dimethyl 1,4,4a,5,8,8a-hexahydro-4a,8a-naphtalenedicarboxylate (2 g, 8.0 mmol) in a 10% methanolic solution of KOH (20 mL) was heated at reflux for 3 h. Water (20 mL) was added to the reaction and it was heated for 3 hours more. The reaction was cooled down and the aqueous layer was washed with $Et_2O$ (4 x 45 mL). The aqueous layer was acidified with concentrated HCl until pH=1. The reaction was concentrated in vacuo and the residue extracted in hot $Et_2O$ (4 x 45 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain diacid **209** (1.43 g, 81% yield) as a white solid. The analytical data coincide with the reported.[207]

**Synthesis of 12-azatricyclo[4.4.3.0$^{1,6}$]trideca-3,8-dien-11,13-dione, 210.**

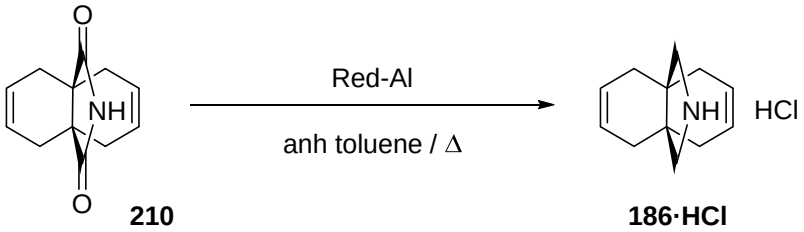



**209** **210**

In a 100 mL round-bottomed flask equipped with a condenser and a gas outlet, a mixture of diacid **209** (1 g, 4.5 mmol) and urea (1.42 g, 95% purity, 22.5 mmol) was stirred an heated at 220 $^{o}$C for 30 min. The reaction was cooled down and the residue was dissolved in water (40 mL) and extracted with $CH_2Cl_2$ (6 x 40 mL). The combined organic extracts were dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuo obtaining imide **210** as a yellowish solid (740 mg, 81% yield). The analytical sample was obtained from crystallization of $CH_2Cl_2$/pentane. The analytical data coincide with the reported.[208]

[207] Spur, P.; Hamon, D. *J. Am. Chem. Soc.* **1983**, *105*, 4734-4739.
[208] Wagner, E.; Rudzik, A. *J. Med. Chem.* **1967**, *10*, 607-611.

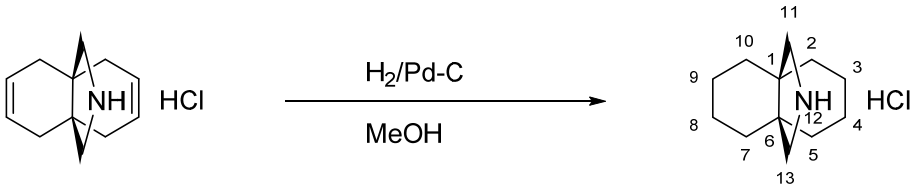
**Synthesis of 12-azatricyclo[4.4.3.0$^{1,6}$]trideca-3,8-diene hydrochloride, 185·HCl.**




**210** **186·HCl**

In a 3-necked 50 mL round-bottomed flask equipped with a condenser, argon atmosphere and magnetic stirring, the imide **210** (630 mg, 3.1 mmol) was dissolved in anhydrous toluene (24 mL). Red-Al® (4.4 mL, 65% in toluene, 15.5 mmol) was added dropwise. The reaction was heated under reflux overnight. The reaction was cooled down and quenched with a 30% aqueous solution of KOH (20 mL).The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (3 x 30 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and an excess of HCl/MeOH was added. The solution was concentrated under vacuo to obtain **186·HCl** (300 mg, 46% yield) as a white solid. An analytical sample was obtained by crystallization from MeOH/$Et_2O$. The analytical data coincide with the reported.[206]

**Synthesis of 12-azatricyclo[4.4.3.0$^{1,6}$]tridecane hydrochloride, 187·HCl.**




**186·HCl** **187·HCl**

A solution of amine **186·HCl** (596 mg, 2.81 mol) and Pd/C at 10% (60 mg) in MeOH (80 mL) was hydrogenated at room temperature and atmospheric pressure until no more hydrogen absorption was observed. The resulting suspension was filtered and the residue washed with methanol. The solvent was removed under vacuo to obtain amine **187·HCl** (567 mg, 93% yield) as a white solid.

Analytic and spectroscopic data of the amine **187·HCl**.

Melting point: 272-273 $^{o}$C (MeOH).

IR (KBr) ν: 2927, 2858, 2792, 2692, 2633, 2552, 2525, 2455, 1865, 1592, 1458, 1218, 1184, 1014, 973, 942, 916, 891, 844, 805, 705, 615 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.43-1.49 [complex signal, 4 H, 3(4,8,9,)-$H_{endo}$], 1.54-1.61 (complex signal, 12 H) [3(4,8,9,)-$H_{exo}$] and [2(5, 7, 10)], 3.22 [bs, 4 H, C2(4)].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 22.3 [$CH_2$, C7(8,11,12)], 31.2 [$CH_2$, C6(9,10,13)], 43.2 [C, C1(5)], 54.8 [$CH_2$, C2(4)].

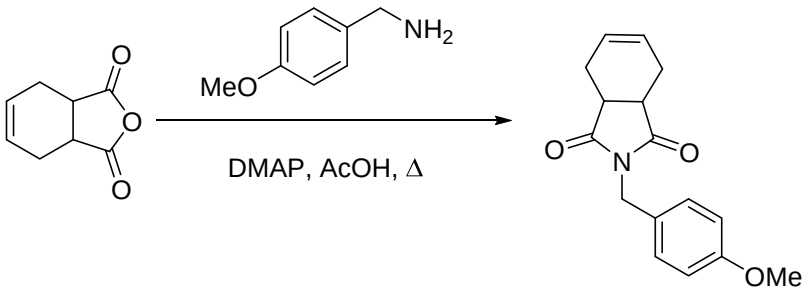
MS (EI), m/e (%); main ions: 179 [$(M\text{-}HCl)^{\cdot+}$, 100], 122 (14), 93 (25), 91 (28), 79 (33), 67 (28).

Accurate mass:

Calculated for [$C_{12}H_{21}N+H^+$]: 180.1747

Found: 180.1745

**Synthesis of *N*-(*p*-methoxybenzyl)-4-cyclohexene-1,2-dicarboximide, 211.**

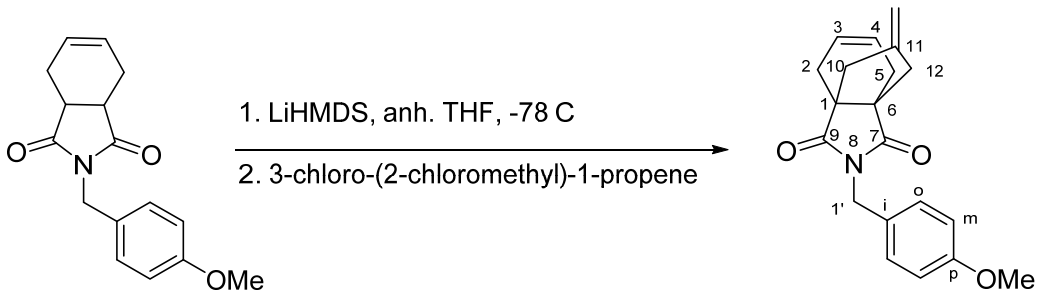



**211**

In a 250 mL round-bottomed flask equipped with a condenser and magnetic stirring, a solution of *cis*-4-cyclohexene-1,2-dicarboxylic anhydride (5.0 g, 32.9 mmol), DMAP (321 mg, 2.63 mmol) and *p*-methoxybenzylamine (4.3 mL, 32.9 mmol) in glacial acetic acid (125 mL) was heated under reflux for 4 days. The reaction was cooled down and concentrated in vacuo. The solid was dissolved in $CH_2Cl_2$ (150 mL) and washed with a saturated solution of $NaHCO_3$ (3 x 50 mL) and a 1 N solution of HCl (3 x 50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the imide **211** (4.6 g, 51% yield), which spectroscopical data coincide with the bibliographic ones.[209]

[209] Costa, B. B. C.; Corrêa, R.; Souza, M. M.; Pretto, J. B.; Ardenghi, J. V.; Campos-Buzzi, F.; Filho, V. C. *Z. Naturforsch. C: J. Biosci.* **2007**, *62*, 201-206.

**Synthesis of *N*-(*p*-methoxybenzyl)-11-methylene-8-azatricyclo[4.3.3.0$^{1,6}$]dodec-3-en-7,9-dione, 212.**




**211** **212**

In a 250 mL 3-necked round-bottomed flask equipped with a pressure equalizing addition funnel, argon atmosphere, gas outlet and magnetic stirring, a solution of hexamethyldisilazane (8.1 mL, 38.3 mmol) in anhydrous THF (95 mL) was cooled to -78 $^{o}$C. *n*BuLi (15.3 mL, 2.5 M in hexanes, 38.3 mmol) was added dropwise and the reaction was stirred 1 hour at -78 $^{o}$C. A solution of imide **211** (3.3 g, 12.2 mmol) in anhydrous THF (48 mL) was added dropwise to the reaction. The solution was stirred 15 min at -78 $^{o}$C and 1 hour at room temperature. The reaction was cooled again to -78 $^{o}$C and 3-chloro-(2-chloromethyl)-1-propene (3.0 mL, 25.6 mmol) was added dropwise and the reaction was stirred at room temperature for 4 days. The reaction was quenched with a 1 N solution of HCl (125 mL). The layers were separated and the aqueous was extracted with $Et_2O$ (3 x 100 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo. The crude was purified by column chromatography (silica gel, hexane:EtOAc, 8:2) obtaining the imide **212** (2.77 g, 70% yield) as a white solid.

Analytical and spectroscopical data of **212**.

Melting point: 80-81 $^{o}$C (ethyl acetate).

IR (KBr) *v*: 3037, 2966, 2838, 1772, 1694, 1614, 1588, 1515, 1444, 1431, 1394, 1362, 1342, 1300, 1269, 1242, 1180, 1152, 1034, 988, 932, 902, 808, 700, 678 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CDCl_3$) δ: 2.20 [dd, *J* = 15.0 Hz, *J'* = 1.6 Hz, 2 H, 2(5)-$H_a$], 2.52 [dt, *J* = 16.4 Hz, *J'* = 2.0 Hz, 2 H, 10(12)-$H_a$], 2.80 [(dq, *J* = 15.0 Hz, *J'* = 2.0 Hz, 2 H, 2(5)-$H_b$], 2.85 [d, *J* = 16.0 Hz, 2 H, 10(12)-$H_b$], 3.88 (s, 3 H, O-C$\underline{H}_3$), 4.64 (s, 2 H, 1'-$H_2$), 4.87 (m, 2 H, 11C=C$\underline{H}_2$), 5.99 [m, 2 H, 3(4)], 6.90 (d, *J* = 9.2 Hz, 2 H, Ar-$H_{meta}$), 7.27 (d, *J* = 9.2 Hz, 2 H, Ar-$H_{ortho}$).

$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 31.1 [$CH_2$, C2(5)], 42.0 ($CH_2$, C1'), 43.9 [$CH_2$, C10(12)], 54.8 [C, C1(6)], 55.2 ($CH_3$, O-$\underline{C}H_3$), 108.9 ($CH_2$, C11=$\underline{C}H_2$), 113.7 (CH, $C_{meta}$), 128.1 (C, C11), 128.6 [CH, C3(4)], 129.2 (CH, $C_{ortho}$), 145.2 (C, $C_{ipso}$), 159.0 (C, $C_{para}$), 181.7 (C, C=O).

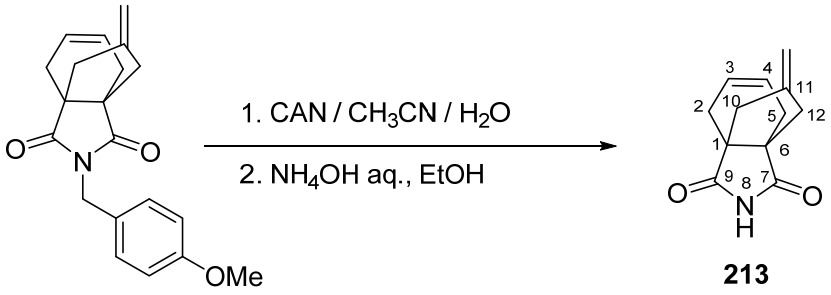
MS (EI), m/e (%); main ions: 323 ($M^{\cdot+}$, 45), 121 (100), 91 (11).

Accurate mass:

Calculated for $[C_{20}H_{21}NO_3+H]^+$ 324.1594

Found 324.1591

**Synthesis of 11-methylene-8-azatricyclo[4.3.3.0$^{1,6}$]dodec-3-en-7,9-dione, 213.**




**212** **213**

In a 250 mL round-bottomed flask equipped with magnetic stirring, a solution of CAN (19.52 g, 35.6 mmol) in $H_2O$ (45 mL) was added dropwise to a solution of imide **212** (2.77 g, 8.6 mmol) in $CH_3CN$ (45 mL). The reaction was stirred at room temperature for 2 h. Water (100 mL) was added and the solution was extracted with EtOAc (3 x 150 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was dissolved in absolute EtOH (45 mL) and $NH_4OH$ 50% v/v (45 mL) in a sealed tube. The solution was heated at 100 $^oC$ for 2 h and after cooling down, the solvent was concentrated in vacuo. The residue was extracted with hot EtOAc (2 x 200 mL) and the solvent filtered and concentrated in vacuo. The crude was purified by column chromatography (silica gel, hexane:EtOAc, 7:3) to obtain imide **213** (1.22 g, 70% yield). An analytical sample was obtained by crystallization from $CH_2Cl_2$/pentane.

Analytical and spectroscopical data of the imide **213**.

Melting point: 179-180 $^oC$ (dichloromethane/pentane).

IR (KBr) *v*: 3163, 3053, 2961, 2847, 1771, 1698, 1450, 1435, 1398, 1355, 1317, 1270, 1185, 1167, 1145, 1129, 1079, 1046, 935, 916, 891, 856, 708, 675, 657, 630 cm$^{-1}$.

$^1$H-RMN (400 MHz, $CDCl_3$) δ: 2.08 [dd, *J* = 14.6 Hz, *J'* = 1.2 Hz, 2 H, 2(5)-$H_a$], 2.42 [dt, *J* = 15.6 Hz, *J'* = 2.4 Hz, 2 H, 10(12)-$H_a$], 2.69 [ddd, *J* = 14.6 Hz, *J'* = 4.0 Hz, *J''* = 2.0 Hz, 2 H, 2(5)-$H_b$], 2.78 [dd, *J* = 15.6 Hz, *J'* = 0.8 Hz, 2 H, 10(12)-$H_b$], 4.85 (m, 2 H, 11C=C$\underline{H_2}$), 5.96 [m, 2 H, 3(4)-H], 8.64 (bs, 1 H, NH).

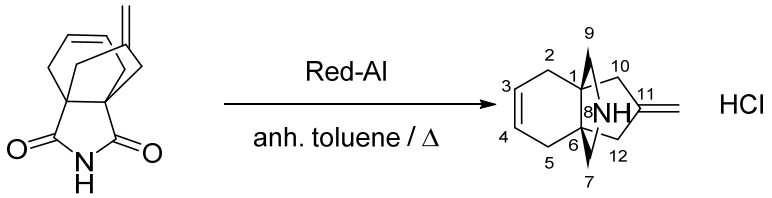
$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 30.9 [$CH_2$, C2(5)], 43.9 [$CH_2$, C10(12)], 56.1 [C, C1(6)], 109.1 ($CH_2$, C11=$\underline{C}H_2$), 128.5 [CH, C3(4)], 145.0 (C, C11), 182.4 (C, C=O).

MS (EI), m/e (%); main ions: 203 ($M^{\cdot+}$, 100), 149 (16), 148 (55), 132 (21), 131 (35), 130 (39), 129 (11), 117 (48), 116 (12), 115 (26), 105 (63), 104 (10), 91 (44), 78 (12), 77 (18), 65 (12), 56 (11), 51 (11).

Accurate mass:

| | |
|---|---|
| Calculated for $[C_{12}H_{13}NO_2+H]^+$ | 204.1019 |
| Found | 204.1023 |

**Synthesis of 11-methylene-8-azatricyclo[4.3.3.0$^{1,6}$]dodec-3-ene hydrochloride, 188·HCl.**




**213** **188·HCl**

In a 3-necked 25 mL round-bottomed flask equipped with a condenser, argon atmosphere and magnetic stirring, the imide **213** (830 mg, 4.1 mmol) was dissolved in anhydrous toluene (35 mL). Red-Al® (6.2 mL, 65% in toluene, 20.5 mmol) was added dropwise and the reaction was heated under reflux overnight. Then, the mixture was cooled down and quenched with a 30% aqueous solution of KOH (35 mL). The layers were separated and the aqueous one was extracted with $CH_2Cl_2$ (3 x 30 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$ and filtered. To the filtrate was added an excess of HCl/$Et_2O$ and the solvent was concentrated under vacuo to obtain amine **188·HCl** (565 mg, 74% yield) as a white solid. An analytical sample was obtained by crystallization from MeOH/$Et_2O$.

Analytical and spectroscopical data of **188·HCl**.

Melting point: 167-168 $^{o}$C (methanol/diethyl ether).

IR (KBr) *v*: 2877, 2783, 2686, 2602, 2564, 2488, 2438, 2388, 1596, 1471, 1442, 1427, 1336, 1154, 1109, 1077, 984, 886, 698, 671, 660 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 2.14 [dm, *J* = 16.0 Hz, 4 H, 2(5)-$H_a$], 2.22 [dm, *J* = 16.0 Hz, 4 H, 2(5)-$H_b$], 2.37 [dq, *J* = 16.0 Hz, *J'* = 2.0 Hz, 2 H, 10(12)-$H_a$], 2.54 [dq, *J* = 16.0 Hz, *J'* = 2.0 Hz, 2 H, 10(12)-$H_b$], 3.19 [d, *J* = 12.0 Hz, 4 H, 7(9)-$H_a$], 3.24 [d, *J* = 12.0 Hz, 4 H, 7(9)-$H_b$], 5.01 (q, *J* = 2.0 Hz, C=C$\underline{H}_2$), 5.76 [quint, *J* = 2.0 Hz, 2 H, 3(4)-H].

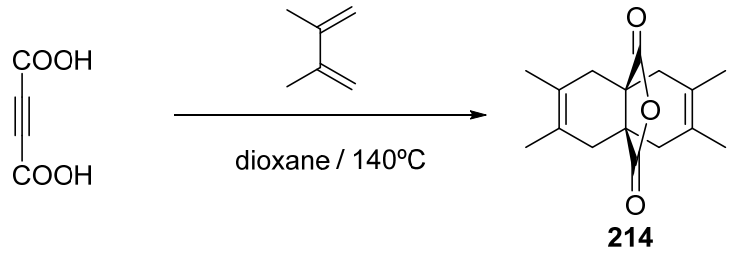
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 31.4 [$CH_2$, C2(5)], 44.3 [$CH_2$, C10(12)], 50.8 [C, C1(6)], 56.2 [$CH_2$, C7(9)], 110.5 ($CH_2$, C11=$\underline{C}H_2$), 126.1 [CH, C3(4)], 147.7 (C, C11).

MS (EI), m/e (%); main ions: 175 ($M^{\cdot+}$, 51), 144 (16), 132 (23), 131 (19), 129 (21), 128 (15), 121 (19), 120 (100), 118 (36), 117 (27), 115 (23), 91 (66), 80 (26), 79 (15), 77 (28), 65 (21), 53 (17).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{12}H_{17}N\cdot HCl$: | C 68.07% | H 8.57% | N 6.62% | Cl 16.74% |
| Found: | C 67.95% | H 8.50% | N 6.60% | Cl 16.98% |

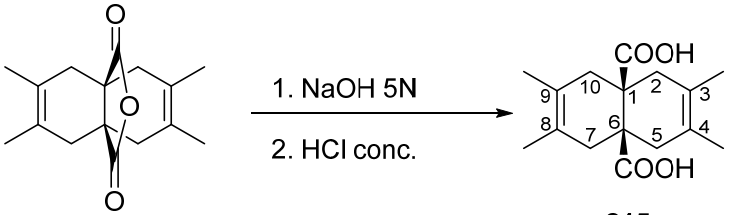
**Synthesis of 3,4,8,9-tetramethyl-12-oxatricyclo[4.4.3.0$^{1,6}$]trideca-3,8-dien-11,13-dione, 214.**




**214**

A solution of acetylenedicarboxylic acid (10 g, 87.7 mmol) and 2,3-dimethyl-1,3-butadiene (30 mL, 263 mmol) was heated in a 100 ml sealed tube at 140 ºC for 20 h. The mixture was concentrated in vacuo and the residue was crystallized from $CH_2Cl_2$ to obtain pure the anhydride **214** (14.82 g, 65% yield). The spectroscopic data coincide with the reported.[210]

**Synthesis of 3,4,8,9-tetramethyl-*cis*-bicyclo[4.4.0]decan-3,8-diene-1,6-dicarboxylic acid, 215.**




**214** **215**

In a 100 mL round-bottomed flask equipped with a condenser and magnetic stirring, a solution of anhydride **214** (5.59 g, 21.5 mmol) in a 5 N aqueous solution of NaOH (56 mL) was heated at reflux overnight. The reaction was cooled down and acidified with concentrated HCl. The precipitate was filtered off to obtain diacid **215** (4.84 g, 81% yield) as a light brown solid.

[210] Avila, W.; Silva R. *J. Chem. Soc. D: Chem. Comm.* **1970**, *2*, 94-95.

Analytical and spectroscopical data of **215**.

Melting point: 223-224 ºC (water).

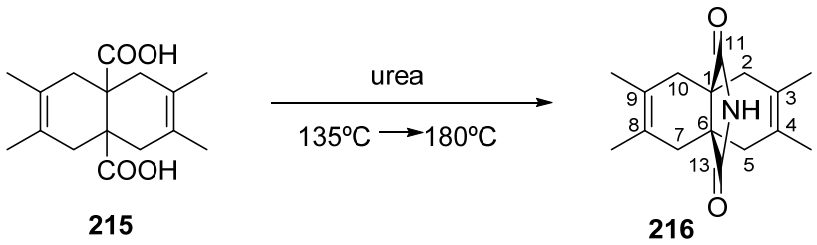
IR (KBr) v: 2915, 2865, 2680, 1697, 1454, 1438, 1400, 1372, 1325, 1291, 1219, 1139, 1116, 1024, 929, 882, 837, 779, 725, 694, 585 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.59 [s, 12 H, 3(4,8,9)-C<u>H</u>$_3$], 2.12 [d, *J* = 16.8 Hz, 4 H, 2(5,7,10)-$H_a$] 2.52 [d, *J* = 16.8 Hz, 4 H, 2(5,7,10)-$H_b$].

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 18.7 [$CH_3$, 3(4,8,9)-<u>C</u>$H_3$], 41.1 [$CH_2$, C2(5,7,10)], 46.3 [C, C1(6)], 123.6 [CH, C3(4,8,9)], 179.6 (C, C=O).

MS (EI), m/e (%); main ions: 260 [$(M-H_2O)^{\cdot+}$, 50], 232 (32), 188 (58), 187 [$(C_{14}H_{19})^+$, 100], 173 (56), 171 (28), 163 (22), 145 (40), 131 (25), 119 (51), 105 (19), 91 (38), 82 (46), 77 (24), 67 (20).

**<u>Synthesis of 3,4,8,9-tetramethyl-12-azatricyclo[4.4.3.0$^{1,6}$]trideca-3,8-dien-11,13-dione, 216.</u>**




**215** **216**

In a 100 mL round-bottomed flask equipped with a condenser and a gas outlet, a mixture of diacid **215** (4.84 g, 17.4 mmol) and urea (5.22 g, 95% purity, 86.9 mmol) was stirred an heated at 220 ºC for 30 min. The reaction was cooled down and the residue was dissolved in water (30 mL) and extracted with $CH_2Cl_2$ (6 x 150 mL). The combined organic extracts were dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuo obtaining imide **216** as a yellowish solid (3.62 g, 80% yield). The analytical sample was obtained by crystallization from $CH_2Cl_2$/pentane.

Analytical and spectroscopical data of **216**.

Melting point: 197-198 ºC (dec).

IR (KBr) v: 3202, 3072, 2983, 2928, 2859, 1775, 1706, 1659, 1439, 1364, 1322, 1186, 1106, 982, 829, 739, 677 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.63 [d, *J* = 1.2 Hz, 12 H, 3(4,8,9)-C<u>H</u>$_3$], 2.07 (broad d, *J* = 14.0 Hz, 4 H) and 2.35 (d, *J* = 14.4 Hz, 4 H) [2(5,7,10)-$H_2$], 8.23 (broad s, 1 H, NH).

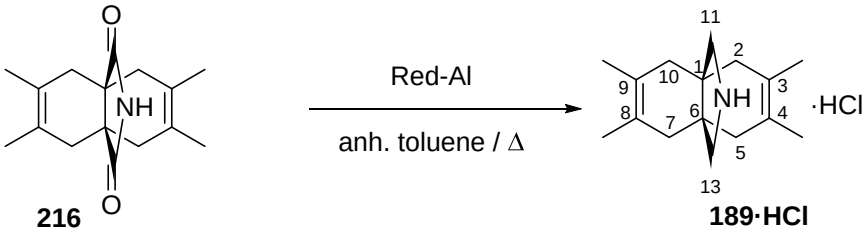
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 19.0 [$CH_3$, C3(4,8,9)-$\underline{C}H_3$], 38.5 [$CH_2$, C2(5,7,10)], 52.8 [C, C1(6)], 127.3 [CH, C3(4,8,9)], 182.7 (C, C=O).

MS (EI), m/e (%); main ions: 259 ($M^{\cdot+}$, 49), 188 (24), 177 (25), 173 (15), 162 (100), 144 (14), 133 (16), 119 (18), 91 (13).

Accurate mass:

| | |
|---|---|
| Calculated for $[C_{16}H_{21}NO_2+H]^+$ | 260.1645 |
| Found | 260.1649. |

**Synthesis of 3,4,8,9-tetramethyl-12-azatricyclo[4.4.3.0$^{1,6}$]trideca-3,8-diene hydrochloride, 189·HCl.**




**216** **189·HCl**

In a 3-necked 50 mL round-bottomed flask equipped with a condenser, argon atmosphere and magnetic stirring, the imide **216** (1 g, 3.9 mmol) was dissolved in anhydrous toluene (35 mL). Red-Al® (5.9 mL, 65% in toluene, 19.3 mmol) was added dropwise. The reaction was heated under reflux overnight. The reaction was cooled down and quenched with a 30% aqueous solution of KOH (30 mL). The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (3 x 30 mL). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and filtered. An excess of HCl/MeOH was added to the filtrate and the solvent was removed under vacuo to obtain **189·HCl** (414 mg, 40% yield) as a brown solid. An analytical sample was obtained by crystallization from MeOH/$Et_2O$.

Analytical and spectroscopical data of **189·HCl**.

Melting point: 209-210 ºC (methanol/diethyl ether).

IR (KBr): 2869, 2833, 2677, 2455, 2357, 1594, 1557, 1443, 1404, 1375, 1286, 1243, 1223, 1175, 1137, 969, 884, 675 cm$^{-1}$.

$^1$H-RMN (400 MHz, $CDCl_3$) δ: 1.63 [s, 12 H, C3(4,8,9)-C$\underline{H}_3$], 1.95 (d, *J* = 16.8 Hz, 4 H) and 2.06 (d, *J* = 16.8 Hz, 4 H) [2(5,7,10)-$H_2$], 3.14 [s, 4 H, 11(13)-$H_2$].

$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 18.7 [$CH_3$, C3(4,8,9)-$\underline{C}H_3$], 38.6 [$CH_2$, C2(5,7,10)], 41.9 [C, C1(6)], 55.1 [$CH_2$, C11(13)], 122.9 [C, C3(4,8,9)].

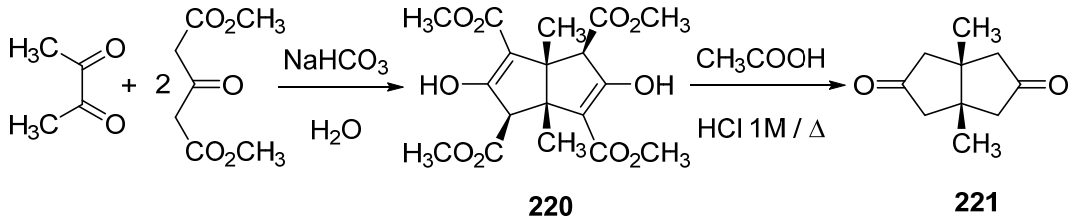
GC-MS (EI), m/e (%); main ions: 231 ($M^{\cdot+}$, 100), 230 (29), 216 (16), 188 (23), 187 (22), 186 (35), 185 (18), 173 (18), 171 (68), 159 (24), 157 (16), 149 (23), 148 (98), 146 (44), 145 (36), 134 (23), 132 (26), 131 (20), 119 (48), 107 (17), 105 (20), 95 (16), 91 (25).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{16}H_{25}N{\cdot}HCl$: | C 71.75% | H 9.78% | N 5.23% | Cl 13.24% |
| Calculated for $C_{16}H_{25}N{\cdot}HCl{\cdot}0.66\ H_2O{\cdot}0.1CH_2Cl_2$: | C 67.09% | H 9.62% | N 4.86% | Cl 14.76% |
| Found: | C 67.00% | H 9.31% | N 4.54% | Cl 14.63% |

# 5. SYNTHESIS OF (TRICYCLO[3.3.0.0$^{3,7}$]OCT-1-YL)AMINES, 3-AZATETRACYCLO[5.2.1.1$^{5,8}$.0$^{1,5}$]UNDECANES AND 7,8-DIMETHYL-3-AZATETRACYCLO[5.2.1.1$^{5,8}$.0$^{1,5}$]UNDECANES

**Synthesis of *cis*-1,5-dimethylbicyclo[3.3.0]octane-3,7-dione, 221.**




**220** **221**

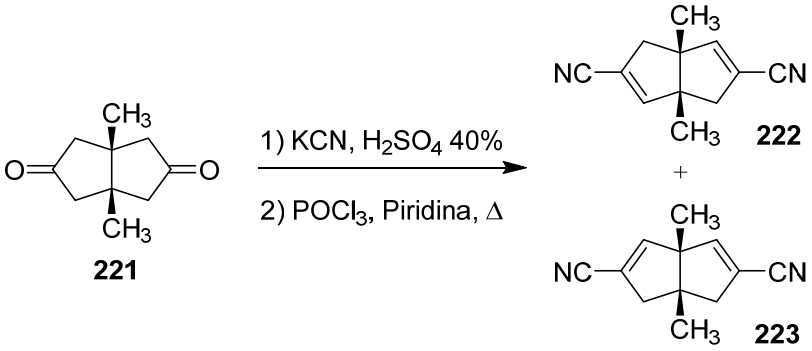
**a)** Synthesis of tetramethyl (1α,4α,5α,8α)-3,7-dihydroxi-1,5-dimethylbicyclo[3.3.0]octa-2,6-diene-2,4,6,8-tetracarboxylate, **220**:

In a 2 L round-bottomed flask equipped with magnetic stirring, a solution of sodium bicarbonate (12 g) in water (850 ml) was prepared, then, dimethyl 1,3-acetonedicarboxylate (150 g, 0.86 mol) and biacetyl (37 g, 0.43 mol) were added and the mixture was stirred at room temperature for 24 hours. The white precipitate was filtered out under vacuo, washed with water and dryed under vacuo obtaining the tetraester **220** (143 g, 84% of yield), which was used in the next step without purification.

**b)** *Cis*-1,5-dimethylbicyclo[3.3.0]octane-3,7-dione, **221**:

In a 2L round-bottomed flask equipped with two condensers and magnetic stirring a solution of HCl 1N (1 L), glacial AcOH (240 mL) and the tetraester **220** (143 g, 0.36 mol) was prepared and the mixture was heated under reflux for 6 hours. The resulting solution was cooled with an ice/water bath and extracted with $CH_2Cl_2$ (6x300 mL). The combined organic layers were concentrated under vacuo. The obtained residue was dissolved in $CH_2Cl_2$ (300 mL), washed with a saturated solution of sodium bicarbonate until reaching basic pH, dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo to obtain the dicetone **221** (57 g, 96% yield), which spectroscopic data coincide with the bibliographic ones.[183]

**Synthesis of a mixture of *cis*-1,5-dimethylbicyclo[3.3.0]octa-2,6-diene-3,7-dicarbonitrile, 222 and *cis*-1,5-dimethylbicyclo[3.3.0]octa-2,7-diene-3,7-dicarbonitrile, 223.**




**a)** Synthesis of the stereoisomeric mixture of bis-cianohydrins:

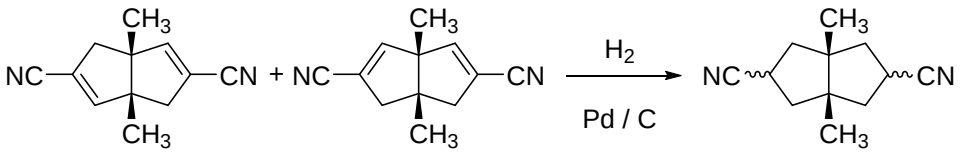
In an 1 L round-bottomed flask equipped with a pressure-equalizing dropping funnel, magnetic stirring and a gas outlet, a suspension of KCN (120 g, 1.8 mol) and the diketone **221** (43 g, 0.26 mol) in water (200 mL) was prepared and cooled with an ice bath. A solution of sulfuric acid 40% (363 mL) was added dropwise over 4 hours, keeping the internal temperature between 10 and 15 $^{o}$C. After the addition, water (300 mL) was added and the aqueous layer was extracted with AcOEt (6x225 mL). The combined organic layers were washed with water (2x200 mL), dryed over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining a stereoisomeric mixture of bis-cyanohydrins (54.7 g), used without purification in the next step of the synthesis.

**b)** Dehydration of bis-cyanohydrins obtaining a mixture of *cis*-1,5-dimethylbicyclo[3.3.0]octa-2,6-diene-3,7-dicarbonitrile, **222** and *cis*-1,5-dimethylbicyclo[3.3.0]octa-2,7-diene-3,7-dicarbonitrile, **223**:

In a 2 L three necked round-bottomed flask equipped with a condenser, a pressure-equalizing dropping funnel and a magnetic stirring, a solution of the previous crude (54.7 g) in pyridine (760 mL) was prepared. The solution was heated under reflux and $POCl_3$ (150 mL) was added dropwise. Once the addition was finished, the reaction was heated under reflux over 6 hours. The mixture was tempered and poured, carefully, over concentrated HCl (475 mL) and crushed ice (625 g). The obtained precipitated was filtered out and washed with diluted HCl and water. The solid was dissolved in $CH_2Cl_2$ (750 mL), the solution was washed with water (2x150 mL), dryed over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining a black solid (27.9 g) which was dissolved in refluxing $Et_2O$, bleached with activated charcoal and filtered through Celite®. The residue was concentrated under vacuo obtaining a a mixture of

unsaturated dinitriles **222** and **223** (24.6 g, 55% yield), which spectroscopic data coincide which the bibliographic ones.[211]

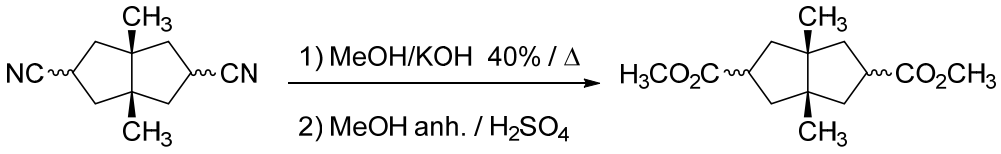
**Synthesis of the stereoisomeric mixture *cis*-1,5-dimethylbicyclo[3.3.0]octane-3,7-dicarbonitrile, 224.**




**222** **223** **224**

A solution of the isomeric mixture of unsaturated dinitriles **222** and **223** (22.3 g, 0.12 mol) and Pd/C at 10% (1.25 g) in absolute EtOH (400 mL) was hydrogenated at room temperature, at a pressure of 20 atm. until no more hydrogen absorption was observed. The resulting suspension was filtered and the residue washed with ethanol. The solvent was removed under vacuo and a mixture of the three possible stereoisomers of dinitrile **224** in a relationship of (1α,3α,5α,7α), (1α,3β,5α,7β), (1α,3α,5α,7β) de 2:1.3:1. The spectroscopic data coincide with the bibliographic ones.[211]

**Synthesis of a stereoisomeric mixture of dimethyl *cis*-1,5-dimethylbicyclo[3.3.0]octane-3,7-dicarboxylate, 225.**[184,211]




**224** **225**

**a)** Hydrolisis of a mixture of dinitriles, **224**:

In a 1 L round-bottomed flask the mixture of dinitriles **224** (24 g, 0.12 mol) was dissolved in a solution of KOH in methanol at 40% (180 mL). The mixture was heated under reflux over 3

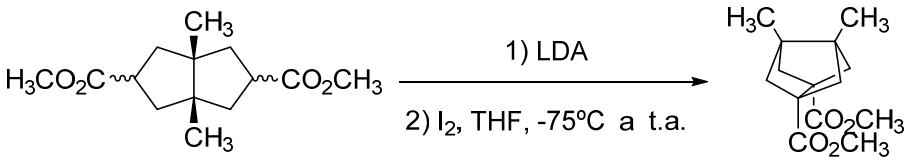
[211] Camps, P.; Iglesias, C.; Rodríguez, M. J.; Grancha, M. D.; Gregori, M. E.; Lozano, R.; Miranda, M. A.; Figueredo, M.; Llinares, A. *Chem. Ber.* **1988**, *121*, 647-654.

hours, then, water was added (270 mL) and the reflux was kept for 3 hours more. The solution was cooled with an ice bath, acidified with concentrated HCl and the solvent was removed under vacuo. The residue was extracted with hot $Et_2O$ (5x100 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$ and filtered. The mother layers were concentrated under vacuo obtaining a stereoisomeric mixture of diacid (25.6 g, 89% yield) used in the next step of the synthesis without purification.

**b)** Synthesis of a stereoisomeric mixture of dimethyl *cis*-1,5-dimethylbicyclo[3.3.0]octane-3,7-dicarboxylate, **225**:

In a 1 L round-bottomed flask equipped with magnetic stirring, a condenser and a $CaCl_2$ tube, the previous crude (25.6 g) was dissolved in anhydrous MeOH (140 mL), concentrated $H_2SO_4$ (15 mL) was added and the solution was refluxed for 18 hours. The mixture was tempered and cooled at 4 $^oC$ overnight, a precipitate appeared which was filtered out and washed with cold methanol. The solid was dissolved in $CH_2Cl_2$ (150 mL) and washed with water (3x50 mL). The organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the diester **225** as a white solid (20.2 g, 70% yield), which spectroscopic data coincide with the bibliographic ones.

**Synthesis of dimethyl 3,7-dimethyltricyclo[3.3.0.0$^{3,7}$]octane-1,5-dicarboxylate, 226.**

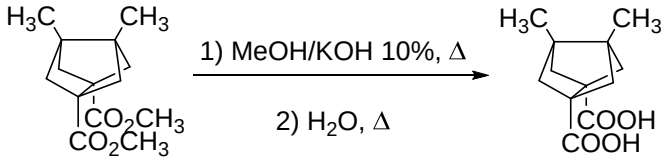



**225** **226**

In a 500 mL three necked round-bottomed flask eqquiped with a gas oulet, low temperature thermometer, a pressure-equalizing dropping funnel, magnetic stirring and argon atmosphere, a solution of LDA 1.5 M in THF (44.6 mL, 66.9 mmol) was diluted in anhydrous THF (87 mL) and cooled to -13 $^oC$ with a dry ice/acetone bath then, a solution of diester **225** (7 g, 30.4 mmol) in anhydrous THF (87 mL) was added dropwise. Once the addition was finished, the temperature was kept for an hour, and the formed suspension was cooled to -70 $^oC$ and a solution of $I_2$ (7.72 g, 30.4 mmol) in anhydrous THF (174 mL) was added dropwise. The red solution was kept at -70 $^oC$ for 1 hour, and tempered to room temperature overnight. Then, the solution was acidified with HCl 6N (20 mL) and it was extracted with $Et_2O$ (5x45 mL), the combined organic layers were washed with a solution of $Na_2S_2O_3$ at 10% (3x100 mL) and brine (3x75 mL). The organic layer was dried over anhydrous $Na_2SO_4$, filtered and concentrated

under vacuo. The tricyclic diester **226** (3.75 g, 54% yield) was obtained by column chromatography in silica (hexane / AcOEt 9:1), which spectroscopic data coincide with the bibliographic ones.[211]

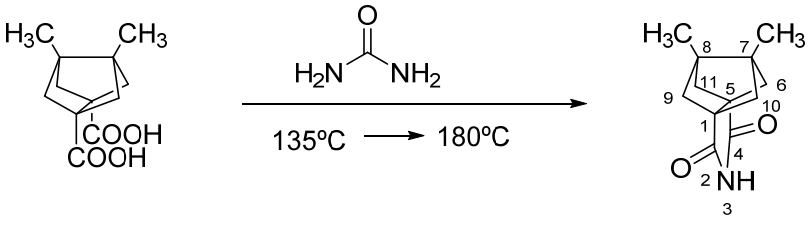
**Synthesis of 3,7-dimethyltricyclo[3.3.0.0$^{3,7}$]octane-1,5-dicarboxylic acid, 217.**




**226** **217**

In a 250 mL round-bottomed flask equipped with a condenser and magnetic stirring, a solution of diester **226** (6.94 g, 27.3 mmol) in a solution of KOH in MeOH at 10% (65 mL) was prepared and heated under reflux for 3 hours. Water (65 mL) was added and the reflux was kept for 3 more hours. The mixture was cooled with an ice bath, acidified with concentrated HCl and the solvent was removed under vacuo. The residue was extracted with hot $Et_2O$ (5x75 mL), and the combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the diacid **217** (5.79 g, 95% yield), which spectroscopic data coincide with the bibliographic ones.[11a]

**Synthesis of 7,8-dimethyl-3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undeca-2,4-dione, 218.**




**217** **218**

In a 100 mL round-bottomed flask equipped with a condenser and magnetic stirring, the diacid **217** (3 g, 13.4 mmol) and urea 95% (4.2 g, 67.0 mmol) were added. The mixture was heated at 135 ºC until the urea melts and then it was heated to 180 ºC for 30 min. The mixture was tempered and dissolved in water (66 mL), the suspension was extracted with $CH_2Cl_2$ (6x40 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the imide **218** (2.07 g, 75% yield).

Analytic and spectroscopic data of the imide **231**.

Melting point: 209-210 $^{o}$C (chloroform).

IR (KBr) v: 3194, 3071, 2969, 2893, 2866, 2767, 1766, 1711, 1480, 1455, 1384, 1344, 1309, 1209, 1152, 1134, 1056, 1033, 963, 840, 728, 614, 507 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CDCl_3$) δ: 1.23 [s, 6 H, C7(8)-$C\underline{H_3}$], 1.88 [dd, *J* = 9.8 Hz, *J'* = 1.9 Hz, 4 H, 6(9,10,11)-$H_\alpha$], 1.92 [dd, *J* = 9.8 Hz, *J'* = 2.0 Hz, 4 H, 6(9,10,11)-$H_\beta$], 8.24 (bs, 1H, NH) .

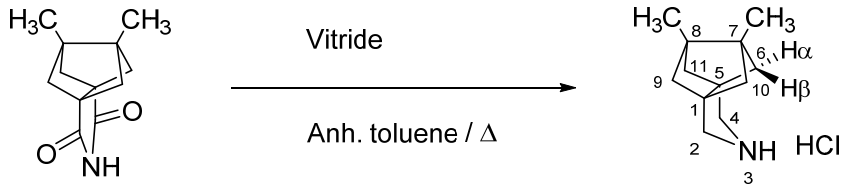
$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 16.1 [$CH_3$, C7(8)-$CH_3$], 51.8 [C, C7(8)], 55.2 [$CH_2$, C6(9,10,11)], 57.5 [C, C1(5)], 177.4 (C, C=O).

MS (EI), m/e (%); main ions: 205 ($M^{\cdot+}$, 17), 134 (83), 120 (100), 119 (53), 150 (48), 117 (10), 105 (17), 92 (22), 91 (38), 79 (15), 77 (23).

Accurate mass:

| | |
|---|---|
| Calculated for $[C_{12}H_{17}NO_2+H]^+$ | 206.1176 |
| Found | 206.1185 |

**Synthesis of 7,8-dimethyl-3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecane hydrochloride, 219·HCl.**




**218** **219·HCl**

In a 25 mL three necked round-bottomed flask equipped with a condenser, high temperature thermometer, argon atmosphere and magnetic stirring, a solution of imide **218** (0.36 g, 1.8 mmol) in anhydrous toluene (8 mL) was prepared. The solution was cooled to 0 $^{o}$C with an ice/water bath and Vitride® 65% in toluene (2.7 mL, 8.8 mmol) was added dropwise and the mixture was heated under reflux for 72 hours. The reaction was tempered and cooled to 0 $^{o}$C with an ice/water bath and a solution of KOH at 30% in water was added dropwise until reaching a basic pH. The aqueous layer was extracted with $CH_2Cl_2$ (3x15 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and a solution of HCl/$Et_2O$ was

added and the solvent removed under vacuo obtaining the amine **219·HCl** (0.41 g, 99% yield). The analytical sample was obtained from crystallization of MeOH/$Et_2O$.

Analytic and spectroscopic data of the amine **219·HCl**.

Melting point: 248-249 ºC (MeOH).

IR (KBr) ν: 3527, 2953, 2883, 2767, 2702, 2588, 2536, 2413, 2241, 1902, 1610, 1594, 1508, 1483, 1457, 1444, 1379, 1363, 1297, 1260, 1183, 983, 871, 645, 477 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.19 [s, 6 H, C7(8)-$CH_3$], 1.67 [m, 8 H, 6(9,10,11)-$H_2$], 3.26 [bs, 4H, C2(4)-$H_2$] .

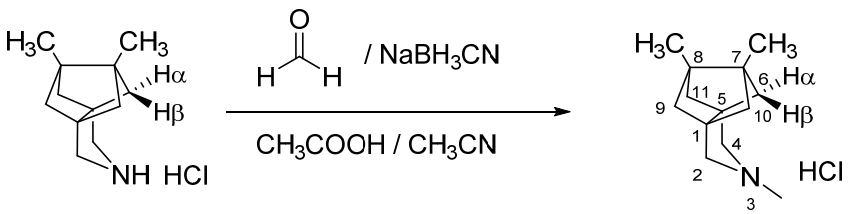
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 16.7 [$CH_3$, C7(8)-$CH_3$], 47.5 [$CH_2$, C2(4)], 52.4 [C, C7(8)], 56.6 [$CH_2$, C6(9,10,11)], 58.7 [C, C1(5)].

MS (EI), m/e (%); main ions: 177 ($M^{\cdot+}$, 6), 135 (21), 134 ($C_{10}H_{14}^{+}$, 100), 133 (35), 122 (29), 119 (22), 107 (22), 106 (35), 105 (48), 93 (32), 92 (21), 91 (64), 80 (23), 79 (24), 77 (34), 55 (21).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{12}H_{20}NCl$: | C 67.43% | H 9.43% | N 6.55% | Cl 16.59% |
| Calculated for $C_{12}H_{20}NCl \cdot 0.5\ H_2O$: | C 64.70% | H 9.50% | N 6.29% | Cl 15.91% |
| Found: | C 64.70% | H 9.19% | N 6.68% | Cl 16.11% |

**Synthesis of 3,7,8-trimethyl-3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecane hydrochloride, 227·HCl.**




**219·HCl** **227·HCl**

In a 25 mL round-bottomed flask equipped with a $CaCl_2$ tube, a suspension of amine **219** (0.5 g, 2.4 mmol), $NaBH_3CN$ 95% purity (0.46 g, 6.9 mmol), formaldehyde 37% (0.6 mL, 7.3 mmol), $CH_3COOH$ (0.4 mL) in acetonitrile (10 mL) was prepared. The reaction was stirred at

room temperature for 8 hours then, more $NaBH_3CN$ 95% purity (0.46 g, 6.9 mmol) and formaldehyde 37% (0.6 mL, 7.3 mmol) were added and the suspension stirred at room temperature overnight. The solvent was removed under vacuo and the residue dissolved in water (20 mL), basified with NaOH 10 N (~4 mL) and extracted with AcOEt (3x20 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, followed by the addition of a solution of $HCl/Et_2O$ and the solvent removed under vacuo obtaining the amine **227·HCl** as a white solid (0.40 g, 73% yield). The analytical sample was obtained from crystallization of 2-propanol.

Analytic and spectroscopic data of the amine **227·HCl**.

Melting point: 280-281 ºC decompose (IPA).

IR (KBr) v: 3384, 3007, 2953, 2882, 2591, 2564, 2474, 2449, 1644, 1480, 1462, 1446, 1419, 1378, 1349, 1293, 1257, 1248, 1193, 1173, 1123, 1105, 1068, 1025, 967, 893, 561, 461 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CDCl_3$) δ: 1.09 (s, 3 H) and 1.11 (s, 3 H) (C7-C$\underline{H_3}$ and C8-C$\underline{H_3}$), 1.47 [d, *J* = 8.5 Hz, 2 H, 9(11)-$H_\alpha$), 1.52 [dd, *J* = 7.2 Hz, *J'* = 2.0 Hz, 2 H, 6(10)-$H_\beta$], 1.63 (d, *J* = 8 Hz, 2 H, 6(10)-$H_\alpha$], 2.18 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 2 H, 9(11)-$H_\beta$], 2.79 [m, 2H, C2(4)-$H_\alpha$], 2.82 (d, *J* = 3.5 Hz, 3 H, N-$CH_3$), 3.64 [d, *J* = 7.7 Hz, 2 H, C2(4)-$H_\beta$] and 12.65 (bs, 1H, NH).

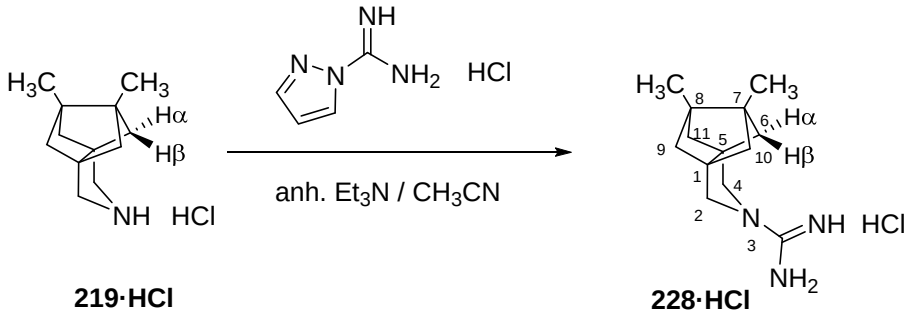
$^{13}$C-RMN (125.7 MHz, $CDCl_3$) δ: 16.0 ($CH_3$) and 16.2 ($CH_3$) (C7-$\underline{C}$H3 and C8-$\underline{C}H_3$), 40.7 ($CH_3$, N-$CH_3$), 50.2 and 51.9 [C, C7(8)], 54.7 ($CH_2$) and 56.3 ($CH_2$) [C6(9,10,11)], 56.5 [$CH_2$, C2(4)], 57.2 [C, C1(5)].

MS (EI), m/e (%); main ions: 192 ($M^{\cdot+}$, 6), 191 (43), 190 (40), 148 (100), 136 ($C_{10}H_{14}^+$, 35), 134 (32), 133 (31), 107 (22), 106 (28), 105 (28), 94 (20), 91 (41), 77 (21), 58 (50), 57 (37).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{13}H_{22}NCl$: | C 68.55% | H 9.74% | N 6.15% | Cl 15.57% |
| Calculated for $C_{12}H_{20}NCl·0.2\ H_2O$: | C 67.48% | H 9.76% | N 6.05% | Cl 15.32% |
| Found: | C 67.57% | H 9.57% | N 6.05% | Cl 15.32% |

**Synthesis of 3-amidino-7,8-dimethyl-3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecane hydrochloride, 228·HCl.**




**219·HCl** **228·HCl**

In a 10 mL round-bottomed flask equipped with a condenser and a magnetic stirrer, a suspension of amine **219·HCl** (0.37 g, 1.8 mmol), 1*H*-pyrazole-carboxamidine hydrochloride (0.31 g, 2.1 mmol), anh. triethylamine (0.44 mL) in acetonitrile (5 mL) was prepared. The reaction was heated at 70 ºC for 6 hours. The reaction was cooled down and cooled at 4 ºC overnight. The suspension was filtered out and the filtrate washed with cold acetonitrile obtaining the guanidine **228·HCl** as a beige solid (0.45 g, 61% yield).

Analytic and spectroscopic data of the guanidine **228·HCl**.

Melting point: 276-277 ºC decompose ($CH_3CN$).

IR (KBr) ν: 3295, 3093, 2937, 2876, 2723, 1634, 1531, 1478, 1456, 1361, 1293, 1189, 1161, 1133, 1116, 1045, 955, 737, 604, 540 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.18 [s, 6 H, C7(8)-$CH_3$], 1.63 [d, *J* = 6.9 Hz, 4 H, C6(9,10,11)-$H_\alpha$], 1.69 [d, *J* = 6.8 Hz, 4 H, C6(9,10,11)-$H_\beta$], 3.49 [s, 4 H, C2(4)-$H_2$].

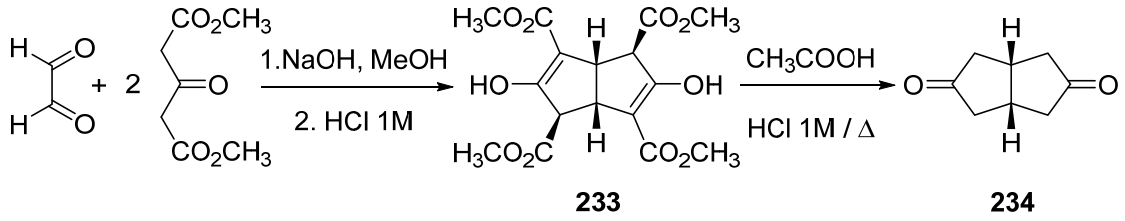
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 16.7 [$CH_3$, C7(8)-$CH_3$], 49.5 [$CH_2$, C2(4)], 52.3 [C, C7(8)], 58.0 [$CH_2$, C6(9,10,11)], 58.4 [C, C1(5)], 156.7 (C, C=NH).

MS (EI), m/e (%); main ions: 220 (54), 219 ($M^{\cdot+}$, 41), 166 (66), 165 (100), 164 (68), 163 (23), 145 (33), 134 ($C_{10}H_{14}^{+}$, 29), 133 (21), 123 (23), 122 (26), 120 (23), 119 (24), 114 (26), 113 (34), 112 (24), 107 (23), 106 (29), 105 (65), 91 (58), 79 (22), 77 (31), 74 (41), 73 (51), 72 (23).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{13}H_{22}N_3Cl$: | C 61.04% | H 8.67% | N 16.43% | Cl 13.86% |
| Calculated for $C_{13}H_{22}N_3Cl·0.33\ Et_2O$: | C 61.37% | H 9.10% | N 14.99% | Cl 12.65% |
| Found: | C 61.70% | H 9.07% | N 14.62% | Cl 13.04% |

**Synthesis of *cis*-bicyclo[3.3.0]octane-3,7-dione, 234.**




**233** **234**

**a)** Synthesis of the sodium salt of tetramethyl (1α,4α,5α,8α)-3,7-dihydroxi-bicyclo[3.3.0]octa-2,6-diene-2,4,6,8-tetracarboxylate, **233**:

In a 2 L three necked round-bottomed flask equipped with magnetic stirring, thermometer, a pressure-equalizing dropping funnel and a condenser a solution of sodium hydroxide (58 g) in methanol (1 L) was prepared and cooled with an ice bath. Then, dimethyl 1,3-acetonedicarboxylate (150 g, 1.3 mol) was added dropwise, followed by the addition of a solution of glyoxal at 40 % (107.25 g, 0.74 mol) over 15-20 min keeping the internal temperature at 65 $^{o}$C. The reaction was stirred at room temperature overnight. The yellow precipitate was filtered out under vacuo, washed with cool methanol and dried under vacuo obtaining the sodium salt of the tetraester **233** (266 g, 56% of yield), which was used in the next step without purification.

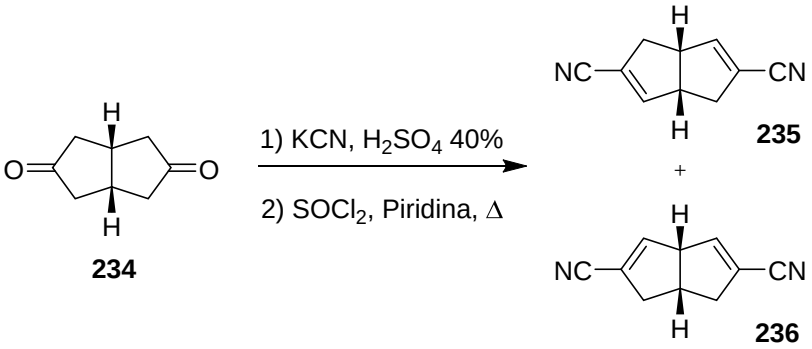
**b)** Synthesis of tetramethyl (1α,4α,5α,8α)-3,7-dihydroxi-bicyclo[3.3.0]octa-2,6-diene-2,4,6,8-tetracarboxylate, **233**:

In a 2 L round-bottomed flask equipped with magnetic stirring a solution the disodium salt of **233** (266 g, 181 mmol) in water (1 L) and $CH_2Cl_2$ (350 mL) was prepared and cooled with an ice bath. Then, a solution of HCl 5N (375 mL) was added while stirring vigorously. The two layers were separated and the aqueous was extracted with $CH_2Cl_2$ (2x250 mL). The combined organic layers were washed with brine (2x100 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the tetraester **233** (144 g, 62% of yield) as a yellow solid, which was crystallized of a mixture of hexane/AcOEt 2:1 an used in the next step of the synthetic route.

**c)** Synthesis of bicyclo[3.3.0]octane-3,7-dione, **234**:

In a 2 L round-bottomed flask equipped with two condensers a solution of HCl 1N (545 mL), glacial AcOH (58 mL) and the tetraester **233** (116 g, 0.36 mol) was prepared and the mixture was heated under reflux for 2.5 hours. The resulting solution was cooled with an ice/water bath and extracted with $CH_2Cl_2$ (5x400 mL). The combined organic layers were concentrated under vacuo. The obtained residue was dissolved in $CH_2Cl_2$ (250 mL), washed with a saturated solution of sodium bicarbonate until reaching basic pH, dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo to obtain the dicetone **234** (26.7 g, 54% yield), which spectroscopic data coincide with the bibliographic ones.[183]

**Synthesis of a mixture of *cis*-1,5-dimethylbicyclo[3.3.0]octa-2,6-diene-3,7-dicarbonitrile, 235 and *cis*-1,5-dimethylbiciclo[3.3.0]octa-2,7-diene-3,7-dicarbonitrile, 236.**



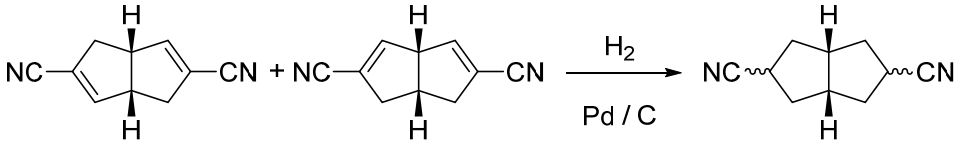

**a)** Synthesis of the stereoisomeric mixture of bis-cianohydrines:

In an 1 L round-bottomed flask equipped with a pressure-equalizing dropping funnel, magnetic stirring and a gas outlet, a suspension KCN (77 g, 1.2 mol) and the dicetone **234** (27 g, 0.19 mol) in water (200 mL) was prepared and cooled with an ice bath. A solution of sulfuric acid 40% (306 mL) was added dropwise over 4 hours, keeping the internal temperature between 10 and 15 $^oC$. After the addition, water (300 mL) was added and the aqueous layer was extracted with $Et_2O$ (6x320 mL). The combined organic layers were washed with water (2x300 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining a stereoisomeric mixture of bis-cyanohidrins (16 g), used without purification in the next step of the synthesis.

**b)** Dehydration of bis-cyanohydrins and obtaining a mixture of *cis*-bicycle[3.3.0]octa-2,6-diene-3,7-dicarbonitrile, **235** and *cis*-bicyclo[3.3.0]octa-2,7-diene-3,7-dicarbonitrile, **236**:

In a 2 L three necked round-bottomed flask equipped with a condenser, pressure-equalizing dropping funnel and a magnetic stirring, a solution of the previous crude (16.0 g) in pyridine (103 mL). $SOCl_2$ (21 mL) was added dropwise to the solution. Once the addition was finished, the reaction was heated under reflux over 5 hours. The mixture was tempered overnight. A solution of HCl 5N (150 mL) was poured, carefully, over the reaction reaching acid pH. The formed precipitated was filtered out and washed with diluted HCl (150 mL) and water (150 mL). The solid was extracted with hot $CH_2Cl_2$ (3x100 mL) and filtered. the solution was washed with water (2x150 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining a black solid (27.9 g) which was dissolved in refluxing $Et_2O$, bleached with activated charcoal and filtered through Celite®. The residue was concentrated under vacuo obtaining a a mixture of unsaturated dinitriles **235** and **236** (24.6 g, 55% yield), which spectroscopic data coincide which the bibliographic ones.[211]

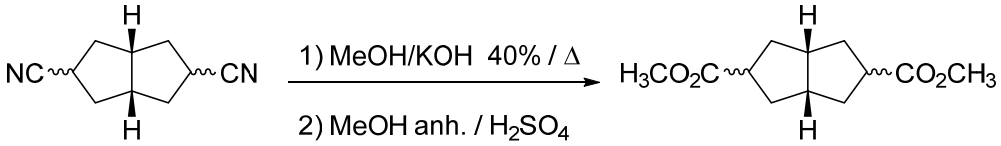
**Synthesis of the stereoisomeric mixture *cis*-bicyclo[3.3.0]octane-3,7-dicarbonitrile, 237.**




**235** **236** **237**

A solution of the isomeric mixture of unsaturated dinitriles **235** and **236** (40 g, 0.26 mol) and Pd/C at 10% (8 g) in absolute MeOH (400 mL) was hydrogenated at room temperature, at a pressure of 20 atm. until no more hydrogen absorption was observed. The resulting suspension was filtered and the residue washed with methanol. The solvent was removed under vacuo and a mixture of the three possible stereoisomers of dinitrile **237** in a relationship of (1α,3α,5α,7α), (1α,3β,5α,7β), (1α,3α,5α,7β) de 2:1.3:1. The spectroscopic data coincide with the bibliographic ones.[211]

**Synthesis of a stereoisomeric mixture of dimethyl *cis*-bicyclo[3.3.0]octane-3,7-dicarboxylate, 238.**




**237** **238**

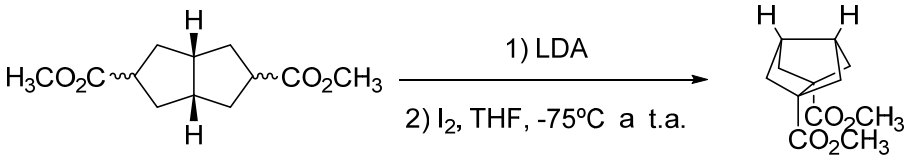
**a)** Hydrolysis of a mixture of dinitriles, **237**:

In a 1 L round-bottomed flask equipped with a condenser the mixture of dinitriles **237** (24 g, 0.15 mol) was dissolved in a solution of KOH in methanol at 40% (180 mL). The mixture was heated under reflux over 3 hours, and water was added (270 mL) keeping the reflux for 3 hours. The solution was cooled with an ice bath, acidified with concentrated HCl and the solvent was removed under vacuo. The residue was extracted with hot $Et_2O$ (5x100 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$ and filtered. The mother layers were concentrated under vacuo obtaining a stereoisomeric mixture of diacid (25 g, 84% yield) used in the next step of the synthesis without purification.

**b)** Synthesis of a stereoisomeric mixture of dimethyl *cis*-1,5-dimethylbicyclo[3.3.0]octane-3,7-dicarboxylate, **238**:

In a 1 L round-bottomed flask equipped with magnetic stirring, a condenser and a $CaCl_2$ tube, the previous crude (25 g, 0.13 mol) was dissolved in anhydrous MeOH (100 mL), concentrated $H_2SO_4$ (10 mL) was added and the solution was refluxed for 18 hours. The mixture was cooled down and cooled at 4 $^oC$ overnight, a precipitate appeared which was filtered out and washed with cold methanol. The solid was dissolved in $CH_2Cl_2$ (150 mL) and washed with water (3x50 mL). The organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the diester **238** as a white solid (11.6 g, 39% yield), which spectroscopic data coincide with the bibliographic ones. [184,211]

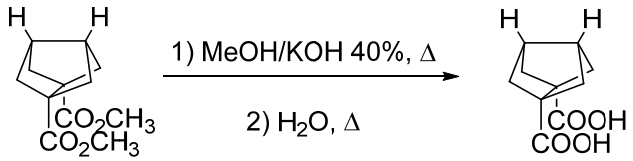
**Synthesis of dimethyl tricyclo[3.3.0.0$^{3,7}$]octane-1,5-dicarboxylate, 239.**




**238** **239**

In a 500 mL three necked round-bottomed flask equipped with a gas outlet, low temperature thermometer, a pressure-equalizing dropping funnel, magnetic stirring and argon atmosphere, a solution of LDA 1.5 M in THF (80.8 mL, 113.1 mmol) was diluted in anhydrous THF (116 mL) and cooled to -13 ºC with a dry ice/acetone bath and a solution of diester **238** (11.6 g, 51.4 mmol) in anhydrous THF (116 mL) was added dropwise. Then, the temperature was kept for an hour, and the formed suspension was cooled to -70 ºC and a solution of $I_2$ (13.06 g, 51.4 mmol) in anhydrous THF (309 mL) was added dropwise. The red solution was kept at – 70ºC for 1 hour, and tempered to room temperature overnight. Then, the solution was acidified with HCl 5N (40 mL) and extracted with $Et_2O$ (5x150 mL), the combined organic layers were washed with a solution of $Na_2S_2O_3$ at 10% (3x150 mL) and brine (3x150 mL). The organic layer was dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo. The tricyclic diester **239** (4.95 g, 43% yield) was obtained by column chromatography in silica (hexane / AcOEt 9:1), which spectroscopic data coincide with the bibliographic ones.[211]

**Synthesis of tricyclo[3.3.0.0$^{3,7}$]octane-1,5-dicarboxylic acid, 240.**

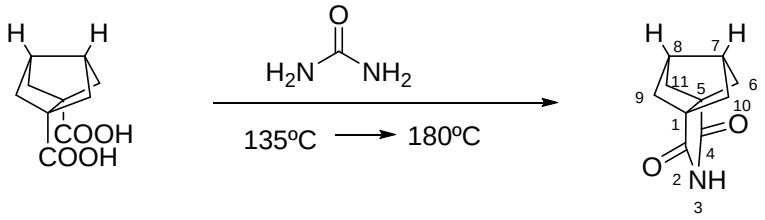



**239** **240**

In a 250 mL round-bottomed flask equipped with a condenser and magnetic stirring, a solution of diester **239** (4.95 g, 22.1 mmol) in a solution of KOH in MeOH at 40% (37 mL) was prepared and heated under reflux for 3 hours. Water (37 mL) was added and the reflux was kept for 3 more hours. The mixture was cooled with an ice bath, acidified with concentrated HCl and the solvent was removed under vacuo. The residue was extracted with hot $Et_2O$ (5x75 mL), and the combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and

concentrated under vacuo obtaining the diacid **240** (3.95 g, 87% yield), which spectroscopic data coincide with the bibliographic ones.[185]

**Synthesis of 3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undeca-2,4-dione, 245.**




**240** **245**

In a 100 mL round-bottomed flask equipped with a condenser and magnetic stirrer, the diacid **240** (0.5 g, 2.6 mmol) and urea 95% (0.8 g, 12.7 mmol) were added. The mixture was heated at 135 ºC until the urea melts and then heated to 180 ºC for 30 min. The mixture was tempered and dissolved in water (10 mL), the suspension was extracted with $CH_2Cl_2$ (6x10 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the imide **245** (0.33 g, 73% yield).

Analytic and spectroscopic data of the imide **245**.

Melting point: 131-132 ºC (dichloromethane).

IR (KBr) v: 3189, 2982, 1749, 1715, 1346, 1308, 1135, 1054, 833 cm$^{-1}$.

$^1$H-RMN (400 MHz, $CDCl_3$) δ: 1.85 [dd, *J* = 9.6 Hz, *J'* = 2.4 Hz, 4 H, 6(9,10,11)-H$_\alpha$], 2.05 [dd, *J* = 7.6 Hz, *J'* = 1.6 Hz, 4 H, 6(9,10,11)-H$_\beta$], 2.68 [m, 2 H, 7(8)-H], 7.74 (bs, 1H, NH).

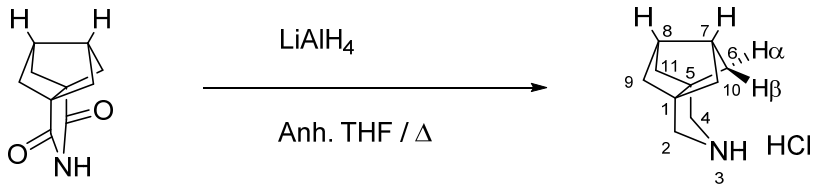
$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 40.3 [CH, C7(8)], 49.5 [$CH_2$, C6(9,10,11)], 57.1 [C, C1(5)], 176.8 (C, C=O).

MS (EI), m/e (%); main ions: 177 ($M^+$, 3), 106 (100), 105 (21), 93 (13), 92 (22), 91 (39), 79 (10), 78 (22), 77 (12), 65 (16).

Accurate mass:

| | |
|---|---|
| Calculated for $[C_{10}H_{13}NO_2\text{-}H]^-$ | 176.0717 |
| Found | 176.0725 |

**Synthesis of 3-azatetracycl0[5.2.1.1$^{5,8}$.0$^{1,5}$]undecane hydrochloride, 246·HCl.**




**245** **246·HCl**

In a 50 mL three necked round-bottomed flask equipped with a condenser, high temperature thermometer, argon atmosphere and magnetic stirring, a solution of imide **245** (0.41 g, 2.3 mmol) in anhydrous THF (15 mL) was prepared. The solution was cooled to 0ºC with an ice/water bath and $LiAlH_4$ (0.9 g, 23.1 mmol) was added in portions and the mixture was heated under reflux for 72 hours. The reaction was tempered and cooled to 0 ºC with an ice/water bath and a solution of NaOH 10N (8 mL) was added dropwise until reaching a basic pH. The reaction was stirred at room temperature for an hour. Then, the mixture was filtered through Celite® and the filtrate was washed with $CH_2Cl_2$, abundantly. The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, followed by the addition of a solution of HCl/$Et_2O$ and the solvent was removed under vacuo obtaining the amine **246·HCl** (0.32 g, 75% yield) as a white solid. The analytical sample was obtained from crystallization of MeOH/$Et_2O$.

Analytic and spectroscopic data of the amine **246·HCl**.

Melting point: 218-219 ºC (MeOH).

IR (KBr) ν: 3421, 2989, 2961, 2887, 2739, 2530, 2493, 1560, 1481, 1458, 1450, 1420, 1388, 1353, 1294, 1248, 1228, 1115, 1082, 1007, 988, 949, 924, 901, 881, 853, 787, 771, 657, 604, $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.60 [broad d, *J* = 7.6 Hz, 4 H, C6(9,10,11)-$H_\alpha$], 1.76 [dd, *J* = 7.6 Hz, *J'* = 2.0 Hz, 4 H, C6(9,10,11)-$H_\beta$], 2.48 [m, 2H, C7(8)-H], 3.32 (bs, 4H, C2(4)-$H_2$) .

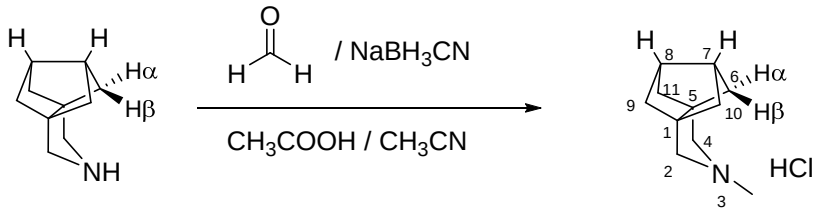
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 41.3 [CH, C7(8)], 47.0 [$CH_2$, C2(4)], 50.2 [$CH_2$, C6(9,10,11)], 58.3 [C, C1(5)].

MS (EI), m/e (%); main ions: 149 ($M^{\cdot+}$, 29), 108 (53), 107 ($C_8H_{11}^+$, 100), 106 (29), 105 (30), 95 (31), 94 (86), 92 (26), 91 (46), 80 (27), 79 (62), 77 (37), 71 (21), 70 (20).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{10}H_{16}NCl$: | C 64.68% | H 8.68% | N 7.54% | Cl 19.09% |
| Calculated for $C_{12}H_{20}NCl \cdot 0.35\ H_2O$: | C 62.56% | H 8.77% | N 7.30% | Cl 18.46% |
| Found: | C 62.67% | H 9.08% | N 7.44% | Cl 18.46% |

**Synthesis of 3-methyl-3-azatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecane hydrochloride, 247·HCl.**




**246·HCl** **247·HCl**

In a 25 mL round-bottomed flask equipped with a $CaCl_2$ tube, a suspension of amine **246·HCl** (0.32 g, 2.1 mmol), $NaBH_3CN$ 95% purity (0.28 g, 4.3 mmol), formaldehyde 37% (1.1 mL, 14.1 mmol), $CH_3COOH$ (1.2 mL) in acetonitrile (13 mL) was prepared. The reaction was stirred at room temperature for 8 hours, then more $NaBH_3CN$ 95% purity (0.28 g, 4.3 mmol) and formaldehyde 37% (1.1 mL, 14.1 mmol) were added and the suspension stirred at room temperature overnight. The solvent was removed under vacuo and the residue dissolved in water (20 mL) and basified with NaOH 10 N (~4 mL) and the aqueous layer was extracted with AcOEt (3x26 mL). The combined organic layers were washed with brine (2x25 mL), dried over anhydrous $Na_2SO_4$, filtered, then $HCl/Et_2O$ was added and the solvent was removed under vacuo obtaining the amine **247·HCl** as a white solid (0.24 g, 70% yield). The analytical sample was obtained from crystallization of $MeOH/Et_2O$.

Analytic and spectroscopic data of the amine **247·HCl**.

Melting point: 223-224 $^oC$ decompose (MeOH).

IR (KBr) ν: 3003, 2962, 2889, 2572, 2462, 1628, 1508, 1482, 1465, 1349, 1293, 1269, 1228, 1177, 1129, 1106, 1079, 1060, 965, 890, 837, 607, 464 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.61 (dd, *J* = 8.2 Hz, *J'* = 3.3 Hz, 2 H), 1.60-1.73 (complex signal, 4 H) and 1.79 (dd, *J* = 8.5 Hz, *J* = 2.4 Hz, 2H) [C6(10)-$H_2$ and C9(11)-$H_2$], 2.46 [m, 2H, C7(8)-H], 2.96 (s, 3H, N-$CH_3$), 3.18 (d, *J* = 12.0 Hz, 2H) and 3.70 [d, *J* = 12.0 Hz, 2 H) [C2(4)-$H_2$].

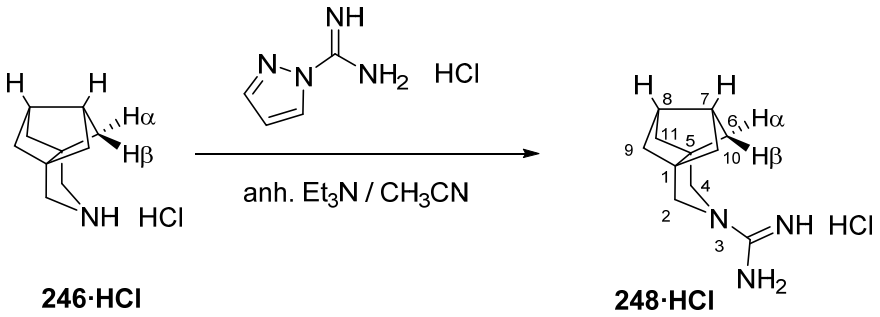
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 39.9 [CH, C7(8)], 40.7 ($CH_3$, N-$CH_3$), 41.7 [CH, C7(8)], 49.7 ($CH_2$) and 50.5 ($CH_2$) [C6(9,10,11)], 57.3 [$CH_2$, C2(4)], 58.0 [C, C1(5)].

MS (EI), m/e (%); main ions: 163 ($M^{+\cdot}$, 57), 162 (100), 121 (26), 120 (35), 108 (30), 105 (11), 94 (13), 91 (19), 79 (17), 77 (14), 58 (22).

Accurate mass:

| | |
|---|---|
| Calculated for $[C_{11}H_{17}N+H]^+$ | 164.1434 |
| Found | 164.1432 |

**Synthesis of 3-amidinotetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undecane hydrochloride, 248·HCl.**




**246·HCl** **248·HCl**

In a 10 mL round-bottomed flask equipped with a condenser and a magnetic stirrer, a suspension of amine **246·HCl** (0.13 g, 0.7 mmol), 1*H*-pyrazole-carboxamidine hydrochloride (0.12 g, 0.8 mmol), anh. triethylamine (0.17 mL) in acetonitrile (5 mL) was prepared. The reaction was heated at 70 ºC for 6 hours. The reaction was tempered and cooled at 4ºC overnight. The suspension was filtered out and the filtrate washed with cold acetonitrile obtaining the guanidine **248·HCl** as a beige solid (0.15 g, 96% yield). The analytical data was obtained from a crystallization of $CH_2Cl_2$.

Analytic and spectroscopic data of the guanidine **248·HCl**.

Melting point: 254-255 ºC decompose ($CH_2Cl_2$).

IR (KBr) ν: 3401, 3323, 3153, 2981, 2966, 2933, 2886, 1652, 1635, 1615, 1471, 1456, 1373, 1297, 1196, 1085, 790, 565 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.55 [broad d, *J* = 7.2 Hz, 4 H, C6(9,10,11)-$H_\alpha$], 1.79 [broad d, *J* = 8.8 Hz, 4 H, C6(9,10,11)-$H_\beta$], 2.44 [s, 2 H, C7(8)], 3.55 [s,4 H, C2(4)-$H_2$].

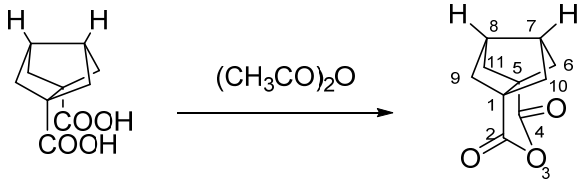
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 41.2 [CH, C7(8)], 48.9 [$CH_2$, C2(4)], 51.5 [$CH_2$, C6(9,10,11)], 57.8 [C, C1(5)], 156.7 (C, C=NH).

MS (EI), m/e (%); main ions: 194 (43), 193 (40), 192 [$(M+H)^{\cdot+}$, 14], 153 (22), 152 (84), 151 (67), 150 (28), 139 (100), 138 (62), 117 (44), 115 (53), 114 (35), 109 (27), 108 (29), 107 (53), 106 (29), 105 (23), 96 (32), 95 (54), 94 (26), 92 (25), 91 (73), 81 (25), 80 (24), 79 (54), 78 (28), 77 (65), 75 (32), 74 (23), 71 (20), 65 (24), 63 (25), 53 (26).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{11}H_{18}NCl$: | C 58.01% | H 7.97% | N 18.45% | Cl 15.57% |
| Calculated for $C_{11}H_{18}NCl \cdot 0.75\ HCl$: | C 51.80% | H 7.41% | N 16.47% | Cl 24.32% |
| Found: | C 52.03% | H 7.72% | N 16.79% | |

**Synthesis of 3-oxatetracyclo[5.2.1.1$^{5,8}$.0$^{1,5}$]undeca-1,2-dione, 241.**




**240** **241**

In a 250 mL round-bottomed flask equipped with a condenser and a magnetic stirrer a solution of the diacid **240** (2.5 g, 12.7 mmol) in acetic anhydride (70 mL) was prepared. The solution was heated under reflux for 90 minutes. The solvent was removed under vacuo, obtaining a dark residue which was purified by sublimation at 105-110 $^{o}$C/2 Torr obtaining the pure anhydride **241** as a white solid (1.7 g, 75% yield).

Analytical and spectroscopical data of the anhydride **241**.

Melting point: 186-187 $^{o}$C (chloroform).

IR (KBr) ν: 3587, 3004, 2955, 2906, 2585, 1957, 1850, 1811, 1774, 1484, 1429, 1289, 1143, 1121, 1045, 1015, 987, 909, 797, 780, 759, 725, 618, 602 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CDCl_3$) δ: 2.02 [m, 4 H, 6(9,10,11)-$H_2$], 2.11 [m, 4 H, 6(9,10,11)-$H_2$], 2.75 [m, 2 H, C7(8)-H].

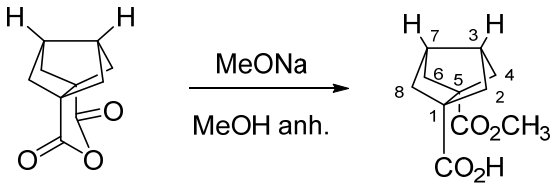
$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 40.7 [CH, C7(8)], 50.5 [$CH_2$, C6(9,10,11)], 56.2 [C, C1(5)], 171.0 (C, C=O).

GC/MS (EI), m/e (%); main ions: 179 [$(M+H)^+$, 1], 134 [$(M-CO_2)^{\cdot+}$, 97], 133 (12), 106 [$(C_8H_{10})^{\cdot+}$, 25], 105 (40), 92 (14), 91 (100), 79 (23), 78 (44), 77 (23), 67 (11), 66 (20), 65 (23), 52 (11), 51 (17).

HRMS:

| | |
|---|---|
| Calculated for $[C_{10}H_{10}O_3+H]^+$ | 179.0703 |
| Found | 179.0705 |

**Synthesis of the 5-(methoxycarbonyl)tricyclo[3.3.0.0$^{3,7}$]octane-1-carboxylic acid, 242.**




**241** **242**

In a 250 mL three necked round-bottomed flask equipped with a condenser, argon atmosphere, a $CaCl_2$ tube and magnetic stirring a suspension of the anhydride **241** (1.55 g, 8.7 mmol) and sodium methoxide (2.35 g, 43.5 mmol) in anh. MeOH (120 mL) was heated under reflux overnight. The resulting solution was concentrated under vacuo and the white residue was dissolved in water (70 mL) and washed with EtOAc (2x30 mL). The aqueous layer was acidified with concentrated HCl and extracted with $CH_2Cl_2$ (3x30 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining a beige solid that was crystallized from EtOAc obtaining the hemiester **242** (1.55 g, 90% yield).

Analytical and spectroscopical data of the hemiester **242**.

Melting point: 90-91 $^{o}$C (EtOAc).

IR (KBr) ν: 3553, 2999, 2948, 2901, 2703, 2649, 2582, 1736, 1701, 1508, 1481, 1438, 1420, 1308, 1289, 1261, 1250, 1187, 1155, 1084, 1062, 985, 946, 922, 906, 808, 745, 571, 481 $cm^{-1}$.

$^{1}$H-RMN (400 MHz, $CDCl_3$) δ: 1.8-1.9 [complex signal, 8 H, C2(4,6,8)-$H_2$], 2.49 [broad s, 2 H, C3(7)-H], 3.70 (s, 3 H, $CO_2CH_3$), 10.60 (bs, 1 H, $CO_2H$) .

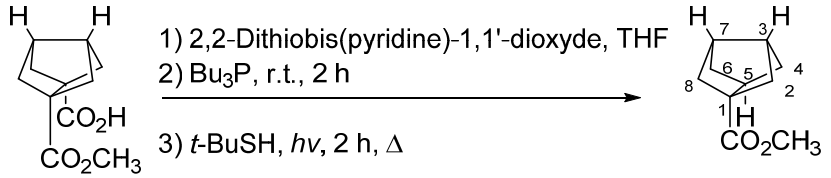
$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 37.2 [CH, C3(7)], 50.1 and 50.3 [$CH_2$, C2(4,6,8)], 51.8 ($CH_3$, $CO_2\underline{C}H_3$), 57.2 and 57.5 (C, C1 and C5), 173.5 (C, $\underline{C}O_2CH_3$), 179.5 (C, $\underline{C}O_2H$).

GC/MS (EI), m/e (%); main ions: 210 ($M^{\cdot+}$, 1), 192 [$(M-H_2O)^{\cdot+}$, 23], 179 [$(M-CH_3O)^+$, 32), 178 (22), 164 (38), 151 [$(M-CO_2CH_3)^{\cdot+}$, 43), 150 (40), 137 (23), 133 (35), 132 (23), 125 (51), 124 (21), 106 (21), 105 (100), 104 (24), 93 (53), 91 (28), 79 (47), 77 (37), 59 (21).

HRMS:

| | |
|---|---|
| Calculated for $[C_{11}H_{14}O_4+H]^+$ | 211.0965 |
| Found | 211.0965 |

**Synthesis of methyl tricyclo[3.3.0.0$^{3,7}$]octane-1-carboxylate, 243.**



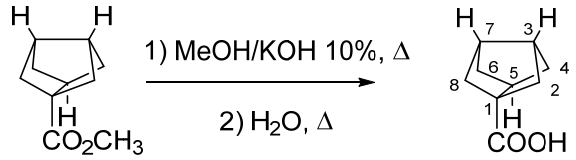

**242** **243**

In a 100 mL three necked round-bottomed flask equipped with a thermometer, magnetic stirring and argon atmosphere a solution of hemiester **242** (1.6 g, 7.4 mmol) and 2,2-dithiobis(pyridine)-1,1'-dioxide (2.37 g, 9.4 mmol) in anh. THF (55 mL) was prepared, the round-bottomed flask was wrapped with aluminium foil and the reaction was cooled to 0 ºC with an ice bath. Then, *n*-tributylphosphine (3.8 mL, 15.1 mmol) was added and the reaction was stirred at room temperature for 2 hours. Then, *t*-butylthiol (4.1 mL, 36.8 mmol) was added, the aluminium foil was removed and the reaction was irradiated with a 60 W bulb for 2 hours. $Et_2O$ (50 mL) was added to the resulting solution and the organic layer was washed with a saturated solution of $NaHCO_3$ (3x35 mL), aqueous 5 M HCl (3x35 mL), water (3x35 mL) and brine (3x35 mL). The organic layer was dried over anh. $Na_2SO_4$, filtered and concentrated by distillation of the solvent obtaining a mixture of ester **243** and *n*-tributylphosphine (in the ratio 6/4). This mixture was used without purification in the next step of the synthesis due to the volatility of the ester.[212]

[212] The ester **243** and the acid **230** had been synthesized using a very different approach by Vogt et al. However, the compounds were not characterized. Vogt, B. R.; Suter, S. R.; Hoover, J. R. E. *Tetrahedron Lett.* **1968**, *9*, 1609-1612.

GC/MS (EI), m/e (%); main ions: 166 ($M^{\cdot+}$, 3), 151 (10), 137 (23), 135 (30), 134 (23), 125 (40), 124 (33), 107 [($C_8H_{11}^{\cdot+}$), 55], 106 (43), 104 (37), 99 (40), 93 (49), 91 (56), 80 (44), 79 (71), 78 (36), 77 (53), 67 (100), 66 (29), 65 (57), 59 (48), 53 (34).

**Synthesis of tricyclo[3.3.0.0$^{3,7}$]octane-1-carboxylic acid, 230.**




**243** **230**

In a 100 mL round-bottomed flask equipped with a condenser and magnetic stirring a suspension of impure ester **243** (1.3 g, aprox. 7.4 mmol) in a 10% methanol solution of KOH (15 mL) was heated under reflux for 3 hours. Water (15 mL) was added and the reaction was refluxed for 3 hours. The solvent was removed under vacuo and the residue was dissolved in water (30 mL), the aqueous layer was washed with EtOAc (3x30 mL) and it was acidified with 6 N HCl til pH=1. The aqueous layer was extracted with EtOAc (4x30 mL). The combined organic layers were washed with brine (3x30 mL), dried over anh. $Na_2SO_4$, filtered and concentrated under vacuo. The beige solid was purified by sublimation at 65-70 $^oC$/ 2 Torr obtaining the acid **230** as a white solid (0.4 g, 36% yield overall from hemiester **242**).

Analytical and spectroscopical data of the acid **230**.

Melting point: 97-98 $^oC$ ($CH_2Cl_2$).[212]

IR (KBr) ν: 2990, 2944, 2895, 2689, 2601, 2577, 1686, 1508, 1482, 1423, 1321, 1292, 1267, 1189, 1146, 1123, 1087, 1052, 1008, 994, 945, 910, 751, 564, 470 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CDCl_3$) δ: 1.45 [dm, *J* = 8.6 Hz, 2 H, C4(6)-$H_2$], 1.54 [dt, *J* = 11.4 Hz, *J'* = 2.8 Hz, 2 H, C2(8)-$H_2$], 1.70 [dd, *J* = 11.3 Hz, *J'* = 3.1 Hz, 2 H, C2(8)-$H_2$], 1.78 [dm, *J* = 7.8 Hz, 2 H, C4(6)-$H_2$], 2.41 [m, 2 H, C3(7)-H], 2.70 (quint, *J* = 2.6 Hz, 1 H, C5-H), 10.42 (bs, 1 H, $CO_2$H).

$^{13}$C-RMN (100.6 MHz, $CDCl_3$) δ: 37.1 [CH, C3(7)], 41.9 (CH, C5), 46.8 [$CH_2$, C2(8)]*, 51.0 [$CH_2$, C4(6)]*, 51.9 (C, C1), 182.4 (C, $\underline{C}O_2H$).

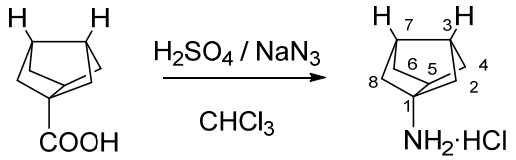
MS (EI), m/e (%); main ions: 152 ($M^{\cdot+}$, 1), 124 (10), 111 (17), 109 (14), 107 [($C_8H_{11}$)$^+$, 27], 91 (17), 80 (14), 79 (45), 77 (19), 67 (100), 66 (25), 65 (24), 54 (12).

HRMS (ESI neg):

Calculated for [$C_9H_{12}O_2$-H]$^-$ 151.0765

Found 151.0765

**Synthesis of (tricyclo[3.3.0.0$^{3,7}$]oct-1-yl)amine hydrochloride, 231·HCl.**




**230** **231·HCl**

In a 10 mL two necked round-bottomed flask equipped with a condenser, a gas outlet, and magnetic stirring, a solution of the acid **230** (87 mg, 0.62 mmol) in $CHCl_3$ (5 mL) was prepared, concentrated $H_2SO_4$ (0,25 mL) was added and the reaction was heated to 50 $^oC$. Then, $NaN_3$ (87 mg, 1.34 mmol) was carefully added portionwise. Then the reaction was kept at 50 $^oC$ for 1.5 hours. The reaction was tempered and cooled with an ice bath. Crushed ice (1 g) was added to the reaction and the aqueous layer was basified with 2 N NaOH (5 mL). The basic aqueous layer was extracted with EtOAc (4x5 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$ and filtered and a solution of HCl/$Et_2O$ was added and the solvent removed under vacuo obtaining the amine **231·HCl** (47 mg, 47% yield).[213]

Analytical and spectroscopical data of the amine **231·HCl**.

Melting point: 226-227 $^oC$ (EtOAc).

IR (KBr) ν: 3419, 2948, 2763, 2176, 2042, 1624, 1478, 1311, 1206, 1162, 1059, 1004 $cm^{-1}$.

$^1$H-RMN (400 MHz, $CD_3OD$) δ: 1.56 [dm, *J* = 8.8 Hz, 2 H, C4(6)-$H_2$], 1.63 [dd, *J* = 11.2 Hz, *J'* = 3.4 Hz, 2 H, C2(8)-$H_2$], 1.70 [dt, *J* = 11.6 Hz, *J'* = 2.8 Hz, 2 H, C2(8)-$H_2$], 1.83 [dm, *J* = 8.0 Hz, 2 H, C4(6)-$H_2$], 2.38 (quint, *J* = 2.8 Hz, 1 H, C5-H), 2.45 [m, 2 H, C3(7)-H].

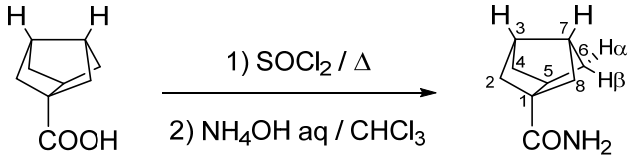
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 37.3 [CH, C3(7)], 42.6 (CH, C5), 47.3 [$CH_2$, C2(8)]*, 50.5 [$CH_2$, C4(6)]*, 61.3 (C, C1).

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_8H_{13}N$·HCl: | C 60.18% | H 8.84% | N 8.77% |
| Calculated for $C_8H_{13}N$·1.25 HCl: | C 56.93% | H 8.51% | N 8.30% |
| Found: | C 56.93% | H 8.44% | N 8.41% |

[213] Amines **231** and **232** had been disclosed, without any characterization, in a patent that claimed antiviral activity for both amines without any detailed information. See, Hoover, J. R. E. (Smith Kline & French), US 3496228 (**1970**).

**Synthesis of (tricyclo[3.3.0.0$^{3,7}$]oct-1-yl)carboxamide, 244.**




**230** **244**

In a 25 mL round-bottomed flask equipped with a condenser, magnetic stirring and $CaCl_2$ tube a solution of the acid **230** (0.24 g, 1.77 mmol) in $SOCl_2$ (10 mL, 0.09 mol) was heated under reflux for 2 hours. The liquid was removed under vacuo, and the residue was dissolved in toluene (2x5 mL) and concentrated under vacuo obtaining the desired acid chloride as yellowish oil. The oil was dissolved in $CH_2Cl_2$ (2 mL), cooled to 0 ºC and aq. 70% solution of $NH_4OH$ (5.4 mL) was added dropwise. The reaction was stirred vigorously at room temperature overnight. The obtained mixture was extracted with $CH_2Cl_2$ (4x10 mL). The combined organic layer were washed with brine (2x10 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo obtaining the desired amide **244** (252 mg, 99 % yield).

Analytical and spectroscopical data of the amine **244**.

Melting point: 196-197 ºC ($CH_2Cl_2$).

IR (KBr) ν: 3375, 3195, 2993, 2968, 2941, 2889, 2773, 1653, 1617, 1478, 1410, 1301, 1270, 1194, 1142, 1115, 807, 687, 617, 470 $cm^{-1}$.

$^1$H-RMN (400 MHz, DMSO-$d_6$) δ: 1.35 [dm, *J* = 8.2 Hz, 2 H, C4(6)-$H_2$], 1.45 [dt, *J* = 11.2 Hz, *J'* = 2.8 Hz, 2 H, C2(8)-$H_2$], 1.50 [dd, *J* = 8.4 Hz, *J'* = 3.1 Hz, 2 H, C2(8)-$H_2$], 1.59 [dm, *J* = 8.5 Hz, 2 H, C4(6)-$H_2$], 2.30 [m, 2 H, C3(7)-H], 2.45 (quint, *J* = 2.6 Hz, 1 H, C5-H), 6.8 (bs, 1 H) and 7.0 (bs, 1 H) (CON$\underline{H_2}$).

$^{13}$C-RMN (100.6 MHz, DMSO-$d_6$) δ: 36.0 [CH, C3(7)], 40.4 (CH, C5), 45.8 [$CH_2$, C2(8)]*, 49.7 [$CH_2$, C4(6)]*, 52.6 (C, C1) and 175.5 (C, $\underline{C}ONH_2$).

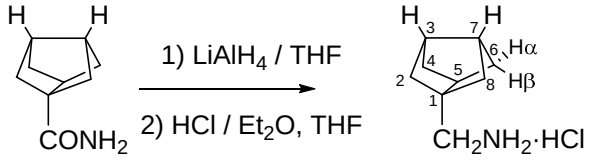
GC/MS (EI), m/e (%); main ions: 151 ($M^{+}$, 6), 123 (12), 122 (19), 110 (25), 107 ($C_8H_{11}^{+}$, 22), 91 (24), 86 (13), 85 (100), 80 (13), 79 (42), 77 (21), 72 (11), 67 (72), 66 (17), 65 (20).

Accurate mass:

Calculated for $[C_9H_{13}NO+H]^+$ 152.1070

Found 152.1068

**Synthesis of [(tricyclo[3.3.0.0$^{3,7}$]oct-1-yl)methyl]amine hydrochloride, 232·HCl.**




**244** **232·HCl**

In a 100 mL three necked round-bottomed flask equipped with a condenser, argon atmosphere and magnetic stirring a solution of the amide **244** (232 mg, 1.54 mmol) in anhydrous THF (35 mL) was cooled to 0 $^{o}$C and $LiAlH_4$ (175 mg, 4.6 mmol) was carefully added portionwise. The suspension was heated under reflux for 15 hours. The resulting mixture was cooled to 0 $^{o}$C and a solution of aq 10 N NaOH (2.5 mL) was added dropwise until reaching basic pH and the reaction was stirred at room temperature for one hour. The resulting suspension was filtered through Celite® and the solid was washed with $CH_2Cl_2$ (3x25 mL). The organic layers were dried over anh. $Na_2SO_4$ and filtered, a solution of HCl/$Et_2O$ was added and the solvent removed under vacuo obtaining the desired amine **232** as its hydrochloride (201 mg, 76% yield). An analytical sample of **232·HCl** was obtained by crystallization from MeOH/$Et_2O$.[213]

Analytical and spectroscopical data of the amine **232·HCl**.

Melting point: 169-170 $^{o}$C dec. (MeOH).

IR (KBr) v: 2959, 2886, 1574, 1480, 1392, 1319, 1277, 1168, 1148, 1071, 1026, 999, 974, 933, 587 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.40 (dd, *J* = 11.3 Hz, *J'* = 3.3 Hz, 2 H) and 1.45-1.57 (complex signal, 6H) [C2(4,6,8)-$H_2$], 2.25 (quint, *J* = 2.6 Hz, 1 H, C5-H), 2.40 [m, 2 H, C3(7)-H], 3.27 (s, 2 H, $\underline{CH_2}NH_2$).

$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 38.2 [CH, C3(7)], 41.2 (CH, C5), 43.6 ($CH_2$, $\underline{CH_2}NH_2$), 47.8 [$CH_2$, C2(8)]*, 48.6 (C, C1), 50.4 [$CH_2$, C4(6)]*.

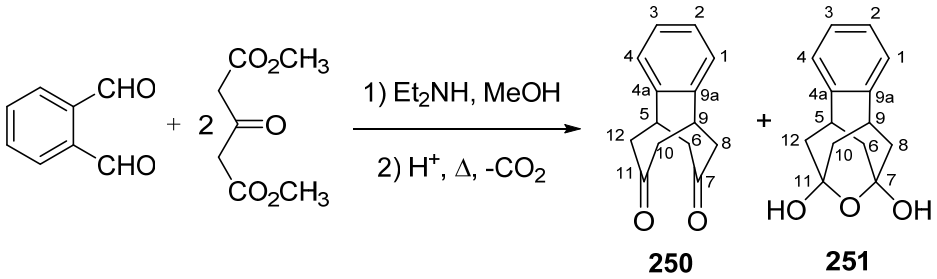
MS (EI), m/e (%); main ions: 138 [$(M+1)^+$, 6], 137 ($M^{\cdot+}$, 12), 109 (30), 108 (25), 105 (27), 97 (25), 96 (37), 95 (44), 94 (34), 92 (37), 91 (55), 79 (100), 78 (26), 77 (36), 70 (32), 69 (25), 67 (21), 57 (35), 56 (30).

Elemental analysis:

| | | | |
|---|---|---|---|
| Calculated for $C_9H_{15}N \cdot HCl$: | C 62.24% | H 9.29% | N 8.06% |
| Found: | C 62.33% | H 9.37% | N 8.10% |

# 6. SYNTHESIS OF 6,7,8,9,10,11-HEXAHYDRO-9-METHYL-5,7:9,11-DIMETHANO-5*H*-BENZOCYCLONONEN-7-AMINES

**Synthesis of 5,6,8,9-tetrahydro-5,9-propanobenzocycloheptane-7,11-dione, 250 in a mixture with 7,11-epoxy-6,7,8,9-tetrahydro-5,9-propano-5*H*-benzocycloheptane-7,11-diol, 251.**




a) Synthesis of the tetraester:

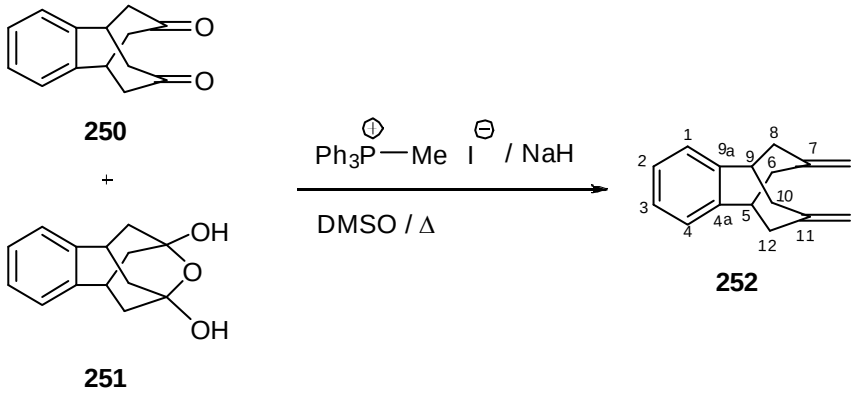
In a 500 mL round-bottomed flask equipped with a condenser and magnetic stirring a solution of *o*-ftaldialdehyde (9.80 g, 73.1 mmol) and dimethyl 1,3-acetondicarboxylate (25.9 g, 149 mmol) in MeOH (200 mL) was prepared. 15 drops of diethylamine were added and the reaction was heated at reflux for 1.5 h, the reaction was cooled down and 12 drops more were added and the reaction was stored at 4 $^{o}$C overnight. The precipitate was filtered off under vacuo and was washed with cold methanol (25 mL), obtaining the corresponding tetraester as white needles (23.73 g, 73% yield).

b) Synthesis of diketone **250** and its corresponding hydrate **251**:

In a 500 mL round-bottomed flask equipped with a condenser and magnetic stirring a solution of the previous tetraester (23.73 g, 53.3 mmol) in acetic acid (135 mL) and conc. HCl (38 mL) was heated at reflux for 12 hours. The solvent was removed under vacuo and the solid residue was diluted with hot $Et_2O$ (160 mL) over 15 minutes and the solution was stored at 4 $^{o}$C overnight. The residue was filtered off obtaining a white solid (11.4 g) that was constituted by a mixture of diketone **250** and its hydrate **251** in an approximate relationship of 1:3.

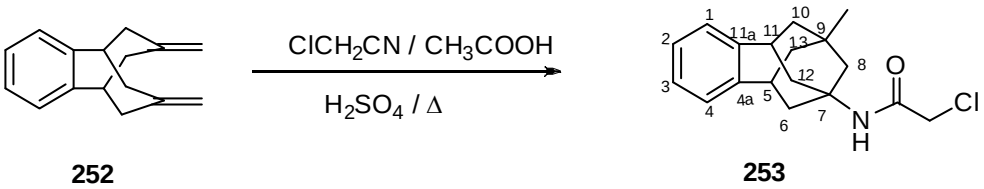
The pure diketone **250** was obtained by sublimation at 160 $^{o}$C / 0.5 (9.69 g, 85% yield), which spectroscopical data coincide with the reported.[190]

**Synthesis of 5,6,8,9-tetrahydro-5,9-propanobenzocycloheptano-7,11-diene, 252**




In a 500 ml 3-necked round-bottomed flask equipped with magnetic stirring and argon atmosphere, a suspension of NaH (6.90 g, 55 % purity, 157.5 mmol) in anhydrous DMSO (89.6 mL) was heated at 75 $^{o}$C over 45 min. The green suspension was cooled down to room temperature and triphenylphosphonium iodide (63.70 g, 157.5 mmol) diluted in anhydrous DMSO (134 mL) and the mixture of diketone **250** and the hydrate **251** (4.00 g, 17.5 mmol) diluted in anhydrous DMSO (37.6 mL) were added sequentially. The resulting mixture was heated at 90 $^{o}$C overnight. The reaction was cooled down and poured into water (250 mL) The aqueous layer was extracted with hexane (5 x 200 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated in vacuo to obtain an ochre wax. The crude was purified making a slurry with alumina that was extracted with pentane (3 x 150 mL). The solvent was concentrated in vacuo and the residue was used in the next synthetic step without further purification.[193]

**Synthesis of *N*-(6,7,8,9,10,11-Hexahydro-9-methyl-5,7:9,11-dimethano-5H-benzocyclononen-7-yl)chloroacetamide, 253**




A suspension of diene **252** (5.3 g, 25.2 mmol), chloroacetonitrile (6.5 mL) and acetic acid (17 mL) was cooled to 0 $^{o}$C and concentrated $H_2SO_4$ (8.2 mL, 151.2 mmol) was added dropwise (T < 10 $^{o}$C). The mixture was allowed to reach room temperature and was stirred overnight. The suspension was added to ice (120 g) and a yellow solid precipitated. The precipitate was filtered and solved in dichloromethane (100 mL). The organic phase was dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to give **253** as a yellow solid (5.44 g, 71% yield). The analytical sample of **253** was obtained by crystallization from $CH_2Cl_2$.

Analytical and spectroscopical data of **253**.

Melting point: 166-168 ºC ($CH_2Cl_2$)

IR (KBr) *v*: 3320, 3301, 3062, 2949, 2910, 2861, 1659, 1651, 1540, 1492, 1451, 1431, 1361, 1328, 1312, 1210, 1145, 1091, 1009, 971, 943, 765, 747, 694, 651, 610, 507, 478 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CDCl_3$) δ: 0.94 (s, 3H, $CH_3$), 1.56 [d, *J* = 13.5 Hz, 2H, 10(13)-$H_b$], 1.67 [ddd, *J* = 13.5 Hz, *J'* = 6.0 Hz, *J''* = 2.0 Hz, 2H, 10(13)-$H_a$], 1.85 (s, 2H, 8-$H_2$), 2.09 [d, *J* = 13 Hz, 2H, 6(12)-$H_b$], 2.17 [ddd, *J* = 13 Hz, *J'* = 6.0 Hz, *J''* = 2.0 Hz, 2H, 6(12)-$H_a$], 3.09 (tt, *J* = 6.0 Hz, *J'* = 2.0 Hz, 2H, 5(11)-H], 3.93 (s, 2H, C$H_2$Cl), 6.31 (bs, 1H, NH), 7.04–7.10 [complex signal, 4H, 1(4)-H and 2(3)-H].

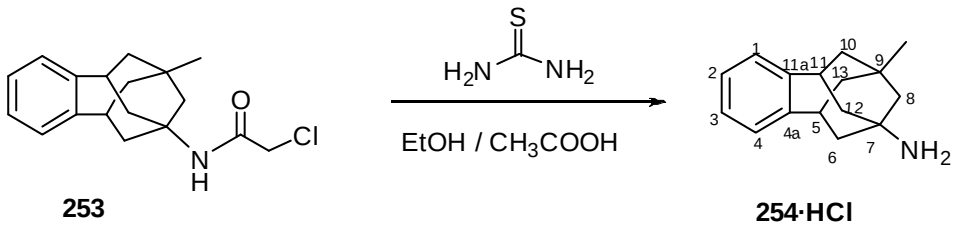
$^{13}$C-RMN (75.4 MHz, $CDCl_3$) δ: 32.1 ($CH_3$, C9-$CH_3$), 33.6 (C, C9), 38.7 [$CH_2$, C6(12)], 40.9 [CH, C5(11)], 41.0 [$CH_2$, C10(13)], 42.9 ($CH_2$, $CH_2$Cl), 46.9 ($CH_2$, C8), 54.9 (C, C7), 126.4 [CH, C2(3)], 128.0 [CH, C1(4)], 146.0 [C, C4a(C11a)], 164.5 (C, C=O).

MS (IE), m/e (%); main ions: 303 ($M^{+}$, 31), 268 (18), 211 [$(C_{16}H_{19})^{+}$, 29], 210 (100), 195 (28), 182 (13), 181 (15), 167 (11), 156 (15), 155 (82), 154 (20), 143 (14), 142 (13), 141 (20), 129 (19), 128 (23), 115 (17).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{18}H_{22}ClNO$: | C 71.16% | H 7.30% | N 4.61% | Cl 11.67% |
| Found: | C 71.07% | H 7.28% | N 4.50% | Cl 11.40% |

**Synthesis of 6,7,8,9,10,11-Hexahydro-9-methyl-5,7:9,11-dimethano-5H-benzocyclononen-7-amine hydrochloride, 254·HCl**




**253** **254·HCl**

A mixture of chloroacetamide **253** (7.21 g, 23.7 mml), thiourea (2.16 g, 28.44 mmol), acetic acid (8.7 mL) in ethanol (44 mL) was stirred under reflux for 18 h. To the cold solution water (250 mL), and 10 N NaOH (80 mL) were added. The base aqueous solution was extracted with EtOAc (3 × 300 mL). The combined organic extracts were dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to give a brown oily residue (7.19 g). The residue was taken in EtOAc and the amine **254** was precipitated as its hydrochloride by adding an excess of

$Et_2O$·HCl (3.87 g, 62% yield). The analytical sample was obtained by crystallization from 2-propanol.

Analytical and spectroscopical data of **254·HCl**.

Melting point: 287-288 ºC (2-propanol)

IR (KBr) *v*: 3499, 2985, 2944, 2907, 2860, 2717, 2680, 2622, 2581, 2548, 2061, 1606, 1509, 1495, 1454, 1362, 757, 578, 471 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CDCl_3$) δ: 0.87 (s, 3H, $CH_3$), 1.38 [d, *J* = 13.5 Hz, 2H, 10(13)-$H_b$], 1.54 [dd, *J* = 13.5 Hz, *J'* = 6.0 Hz, 2H, 10(13)-$H_a$], 1.58 (s, 2H, 8-$H_2$), 1.73 [d, *J* = 13.0 Hz, 2H, 6(12)-$H_b$], 1.96 [dd, *J* = 13.0 Hz, *J'* = 6.0 Hz, 2H, 6(12)-$H_a$], 3.09 [t, *J* = 6.0 Hz, 2H, 5(11)-H], 7.05 (s, 4H, Ar-H), 8.31 (bs, 3H, $NH_3$).

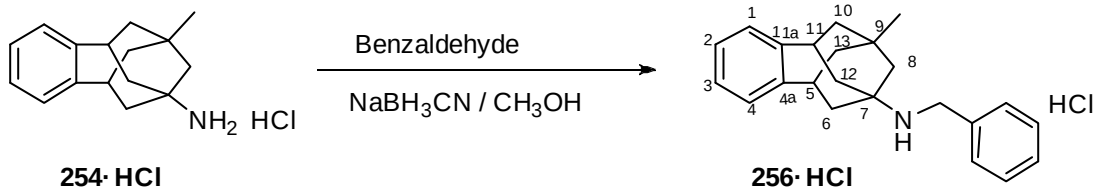
$^{13}$C-RMN (75.4 MHz, $CDCl_3$) δ: 32.3 ($CH_3$, C9-$\underline{C}H_3$), 34.7 (C, C9), 39.3 [$CH_2$, C6(12)], 41.6 [$CH_2$, C10(13)], 41.7 [CH, C5(11)], 47.2 ($CH_2$, C8), 55.5 (C, C7), 128.0 [CH, C2(3)], 129.2 [CH, C1(4)], 146.4 [C, C4a(C11a)].

MS (IE), m/e (%); main ions: 227 ($M^{\cdot+}$, 100), 212 (28), 185 (65), 171 (12), 170 (52), 157 (15), 156 (58), 155 (32), 153 (11), 144 (21), 143 (15), 141 (17), 130 (12), 129 (18), 128 (25), 115 (22), 108 (22), 94 (27).

Accurate Mass

| | |
|---|---|
| Calculated for $[C_{16}H_{22}N+H]^+$: | 228.1746 |
| Found: | 228.1743 |

**Synthesis of *N*-Benzyl-6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5*H*-benzocyclononen-7-amine hydrochloride, 256·HCl.**




**254· HCl** **256· HCl**

To a solution of amine **254·HCl** (200 mg, 0.76 mmol) in MeOH (10 mL), $NaBH_3CN$ (104 mg, 1.65 mmol), AcOH (0.25 mL) and benzaldehyde (80.4 mg, 0.76 mmol) were added and the mixture was stirred at room temperature for 18 h and concentrated in vacuo to dryness. Water (10 mL) was added to the residue, and the mixture was extracted with $Et_2O$ (4 × 15 mL). The combined organic extracts were washed with 2 N NaOH (3 × 25 mL), brine (2 × 25mL), dried with anhyd $Na_2SO_4$, filtered and concentrated in vacuo. The residue was taken in EtOAc

and the amine **256** was precipitated as its hydrochloride (159 mg, 68% yield) by adding an excess of $Et_2O$·HCl. The analytical sample was obtained by crystallization from methanol/$Et_2O$.

Analytical and spectroscopical data of **256·HCl**.

Melting point: 263-264 ºC (MeOH)

IR (KBr) *v*: 3410, 2948, 2912, 2840, 2733, 2407, 1494, 1455, 1364, 1347, 1307, 1217, 1127, 992, 943, 755, 696, 567, 501 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CDCl_3$) δ: 0.89 (s, 3H, $CH_3$), 1.50 [d, *J* = 13.5 Hz, 2H, 10(13)-$H_b$], 1.66 [dd, *J* = 13.5 Hz, *J'* = 6.0 Hz, 2H, 10(13)-$H_a$], 1.92 [d, *J* = 12.5 Hz, 2H, 6(12)-$H_b$], 2.00 (s, 2H, 8-$H_2$), 2.24 [dd, *J* = 12.5 Hz, *J'* = 6.0 Hz, 2H, 6(12)-$H_a$], 3.08 [m, 2H, 5(11)-H], 3.80 (broad s, 2H, $C\underline{H}_2C_6H_5$), 7.00 [m, 2H, 1(4)-Ar-H], 7.08 [m, 2H, 2(3)-Ar-H], 7.20 (t, *J* = 7.5 Hz, 1, Ar-$H_{para}$), 7.27 (t, *J* = 7.5 Hz, 2H, Ar-$H_{meta}$), 7.61 (d, *J* = 7.5 Hz, 2H, Ar-$H_{orto}$), 9.61 (bs, 2H, $NH_2$).

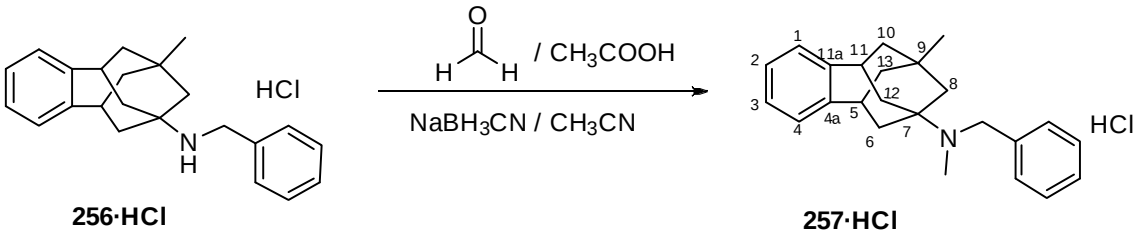
$^{13}$C-RMN (75.4 MHz, $CDCl_3$) δ: 32.3 ($CH_3$, C9-$\underline{C}H_3$), 35.0 (C, C9), 37.0 [$CH_2$, C6(12)], 41.7 [CH, C5(11)], 41.8 [$CH_2$, C10(13)], 45.0 ($CH_2$, $\underline{C}H_2C_6H_5$), 45.4 ($CH_2$, C8), 62.0 (C, C7), 128.0 [CH, C2(3)], 129.2 [CH, C1(4)], 130.3 (CH, Ar-$C_{meta}$), 130.5 (CH, Ar-$C_{para}$), 131.2 (CH, Ar-$C_{ortho}$), 133.1 (C, Ar-$C_{ipso}$), 146.4 [C, C4a(C11a)].

MS (IE), m/e (%); significant ions: 317 ($M^{\cdot+}$, 100), 302 (23), 276 (15), 275 (64), 260 (19), 246 (23), 211 [$(C_{16}H_{19})^+$, 17], 184 (21), 169 (11), 155 (28), 147 (16), 146 (14), 143 (18), 141 (18), 129 (18), 128 (19), 115 (15), 91 (82).

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{23}H_{28}ClN$: | C 78.05% | H 7.97% | N 3.96% | Cl 10.02% |
| Calculated for $C_{23}H_{28}ClN$·0.1 HCl· 0.3 $H_2O$: | C 76.11% | H 7.97% | N 3.86% | Cl 10.74% |
| Found: | C 76.42% | H 7.65% | N 3.62% | Cl 10.36% |

**Synthesis of N-Benzyl-N-methyl-6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5H-benzocyclononen-7-amine hydrochloride, 257·HCl.**




**256·HCl** **257·HCl**

To a solution of **256·HCl** (327 mg, 0.96 mmol) in acetonitrile (10 mL), formaldehyde (0.75 mL, 37% wt. in water solution, 9.60 mmol) and $NaBH_3CN$ (180 mg, 2.88 mmol) were added. The mixture was stirred for 30 min at room temperature, acetic acid (0.5 mL) was added and the mixture was stirred at room temperature for 2 h. An additional portion of $NaBH_3CN$ (180 mg, 2.88 mmol) was added and the mixture was further stirred at room temperature for 2 h. The mixture was concentrated to dryness, 2 N NaOH (10 mL) was added and the suspension was extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic phases were washed with $H_2O$ (2×10 mL), dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to give **257** as an oil. Its hydrochloride was obtained by adding an excess of $Et_2O$·HCl to a solution of the amine in EtOAc, followed by filtration of the white solid precipitate (194 mg, 55% yield). The analytical sample was obtained by crystallization from methanol/$Et_2O$.

Analytical and spectroscopical data of **257·HCl**.

Melting point: 250-251 $^oC$ (MeOH)

IR (KBr) *v*: 4044, 3411, 3060, 3016, 2963, 2943, 2914, 2865, 2844, 2461, 2375, 1948, 1624, 1492, 1452, 1421, 1384, 1308, 1277, 1264, 1214, 1177, 1156, 1121, 1101,1052, 1005, 973, 756, 698 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CDCl_3$) $\delta$: 1.00 (s, 3H, $CH_3$), 1.59 [d, *J* = 13.5 Hz, 2H, 10(13)-$H_b$], 1.73 [ddm, *J* = 13.5 Hz, *J'* = 6.0 Hz, 2H, 10(13)-$H_a$], 2.01 (d, *J* = 12 Hz, 1H, 6-$H_b$ or 12-$H_b$), 2.06 (complex signal, 2 H, 8-$H_a$ and 12-$H_b$ or 6-$H_b$), 2.25 (d, *J* = 12.0 Hz, 1H, 8-$H_b$), 2.38 (d, *J* = 5.0 Hz, 3 H, N-C<u>H</u>$_3$), 2.55 (m, 1 H) and 2.65 (m, 1 H) (6-$H_a$ and 12-$H_a$), 3.25 (t, *J* = 6.0 Hz, 1H) and 3.27 (t, *J* = 6.0 Hz, 1H) (5H and 11-H), 3.65 (dd, *J* = 12.5 Hz, *J'* = 9.0 Hz, 1H, C<u>H</u>$_2$-$C_6H_5$), 4.66 (dd, *J* = 12.5 Hz, *J'* = 3.5 Hz, 1H, C<u>H</u>$_2$-$C_6H_5$), 7.06 [m, 2H, 1(4)-Ar-H], 7.11 [m, 2H, 2(3)-Ar-H], 7.33-7.39 (complex signal, 3H, Ar-$H_{meta}$ and $Ar_{para}$), 7.69 (dd, *J* = 7.5 Hz, *J*= 2.0 Hz, 2H, Ar-$H_{ortho}$), 11.9 (broad s, 2H, $NH_2$).

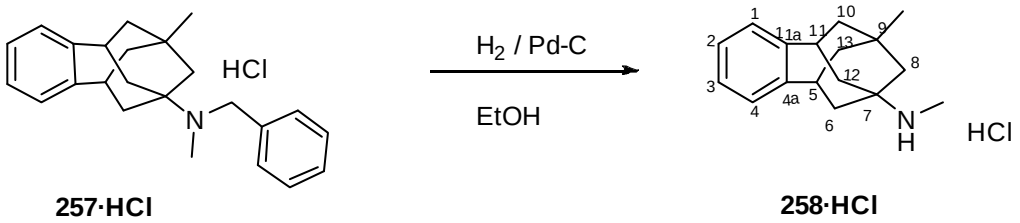
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) $\delta$: 31.7 ($CH_3$, <u>C</u>$H_3$-N), 31.9 ($CH_3$, C9-<u>C</u>$H_3$), 33.1 ($CH_2$) and 33.5 ($CH_2$) (C6 and C12), 34.5 (C, C9), 40.1 ($CH_2$), and 40.28 ($CH_2$) (C10 and C13), 40.31 [CH, C5(11)], 43.2 ($CH_2$, C8), 52.5 ($CH_2$, <u>C</u>$H_2C_6H_5$), 67.9 (C, C7), 127.0 (CH, Ar-$C_{meta}$), 128.0 [CH, C2(3)], 129.0 [CH, C1(4)], 129.2 (C, Ar-$C_{ipso}$), 129.7 (CH, Ar-$C_{para}$), 131.7 (CH, Ar-$C_{ortho}$), 144.89 (C), and 144.92 (C) (C4a and C11a).

MS (EI), m/z (%): 331 ($M^{+}$, 100), 316 (33), 290 (18), 289 (77), 288 (12), 274 (12), 260 (10), 246 (11), 240 (10), 211 [$(C_{16}H_{19})^{+}$, 11], 198 (22), 169 (10), 161 (10), 160 (16), 155 (30), 143 (18), 141 (18), 129 (18), 128 (18), 115 (13), 91 (61)

Elemental analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{24}H_{29}N\cdot HCl$: Cl 9.63% | C 78.34% | H 8.22% | N 3.81% |
| Calculated for $C_{24}H_{29}N\cdot HCl\cdot 0.3H_2O$: Cl 9.50% | C 77.21% | H 8.26% | N 3.75% |
| Found: Cl 9.89% | C 76.99% | H 8.05% | N 3.66% |

**Synthesis of *N*-Methyl-6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5H-benzocyclononen-7-amine hydrochloride, 258·HCl.**




**257·HCl** **258·HCl**

A suspension of **257·HCl** (184 mg, 0.50 mmol) and 5% Pd/C (50% in water, 50 mg) in absolute EtOH (25 mL) was hydrogenated at 1 atm and 90 $^{o}$C for 24 h. The suspension was filtered, the residue was washed with EtOH and to the combined organic filtrates an excess of $Et_2O\cdot HCl$ was added. Evaporation of the solvents from the filtrate in vacuo followed by crystallization of the residue from methanol/$Et_2O$ gave **258·HCl** (102.8 mg, 74% yield) as a white solid.

Analytical and spectroscopical data of **258·HCl**.

Melting point: >225 $^{o}$C (decompose)

IR (KBr) *v*: 3419, 2910, 2846, 2692, 2133, 2094, 2061, 1867, 1447, 1367, 1215, 1172, 1126, 1072, 1007, 957, 773, 574, 509 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 1.00 (s, 3H, C9-C$\underline{H}_3$), 1.56 [d, *J* = 13.5 Hz, 2H, 10(13)-$H_b$], 1.70 (s, 2H, 8-$H_2$), 1.73 [dd, *J* = 13.5 Hz, *J'* = 6.0 Hz, 2H, 10(13)-$H_a$], 1.90 [d, *J* = 12.5 Hz, 2H, 6(12)-$H_b$],

2.07 [dd, *J* = 12.5 Hz, *J'* = 6.5 Hz, 2H, 6(12)-$H_a$], 2.61 (s, 3 H, N-C$\underline{H}_3$), 3.24 [m, 2H, 5(11)-H], 7.10 (s, 4H, Ar-H).

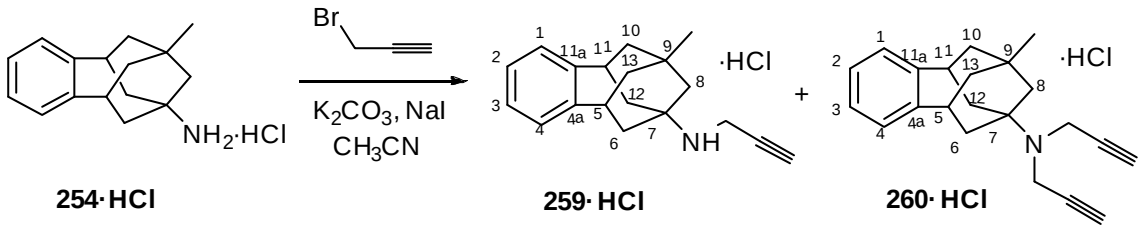
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 25.6 ($CH_3$, $\underline{C}H_3$-N), 32.3 ($CH_3$, C9-$\underline{C}H_3$), 34.9 (C, C9), 36.8 [$CH_2$, C6(12)], 41.5 [CH, C5(11)], 41.7 [$CH_2$, C10(13)], 45.3 ($CH_2$, C8), 60.3 (C, C7), 128.0 [CH, C2(3)], 129.2 [CH, C1(4)], 146.3 [C, C4a(C11a)].

MS (EI), m/z (%): 241 ($M^{\cdot+}$, 100), 226 (35), 200 (12), 199 (72), 198 (12) 184 (40), 171 (14), 170 (47), 169 (13), 158 (14), 156 (11), 155 (30), 153 (11), 144 (11), 143 (12), 141 (23), 129 (22), 128 (28), 122 (21), 115 (27), 108 (29), 91 (10), 71 (28), 57 (10), 56 (15).

Elemental analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{17}H_{23}N\cdot HCl$: | C 73.49% | H 8.71% | N 5.04% |
| Calculated for $C_{17}H_{23}N\cdot 1.6\ HCl$: | C 68.13% | H 8.27% | N 4.67% |
| Found: | C 67.81% | H 8.37% | N 4.34% |

**Synthesis of *N*-Propargyl-6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5*H*-benzocyclononen-7-amine hydrochloride, 259·HCl, and N,N-dipropargyl-6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5*H*-benzocyclononen-7-amine hydrochloride, 260·HCl**




**254·HCl** **259·HCl** **260·HCl**

A suspension of **254·HCl** (400 mg, 1.52 mmol), $K_2CO_3$ (246 mg, 1.78 mmol), propargyl bromide (0.22 mL, 80% solution in toluene, 1.98 mmol) and NaI (21 mg, 0.14 mmol) in acetonitrile (15 mL) was heated under reflux for 18 h. After concentration to dryness in vacuo, the residue was taken in $CH_2Cl_2$ (20 mL) and the solution was washed with water (3 × 10 mL). The organic layer was dried with anhyd $Na_2SO_4$, filtered and concentrated in vacuo to give a residue which was subjected to column chromatography (hexane/EtOAc mixtures) affording **260** (hexane/EtOAc 99/1, 220 mg, 48% yield) and **259** (hexane/EtOAc 97/3, 70 mg, 17 % yield). The hydrochlorides of **259** and **260** were obtained by adding an excess of $Et_2O\cdot HCl$ to a solution of the corresponding amine in EtOAc, followed by filtration of the formed precipitate. The analytical samples were obtained by crystallization from methanol/$Et_2O$.

Analytical and spectroscopical data of **259·HCl**.

Melting point: 175-176 ºC (MeOH)

IR (KBr) *v*: 3500-2300 (max. a, 3487, 3221, 2908, 2446, 2366), 2125, 1628, 1451, 850, 761, 548 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 1.00 (s, 3H, C9-C$\underline{H}_3$), 1.56 [d, *J* = 13.5 Hz, 2H, 10(13)-$H_b$], 1.71 (s, 2H, 8-$H_2$), 1.73 [ddd, *J* = 13.5 Hz, *J'* = 6.0 Hz, *J'* = 2.0 Hz, 2H, 10(13)-$H_a$], 1.93 [d, *J* = 12.5 Hz, 2H, 6(12)-$H_b$], 2.08 [dm, *J* = 12.5 Hz, 2H, 6(12)-$H_a$], 3.20 (t, *J* =2.5 Hz, 1H, $CH_2C{\equiv}C\underline{H}$), 3.23 [tm, *J'* = 6.0 Hz, 2H, 5(11)-H], 3.94 (d, *J* = 2.5 Hz, 2H, $C\underline{H}_2C{\equiv}CH$), 7.10 (s, 4H, Ar-H).

$^{13}$C-RMN (75.4 MHz, $CD_3OD$) δ: 30.5 ($CH_2$, $\underline{C}H_2C{\equiv}CH$), 32.3 ($CH_3$, C9-$\underline{C}H_3$), 34.9 (C, C9), 37.0 [$CH_2$, C6(12)], 41.5 [CH, C5(11)], 41.6 [$CH_2$, C10(13)], 45.3 ($CH_2$, C8), 61.7 (C, C7), 75.5 (CH, $CH_2C{\equiv}\underline{C}H$), 78.7 (C, $CH_2\underline{C}{\equiv}CH$), 128.0 [CH, C2(3)], 129.2 [CH, C1(4)], 146.2 [C, C4a(C11a)].

Elemental analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{19}H_{22}N{\cdot}HCl$: | C 75.60% | H 8.01% | N 4.64% |
| Calculated for $C_{19}H_{22}N{\cdot}1.5HC$: | C 71.30% | H 7.71% | N 4.38% |
| Found: | C 71.55% | H 7.81% | N 4.18% |

Accurate mass:

| | |
|---|---|
| Calculated for $[C_{19}H_{22}N+H]^+$ | 266.1903 |
| Found | 266.1898 |

Analytical and spectroscopical data of **260·HCl**.

Melting point: 199-200 ºC (MeOH)

IR (KBr) *v*: 3305, 3239, 2993, 2951, 2907, 2840, 2346, 2118, 1493, 1474, 1455, 1435, 1380, 1355, 1308, 1212, 1166, 1120, 1094, 1039, 1007, 959, 755, 639 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 1.03 (s, 3H, C9-C$\underline{H}_3$), 1.54 [d, *J* = 13.5 Hz, 2H, 10(13)-$H_b$], 1.73 [dd, *J* = 13.5 Hz, *J'* = 6.0 Hz, 2H, 10(13)-$H_a$], 1.90 (s, 2H, 8-$H_2$), 2.13 [d, *J* = 12.0 Hz, 2H, 6(12)-$H_b$], 2.30 [dd, *J* = 12.0 Hz, *J'* = 6.5 Hz, 2H, 6(12)-$H_a$], 3.27 [t, *J'* = 6.0 Hz, 2H, 5(11)-H], 4.32 (broad s, 4H, $C\underline{H}_2C{\equiv}CH$), 7.10 (s, 4H, Ar-H). The signal corresponding to the terminal ≡C$\underline{H}$ was not observed.

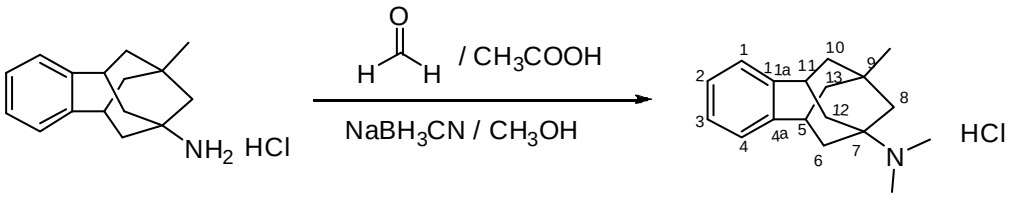
$^{13}$C-RMN (75.4 MHz, $CD_3OD$) δ: 32.5 ($CH_3$, C9-$\underline{C}H_3$), 35.9 (C, C9), 36.0 [$CH_2$, C6(12)], 38.4 ($CH_2$, $\underline{C}H_2C{\equiv}CH$), 41.4 [$CH_2$, C10(13)], 41.9 [CH, C5(11)], 45.0 ($CH_2$, C8), 71.7 (C, C7), 74.8 (CH, $CH_2C{\equiv}\underline{C}H$), 80.9 (C, $CH_2\underline{C}{\equiv}CH$), 128.1 [CH, C2(3)], 129.2 [CH, C1(4)], 146.3 [C, C4a(C11a)].

MS (EI), m/z (%): 303 ($M^{+}$, 87), 302 (35), 288 (28), 260 (38), 246 (19), 232 (10), 218 (12), 211 (25), 206 (27), 169 (21), 167 (11), 165 (11), 156 (15), 155 (77), 153 (15), 144 (11), 143 (42), 142 (13), 141 (40), 133 (63), 132 (100), 130 (18), 129 (46), 128 (35), 127 (11), 118 (11), 117 (16), 115 (30), 91 (15)

Elemental analysis

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{22}H_{25}N{\cdot}HCl$: | C 77.74% | H 7.71% | N 4.12% | Cl 10.43% |
| Found: | C 77.86% | H 7.43% | N 3.84% | Cl 10.06% |

**Synthesis of *N,N*-Dimethyl-6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5*H*-benzocyclononen-7-amine hydrochloride, 261·HCl.**




**254·HCl** **261·HCl**

To a solution of **254·HCl** (300 mg, 1.14 mmol) in MeOH (10 mL), $NaBH_3CN$ (205 mg, 3.27 mmol), AcOH (0.3 mL) and formaldehyde (0.26 mL, 37% wt. in water solution, 3.48 mmol) were added and the mixture was stirred at room temperature for 8 h. An additional portion of $NaBH_3CN$ (205 mg, 3.27 mmol) and formaldehyde (0.26 mL, 37 % wt. in water solution, 3.48 mmol) were added, the mixture was stirred at room temperature for 18 h and then it was concentrated in vacuo to dryness. Water (20 mL) was added to the residue, the suspension was basified with 1 N NaOH (10 mL) and was extracted with EtOAc (4 × 15 mL). The combined organic extracts were washed with brine (2 × 10 mL), dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuo to give **261** as a yellow oil. Its hydrochloride was obtained by adding an excess of $Et_2O{\cdot}HCl$ to a solution of the amine in EtOAc, followed by filtration of the white solid precipitate (249 mg, 75% yield).

Analytical and spectroscopical data of **261·HCl**.

Melting point: 226-227 ºC (MeOH)

IR (KBr) *v*: 3468, 3411, 3244, 3063, 3022, 2945, 2917, 2892, 2865, 2846, 2670, 2462, 2071, 1635, 1492, 1458, 1449, 1409, 1369, 1309, 1215, 1176, 1155, 1125, 1096, 1051, 980, 903, 756, 584 $cm^{-1}$.

$^{1}$H-RMN (500 MHz, $CDCl_3$) δ: 0.97 (s, 3H, C9-C$\underline{H}_3$), 1.56 [d, *J* = 13.5 Hz, 2H, 10(13)-$H_b$], 1.66 [dd, *J* = 13.5 Hz, *J'* = 6.0 Hz, 2H, 10(13)-$H_a$], 1.88 (s, 2H, 8-$H_2$), 1.92 [d, *J* = 12.0 Hz, 2H, 6(12)-$H_b$], 2.17 (broad s, 1 H, NH), 2.25 [dd, *J* = 12.0 Hz, *J'* = 6.0 Hz, 2H, 6(12)-$H_a$], 2.69 (s, 6H, N-C$\underline{H}_3$), 3.20 [t, *J* = 6.0 Hz, 2H, 5(11)-H], 7.04 [m, 2H, 1(4)-Ar-H], 7.10 [m, 2H, 2(3)-Ar-H].

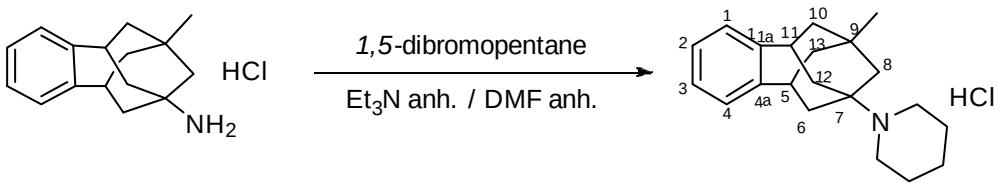
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 31.8 ($CH_3$, C9-$\underline{C}H_3$), 33.2 [$CH_2$, C6(12)], 34.3 (C, C9), 36.4 ($CH_3$, $\underline{C}H_3$-N), 40.0 [$CH_2$, C10(13)], 40.1 [CH, C5(11)], 43.6 ($CH_2$, C8), 65.3 (C, C7), 127.1 [CH, C2(3)], 128.2 [CH, C1(4)], 144.8 [C, C4a(C11a)].

MS (EI), m/z (%): main ions: 255 ($M^{\cdot+}$, 100), 240 (39), 214 (15), 213 (88), 212 (17), 198 (29), 185 (11), 184 (22), 170 (13), 169 (10), 155 (35), 153 (10), 143 (12), 141 (22), 136 (12), 129 (17), 128 (23), 122 (22), 115 (20), 85 (24).

Elemental analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{18}H_{25}N$·HCl: | C 74.08% | H 8.98% | N 4.80% |
| Cl 12.15 | | | |
| Calculated for $C_{18}H_{25}N$·HCl·1.3 $H_2O$: | C 68.65% | H 9.14% | N 4.45% |
| Cl 11.26% | | | |
| Found: | C 68.29% | H 8.84% | N 4.52% |
| Cl 11.65% | | | |

**Synthesis of *N*-(6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5*H*-benzocyclononen-7-yl)piperidine hydrochloride, 262·HCl.**




**254·HCl** **262·HCl**

To a solution of **254·HCl** (288 mg, 1.09 mmol) in DMF (4.0 mL), anhydrous $Et_3N$ (0.45 mL, 3.27 mmol) was added and the suspension was stirred at room temperature for 2 h. 1,5-dibromopentane (0.18 mL, 1.3 mmol) was added and the mixture was heated at 60 $^oC$ for 26 h. To the cold mixture, water (15 mL) and 2 N NaOH (10 mL) were added and the solution was extracted with EtOAc (6 × 15 mL). The combined organic extracts were washed with water (3 × 15 mL), dried with anhydrous $Na_2SO_4$ and filtered. Excess of $Et_2O$·HCl was added and the solution was concentrated in vacuo to dryness to give **262·HCl** (125 mg, 35% yield). The analytical sample of **262·HCl** was obtained by crystallization from methanol/$Et_2O$.

Analytical and spectroscopical data of **262·HCl**.

Melting point: 280 $^oC$ (sublimates)

IR (KBr) *v*: 3427, 3023, 2951, 2924, 2900, 2858, 2661, 2530, 2014, 1941, 1455, 1374, 1297, 1218, 1122, 1094, 973, 752, 551 $cm^{-1}$.

$^1$H-RMN (500 MHz, $CDCl_3$) δ: 1.02 (s, 3H, C9-C$\underline{H}_3$), 1.52 (broad s, 1H, piperidine 4-$H_{ax}$), 1.55 [d, *J* = 13.5 Hz, 2H, 10(13)-$H_b$], 1.73 [dd, *J* = 12.0 Hz, *J'* = 6.5 Hz, 2H, 10(13)-$H_a$], 1.83 (s, 2H, 8-$H_2$), 1.83–1.98 [complex signal, 5H, piperidine 4-$H_{eq}$, 3(5)-$H_{ax}$ and 3(5)-$H_{eq}$], 2.04 [d, *J* = 12.5 Hz, 2H, 6(12)-$H_b$], 2.18 [dd, *J* = 12.5 Hz, *J'* = 6.5 Hz, 2H, 6(12)-$H_a$], 2.92 [broad s, 2H, piperidine 2(6)-$H_{ax}$], 3.27 [tm, *J'* = 6.5 Hz, 2H, 5(11)-H], 3.68 [broad s, 2H, piperidine 2(6)-$H_{eq}$], 7.10 (s, 4H, Ar-H).

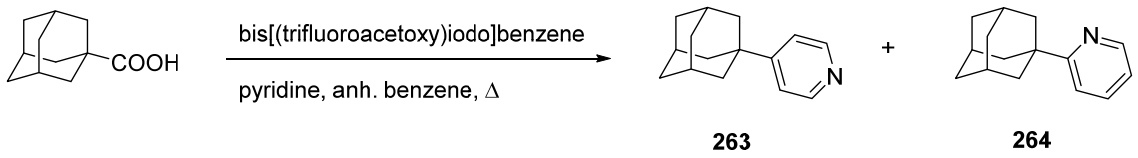
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 23.2 ($CH_2$, piperidine C4), 25.0 [$CH_2$, piperidine C3(5)], 32.5 ($CH_3$, C9-C$\underline{H}_3$), 34.9 [$CH_2$, C6(12)], 35.6 (C, C9), 41.5 [$CH_2$, C10(13)], 41.8 [CH, C5(11)], 44.3 ($CH_2$, C8), 47.7 [$CH_2$, piperidine C2(6)], 68.8 (C, C7), 128.0 [CH, C2(3)], 129.2 [CH, C1(4)], 146.4 [C, C4a(C11a)].

Elemental analysis

| | | | |
|---|---|---|---|
| Calculated for $C_{21}H_{29}N$·HCl: | C 75.99% | H 9.11% | N 4.22% |
| Calculated for $C_{21}H_{29}N$·HCl·0.5$H_2O$: | C 73.98% | H 9.16% | N 4.11% |
| Found: | C 73.79% | H 8.93% | N 3.91% |

# 7. SYNTHESIS OF 4-(1-ADAMANTYL)PIPERIDINES

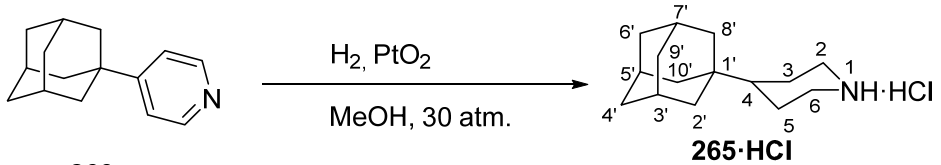
**Synthesis of 4-(1-adamantyl)pyridine, 263 and 2-(1-adamantyl)pyridine, 264.**




**263** **264**

In a 250 mL round-bottomed flask equipped with a condenser and argon atmosphere, a solution of 1-adamantyl carboxylic acid (11.35 g, 63.0 mmol), pyridine (5.1 mL, 63.0 mmol), bis[(trifluoroacetoxy)iodo]benzene (9.34 g, 21.8 mmol) in anhydrous benzene (100 mL) was heated under reflux overnight. The reaction was cooled down and the organic phase was washed with a 10 N aqueous solution of NaOH (2 x 100 mL) and the aqueous layer was washed with AcOEt (2 x 100 mL). The combined organic extracts were washed with a 1 N solution of HCl (3 x 100 mL) and the aqueous was neutralized to pH = 14 with a 10 N solution of NaOH. Then, the aqueous layer was extracted with AcOEt (3 x 100 mL) and the organic layer was dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuo to obtain a mixture of pyridines **263** and **264**. This regioisomeric mixture was purified by silica coloumn chromatography. Compound **263** was obtained ($CH_2Cl_2$:MeOH 98.5:1.5, 830 mg, 9.3% yield) as a beige solid. Compound **264** was obtained ($CH_2Cl_2$:MeOH 99.5:0.5, 830 mg, 27.4% yield) as a white solid. The sprectroscoopical data coincide with the reported one.[185]

**Synthesis of 4-(1-adamantyl)piperidine hydrochloride, 265·HCl.**




**263** **265·HCl**

A solution of compound **263** (830 mg, 3.9 mol) and $PtO_2$ at 10% (83 mg) in absolute MeOH (100 mL) was hydrogenated at room temperature, at a pressure of 30 atm. until no more hydrogen absorption was observed. The resulting suspension was filtered and the residue washed with methanol. The solvent was removed under vacuo to obtain amine **265·HCl** (970 mg, 97% yield) as a white solid. The solid was dissolved in MeOH and an excess of HCl/$Et_2O$ was added. The solid was filtrated in vacuo and recrystallized from 2-propanol to obtain the analytical sample.

Analytic and spectroscopic data of the amine **265·HCl**.

Melting point: 272-273 $^{o}$C (decompose).

IR (KBr) v: 3674, 2900, 2846, 2728, 2671, 2503, 1597, 1446, 1396, 1344, 1075, 1023, 960 cm$^{-1}$.

$^{1}$H-RMN (500 MHz, $CD_3OD$) δ: 1.20 (tt, *J* = 12.3 Hz, *J'* = 3.2 Hz, 1 H, 4-H), 1.49 [qd, *J* = 14.0 Hz, *J'* = 4.0 Hz, 2 H, 3(5)-$H_{ax}$], 1.57 [d, *J* = 2.6 Hz, 6 H, 2'(8', 10')], 1.68 (d, *J* = 11.3 Hz, 3 H) and 1.77 (d, *J* = 12.1 Hz, 3 H) [4'(6', 9')-$H_{exo}$ and $H_{endo}$], 1.94 [bs, 2 H, 3(5)-$H_{eq}$], 1.99 [bs, 3 H, 3'(5', 7')-H], 2.91 [td, *J* = 13.1 Hz, *J'* = 2.9 Hz, 2 H, 2(6)-$H_{ax}$], 3.42 [dt, *J* = 12.4 Hz, *J'* = 2.2 Hz, 2 H, 2(6)-$H_{eq}$].

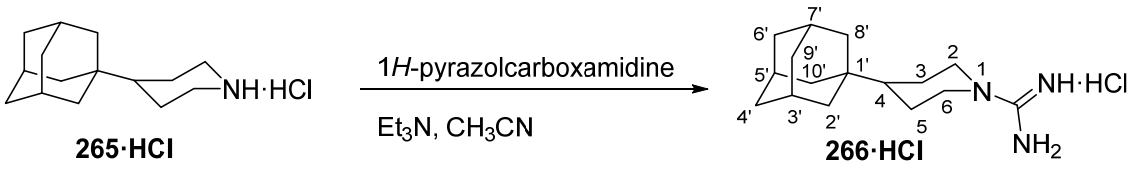
$^{13}$C-RMN (125.7 MHz, $CD_3OD$) δ: 23.6 [$CH_2$, C3(5)], 30.1 [CH, C3'(5', 7')], 35.0 (C, 1'), 38.3 [$CH_2$, C4'(6', 9')], 40.3 [$CH_2$, C2'(8', 10')], 45.8 (CH, C4), 46.0 [$CH_2$, C2(6)].

ME (EI), m/e (%); main ions: 220 ($M^{+}$, 95), 219 (100), 218 (21), 205 (60), 163 (15), 135 ($C_{10}H_{15}^{+}$, 83), 93 (27), 91 (19), 85 (83), 84 (50), 83 (21), 79 (33), 77 (16), 58 (18), 57 (48), 56 (25), 55 (21).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{12}H_{20}NCl$: | C 70.42% | H 10.24% | N 5.48% | Cl 13.86% |
| Found: | C 70.30% | H 10.42% | N 5.53% | Cl 13.90% |

**Synthesis of *N*-amidyl-4-(1-adamantyl)piperidine hydrochloride, 266·HCl.**




In a 10 mL round-bottomed flask equipped with a condenser and a magnetic stirrer, a suspension of amine **265·HCl** (484 mg, 1.9 mmol), 1*H*-pyrazole-carboxamidine hydrochloride (333 mg, 2.3 mmol), anhydrous triethylamine (0.47 mL) in acetonitrile (5 mL) was prepared. The reaction was heated at 70ºC for 6 hours. The reaction was cooled down and stored at 4ºC overnight. The suspension was filtered out and the filtrate washed with cold acetonitrile obtaining the guanidine **266·HCl** as a beige solid (428 mg, 76% yield). The solid was crystallized from 2-propanol to obtain the analytical sample.

Analytic and spectroscopic data of the guanidine **266·HCl**.

Melting point: >300 $^{o}$C (decompose).

IR (KBr) v: 3112, 2898, 2845, 1639, 1597, 1526, 1446, 1345, 1195, 1160, 967, 743 cm$^{-1}$.

$^{1}$H-RMN (400 MHz, $CD_3OD$) δ: 1.18 (tt, *J* = 12.4 Hz, *J'* = 3.2 Hz, 1 H, 4-H), 1.31 [qd, *J* = 13.2 Hz, *J'* = 3.6 Hz, 2 H, 3(5)-$H_{ax}$], 1.57 [d, *J* = 2.4 Hz, 6 H, 2'(8', 10')], 1.68 (d, *J* = 12.0 Hz, 3 H) and 1.76 (d, *J* = 12.0 Hz, 3 H) [4'(6', 9')-$H_{exo}$ and $H_{endo}$], 1.83 [dd, *J* = 13.2 Hz, *J'* = 1.6 Hz, 2 H, 3(5)-$H_{eq}$], 1.98 [bs, 3 H, 3'(5', 7')-H], 2.98 [td, *J* = 13.2 Hz, *J'* = 2.0 Hz, 2 H, 2(6)-$H_{ax}$], 3.93 [dt, *J* = 13.2 Hz, *J'* = 2.4 Hz, 2 H, 2(6)-$H_{eq}$].

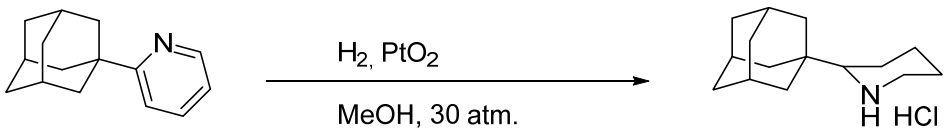
$^{13}$C-RMN (100.6 MHz, $CD_3OD$) δ: 26.1 [$CH_2$, C3(5)], 30.1 [CH, C3'(5', 7')], 35.0 (C, 1'), 38.3 [$CH_2$, C4'(6', 9')], 40.5 [$CH_2$, C2'(8', 10')], 47.5 (CH, C4), 47.6 [$CH_2$, C2(6)], 157.4 (C, C=NH).

ME (EI), m/e (%); main ions: 262 ($M^{\cdot+}$, 10), 219 (13), 135 ($C_{10}H_{15}^{+}$, 30), 129 (96), 128 (100), 127 (38), 101 (11), 93 (17), 91 (16), 88 (16), 87 (17), 86 (15), 85 (15), 79 (23), 77 (12), 75 (13), 74 (14), 67 (11), 57 (24), 56 (18), 55 (16).

Elemental analysis:

| | | | | |
|---|---|---|---|---|
| Calculated for $C_{16}H_{28}N_3Cl$: | C 64.52% | H 9.47% | N 14.11% | Cl 11.90% |
| Found: | C 64.32% | H 9.63% | N 13.93% | Cl 12.01% |

**Synthesis of 2-(1-adamantyl)piperidine hydrochloride, 267·HCl.**




**264** **267·HCl**

A solution of compound **264** (2.25 g, 10.5 mol) and $PtO_2$ at 10% (225 mg) in absolute MeOH (100 mL) was hydrogenated at room temperature, at a pressure of 30 atm. until no more hydrogen absorption was observed. The resulting suspension was filtered and the residue washed with methanol. The solvent was removed under vacuo to obtain amine **267·HCl** (970 mg, 97% yield) as a white solid. The solid was dissolved in MeOH and an excess of HCl/$Et_2O$ was added. The spectroscopical data of compound **267·HCl** coincided with the reported ones.[214]

[214] Fytas, G.; Stamatiau, G.; Foscolos, G. B.; Kolocouris, A.; Kolocouris, N.; Witvrouw, N.; Pannecouque, C.; DeClerq, E. *Bior. Med. Chem. Lett* **1997**, *7*, 1887-1890.

**REFERENCES**

1. Hughes, S. S. 'The virus: A History of the concept' **1977**, Heinemann Educational Books, London, United Kingdom.

2. Flint, S. J.; Enquist, L. W.; Krug, R. M.; Racaniello, V. R.; Skalka, A. M. 'Principles of virology: Molecular Biology, Pathogenesis and Control' **2000**, American Society for Microbiology, Washington, United States of America.

3. Crick, F. H.; Watson, J. D. *Nature* **1956**, *177*, 473-475.

4. Baltimore, D. *Bacteriol. Rev.* **1971**, *35*, 235-241.

5. Taken from Dr. Jeff Young, Biology Department, Western Washington University: http://biol.wwu.edu/young/321/stuff/collection/baltimore_classification.html (accessed on July, 8th 2013).

6. Taken from: Chapter 33 (Disease summaries), pp 367-392 in: Fisher, B.; Harvey, R.; Champe, P. *Lippincott's Illustrated Reviews: Microbiology* Hagerstown, MD: Lippincott Willims & Wilkins; **2007**. pp 367-392.

7. Whitley, R. J.; Roizman, B. *Lancet* **2001**, *357*, 1513-1518.

8. Gao, F.; Bailes, E.; Robertson, D. L. *Nature* **1999**, *397*, 436-441.

9. Mawar, N.; Saha, S.; Pandit, A.; Mahajan, U. *Indian J. Med. Res.* **2005**, *122*, 471-484.

10. Lane, J. M. *Curr. Top. Microbiol. Immunol.* **2006**, *304*, 17-29.

11. a) Camps, P.; Font-Bardia, M.; Pérez, F.; Solans, X.; Vázquez, S. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 912–914. b) Camps, P.; Fernández, J. A.; Vázquez, S.; Font-Bardia, M.; Solans, X. *Angew. Chem. Int. Ed.* **2003**, *42*, 4049–4051. c) Ayats, C.; Camps, P.; Fernández, J. A.; Vázquez, S. *Chem. Eur. J.* **2007**, *13*, 1522–1532.

12. Byrd, C. M.; Page, J.; Hruby, D. E.; Jordan, R. *Drugs Future* **2008**, *33*, 875-890.

13. Moss, B. *Fields Virology 5th ed.* (Ed. D. M. Knipe, P. M. Howley), Lippincott Williams & Wilkins, Philadelphia, **2007**, 2905-2946.

14. Harrison, S. C.; Alberts, B.; Ehrenfeld, E.; Enquist, L.; Fineberg, H.; McKnight, S. L.; Moss, B.; O'Donnell, M.; Ploegh, H.; Schmid, S. L.; Walter, K. P.; Theriot, J. *Proc. Nat. Acad. Sci.* **2004**, *101*, 11178-11192.

15. Bray, M.; Buller, M. *Clin. Infect. Dis.* **2004**, *38*, 882-889.

16. Bolken, T. C.; Hruby, D. E. *Antivir. Res.* **2008**, *77*, 1-5.

17. Duraffour, S.; Vigne, S.; Vermeire, K.; Garcel, A.; Vanstreels, E.; Daelemans, D.; Yang, G.; Jordan, R.; Hruby, D. E.; Crance, J. M.; Garin, D.; Andrei, G.; Snoeck, R. *Antivir. Ther.* **2008**, *13*, 977-990.

18. Sliva, K.; Schnierle, B. *Virology J.* **2007**, 4:8.

19. Jordan, R.; Hruby, D. *Expert Rev. Anti Infect. Ther*. **2006**, *4*, 277-289.

20. Henderson, A. D.; Inglesby, T. V.; Bartlett, J. G.; Ascher, M.S.; Eitzen, E.; Jahrling, P. B.; Hauer, J.; Layton, M.; McDade, J.; Osterholm, M. T.; O'Toole, T.; Parker, G.; Russell, P. T.; Tonat, K. *J. Am. Med. Ass.* **1999**, *281*, [1] Jacobs B. L.; Langland J. O.; Kibler K. V.; Denzler K. L.; White S. D.; Holechek S. A.; Wong S.; Huynh T.; Baskin C. R. *Antiviral Res.* **2009**, *84*, 1-13.

21. Jacobs B. L.; Langland J. O.; Kibler K. V.; Denzler K. L.; White S. D.; Holechek S. A.; Wong S.; Huynh T.; Baskin C. R. *Antiviral Res.* **2009**, *84*, 1-13.

22. a) Lane J. M.; Ruben F. L.; Abrutyn E.; Millar J. D. *J. Am. Med. Ass.* **1970**, *126*, 160-168; b) Baggs J.; Chen R. T.; Damon I. K.; Rotz L.; Allen C.; Fullerton K. E.; Casey C.; Nordenberg D.; Mootrey G. *Clin. Infect. Dis.* **2005**, *40*, 1133-1140; c) Schwartz B.; Lebwohl M. *Int. J. Dermatol.* **2005**, *44*, 289-292; d) Reif D. M.; McKinney B. A.; Motsinger A. A.; Chanock S. J.; Edwards K. M.; Rock M. T.; Moore J. H.; Crowe J. E.; *J. Infect. Dis.* **2008**, *198*, 16-22.

23. Omari K.; Stammers D. K. *Expert Opin. Drug Discov.* **2007**, *2*, 1263-1272.

24. Henderson, D. A. *Smallpox: The Death of a Disease*, Prometheus Books, **2009**.

25. Whitley R. J. *Antivir. Res.* **2003**, *57*, 7-12.

2127-2137.

26. a) Stittelaar K. J.; Neyts J.; Naesens L.; Amerongen G.; Lavieren R. F.; Holý A.; De Clercq E.; Niesters H. G.; Fries E.; Maas C.; Mulder P. G.; Zeijst B. A.; Osterhaus A. D. *Nature* **2006**, *439*, 745-748; b) Cowpox infections are being increasingly reported through Eurasia; see, for example: Duraffour, S.; Mertens, B.; Meyer, H.; van der Oord, J. J.; Mitera, T.; Matthys, P.; Snoeck, R.; Andrei, G. *PLoS One* **2013**, *8*(2); e55808.

27. a) In this regard, it is very interesting to read the following news: "Israel taps Siga Technologies' ST-246 to combat smallpox in simulated bioterror attack", available in www.investar.siga.com/releasedetail.cfm?.releaseID=438564 (accessed on 25-05-2013); ; b) For recent advances on anti-orthopoxviruses agents see, Kolodziej, M.; Joniec, J.; Bartoszcze, M.; Gryko, R.; Kocik, J.; Knap, J. *Ann. Agric. Environ. Med.* **2013**, *20*, 1-7.

28. Hamre D.; Brownlee K. A.; Donovick R. *J. Immunol.* **1951**, *67*, 305-312.

29. Reeves P. M.; Bommarius B.; Lebeis S. *Nature Med*. **2005**, *11*, 731-739.

30. De Clercq E. *Med. Res. Rev.* **2008**, *28*, 929-953.

31. Kern, E. R. in *Antiviral Drug Discovery For Emerging Disease and Bioterrorism Threats,* Torrence, P. F. ed.; Wiley, **2005**, pp 331-351.

32. a) Clerq E. *Antiviral Res.* **2002**, *55*, 1-13; b) Smee D. F.; Sidwell R.W., Kefauver D.; Bray M.; Huggins J. W. *Antimicrob. Agents Chemother*. **2002**, *46*, 1329-1335; c) Hostetler K. Y. *Antivir. Res.* **2009**, *82*, A84-A98.

33. Kesson A. M.; Ferguson J. K.; Rawlinson W. D.; Cunningham A. L. *Clin. Infect. Dis*. **1997**, *25*, 911-914.

34. a) Jordan, R.; Leeds, J. M.; Tyavanagimatt, S.; Hruby, D. E. *Viruses* **2010**, *2*, 2409-2435. b) Grosenbach, D. W.; Jordan, R.; Hruby, D. E. *Future Virol.* **2011**, *6*, 653-671.

35. a) Jordan R., Bailey T. R., Rippin S. R. (Viropharma Inc.), WO 2008/130348, **2008**; b) Jordan R., Bailey T. R., Rippin S. R., Dai D.(SIGA Technologies, Inc.), WO 2004/112718, **2004**.

36. a) Bailey, T. R.; Rippin, S. R.; Opsitnick, E.; Burns, C. J.; Pevear, D. C.; Collett, M. S.; Rhodes, G.; Tohan, S.; Huggins, J. W.; Baker, R. O.; Kern, E. R.; Keith, K. A.; Dai, D.; Yang, G.; Hruby, D.; Jordan, R. *J. Med. Chem.* **2007**, *50*, 1442−1444; b) Grosenbach, D. W.; Berhanu, A.; King, D. S.; Mosier, S.; Jones, K. F.; Jordan, R. A.; Bolken, T. C.; Hruby, D. E.; *Proc. Nat. Acad. Sci.* **2010**, *107*, 838−843.

37. a) Leitich, J.; Sprintschnik, G. *Chem. Ber.* **1986**, *119*, 1640-1660; b) Alder, K.; Jacobs, G. *Chem. Ber.* **1953**, *86*, 1528-1539.

38.a) De Clercq, E. *Med. Res. Rev.* **2008**, *28*, 929-953; b) De Clercq, E. *Med. Res. Rev.* **2009**, *29*, 571-610; c) De Clercq, E. *Med. Res. Rev.* **2009**, *29*, 611-645; d) De Clercq, E. *Med. Res. Rev.* **2010**, *30*, 667-707; e) De Clercq, E. *Med. Res. Rev.* **2011**, *31*, 118-160.

39. Delgado, A.; Minguillón, C.; Joglar, J. *Introducción a la Química Terapéutica 2ª ed*., **2003**, 167-168.

40. Topliss, J. G.; *J. Med. Chem*. **1972**, *15*, 1006-1011.

41. Topliss, J. G.; *J. Med. Chem*. **1977**, *20*, 463-469.

42. A search in the ISI Web of knowledge (July, 9th 2013) revealed that reference 40 has been cited more than 300 times, while reference 41 has been cited more than 170 times.

43. Camps, P.; Pujol, X.; Rossi, R. A.; Vázquez, S. *Synthesis* **1999**, 854-858.

44. a) Paquette, L. A.; Wyvratt, M. J. *J. Am. Chem. Soc.* **1974**, *96*, 4671-4673. b) Neil, D.; Vogt, B. R.; Sudol, J. J.; Theodoropulos, S.; Hedaya, E. *J. Am. Chem. Soc.* **1974**, *96*, 4673-4674.

45. Review of tandem Diels-Alder reactions: Winkler, J. D. *Chem. Rev.* **1996**, *96*, 167-176.

46. For a theorical study of this process: Domingo, L. R.; Arnó, M.; Andrés, J. *Tetrahedron Lett.* **1996**, *37*, 7573-7576.

47. Taylor, R. J.; Welter, M. W.; Paquette, L. A. *Org. Synth. Coll. VIII* **1993**, 298-302.

48. Welch, D. E.; Baron, R. R.; Burton, B. A. *J. Org. Chem.* **1969**, *12*, 299-302.

49. Baumstark, A. L.; McCloskey, C. J. *Tetrahedron Lett* **1987**, *28*, 3311-3314.

50. Adam, W.; Curci, R.; Edwards, J. O. *Acc. Chem. Res*. **1989**, *22*, 205-211.

51. Adam, W.; Smerz, A. K. *J. Org. Chem*. **1996**, *61*, 3506-3510.

52. Shi, Y. *Acc. Chem. Res*. **2004**, *37*, 488-496.

53. a) Kottwitz, J.; Vorbrüggen, H. *Synthesis* **1975**, 636-637. b) Illa, O.; Rodríguez-García, C.; Acosta-Silva, C.; Avier, I.; Picurelli, D.; Oliva, A.; Gómez, M.; Branchadell, V.; Ortuño, R. M. *Organometallics* **2007**, *26*, 3306-3314.

54. Straub, B. F. *J. Am. Chem. Soc*. **2002**, *124*, 14195-14201.

55. For methods: Derudas, M.; Brancale, A.; Naesens, L.; Neyts, J.; Balzarini, J.; McGuigan, C. *Bioorg. Med. Chem.* **2010**, *18*, 2748-2755.

56. Beigel, J.; Bray, M. *Antiviral Res.* **2008**, *78*, 91-102.

57. Taubenberger, J. K.; Morens, D. M. *Emerging Infect. Dis.* **2006**, *12*, 15-22. Although it is not known for sure, the Spanish flu is considered to be started in Kansas and the American soldiers sent to fight in the First World War transmitted the virus. Since Spain was one of the few neutral countries, was there where the press informed about the pandemic without censoring, this is why it is called Spanish Flu.

58. Oxford, J. S.; Lambkin, R.; Elliot, A.; Daniels, R.; Sefton, A.; Gill, D. *Vaccine* **2006**, *24*, 6742-6746.

59. a) Laver, W. G.; Bischofberger, N.; Webster, R. G. *Sci. Am.* **1999**, *280*, 78-87. b) Reid, A. H. *Microbes Infect*. **2001**, *3*, 81-87. c) Belshe, R. B. *N. Engl. J. Med.* **2005**, *353*, 2209-2211. d) Kilbourne, E. D. *Emerging infect. Dis.* **2006**, *12*, 9-14.

60. Abdel-Ghafar, A. N; Chotpitayasunondh, T.; Gao, Z.; Hayden, F. G.; Nguyen, D. H.; de Jong, M. D.; Naghdaliyev, A.; Peiris, J. S.; Shindo, N.; Soeroso, S.; Uyeki, T. M. *N. Engl. J. Med.* **2008**, *358*, 261-273.

61. a) Neumann, G.; Noda, T.; Kawaoka, Y. *Nature* **2009**, *459*, 931-939. b) Michaelis, M.; Doerr, H. W., Cinatl, J., Jr. *Curr. Mol. Med.* **2009**, *9*, 131-151.

62. Brankston, G.; Gitterman, L.; Hirji, Z.; Lemieux, C.; Gardam, M. *Lancet Infect Dis* **2007**, *7*, 257-265.

63. Weber, T.P.; Stilianakis, N. I. *J. Infect* **2008**, *57*, 361-373.

64. Lowen, A. C.; Mubareka, S.; Steel J.; Palese P. *PLoS Pathog* **2007**, *3(10)*, e151.

65. Weinstock, D. M.; Zuccotti, G. *J. Am. Med. Assoc.* **2006**, *295*, 934-936.

66. Palese, P. *Nature Med.* **2004**, *10*, S82-S87.

67. a) Lamb, R. A. *Am. J. Respir. Crit. Care Med.* **2013**, *188*, 1-2. b) Morens, D. M.; Taubenberger, J. K.; Fauci, A. S. *MBio* **2013**, *4*, e00445-13. c) Butler, D. *Nature* **2013**, *496*, 145-146. d) Horby, P. *Nature* **2013**, *496*, 399-399.

68. De Clercq, E. *Nat. Rev*. *Drug Discov.* **2006**, *5*, 1015-1025.

69. Molinari, N. A.; Ortega-Sánchez, I. R.; Messonier, M. L.; Thompson, W. W.; Wortley, P. M.; Weintraub, E.; Bridges, C. B. *Vaccine* **2007**, *25*, 5086-5096.

70. Wright, P. F.; Webster, R. G. 'Orthomyxoviruses' Knipe, D. M., Howley, P. M. (Eds.), Fields Virology, fourth ed. Lippincott Williams & Wilkins, Philadelphia, **2001**, 1533-1579.

71. Bouvier, N. M.; Palese, P. *Vaccine* **2008**, *26S*, D49-D53.

72. Ghedin, E.; Sengamalay, N. A.; Shumway, M.; Zaborsky, J.; Feldblyum, T.; Subbu, V.; Spiro, D. J.; Sitz, J. *Nature* **2005**, *437*, 1162-1166.

73. Taken from: http://www.ifpma.org/uploads/RTEmagicP_diagram_virus.jpg (accessed on July, 14th 2013).

74. Du, J.; Cross, T. A.; Zhou, H. *Drug Discovery Today* **2012**, *17*, 1111-1120.

75. a) Zhirnov, O.P. *Virology* **1990**, *176*, 271-279. b) Cheung, T. K. W.; Poon, L. L. *Ann. N. Y. Acad. Sci.* **2007**, *1102*, 1-25.

76. Das, K.; Aramini, J.M.; Ma, L.; Krug, R. M.; Arnold, E. *Nat. Struct. Mol. Biol*. **2010**, *17*, 530-538.

77. Sieczkarski, S. B.; Whittaker, G. R. *Curr. Top. Microbiol. Immunol*. **2005**, *285*, 1-23.

78. Rossman, J. S.; Lamb, R. A. *Virology* **2011**, *411*, 229-236.

79. a) Lamb, R. A.; Zebedee, S. L.; Richardson, C.D. *Cell* **1985**, *40*, 627-633. b) Holsinger, L. J.; Lamb, R. A. *Virology* **1991**, *183*, 32-43. c) Sugrue, R. J., Hay, A. *J. Virology* **1991**, *180*, 617-624. d) Park, E. K.; Castrucci, M. R.; Portner, A.; Kawaoka, Y. *J. Virol.* **1998**, *72*, 2449-2455. e) Kochendoerfer, G. G.; Salom, D.; Lear, J. D.; Wilk-Orescan, R.; Kent, S. B.; DeGrado, W. F. *Biochemistry* **1999**, *38*, 11905-11913. f) Rossman, J. S.; Jing, X.; Leser, G. P.; Lamb, R. A.; *Cell* **2010**, *142*, 902-913.

80. a) Sakaguchi, T.; Leser, G. P.; Lamb, R. A. *J. Cell Bio.* **1996**, *133*, 733-747. b) Pinto, L. H., Holsinger, L. J., Lamb, R. A. *Cell* **1992**, *69*, 517-528.

81. Hu, J.; Fu, R.; Cross, T. A. *Biophys. J.* **2007**, *93*, 276-283.

82. Hoffmann, H.; Palese, P.; Shaw, M. L. *Anti. Res.* **2008**, *80*, 124-134.

83. Ma, C.; Polishchuk, A. L.; Ohigashi, Y.; Stouffer, A. L., Schön, A., Magavern, E.; Jing, X., Lear, J. D.; Freire, E.; Lamb, R. A.; DeGrado, W. F.; Pinto, L. H. *Prod. Nat. Acad. Sci.* **2009**, *106*, 12283-12288.

84. a) Schnell, J. R.; Chou, J. J. *Nature* **2008**, *451*, 591-596. b) Fiorin, G.; Carnevale, V.; DeGrado, W. F. *Science* **2010**, *330*, 456-458. c) Hu, F.; Luo, W.; Hong, M. *Science* **2010**, *330*, 505-508.

85. Hu, J.; Asbury, T.; Achuthan, S.; Li, C.; Bertram, R.; Quine, J. R.; Fu, R.; Cross T. A. *Biophys. J.* **2007**, *92*, 4335-4343.

86. Hong. M.; DeGrado, W. F. *Protein Science* **2012**, *21*, 1620-1633.

87. Ivanovic, T.; Rozendaal, R.; Floyd, D. L.; Popovic, M.; Van Oijen, A. M.; Harrison, S. C. *PLos One* **2012**, *7*, e31566, 1-9.

88. Okada, A.; Miura, T.; Takeuchi, H. *Biochemistry* **2001**, *40*, 6053-6060.

89. a) Sharma, M.; Yi, M.; Dong, H.; Qin, H.; Peterson, E.; Busath, D. D.; Zhou, H.; Cross, T. A. *Science* **2010**, *330*, 509-512. b) Carnevale, V.; Fiorin, G.; Levine, B. G.; DeGrado, W. F.; Klein, M. L. *J. Phys. Chem C* **2010**, *114*, 20856-20863. c) Dong, H.; Yi, M.; Cross, T. A.; Zhou, H.-X. *Chem.*

*Sci.* **2013**, *4*, 2776-2787. d) Williams, J. K.; Zhang, Y.; Schmidt-Rohr, K.; Hong, M. *Biophys. J.* **2013**, *104*, 1698-1708.

90. Stouffer, A. L.; Acharya, R.; Salom, D.; Levine, A. S.; Di Costanzo, L.; Soto, C. S.; Tereshko, V.; Nanda, V.; Stayrook, S.; DeGrado, W. F. *Nature* **2008**, *451*, 596-599.

91. a) Varghese, J. N.; Laver, W. G.; Colman, P. M. *Nature* **1983**, *303*, 35-40. b) Colman, P. M.; Varghese, J. N.; Laver, W. G. *Nature* **1983**, *303*, 41-44.

92. Von Itzstein, M.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Van Phan, T.; Smythe, M L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. H.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418-423.

93. Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681-690.

94. a) Bantia, S.; Arnold, C. S.; Parker, C. D.; Upshaw, R.; Chand, P. *Antiviral Res*. **2006**, *69*, 39-45. b) Shetty, A. K.; Peek, L. A. *Expert Rev. Anti-infect. Ther.* **2012**, *10*, 123-143.

95. Lagoja, I. M.; De Clercq, E. *Med. Res. Rev.* **2008**, *28*, 1-38.

96. Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kaneko, S.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921-1924.

97. a) Yamashita, M.; Tomozawa, T.; Kakuta, M.; Tokumitsu, A. Sugaya, N.; Ohashi, Y. *Antimicrob. Agents Chemoter.* **2010**, *54*, 2575-2582. b) Kubo, S. *Antimicrob. Agents Chemother.* **2009**, *53*, 186-192. c) Ikematsu, H.; Kawai, N. *Expert Rev. Anti-infect. Ther.* **2011**, *9*, 851-857.

98. Aoki, F. Y.; Macleod, M. D.; Paggiaro, P.; Carewicz, O.; El Sawy, A.; Wat, C.; Griffiths, M.; Waalberg, E.; Ward, P. *J. Antimicrob. Chemother.* **2003**, *51*, 123-129.

99. a) Wang, G. T.; Chen, Y.; Wang, S.; Gentles, R.; Sowin, T.; Kati, W.; Muchmore, S.; Giranda, V.; Stewart, K.; Sham, H.; Kempf, D.; Laver, G. *J. Med. Chem.* **2001**, *44*, 1192-1201. b) Abdel, M. A. F.; Maryanoff, C.A.; Mehrman, S. J. *Curr. Opin. Drug Discover. Develop.* **2001,** *4,* 776-791. c) Hanessian, S.; Bayrakdarian, M.; Luo, X. *J*. Am. Chem. Soc. **2002***, 124,* 4716-4721. d) Maring, C. J.; Stoll, V. S.; Zhao, C.; Sun, M.; Krueger, A. C.; Stewart, K. D.; Madigan, D. L.; Kati, W. M.; Xu, Y.; Carrick, R. J.; Montgomery, D. A.; Kempf-Grote, A.; Marsh, K. C.; Molla, A.; Steffy, K. R.; Sham, H. L.; Laver, W. G.; Gu, Y.; Kempf, D. J.; Kohlbrenner, W. E. *J. Med. Chem.* **2005**, *48*, 3980-3990. e) Momose, T.; Hama, N.; Higashino, C.; Sato, H.; Chida, N. *Tet. Lett.* **2008**, *49*, 1376-1379. f) Liu, Y.; Zhang, J.; Xu, W. *Curr. Med. Chem.* **2007**, *14*, 2872-2891.

100. a) Malakhov, M. P.; Aschenbrenner, L. M.; Smee, D. F.; Wandersee, M. K.; Sidwell, R. W.; Gubareva, L. V.; Mishin, V. P.; Hayden, F. G.; Kim, D. H.; Ing, A.; Campbell, E. R.; Yu, M.; Fang, F. *Antimicrob. Agents Chemother.* **2006**, *50*, 1470-1479 b) Triana-Baltzer, G. B.; Gubareva, L. V.;

Klimov, A. I.; Wurtman, D. F.; Moss, R. B.; Hedlund, M.; Larson, J. F.; Belshe, R. B.; Fang, F. *PLOS one* **2009**, *4*, e7838.

101. a) Bodian, D. L.; Yamasaki, R. D.; Buswell, R. L.; Stearns, J. F.; White, J. M.; Kuntz, I. D.; *Biochemistry* **1993**, *32*, 2967-2978. b) Staschke, K. A.; Hatch, S. D.; Tang, J. C.; Hornback, W. J.; Munroe, J. E.; Colacion, J. M.; Muesing, M. A. *Virology* **1998**, *248*, 264-274. c) Deshpande, M. S.; Wei, J.; Luo, G.; Cianci, C.; Danetz, S.; Torri, A.; Tiley, L.; Krystal, M.; Yu, K.; Huang, S.; Gao, Q.; Meanwell, N. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2393-2396. d) Russell, R. J.; Kerry, P. S.; Stevens, D. J.; Steinhauer, D. A.; Martin, S. R.; Gamblin, S. J.; Skehel, J. J. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17736-17741.

102. Tang, G.; Qiu, Z.; Lin, X.; Li, W.; Zhu, L.; Li, S.; Li, H.; Wang, L.; Chen, L.; Wu, J. Z.; Yang, W. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3507-3510.

103. Luo, G.; Torri, A.; Harte, W. E.; Danetz, S.; Cianci, C.; Tiley, L.; Day, S:; Mullaney, D.; Yu, K. L.; Ouellet, C.; Dextraze, P.; Meanwell, N.; Colonno, R.; Krystal, M. *J. Virol.* **1997**, *71*, 4062-4070. b) Cianci, C.; Yu, K. L.; Dischino, D. D.; Harte, W.; Deshapnde, M.; Luo, G.; Colonno, R. J.; Meanwell, N. A.; Krystal, M. *J. Virol.* **1999**, *73*, 1785-1794.

104. a) I. A. Leneva, N. I. Fadeeva, I. T. Fedyakina *Antivir. Res.* **1994**, *23*, 187. b) I. A. Leneva, N. I. Fadeeva, I. T. Fedyakina, T. A. Guskova, M. L. Khristova, M. V. Sokolova, I. G. Kharitonenkov *Chem. Pharm. J.* **1994**, *9*, 4-15. c) Boriskin, Y. S.; Leneva, I. A.; Pécheur, E. I.; Polyak, S. J. *Curr. Med. Chem.* **2008**, *15*, 997-1005. d) Leneva, I.A.; Russell, R. J.; Bariskin, Y. S.; Hay, A. J. *Antiviral Res.* **2009**, *81*, 132-140.

105. Davies, W. L.; Grunert, R. R.; Haff, R. F.; McGahen, J. W.; Neumayer, E. M.; Paulshock, M.; Watts, J. C.; Wood, T. R.; Hermann, E. C.; Hoffmann, C. E. *Science* **1964**, *144*, 862-863.

106. a) Oxford, J.S., Galbraith, A. *Pharmacol. Ther.* **1980**, *11*, 181. b) Belshe, R. B.; Burk, B.; Newman, F.; Cerruti, R. L.; Sim, I. S. *J. Infect. Dis.* **1989**, *159*, 430.

107. Hubsher, G.; Haider, M.; Okun, M. S. *Neurology* **2012**, *78*, 1096-1099.

108. Czabotar, P. E.; Martin, S. R.; Hay, A. J. *Virus Res.* **2004**, *99*, 57-61.

109. a) Rosenberg, M. R.; Casarotto, M. G. *Proc. Nat. Acad. Sci. USA.* **2009**, *106*, 13866-13871. b) Gkeka, P.; Eleftheratos, S.; Kolocouris, A.; Ournia, Z. *J. Chem. Theory Comput.* **2013**, *9*, 1272-1281. c) Gu, R.-X.; Liu, L. A.; Wang, Y.-H.; Xu, Q.; Wei, D.-Q. *J. Phys. Chem. B* **2013**, *117*, 6042-6051. d) Andreas, L. B.; Barnes, A. B.; Corzilius, B.; Chou, J. J.; Miller, E. A.; Caporini, M.; Rosay, M.; Griffin, R. G. *Biochemistry* **2013**, *52*, 2774-2782.

110. Wang, J.; Ma, C.; Fiorin, G.; Carnevale, V.; Wang T.; Hu, F.; Lamb, R. A.; Pinto, L. H.; Hong, M.; Klein, M. L.; DeGrado, W. F. *J. Am. Chem. Soc.* **2011**, *133*, 12834-12841.

111. a) Pielak, R. M.; Schnell, J. R.; Chou, J. J. *Proc. Nat. Acad. Sci.* **2009**, *106*, 7379-7384. b) Acharya, A.; Carnevale, V.; Fiorin, G.; Levine, B. G.; Polishchuk, A.; Balannick, V.; Samish, I.;

Lamb, R. A.; Pinto, L. H.; DeGrado, W. F.; Klein, M. L. *Proc. Nat. Acad. Sci. USA.* **2010**, *107*, 15075-15080.

112. a) Pinto, L. H.; Lamb, R. A. *J. Biol. Chem.* **2006**, *14*, 8997-9000; b) Zhang, Y.; Shen, H.; Zhang, H.; Li, G.; *J. Phys. Chem. B* **2013**, *117*, 982-988.

113. a) Bright, R. A.; Medina, M. J.; Xu, X.; Perez-Oronoz, G.; Wallis, T. R.; Davis, X. M.; Povinelli, L.; Cox, N. J.; Klimov, A. I. *Lancet* **2005**, *366*, 1175-1181. b) Saito, R.; Sakai, T.; Sato, I.; Sano, Y.; Oshitani, H.; Sato, M.; Suzuky, H. *J. Clin. Microbiol.* **2003**, *41*, 2164-2165.

114. a) Bright, R. A.; Shay, D. K.; Shu, B.; Cox, N. J.; Klimov, A. *J. Am. Med. Assoc.* **2006**, *295*, 891-894. b) Deyde, V. M.; Xu, X. Y.; Bright, R. A.; Shaw, M.; Smith, C. B.; Zhang, Y.; Shu, Y. L.; Gubareva, L. V. Cox, N. J.; Klimov, A. *J. Infect. Dis.* **2007**, *196*, 249-257. c) Bright, R. A. *Morb. Mortal. Weekly Rep.* **2006**, *55*, 44-46.

115. Furuse, Y.; Suzuki, A.; Oshitani, H. *Antimicrob. Agents Chemoter.* **2009**, *53*, 4457-4463.

116. Balannik, V.; Wang, J.; Ohigashi, Y.; Jing, X. H.; Magavern, E.; Lamb, R. A.; DeGrado, W. F.; Pinto, L. H. *Biochemistry* **2009**, *48*, 11872-11882.

117. a) Geluk, H. W.; Schut, J.; Schlatmann, J. L. M. A. *J. Med. Chem.* **1969**, *12*, 712. b) Manchand, P. S.; Cerruti, R. L.; Maritn, J. A.; Hill, C. H., Merrett, J. H.; Keech, E., Belshe, R. B.; Connell, E. V.; Sim, I. S. *J. Med. Chem.* **1990**, *33*, 1992-1995. c) Wang, J., Ma, C.; Balannik, V., Pinto, L. H.; Lamb, R. A.; DeGrado, W. F. *ACS Med. Chem. Lett* **2011**, *2*, 307-312. d) Zoidis, G.; Fytas, C.; Papanastasiou, I.; Foscolos, G. B., Fytas, G.; Padalko, E., De Clerq, E.; Naesens, L.; Neyts, J.; Kolocouris, N. *Bioorg. Med. Chem.* **2006**, *14*, 3341.

118. Tataridis, D.; Fytas, G.; Kolocouris, A.; Fytas, C.; Kolocouris, N.; Foscolos, G. B., Padalko, E.; Neyts, J.; De Clercq, E. *Bioorg. Med. Chem.* **2007**, *17*, 692-696.

119. Zoidis, G.; Kolocouris, N.; Kelly, J. M.; Prathalingam, S. R.; Naesens, L.; DeClerq, E. Eur. *J. Med. Chem.* **2010**, *45*, 5022-5030.

120. Setaki, D.; Tataridis, D.; Stamatiou, G.; Kolocouris, A.; Foscolos, G. B.; Fytas, G.; Kolocouris, N.; Padalko, E.; Neyts, J.; De Clerq, E. *Bioorg. Chem.* **2006**, *34*, 248-273.

121. a) Beare, A. S.; Hall, T. S.; Tyrrel, D. A. *Lancet* **1972**, *299*, 1039. b) Mathur, A.; Beare, A.; Reed, S. E. *Antimicrob. Agents Chemoter.* **1973**, *4*, 421-426. c) Togo, Y. *Antimicrob. Agents Chemoter.* **1973**, *4*, 641-642.

122. Kolocouris, N.; Foscolos, G. B.; Kolocouris, A.; Marakaos, P.; Pouli, N.; Fytas, G.; Ikeda, S.; De Clerq, E. *J. Med. Chem.* **1994**, *37*, 2896-2902.

123. a) Zoidis, G.; Tsotinis, A.; Kolocouris, N.; Kelly, J. M.; Prathalingam, S. R.; Kolocouris, N.; Naesens, L.; De Clerq, E. *Org. Biomol. Chem.* **2008**, *6*, 3177-3185. b) Zoidis, G.; Kolocouris, N.; Naesens, L.; De Clerq, E. *Bioorg. Med. Chem.* **2009**, *17*, 1534-1541.

124. Duque, M. D.; Torres, E.; Valverde, E.; Barniol, M.; Guardiola, S.; Rey, M.; Vázquez, S. *Rec. Adv. Pharm. Sci.* **2011**, *19*, 1655-1663.

125. Cady, S. D.; Schmidt-Rohr, K.; Wang, J.; Soto, C. S.; DeGrado, W. F.; Hong, M. *Nature* **2010**, *463*, 689-693.

126. Wang, J.; Wu, Y.; Ma, C.; Fiorin, G.; Wang, J.; Pinto, L. H.; Lamb, R. A.; Klein, M. L.; DeGrado, W. *Proc. Nat. Acad. Sci*. **2013**, *110*, 1315-1320. b) Williams, J. K.; Tietze, D.; Wang, J.; Wu, Y.; DeGrado, W.; Hong, M. *J. Am. Chem. Soc.* **2013**, 135, 9885-9897.

127. Pielak, R. M.; Chou, J. J. *Biochem. Biophys. Res. Commun.* **2010**, *401*, 58-63.

128. Pielak, R. M.; Oxenoid, K.; Chou, J. J: *Structure* **2011**, *19*, 1655-1663.

129. Kurts, S.; Luo, G. X.; Hahnenberger, K. M.; Brooks, C.; Gecha, O.; Ingalls, K.; Numata, K. I.; Krystal, M. *Antimicrob. Agents Chemoter.* **1995**, *39*, 2204.

130. Wang, J.; Cady, S. D.; Balannik, V.; Pinto, L. H.; DeGrado, W. F.; Hong, M. *J. Am. Chem. Soc.* **2009**, *131*, 8066.

131. Hu, W.; Zeng, S.; Li, C.; Jie, Y.; Li, Z.; Chen, L. *J. Med. Chem.* **2010**, *53*, 3831-3834.

132. Zhao, X.; Li, C.; Zeng, S.; Hu, W. *Eur. J. Med. Chem.* **2011**, *46*, 52-57.

133. Wenjuan, Z.; Jing, X.; Fang, L.; Chufang, L.; Yanling, J.; Shaopeng, C.; Zhiyuan, L.; Jinsong, L.; Ling, C.; Guochun, Z. *Chin. J. Chem.* **2010**, *28*, 1417-1423.

134. Wang, J.; Ma, C.; Wu, Y.; Lamb, R. A.; Pinto, L. H.; DeGrado, W. F. *J. Am. Chem. Soc.* **2011**, *133*, 13844-13847.

135. DeGrado, W. F.; Wang, J. (University of Pennsylvania), **2011**, WO2011/022191.

136. Wang, J.; Ma, C.; Wang, J.; Jo, H.; Canturk, B.; Fiorin, G.; Pinto, L. H.; Lamb, R.; Klein, M.; DeGrado, W.F. *J. Med. Chem.* **2013**, *56*, 2804-2812.

137. However, some analogs containing some substitution in the position 2 have been described, see for example: Djaidi, D.; Leung, I. S. H.; Bishop, R.; Craig, D. C.; Scudder, M. L. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2037-2042.

138. Stoelting, D. T.; Shier, V. J. Jr. *J. Am. Chem. Soc.* **1993**, *115*, 1695-1705.

139. Kürti, L.; Czakó, B. "Strategic Applications of Named Reactions in Organic Sythesis" Elsevier: **2005**, pp 382-383.

140. a) Colombo, M. I.; Bohn, M. L.; Rúveda, E. *A. J. Chem. Ed.* **2002**, *79*, 484-485. b) Gerasimova, N. P.; Nozhnin, N. A.; Ermolaeva, V. V.; Ovchinnikova, A. V.; Moskvichev, Y.; Alov, E. M.; Danilova, A. S. *Mendeleev Commun.* **2003**, *13*, 82-83.

141. Moss, R. A.; Sauers, R. R.; Sheridan, R. S.; Tian, J.; Zuev, P. S. *J. Am. Chem. Soc.* **2004**, *126*, 10196-10197.

142. Duque, M. D.; Camps, P.; Torres, E.; Valverde, E.; Sureda, F. X.; López-Querol, M.; Camins, A.; Prathalingam, S. R.; Kelly, J. M.; Vázquez, S. *Bioorg. Chem. Med.* **2010**, *18*, 46-57.

143. Jirgensons, A.; Kauss, V.; Kalvinsh, I.; Gold, M. R. *Synthesis* **2000**, 1709-1712.

144. a) Schwertfeger, H.; Würtele, C.; Serafin, M.; Hausmann, H.; Carlson, R. M. K.; Dahl, J. E. P.; Schreiner, P. R. *J. Org. Chem.* **2008**, *73*, 7789-7792. b) Fokin, A. A.; Merz, A.; Fokina, N. A.; Schwertfeger, H.; Liu, S. L.; Dahl, J. E. P.; Carlson, R. M. K.; Schreiner, P. R. *Synthesis* **2009**, 909-912.

145. a) Stamatiou, G.; Foscolos, G. B.; Fytas, G.; Kolocouris, A.; Kolocouris, N.; Pannecouque, C.; Witvrouw, M.; Padalko, E.; Neyts, J.; De Clercq, E. *Bioorg. Med. Chem.* **2003**, *11,* 5485-5492. b) Kolocouris , A.; Tataridis, D.; Fytas, G.; Mavromoustakos, T.; Foscolos, G. B.; Kolocouris, N.; De Clercq, E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3465-3470.

146. a) Lane, C. F. *Synthesis* **1974**, 135-146. b) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897-2904.

147. María D. Duque, Doctoral Thesis (Universitat de Barcelona, 2010).

148. The reductive ability of Grignard reagents in sterically encumbered ketones is well precedented: Brucker, R. "Reaction Mechanism. Stereochemistry and Synthesis." Springer-Verlag; Berlin; **2010**, pp 328-330.

149. Kharasch, M. S.; Weinhouse, S. *J. Org. Chem.* **1936**, *1*, 209-230.

150. a) Shokova, E.; Mousoulou, T.; Luzikov, Y.; Kovalev, V. *Synthesis* **1997**, *9*, 1034-1040. b) Moiseev, I. K.; Makarova, N. V.; Zemtsova, M. N. *Russ. Chem. Rev.* **1999**, *68*, 1001-1020.

151. Sosnowski, J. J.; Rheingold, A. L.; Murray, R. K. Jr. *J. Org. Chem.* **1985**, *50*, 3788-3791.

152. Camps, P.; Lukach, A. E.; Rossi, R. A. *J. Org. Chem.* **2001**, *66*, 5366-5373.

153. Kolocouris, A.; Spearpoint, P.; Martin, S. R.; Hay, A. J.; López-Querol, M.; Sureda, F. X.; Padalko, E.; Neyts, J.; De Clercq, E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6156-6160.

154. a) Schaber, P. M.; Colson, J.; Higgins, S.; Thielen, D.; Anspach, B.; Brauer, *J. Therm. Ac.* **2004**, *424*, 131-142. b) Bernhard, A. M.; Peitz, D.; Elsener, M.; Wokaun, A.; Kröcher, O. *App. Cat. B: Env.* **2012**, *115-116*, 129-137.

155. Clayden, Greeves, Warren, Wothers "Organic Chemistry", 2nd edition, Oxford University Press: Oxford 2012, 174-175.

156. a) Black, R. M.; Gill, G. B.; *J. Chem. Soc. D, Chem. Commun.* **1970**, 972-973. b) Janjatović, J.; Majerski, Z. *J. Org. Chem.* **1980**, *45*, 4892-4898.

157. a) Bernatowiez, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497-2502. b) Makovec, F.; Artusi, R.; Zanzola, S.; Rovati, C. Patent US 2005/0049312, **2005**.

158. Delgado, A.; Minguillón, C.; Joglar, J. 'Introducción a la química terapéutica.' Díaz de Santos; Madrid; **2003**, pp 130-140.

159. Jun, J.; Mundy, B. P. *Bull. Korean Chem. Soc.* **1987**, *8*, 310-313.

160. Sortino, M.; Garibotto, F.; Cechinel Filho, V.; Gupta, M.; Enriz, R.; Zacchino, S. *Biorg. Med. Chem.* **2011**, *19*, 2823-2834.

161. Camps, P.; Fernández, J. A.; Rull, J.; Vázquez, S. *Eur. J. Org. Chem.* **2009**, 3081-3087.

162. Kalo, J.; Ginsburg, D.; Vogel, E. *Tetrahedron* **1977**, *33*, 1177-1182.

163. Verschueren, W. G., Dierynck, I.; Amssons, K. I. E.; Hu, L.; Boonants, P.; Pille, G.; Daeyaert, F.; Hertogs, K.; Surleraux, D.; Wigerinck, P. *J. Med. Chem* **2005**, *48*, 1930-1940.

164. Ishibashi, H.; Nakaharu, T.; Nishimura, M.; Nishikawa, A. Kameoka, C.; Ikeda, M. *Tetrahedron* **1995**, *51*, 2929-2938.

165. Sample, T. E.; Hatch, L. F. *J. Chem. Educ.* **1968**, *45*, 55-56.

166. Amith, C.; Kalo, J.; North, B. E.; Ginsburg, D. *Tetrahedron* **1974**, *30*, 479-481.

167. Daniels, R. S.; Downie, J. C.; Hay, A. J.; Knossow, M.; Skehel, J. J.; Wang, M. L.; Wiley, D. C.; *Cell* **1985**, *40*, 431-439.

168. Gamblin, S. J.; Haire, L. F.; Russell, R. J.; Stevens, D. J.; Xiao, B.; Ha, Y.; Vasisht, N.; Steinhauer, D. A.; Daniels, R. S.; Elliot, A.; Wiley, D. C.; Skehel, J. J. *Science* **2004**, *303*, 1838-1842.

169. a) Bao, Y.; Bolotov, P.; Dernovoy, D.; Kiryutin, B.; Zaslavsky, L.; Tatusova, T.; Ostell, J.; Lipman, D. *J. Virol.* **2008**, *82*, 596-601. b) http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html

170. Vanderlinden E.; Göktas F.; Cesur Z.; Froeyen M.; Reed M. L.; Russell, C. J.; Cesur, N.; Naesens L. *J. Virol.* **2010**, *84*, 4277-4288.

171. Cross, K. J.; Burleigh, L. M.; Steinhauer, D. A., *Exp. Rev. Mol. Med.* **2001**, *3*, 1-18.

172. Scholtissek, C.; Quack, G.; Klenk, H. D.; Webster, R. G. *Antiviral Res.* **1998**, *37*, 83-95.

173. Plotch, S. J.; O'Hara, B.; Morin, J.; Palant, O.; LaRocque, J.; Bloom, J. D.; Lang, S. A., Jr.; DiGrandi, M. J.; Bradley, M.; Nilakantan, R.; Gluzman, Y. *J Virol.* **1999**, *73*, 140-151.

174. Korte, T.; Ludwig, K.; Booy, F. P.; Blumenthal, R.; Herrmann, A. *J Virol.* **1999**, *73*, 4567-4574.

175. Rachakonda, P. S.; Veit, M.; Korte, T.; Ludwig, K.; Böttcher, C.; Huang, Q.; Schmidt, M. F.; Herrmann, A. *FASEB J.* **2007**, *21*, 995-1002.

176. Stevens, J.; Corper, A. L.; Basler, C. F.; Taubenberger, J. K.; Palese, P.; Wilson, I. A. *Science* **2004**, *303*, 1866-1870.

177. Zhang, W.; Qi, J.; Shi, Y.; Li, Q.; Gao, F.; Sun, Y.; Lu, X.; Lu, Q.; Vavricka, C. J.; Liu, D.; Yan, J.; Gao, G. F. *Protein Cell*. **2010**, *1*, 459-467.

178. a) Stevens, J.; Blixt, O.; Tumpey, T. M.; Taubenberger, J. K.; Paulson J. C.; Wilson, I. A. *Science* **2006**, *312*, 404-410. b) Biosafety regulations do not allow Prof. Naesens' group to work with the Influenza A/H5N1 viruses.

179. Qin, J.; Yu, K.; Shi, T.; Luo, C.; Li, G.; Zhu, W.; Jiang, H. *J. Phys. Chem. B* **2010**, *114*, 8487-8493.

180. Duque, M. D.; Ma, C.; Torres, E.; Wang, J.; Naesens, L.; Juárez-Jiménez, J.; Camps, P.; Luque, F. J.; DeGrado, W. F.; Lamb, R. A.; Pinto, L. H.; Vázquez S. *J. Med. Chem.* **2011**, *54*, 2646-2657.

181. Camps, P.; Iglesias, C.; Rodríguez, M. J.; Grancha, M. D.; Gregori, M. E.; Lozano, R.; Miranda, M. A.; Figueredo, M.; Linares, A. *Chem. Ber.* **1988**, *121*, 647-654.

182. a) Fu, X.; Cook, J. M. *Aldrichimica Acta* **1992**, *25*, 43-54. b) Gupta, A. K.; Fu, X.; Snyder, J. P.; Cook, J. M. *Tetrahedron* **1991**, *47*, 3665-3710.

183. Bertz, S. H.; Cook, J. M.; Gawish, A.; Weiss, U. *Org. Synth. Coll. Vol. VII*, Wiley: New York, **1990**, pp 50-56.

184. Xavier Pujol, Tesis Doctoral, Universidad de Barcelona, 2001.

185. Camps, P.; Luque, F. J.; Orozco, M.; Pérez, F.; Vázquez, S. *Tetrahedron Lett.* **1996**, *37*, 8605-8608.

186. a) Barton, D. H. R.; Crich, D.; Motherwell, W. B. *Tetrahedron* **1985**, *41*, 3901-3924. b) Barton, D. H. R.; Samadi, M. *Tetrahedron* **1992**, *48*, 7083-7090.

187. Kürti, L.; Czakó, B. "Strategic Applications of Named Reactions in Organic Sythesis" Elsevier: 2005, pp 396-397.

188. Jawdosiuk, M.; Kovacic, P. *Synth. Commun.* **1983**, *13*, 53-62.

189. In July, 2011, DeGrado and coworkers published the first submicrolar inhibitor of the V27A mutant M2 channel ($EC_{50}$= 0.30 μM). However, their compound, **91**, is less potent against the wt channel ($EC_{50}$= 18.7 μM). See Figure 38 and references 110 and 135.

190. a) Bishop, R.; Landers, A. E. *Aust. J. Chem.* **1979**, *32*, 2675–2679. b) Amini; Bishop, R. *Aust. J. Chem.* **1983**, *36*, 2465–2472. c) Amini; Bishop, R.; Burgess, G.; Craig, D. C.; Dance, I. G.; Scudder, M. L. *Aust. J. Chem.* **1989**, *42*, 1919-1928.

191. a) Föhlisch, B.; Widmann, E.; Schupp, E. *Tetrahedron Lett.* **1969**, 2355-2358. b) Föhlisch, B.; Dukek, U.; Graeble, I.; Novotny, B.; Schupp, E.; Schwaiger, G.; Widmann, E. *Liebigs Ann. Chem.* **1973**, 1839-1850.

192. Alternatively, refluxing the mixture in toluene overnight in a Dean-Stark system also led to pure **250**.

193. a) Greenwald R.; Chaykovsky M.; Corey E. J. *J. Org. Chem*. **1963**, *28*, 1128-1129. b) Dehmlow E. V.; Gröning C. *J. Chem. Res.*, **1992**, 108-109.

194. a) Ishiyama, J.; Senda, Y.; Imaizumi, S. *Chem. Lett.* **1983**, 771-774. b) Chow, T. J.; Li, L. *Tetrahedron* **1999**, *55*, 6067-6074. c) Bishop, R. *Aust. J. Chem.* **1984**, *37*, 319-325. d) Olah, G. A.; Krishnamurti, R.; Prakash S. *Synthesis* **1990**, 646-648.

195. The Ritter reaction of diene **252** with acetonitrile had been described in reference 190c. However, in our hands, the hydrolysis of this acetamide with aq. HCl gave amine **254** in low yield. Only under very drastic basic conditions (NaOH, diethyleneglycol at 210 °C for 24 hours) **254** was obtained in high yield (89%).

196. Togo, H.; Aoki, M.; Kuramochi, M.; Yokoyama, M. *J. Chem. Soc. Perkin Trans. 1* **1993**, 2417-2427.

197. Hachiya, S.; Oku, M.; Mukai, H.; Shin, T.; Matsuura, K.; Seo, R.; Kamikubo, T.; Terada, Y.; Sanagi, M.; Yoshihara, K.; Takahashi, T. (Astellas Pharma Inc.), WO2006/123725, **2006**.

198. Tanabe, Y.; Matsuo, N.; Ohno, N. *J. Org. Chem* **1988**, *53*, 4582-4585.

199. Rando, D. G.; Avery, M. A.; Tekwani, B. L.; Khan, S. I.; Ferreira, E. I. *Bioorg Med. Chem.* **2008**, *16*, 6724-6731.

200. Verschueren, W.; Dierynck, I.; Amssoms, K.; Hu, L.; Boonants, P.; Pille, G.; Daeyaert, F.; Hertogs, K.; Surleraux, D.; Wigerinck, P. *J. Med. Chem.* **2005**, *48*, 1930-1940.

201. Thesis of Dr. Jordi Rull, Universitat de Barcelona, **2010**.

202. Altman, J.; Babad, E.; Itzchaki, J.; Ginsburg, D. *Tetrahedron*, **1966**, *22*, Supp. 8, 279-304.

203. Camps, C.; Figueredo, M. *Can. J. Chem.* **1984**, *62*, 1184-1193.

204. Wagner, E.; Rudzick, A. D. *J. Med. Chem.* **1967**, *10*, 607-611.

205. Alder, K. *Ber. Dtsch. Chem. Ges.* **1938**, *71B*, 2199-2209.

206. Wagner, E.; Davisson, J. *J. Med. Chem.* **1968**, *11*, 805-807.

207. Spur, P.; Hamon, D. *J. Am. Chem. Soc.* **1983**, *105*, 4734-4739.

208. Wagner, E.; Rudzik, A. *J. Med. Chem.* **1967**, *10*, 607-611.

209. Costa, B. B. C.; Corrêa, R.; Souza, M. M.; Pretto, J. B.; Ardenghi, J. V.; Campos-Buzzi, F.; Filho, V. C. *Z. Naturforsch. C: J. Biosci.* **2007**, *62*, 201-206.

210. Avila, W.; Silva R. *J. Chem. Soc. D: Chem. Comm.* **1970**, *2*, 94-95.

211. Camps, P.; Iglesias, C.; Rodríguez, M. J.; Grancha, M. D.; Gregori, M. E.; Lozano, R.; Miranda, M. A.; Figueredo, M.; Llinares, A. *Chem. Ber.* **1988**, *121*, 647-654.

212. The ester **243** and the acid **230** had been synthesized using a very different approach by Vogt et al. However, the compounds were not characterized. Vogt, B. R.; Suter, S. R.; Hoover, J. R. E. *Tetrahedron Lett.* **1968**, *9*, 1609-1612.

213. Amines **231** and **232** had been disclosed, without any characterization, in a patent that claimed antiviral activity for both amines without any detailed information. See, Hoover, J. R. E. (Smith Kline & French), US 3496228 (**1970**).

214. Fytas, G.; Stamatiau, G.; Foscolos, G. B.; Kolocouris, A.; Kolocouris, N.; Witvrouw, N.; Pannecouque, C.; DeClerq, E. *Bior. Med. Chem. Lett* **1997**, *7*, 1887-1890.