Synthesis of 1*S*-ethyl-4-substituted quinolizidines and other potentially bioactive compounds

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Programa de Doctorado:
Química Orgánica en la Industria Químico-Farmacéutica
Bienio 2006-2008

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Memoria presentada por Vladislav Semak
para optar al título de Doctor por la Universitat de Barcelona

Dirigida por:

Dra. Carmen Escolano Mirón

Vladislav Semak
Barcelona, 2012

El trabajo experimental recogido en esta memoria se ha realizado en el Laboratorio de la Unidad de Química Orgánica de la Facultad de Farmacia de la Universitat de Barcelona bajo la dirección de la Dra. Carmen Escolano Mirón.

Esta Tesis Doctoral ha sido posible gracias a la concesión de una beca predoctoral de *Formación de Profesorado Universitario* (FPU No. AP2006-03468) por parte del *Ministerio de Educación y Ciencia*, al cual quiero expresar mi gratitud.

El presente trabajo ha sido financiado por el *Ministerio de Educación y Ciencia* (proyectos CTQ2006-02390/BQU y CTQ2009-07021/BQU) y por el DURSI de la *Generalitat de Catalunya* (proyectos 2005-SGR-0603 y 2009-SGR-111).

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[*] *Introduction, Summary & Conclusion sections are written in Spanish according to the current UB rules.*

DIFFUSION OF THE RESULTS

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MANUSCRIPTS (chronological order)

1. A practical procedure for the removal of the phenylethanol moiety from phenylglycinol-derived lactams. Semak, V.; Escolano, C.; Arróniz, C.; Bosch, J.; Amat, M. *Tetrahedron: Asymmetry* **2010**, *21*, 2542–2549.

2. Dauben-Michno oxidative transposition of allylic cyanohydrins - Enantiomeric switch of (-)-carvone to (+)-carvone. Hudlicky, R. J.; Werner, L.; Semak, V.; Simionescu, R.; Hudlicky, T. *Can. J. Chem.* **2011,** *89*, 535-543.

3. Toluene dioxygenase (TDO) mediated oxidation of halogen-substituted benzoate esters. Semak, V.; Metcalf, T. A.; Endoma-Arias, M. A. A.; Mach, P.; Hudlicky, T. *Org. Biomol. Chem.* **2012**, *10*, 4407-4416.

4. Enantioselective, protecting group-free synthesis of 1*S*-ethyl-4-substituted quinolizidines. Amat, M.; Semak, V.; Escolano, C.; Molins, E., Bosch, J. *Org. Biomol. Chem.* **2012**, *10*, 6866-6875.

5. Synthesis of triheptanoin and formulation as a solid diet for rodents. Semak, V.; Semakova, J.; Halbaut, L.; Asó, E.; Ferrer, I.; Calpena, A.; Escolano, C.; Perales, J. C. *Eur. J. Lipid Sci. Technol.* **2012***, 114*, 889-895.

6. Triheptanoin supplementation to ketogenic diet curbs cognitive impairment in APP/PS1 mice used as a model of familial Alzheimer's disease. Asó, E.; Semakova, J.; Joda, L.; Semak, V.; Halbaut, L.; Calpena, A.; Escolano, C.; Perales, J. C.; Ferrer, I. *Curr. Alzheimer Res.* **2012***, ASAP*.

CONGRESS CONTRIBUTIONS

- Arróniz, C.; Gil-González, A.; Semak, V.; Escolano, C., Bosch, J., Amat, M.: *Rapid Construction of Enantioenriched 2,3-Dihydropyrroles by a Cooperative Brønsted Base/Lewis Acid Cascade Catalysis*. **16th French-Spanish Meeting of Organic Chemistry**, Burgos, Spain. 06/2011. Poster presentation.

- Semak, V.; Amat, M.; Arróniz, C.; Bosch, J.; Escolano, C.: *New Approach to the Conversion of Chiral Phenylglycinol Derived Lactams into Piperidones.* **14th Symposium on the Latest Trends in Organic Synthesis**, St. Catharines, Ontario, Canada. 07/2010. Poster presentation.

- Semak, V.; Escolano, C.; Amat, M.; Bosch, J.: *An Appeal to Synthetic Organic Chemistry to Resolve the Supply of TRIHEPTANOIN*. **11th International Child Neurology Congress,** El Cairo, Egypt. 05/2010. Poster presentation.

- Semak, V.; Amat, M.; Escolano, C., Bosch, J.: *Towards the First Enantioselective Synthesis of Quinolizidine Alkaloid (-)-207I.* **Symposium of Organic Chemistry**; Research Groups: Prof. J. Bosch & Prof. M. Amat (University of Barcelona); Prof. J. Cossy (ESPCI, Paris) and Prof. J. Mulzer (University of Vienna); Paris, France. 02/2009. Poster presentation.

- Semak, V.; Amat, M.; Escolano, C., Bosch, J.: *Towards the First Enantioselective Synthesis of Quinolizidine Alkaloid (-)-207I.***: Acção Integrada Luso-Espanhola FCT/UNL – FF/UB**. Lisboa, Portugal. 2008. Poster presentation.

INTERNATIONAL STUDENTSHIPS

06/2010 - 10/2010 **Short-Stay FPU** (granted by Spanish Ministry of Education)
Center: Chemistry Department, Brock University, St. Catharines, Ontario, Canada
Supervisor: Prof. Tomáš Hudlický, PhD

INTRODUCCIÓN

INTRODUCCIÓN

Esta Tesis Doctoral se presenta como *Compendio de publicaciones*. De acuerdo con la normativa vigente, la memoria está formada por un resumen global de los resultados obtenidos, cuatro capítulos que incluyen cinco publicaciones, un apartado dedicado a las conclusiones generales de la Tesis y un anexo que recoge el material suplementario de las diferentes publicaciones.[1] En cada artículo hay una sección dedicada a la introducción del tema, a los resultados y discusión, a la parte experimental y conclusiones. Asimismo, la bibliografía queda recogida en los deferentes artículos que conforman la tesis.

Los capítulos en los que se ha dividido la presente Tesis son los siguientes:

El capítulo 1 se centra en la publicación *"Enantioselective, protecting group-free synthesis of 1S-ethyl-4-substituted quinolizidines"* (Amat, M.; Semak, V.; Escolano, C.; Molins, E.; Bosch, J. *Org. Biomol. Chem.* **2012**, *10*, 6866-6875). Como resultado de la síntesis publicada en este artículo se puso a punto una nueva metodología para la desbencilación de lactamas bicíclicas derivadas del fenilglicinol que permitió redactar un manuscrito que fue aceptado para su publicación, "*A practical procedure for the removal of the phenylethanol moiety from phenylglycinol-derived lactams"* (V. Semak; C, Escolano; C. Arróniz; J. Bosch; M. Amat *Tetrahedron: Asymmetry* **2010**, *21*, 2542-2549).

En capítulo 2 se incluye la publicación "*Synthesis of triheptanoin and formulation as a solid diet for rodents*" (Semak, V.; Semakova, J.; Halbaut, L.; Asó, E.; Ferrer, I.; Calpena, A.; Escolano, C.; Perales, J. C. *Eur. J. Lipid Sci. Technol*. **2012**, *114*, 889-895). Esta publicación es fruto de una colaboración con los tres grupos de investigación de la Universidad de Barcelona: *i*) Dra. Ana Calpena (Departamento de Farmacia y Tecnología Farmacéutica), *ii*) Dr. José Carlos Perales (Departamento de Ciencias Fisiológicas – II, Facultad de Medicina, UB) y *iii*) Dr. Isidro Ferrer (Instituto de Neuropatología, Hospital Universitario de Bellvitge, UB).

[1] NOTA: En la presente tesis doctoral se utilizan las abreviaturas y acrónimos estándar de acuerdo con la revista *The Journal of Organic Chemistry* (ACS); http://pubs.acs.org/paragonplus/submission/joceah/joceah_authguide.pdf

El capítulo 3 recoge la publicación "*Toluene dioxygenase (TDO) mediated oxidation of halogen-substituted benzoate esters*" (Semak, V.; Metcalf, T. A.; Endoma-Arias, M. A. A.; Mach, P.; Hudlicky, T. *Org. Biomol. Chem*. **2012**, *10*, 4407-4416).

Y por último el capítulo 4 se centra en el artículo "*Dauben–Michno oxidative transposition of allylic cyanohydrins. Enantiomeric switch of (–)-carvone to (+)-carvone*" (Hudlicky, J. R.; Werner, L.; Semak, V.; Simionescu, R.; Hudlicky, T. *Can. J. Chem.* **2011**, *89*, 535-543).

Estos dos últimos artículos son consecuencia de una estancia breve realizada en el Departamento de Química de la Brock University (Canadá) bajo de la supervisión del Prof. Tomáš Hudlický y financiada por la beca FPU No. AP2006-03468ESTANCIA-2010.

SUMMARY

SUMMARY

CHAPTER 1 – PART A

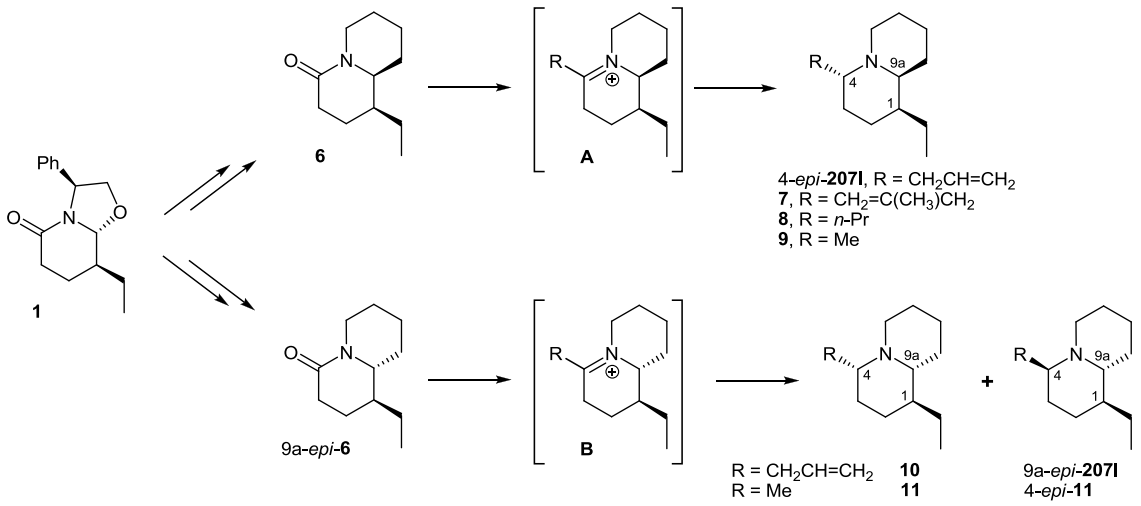
Enantioselective, Protecting Group-Free Synthesis of 1*S*-Ethyl-4-Substituted Quinolizidines

Amat, M.; Semak, V.; Escolano, C.; Molins, E.; Bosch, J. *Org. Biomol. Chem.* **2012**, *10*, 6866-6875.

Abstract

A practical enantioselective protecting group-free four-step route to the key quinolizidinone **6** from phenylglycinol-derived bicyclic lactam **1** is reported. The organometallic addition reaction upon **6** takes place stereoselectively to give 1-ethyl-4-substituted quinolizidines 4-*epi*-**207l** and **7**-**9**. Following a similar synthetic sequence, 9a-*epi*-**6** is also accessed. However, the addition of Grignard reagents upon 9a-*epi*-**6** proceeds in a non-stereoselective manner.

In order to gain insight into the different stereochemical outcome in the two series, theoretical calculations on the iminium salts **A** and **B** have been performed. The study concludes that the addition of the hydride, which is the step that determines the configuration of the final products, occurs in a stereoelectronic controlled manner.



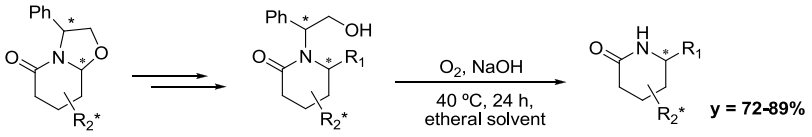
CHAPTER 1 – PART B

A practical procedure for the removal of the phenylethanol moiety from phenylglycinol-derived lactams

V. Semak; C, Escolano; C. Arróniz; J. Bosch; M. Amat *Tetrahedron: Asymmetry* **2010**, *21*, 2542-2549.

Abstract

Chiral non-racemic bicyclic lactams derived from phenylglycinol have been appointed as key building blocks for the preparation of enantiopure nitrogen compounds. The removal of the chiral inductor leading to substituted piperidones by using air or oxygen in basic media is presented in this chapter.



CHAPTER 2

Synthesis of triheptanoin and formulation as a solid diet for rodents

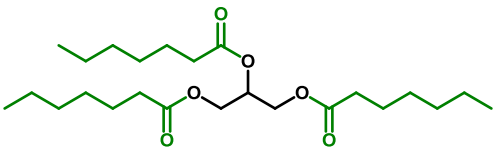
Semak, V.; Semakova, J.; Halbaut, L.; Asó, E.; Ferrer, I.; Calpena, A.; Escolano, C.; Perales, J. C.

Eur. J. Lipid Sci. Technol. **2012**, *114*, 889-895.

Abstract

In the present study, we successfully synthesized triheptanoin to the highest standards of purity from glycerol and heptanoic acid, using sulfonated charcoal as a catalyst. Triheptanoin oil was then formulated as a solid, stable and palatable preparation using a ketogenic base and a combination of four commercially available formulation agents: hydrophilic fumed silica, hydrophobic fumed silica, microcrystalline cellulose, and talc. Diet compliance and safety was tested on C57Bl/6 mice over a 15-week period, comparing overall status and body weight change.

This work provides a complete description of (*i*) an efficient and cost-effective synthesis of triheptanoin and (*ii*) its formulation as a solid, stable, and palatable ketogenic diet (triheptanoin-rich; 39% of the caloric intake) for rodents. Triheptanoin-rich diets will be helpful on pre-clinical experiments testing the therapeutic efficacy of triheptanoin in different rodent models of human diseases.



Triheptanoin, (Glycerol triheptanoate)

CHAPTER 3

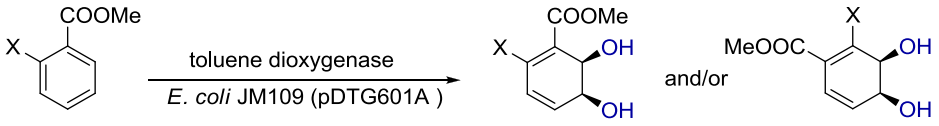
Toluene dioxygenase (TDO) mediated oxidation of halogen-substituted benzoate esters

Semak, V.; Metcalf, T. A.; Endoma-Arias, M. A. A.; Mach, P.; Hudlicky, T.

Org. Biomol. Chem. **2012**, *10*, 4407-4416.

Abstract

A series of *ortho-, meta-,* and *para*-halogen-substituted methyl benzoate esters was subjected to enzymatic dihydroxylation *via* the whole-cell fermentation with *E. coli* JM109 (pDTG601A). Only *ortho*-substituted benzoates were metabolized. Methyl 2-fluorobenzoate yielded one diol regioselectively whereas methyl 2-chloro-, methyl 2-bromo- and methyl 2-iodobenzoates each yielded a mixture of regioisomers. Absolute stereochemistry was determined for all new metabolites. Computational analysis of these results and a possible rationale for the regioselectivity of the enzymatic dihydroxylation is advanced.



X = F, Cl, Br, I

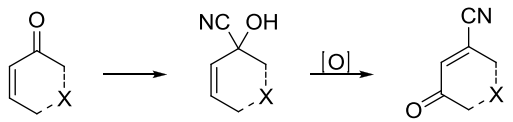
CHAPTER 4

Dauben–Michno oxidative transposition of allylic cyanohydrins
Enantiomeric switch of (–)-carvone to (+)-carvone

Hudlicky, J. R.; Werner, L.; Semak, V.; Simionescu, R.; Hudlicky, T. *Can. J. Chem.* **2011**, *89*, 535-543.

Abstract

Allylic cyanohydrins were subjected to Dauben–Michno oxidation at low temperatures to provide β-cyanoenones in good to excellent yields. The potential of this oxidative transposition as a means of an enantiomeric switch of enones containing a latent plane of symmetry was tested by conversion of (–)-carvone to its enantiomer.



Acyclic and cyclic enones
X = 0, CH_2, $(CH_2)_2$,

RESUMEN

RESUMEN

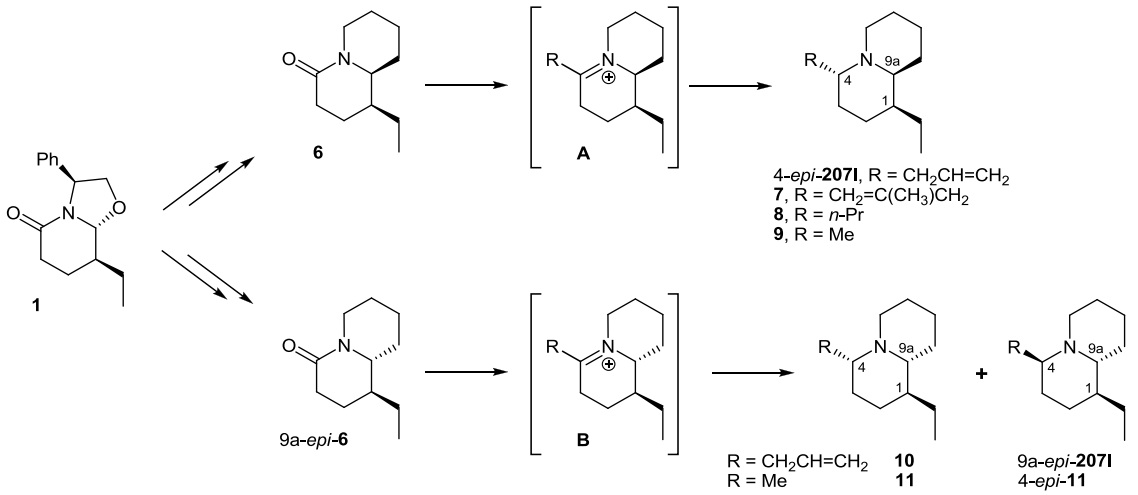
CAPÍTULO 1 – PARTE A

Enantioselective, Protecting Group-Free Synthesis of 1*S*-Ethyl-4-Substituted Quinolizidines

Amat, M.; Semak, V.; Escolano, C.; Molins, E.; Bosch, J. *Org. Biomol. Chem.* **2012**, *10*, 6866-6875.

Se ha descrito una síntesis enantioselectiva que no implica grupos protectores para acceder a la quinolizidina clave **6** a partir de la lactama bicíclica derivada de fenilglicinol **1**. La adición de un reactivo organometálico a **6** ocurre de manera estereoselectiva para conducir a las quinolizidinas 1*S*-etil-4-sustituidas 4-*epi*-**207l** y **7**-**9**. Siguiendo una secuencia sintética análoga, se preparó el compuesto 9a-*epi*-**6**. Sin embargo, la adición de reactivos de Grignard al compuesto 9a-*epi*-**6** no ocurre de manera estereoselectiva.

Para explicar las diferencias en el comportamiento estereoquímico de las dos series se han llevado a cabo estudios teóricos centrados en las sales de iminio **A** y **B**. El estudio concluye que la adición del ión hidruro, que es el paso determinante en la configuración del producto final, ocurre de una manera estereocontrolada.

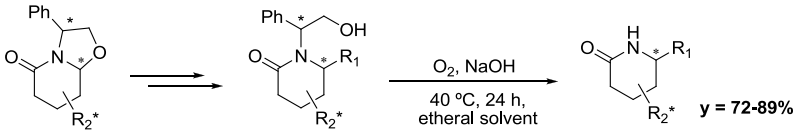


CAPÍTULO 1 – PARTE B

A practical procedure for the removal of the phenylethanol moiety from phenylglycinol-derived lactams

V. Semak; C, Escolano; C. Arróniz; J. Bosch; M. Amat *Tetrahedron: Asymmetry* **2010**, *21*, 2542-2549.

Las lactamas bicíclicas no racémicas derivadas de fenilglicinol se han relevado como intermedios clave en la preparación de compuestos nitrogenados enantiopuros. En este capítulo se describe la eliminación del inductor quiral de piperidonas sustituidas utilizando aire u oxígeno en medio básico.



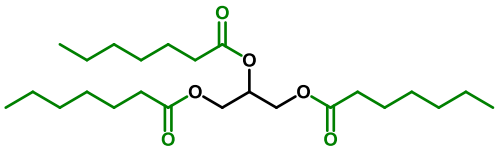
CAPÍTULO 2

Synthesis of triheptanoin and formulation as a solid diet for rodents

Semak, V.; Semakova, J.; Halbaut, L.; Asó, E.; Ferrer, I.; Calpena, A.; Escolano, C.; Perales, J. C.
Eur. J. Lipid Sci. Technol. **2012**, *114*, 889-895.

En el presente estudio se describe la síntesis eficaz de triheptanoina de elevada pureza a partir de glicerol y ácido heptanoico, en presencia de carbono sulfonado como catalizador. La triheptanoina se formula como un sólido estable para que constituya la base de una dieta cetogénica mediante la combinación de custro gentes de formulación comerciales; dos tipos de sílica, celulosa microcristalina y talco. La adecuación de la dieta se prueba en ratones C57Bl/6 en un periodo de 15 días, comparando el estado general y el cambio de peso corporal.

El trabajo implica la descripción de (*i*) una síntesis efectiva y de reducido coste de triheptanoina y (*ii*) su formulación como un sólido estable e integrante de una dieta cetogénica para roedores (dieta rica en tripetanoina implica el 39% del consumo calórico). Las dietas ricas en triheptanoina son útiles para los experimentos preclínicos que sirven para testar la eficacia terapéutica de la triheptanoina en diferentes modelos animales relacionados con enfermedades humanas.



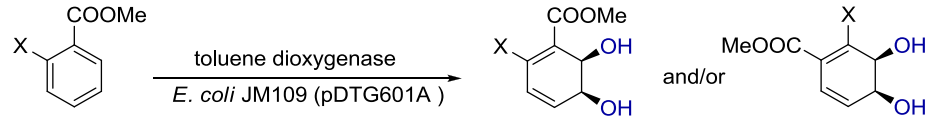
Triheptanoin, (Glycerol triheptanoate)

CAPÍTULO 3

Toluene dioxygenase (TDO) mediated oxidation of halogen-substituted benzoate esters

Semak, V.; Metcalf, T. A.; Endoma-Arias, M. A. A.; Mach, P.; Hudlicky, T.
Org. Biomol. Chem. **2012**, *10*, 4407-4416.

Una serie de esteres benzoicos metílicos sustituidos en *o*-, *m*- y *p*- se han sometido a hidroxilación enzimática *via* fermetación con *E. coli* JM109 8pDTG601A). Solo se metabolizaron los benzoatos sustituidos en orto. El 2-fluorobenzoato de metilo produjo regioselectivamente solo un diol mientras que el 2-cloro, 2-bromo y 2-iodobenzoato de metilo proporciona una mezcla de regioisómeros. La configuración absoluta se determinó a partir de nuevos metabolitos. Se incluye el análisis computacional para racionalizar la regioselectividad enzimática de la reacción de hidroxilación.



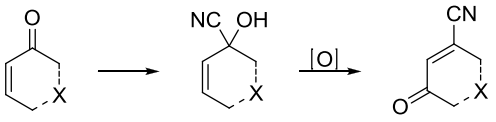
X = F, Cl, Br, I

CAPÍTULO 4

Dauben–Michno oxidative transposition of allylic cyanohydrins
Enantiomeric switch of (–)-carvone to (+)-carvone

Hudlicky, J. R.; Werner, L.; Semak, V.; Simionescu, R.; Hudlicky, T. *Can. J. Chem.* **2011**, *89*, 535-543.

Cuando cianohidrinas alílicas se someten a la reacción de oxidación de Dauben-Michno a bajas temperaturas se accede a β-cianoenonas en buenos a excelentes rendimientos. El potencial de esta trasposición oxidativa se pone de manifiesto en enonas que contienen un plano latente de simetría como por ejemplo la conversión de la (-)-carvona en su enantiomero.



Acyclic and cyclic enones
X = 0, CH_2, $(CH_2)_2$,

CHAPTER 1 – PART A

Enantioselective, Protecting Group-Free Synthesis of 1*S*-Ethyl-4-Substituted Quinolizidines

Amat, M.; Semak, V.; Escolano, C.; Molins, E.; Bosch, J.
Org. Biomol. Chem. **2012**, *10*, 6866-6875.

Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 6866

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PAPER

Enantioselective, protecting group-free synthesis of 1*S*-ethyl-4-substituted quinolizidines†

Mercedes Amat,*[a] Vladislav Semak,[a] Carmen Escolano,*[a] Elies Molins[b] and Joan Bosch[a]

Received 23rd February 2012, Accepted 30th March 2012
DOI: 10.1039/c2ob25392e

A practical enantioselective protecting group-free four-step route to the key quinolizidinone **6** from phenylglycinol-derived bicyclic lactam **1** is reported. The Grignard addition reaction to **6** takes place stereoselectively to give 1-ethyl-4-substituted quinolizidines 4-*epi*-**207I** and **7–9**. Following a similar synthetic sequence, 9a-*epi*-**6** is also accessed. However, the addition of Grignard reagents to 9a-*epi*-**6** proceeds in a non-stereoselective manner. In order to gain insight into the different stereochemical outcome in the two series, theoretical calculations on the iminium salts **A** and **B** have been performed. The study concludes that the addition of the hydride, which is the step that determines the configuration of the final products, occurs in a stereoelectronic controlled manner. The theoretical study is in agreement with the experimental results.

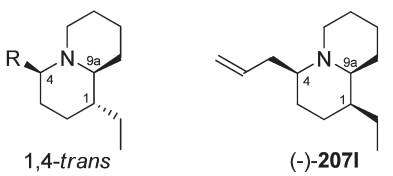
Introduction

The extracts from the skin of certain poisonous frogs and toads contain alkaloids showing promising biomedical activities. So far, none of these alkaloids has been reported from any other natural source. Most of them contain as a common structural feature an azabicyclic "izidine" nucleus, *e.g.* disubstituted pyrrolizidines, di- and trisubstituted indolizidines, or disubstituted quinolizidines.[1]

Among them, 1,4-disubstituted quinolizidines[2] are a relatively new class of alkaloids that have been isolated in minute quantities. Their structures have been partially elucidated based on the GC-MS and GC-FTIR spectra, the latter showing significant Bohlmann bands[3] indicating that the hydrogens at positions 4 and 9a are *cis*. The relative configuration at position 1 is only tentative and the absolute configuration of the stereocenters is unknown. As total synthesis is required for structural proof, it would be highly desirable to develop general asymmetric methodologies to easily access these biologically interesting compounds.

There are currently about 20 compounds assigned to this particular structural family of amphibian alkaloids[4] including seven 1-ethylquinolizidine representatives (Fig. 1). Among them, six show a 1,4-*trans* relative configuration while alkaloid (−)-**207I** is unique, with substituents being 1,4-*cis*.

The relative stereochemistry of natural quinolizidine (−)-**207I** was determined in 1997 by Momose's group by comparison of the GC-MS and GC-FIT spectra of the 1-*epi*-**207I** isomer synthesized by them and the natural alkaloid.[5] In 2003, Toyooka and co-workers published the first enantioselective synthesis of (+)-**207I**, the enantiomer of the alkaloid, thus determining the absolute stereochemistry of the natural product.[6] More recently, the same research team reported the synthesis of **233A**, **235U** and **251AA** alkaloids.[7] Although pioneering, these syntheses suffer from the drawback of requiring a considerable number of synthetic steps, leading to a low yield of the final product, partly due to the use of protecting groups. Therefore, a general and



R = C_4H_7, **221X**
R = $CH_2CH{=}CHC{\equiv}CH$, **231A**
R = $(CH_2)_3C{\equiv}CH$, **233A**
R = $(CH_2)_3CH{=}CH_2$, **235U**
R = $(CH_2)_4C{\equiv}CH$, **247D**
R = n-C_6H_{13}, **251AA**

Fig. 1 1-Ethyl-4-substituted quinolizidines.

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†Electronic supplementary information (ESI) available: Additional experimental information. Copies of ^{1}H and ^{13}C NMR spectra of new products. Cartesian coordinates and total energies for compounds **9**, 4-*epi*-**9** and iminium salts **A** and **B**. CCDC 861638. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob25392e

efficient procedure providing access to different 1,4-disubstituted quinolizidine alkaloids would be of interest.

Interestingly from the biological point of view, 1-*epi*-**207I** selectively blocks α7 nicotinic receptors (IC_{50} = 0.6 μM).[8] In contrast, alkaloids **233A**, **235U** and **251AA** block the responses mediated by α7 and α4β2 nicotinic receptors without any selectivity observed between both subtypes. Comparing the latter compounds with 1-*epi*-**207I** led the authors to conclude that the α7 subtype selectivity of 1,4-disubstituted quinolizidines is remarkably dependent on the structure of the C4 side chain. Increasing the length of the 4-moiety beyond three carbons appears to markedly reduce potency and selectivity at the α7 receptor.

In this paper we disclose a general protocol for the enantioselective construction of these biologically attractive structural motifs using chiral bicyclic lactams as enantiomeric scaffolds.[9] Taking into account the aforementioned biological studies, we planned to attach a 1 to 3 carbon chain at the C4 position of the azabicyclic nucleus.

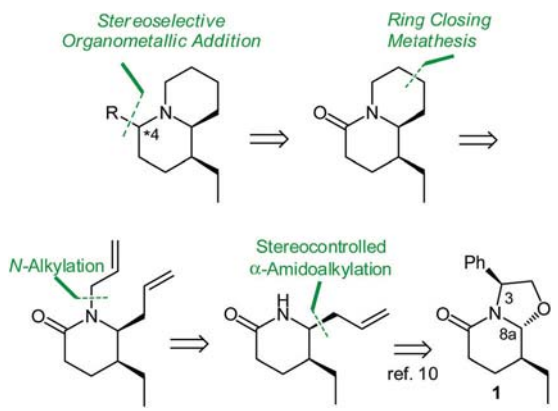
Results and discussion

Retrosynthetic analysis

Our retrosynthetic analysis is briefly outlined in Scheme 1. We envisioned that the alkyl group at C-4 could be installed by a stereoselective addition of an organometallic reagent to the carbonyl amide group of a 1-ethyl quinolizidine derivative. This bicyclic lactam was surmised to be constructed by a ring-closing metathesis reaction of a monocyclic diallylated derivative. In turn, the required trisubstituted 2-piperidone would be obtained by alkylation of (5*S*,6*S*)-6-allyl-5-ethyl-2-piperidone, whose enantiomer had been previously synthesized by our group by an α-amidoalkylation reaction[10] of the enantiomer of the chiral bicyclic lactam **1**.[11]

Preparation of (5*S*,6*S*)-6-allyl-5-ethyl-2-piperidone (2)

As previously reported by our group in the enantiomeric series, the $TiCl_4$-promoted addition of allyltrimethylsilane to **1** occurs stereoselectively, with inversion of the configuration at C8a to afford a 9 : 1 mixture of *cis*-6-allyl-5-ethyl disubstituted lactam **2** and its C6 epimer, 6-*epi*-**2**. The absolute stereochemistry of **2** was unambiguously confirmed by X-ray crystallographic analysis (Fig. 2). The configuration of the 5 and 6 stereocenters remains in the final product since both of them are configurationally stable in the subsequent synthetic transformations (Scheme 2).



Scheme 1 Retrosynthetic analysis.

Preparation of azabicyclic compound 6

To construct the second six-membered ring from **2**, removal of the 2-phenylethanol moiety and installation of a second allyl moiety was required. To this end, treatment of **2** with Na/liquid NH_3 afforded **3** in 81% yield. Transformation of **3** into the diolefinic compound **4** was accomplished by deprotonation with NaH followed by alkylation with allyl bromide. More conveniently, we developed a one-pot two-step process from **2** to **4**

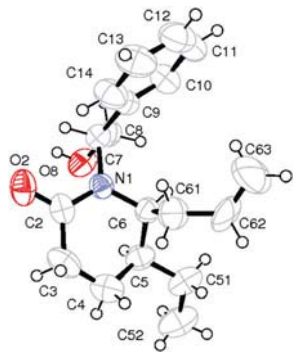
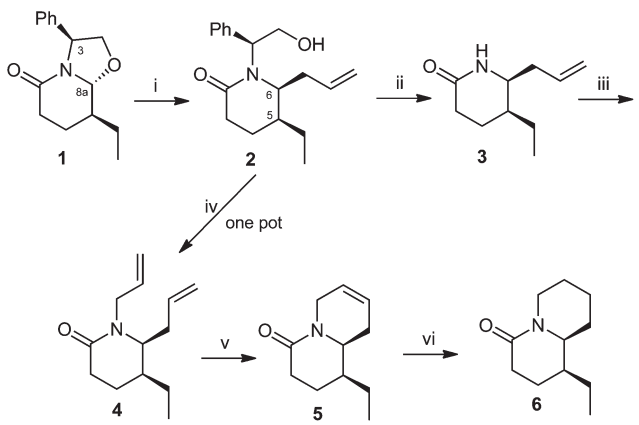


Fig. 2 X-Ray structure of (5*S*,6*S*)-6-allyl-5-ethyl-1-[(1*S*)-2-hydroxy-1-phenylethyl]-2-piperidone (**2**). Molecules are linked along the *a*-axis of the crystal through the hydrogen bond O8–H8···O2^{i} (*d*(O···O) = 2.722(3) Å, angle(O–H···O) = 171.2°, *i* = [$x - 1\frac{1}{2}$, $\frac{1}{2} - y$, z]) forming zigzag motifs.



Scheme 2 Reagents and conditions: (i) allyltrimethylsilane (2.0 equiv), $TiCl_4$ (4.0 equiv), CH_2Cl_2, 0 °C to rt, 16 h, **2** (77%) and 6-*epi*-**2** (7%); (ii) Na (metal), NH_3 (*l*), 30 min, −33 °C, 81%; (iii) NaH (2.0 equiv), allyl bromide (1.1 equiv), THF, 0 °C to rt, 24 h, 75%; (iv) (a) NaOH (10 equiv), O_2 (1 atm), MTBE, 40 °C, 24 h; (b) allyl bromide (1.25 equiv), rt, 19 h, 69% overall; (v) second-generation Grubbs cat. (2.5 mol%), CH_2Cl_2, rt, 8 h, 87%; (vi) H_2 (1 atm), 10% Pd/C, MeOH, rt, 18 h, 87%.

involving removal of the 2-phenylethanol moiety and alkylation in the same vessel in basic media by sequential treatment with O_2 and allyl bromide (69% overall yield).[12] With all the carbon atoms installed in compound **4** a ring-closing metathesis reaction could be performed and the required six-membered piperidine ring accessed. A second-generation ruthenium Grubbs catalyst mediated this reaction from **4** to generate **5** (87%) under mild conditions.[13] Chemoselective reduction of **5** employing catalytic hydrogenation was accomplished to give **6**, which is a valuable synthetic intermediate for the formation of the targeted diversely 4-substituted 1-ethylquinolizidines.

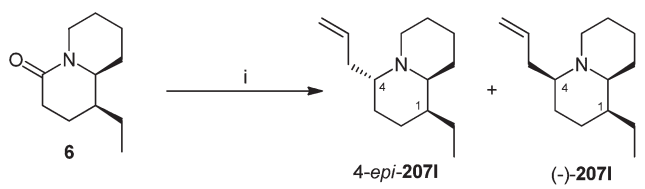
Addition of Grignard reagents to azabicyclic compound 6

With bicyclic lactam **6** in hand, the stage was set to execute the installation of an alkyl substituent at carbon 4. To this end, a one-pot procedure, involving the reaction of **6** with Grignard reagents followed by dehydration to the corresponding iminium salts and reduction to the final products, was studied.

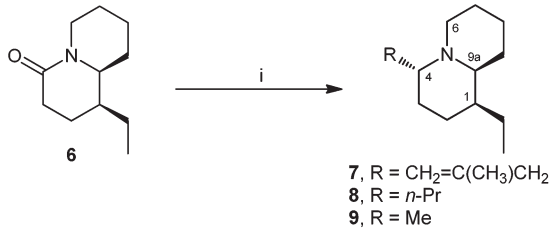
In order to access alkaloid (−)-**207I** we first considered the addition of allylmagnesium bromide. Interestingly, while there are several reports dealing with the addition of organometallic reagents to the 2-quinolizidinone nucleus,[14] to the best of our knowledge, the addition of an allylmetallic reagent is unprecedented.[15] Only after much experimentation did we find that the reaction indeed occurs using an excess of Grignard reagent (4 equiv) in the presence of anhydrous $CeCl_3$.[16]

Being aware that the reduction step would be responsible for the stereochemistry of the final product, different reducing agents were evaluated. While reduction with $NaBH_3CN$ or $NaBH(AcO)_3$ gave inseparable mixtures of (−)-**207I** and its C4-epimer, 4-*epi*-**207I**, (34 : 66 and 23 : 77, respectively), $NaBH_4$ and DIBAL-H furnished 4-*epi*-**207I** with a high degree of diastereoselectivity (3 : 97 and 4 : 96, respectively) (Scheme 3).[17]

In order to access C4 analogues of the alkaloid (−)-**207I**, the addition of different Grignard reagents was studied. In all cases, the alkyl chain introduced was limited to a length of up to three carbon atoms, taking into account previous biological studies.[7,8] To this end, an excess of (2-methylallyl)magnesium bromide was added to **6**, followed by treatment with $NaBH_4$ to give **7** and 4-*epi*-**7** (96 : 4) in 56% yield. Following similar experimental conditions, a propyl and a methyl chain were also introduced to stereoselectively furnish **8** and **9** in 64 and 69% yield, respectively (Scheme 4).



Scheme 3 Reagents and conditions: (i) (a) $CeCl_3$ (4.0 equiv), allylmagnesium bromide (4.0 equiv), THF, rt, 18 h; (b) $NaBH_4$ (1.25 equiv), MeOH, AcOH, −78 °C, 30 min, 58%, d.r. = 97 : 3.



Scheme 4 Reagents and conditions: (i) (a) $CeCl_3$ (2.0 equiv), 2-methylallylmagnesium chloride (8.0 equiv), propylmagnesium bromide (4.0 equiv) or methylmagnesium bromide (4.0 equiv), THF, rt, 18 h; (b) $NaBH_4$ (1.25 equiv), MeOH, AcOH, −78 °C, 30 min, 56%, **7**; 64% **8**; 69%, **9**.

Table 1 Significant ^{13}C NMR data for bicyclic amines

Carbon	(−)-**207I**[a]	4-*epi*-**207I**	**7**	**8**	**9**
1	40.6	41.9	41.8	42.1	41.8
4	64.2	50.9	49.7	51.2	46.7
9a	66.7	61.1	61.1	61.2	60.8
6	53.1	50.1	50.3	50.2	50.1

[a] δ values from ref. 6.

Table 2 Significant ^{1}H NMR data for bicyclic amines

Carbon	(−)-**207I**[a]	4-*epi*-**207I**	**7**	**8**	**9**
1	1.55	1.53	1.54	1.52	1.58
4	1.88	3.00	3.03	2.88	2.98
9a	1.96	2.93	2.94	2.93	2.92
6_{eq}	3.33	3.34	3.32	3.30	3.27
6_{ax}	1.54	2.71	2.69	2.66	2.67

[a] δ values from ref. 6.

Structural elucidation of the final bicyclic amines

The absolute configuration of the C4 stereogenic center in (−)-**207I** and 4-*epi*-**207I** was assigned by correlation of the NMR data of these two compounds with those reported for the previously synthesized enantiomer, (+)-**207I**.[6]

It is worthy of note that in 4-*epi*-**207I** the peaks corresponding to C-6 and C-9a are about 5 and 3 ppm more shielded, respectively, than in (−)-**207I** (Table 1). This shielding probably reflects the γ-gauche effect due to an axial disposition of the allyl substituent in 4-*epi*-**207I**. Comparison of the ^{13}C NMR and ^{1}H NMR (Table 2) of compounds **7–9** with that of 4-*epi*-**207I** led to the depicted configuration being assigned to the C-4 stereocenter of these new products (Scheme 4).[18]

In fact, full geometry optimization of **9** and its hypothetical C4 epimer (4-*epi*-**9**), performed with the B3LYP density functional method using the 6-31G(d) basis set, revealed that while both compounds were in a chair–chair conformation, only **9** displayed its 4-methyl group in an axial manner (Fig. 3), while the methyl group in 4-*epi*-**9** was in an equatorial disposition (Fig. 4). Thus, theoretical calculations are in concordance with the ^{13}C-NMR observations.

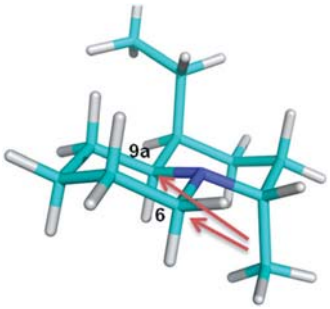


Fig. 3 The most stable conformation of compound **9** (γ-gauche effects are indicated).

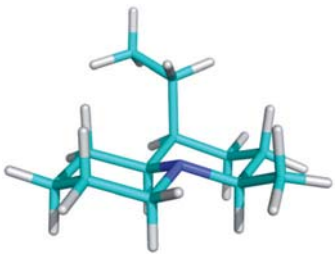


Fig. 4 The most stable conformation of hypothetical compound 4-*epi*-**9**.

Synthesis of 9a-*epi* derivatives of 4-substituted 2-ethylquinolizidines

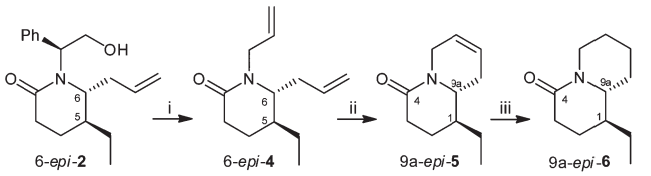
With compounds 6-*epi*-**2** in hand (Scheme 2), we decided to go a step further and apply the developed procedure to prepare 9a-*epi*-**6** to study the stereochemical outcome of the Grignard addition reactions in compounds with a C9a *R* configuration. Thus, the introduction of an allyl group on the piperidone nitrogen of 6-*epi*-**2** gave compound 6-*epi*-**4** in 56% yield. Subsequent treatment with Grubbs second-generation catalyst furnished 9a-*epi*-**5**, which was hydrogenated to give 9a-*epi*-**6** with yields comparable to those obtained in the previous series (Scheme 5).

The addition reaction of allylmagnesium bromide in the presence of anhydrous $CeCl_3$ to 9a-*epi*-**6**, followed by $NaBH_4$ reduction, occurs in a non-stereoselective manner to give a 1 : 1 mixture of **10** and 9a-*epi*-**207I**. Similar results were observed in the reaction of 9a-*epi*-**6** with methylmagnesium bromide, yielding an equimolecular mixture of the two possible products (Scheme 6). These results are in striking contrast with the stereochemical behaviour of the additions previously studied in **6**, which occurred with very high stereoselectivity.

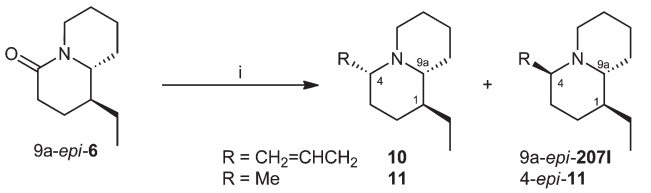
The structural assignation of these quinolizidines was done by comparison with the spectroscopic data of compound **10** whose enantiomer had been previously described in the literature.[5,19]

Theoretical considerations of the stereochemical outcome in the introduction of the C-4 substituent

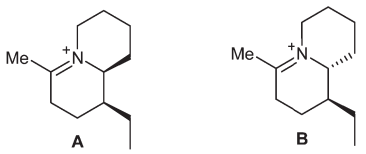
As previously mentioned, the stereochemistry of the organometallic addition reaction is determined by the attack of the hydride to the iminium salt. In order to gain insight into the stereochemistry of the final products, studies on the relative



Scheme 5 Reagents and conditions: (i) (a) NaOH (10 equiv), O_2 (1 atm.), MTBE, 40 °C, 24 h; (b) allyl bromide (1.25 equiv), rt, 19 h, 56% overall; (ii) second-generation Grubbs cat. (3 mol %), CH_2Cl_2, rt, 6 h, 90%; (iii) H_2 (1 atm.), 10% Pd/C, MeOH, rt, 16 h, 98%.



Scheme 6 Reagents and conditions: (i) (a) $CeCl_3$ (2.0 equiv), allylmagnesium bromide or methylmagnesium bromide (4.0 equiv), THF, rt, 18 h; (b) $NaBH_4$ (1.25 equiv), MeOH, AcOH, −78 °C, 30 min, **10** and 9a-*epi*-**207I** (54%); **11** and 4-*epi*-**11** (48%).



Scheme 7 Intermediate iminium salts.

stability of different conformers of the iminium ion intermediates when a methyl substituent is attached were considered (Scheme 7). Indeed, exploration of the different conformational states for the iminium salts **A** and **B**, at the B3LYP/6-31 (G) level, gave two main possible conformations, depending on the disposition adopted by the ethyl substituent. Starting from **A**, the non-substituted six-membered ring of the quinolizidine adopts a chair conformation in both iminium forms. As expected, the substituted ring adopts a half-chair conformation with the C3, C4, N and C9a atoms in the same plane, with the ethyl group either in an equatorial, **AI**, or axial disposition, **AII** (Fig. 5 and 6), **AI** being more stable by 3.1 kcal mol^{-1}. This fact could be ascribed to a 1,3-diaxial destabilizing interaction in **AII** between the axial ethyl substituent and the axial hydrogens at positions 3 and 9. On the basis of these findings, the stereochemical outcome of the addition reaction can be explained considering an axial attack of the hydride, under stereoelectronic control,[20] at the electrophilic carbon of the lowest-energy iminium ion intermediate, **AI**. This directed nucleophile attack dictates an *S* configuration in the newly created stereocenter, as depicted in Fig. 5.

In a similar fashion, in the two possible conformations of the iminium salt **B**, the non-substituted ring adopts a chair conformation, whereas the substituted ring shows a half-chair conformation with the C3, C4, N and C9a atoms in the same plane. However, in the iminium salt **B** both possible conformations, **BI**, with the ethyl group in an equatorial disposition, and **BII**, with the ethyl group in an axial disposition, are energetically similar

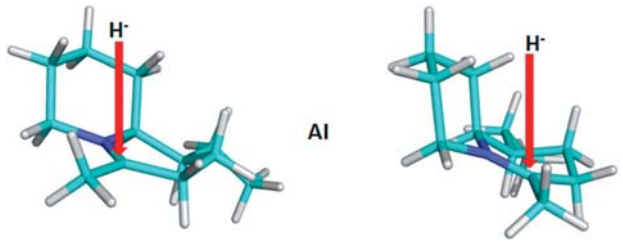


Fig. 5 Two views of the most stable conformation of the iminium salt intermediate **AI** and indication of the hydride stereocontrolled addition.

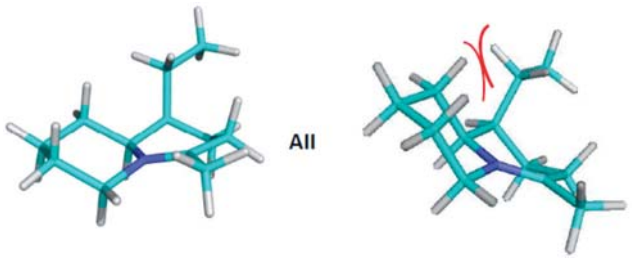


Fig. 6 Two views of the most stable conformation of the iminium salt intermediate **AII**. 1,3-Diaxial destabilizing interactions are indicated.

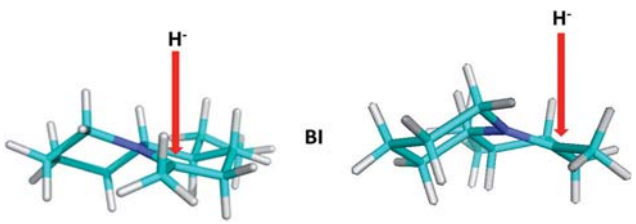


Fig. 7 Two views of the most stable conformation of the iminium salt intermediate **BI** and indication of the hydride stereocontrolled addition.

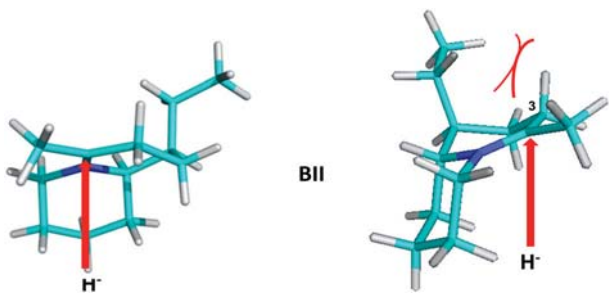


Fig. 8 Two views of the most stable conformation of the iminium salt intermediate **BII** and indication of the hydride stereocontrolled addition. 1,3-Diaxial destabilizing interaction is indicated.

(ΔE < 1 kcal mol^{-1}) (Fig. 7 and 8). The smaller energy gap between both conformations may be indicative of a relative stabilization of the conformation **BII** in comparison with **AII** because only one 1,3-diaxial destabilizing interaction (ax-Et/ax-H_3) is found in the former. Consequently, as both conformations are of similar energies, the stereoelectronic controlled addition of the hydride upon the iminium salt can occur on both **BI** and **BII**, yielding compounds **11** and 4-*epi*-**11** in nearly equal amounts.

Conclusions

In conclusion, we have developed a straightforward enantioselective synthesis of potentially biologically interesting 1-ethyl-4-substituted quinolizidines without using protecting groups. The stereochemistry of the stereocenters is defined by the use of (*S*)-phenylglycinol as the source of chirality and by two stereocontrolled reactions, an α-amidoalkylation and an organometallic addition. Compounds in the 9a-*epi* series have been efficiently obtained following the synthetic sequence developed in the original series. However, the final organometallic addition reaction proved to be not stereoselective. In order to rationalize the different stereochemical outcome in the Grignard addition reactions in the two series, theoretical calculations on the iminium salts were performed indicating that the addition of the hydride occurs in a stereoelectronic controlled fashion.

Experimental section

General methods

NMR spectra were recorded in $CDCl_3$ at 300 or 400 MHz (^{1}H) and 75.4 or 100.6 MHz (^{13}C), and chemical shifts are reported in δ values downfield from TMS or relative to residual chloroform (7.26 ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, multiplicity, coupling constant (*J*) in hertz (Hz), integrated intensity. Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; br s, broad signal, app, apparent. Evaporation of solvents was accomplished with a rotatory evaporator. Melting points were determined in a capillary tube and are uncorrected. Thin-layer chromatography was done on SiO_2 (silica gel 60 F_{254}), and the spots were located by UV, 1% aqueous $KMnO_4$ or iodoplatinate (for tertiary amines). Chromatography refers to flash column chromatography and was carried out on SiO_2 (silica gel 60, SDS, 230–400 mesh) or Al_2O_3 (Aluminium oxide 90 active basic, Merck). Mass spectra were recorded on a LTQ spectrometer using electrospray (ES^+) ionization techniques.

(5*S*,6*S*)-6-Allyl-5-ethyl-1-[(1*S*)-2-hydroxy-1-phenylethyl]-2-piperidone, (2). $TiCl_4$ (20 mL, 182.12 mmol) was slowly added to a cooled (0 °C) solution of **1** (11.17 g, 45.53 mmol) in anhydrous CH_2Cl_2 (90 mL) and the mixture was stirred for 15 min. Allyltrimethylsilane (14.5 mL, 91.06 mmol) was added in 3 portions and the resulting mixture was warmed at rt and stirred for 16 h. The mixture was poured onto ice and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried, filtered and concentrated to give a 90 : 10 (^{1}H NMR) mixture of **2** and 6-*epi*-**2**, which was purified by column chromatography (Biotage Si 40M 2197-1, CH_2Cl_2–MeOH 99.5 : 0.5 to 97 : 3) to yield **2** (10.02 g, 77%) and C6-*epi*-**2** (0.87 g, 7%). **2**:[10] m.p. 75.0–76.0 °C (cyclohexane/pentane); $[\alpha]_D^{22}$ = +21.3 (*c* 0.45, $CHCl_3$). Anal. Calcd for $C_{18}H_{25}NO_2$: C, 75.22; H, 8.77; N, 4.87. Found: C, 75.29; H, 8.68; N, 4.94%.

(5*S*,6*R*)-6-Allyl-5-ethyl-1-[(1*S*)-2-hydroxy-1-phenylethyl]-2-piperidone, (6-*epi*-2). IR (NaCl) 3383, 2958, 1624 (s, NCO), 1452 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.62 (t, *J* = 8.2 Hz, 3H, CH_2CH_3), 1.01 (m, 1H, CH_2CH_3), 1.17 (m, 1H, CH_2CH_3),

1.51 (m, 1H, H-4), 1.61 (m, 1H, H-5), 1.99 (m, 1H, H-4), 2.28 (m, 1H, CH_2CH═), 2.41–2.46 (m, 3H, H-3, CH_2CH═), 3.06 (dm, J = 10.5 Hz, 1H, H-6), 3.96 (br s, 1H, OH), 4.16 (m, 1H, H-2′), 4.24 (m, 1H, H-2′), 5.01 (d, J = 4.5 Hz, 1H, CH═CH_2), 5.05 (m, 1H, CH═CH_2), 5.41 (dd, J = 8.1, 5.1 Hz, 1H, H-1′), 5.57 (m, 1H, CH═CH_2), 7.26–7.37 (m, 5H, H–Ar); ^{13}C NMR (100.6 MHz) δ 11.3 (CH_2CH_3), 22.2 (C-4), 24.3 (CH_2CH_3), 28.5 (C-3), 35.4 (C-5), 39.3 (CH_2CH═), 59.1 (C-6), 62.7 (C-1′), 63.6 (C-2′), 117.7 (CH═CH_2), 127.8 (CHAr),128.3 (2CHAr), 128.5 (2CHAr), 134.0 (CH═CH_2), 136.9 (CAr), 172.4 (NCO); $[\alpha]_D^{22}$ −19.6 (c 1.0, $CHCl_3$); HRMS $C_{18}H_{26}NO_2$ $[M + H]^+$ 288.1958; found, 288.1958.

(5*S*,6*S*)-6-Allyl-5-ethyl-2-piperidone, (3). Into a three-necked, round-bottomed flask equipped with a cold-finger condenser charged with dry ice–acetone was condensed NH_3 (*ca*. 150 mL) at −78 °C. The temperature was allowed to rise to −33 °C and a solution of alcohol **2** (1.00 g, 3.48 mmol) in THF (5 mL) was added, followed by the addition of sodium metal in small portions until the blue colour persisted. After the mixture was stirred at −33 °C for 30 min, the reaction was carefully quenched by the addition of solid NH_4Cl until the blue colour disappeared. The mixture was stirred at rt overnight, the residue was partitioned between H_2O and CH_2Cl_2, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried, filtered and concentrated. The resulting residue was chromatographed (SiO_2, hexane–EtOAc 1 : 1 to 1 : 2) to give **3** as a white solid (0.47 g, 81%) and **2** (0.07 g, 7%). IR (NaCl) 3208, 2959, 1664 (s, NCO) cm^{-1}; ^{1}H NMR (300 MHz, COSY) δ 0.96 (t, J = 7.4, 3H, (CH_2CH_3), 1.52–1.24 (m, 2H, CH_2CH_3), 1.70–1.86 (m, 3H, H-4, H-5), 2.02–2.12 (m, 1H, CH_2CH═), 2.21–2.40 (m, 3H, CH_2CH═, H-3), 3.39–3.50 (m, 1H, H-6), 5.16 (d, J = 7.6, 1H, CH═CH_2), 5.20 (d, J = 0.7, 1H, CH═CH_2), 5.65–5.82 (m, 1H, CH═CH_2), 5.83 (br s, 1H, NH); ^{13}C NMR (75.4 MHz) δ 11.6 (CH_2CH_3) 20.7 and 22.7 (C-4/CH_2CH_3), 29.1 (C-3), 36.3 (CH_2CH═), 37.3 (C-5), 54.8 (C-6), 119.1 (CH═CH_2), 134.0 (CH═CH_2), 172.0 (NCO); $[\alpha]_D^{22}$ = −66.32 (c 1.03, MeOH); HRMS $C_{10}H_{18}NO$ $[M + H]^+$ 168.1383; found, 168.1382.

(5*S*,6*S*)-1,6-Diallyl-5-ethyl-2-piperidone, (4). *Method A*. A solution of **3** (656 mg, 3.93 mmol) in THF (20 mL) was added *via* cannula to NaH (320 mg, 7.85 mmol, 60% dispersion in mineral oil). After 15 min, the reaction mixture was cooled (0 °C) and allyl bromide (380 μL, 4.32 mmol) was added with a syringe pump over 60 min. Then, the mixture was warmed up at rt and stirred for 24 h. The reaction mixture was cooled (0 °C), quenched by the addition of water (10 mL), and extracted with EtOAc. The combined organic extracts were dried and concentrated to give a residue, which was chromatographed (hexane–EtOAc 4 : 1) to furnish **4** (613 mg, 75%) as a yellow oil.

Method B. In a round-bottomed flask equipped with a 1 gallon gas bag of O_2, an excess of freshly ground NaOH (2.78 g, 69.6 mmol) was added to a solution of **2** (2.00 g, 6.96 mmol) in MTBE (20 mL). The mixture was heated at 40 °C and stirred slowly at this temperature for 24 h. The progress of reaction was monitored by TLC and, when **2** was consumed, the gas bag was disconnected and the reaction mixture was cooled to rt. Allyl bromide (0.75 mL, 8.70 mmol) was slowly added and stirring was continued for an additional 19 h at rt. The solvent was removed, the residue was partitioned between H_2O and CH_2Cl_2, and the aqueous layer was extracted with CH_2Cl_2. The organic extracts were washed with saturated NH_4Cl solution, dried and concentrated to give a residue, which was purified by column chromatography (hexane–EtOAc 4 : 1) to yield **4** (994 mg, 69%) as a yellow oil. IR (NaCl film) 2932, 1642 (s, NCO) cm^{-1}; ^{1}H NMR (300 MHz, COSY, HSQC) δ 0.93 (t, J = 7.4 Hz, 3H CH_2CH_3), 1.31–1.42 (m, 2H, –CH_2CH$_3$), 1.58–1.83 (m, 3H, H-4 and H-5), 2.23 (m, 1H, H-1′), 2.36–2.49 (m, 3H, H-3 and H-1′), 3.35 (m, 1H, H-1″), 3.40 (m, 1H, H-6), 4.71 (dddd, J = 15.3, 4.2, 1.5, 1.5 Hz, 1H, H-1″), 5.04–5.16 (m, 4H, H-3′, H-3″), 5.69–5.90 (m, 2H, H-2′, H-2″); ^{13}C NMR (100.6 MHz) δ 11.8 (CH_2CH_3), 22.6 (CH_2CH$_3$), 25.4 (C-4), 30.6 (C-3), 34.1 (C-1′), 40.6 (C-5), 49.0 (C-1″), 58.7 (C-6), 116.9 (═CH_2), 117.4 (═CH_2), 133.2 (CH═CH$_2$), 135.7 (CH═CH$_2$), 170.0 (CON); $[\alpha]_D^{22}$ = –95.56 (c 1.0, MeOH); HRMS $C_{13}H_{22}NO$ $[M + H]^+$ 208.1696; found, 208.1696. Anal. Calcd for $C_{13}H_{21}NO\cdot1/4$ H_2O: C, 73.72; H, 10.23; N, 6.61. Found: C, 73.96; H, 10.18; N, 6.18%. Note: Trace amounts of **(5*S*,6*S*)-6-allyl-1-[(*S*)-2-(allyloxy)-1-phenylethyl)]-5-ethylpiperidin-2-one** were also isolated. IR (NaCl) 2961, 1641 (s, NCO), 1452 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, HSQC, COSY) δ 0.88 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.24–1.37 (m, 2H, CH_2CH$_3$), 1.60 (m, 1H, H-4), 1.73–1.87 (m, 2H, H-4, H-5), 1.96 (dddt, J = 14.6, 7.2, 4.5, 1.3 Hz, 1H, CH_2CH═), 2.21 (m, 1H, CH_2CH═), 2.45–2.54 (m, 2H, H-3), 3.46 (m, 1H, H-6), 4.01 (ddt, J = 7.0, 5.5, 1.4 Hz, 2H, OCH_2CH═), 4.08 (dd, J = 6.1, 1.6 Hz, 2H, H-2′), 4.83 (m, 1H, C6-CH$_2$CH═CH_2), 4.87 (app t, J = 1.4 Hz, 1H, C6CH$_2$CH═CH_2), 5.16 (m, app dq, J = 10.4, 1.3 Hz, 1H, OCH$_2$CH═CH_2), 5.23–5.28 (m, 2H, H-1′, OCH$_2$CH═CH_2), 5.48 (m, 1H, CH$_2$CH═), 5.89 (ddt, J = 17.2, 10.4, 5.5 Hz, 1H, OCH$_2$CH═CH$_2$), 7.24–7.36 (m, 5H, H–Ar); ^{13}C NMR (100.6 MHz) δ 11.9 (CH_2CH_3), 22.2 (C-4), 25.7 (CH_2CH$_3$), 30.6 (C-3), 34.8 (CH_2CH═), 41.3 (C-5), 58.8 (C-6), 60.1 (C-1′), 70.6 (C-2′), 71.9 (OCH_2CH═CH$_2$), 116.5 (CH═CH_2), 116.8 (OCH$_2$CH═CH_2), 127.6 (CHAr), 128.4 (4CHAr), 134.6 (CH$_2$CH═), 135.6 (C6CH$_2$CH═CH$_2$), 138.4 (CAr), 171.0 (NCO); HRMS $C_{21}H_{30}NO_2$ $[M + H]^+$ 328.2271; found, 328.2270.

(1*S*,9a*S*)-1-Ethyl-4-oxo-1,2,3,6,9,9a-hexahydro-4*H*-quinolizine, (5). Ruthenium catalyst (Grubbs 2nd generation, 245 mg, 0.29 mmol) was added to a solution of **4** (2.40 g, 11.58 mmol) in CH_2Cl_2 (300 mL) and the solution was stirred at rt for 8 h. The mixture was concentrated and the resulting residue was purified by column chromatography (Al_2O_3, hexane–EtOAc 3 : 1 to 3 : 2) to afford **5** (1.80 g, 87%), which was unstable. IR (NaCl) 2960, 2932, 1662 (s, NCO), 1634 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, COSY, HSQC) δ 0.96 (t, 3H, J = 7.4 Hz, –CH$_2$CH_3), 1.36 (m, 2H, –CH_2CH$_3$), 1.50–1.75 (m, 3H, 2 × H2 and H-1), 1.80–2.22 (m, 2H, H-9), 2.30–2.55 (m, 2H, H-3), 3.39 (app d, J = 18.0 Hz, 1H, H-6), 3.58 (td, J = 11.6, 4.5 Hz, 1H, H-9a), 4.95 (app d, J = 18.0 Hz, 1H, H-6), 5.68 (m, 1H, H-7), 5.73 (m, 1H, H-8); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 11.8 (CH_2CH_3), 22.1 (C-2), 24.3 (CH_2CH$_3$), 26.3 (C-9), 32.3 (C-3), 38.3 (C-1), 43.1 (C-6), 55.9 (C-9a), 124.8 and 125.0 (C-7/C-8), 168.8 (NCO); $[\alpha]_D^{22}$ = –44.3 (c = 1.20, MeOH); HRMS $C_{11}H_{18}NO$ $[M + H]^+$ 180.1383; found, 180.1379.

(1*S*,9a*S*)-1-Ethyl-4-oxo-1,2,3,6,7,8,9,9a-octahydro-4*H*-quinolizine, (6). 10% Pd/C (60 mg) was added to a solution of **5** (1.20 g, 6.69 mmol) in MeOH (20 mL) and the mixture was stirred under hydrogen atmosphere for 18 h. Then, the crude mixture was filtered through Celite® and the solvent was evaporated to afford a residue, which was purified by bulb-to-bulb distillation (6 Torr, 160–170 °C) to obtain **6** (1.05 g, 87%) as a colourless liquid. IR (NaCl) 2934, 2863, 1642 (s, NCO), 1466 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.87 (t, 3H, J = 7.4 Hz, CH_2CH_3), 1.16–1.64 (m, 9H, CH_2CH_3, H-2, H-7, H-8, H-9), 1.78 (m, 1H, H-1), 1.87 (m, H7), 2.27 (m, 1H, H-3), 2.30–2.41 (m, 2H, H-3, H-6), 3.24 (dd, 1H, J = 11.8, 5.1 Hz, H-9a), 4.65 (ddd, 1H, J = 12.8, 3.9, 1.9 Hz, H-6); ^{13}C NMR (100.6 MHz) δ 11.7 (CH_2CH_3), 22.9 (C-8), 24.1 (CH_2CH_3), 25.1 (C-7), 25.7 (C-2), 26.2 (C-9), 32.3 (C-3), 38.7 (C-1), 44.6 (C-6), 60.6 (C-9a), 168.6 (NCO); $[\alpha]_D^{22}$ = –49.1 (*c* 2.0, $CHCl_3$); MS-EI *m*/*z* 181 M^+ (37), 125 (51), 97 (100), 83 (43), 55 (30); HRMS $C_{11}H_{20}NO$ $[M + H]^+$, 182.1539; found, 182.1541. Anal. Calcd for $C_{11}H_{19}NO{\cdot}1/3\ H_2O$: C, 70.57; H, 10.58; N, 7.48. Found: C, 70.23; H, 10.14; N, 7.32%.

(5*S*,6*R*)-1,6-Diallyl-5-ethyl-2-piperidone, (6-*epi*-4). Following the procedure for the preparation of **4** (method B), from lactam 6-*epi*-**2** (2.10 g, 7.27 mmol), NaOH (2.91 g, 72.7 mmol), MTBE (25 mL), and allyl bromide (0.79 mL, 9.09 mmol), compound 6-*epi*-**4** (0.84 g, 56%) was obtained after column chromatography (hexane–EtOAc 4 : 1). IR (NaCl) 2932, 1642 (s, NCO) cm^{-1}; ^1H NMR (400 MHz, COSY, HSQC) δ 0.91 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.32–1.43 (m, 2H, CH_2CH_3), 1.58 (m, 1H, H-4), 1.69 (m, 1H, H-5), 1.98 (m, 1H, H-4), 2.22–2.51 (m, 4H, 2 × H-3 and 2 × H-1′), 3.20 (dt, J = 9.3, 3.3 Hz, 1H, H-6), 3.37 (dd, J = 15.0, 7.7 Hz, 1H, H-1″), 4.67 (ddt, J = 15.0, 4.7, 1.6 Hz, 1H, H-1″), 5.07–5.21 (m, 4H, H-3′ and H-3″), 5.62–5.82 (m, 2H, H-2′ and H-2″); ^{13}C NMR (100.6 MHz) δ 11.3 (CH_2CH_3), 20.3 (C-4), 24.1 (–CH_2CH_3), 27.9 (C-3), 35.4 (C-5), 37.3 (C-1′), 47.5 (C-1″), 59.3 (C-6), 117.3 (C-3′ or C-3″), 117.8 (C-3′ or C-3″), 133.2 (C-2″ or C-2′), 135.9 (C-2″ or C-2′), 169.6 (CON); $[\alpha]_D^{22}$ = +44.1 (*c* 1.0, $CHCl_3$); HRMS calcd for $C_{13}H_{22}NO$ $[M + H]^+$, 208.1696; found, 208.1695. Anal. Calcd for $C_{13}H_{21}NO{\cdot}1/5\ H_2O$: C, 74.07; H, 10.23; N, 6.64. Found: C, 74.07; H, 10.40; N, 6.42%.

(1*S*,9a*R*)-1-Ethyl-4-oxo-1,2,3,6,9,9a-hexahydro-4*H*-quinolizine, (9a-*epi*-5). Following the procedure for the preparation of **5**, from lactam 6-*epi*-**4** (0.62 g, 2.99 mmol) and 2nd generation Grubbs catalyst (76 mg, 0.09 mmol, 0.03 equiv) in CH_2Cl_2 (100 mL), compound 9a-*epi*-**5** (0.48 g, 90%) was obtained after column chromatography (Al_2O_3, hexane–EtOAc 1 : 1 to 0 : 1). 9a-*epi*-**5** was unstable. ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.96 (t, 3H, J = 7.3 Hz, CH_2CH_3), 1.36 (m, 1H, CH_2CH_3), 1.45–1.55 (m, 2H, H-1, H-2), 1.60 (m, 1H, CH_2CH_3), 1.92 (m, 1H, H-2), 2.11 (m, 1H, H-9), 2.26 (m, 1H, H-9), 2.32 (m, 1H, H-3), 2.45 (m, 1H, H-3), 3.21 (ddd, J = 9.9, 5.8, 3.8 Hz, 1H, H-9a), 3.40 (app d, J = 18.5 Hz, 1H, H-6), 4.83 (app d, J = 18.5 Hz, 1H, H-6), 5.68 (m, 1H, H-7), 5.77 (m, 1H, H-8); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 11.3 (CH_2CH_3), 22.9 (C-2), 25.1 (CH_2CH_3), 30.7 (C-3), 32.5 (C-9), 40.4 (C-1), 42.5 (C-6), 57.7 (C-9a), 124.1 and 124.3 (C-7/C-8), 169.5 (NCO); HRMS calcd for $C_{11}H_{18}NO$ $[M + H]^+$, 180.1383; found, 180.1380.

(1*S*,9a*R*)-1-Ethyl-4-oxo-1,2,3,6,7,8,9,9a-octahydro-4*H*-quinolizine, (9a-*epi*-6). Following the procedure for the preparation of **6**, from lactam 6-*epi*-**5** (315 mg, 1.76 mmol), 10% Pd/C (32 mg) and MeOH (9.0 mL), compound 9a-*epi*-**6** (310 mg, 98%) was obtained as a colourless liquid after bulb-to-bulb distillation (6 Torr, 160–170 °C). IR (NaCl) 2933, 1643 (s, NCO), 1463 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.95 (t, 3H, J = 7.4 Hz, CH_3CH_2–), 1.14–1.47 (m, 6H, CH_2CH_3), H-1, H-2, H-8, H-9), 1.55–1.68 (m, 2H, CH_2CH_3, H-9), 1.83–1.97 (m, 3H, H-2, H-7, H-8), 2.23 (m, 1H, H-3), 2.35 (td, 1H, J = 12.8, 2.9 Hz, H-6), 2.44 (dt, 1H, J = 17.3, 4.4 Hz, H-3), 2.89 (ddd, 1H, J = 11.3, 6.9, 2.4 Hz, H-9a), 4.81 (ddt, 1H, J = 12.8, 4.0, 2.9 Hz, H-6); ^{13}C NMR (100.6 MH) δ 11.3 (CH_2CH_3), 23.5 (C-2*), 24.7 (C-8*), 25.3 (CH_2CH_3), 25.4 (C-9*), 31.4 (C-3), 33.0 (C-7), 41.4 (C-1), 42.9 (C-6), 61.9 (C-9a), 169.0 (NCO); $[\alpha]_D^{22}$ = −27.5 (*c* 1.0, $CHCl_3$); HRMS calcd for $C_{11}H_{20}NO$ $[M + H]^+$ 182.1539; found, 182.1538. Anal. Calcd for $C_{11}H_{19}NO{\cdot}1/4\ H_2O$: C, 71.12; H, 10.58; N, 7.54. Found: C, 71.36; H, 10.49; N, 6.94%.

General procedure for the addition of organometal reagents

Anhydrous $CeCl_3$[21] (2.0 equiv) was added to a solution of **6** (1 equiv) in anhydrous THF. The resulting suspension was vigorously stirred at rt for 1 h. Grignard reagent was added dropwise (4.0–8.0 equiv) over 30 min and the mixture was stirred for additional 18 h. MeOH was added to quench the reaction and the mixture was cooled at −78 °C. $NaBH_4$ (1.25 equiv) and AcOH (0.3 mL) were added and the mixture was stirred at −78 °C for 30 min. The mixture was concentrated and the residue was partitioned between Et_2O and 1 N HCl solution. Then, the acidic aqueous phase was washed with Et_2O and basified with 4 N NaOH solution (pH = 12–14). The resulting suspension was centrifuged (800*g* for 30 min at rt) and the supernatant was extracted with CH_2Cl_2.[22] The combined organic extracts were dried, filtered, concentrated and analysed by GC-MS. The crude product was purified by flash chromatography (Al_2O_3).

(1*S*,4*R*,9a*S*)-4-Allyl-1-ethylquinolizidine, (4-*epi*-207I), (1*S*,4*S*,-9a*S*)-4-allyl-1-ethylquinolizidine, (−)-207I and (1*S*,9a*S*)-4,4-diallyl-1-ethylquinolizidine. Following the general procedure, from lactam **6** (300 mg, 1.66 mmol), $CeCl_3$ (1.63 g, 6.62 mmol), allylmagnesium bromide (7.0 mL, 7.0 mmol, 1.0 M solution in Et_2O), THF (7 mL), and $NaBH_4$ (78 mg, 2.7 mmol), a 97 : 3 diastereomeric mixture of 4-*epi*-**207I** and (−)-**207I** (GC-MS) was obtained. Traces of diallylated quinolizidine were also detected. 4-*epi*-**207I** (198 mg, 58%) was obtained after column chromatography (Al_2O_3, hexanes–EtOAc 95 : 5 to 85 : 15). 4-*epi*-**207I**. IR (NaCl) 2957, 2928, 2856, 1456, 908 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.87 (t, 3H, J = 7.4 Hz, CH_3), 1.13–1.74 (m, 12H, CH_2CH_3, H-1, H-2, H-3, H-7, H-8, H-9), 1.88 (m, app dd, 1H, J = 12.7, 2.5 Hz, H-7), 2.14 (m, 1H, CH_2CH═), 2.37 (m, 1H, CH_2CH═), 2.71 (td, 1H, J = 13.6, 2.5 Hz, H-$6_{ax.}$), 2.93 (m, 1H, H-9a), 3.00 (m, 1H, H-4), 3.34 (app d 1H, J = 13.6 Hz, H-$6_{eq.}$), 4.98–5.10 (m, 2H, –CH═CH_2), 5.83 (dddd, J = 16.8, 10.2, 7.7, 6.6 Hz, 1H, CH═CH_2); ^{13}C NMR (75.4 MHz) δ 11.8 (CH_3), 18.0 (CH_2), 19.3 (CH_2), 25.2 (CH_2), 25.7 (C-7), 29.7 (CH_2), 31.3 (CH_2), 36.9 (CH_2CH═), 41.9

(C-1), 50.1 (C-6), 50.9 (C-4), 61.1 (C-9a), 116.5 (CH=CH_2), 135.7 (CH=CH_2); $[\alpha]_D^{22}$ = +14.4 (*c* 0.5, CH_2Cl_2); MS-EI *m/z* 206 (1), 167 (14), 166 (100), 110 (12), 55 (3); HRMS calcd for $C_{14}H_{26}N$ $[M + H]^+$ 208.2060; found, 208.2061. (−)-**207I**. ^{13}C NMR spectra of (−)-**207I** (from a mixture of 4-*epi*-**207I** and (−)-**207I**) was coincident with that described previously in the literature;[6] MS-EI *m/z* 206 M^+ (1), 167 (12), 166 (100), 136 (4), 110 (8), 55 (3).

(1*S*,9a*S*)-4,4-Diallyl-1-ethylquinolizidine. ^1H NMR (300 MHz, $CDCl_3$, COSY) δ 0.89 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 1.12–1.94 (m, 13H, CH_2CH_3, H-1, H-2, H-3, H-7, H-8, H-9), 2.24 (app d, 4H, *J* = 7.4 Hz, CH_2CH=), 2.54 (m, 1H, H-9a), 2.61 (app t, 1H, *J* = 11.4 Hz, H-$6_{ax.}$), 3.12 (app d, 1H, *J* = 11.4 Hz, H-$6_{eq.}$), 5.03–5.19 (m, 4H, CH_2CH=CH_2), 5.86 (m, 2H, CH_2CH=CH_2); ^{13}C NMR (75.4 MHz) δ 12.0 (CH_2CH_3), 22.7 (CH_2), 25.1 (CH_2), 26.5 (CH_2), 28.7 (CH_2), 36.9 (CH_2), 43.6 (2 × CH_2CH=), 44.1 (CH_2), 45.5 (C-1), 47.5 (C-6), 59.1 (C-9a), 73.5 (C-4), 118.3 (2 × CH=CH_2), 134.0 (2 × CH=CH_2); MS-EI *m/z* 224 (34), 110 (3), 85 (6), 84 (100), 69 (5), 56 (6), 55 (6); HRMS calcd for $C_{14}H_{32}NO$ $[M + H_2O]^+$ 266.2478; found, 266.2478.

(1*S*,4*R*,9a*S*)-1-Ethyl-4-(2-methylallyl)quinolizidine, (7). Following the general procedure, from lactam **6** (50 mg, 0.28 mmol), $CeCl_3$ (140 mg, 0.55 mmol), 2-methylallylmagnesium chloride (3 mL, 2.20 mmol, 0.7 M solution in THF), THF (1.5 mL), and $NaBH_4$ (14 mg, 0.35 mmol), a 96 : 4 diastereomeric mixture of **7** and 4-*epi*-**7** (GC-MS) was obtained. Pure **7** (34 mg, 56%) was obtained after column chromatography (Al_2O_3, hexane–EtOAc 90 : 10 to 75 : 25). **7**. IR (NaCl) 2956, 2926, 2855, 1734, 1460, 1377 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.87 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 1.10–1.70 (m, 12H, CH_2CH_3, H-1, H-2, H-3, H-7, H-8, H-9), 1.73 (s, 3H, CCH_3), 1.81–1.90 (m, 2H, H-7, CH_2C), 2.52 (dd, 1H, *J* = 13.0, 3.7 Hz, CH_2C), 2.69 (td, 1H, *J* = 13.6, 2.6 Hz, H-$6_{ax.}$), 2.94 (app d, 1H, *J* = 12.7 Hz, H-9a), 3.03 (m, 1H, H-4), 3.32 (app d, *J* = 13.6 Hz, H-$6_{eq.}$), 4.71 (br s, 1H,=CH_2), 4.77 (br s, 1H,=CH_2); ^{13}C NMR (100.6 MHz) δ 11.9 (CH_2CH_3), 19.7 (CH_2), 22.7 (CCH_3), 24.8 (CH_2), 25.4 (CH_2), 25.6 (C-7), 29.7 (CH_2), 30.3 (CH_2), 40.7 (CH_2C), 41.8 (C-1), 49.7 (C-4), 50.3 (C-6), 61.1 (C-9a), 112.5 (=CH_2), 143.7 (CH_2C); MS-EI *m/z* 220 (1), 167 (13), 166 (100), 110 (10), 55 (3); HRMS calcd for $C_{15}H_{27}N$ $[M + H]^+$, 222.2216; found, 222.2219. 4-*epi*-**7**: MS-EI *m/z* 221 (13), 220 (12), 206 (10), 192 (18), 167 (12), 166 (100), 164 (16), 136 (11), 110 (14), 84 (35), 83 (11), 82 (15), 67 (10), 55 (10).

(1*S*,4*S*,9a*S*)-1-Ethyl-4-propylquinolizidine, (8). Following the general procedure, from lactam **6** (90 mg, 0.50 mmol), $CeCl_3$ (250 mg, 1.0 mmol), propylmagnesium bromide (1.0 mL, 2.0 mmol, 2.0 M solution in Et_2O), THF (2.5 mL) and $NaBH_4$ (25 mg, 0.63 mmol), a 99 : 1 diastereomeric mixture of **8** and 4-*epi*-**8** (GC-MS) was obtained. Pure amine **8** (67 mg, 64%) was isolated after column chromatography (Al_2O_3, hexane–EtOAc 95 : 5 to 80 : 20). **8**. IR (NaCl) 2958, 2928, 2858, 1461, 1276 cm^{-1}; ^1H NMR: (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.86 (t, 3H, *J* = 7.4 Hz, CH_3CH_2), 0.91 (t, 3H, *J* = 7.1 Hz, $CH_3CH_2CH_2C4$), 1.10–1.30 (m, 10H), 1.36–1.75 (m, 6H), 1.87 (app d, 1H, *J* = 14.6, H-7), 2.66 (td, 1H, *J* = 13.5, 2.7, H-$6_{ax.}$), 2.88 (m, 1H, H-4), 2.93 (app d, 1H, *J* = 14.1, H-9a), 3.30 (app d, 1H, *J* = 13.5, H-$6_{eq.}$); ^{13}C NMR (100.6 MHz) δ 11.8 (CH_2CH_3), 14.7 ($CH_2CH_2CH_3$), 18.5 (CH_2), 18.5 (CH_2), 19.3 (CH_2), 25.3 (CH_2), 25.8 (C-7), 25.9 (CH_2), 29.7 (CH_2), 31.3 (CH_2), 34.6 (CH_2), 42.0 (C-1), 50.0 (C-6), 50.8 (C-4), 61.1 (C-9a); MS-EI *m/z* 209 M^+ (2), 167 (13), 166 (100), 110 (7), 84 (4); $[\alpha]_D^{22}$ = +22.2 (*c* 1.0, $CHCl_3$); HRMS calcd for $C_{14}H_{27}N$ $[M + H]^+$ 210.2216; found, 210.2218. 4-*epi*-**8**. MS-EI *m/z* 209 (2), 167 (21), 166 (100), 138 (6), 110 (12), 84 (5), 55 (6).

(1*S*,4*S*,9a*S*)-1-Ethyl-4-methylquinolizidine, (9). Following the general procedure, from lactam **6** (90 mg, 0.50 mmol), $CeCl_3$ (250 mg, 1.0 mmol), methylmagnesium bromide (0.7 mL, 2.0 mmol, 3.0 M solution in Et_2O), THF (2.5 mL) and $NaBH_4$ (25 mg, 0.63 mmol), only one diastereomer (**9**) was detected by GC-MS. Pure **9** (62 mg, 69%) was obtained after filtration thought a pad of Al_2O_3 (hexane–EtOAc 95 : 5). IR (NaCl) 2958, 2935, 2877, 1665, 1463 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.82 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 1.02 (d, *J* = 6.2 Hz, 3H, CH_3C4), 1.07–1.31 (m, 6H, CH_3CH_2, H-3, H-8, H-9), 1.36–1.66 (m, 6H, H-1, H-2, H-7, H-8, H-9), 1.83 (app d, *J* = 12.7 Hz, 1H, H-7), 2.67 (td, *J* = 13.7, 2.7 Hz, 1H, H-$6_{ax.}$), 2.92 (app d, *J* = 12.6 Hz, 1H, H-9a), 2.98 (m, 1H, H-4), 3.27 (app d, *J* = 13.7 Hz, 1H, H-$6_{eq.}$); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 11.7 (CH_2CH_3), 17.8 (CH_2), 18.8 (CH_2), 19.3 (CH_3C4), 25.2 (CH_2), 25.4 (CH_2), 25.8 (CH_2), 34.8 (CH_2), 41.8 (C-1), 46.7 (C-4), 50.1 (C-6), 60.8 (C-9a); MS-EI *m/z* 181 M^+ (10), 167 (13), 166 (100), 152 (13), 110 (11), 83 (17); $[\alpha]_D^{22}$ = −23.8 (*c* 1.0, $CHCl_3$); HRMS calcd for $C_{12}H_{24}N$ $[M + H]^+$ 182.1903; found, 182.1907.

(1*S*,4*R*,9a*R*)-4-Allyl-1-ethylquinolizidine, (10) and (1*S*,4*S*,9a*R*)-4-allyl-1-ethylquinolizidine, (9a-*epi*-207I). Following the general procedure, from lactam 9a-*epi*-**6** (124 mg, 0.6840 mmol), $CeCl_3$ (340 mg, 1.37 mmol), allylmagnesium bromide (2.75 mL, 2.75 mmol, 1.0 M solution in Et_2O), THF (3.0 mL) and $NaBH_4$ (33 mg, 0.86 mmol), a 1 : 1 diastereomeric mixture of **10** and 9a-*epi*-**207I** (GC-MS) was obtained (75 mg, 54%). Compound 9a-*epi*-**207I** was isolated by column chromatography (Al_2O_3, hexane–EtOAc 95 : 5). **10**:[5] MS-EI *m/z* 206 (1), 167 (42), 166 (100), 110 (54), 84 (20), 67 (23), 55 (42), 54 (20). 9a-*epi*-**207I**. IR (neat) 2958, 2928, 2856, 1450, 1119 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, TMS, NOE, COSY, HSQC) δ = 0.86 (t, 3H, *J* = 7.3 Hz, CH_2), 1.13–1.74 (m, 13H, CH_2CH_3, H-1, H-2, H-3, H-7, H-8, H-9), 2.04 (td, 1H, *J* = 9.6, 3.0 Hz, 1H, H-9a), 2.17 (m, 1H, CH_2CH=), 2.42 (m, 1H, CH_2CH=), 2.50 (td, 1H, *J* = 11.3, 3.7 Hz, H-$6_{ax.}$), 2.66 (m, app dt, 1H, *J* = 11.3, 4.4 Hz, H-$6_{eq.}$), 2.81 (m, 1H, H-4), 4.97–5.06 (m, 2H, CH=CH_2), 5.71 (dddd, *J* = 17.0, 10.1, 8.4, 6.1 Hz, 1H, CH_2CH=); ^{13}C NMR (100.6 MHz) δ 10.8 (CH_2CH_3), 23.4 (CH_2), 24.9 (CH_2), 25.0 (CH_2), 26.1 (C-7), 27.3 (CH_2), 27.4 (CH_2CH=), 31.1 (CH_2), 43.2 (C-1), 52.9 (C-6), 58.4 (C-4), 59.8 (C-9a), 115.8 (=CH_2), 137.5 (–CH_2CH=); $[\alpha]_D^{22}$ = +57.5 (*c* 0.75, $CHCl_3$); MS-EI *m/z* 206 M (1), 167 (27), 166 (100), 110 (55), 84 (17), 67 (21), 55 (37), 54 (20). HRMS calcd for $C_{14}H_{26}N$ $[M + H]^+$, 208.2060; found, 208.2058.

(1*S*,4*S*,9a*R*)-1-Ethyl-4-methylquinolizidine, (11) and (1*S*,4*R*,9a*R*)-1-ethyl-4-methylquinolizidine, (4-*epi*-11). Following the general procedure, from lactam 9a-*epi*-**6** (90 mg, 0.50 mmol), $CeCl_3$

(250 mg, 1.0 mmol), methylmagnesium bromide (0.70 mL, 2.0 mmol, 3.0 M solution in Et_2O), THF (3.0 mL) and $NaBH_4$ (25 mg, 0.63 mmol), a 1 : 1 diastereomeric mixture of **11** and 4-*epi*-**11** (GC-MS) was obtained (46 mg, 48%). The amines proved to be unstable under chromatography conditions. **11***. MS-EI: *m*/*z* 181 M^+ (34), 180 (29), 166 (100), 152 (46), 138 (35), 124 (47), 110 (48), 96 (49), 83 (90), 67 (30), 55 (74). C4-*epi*-**11***. MS-EI *m*/*z* 181 M^+ (29), 180 (30), 166 (100), 152 (47), 138 (37), 124 (46), 110 (42), 96 (50), 83 (88), 67 (32), 55 (78). Dimethylated product was detected in the GC-MS MS-EI *m*/*z* 195 M (17), 181 (16), 180 (100), 124 (20), 110 (20), 84 (43), 83 (43), 82 (21), 56 (23), 55 (37).

Theoretical calculations

Initial geometries were obtained using the PCMODEL program.[23] Further geometry optimizations were carried out using the Gaussian 03 suite of programs on an Compaq HPC320 computer,[24] at the Hartree–Fock (HF) level,[25] and at the Becke's three-parameter hybrid functional with the Lee, Yang and Parr correlation functional (B3LYP) level,[26] using the 6-31G(d) basis set.[27] Analytical energy second derivatives were calculated at all optimized structures to confirm that these were minima.

Acknowledgements

Financial support from the Ministerio de Ciencia e Innovación (MICINN), Spain (Projects CTQ2009-12520-C03-03 and CTQ2009-07021/BQU), and the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), Generalitat de Catalunya (Grants 2009SGR-203 and 2009-SGR-1111) is gratefully acknowledged. Thanks are also due to the MICINN (Spain) for a fellowship to V. S. We are indebted to Dr Pilar Franco for her helpful discussions and experimental efforts to separate (−)-**207I** and 4-*epi*-**207I** by HPLC chromatography. We are deeply grateful to Dr Santiago Vázquez for his invaluable advice and help in carrying out molecular calculations.

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CHAPTER 1 – PART B

A practical procedure for the removal of the phenylethanol moiety from phenylglycinol-derived lactams

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Tetrahedron: Asymmetry **2010**, *21*, 2542-2549.

A practical procedure for the removal of the phenylethanol moiety from phenylglycinol-derived lactams

Foreword

Elimination of chiral inductor is unavoidable step in enantioselective synthesis of chiral piperidines (and/or pyrrolidines) *via* chiral oxazolopiperidone lactams. Debenzylation reaction carried out under Birch conditions[1] is a commonly used method for removal of chiral inductor from 1,2 amino alcohols with general structures **A** (Figure 1). Reactions performed under Birch conditions have some limitations, *e.g.* it is experimentally difficult and dangerous protocol, progress of reaction cannot be monitored by any common analytical method, the reaction cannot be scaled-up (more than 0.5 g of substrate), it requires extreme basic conditions, *etc*. The main advantage of this protocol is reaction time, usually shorter than 30 min. Crude reaction mixture after aqueous work-up contains phenylethanol (compound with typical odour) and liberated lactam. These two products must be separated by column chromatography.

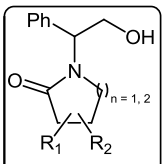
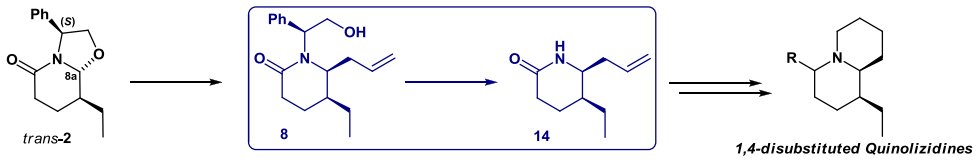


Figure 1 **A**

In case of amines, the phenylethanol moiety can be easily cleaved by hydrogenolysis, however if conservation of unsaturated bonds is required, this alternative is irrelevant.[2]

Introduction

To succeed in our synthesis of 1*S*-ethyl-4-substituted quinolizidines (see previous section), new scalable protocol for debenzylation of aminoalcohol **8** had to be established (Scheme 1).



Scheme 1 Overview of the synthesis of 1*S*-ethyl-4-substituted quinolizidines from oxazolopiperidone lactam.

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[1] Dissolved alkali metal (usually Na or Li) in liquid ammonia at -33 ºC: (a) ref. 4 in presented article. For some examples from other groups see: (b) Vonhoff, S.; Heightman, T.; Vasella, A. *Helv. Chim. Acta* **1998**, *81*, 1710-1725. (c) Lee, S.-G.; Lim, Ch. W.; Kim, D. Ch.; Lee, J. K. *Tetrahedron Lett.* **2000**, *11*, 1455-1458. (d) Steven D. Bull, Davies, S. G.; Kelly, P. M.; Gianotti, M.; Smith, A. D. *J. Chem. Soc.*, *Perkin Trans. 1* **2001**, 3106–3111.

[2] (a) Nishimura, S. *Handbook of Heterogeneous Catalytic Hydrogenation for Organic Synthesis*,1st ed.; A Wiley-Interscience Publication: New York, 2001; pp 587-586. For representative example, see: (b) Amat, M.; Griera, R.; Fabregat, R.; Molins, E.; Bosch, J. *Angew. Chem. Int. Ed.* **2008**, *47*, 3348–3351. (c) Tertiary amines can be also debenzylated by oxidation with singlet oxygen, *e.g.*: Lindner, J. H. E.; Kuhn, H. J.; Gollnick, K. *Tetrahedron Lett.* **1972**, *13*, 1705-1706.

Objective

Development of alternative procedure (to Birch conditions) for the removal of phenylethanol moiety from phenylglycinol-derived lactams with different features.
It has to be:

i) scalable (at least to gram scale – future application in the sequence of linear synthesis),

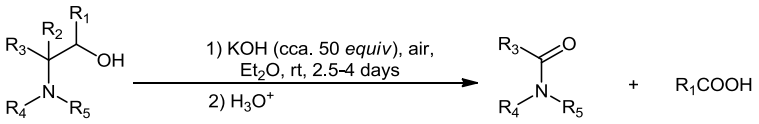
ii) safe (especially with bigger set-up),

iii) cost-effective,

iv) with easy set-up & work-up.

State of Art

Selection of the alternative approach was focused on scalable and easy to set-up protocols. As a promising hit we found a versatile method for oxidation of 1,2-amino alcohols and 1,2-amino ketones to amides, published by Pedrosa and co-workers[3]. General conditions and reagents are presented in Scheme 2. Reaction mechanism was not discussed in detail, however radical pathway was expected. EPR studies of reaction mixtures at different times confirmed radical mechanism, but collected spectra were too complicated to verify exact structure of intermediates.



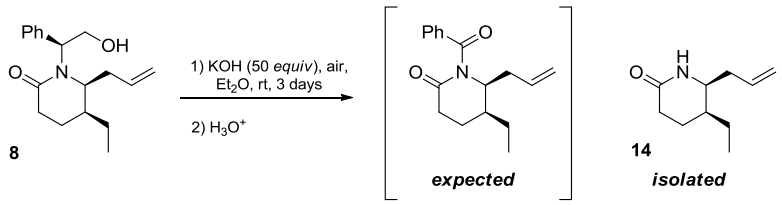
R_1=H, Me, Ph; R_2=H,Me; R_3=H, Me, Et, Ph, -$(CH_2)_4$-;
R_4=Me,Bn,*n*-Bu,-$(CH_2)_4$-; R_5=Me,Bn,*n*-Bu.

Scheme 2 Pedrosa's original conditions.

Preliminary results

To explore universality of this procedure we set-up reactions under Pedrosa's conditions with two available lactams (**8** and C1'-*epi*-**5**). Unexpectedly, totally liberated δ-valerolactams (**14** and **11**) and benzoic acid were isolated (Scheme 3). These two products were easily separated by acid-base extraction (purification by column chromatography was not necessary). Separated products were synthetically pure (^{1}H-NMR purity = 95+%).

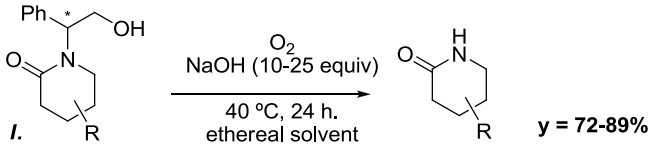
[3] García-Valverde, M.; Pedrosa, R.; Vicente, M. *Synlett* **2002**, *12*, 2092-2094.



Scheme 3 Unexpected elimination of chiral inductor under Pedrosas's conditions.

Results and discussion

A promising preliminary result permits us to explore the potential of the reaction in the context of piperidine-2-one chemistry. Reaction conditions, scope, selectivity and limitations of the new protocol were extensively studied. Potential of the reaction was demonstrated on the successful debenzylation of six different piperidine-2-ones with general structure ***I*** (Scheme 4).



Scheme 4 Cleavage of the 2-hydroxy-1-phenylethyl moiety.

A putative mechanism of the reaction was proposed based on the isolation of intermediates of the reaction. Structure of the intermediates was confirmed by comparison of authentic samples prepared by independent pathway.

Conclusions

A new easy to handle method for cleaving the *N*-(2-hydroxy-1-phenyl)ethyl moiety from phenylglycinol-derived oxazolopiperidone lactams was developed. In comparison with a classical protocol preformed under Birch conditions (Na/liquid NH_3) this method is scalable, safer, cost effective and easy to set-up. As the only disadvantage, a considerable longer reaction time is needed in comparison with Na/NH_3 protocol. The reaction work-up only requires an acid-base extraction to eliminate the benzoic acid formed, and the desired debenzylated product can be used for synthetic purposes without further purification.

This article was highlighted in ChemInform (Wiley online library) as a: *ChemInform* **2011**, *42*, 17-151.

Tetrahedron: *Asymmetry* 21 (2010) 2542–2549

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

A practical procedure for the removal of the phenylethanol moiety from phenylglycinol-derived lactams

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ARTICLE INFO

Article history:
Received 14 September 2010
Accepted 29 September 2010
Available online 26 October 2010

ABSTRACT

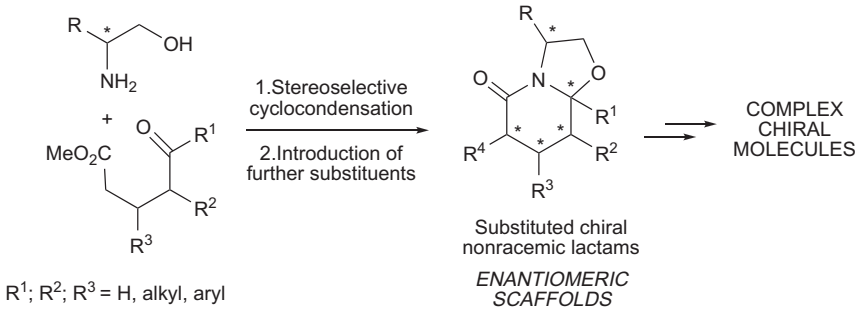
Chiral non-racemic bicyclic lactams derived from phenylglycinol have been appointed as key building blocks for the preparation of enantiopure nitrogen compounds. The removal of the chiral inductor leading to substituted piperidones by using air or oxygen in basic media is presented.

1. Introduction

Chiral aminoalcohol-derived bicyclic lactams[1] are considered as an outstanding example of organic enantiomeric scaffolds[2] for the enantiocontrolled synthesis of complex molecules. These compounds are easily accessible via cyclocondensation reactions of racemic or prochiral δ-oxo(di)acid derivatives with chiral nonracemic amino alcohols. Amongst them, phenylglycinol-derived oxazolopiperidone lactams (R = Ph) are exceptionally versatile building blocks for the enantioselective construction of structurally diverse piperidine-containing natural products and bioactive compounds (Scheme 1).[3]

Typically, bicyclic lactams with the general structure **A** are transformed into enantiopure polysubstituted 2-piperidones by removing the chiral inductor in two steps: (1) opening of the oxazolidine ring, which is accomplished either by an α-amidoalkylation reaction involving the introduction of a substituent at the α-position, or by reductive treatment with Et_3SiH in the presence of a Lewis acid ($TiCl_4$); and (2) debenzylation by treatment under dissolving metal conditions (sodium in liquid ammonia)[4] or multistep processes.[5] This methodology provides versatile diversely substituted enantiopure piperidones, which are key intermediates in the construction of nitrogen compounds (Scheme 2).

Alternatively, removal of the chiral inductor to afford piperidine derivatives can be performed by the initial reduction ($LiAlH_4$, BH_3, etc.) of the lactam carbonyl followed by debenzylation by hydrogenolysis.[3]



Scheme 1. General strategy.

Conventional Birch debenzylation reductions[6] are performed with alkali metals in liquid ammonia at −33 °C. Despite its utility, this method has several undesirable drawbacks that limit its use, particularly on a large scale,[7] such as the inherent dangers of handling elemental alkali metals and the toxicity of ammonia, and also the nuisance and hazards of running cryogenic reactions.[8] Moreover, in the particular case of phenylglycinol-derived lactams, the

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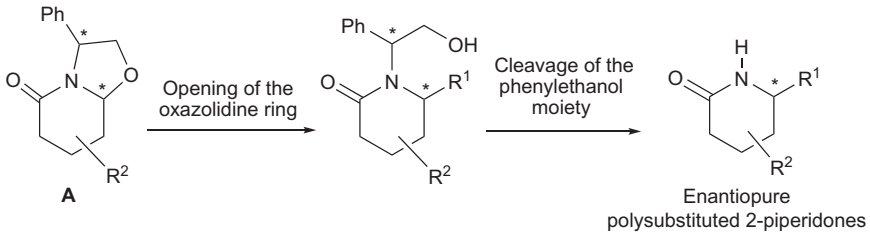
doi:10.1016/j.tetasy.2010.09.014



Scheme 2. Steps to remove the chiral inductor from lactams **A**.

crude reaction mixture after the aqueous work-up contains phenylethanol, which has to be separated from the final N-unsubstituted piperidones by column chromatography.

In 1983, Gigg reported the use of potassium *tert*-butoxide/DMSO and O_2 for the rapid *N*-debenzylation of nitrogen-functionalized glucopyranosides.[9] Nearly twenty years later, the same reagents in the hands of Deaton–Rewolinski allowed the *N*-debenzylation of aromatic heterocycles.[10] Simultaneously, a novel and efficient oxidation of 1,2-aminoalcohols to dialkylamides with potassium hydroxide under aerobic conditions was accomplished by Pedrosa.[11]

Inspired by this previous work, and in an effort to avoid the drawbacks of using sodium in ammonia or multistep sequences, we herein report an easy, safe and cost-effective procedure for the removal of the 2-hydroxy-1-phenylethyl moiety from the phenylglycinol chiral auxiliary in chiral oxazolopiperidone bicyclic lactams. Essentially, it consists of the use of oxygen as an oxidant under basic conditions.

2. Results and discussion

2.1. Preparation of *N*-(2-hydroxy-1-phenylethyl)-2-piperidones 5–10

Enantiopure lactams *cis*-**1** and *cis*-**2** are easily accessible by cyclocondensation of (*S*)-phenylglycinol with methyl 5-oxopentanoate[12] or methyl 4-formylhexanoate,[13] respectively, under neutral conditions. Isomerization of *cis*-**1** and *cis*-**2** under acidic conditions gave access to H-3/H-8a trans lactams *trans*-**1**[12] and *trans*-**2**,[4d] respectively (Scheme 3).

In order to study the scope of the proposed debenzylation reaction, a variety of diversely substituted *N*-(2-hydroxy-1-phenylethyl)-2-piperidones were prepared by diastereoselective enolate alkylation and/or α-amidoalkylation procedures, following the experimental conditions previously described by our group.

In previous studies all attempts to carry out α-amidoalkylation reactions from lactams *cis*-**1** or *cis*-**2** have been unsuccessful. In all cases, only the starting material was recovered, thus confirming the reluctance of *cis* H3–H8a phenylglycinol-derived lactams to undergo α-amidoalkylation reactions.[14] However, the opening of the oxazolidine ring in lactams with a *trans* relationship between H-3/H-8a has been performed easily. Thus, *trans*-**1** and *trans*-**2** lactams were chosen as starting materials.

The diastereoselective alkylation of non-substituted oxazolopiperidone *trans*-**1** with EtI gave exclusively α-ethyl substituted oxazolopiperidone **3**,[12a] and the dialkylation reaction with allylbromide led to bicyclic lactam **4**.[15]

By choosing the appropriate organometallic reagent, the stereocontrolled formation of a C–C bond at the C-6 position of the piperidone can be ensured.[14] The opening of the oxazolidine ring by Grignard reagents (MeMgBr and PhMgBr) from *trans*-**1** took place stereoselectively with retention of configuration at the C-8a stereocentre, to afford the corresponding compounds **5** and **6**.[14] The reaction of *trans*-**1** with indole in the presence of $TiCl_4$ led to a 3:1 mixture of 6-(3-indolyl)-2-piperidones **7** and 6-*epi*-**7**. The *N*-MEM-indole derivative **7a** was prepared from **7** by silylation of the hydroxy group, followed by reaction with MEMCl, and desilylation with TBAF (73% overall yield).[16]

The stereochemical outcome changed when a Lewis acid such as $TiCl_4$ was used, and introduction of allyltrimethylsilane to *trans*-**2** and **3** took place with inversion of the configuration at the C-8a stereocenter to give the respective *cis*-5,6-disubstituted piperidone **8** and 3,6-disubstituted piperidone **9**.

Finally, reductive cleavage of the C–O bond of the oxazolidine ring of **4** with Et_3SiH–$TiCl_4$ gave compound **10** in 78% yield (Scheme 4).

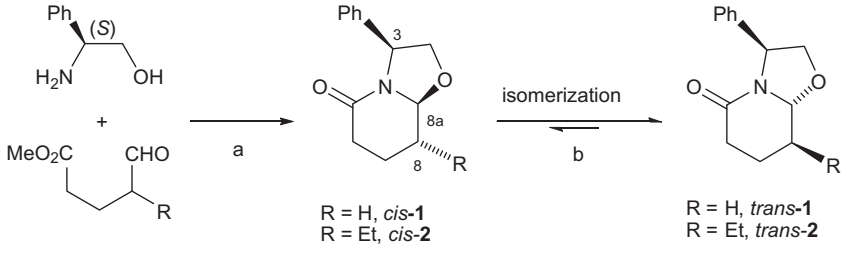
2.2. Debenzylation reaction of lactams 5–10

2.2.1. Reaction conditions

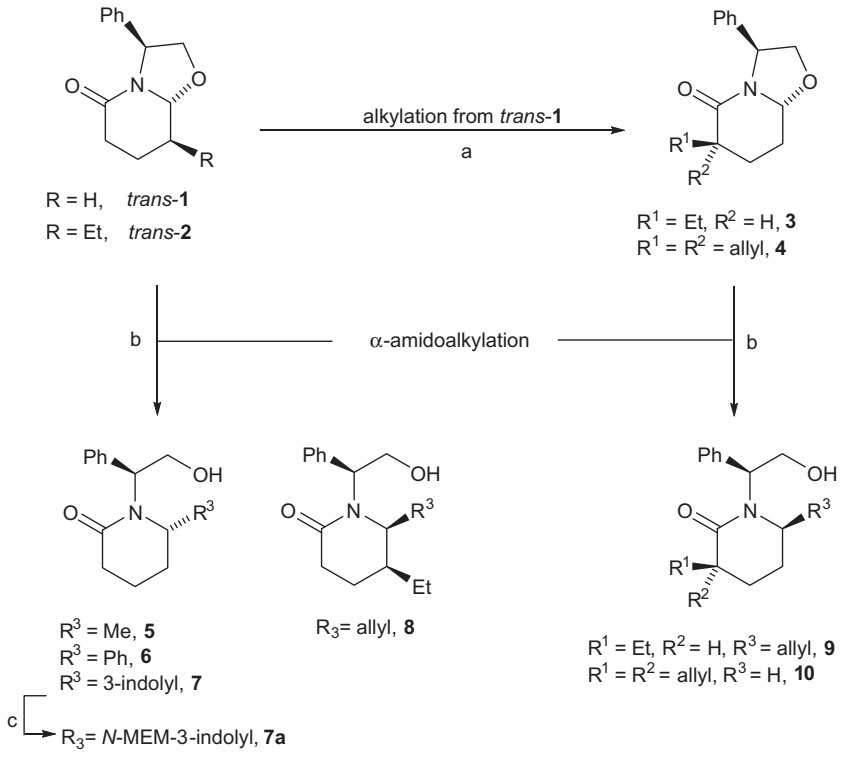
With a variety of diversely substituted *N*-(2-hydroxy-1-phenylethyl)-2-piperidones in hand, we first decided to set up new oxidative reaction conditions from the 5,6-*cis* disubstituted lactam **8** (Table 1).

To start with, we considered the conditions used by Pedrosa in the oxidation of amino alcohols and amino ketones to dialkylamides and carboxylic acids.[11] Treatment of **8** with an excess of KOH in Et_2O at room temperature under aerobic conditions led to **14** in 65% yield (entry 1), which increased to 80% under an oxygen atmosphere (entry 2). Remarkably, benzoic acid was formed in these reactions.

In order to fine tune the reaction conditions, we then studied possible modifications of different factors such as the base, solvent, atmosphere, and sun lamp/heating. As mentioned earlier, one of

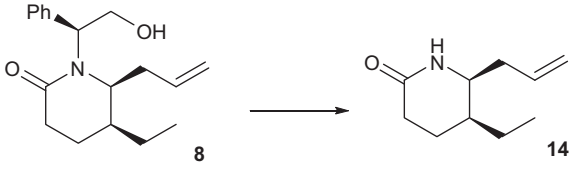


Scheme 3. Synthesis of the starting lactams. Reagents and conditions: (a) toluene, reflux, 36 h, 86%, *cis*-**1**/*trans*-**1** 85:15; toluene, reflux, 18 h, 80%, *cis*-**2**/*trans*-**2**/*epi*-**8**-*trans*-**2** 63:25:12; (b) TFA–CH_2Cl_2 64 h, 25 °C, from *cis*-**1**, 14:86 *cis*-**1**/*trans*-**1**; MeOH–HCl 3 M, 25 °C, 25 h, from *cis*-**2**, *cis*-**2**/*trans*-**2**/*epi*-**8**-*trans*-**2** 28:70:2.



Scheme 4. Synthesis of starting lactams. Reagents and conditions: (a) (i) LiHMDS, −78 °C, 1 h; (ii) EtI, 2 h, 83%, **3**; (b) MeMgBr, Et_2O, 0 °C, 8 h, 62%, **5**. PhMgBr, 0 °C, 8 h, 72%, **6**. Indole, $TiCl_4$, 25 °C, 30 min, 80%, 3:1, **7**: 6-*epi*-**7**. $AllylSiMe_3$, $TiCl_4$, CH_2Cl_2, rt, 23 h, 83%, **8**. $AllylSiMe_3$, $TiCl_4$, CH_2Cl_2, 0 °C, 2 h and rt, 16 h, 91%, **9**. Et_3SiH, $TiCl_4$, CH_2Cl_2, rt, 18 h, 78%, **10**; (c) (i) *t*$BuMe_2SiCl$, imidazole, DMF, 35 °C; (ii) KH, $ClCH_2OCH_3$, THF, 0 °C, 81%; (iii) Bu_4NF, THF, 4 h, rt, 90%, **7a**.

Table 1
Debenzylation conditions of lactam **8**



Entry	Base	Equiv	Solvent	Time (h)	Conditions	Yield (%)
1	KOH	50	Et_2O	16	Air, rt	65
2	KOH	50	Et_2O	16	O_2, rt	80
3	NaOH	50	THF	20	O_2, lamp[a]	60
4	NaOH	25	THF	24	O_2, lamp[a]	66
5	NaOH	25	PhMe	65	O_2, lamp[a]	49
6	NaOH	25	MTBE	22	O_2, lamp[a]	57
7	NaOH	25	MTBE	16	O_2, 40 °C	71
8	NaOH	10	MTBE	16	O_2, 40 °C	83
9	NaOH	5	MTBE	16	O_2, 40 °C	40
10	NaOH	10	MTBE	16	air, 40 °C	60[b]
11	NaOH	10	MTBE	16	O_2, rt	68[b]

[a] A 250 W sun lamp was used at 25 cm of the reaction flask.
[b] 27% of **8** was recovered (entry 10), 10% of **8** was recovered (entry 11).

our concerns was to develop a methodology that would tackle the scale-up problems of other debenzylation methods.

2.2.1.1. Base. The number of equivalents of the base and its solubility were improved by replacing KOH with NaOH and Et_2O with THF, respectively. Thus, when the reaction was carried out using NaOH as the base in THF under oxygen atmosphere in the presence of a sun lamp (20 h), the final product **14** was isolated in 60% yield (entry 3). Halving the NaOH (25 equiv) had no effect on the yield (entry 4).[17]

2.2.1.2. Solvent. As the use of Et_2O or THF under aerobic conditions can result in the formation of dangerous peroxides, other solvents such as toluene and *tert*-butylmethylether (MTBE) were tested.[18] The use of toluene required increasing the reaction time to 65 h to complete the conversion of **8** into the final product, with the yield being lower (49%) (entry 5).

In contrast, MTBE, which has a distinct advantage over most ethers in showing no tendency to form explosive organic peroxides, proved to be a good media for the debenzylation reaction (entry 6). Changes in the lactam concentration had no influence on the final yield.

2.2.1.3. Sun lamp/heat. Based on a previous work suggesting a radical mechanism in debenzylation reactions by oxidative procedures involving oxygen, we included a sun lamp in the above experimental process (entries 3–6).[19]

To determine if the heat provided by the sun lamp was the real accelerator, the following reactions were carried out at 40 °C in the dark (entries 7–11). Indeed, the new conditions gave a good yield (71%) of the pure final product (entry 7). Reduction of the excess of base to 10 equiv resulted in an 83% yield (entry 8), although a further reduction to 5 equiv resulted in a lower yield (entry 9).

When the reaction was carried out under aerobic conditions, 27% of the starting material was recovered (entry 10). At room temperature, the debenzylated product **14** was obtained in 68% yield, with recovery of 10% of the starting material **8** (entry 11).

From the above experiments, the best debenzylation conditions for lactam **8** were the use of NaOH (10 equiv) in MTBE as the solvent at 40 °C in the presence of oxygen (entry 8).

To compare these new debenzylation conditions with the Birch procedure, we subjected compound **8** to the Na/liquid NH_3 reaction conditions. The best yield (81%, **14**; 7%, **8**) was obtained when the reaction was performed at −33 °C starting from 1 g of **8**. When the

reaction was scaled up, the starting product **8** (3 g) was consumed but the formation of **14** was not observed.

The experimental conditions involved in the Na/liquid NH_3 methodology are somewhat difficult to control. For example, Na metal is added in small portions to a solution of the lactam in NH_3 until a blue colour persists, but as the surface of Na is rapidly damaged by the air, the quantities of metal used are variable. Moreover, the basic conditions can affect some functional groups.

In contrast, the reagents and conditions involved in the oxygen-mediated debenzylation reaction are controllable, the consumption of the starting material is easily monitored by TLC and the reaction can be carried out on a multigram scale.

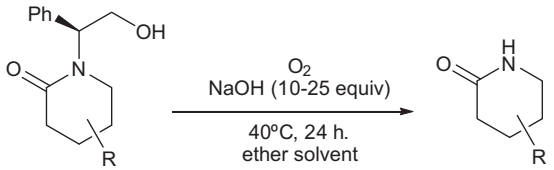
2.2.2. Debenzylation of lactams 5–10

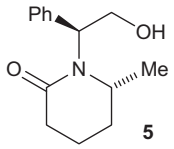
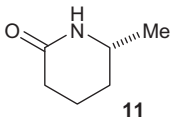
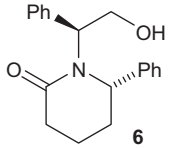
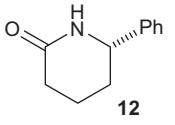
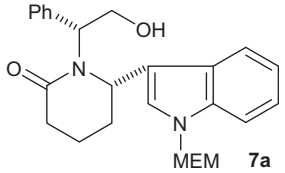
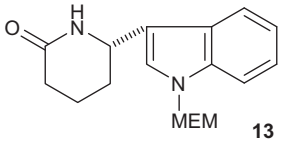
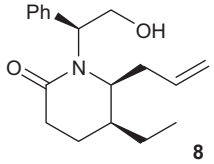
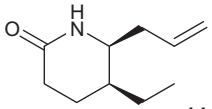
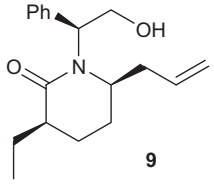
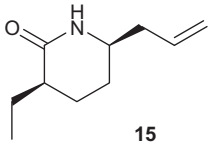
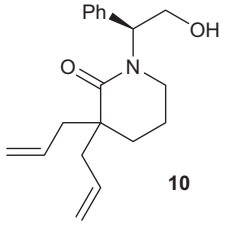
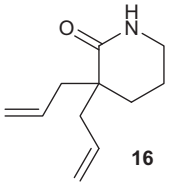
With the aim of evaluating the scope of the reaction, the cleavage of the 2-hydroxy-1-phenylethyl moiety in different substituted lactams **5**, **6**, **7a**, **9** and **10** was undertaken (Table 2).

When the 6-methyl substituted lactam **5** was treated under the standard basic oxidative conditions using THF or MTBE as the solvent, debenzylation occurred in good yield (87%) to give 6-methyl-2-piperidone **11** (entry 1). Notably, in some runs enamide **17** and imide **18** were formed in low yield (Scheme 5). These products are key intermediates to postulate the mechanism of the reaction (see Section 3).

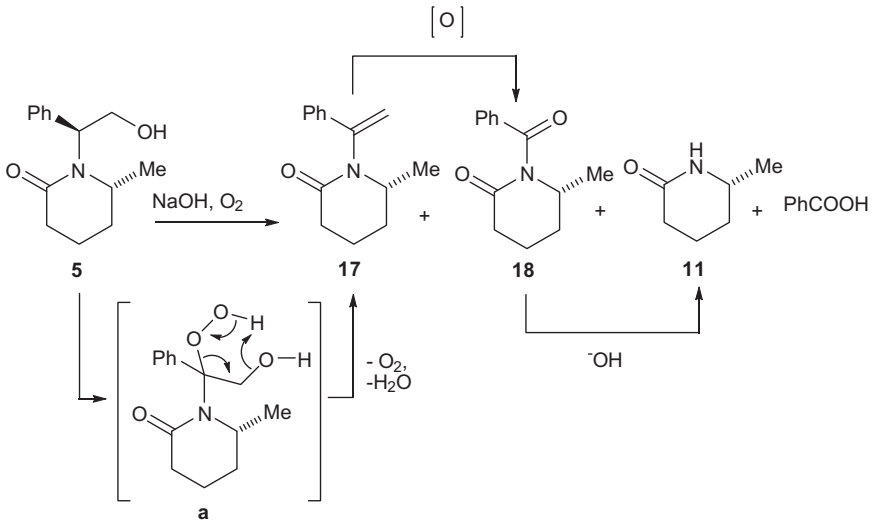
Similarly, 6-phenyl lactam **6** was transformed into **12** in good yield (entry 2; 81%). In this case THF was used as a solvent due

Table 2
Cleavage of the 2-hydroxy-1-phenylethyl moiety



Entry	Starting material	Product	Yield (%)
1	 **5**	 **11**	87
2	 **6**	 **12**	81
3	 **7a**	 **13**	72
4	 **8**	 **14**	85
5	 **9**	 **15**	89[a]
6	 **10**	 **16**	88

[a] Epimerization was observed at the C-3 position to furnish a 1:1 mixture of **15** and 3-*epi* **15**.



Scheme 5. Postulated mechanism of O_2/OH^- mediated debenzylation process.

to the low solubility of the starting material in MTBE. Notably, this transformation involves the chemoselective cleavage of the exocyclic *N*–CHPh bond.

When the reaction was performed from the 6-*N*-MEM-indolyl derivative **7a**, the N-unsubstituted lactam **13** was isolated in 72% yield (entry 3). Debenzylation from the N-unprotected indole derivative **7** was unsuccessful.

As mentioned above, the reaction of 5,6-*cis*-disubstituted lactam **8** (3 g) under standard conditions furnished **14** in 85% yield after 24 h (entry 4).

When 3,6-disubstituted lactam **9** was subjected to the standard debenzylation conditions, a 1:1 mixture of lactams **15** and 3-*epi*-**15** was obtained (entry 5). The same result was observed when the reaction was carried out at room temperature. Epimerization of lactam **15** occurred after treatment with base under an inert atmosphere, indicating the lability of the acidic proton. However, lactam **9** remained unchanged under the same conditions.

Finally, the debenzylation of 3,3-diallyl substituted derivative **10** gave **16** in 88% yield (entry 6).

3. Mechanistic considerations

When the reaction from **8** was conducted in the absence of oxygen or air, or when the base was not included in the experimental procedure, only the starting material was recovered; this demonstrates the crucial role that both factors play in the process.

As aforementioned, when the debenzylation of **5** was conducted in MTBE with 25 equiv of NaOH, enamide **17** was isolated as a by-product (6% yield)[20] and trace amounts of imide **18** were detected. The structure of **18** was confirmed unequivocally by comparison with an authentic sample prepared by acylation of N-unsubstituted lactam **11** with benzoyl chloride.[21]

It is well known that molecular oxygen can oxidize benzylic positions[22] so in the reaction of **5** an intermediate such as **a** could be considered. Extrusion of O_2 and H_2O from hydroperoxide **a** would furnish enamide **17**. Further oxidation of enamide **17** would give imide **18**,[23] which in turn would undergo hydrolysis under basic conditions to afford N-unsubstituted lactam **11** and benzoic acid (Scheme 5).

As an additional test, two different N-substituted lactams were examined. When either *N*-benzyl-2-piperidone or *N*-(2-hydroxyethyl)-2-piperidone was subjected to the $NaOH/O_2$ conditions described, only the starting material was recovered. Therefore, in support of the proposed mechanism, the *N*-2-hydroxyethyl-1-phenyl moiety was proven to be an essential structural requirement for the progress of the reaction.

4. Conclusion

In conclusion, as an alternative to classical Na/liquid NH_3 conditions, we have provided an environmentally friendly, easy to handle, and cost-effective method for cleaving the *N*-(2-hydroxy-1-phenyl)ethyl moiety from phenylglycinol-derived oxazolopiperidone lactams. Advantageously, air (diluted oxygen) or oxygen, which are the cheapest oxidants, was used without irradiation or catalysts. The reaction work-up only requires an acid-base extraction to eliminate the benzoic acid formed, and the desired debenzylated product can be used for synthetic purposes without further purification. Moreover, a putative mechanism for this transformation has been proposed based on the isolation of possible intermediates in the process.

5. Experimental

5.1. General

All non-aqueous reactions were performed under an inert atmosphere with dry, freshly distilled solvents using standard procedures. Solvents for the debenzylation reaction (MTBE, Et_2O and PhMe) were used without purification. THF was distilled prior to use, to avoid possible interaction of BHT (antioxidant stabilizer). Commonly available hydroxides (NaOH, KOH) were used without any purification. Drying of organic extracts during the work-up of reactions was performed over anhydrous Na_2SO_4 or $MgSO_4$. Evaporation of solvents was accomplished with a rotatory evaporator. For reactions that need an oxygen atmosphere the flask was equipped with 1 gallon gas bag (heavy natural rubber, Aldrich Z186740). In experiments with irradiation, a commercial lamp (Philips POWERTONE, ML 250 W E40 225–235 V) was installed ca. 25 cm from the reaction flask. Thin layer chromatography was done on SiO_2 (Silica Gel 60 F_{254}) and the spots were located by UV or 1% $KMnO_4$ solution. Chromatography refers to flash column chromatography and was carried out on SiO_2 (Silica Gel 60, 230–400 mesh). Melting points were determined in a capillary tube and are uncorrected. Unless otherwise indicated, NMR spectra were recorded in $CDCl_3$ at 300 or 400 MHz (^1H) and 75.4 or 100.6 MHz (^{13}C), and chemical shifts are reported in δ values downfield from TMS or relative to residual chloroform (7.26 ,

77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, multiplicity, coupling constant (J) in hertz (Hz), integrated intensity. Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad signal.

5.2. (6*S*)-1-[(1*R*)-2-Hydroxy-1-phenylethyl]-6-[1-(methoxymethyl)indol-3-yl]-piperidin-2-one 7a

TBAF (0.6 mL, 0.58 mmol, 1.0 M THF) was added dropwise to a solution of (6*S*)-1-[(1*R*)-(*tert*-butyldimethylsilyl)oxy-1-phenylethyl]-6-[1-(methoxymethyl)indol-3-yl]piperidin-2-one[16] (190 mg, 0.38 mmol) in THF (1 mL). The mixture was stirred at rt for 4 h. Then EtOAc and 0.5 M HCl solution were added to the reaction mixture. The organic phase was washed with brine, dried and concentrated. The residue was purified by column chromatography (EtOAc to 99:1 EtOAc–MeOH) to yield **7a** (131 mg, 90%). IR (KBr) 3386, 2944, 1621, 1465 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 1.67 (m, 1H, H-4), 1.80 (m, 1H, H-4), 2.01 (m, 1H, H-5), 2.11 (m, 1H, H-5), 2.56 (m, 1H, H-3), 2.70 (m, 1H, H-3), 3.27 (s, 3H, OCH_3), 3.98 (dap, J = 12.0 Hz, 1H, CH_2OH), 4.16 (dt, J = 12.0, 8.2 Hz, 1H, CH_2OH), 4.43 (br s, J = 6.8 Hz, 1H, CHPh), 4.83 (t, J = 4.0 Hz, 1H, H-6), 4.88 (br s, 1H, OH), 5.43 (s, 2H, NCH_2OMe), 7.10–7.19 (m, 2H, Hind), 7.23–7.34 (m, 6H, Hind), 7.44 (d, J = 7.9 Hz, 1H, H-7ind), 7.50 (d, J = 8.3 Hz, 1H, H-4ind); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.8 (C-4), 29.6 (C-5), 32.9 (C-3), 56.0 (OMe), 56.4 (C-6), 64.9 (CH_2OH), 68.2 (CHPh), 77.4 (NCH_2OMe), 110.3 (CHind), 115.8 (Cind), 118.6 (CHind), 120.3 (CHind), 122.8 (CHind), 125.6 (CHind), 127.4 (2 × CHind), 127.5 (CHind), 128.5 (2 × CHind), 137.1 (Cind), 137.6 (Cind), 172.5 (NCO); $[\alpha]_D^{22}$ = +25.5 (c 2.56, $CHCl_3$); MS-EI m/z 378 (M^+, 15), 347 (17), 258 (32), 241 (52), 210 (100), 200 (61), 168 (54), 106 (62); HMRS $C_{23}H_{27}N_2O_3$ (M^++1), 379.2016; found, 379.2020.

5.3. (3*R*,6*R*)-6-Allyl-3-ethyl-1-[(1*S*)-2-hydroxy-1-phenylethyl]-piperidin-2-one 9

Allyltrimethylsilane (0.83 mL, 5.18 mmol) was added to a cooled solution (0 °C) of **3**[12a] (635 mg, 2.59 mmol) in CH_2Cl_2 (10 mL). Then, $TiCl_4$ (9.1 mL of a 1 M solution in CH_2Cl_2, 9.1 mmol) was added dropwise in 2 h by a syringe pump. The reaction mixture was warmed up to rt and stirred for an additional 16 h. The mixture was poured onto ice, and the organic layer was separated, washed with brine, dried, filtered and concentrated. The residue was purified by column chromatography (1:1 to 4:1 EtOAc–hexane) to afford **9** (675 mg, 91%). IR (NaCl) 3387, 2952, 2874, 1617, 1449 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.96 (m, 3H, CH_3), 1.64–1.73 (m, 3H, CH_2CH_3, H-4, H-5), 1.80 (m, 1H, CH_2CH_3), 1.87–2.00 (m, 2H, H-4, H-5), 2.20–2.34 (m, 2H, CH_2–CH=), 2.41 (m, 1H, H-3), 3.34 (m, 1H, H-6), 4.03 (dd, J = 12.0, 3.0 Hz, 1H, CH_2OH), 4.27 (m, 1H, CH_2OH), 4.51 (br s, 1H, OH), 4.63 (dd, J = 7.0, 3.0 Hz, 1H, CHAr), 5.04 (dd, J = 10.5, 1.0 Hz, =CH_2), 5.08 (m, 1H, =CH_2), 5.60 (m, 1H, CH=), 7.25–7.35 (m, 5H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 10.8 (CH_3CH_2), 20.9 (CH_2CH_3), 25.0 (C-5), 25.5 (C-4), 37.4 (CH_2–CH=), 43.1 (C-3), 57.8 (C-6), 64.5 (CH_2OH), 66.5 (CHAr), 118.0 (=CH_2), 127.4 (2 × CHAr), 127.5 (CHAr), 128.5 (2 × CHAr), 134.0 (CH=), 137.2 (C-*i*), 174.5 (NCO); $[\alpha]_D^{22}$ = −63.1 (c 1.25, $CHCl_3$). HMRS $C_{18}H_{26}NO_2$ (M^++1), 288.1958; found, 288.1958. Anal. Calcd for $C_{18}H_{25}NO_2 \cdot 1/4H_2O$: C, 74.06; H, 8.80; N, 4.80. Found: C, 74.20; H, 8.88; N, 4.71.

5.4. 3,3-Diallyl-1-[(1*S*)-2-hydroxy-1-phenylethyl]piperidin-2-one 10

$TiCl_4$ (8.0 mL of 1.0 M solution in CH_2Cl_2, 8.0 mmol) and Et_3SiH (0.80 mL, 5.0 mmol) were added dropwise to a cooled solution (0 °C) of **4**[15] (595 mg, 2.0 mmol) in anhydrous CH_2Cl_2 (7 mL). The mixture was allowed to warm up to rt, stirred for additional 18 h, and poured into ice. The combined organic layers were washed with 5% aqueous $NaHCO_3$ solution, dried and concentrated. The resulting residue was chromatographed (1:4 to 1:1 EtOAc–hexane) to give **10** (470 mg, 78%). IR (NaCl) 3398, 2939, 1611 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 1.59–1.81 (m, 4H, H-4, H-5), 2.18 (td, J = 13.0, 8.0 Hz, 2H, CH_2–CH=), 2.54–2.67 (m, 2H, CH_2–CH=), 2.89 (m, 1H, H-6), 3.13 (m, 1H, H-6), 3.78 (br s, 1H, OH), 4.02 (m, 1H, CH_2OH), 4.14 (m, 1H, CH_2OH), 5.08–5.09 (m, 4H, =CH_2), 5.70–5.88 (m, 2H, CH=), 5.93 (dd, J = 9.0, 5.5 Hz, 1H, CHPh), 7.20–7.35 (m, 5H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.9 (C-5), 28.8 (C-4), 43.6 (C-6), 43.8 (CH_2–CH=), 43.9 (CH_2–CH=), 45.5 (C-3), 58.1 (CHAr), 61.1 (CH_2OH), 118.0 (=CH_2), 118.1 (=CH_2), 127.4 (CHAr), 127.6 (2 × CHAr), 128.4 (2 × CHAr), 134.2 (CH=), 134.4 (CH=), 137.1 (C-*i*), 175.4 (NCO); $[\alpha]_D^{22}$ = +1.4 (c 1.0, MeOH); MS-EI m/z 299 (M^+, 6), 268 (100), 257 (24), 240 (25), 180 (15), 91 (30); HMRS $C_{19}H_{26}NO_2$ (M^++1), 300.1958; found, 300.1955. Anal. Calcd for $C_{19}H_{25}NO_2 \cdot 1/2H_2O$: C, 73.99; H, 8.50; N, 4.54. Found: C, 74.12; H, 8.23; N, 4.30.

5.5. Na/liquid NH_3 debenzylation procedure: (5*S*,6*S*)-6-allyl-5-ethylpiperidin-2-one 14

Into a three-necked, round bottomed flask equipped with a cold-finger condenser charged with dry ice-acetone was condensed NH_3 (~150 mL) at −78 °C. The temperature was allowed to raise to −33 °C (reflux of liquid NH_3), and a solution of lactam **8**[4d] (1 g, 3.48 mL) in THF (5 mL) was added, followed by the addition of sodium metal in small portions until the dark blue colour persisted. After the mixture was stirred at -33 °C for 30 min, the reaction was carefully quenched by the addition of solid NH_4Cl until the blue colour disappeared. The mixture was stirred at rt overnight giving a white solid that was partitioned between water (50 mL) and CH_2Cl_2 (100 mL). The aqueous phase was extracted with CH_2Cl_2, and the combined organic extracts were dried, filtered and concentrated. The resulting residue was chromatographed (1:1 to 1:2 hexane–EtOAc) to give **14** as a white solid (470 mg, 81%) and starting lactam **8** (70 mg, 7%).

5.6. (5*S*,6*S*)-6-Allyl-5-ethylpiperidin-2-one 14

Table 1, entry 2. Freshly ground KOH (16.6 g, 296 mmol) was added to a solution of **8** (1.70 g, 5.92 mmol) in Et_2O (60 mL). The round- bottomed flask was equipped with a 1 gallon gas bag of O_2 and stirring was continued for 16 h at rt. The mixture was partitioned between H_2O and EtOAc. The aqueous phase was extracted with EtOAc (or CH_2Cl_2), and the combined organic phases were dried and concentrated. The resulting residue was purified by flash column chromatography (1:1 EtOAc–hexane) to yield **14** (790 mg, 80%). $[\alpha]_D^{22}$ = −23.7 (c 2.0, $CHCl_3$); HMRS $C_{10}H_{18}NO$ (M^++1), 168.1383; found, 168.1382.[4d]

Table 1, entry 4. Freshly ground NaOH (2.37 g, 59.2 mmol) was added to a solution of **8** (680 mg, 2.37 mmol) in THF (30 mL). The round bottomed flask was equipped with a 1 gallon gas bag of O_2 and a sun lamp, and stirring was continued for 24 h. The mixture was concentrated to dryness. Work up as above gave **14** (261 mg, 66%).

Table 1, entry 8. Freshly ground NaOH (400 mg, 10 mmol) was added to a solution of **8** (288 mg, 1 mmol) in MTBE (5 mL). The round bottomed flask was equipped with a 1 gallon gas bag of O_2 and the mixture was warmed at 40 °C for 16 h. Work up as above gave **14** (138 mg, 83%).

Table 2, entry 4. The reaction was performed from **8** (3.07 g, 10.67 mmol), NaOH (4.27 g, 106.7 mmol) and MTBE (22 mL) for 24 h. Work up as above gave **14** (1.51 g, 85%).

5.7. General procedure for the cleavage of the 2-hydroxy-1-phenylethyl moiety from lactams 5, 6, 7a, 9 and 10

In a round bottomed flask equipped with a 1 gallon gas bag of O_2, an excess (10–25 equiv) of freshly ground NaOH was added to a solution of lactam **5**, **6**, **7a**, **9**, or **10** (1 equiv) in MTBE (or THF). The mixture was heated at 40 °C and stirred slowly at this temperature for 24 h. The solvent was removed, and the residue was partitioned between H_2O and CH_2Cl_2. The combined organic phases were dried and concentrated to give the respective compounds **11–16**.

5.7.1. (*R*)-6-Methylpiperidin-2-one 11[4a]

Following the general procedure, from lactam **5** (70 mg, 0.30 mmol) and NaOH (600 mg, 15.0 mmol) in THF (15 mL) for 16 h, 48 mg of a 9:1 mixture of **11** and enamide **17** were obtained.[24] This crude mixture (48 mg) in Et_2O (25 mL) and 5 N aqueous H_2SO_4 acid (10 mL) was heated at reflux temperature for 1 h. The mixture was cooled and diluted with water (15 mL). The aqueous phase was neutralised to pH 7–8 with $NaHCO_3$ and extracted with EtOAc. The organic extracts were dried and concentrated. The residue was purified by column chromatography (1:1 hexane–EtOAc to EtOAc) to yield lactam **11**[4a] (30 mg, 87%). An analytical sample of **17** was obtained from the mixture by column chromatography (1:1 hexane–EtOAc to EtOAc) (*R*)-6-Methyl-1-(1-phenylvinyl)piperidin-2-one **17**: IR (NaCl) 2936, 1649 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 1.19 (d, J = 7.0 Hz, 3H, CH_3), 1.67 (m, 1H, H-5), 1.83 (m, 1H, H-4), 1.95–2.06 (m, 2H, H-4, H-5), 2.56 (m, 2H, H-3), 3.64 (dd, J = 11.2, 4.9 Hz, 1H, H-6), 5.22 (s, 1H, C=CH_2), 5.84 (s, 1H, C=CH_2), 7.31–7.38 (m, 5H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 18.4 (C-4), 20.6 (CH_3), 30.6 (C-5), 32.7 (C-3), 53.6 (C-6), 113.9 (=CH_2), 125.7 (2 × CHAr), 128.4 (CHAr), 128.5 (2 × CHAr), 136.1 (C-*i*), 145.3 (C=), 170.2 (NCO). MS-EI m/z 215 (M^+, 100), 186 (40), 172 (40), 118 (40), 103 (53), 77 (47), 55 (38).

5.7.2. (*S*)-6-Phenyl-2-piperidone 12

Following the general procedure, from lactam **6** (200 mg, 0.68 mmol) and NaOH (270 mg, 6.77 mmol) in THF (3.4 mL) for 24 h, compound **12**[25] (96 mg, 81%) was obtained.[26]

5.7.3. (6*S*)-6-[1-(Methoxymethyl)indol-3-yl]piperidin-2-one 13

Following the general procedure, from lactam **7a** (185 mg, 0.49 mmol) and NaOH (490 mg, 12.2 mmol) in MTBE (2.5 mL) for 24 h, compound **13** (91 mg, 72%) was obtained after column chromatography (99:1 to 98:2 CH_2Cl_2–MeOH). IR (KBr) 3241, 2953, 1662, 1081 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 1.83 (m, 1H, H-4), 1.90–2.03 (m, 2H, H-4, H-5), 2.20 (m, 1H, H-5), 2.49 (m, 2H, H-3), 3.25 (s, 3H, OCH_3), 4.91 (dd, J = 7.9, 4.6 Hz, 1H, H-6), 5.41 (d, J = 2.3 Hz, 2H, NCH_2OMe), 6.04 (br s, 1H, NH), 7.13 (s, 1H, H-2ind), 7.17 (t, J = 7.5 Hz, H-5ind), 7.28 (t, J = 7.9 Hz, 1H, H-6ind), 7.48 (d, J = 8.2 Hz, 1H, H-7ind), 7.59 (d, J = 7.9 Hz, 1H, H-4ind); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.6 (C-4), 30.0 (C-5), 31.4 (C-3), 50.6 (C-6), 56.0 (OMe), 77.4 (NCH_2OMe), 110.3 (C-7ind), 117.8 (C-3ind), 118.9 (C-4ind), 120.3 (C-5ind), 122.8 (C-6ind), 125.3 (C-2ind), 126.2 (C-3ind), 137.1 (C-7ind), 172.3 (NCO). $[\alpha]_D^{22} = -55.5$ (c 0.6, MeOH); MS-EI m/z 258 (M^+, 100), 227 (46), 188 (34), 157 (32), 130 (29); HMRS $C_{15}H_{19}N_2O_2$ (M^++1), 259.1441; found, 259.1443. Anal. Calcd for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.34. Found: C, 69.24; H, 7.04; N, 10.31.

5.7.4. (3*R*,6*R*)-6-Allyl-3-ethylpiperidin-2-one 15

Following the general procedure, from lactam **9** (200 mg, 0.70 mmol) and NaOH (280 mg, 7 mmol) in MBTE (3.5 mL) for 24 h, a 1:1 mixture of **15** and 3-*epi*-**15** (104 mg, 89%) was obtained. Epimers were separated by column chromatography (4:1 to 1:1 hexane–EtOAc). **15.** IR (NaCl) 3204, 2935, 1663, 1459 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.94 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.66–1.80 (m, 3H, H-4, 2 × H-5), 1.85 (m, 1H, CH_2CH_3), 2.07–2.30 (m, 3H, H-3, H-5, 2 × CH_2–CH=), 3.37 (m, 1H, H-6), 5.10 (m, 2H, =CH_2), 5.13 (m, 2H, =CH_2), 5.70 (m, 1H, CH=), 6.11 (br s, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 11.8 (CH_2CH_3), 22.8 (C-4), 24.7 (CH_2CH_3), 25.0 (C-5), 41.1 (CH_2–CH=), 41.7 (C-3), 51.7 (C-6), 118.8 (=CH_2), 133.4 (=CH), 175.4 (NCO); $[\alpha]_D^{22} = +20.1$ (c 0.63, MeOH); HMRS $C_{10}H_{18}NO$ (M^++1), 168.1383; found, 180.1382. Anal. Calcd for $C_{10}H_{17}NO{\cdot}1/4H_2O$: C, 69.93; H, 10.27; N, 8.16. Found: C, 69.80; H, 10.25; N, 7.98. (3*S*,6*R*)-6-Allyl-3-ethyl-2-piperidin-2-one 3-*epi*-**15**. IR (NaCl) 3211, 2933, 1656, 1450 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 0.93 (m, J = 7.5 Hz, 3H, CH_2CH_3), 1.39–1.48 (m, 2H, H-4, H-5), 1.56 (m, 1H, CH_2CH_3), 1.90–1.98 (m, 3H, H-4, H-5, CH_2CH_3), 2.09 (m, 1H, CH_2–CH=), 2.18 (m, 1H, H-3), 2.32 (m, 1H, CH_2–CH=), 3.37 (m, 1H, H-6), 5.14 (m, 1H, =CH_2), 5.18 (m, 1H, =CH_2), 5.71 (m, 1H, CH=), 5.78 (br s, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 11.0 (CH_2CH_3), 24.1 (CH_2CH_3), 25.4 (C-4), 28.9 (C-5), 41.5 (CH_2–CH=), 42.2 (C-3), 52.3 (C-6), 119.1 (=CH_2), 133.3 (=CH), 174.5 (NCO); $[\alpha]_D^{22} = -5.4$ (c 0.7, MeOH); MS-EI m/z 168 (M^++1, 1), 126 (100), 98 (9), 83 (69), 55 (29); HMRS $C_{10}H_{18}NO$ (M^++1), 168.1383; found, 180.1381.

5.7.4.1. Epimerisation. Freshly ground NaOH (72 mg, 1.79 mmol) was added under an inert atmosphere to a solution of **15** (30 mg, 0.18 mmol) in MTBE (1 mL) and the mixture was heated at 40 °C for 24 h. The solvent was evaporated and the residue was partitioned between H_2O and CH_2Cl_2. The combined organic extracts were washed with aqueous NH_4Cl, dried and evaporated to give a 1:1 mixture of **15** and 3-*epi*-**15** (23 mg, 77%).

5.7.5. 3,3-Diallylpiperidin-2-one 16

Following the general procedure, from lactam **10** (150 mg, 0.5 mmol) and NaOH (200 mg, 5.0 mmol) in MTBE (2.5 mL) for 24 h, compound **16** (80 mg, 88%) was obtained after column chromatography (1:1 EtOAc–hexanes to EtOAc). IR (NaCl) 3214, 3074, 2936, 1657, 1489 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 1.66–1.77 (m, 4H, H-4, H-5), 2.15 (dd, J = 13.5, 8.0 Hz, 2H, CH_2–CH=), 2.49 (ddd, J = 13.5, 8.0, 1.0 Hz, 2H, CH_2–CH=), 3.21 (m, 2H, H-6), 5.02 (m, 2H, =CH_2), 5.06 (m, 2H, =CH_2), 5.70–5.81 (m, 2H, CH=), 6.59 (br s, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.4 (C-5), 28.8 (C-4), 42.5 (C-6), 42.7 (2 × CH_2–CH=), 44.7 (C-3), 118.0 (2 × =CH_2), 134.2 (2 × =CH), 176.3 (NCO); HMRS $C_{11}H_{18}NO$ (M^++1), 180.1383; found, 180.1383.

5.8. (*R*)-1-Benzoyl-6-methylpiperidin-2-one 18[21]

6-Methylpiperidone **11** (100 mg, 0.75 mmol) in THF (1 mL) was added via cannula to a solution of *n*-BuLi (0.54 mL of a 1.6 M solution in hexane, 0.86 mmol) in THF (1 mL) at −78 °C. After stirring at −78 °C for 1.5 h, benzoyl chloride (87 μL, 0.75 mmol) was added dropwise. The mixture was slowly warmed to rt and stirred for 19 h. Then, the yellowish suspension was poured into a saturated NH_4Cl solution (5 mL) and extracted with Et_2O. The combined organic layers were dried and concentrated, and the resulting residue was chromatographed (9:1 to 1:1 hexane–EtOAc) to afford **18**, which was crystallised from hexane–EtOAc to give a white solid (80 mg, 53%). IR (NaCl) 2953, 1675 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC) δ 1.81 (m, 1H, H-5), 1.90 (m, 1H, H-4), 2.02–2.56 (m, 2H, H-4, H-5), 2.52–2.56 (m, 2H, H-3), 4.53 (m, 1H, H-5), 7.36–7.40 (m, 2H, ArH), 7.44 (m, 1H, ArH), 7.54–7.56 (m, 2H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 17.8 (C-4), 20.3 (CH_3), 29.4 (C-5), 34.2 (C-3), 51.6 (C-6), 127.8 (2 × CHAr), 128.2 (2 × CHAr), 131.6 (CHAr), 136.4 (C-*i*), 174.0 (NCO), 174.5 (NCO); mp = 74–76 °C (hexane–EtOAc); HMRS $C_{13}H_{16}NO_2$ (M^++1), 218.1176; found, 218.1174.

Acknowledgements

We thank Dr. Pavel Mach (Comenius University of Bratislava) for fruitful discussions. Financial support from the Ministry of Science and Innovation, Spain (Project CTQ2009-07021/BQU), and the AGAUR, Generalitat de Catalunya (Grant 2009-SGR-111) is gratefully acknowledged. Thanks are also due to the Ministry of Education and Science for fellowships to V.S. and C.A.

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Pyridine derivatives

R 0380

DOI: 10.1002/chin.201117151

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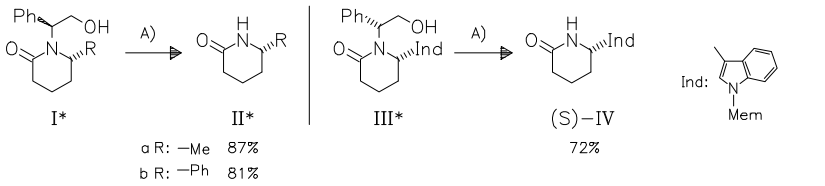
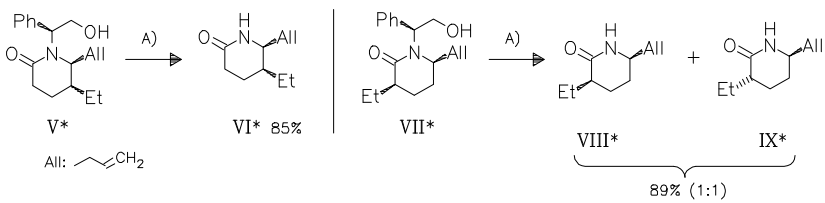
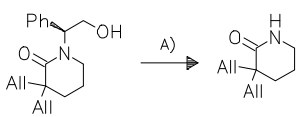
A Practical Procedure for the Removal of the Phenylethanol Moiety from Phenylglycinol-Derived Lactams. — The reaction proceeds without irradiation or catalysis with complete retention of configuration. Only in the case of (VII) epimerization takes place. — (SEMAK, V.; ESCOLANO*, C.; ARRONIZ, C.; BOSCH, J.; AMAT, M.; Tetrahedron: Asymmetry 21 (2010) 20, 2542-2549, http://dx.doi.org/10.1016/j.tetasy.2010.09.014 ; Inst. Recerca Biomed., Univ. Barcelona, E-08028 Barcelona, Spain; Eng.) — Y. Steudel







CHAPTER 2

Synthesis of triheptanoin and formulation as a solid diet for rodents

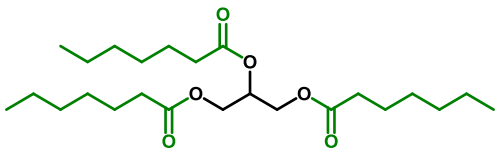
Semak, V.; Semakova, J.; Halbaut, L.; Asó, E.; Ferrer, I.; Calpena, A.; Escolano, C.; Perales, J. C. *Eur. J. Lipid Sci. Technol.* **2012**, *114*, 889-895.

Synthesis of triheptanoin and formulation as a solid diet for rodents

Introduction

Triheptanoin is a synthetic triglyceride composed of three C7 fatty acids (Figure 1). In the past years, triheptanoin has been shown as an effective agent to treat disorders of pyruvate metabolism and fatty acid oxidation.[1] These inborn errors of metabolism are characterized by a significant energy deficit due to deficiencies in pathways responsible for filling up the citric acid cycle (CAC), a central component of aerobic metabolism. Anaplerotic therapy (as it is called) aims to provide alternative metabolites (non-natural C-5 ketone bodies) to feed the CAC, by-passing endogenous pathways blocked by enzyme deficit.

Although triheptanoin has been shown to be a very promising compound for anaplerotic therapy, access to the compound by the basic research community in a sufficient quality and quantity is rather limited.[2] From the chemical viewpoint, quantity and quality of the synthetic product are of crucial importance because triheptanoin is therapeutically used in long-term gram per kilogram dose.



Triheptanoin, (Glycerol triheptanoate)

Figure 1 Triheptanoin [CAS 620-67-7] structure.

[1] Roe, Ch. R.; Mochel, F. *J. Inherit. Metab. Dis.* **2006**, *29*, 332-340.
[2] Sigma-Aldrich®: Glycerol trienanthate [620-67-7]; 25 mL = 456.00 euro; purity 94+%. http://www.sigmaaldrich.com/catalog/product/aldrich/92855?lang=es&region=ES, (07. 10. 2012).

An appeal to synthetic organic chemistry to resolve the supply of triheptanoin

Foreword

Triheptanoin is a molecule which was passed overlooked by modern organic chemists. If we omit its potential for bio-medical research this fact is more than understandable. Triheptanoin has a simple structure, easily predictable chemical-physical properties and also reactivity. In current literature the topic of chemical synthesis of glycerides is limited, *e.g.* in the book "*Esterification*" written by Junzo Otera (1st Ed., 2004) glycerides are only mentioned.[3] That is clearly because the methods of preparation of glycerides are considered as a "classic building block" of organic chemistry. The best proof of this statement is a comprehensive review article titled "*The Chemical Synthesis of Glycerides*" written by Frank A. Norris in 1940 (!) with the almost 150 references (1/3 are patents!).[4] Begin of systematic preparation of glycerides starts in the second half of the 19th century by work of Marcellin Berthelot.[5] The syntheses of glycerides were nearly related with natural product chemistry. In that time, the glycerides were synthesized to confirm the structure of natural occurring oily and fatty compounds. This approach is not limited only to the past. As it is mentioned in the presented article, in 1995 more then 30 examples of triacylglycerols were prepared by Lie Ken Jie and co-workers for the detailed ^{1}H-NMR spectroscopic study.[6] Coupling reagent (*N,N'*-dicyclohexylcarbodiimid, DCC) was used for the preparation of saturated triacylglycerols (type AAA).

Aims & State of Art

The main goal was to develop the high scale synthesis of triheptanoin with high grade of purity. The above mentioned method was directly discarded due to employing of DCC.

First of all we were interested in the procedure described by Ataide and co-workers.[7] Authors were inspired by classical approach to triacylglycerols – two stage

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[4] Norris, F. A. *J. Am. Oil Chem. Soc.* **1940**, *17*, 257-262.
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high temperature synthesis under reduced pressure (1st 160 ºC, 10 mmHg, 8h; 2nd 195 ºC, 10 mmHg, 18h) which was previously employed in the synthesis of the erucic acid triacylglycerols.[8] Ataide claims that the triheptanoin was isolated in the 79% yield (ca. 25 g scale) after flash chromatography. However, when the higher scale was required, the experimental procedure was modified, and the mixture of heptanoic acid (30% molar excess) and glycerol was heated to 130 ºC under reduced pressure for 100 h.[9] The crude product of this reaction consists mainly from triheptanoin and 1,3-diheptanoin in ratio 65 : 35. This mixture was further used as a base for anaplerotic rodent diet. This experimental protocol was discarded, mainly because the optimisation of the reaction conditions was already performed by Dr. Ataide research group and no satisfactory results were achieved. We see even more disadvantages as: a problematic periodical sampling (monitoring of the reaction) and special attention and glassware is required if high scale reactions are performed under reduced pressure. It looks like that this experimental protocol (high temperature synthesis under reduced pressure) is not adequate for middle chain carboxylic acids and works well with higher fatty acids. Probably, the difference between boiling points of the employed acids (and/or products) and reaction conditions is important.

Results

We focused our attention to the methods of preparation of triglycerides by esterification of carboxylic acids by glycerol. Triheptanoin prepared in our study is to be used for alimentation purpose. Due to this, all synthetic methods which require any activation of carboxylic acid *via* haloderivative or anhydride were discarded.

Sulfonated charcoal was the catalyst of the choice. It is cheap, heterogeneous (simple elimination from the reaction mixture), easy to prepare, safe and can be recycled. This catalyst was extensively studied in the (trans-)esterification reactions[10] and was also employed in the synthesis of the triacylglycerols with short (up to 5 carbons) carboxylic acids.[11]

By employing sulfonated charcoal catalyst (2.3% weight) we were able to prepare triheptanoin in the scale of 800g with purity more than 99% according to GC-MS and ^{1}H-NMR. The synthetic product successfully passed elementary analysis without any additional purification step. Reaction was easy to set-up and no special glassware was needed. Reaction was scaled up from 5 g up to 800 g without any

[8] Grewal, V. S.; Ramamurthi, S.; McCurdy, A. R. *J. Am. Oil Chem. Soc.* **1993**, *70*, 955-959.
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difficulty and no decrease of purity or yield was observed. The product was isolated from the reaction mixture after filtration and alkali refining.

Conclusion

Experimental protocol for preparation of triheptanoin with outstanding level of purity was developed in our laboratory. Procedure involves usage of sulfonated charcoal catalyst. Reaction was scaled up to 800 g and only environmental friendly solvents and reagents were employed.

Triheptanoin was incorporated to the ketogenic rodent diet. By adding of four formulation aids (hydrophilic fumed silica, hydrophobic fumed silica, microcrystalline cellulose, talc) a new solid form of anaplerotic rodent diet was prepared. In the pilot animal experiment presented in the article manuscript, compliance and safety of our formulation was studied in 15 weeks long trial on mice.

Prepared anaplerotic diet was used in the treatment of the Alzheimer disease in mice model (Dr. Isidre Ferrer's group, University Hospital /Bellvitge Campus/ UB & Dr. Jose C. Perales's group, Faculty of Medicine UB). Results of this work were collected in the research article titled: *"Triheptanoin supplementation to ketogenic diet curbs cognitive impairment in APP/PS1 mice used as a model of familial Alzheimer's disease."* Asó, E.; Semakova, J.; Joda, L.; Semak, V.; Halbaut, L.; Calpena, A.; Escolano, C.; Perales, J. C.; Ferrer, I. accepted for the publication in the journal *Current Alzheimer Research*.

Research Article

Synthesis of triheptanoin and formulation as a solid diet for rodents

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[3] Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain
[4] Institute of Neuropathology, University Hospital (Bellvitge Campus), University of Barcelona, Hospitalet de Llobregat, Spain

Triheptanoin-enriched diets have been successfully used in the experimental treatment of various metabolic disorders. Maximal therapeutic effect is achieved in the context of a ketogenic diet where triheptanoin oil provides 30–40% of the daily caloric intake. However, pre-clinical studies using triheptanoin-rich diets are hindered by the difficulty of administering to laboratory animals as a solid foodstuff. In the present study, we successfully synthesized triheptanoin to the highest standards of purity from glycerol and heptanoic acid, using sulfonated charcoal as a catalyst. Triheptanoin oil was then formulated as a solid, stable and palatable preparation using a ketogenic base and a combination of four commercially available formulation agents: hydrophilic fumed silica, hydrophobic fumed silica, microcrystalline cellulose, and talc. Diet compliance and safety was tested on C57Bl/6 mice over a 15-week period, comparing overall status and body weight change.

Practical applications: This work provides a complete description of (i) an efficient and cost-effective synthesis of triheptanoin and (ii) its formulation as a solid, stable, and palatable ketogenic diet (triheptanoin-rich; 39% of the caloric intake) for rodents. Triheptanoin-rich diets will be helpful on pre-clinical experiments testing the therapeutic efficacy of triheptanoin in different rodent models of human diseases. In addition, using the same solidification procedure, other oils could be incorporated into rodent ketogenic diet to study their dosage and long-term effects on mammal health and development. This approach could be extremely valuable as ketogenic diet is widely used clinically for epilepsy treatment.

Keywords: Anaplerotic diet / Ketogenic diet / Rheological study / Spreading capacity / Triheptanoin synthesis

Received: December 23, 2011 / Revised: February 17, 2012 / Accepted: March 30, 2012

DOI: 10.1002/ejlt.201100425

Supporting information available online

1 Introduction

In mammals, glucose is the main metabolic substrate supplied to peripheral tissues and the brain. However, under certain circumstances such as long-term fasting or ketogenic diet (KD) administration, the liver produces four-carbon (C4) ketone bodies (acetoacetate and β-hydroxybutyrate) that are

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Abbreviations: BM, binary mixture; **KD,** ketogenic diet; **TKD,** triheptanoin-rich ketogenic diet; **TAG,** triacylglyceride

*Both authors contributed equally to this work.

preferentially utilized by peripheral organs and the brain, sparing glucose for glucose-dependent tissues, such as red blood cells and cornea. Since the 1990s, KD has been used clinically to treat refractory epilepsy [1], GLUT-1 deficiency syndrome [2], and pyruvate dehydrogenase deficiency [3].

Triheptanoin (also glycerol trienanthate; 1,2,3-trienanthoylglycerol; glycerol triheptanoate, trienantin) is a special non-natural TAG composed of three heptanoyl chains (C7:0). If added to a KD, it is metabolized in the liver to non-natural ketone bodies such as β-keto-pentanoate and β-hydroxypentanoate. In peripheral organs and the brain, these blood-born five-carbon ketones (C5) can enter directly to the tricarboxylicacid (TCA, also Krebs) cycle via succinyl-CoA and, unlike the ''classical'' C4 ketone bodies, provide *anaplerotic* carbons. Anaplerosis is a process that replenishes TCA cycle intermediates, thus providing building blocks for biosynthetic pathways. Triheptanoin-derived C5 could bypass various steps of intermediary metabolism and directly ''energize'' mitochondria [4]. This strategy has been successfully used in the treatment of metabolic disorders such as mitochondrial fatty acid oxidation defects, pyruvate carboxylase deficiency, and adult polyglucosan body disease [5–7]. Interestingly, according to a recent clinical trial, even patients suffering from Huntington's chorea could benefit from a triheptanoin diet [8]. In recent years, basic research has revealed that secondary derangement of mitochondrial metabolism accompanies and worsens many complex disorders such as cancer, neurodegeneration, and cardiovascular disease [1, 9, 10]. Intensive screening for possible therapeutic effects of triheptanoin in different animal models of these diseases could open new horizons for medical dietary therapy of multiple aging-associated pathologies.

Although triheptanoin has been administered parentally in some studies [11], it has best anaplerotic potential (C5 ketone production) when administered with a KD (high-protein and fat with up to one-third of dietary calories provided by triheptanoin). However, pre-clinical studies using triheptanoin/KDs are hindered by the difficulty of administering them to laboratory animals as a solid foodstuff, due to the low viscosity of triheptanoin. In the present study, we have developed an efficient synthetic protocol to produce triheptanoin from simple precursors and elaborated a palatable, low spreading, and easy to administer solid diet rich in triheptanoin.

Our main goal has been to develop an efficient, economic, and environmentally friendly synthesis that provides considerable quantities of triheptanoin with an outstanding level of purity, of crucial importance because triheptanoin is therapeutically used in long-term, high-dose protocols. To this end, the use of sulfonated charcoal as the catalyst in non-halogenated solvents was considered [12]. Next, we produced a formulation taking into consideration not only the thickening or adsorption capacity of each additive, but also their possible interaction with ketogenesis (i.e., carbohydrate content) and median lethal dose in rodents. To achieve this goal, a combination of four commercially available formulation agents has been used in this work: hydrophilic fumed silica, hydrophobic fumed silica, microcrystalline cellulose, and talc. Finally, compliance and safety was studied in C57Bl/6 mice, comparing overall status and body weight change for 15 weeks.

2 Materials and methods

2.1 Chemicals

Anhydrous glycerol (CAS: [56-81-5]) was purchased from Fluka, heptanoic acid (CAS: [111-14-8]) from Sigma–Aldrich, toluene, sodium hydroxide (NaOH), charcoal, and sulfuric acid (H_2SO_4) from local supplier. Rheological additives (all pharmaceutical grade): hydrophilic fumed silica (AEROSIL® 200) and hydrophobic fumed silica (AEROSIL® R972) were purchased from Evonik Industries; microcrystalline cellulose (VIVAPUR® 101) and talc (Ph Eur./USP quality) from Quimivita S.A.

High protein ketogenic diet for rodents (Test Diet – 5TJR) was purchased from IPS Product Supplies Ltd. and normal rodent chow from Harlan.

2.2 Chemical synthesis of triheptanoin

2.2.1 General methods

NMR spectra were recorded in $CDCl_3$ at 400 MHz (^1H) and 100.6 MHz (^{13}C), and chemical shifts are reported in δ-values downfield from TMS or relative to residual chloroform ($\delta = 7.26$ ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, multiplicity, coupling constant, integrated intensity. Multiplicities are reported using the following abbreviations: dd, doublet of doublets; t, triplet; tt, triplet of triplets; m, multiplet. Evaporation of solvents was accomplished with a rotary evaporator. Thin-layer chromatography was performed on SiO_2 (silica-gel 60 F254), and the spots were located by 1% aqueous $KMnO_4$. Mass spectra were recorded with an LTQ spectrometer using ESI+ techniques.

2.2.2 Preparation of sulfonated charcoal catalyst

A mixture of active charcoal (25 g) and sulfuric acid (75 mL, 96%) was heated at 260°C and vigorously stirred overnight (18 h). Then the mixture was filtered off and washed with distilled water (1 L) until washings gave a negative barium chloride test. The filtrate was dried at 100°C for 24 h [13].

2.2.3 Esterification procedure

Sulfonated charcoal catalyst (4.20 g, $w = 2.3\%$, based on glycerol) was added to a mixture of glycerol (176.85 g, 1.92 mol), heptanoic acid (1000 g, 7.68 mol, 4.0 equiv.), and toluene (350 mL). The reaction mixture was heated under

reflux (internal temperature: 140°C) with water being removed in a Dean–Stark apparatus (Annotation 1) (see Scheme 1).

Work-up; Method A: after 4 days the catalyst was removed by filtration (paper; Albet 400) with the aid of toluene (400 mL). Filtrate was washed with aqueous 15% NaOH (3 × 300 mL) (Annotation 2) and with distilled water (2 × 50 mL). Combined aqueous layers where extracted with toluene (1 × 100 mL) and organic layer was washed with distilled water (2 × 20 mL). Combined organic extracts were dried over sodium sulfate (Na_2SO_4), filtered, and concentrated under reduced pressure to give transparent colorless liquid product. Product was dried in vacuum drying oven (60°C, 20–30 mbar, 48 h) to give transparent colorless oily liquid (802 g, 97.5%) with purity 99+% (GC/MS).

Work-up; Method B: 20 g scale experiment: the resulting filtrated was evaporated and the residue was fractionally distilled to give transparent colorless oil (20.14 g, 94%, 190°C, 1 mm Hg) with purity 99+% (GC/MS).

2.2.4 Analytical data

IR (NaCl): 2930, 1745 (s, COOR), 1162 cm^{-1}. ^1H NMR (400 MHz, COSY, $CDCl_3$): δ(ppm) = 0.89 (t, $\mathcal{J}$ = 6.8 Hz, 9H, 3*x* $-^7CH_3$), 1.25–1.31 (m, 18H, 3*x* $-^4CH_2-^5CH_2-^6CH_2-$), 1.61 (m, 6H, 3*x* $-^3CH_2-$), 2.31 (m, 6H, 3*x* $-^2CH_2-$), 4.15 (dd, $\mathcal{J}$ = 11.9, 6.0 Hz, 2H, 2*x*$H_{A'}$), 4.30 (dd, $\mathcal{J}$ = 11.9, 4.3 Hz, 1H, 2*x*H_A), 5.27 (tt, $\mathcal{J}$ = 6.0, 4.3 Hz, 1H, H_B). ^{13}C NMR (100.6 MHz, HSQC, $CDCl_3$): δ(ppm) = 13.9 (3*x*C-7, CH_3), 22.4 (3*x*C-6, CH_2), 24.7 (3*x*C-3, CH_2), 28.7 (3*x*C-4, CH_2), 31.3 (3*x*C-5, CH_2), 33.9 (2*x*C-2A, CH_2), 34.1 (C-2B, CH), 62.0 (2*x*C-A, CH_2), 68.8 (C-B, CH), 172.7 (C-1B, COOR), 173.1 (2*x*C-1A, COOR) (see Fig. 1). MS-EI *m*/*z* 299 (21; $C_{17}H_{31}O_4^{\bullet}$), 285 (10; $C_{16}H_{29}O_4^{\bullet}$), 113 (100; $C_7H_{13}O^{\bullet}$), 85 (16; $C_6H_{13}^{\bullet}$). Elemental Analysis: anal. calcd for $C_{24}H_{44}O_6$: C 67.26, H 10.35. Found: C 67.12, H 10.64. (Annotation 3) high resolution MS: $C_{22}H_{48}NO_6$ ($M^+ + NH_4^+$) 446.3474, calculated 446.3476. (Annotation 4) UV–Vis (EtOH) = 211 nm (Imax). TLC: R_f = 0.26 (SiO_2, 2% MeOH in CH_2Cl_2).

Annotations to the synthesis:

(i) 106 mL of water was collected, expected amount was 103.7 mL of water formed during the course of reaction and the excess is from the used solvent.
(ii) Error: C = 0.14%; H = 0.29% when 0.40% is permitted.
(iii) 0.42 ppm difference when 5 ppm is permitted.

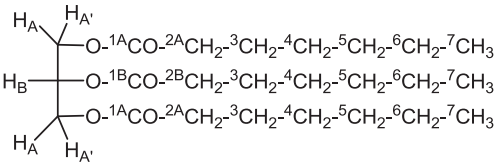


Figure 1. Triheptanoin: atom numeration for NMR assignment.

(iv) When the reaction is finished, mixture contains 1.92 mol of unreacted heptanoic acid. For alkali refines 2 molar excess of base was used, i.e., 3.84 mol (153.6 g). Solution 15% (weight): 153.6 g of NaOH and 870 mL of water. Observation: sodium salt of heptanoic acid can form temporal solid – soap, which is dissolved spontaneously with time.

2.3 Pharmaceutical formulation

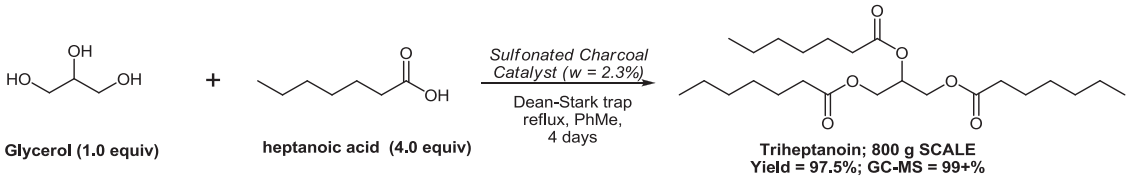
2.3.1 Food mixture preparation and storage

Food mixtures were prepared under normalized conditions as follows: the standard KD was placed into a warm water bath (30°C) while the rheological additives were incorporated at RT to the oily phase (triheptanoin) under gentle stirring. Finally, the thickened oily phase was added to the standard KD and the mixture kneaded until homogeneity. Each sample was transferred to hermetically closed glass recipient. After standing at RT, they were stored in a refrigerator (4 ± 1°C). The mixtures were examined 48 h after their elaboration. All measurements were replicated three times. Results are presented as mean value ± SD.

2.3.2 Rheological characterization

2.3.2.1 Spreading capacity

The spreading capacity of the KD, binary mixture (BM, i.e., 72–28% KD and triheptanoin) and final triheptanoin-rich ketogenic diet (TKD) was assessed. The measurement consisted of determining the area increase of a fixed volume of product (352 mm^3) squashed between two parallel planes, under the effect of a constant weight pressure (200 g) during a fixed period of time (1 min). For this determination an original apparatus developed in our laboratory was used. (For detailed information see Supporting information).



Scheme 1. Esterification reaction.

2.3.2.2 Viscosity determination and viscoelastic behavior

The rheological studies were performed using the HAAKE RheoStress 1 rheometer: Haake*PP60Ti* plate sensor (6 cm diameter), connected to a temperature control Thermo Haake Phoenix II + Haake C25P. Viscosity measurements at 25°C were applied to triheptanoin and BM. They were carried out at three different shear rates (25, 50, and 100 s^{-1}) and recorded during 1 min after the corresponding three ramp-up periods (0–25, 25–50, and 50–100 s^{-1}) within 1 min. Oscillatory tests were applied to the final TKD in order to determine the linear viscoelastic region. Oscillatory stress sweeps between 0.1 and 100 Pa were performed at 1 Hz. Four different gaps between plates were tested (1, 2, 3, or 4 mm). Frequency sweep tests were performed from 0.01 and 10 Hz at a constant shear stress within the linear viscoelastic region in order to determine the related variation of the storage modulus (G') and loss modulus (G'') at different temperatures (25, 30, and 35°C). Both viscoelastic moduli are defined as follows: $G' = \tau_o/\gamma_o \cdot \cos\delta$ and $G'' = \tau_o/\gamma_o \cdot \sin\delta$ (where τ_o and γ_o are the amplitudes of stress and strain and δ is the phase shift between them) [14–16].

2.4 Animals

Thirty-five female 3-month old C57Bl/6 mice from our colony were housed under the standard conditions, with ad libitum access to water. The control group ($n = 12$) received normal chow ad libitum. KD and TKD were stored at 4°C, according to the manufacturer's recommendation. Fresh doses of KD and TKD were offered at 3 g/day/animal twice a week and unconsumed remains were discarded. Animals were observed twice a week and weighed once a week. The study protocol was approved by the University of Barcelona Animal ethical committee.

3 Results and discussion

3.1 Triheptanoin synthesis from glycerol and heptanoic acid: high yield and purity

To the best of our knowledge, the first reported ^{1}H NMR data of triheptanoin was published by Lie Ken Jie and Lam [17]. Saturated TAGs were prepared by Ziegler and Berger [18] by esterification reaction of the corresponding carboxylic acid with glycerol mediated by coupling reagent (dicyclohexylcarbodiimide – DCC) in dichloromethane media.

More recently, Ataide et al. reported the chemical synthesis of tricaproin, triheptanoin, and tricaprylin in the absence of both solvent and catalyst at high temperatures and under vacuum [19]. The process involved two stages under high temperature and vacuum and a 50% molar excess of heptanoic acid. Triheptanoin was isolated in 79% yield after purification by column chromatography (silica gel, hexane/chloroform 1:1).

A few aspects of the above mentioned synthesis of acylglycerols needed to be considered especially those of potential application in the food industry and as nutraceuticals. It was particularly desirable to avoid halogenated solvents either as reacting media or in the purification process. Additional experimental problems were the removal of the excess of reagents and the scaling-up of the reaction carried out under high vacuum and high temperature.

We therefore developed an alternative procedure for the synthesis of triheptanoin that involves the esterification of heptanoic acid (33% molar excess) by glycerol in toluene under heterogeneous catalysis (sulfonated charcoal). The reaction was scaled up from 5 to 800 g without loss of purity and with a yield of 94–97%. In terms of "*green chemistry metrics*" [20] this process can be considered as environmentally friendly, since water is the only subproduct and the catalyst can be recycled [13]. The atom economy (AE) of this process is 88.8% and carbon efficiency (CE) is 75.5%. Mass intensity (MI), rigorous metrics – in which also non-binding reagents and solvents are included – is 1.85 g/g. A very good MI ratio confirmed that our synthesis is highly efficient. This method to obtain highly pure triheptanoin is clean, cost-effective, safe, and easy to set up.

All the analytical data (^{1}H NMR, ^{13}C NMR, IR, etc.) coincided with previously reported data. Moreover, high resolution MS and elemental analysis were performed. Notably, the previous assignation of the ^{13}C NMR should be corrected since bidimensional studies revealed that previously assigned peaks 31.32 (C-5) and 24.70 (C-3) were interchanged.

3.2 The combination of four pharmaceutical additives improves the spreading capacity and viscoelastic parameters of the triheptanoin-rich ketogenic diet (TKD)

In 2008, a research group from the Federal University of Alagoas, Brasil, fed rats during 7 weeks with an anaplerotic diet based on margarine, casein, and triheptanoin with a weight ratio of Fat to Protein + Carbohydrate 3.5:1, but energy ratio close to 7:1. Unfortunately, no details on physical properties of this diet are available [21]. To the best of our knowledge, no formulation of a solid TKD for rodents has been described to date. Direct incorporation of requested amount of triheptanoin (28 wt%, corresponding to 39 calorie%) into a standard rodent KD gives dense liquid consistency, difficult to administer to animals at RT in dose-controlled manner. Triheptanoin oil showed Newtonian behavior and its viscosity at 25°C was 14.15 mPa s. In contrast, BM showed pseudoplastic rheological behavior and its viscosity decreases with shear rate: 5743 ± 38 mPa s (25 s^{-1}), 3407 ± 13 mPa s (50 s^{-1}), and 2105 ± 8 mPa s (100 s^{-1}). Spreading capacity of the KD was 255 ± 7 mm^2 while spreading capacity of the BM was 562 ± 11 mm^2. Four additives have been added; hydrophilic fumed silica (Aerosil®

200), hydrophobic fumed silica (Aerosil® R972), microcrystalline cellulose, and talc (Table 1). The final TKD has a solid pasty texture with spreading capacity 349 ± 24 mm^2 at RT. Additive content already present in KD (powdered cellulose) as well as mouse median lethal dose (LD50) were considered when determining the maximum amount of each additive beside their thickening or adsorption capacity [22–25].

According to the results of oscillatory stress sweeps, a plat gap of 2 mm and a constant shear stress of 2 Pa (20% of the critical value) were selected to perform the frequency sweep tests. Oscillatory measurements applied to TKD sample showed prevalence of the elastic over the viscous behavior ($G'>G''$) at all studied temperatures (25–35°C) in the whole frequency range. No significant differences were detected within the temperature range (Fig. 2).

3.3 Pilot testing in adult mice shows the diet is safe and palatable

Finally, TKD was easy to weight and administer over the cage grid and was well tolerated by the animals. There was no significant difference in body weight variation between the three groups until week nine. In addition, there were no physical signs indicative of disease or not discomfort, such as piloerection, ptosis, or hypolocomotion on any of the experimental groups. After 12 weeks of diet administration, animals in TKD group showed continuously lower weight gain if compared to KD and control group. However, reduced weight gain was not a consequence of reduced caloric intake subsequent to low palatability of the diet. Caloric intake in the TKD group was well within the range of chow diet intake for 8 week old C57Bl/6 (25 g) mice (averaging 470 kcal/kg/day) [26] (Fig. 3).

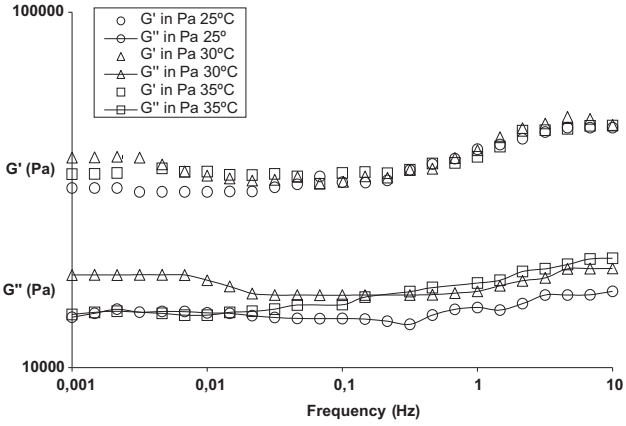


Figure 2. Frequency dependence of the storage and loss moduli for TKD. Frequency sweep tests were performed from 0.01 to 10 Hz at a constant shear stress within the linear viscoelastic region in order to determine the related variation of the storage modulus (G') and loss modulus (G'') at different temperatures (25, 30, and 35°C).

4 Conclusions

From the chemical viewpoint, we have developed a cost-effective procedure of triheptanoin synthesis with evident advantages: (i) just small excess of heptanoic acid is used, (ii) the process is metal free, (iii) only environmentally friendly solvents and catalysts are involved, (iv) no column chromatography or other purification of product is needed. Triheptanoin is isolated in 94–97% yield, with more than 99% purity (CG-MS) and no additional purification step is needed. Reaction was successfully scaled up from 5 up to 800 g.

Table 1. Content of triheptanoin-rich ketogenic diet(TKD)

TKD content	Parts	Mice LD_{50} (mg/g)	Content in daily food offer[a)] (mg)	Maximal daily dose/body weight[b)] (mg/g)	Maximal average (%) mice LD	Caloric content (kcal/g)	Daily energy offer (kcal)	Energy (%)
Ketogenic diet (Test Diet – 5TJR)	72		1993.0	99.7		5.2	10.4	61%
(in which powdered cellulose content 2.89%)		5	57.6	2.9	57.6%			
Triheptanoin oil	28		775.0	38.8		8.5	6.6	39%
Aerosil®200 (Hydrophilic fumed silica)	1.5	3.2	41.5	2.1	65.7%	0	0	0
Aerosil® R972 (Hydrophobic fumed silica)	0.9	5	24.9	1.3	24.9%	0	0	0
Microcrystalline cellulose	2	5	55.4	2.8	55.4%	0	0	0
Talc	4	n.d.	110.7	5.5	n.d.	0	0	0
Total	108.4		3000	150			17.0	

[a)] Daily food offer: 3 g (17.0 kcal).
[b)] Minimal body weight: 20 g.
n.d.: not determined.

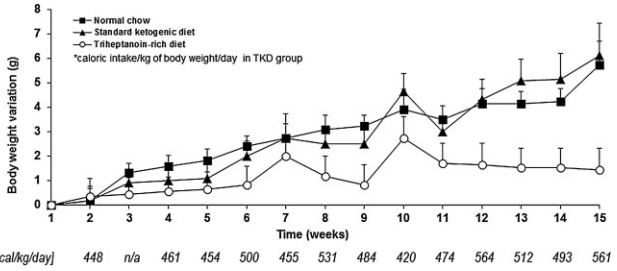


Figure 3. Animal body weight variation. Body weight was measured weekly on C57B/6 mice on various diets. Mice were assigned to each diet randomly and their initial weight was not significantly different. Weight was recorded for a 15-week period. Variation data was analyzed using a one-way ANOVA and bonferroni post-test. Mice on a triheptanoin-rich diet were significantly leaner ($p<0.05$).

A combination of four pharmaceutical additives improved spreading capacity and viscoelastic properties of the pilot BM and resulted in solid, palatable, non-toxic, stable, and easy to handle formulation of rodent TKD. Finally, animal testing showed that this diet is well tolerated and could be used in wide variety of chronic experiments requiring anaplerotic, TKD.

Financial support from the Ministry of Science and Innovation, Spain (Projects BFU2009-07506and CTQ2009-07021/BQU) and the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), Generalitat de Catalunya (Grant 2009-SGR-111) is gratefully acknowledged. V. S. and J. S. were supported by fellowships by the Ministry of Education, Spain. The authors are in debt to D. Sanchez Ahsen for preliminary experiments in pharmaceutical formulation performed as part of his master thesis.

The authors have declared no conflict of interest.

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In this issue

Triheptanoin-based ketogenic diet for pre-clinical research

Eating ketogenic diet mimics some aspects of starvation – the diet has a high fat, adequate protein and low carbohydrate content; such composition favors conversion of fats into fatty acids and ketone bodies which can replace glucose as an energy source in the brain. In patients with epilepsy, this fundamental metabolic change leads to a reduction in the frequency of seizures. Triheptanoin has an anaplerotic potential and has been successfully used in ketogenic diets. This paper provides a complete description of an alternative and efficient synthesis of triheptanoin and its formulation as a solid, stable and palatable ketogenic diet for rodents which should be of interest for pre-clinical animal studies. The described synthesis is compatible with the requirements of the food and nutraceuticals industry.

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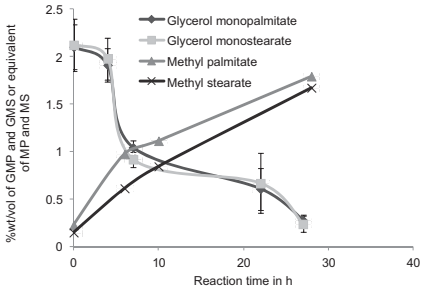
Semak, V. et al. Synthesis of triheptanoin and formulation as a solid diet for rodents. *Eur. J. Lipid Sci. Technol.* 2012, *114*, 889–985

Removing saturated monoacylglycerols from biodiesel

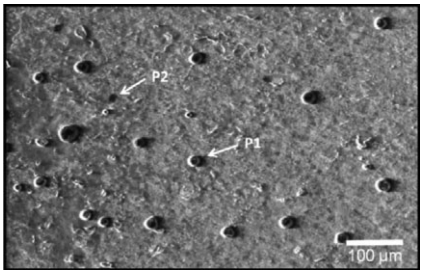


As the demand for renewable fuels increases, the quality of biodiesel is becoming more significant. Using impure biodiesel at low ambient temperatures is often problematic because contaminants crystalize and clog the fuel filter. One such contaminant which strongly impacts the formation of solid particles in biodiesel are saturated monoacylglycerols, SMG. In this study it is shown that lipase G from *Penicillium camemberti*, a mold better known for its use in cheese making, can catalyse the transesterification of SMG to fatty acid methyl ester and glycerol even in the presence of the bulk biodiesel, thus improving its quality. Furthermore, the by-product glycerol can be easily removed by water washing treatment.

Padhi, S. K. et al. Lipase-catalyzed transesterification to remove saturated MAG from biodiesel. *Eur. J. Lipid Sci. Technol.* 2012, *114*, 875–879

Chocolate topology studied by confocal Raman microscopy

Fat migration in chocolate products often leads to the appearance of ''white stuff'' on the chocolate surface. This is known as fat bloom. In this paper, confocal Raman microscopy has been used to study the surface of white chocolate pralines with hazelnut filling to understand the development of fat bloom. The analysis can be performed at ambient conditions, and no special sample preparation or marker molecules are required. It is shown that topological imperfections such as pores and protrusions at the chocolate surfaces are a part of a network of pore structures beneath and at the chocolate surface. The results indicate that the protrusions are connected to channels where a pressure driven convective flow of liquid fat from within the chocolate matrix moves up to the surface or back into the matrix, leaving an empty pore with a shell of fat at the surface. The findings support the hypothesis that the pores are connected to oil migration in filled chocolate products and thus to fat bloom development.



Dahlenborg, H. Study of the porous structure of white chocolate by confocal Raman microscopy. *Eur. J. Lipid Sci. Technol.* 2012, *114*, 919–926

APPENDIX

Anaplerotic & Ketogenic Diet
Biochemical & Metabolic Background for Organic Chemist

For better understanding of this project the biochemical keywords will be explained. Some simplification has to be done to reach this objective. Final goal of this project is therapeutic change of brain metabolism, so the introduction will be focused on the brain.

In the introduction of article manuscript one can find this information:

"In peripheral organs and the brain, these blood-born five-carbon ketones (C5) can enter directly to the tricarboxylicacid (TCA, also Krebs) cycle via succinyl-CoA and, unlike the ''classical'' C4 ketone bodies, provide ana-plerotic carbons. Anaplerosis is a process that replenishes TCA cycle intermediates, thus providing building blocks for biosynthetic pathways. Triheptanoin-derived C5 could bypass various steps of intermediary metabolism and directly ''energize'' mitochondria."

But what does all this mean really?

Normal diet – Metabolism of glucose:

Glucose is a key energy substrate for brain. During digestion, dietary polysaccharides are broken down into glucose, which is absorbed to the blood and then transported around the body. Protein GLUT1 (Glucose transporter 1) facilitates glucose crossing through blood-brain barier (BBB). Once in cytosol of target cell, metabolic pathway called glycolysis (10 reaction sequence) takes place and glucose (C6) is converted into two molecules of pyruvate (C3). Pyruvate dehydrogenase complex catalyzes sequence of reactions (translocation of pyruvate into mitochondria, its decarboxylation and association with *coenzyme-A*) resulting in its conversion to acetyl-CoA (C2). Alternatively, pyruvate can be converted to oxalacetate (C4), *i.e.* provides anaplerotic carbons for TCA cycle = a start of new TCA cycle in mitochondria.

Acetyl-CoA enters into the TCA cycle, where carbons are converted to CO_2 and hydrogens reduce special coenzymes (NAD+, FAD) that enter an electron transport chain, react with O_2, where final product is H_2O and the reaction energy is conserved in conversion of ADP molecules to ATP molecules.

We can schematically present this pathway as follows:

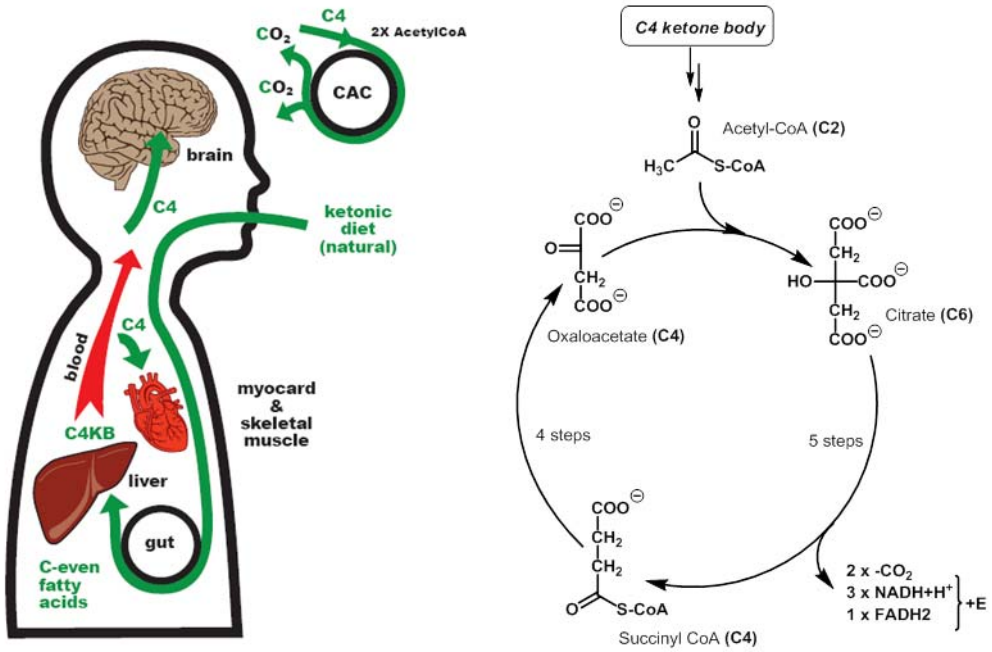
$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + energy conservation in 30-38 molecules of ATP.

Alternative substrate – KETONE BODIES:

During starvation, when source of carbohydrates is drastically suppressed, liver converts fat stored in adipose tissue into small molecules called ketone bodies (acetoacetate, and β-hydroxybutyrate). The state of elevated levels of ketone bodies in blood is called ketosis. Ketone bodies are able to enter into the brain *via* MCT1 (Monocarboxylate transporter 1) and replace glucose as an energy source.

The ketogenic diet is a high-fat, adequate-protein, low-carbohydrate diet. The diet mimics ketogenic aspects of starvation by forcing liver to burn fats and produce ketone bodies. Difference between ketogenic diet and starvation is that there is enough energy intake but the polysaccharide-derived glucose is substituted by fat-derived ketone bodies as a key source of energy for brain.

All naturally occurring fatty acids have even number of carbons. This results in stereotypic four-carbon ketone bodies (C4) production in liver. In brain C4 is converted into two molecules of acetyl-CoA (C2), which are burned in the TCA cycle getting the same result as mentioned previously, CO_2, H_2O and energy conserved in ATP.



Picture 1: Ketogenic diet

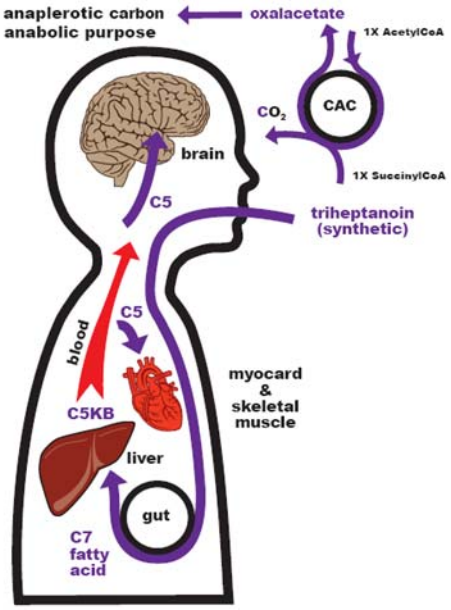
2 x Ac-CoA (2 x C2)

Anaplerosis

If we compare the following two situations, glucose metabolism and ketone bodies metabolism, there is not a big difference in energy produced from one molecule of glucose and one molecule of ketone. However, there is a rate limiting factor we have omitted in previous descriptions, which is an available amount of oxalacetate. First reaction of TCA cycle is condensation of oxalacetate (C4) with acetyl-CoA (2C), forming citrate (C6). In normal conditions, when there is enough glucose available, oxalacetate can be easily formed in adequate amount from glucose-derived pyruvate. This process is called anaplerosis. When ketone bodies are the major energetic substrate, oxalacetate must be formed from different substrates such as aminoacids, resulting in complex changes in brain biochemistry.

Anaplerotic diet

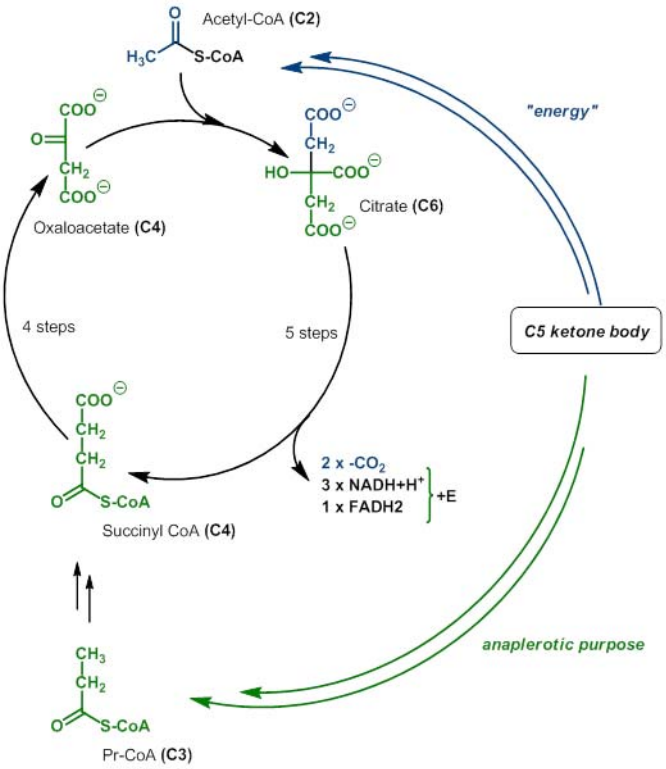
Anaplerotic diet is a special form of ketogenic diet, where significant part of natural even-carbon triglycerides (30-40% of daily caloric intake) are substituted with unnatural trygliceride composed of odd-carbon alkylnoyl chain, *e.g.* triheptanoin. In such case liver starts to produce also non-natural C5 ketone bodies (β-keto-pentanoate and β-hydroxypentanoate). C5 ketone bodies are also able to enter a brain *via* MCT1 transporter. In the target cell, C5 ketone body is converted to one molecule of acetyl-CoA (C2) and one molecule of propionyl-CoA (C3). Acetyl-CoA (C2) is metabolized in TCA cycle in the same way as described previously. On the other hand, the propionyl-CoA (C3) is converted to succinyl-CoA and then to oxalacetate, *i.e.* propionyl-CoA is anaplerotic. It means that anaplerotic diet is able to provide both substrates for TCA cycle, acetyl-CoA and oxalacetate in one molecule of C5 ketone body, resulting in higher metabolic efectivity if compared with C4 ketone body.



Picture 2: Anaplerotic diet

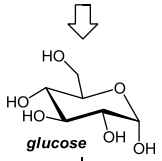
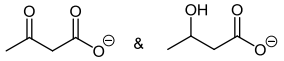
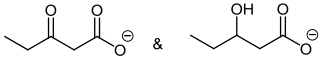
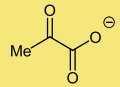
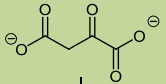
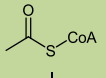
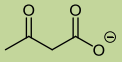
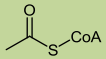
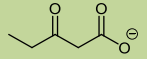
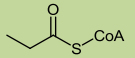
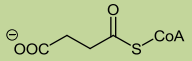
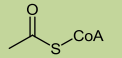
1 x Ac-CoA (1 x C2)

1 x Pr-CoA (1 x C3)



Conclusion

Anaplerotic diet is more complete than the normal ketogenic diet and biochemistry in brain is not dramatically alternated. Schematic overview and comparison of all three diets discussed above is presented in Figure 1. In the figure the same information is presented in two ways: first from the chemical viewpoint and the second from the biological viewpoint.

	Normal diet	Ketogenic diet	Anaplerotic diet
extracellular		***C4 ketone bodies*** 	***C5 ketone bodies*** 
Transporter	GLUT1 (Glucose transporter 1)	MCT1 (Monocarboxylate transporter 1)	
Intracellular (cytosol)	*glycolysis* 2 x ***pyruvate*** (C3)  and/or		
Mitochondrial membreane	PC (Pyruvate carboxylase) PDH (pyruvate dehydrogenase)	BDH (3-hydroxybutyrate dehydrogenase) KBH (Ketone Bodies Utilisation)	
mitochondria	***oxaloacetate*** (C4)  ***TCA cycle (anaplerotic purpose)*** ***Acetyl-CoA*** (C2)  ***TCA cycle (energy)***	***acetoacetat*** (C4)  *OXCT1 (3-oxoacid CoA transferase 1)* ***2 x Acetyl-CoA*** (C2)  ***TCA cycle (energy)***	***3-keto-pentanoate*** (C5)  ***Propyonyl-CoA*** (C3)  ***Succinyl-CoA*** (C4)  ***TCA cycle (anaplerotic purpose)*** ***Acetyl-CoA*** (C2)  ***TCA cycle (energy)***

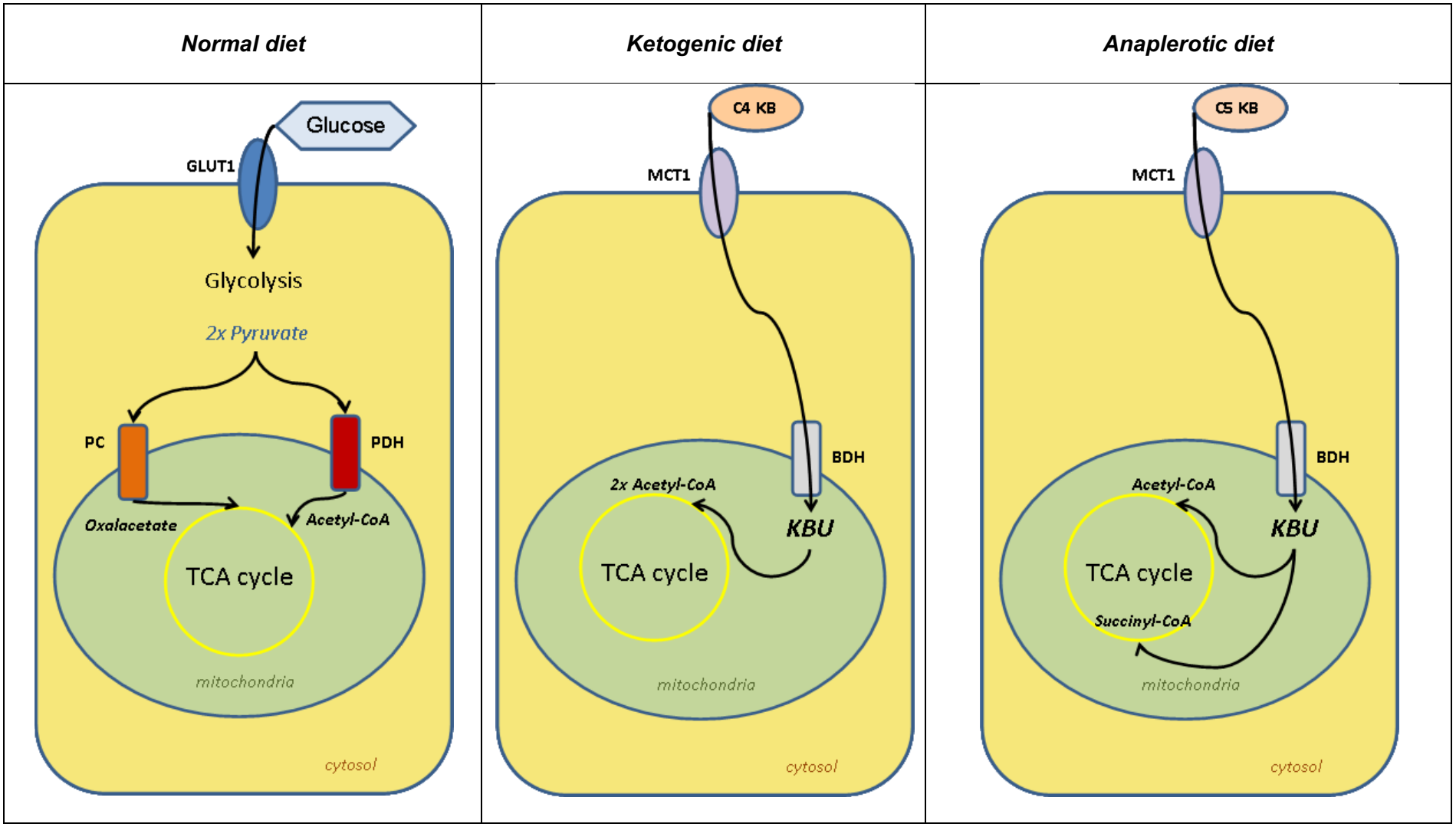


Figure 1 Schematic overview of normal, ketogenic and anaplerotic diet.

CHAPTER 3

Toluene dioxygenase (TDO) mediated oxidation of halogen-substituted benzoate esters

Semak, V.; Metcalf, T. A.; Endoma-Arias, M. A. A.; Mach, P.; Hudlicky, T.
Org. Biomol. Chem. **2012**, *10*, 4407-4416.

Toluene dioxygenase (TDO) mediated oxidation of halogen-substituted benzoate esters

Foreword

Microbial biotransformation is the chemical modification of substrate made by a microorganism. Metabolism or metabolic transformations are terms frequently used for the biotransformation process.[1] The biggest advantage of biotransformations remains in fact that the product(s) of the reaction is(are) usually unavailable by any other known method or its synthesis will be much more complex. For this reason, biotransformations attract the preparative organic chemists working in different fields, *e.g*. carbohydrates, natural products, chemical industry. Enzymes present in microorganism work as a enantioselective chemical catalyst.

Introduction

Enzymatic dihydroxylation of aromatic compounds was discovered by Professor David T. Gibson (University of Iowa) four decades ago.[2] Gibson's disclosure greatly contributed to advancing biocatalysis as a discipline with significant impact on synthesis of optically pure compounds. Impact in the field of enantioselective synthesis is very well documented by various recent reviews.[3]

Objectives

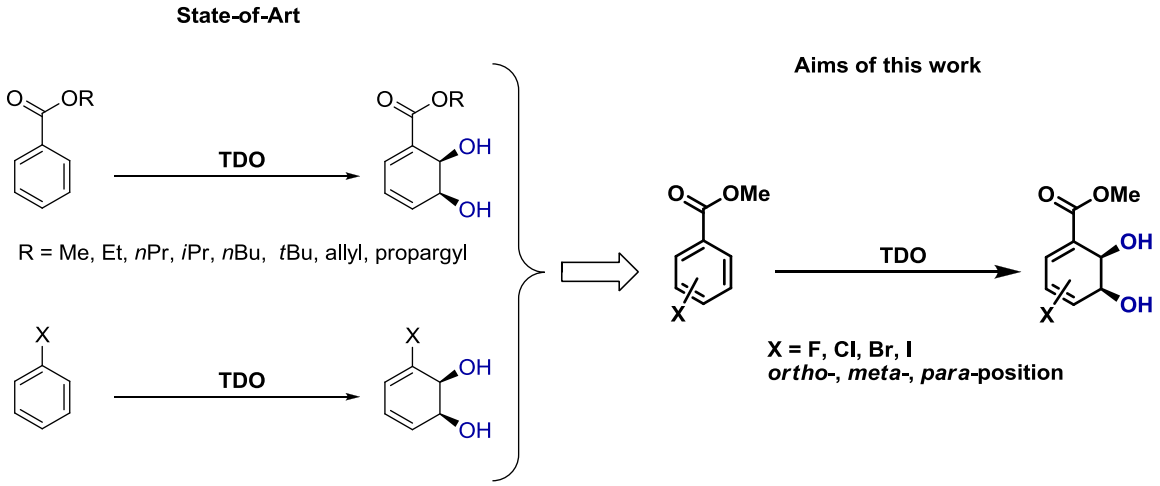
1) Extrapolation of TDO mediated oxidation to halogen-substituted methyl benzoate esters (Scheme 1).
2) Determination of absolute configuration of the prepared metabolites *via* chemical modifications.
3) Possible application of metabolite to an enantioselective synthesis of natural products.

---

[1] The terminology in the area of biotransformations slightly differs from that used in organic chemistry, *e.g.* harvest = work-up, metabolite = product, substrate = starting material, yields are given as an amount of metabolite to volume of media (gL^{-1}), *etc*.

[2] (a) Gibson, D. T.; Koch, J. R.; Kallio, R. E. *Biochemistry* **1968**, *7*, 2653. (b) Gibson, D. T.; Koch, J. R.; Schuld, C. L.; Kallio, R. E. *Biochemistry* **1968**, *7*, 3795.

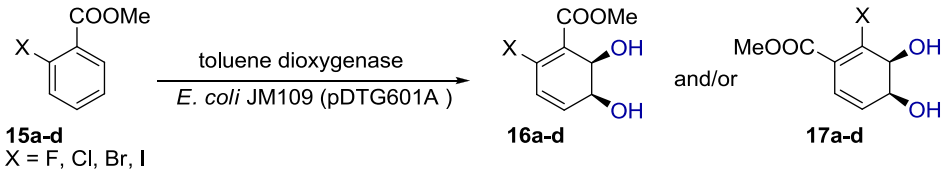
[3] a) Duchek, J.; Adams, D. R., Hudlicky, T. *Chem. Rev.* **2011**, *111*, 4223–4258; b) Hudlicky, T. *Pure Appl. Chem.* **2010**, *82*, 1785–1796; c) Hudlicky, T.; Reed, J. W. *Chem.Soc.Rev.* **2009**, *38*, 3117–3132; d). Hudlicky, T.; Reed, J. W. *Synlett* **2009**, 685-703.



Scheme 1 Enzymatic dihydroxylation of aromatic compounds.[4]

Results

A series of *ortho*-, *meta*-, and *para*-halogen-substituted methyl benzoate esters was subjected to enzymatic dihydroxylation *via* the whole-cell fermentation with *E. coli* JM109 (pDTG601A). Only *ortho*-substituted benzoates were metabolized (Scheme 2). Methyl 2-fluorobenzoate yielded one diol regioselectively (y = 0.50 gL^{-1}) whereas methyl 2-chloro- (y = 0.47 gL^{-1}), methyl 2-bromo- (y = 0.20 gL^{-1}) and methyl 2-iodobenzoates (y = 0.15 gL^{-1}) each yielded a mixture of regioisomers (Table 1 of the full paper).

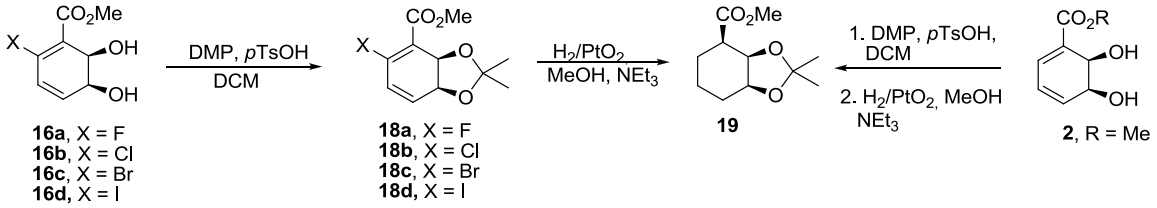


Scheme 2 TDO mediated oxidation of halogen-substituted benzoate esters.

Absolute stereochemistry was determined for all new metabolites **16a-d** and **17b-d** by comparing physical & chemical properties of their chemical derivatives. For example, in case of metabolites **16a-d**; the diol was protected and hydrogenated (dehalogenation & double bond reduction) (Scheme 3). By this way, all diols **16a-d** provide the same product, ester **19**. Identical cyclic ester **19** was alternatively prepared from the well defined diol **2**. All physical & chemical properties of **19** were identical irrwspective of which starting material was employed (**16a**, **16b**, **16c**, **16d** or **2**). By this

[4] a) *Benzoates*: Fabris, F.; Collins, J.; Sullivan, B.; Leisch, H.; Hudlicky, T. *Org. Biomol. Chem.* **2009**, *7*, 2619 –2627. b) *Haloderivatives*: Boyd, D.R.; Sharma, N. D.; Byrne, B.; Hand, M. V.; Malone, J. F.; Sheldrake, G. N.; Blacker, J.; Dalton, H. J. *J Chem Soc, Perkin Trans 1* **1998**, *12*, 1935-1943.

way, absolute configuration of metabolites **16a-d** was determined. Similar strategy was used in case of derivatives **17a-d**.



16a, X = F
16b, X = Cl
16c, X = Br
16d, X = I

18a, X = F
18b, X = Cl
18c, X = Br
18d, X = I

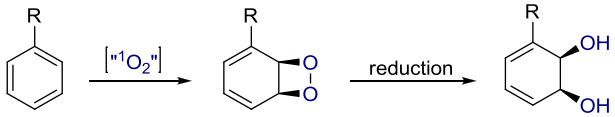
19

2, R = Me

Scheme 3 Absolute stereochemistry determination of diols **16a-d**.

Mechanistic considerations & Discussion

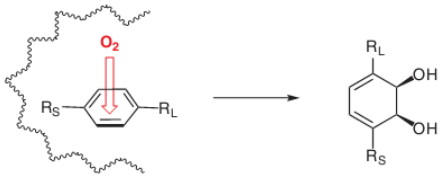
The mechanism of enzymatic dihydroxylation of arenes is unknown. Numerous propositions have been advanced but none has been proven by an experiment.[5] Original Gibson`s proposition for dioxetane intermediates (Scheme 4) is, to date, the only one partially subjected to experimental investigation through studies of kinetic isotope effects in dehydrogenation to cathecols.[6]



Scheme 4 Dioxetane pathway.

Because the actual mechanism is not known, it is logical that all possibilities are equivalent in merit until proven otherwise. For this reason, the computational studies which are discussed in the article are based on the assumption that dioxetanes are intermediates in the reaction, whether formed by singlet oxygen cycloaddition or by internal redox reactions of triplet species.

Based on high number of metabolites and relatively consistent production of similar regio- and stereoisomers from a wide variety of substrates, a model was developed that can be effectively used to predict the out-come of the enzymatic hydroxylation for new and untried compounds, as illustrated in Scheme 5.



Scheme 5 Boyd's empirical model (R_S – small substituent, R_L – large substituent).

[5] For a summary of mechanistic postulates see Ref. 3d and (a) Bui, V. P.; Nguyen, M.; Hansen, J.; Baker, J.; Hudlicky, T. *Can. J. Chem.* **2002**, *80*, 708-713; (b) Hudlicky, J. R.; Hopskins-Hill, J.; Hudlicky, T. *Synlett* , **2011**, 2891–2895.

[6] Jeffrey, A. M.; Yah, H. J. C.; Jerina, D. M.; Patel, T. R.; Davey, J. F.; Gibson, D. T. *Biochemistry* **1975**, *14*, 575–585.

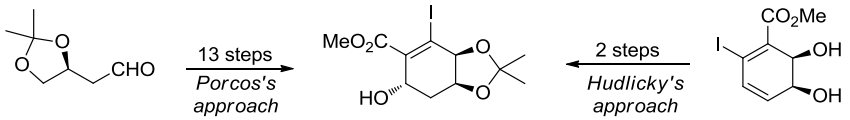
However, this Boyd empirical model, based on Charton steric parameters, cannot satisfactorily explain the observed regioselectivity in TDO-catalysed *cis*-dihydroxylation of the *ortho*-substituted benzoates.[7] Due to this, computational study was performed. Theoretical models indicated that also the charge (positive or negative) of the arene substituents can have influence during the docking of the substrate to the active pocket of the enzyme.

Conclusions

Seven new metabolites from Toluene DiOxygenase (TDO) - catalyzed dihydroxylation of *o*-halobenzoates were identified. In general, benzoate esters are converted to diols in significantly lower yields than halobenzenes, and substituted benzoate esters are somewhat reluctant substrates. However, all of these compounds are accessible and can be prepared in multi-gram amounts by fermentation.

Ab initio calculations were shown to be in good qualitative agreement with the experimental results for microbial dihydroxylation of *ortho*-substituted halobenzoates.

The new metabolites will find widespread use in the preparation of optically pure diols not otherwise available from enzymatic oxidation of the corresponding arenes. The short preparation of the intermediate in the Kibledone C[8] synthesis lends credence to the above investigation (Scheme 6).[9]



Scheme 6 A short route for the preparation of Kibledone C intermediate.

This article was highlighted by Robert A. Hill and Andrew Sutherland (*Nat. Prod. Rep.* **2012**, *29*, 829-833) in the selection of 32 recent papers which covers various aspects of current developments in bioorganic chemistry and novel natural products.

[7] In general, Boyd's empirical rule works well only with spherical substituents.
[8] Kibdelones are hexacyclic tetrahydroxanthones and potent anticancer agents isolated from an Australian microbe *Kibdelosporangium sp*.
[9] Endoma-Arias, M. A. A.; Hudlicky T. *Tetrahedron Lett.* **2011**, *52*, 6632-6634. b) Sloman, D. L.; Bacon, J. W.; Porco, J. A. *J. Am. Chem. Soc.* **2011**, *133*, 9952-9955.

Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 4407

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PAPER

Toluene dioxygenase mediated oxidation of halogen-substituted benzoate esters†

Vladislav Semak,[a] Thomas A. Metcalf,[a] Mary Ann A. Endoma-Arias,[a] Pavel Mach[b] and Tomas Hudlicky*[a]

Received 27th January 2012, Accepted 29th March 2012
DOI: 10.1039/c2ob25202c

A series of *ortho*-, *meta*-, and *para*- halogen-substituted methyl benzoate esters was subjected to enzymatic dihydroxylation *via* the whole-cell fermentation with *E. coli* JM109 (pDTG601A). Only *ortho*-substituted benzoates were metabolized. Methyl 2-fluorobenzoate yielded one diol regioselectively whereas methyl 2-chloro-, methyl 2-bromo- and methyl 2-iodobenzoates each yielded a mixture of regioisomers. Absolute stereochemistry was determined for all new metabolites. Computational analysis of these results and a possible rationale for the regioselectivity of the enzymatic dihydroxylation is advanced.

Introduction

The oxidation of aromatic compounds by toluene dioxygenase (TDO) yields *cis*-dihydrodiols, over 400 of which are known.[1] In 1968 Gibson isolated the first stable arene-*cis*-diol from a fermentation of *Pseudomonas putida* grown in the presence of *para*-chlorotoluene.[2] He later developed a mutant strain that lacked the requisite enzymes to process *cis*-dihydrodiols, allowing the accumulation of these metabolites in the cell broth.[3] The genes encoding TDO were later cloned into a strain of *Escherichia coli* producing the recombinant organism JM109 (pDTG601A), in which the protein synthesis is initiated by isopropylthiogalactose (IPTG) and thus no aromatic inducer is required.[4] This aspect greatly simplifies the identification of any new metabolites as the diol derived from the inducer will be absent from the product mixtures.

The first applications of *cis*-dihydrodiols in organic synthesis were the synthesis of polyphenylene by the ICI group[5] and the preparation of pinitol from the *meso*-diol derived from benzene accomplished by Ley some 20 years after Gibson's disclosure.[6] In 1988 our group published the formal synthesis of a prostaglandin from the diol derived from toluene.[7] Since then, many new metabolites have been discovered, and *cis*-dihydrodiols have enjoyed widespread use in organic synthesis.[8]

In 2009 we reported the microbial oxidation of several benzoate esters **1** in order to probe the limits of substrate size.[9] It was found that TDO oxidized methyl, ethyl, allyl, and propargyl benzoates to their corresponding diols **2** in yields of about 1 g L^{-1}, whereas *n*Pr, and *i*Pr, esters were found to be poor substrates and *n*Bu, and *t*Bu benzoate were not metabolized (Fig. 1). The diol derived from the oxidation of ethyl benzoate was recently used in several generations of synthesis of oseltamivir.[10] In this disclosure, we report the metabolism of halogen-substituted methyl benzoates, provide computational study and rationale for the observed selectivities, and offer further applications for the use of new metabolites.

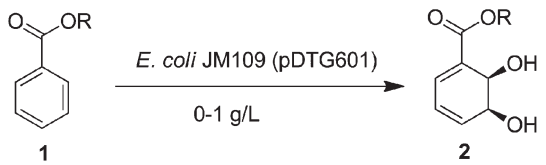

Fig. 1 Microbial dihydroxylation of benzoate esters.

Results and discussion

In a recent paper[9] we detailed the results of the microbial dihydroxylation of benzoate esters **1** and the application of dienediols **2** in the preparation of pseudo-sugars. To further extend the applicability of diols derived from benzoate esters, we subjected methyl halobenzoates to microbial dihydroxylation studies to determine the effect of the halogen substituent on the outcome of bio-oxidation. From the standpoint of synthesis, the halogen group provides an additional reactive functionality that can be further exploited in various radical or transition metal-catalyzed coupling protocols for accessing new optically pure *cis*-diols not

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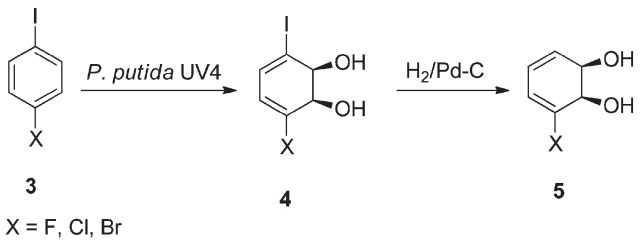
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c2ob25202c

available by enzymatic dihydroxylation of the corresponding aromatic substrates.

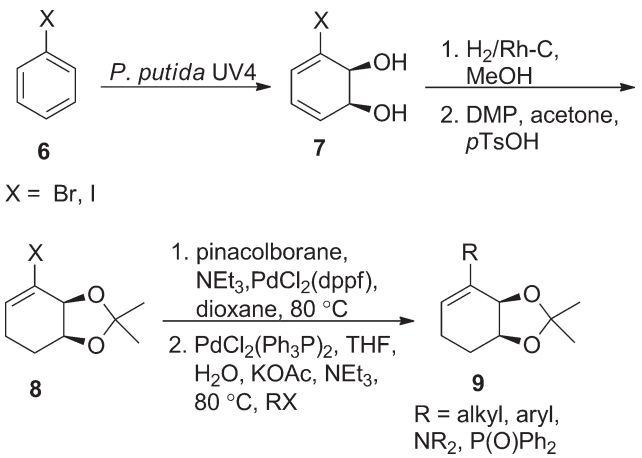
The required methyl halobenzoate esters were prepared from the corresponding commercially available halobenzoic acids by treatment with sodium carbonate and dimethyl sulfate in acetone. The methyl halobenzoate esters were incubated in Fernbach shake flasks (2 L) with *E. coli* JM109 (pDTG601A), grown to an optimum optical density in a 15 L Biostat C fermentor. After incubation for six hours, the broth was extracted with EtOAc and analyzed by TLC and ^{1}H NMR. If a new metabolite was detected, preparative-scale fermentation was undertaken in a 15 L Biostat fermentor as previously described.[11] We found that *meta*- or *para*- substituted benzoate esters, unlike their dihalo- or halo-alkyl counterparts, were not metabolized by TDO.

The utility of the iodine atom as a directing group for bio-oxidation and its subsequent reductive removal has been illustrated in the work of Boyd and co-workers.[12] A series of dihalogenated arene substrates (Scheme 1) was oxidized using *P. putida* UV4. Removal of iodine in the presence of bromine or fluorine was achieved by means of catalytic hydrogenation as shown below for the *para* isomer. The corresponding *ortho* and *meta* isomers were also investigated. This study provided access to *ent*-halo diols, which are not accessible from the corresponding halobenzenes using bacterial dioxygenases.



Scheme 1

Boyd and co-workers[13] have recently demonstrated the versatility of bromo- and iodobenzene-derived diols as starting materials in the preparation of a wide array of other diols either not accessible by fermentation or produced in low yields by bio-oxidation (Scheme 2). The bio-oxidation of bromo- or iodobenzene **6** afforded the enantiomerically pure diols **7**, and selective



Scheme 2

hydrogenation of the less hindered alkene catalyzed by rhodium on carbon under a hydrogen atmosphere provided tetrahydrodiols, which were protected as acetonides **8**. Substitution of Br or I with boronates followed by coupling with carbon, nitrogen, or phosphorus nucleophiles afforded coupled products of type **9**.

The coupling strategy employed by Boyd provides access to a wide variety of optically pure intermediates. Similarly useful would be an approach to diols derived from disubstituted aromatics especially ones containing functionalities of different size, using the Charton steric parameter as an indicator of substituent size. Such substrates are processed according to a model proposed by Boyd *et al.*[8g,14] where the larger of the substituents "directs" the oxidation.

There are 15 known *cis*-dihydrodiols of type **11**, derived from *o*-, *m*-, and *p*-alkyl-/or halo-iodobenzenes, Fig. 2.[12,15] In all cases, the iodine atom "directed" the regiochemistry of dihydroxylation.

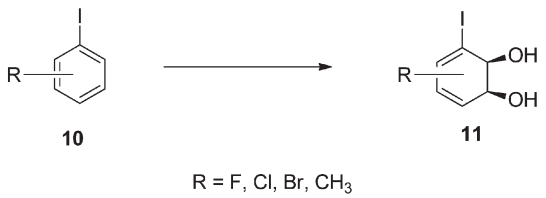


Fig. 2 Metabolites of iodobenzenes.

Nineteen *cis*-dihydrodiols derived from various isomers of alkyl- or halo-benzoic acids have been reported; however, there is no clear trend in regiochemical preferences of dihydroxylation. Most of these substrates give *ipso*[16] diols of type **14** (only a few exceptions provide diols of type **13**).[17]

There are, surprisingly, only seven metabolites derived from various alkyl benzoate esters, as shown in Fig. 1.[9,16a,18] In contrast to the number of metabolites available from the disubstituted arenes, disubstituted alkyl benzoate dihydroxylation has not yet been reported even though the oxidation of substituted benzoic acids is known (Fig. 3).[16,17] We therefore decided to pursue the current study to seek functionalized benzoate-derived diols *via* subsequent coupling protocols for providing additional building blocks not available directly by the enzymatic dihydroxylation.

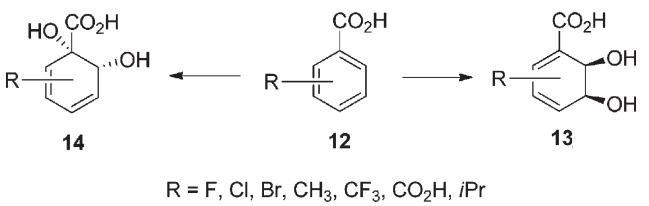
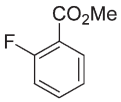
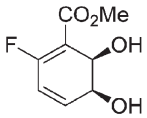
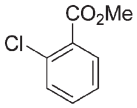
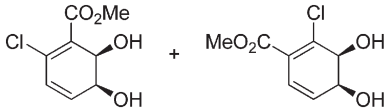
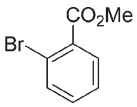
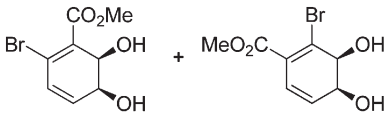
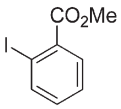
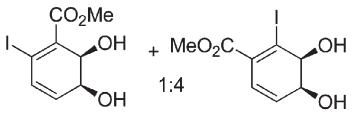


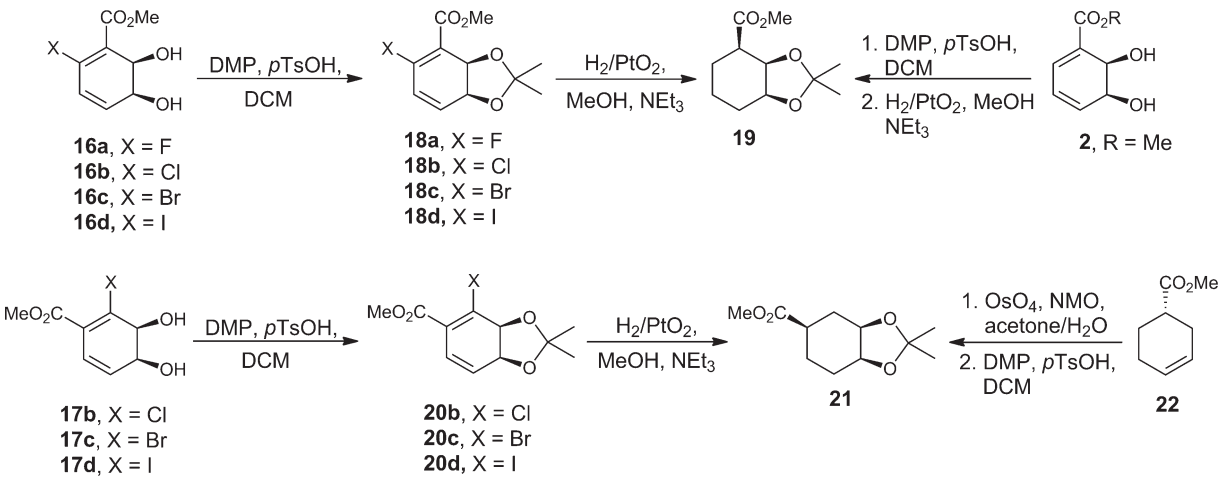
Fig. 3 Metabolites of substituted benzoic acids.

Ortho-halogen substituted methyl benzoate esters **15** gave diols of type **16** or **17**, as shown in Table 1. Methyl 2-fluorobenzoate **15a** furnished a single diol of type **16**, whereas methyl 2-chloro-, methyl 2-bromo- and methyl 2-iodobenzoate gave mixtures of **16** and **17**. These observations are in agreement with Boyd's rules for predicting the regio- and stereochemistry of dihydroxylation by TDO.[8g,14]

Table 1 Microbial dihydroxylation of methyl halobenzoate substrates

Starting material	Conversion (%)	Yield (g L^{-1})	Products[a] (ratio)
 **15a**	49.6	0.50	 **16a**
 **15b**	60.2	0.47	 **16b** 4:1 **17b**
 **15c**	13.3	0.20	 **16c** 3.5:1 **17c**
 **15d**	10.0	0.15	 **16d** 1:4 **17d**

[a] Ratio was obtained using integration of ^{1}H NMR peaks corresponding to olefinic signals in the crude mixture.



Scheme 3

Yields of diols derived from *ortho*-substituted benzoates are given in Table 1, along with the ratios of regioisomers. The stability of diols **16** and **17** above was found to depend strongly on the nature of the X group. The most electronegative substituent, fluorine, confers the highest stability while the least electronegative substituent, iodine, contributes to lower stability. In fact, the diols **16d** and **17c** could not be isolated (these undergo a facile dehydration to the corresponding phenols upon concentration in the rotary evaporator, even at low temperatures) and hence were characterized as the corresponding acetonide derivatives instead. Even the acetonides had a limited stability at room temperature and had to be stored at low temperatures.

For substrates **15a**, **15b** and **15c** the major diol product was the result of methyl carboxylate directing the dihydroxylation. This trend was reversed with substrate **15d** where the iodine atom directed the regiochemistry of the dihydroxylation.

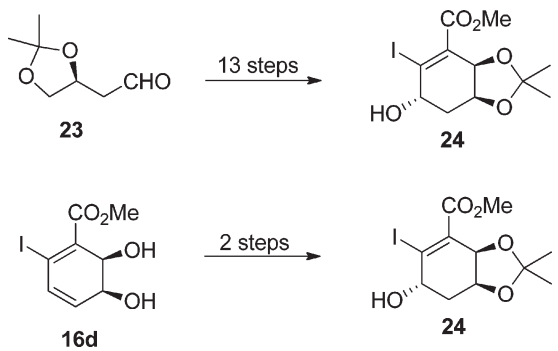
With the exception of methyl 2-fluorobenzoate diol **16a**, these diols readily underwent dehydration at room temperature. They are stable in crystalline form at −78 °C or in pH 8 phosphate buffer at 0 °C. They are less stable than diols **2** derived from non-halogenated alkyl benzoate substrates **1**, for example, methyl benzoate or ethyl benzoate.[9]

In order to determine the relative and absolute stereochemistry, the new metabolites were protected as acetonides followed by hydrogenation as shown below (Scheme 3). The products obtained from diols **16** were matched with the fully hydrogenated diol **19** derived from the known diol **2**[9] obtained previously from methyl benzoate. Similarly, diols **17** were converted to

ester **21**, which was derived from known compound **22**[19] in two steps. These experiments confirmed the relative as well as absolute stereochemistry of the new metabolites as drawn.

Hydrogenation of acetonides **18a**, **18b**, **18c** and **18d** from type **16** diol resulted in a product that was identical in all properties to ester **19**, prepared from the known methyl benzoate diol **2**. Similarly, hydrogenation of acetonide **20b**, **20c** and **20d** resulted in the formation of ester **21** (except for the sign of optical rotation), derived from methyl cyclohexenylcarboxylate ester **22**. The starting ester **22** was obtained in racemic form *via* Diels–Alder reaction of butadiene sulfone and methyl acrylate in toluene at 130 °C performed for two days in a sealed tube. Resolution was achieved using porcine pancreatic lipase (PPL) in phosphate buffer at pH 7.0. Dihydroxylation of **22** followed by acetonide formation afforded the desired standard compound *cis* (relative stereochemistry of ester to acetonide) **21** as a 3 : 4 mixture with its *trans* diastereomer (relative stereochemistry of ester to acetonide), which was separated by silica gel column chromatography.

With respect to potential applications of diols derived from halobenzoates, Porco and co-workers[20] recently determined the absolute stereochemistry of the natural product kibdelone C by way of its total synthesis. A key intermediate, **24**, was prepared in 13 steps starting from commercially available material **23** as shown below (Fig. 4). We have shown[21] that diol **16d** provided a good choice for access to this key intermediate: two *versus* the thirteen steps originally reported. Applications of the metabolites derived from halobenzoates to the synthesis of additional kibdelone derivatives are now in progress with collaboration.



23 24 16d 24

Fig. 4 A short route for the preparation of Kibledone C intermediate **24**.

Computational studies

The Boyd empirical model, based on Charton steric parameters (ν), cannot satisfactorily explain the observed regioselectivity in TDO-catalysed *cis*-dihydroxylation of the *ortho*-substituted benzoates **15a–15d**. The size of the substituents, according to Charton steric parameters, are as follows: COOMe (ν = 1.39) > I (ν = 0.78) > Br (ν = 0.65) > Cl (ν = 0.55) > F (ν = 0.27). This indicates that in all examples the COOMe should be the larger stereodirecting group; however, in the case of iodine derivative **15d**, the iodine atom is clearly dominant over the ester group. As Boyd claims for the case of nonsymmetrical substituents, other considerations, *e.g.*, substituent length and conformation in the vicinity of the active site, should also be taken into consideration.[8g,14]

The mechanism of the enzymatic dihydroxylation of arenes is unknown. Various propositions have been advanced[22] but none has been proven by experiment. The original proposition by Gibson[23] for dioxetane intermediates is, to date, the only one partially subjected to experimental investigation through studies of the kinetic isotope effects in dehydrogenation to cathecols.[24] We have briefly investigated mechanistic options involving radical intermediates but have not arrived at any reasonable mechanistic postulates.[25] Other options include high oxidation state iron peroxides, iron oxo species, and singlet oxygen cycloadditons. Because the actual mechanism is not known, it is logical that all possibilities are equivalent in merit until proven otherwise. For this reason, the computational studies that follow are based on the assumption that dioxetanes are the intermediates in the reaction, whether formed by singlet oxygen cycloaddition or internal redox reactions of triplet species. The results of these computational studies follow.

Methyl benzoates 15a–15d. Taking into account Boyd's observations, we studied the geometry and electrostatic effects of methyl benzoates **15a–15d**. The resulting data were employed in the calculation of energies of proposed dioxetane intermediates. Two rotamers of benzoates **15a–15d** were studied (Fig. 5).

In all cases except for **15a**, the $COOCH_3$ group is rotated out of the plane of benzene ring. As seen from Table 2, this angle varies from 17 to 31°, reducing π-interaction of $COOCH_3$ group with the aromatic ring. For benzoates **15a–15c**, the *anti*-conformer is more stable, but for iodine derivative **15d** the *syn* arrangement is preferred. Quantitatively, the stability difference between *syn* and *anti* is significant only for **15a**. In the case of Cl-, Br- and I-substituted benzoates (**15b–15d**) the barrier for interconversion (1.3–1.5 kcal mol^{-1}), is small (Table 2). Even one weak hydrogen-bond formation can supply enough energy for a conformational change, and the $COOCH_3$ group can easily adopt conformation, as required by the active center of enzyme.

Electrostatic effects can also play a role in substrate docking. Table 2 shows the atomic charges calculated by natural

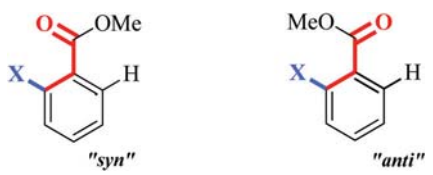


Fig. 5 Rotamers of **15a–15b**.

Table 2 Properties of **15a–15d**

		Out-of-plane angle [°]	$E_{relative}$ [kcal mol^{-1}]	Barrier heights [kcal mol^{-1}]	NPA charge on X [*e*]
15a	*syn*	0.0	1.17	3.9	−0.355
	anti	0.0	0.00		−0.363
15b	*syn*	25.5	0.21	1.5	−0.005
	anti	24.2	0.00		−0.018
15c	*syn*	23.0	0.13	1.3	+0.088
	anti	26.2	0.00		+0.071
15d	*syn*	17.4	0.00	1.3	+0.216
	anti	30.0	0.19		+0.192

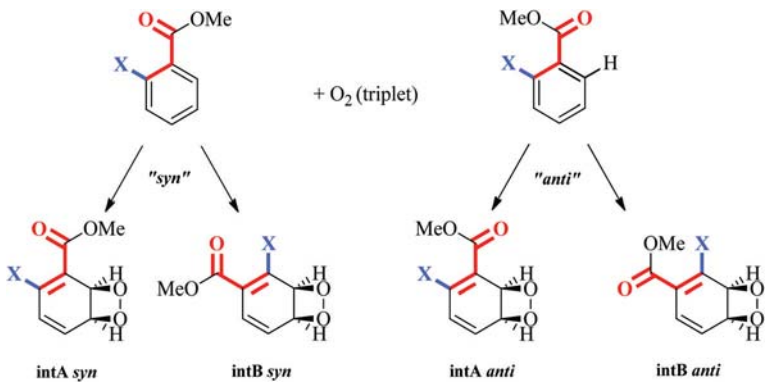
population analysis (NPA)[26] for halogen atoms in *syn* and *anti* conformations. The charges of the oxygen atoms in the acetyl group are negative in each case, but the charge of halogen is variable. This can have a significant influence on the orientation of the benzoate during docking in the active site of the enzyme. This preference can be "matched" or "mismatched" with the preference based on the orientation effect of the functional group (electronic or steric). As seen in Table 2, the charge on the halogen is practically the same in either the *syn* and *anti* conformation.

Formation of dioxetane intermediates: electronic effects

We wished to assess the combined orientational effect of X and COOMe substituents on the specific type of reaction, *i.e.* formation of dioxetane intermediate[25a] (see Scheme 4). Each formed regioisomer of dioxetane intermediate can be in two rotameric forms, *syn* and *anti.* If the COOMe group is regiodirecting, the intermediate must have the **A** type structure, if halogen is in this role, the intermediate is of **B** type. Calculated reaction energies for formations of intermediates **intA** and **intB** in *syn* and *anti* conformations by reaction of benzoate with triplet (ground state) oxygen are listed in Table 3. The idea behind this is that relative energies of these intermediates are related to barrier heights for formation of diols (Hammond's postulate) and thus can serve as a rough guide to assess effect of different halogen substituents.

It is not known whether singlet oxygen or another reactive oxygen species (ROS) takes part in this reaction; however, our calculations were based on oxygen in its ground (triplet) state as we do not know the exact character of the reacting species (singlet oxygen, superoxide anion, peroxy or hydroperoxy radical, or any metal-bound species that may exist in the active site). The proper theoretical description of singlet oxygen is technically more complicated, but for the present study only the relative preference for **A** and **B** in different halo-benzoates is important. We also assume that the intermediate of the oxidation is the dioxetane, as originally proposed by Gibson. This assumption is valid for the comparisons made in the computational study but may not accurately describe the actual intermediates in the enzymatic reaction.

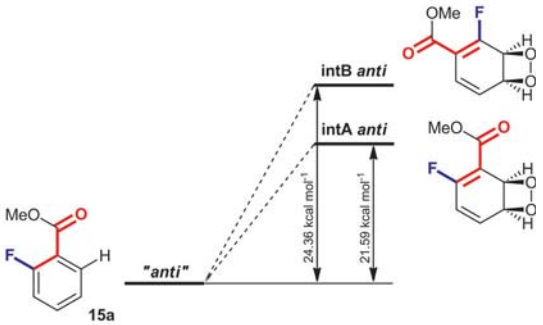
From the energy values in Table 3 we may conclude that the most pronounced orientational effect exists in the case of **15a**. The energy difference between **intA** and **intB** is 3.4 kcal mol^{-1}. In the case of **15d** it drops to just 0.5 kcal mol^{-1}. If there is another orientational effect (steric, electrostatic *etc.*) that competes with this preference for formation of intermediate **intA**,



Scheme 4

Table 3 Reaction energies for formation of intermediates **intA** ("actE A") and **intB** ("actE B")

		"actE" A [kcal mol^{-1}]	"actE" B [kcal mol^{-1}]
15a	*syn*	21.24	24.57
	anti	21.59	24.36
15b	*syn*	22.06	24.18
	anti	22.01	23.48
15c	*syn*	21.73	23.64
	anti	21.95	23.04
15d	*syn*	21.95	23.28
	anti	22.17	22.30



it can easily change this preference for iodine, but not for fluorine. This effect will be very similar for Cl and Br substituents. If we would consider the reacting species to be singlet oxygen (instead of triplet) in the lowest delta state, we can add its experimental energy: +22.5 kcal mol^{-1} (relative to triplet O_2) to the left-hand side of reactions for the formation of intermediates **A** and **B** and thus reduce the reaction energy by this amount. Resulting energies are then close to zero, indicating that these reactions are now almost thermally neutral, *i.e.* reaction enthalpy is ~0 kcal mol^{-1}.

Conclusions

We have identified seven new metabolites from the TDO-catalyzed dihydroxylation of *o*-halo benzoates. In general, benzoate esters are converted to diols in significantly lower yields than halobenzenes, and substituted benzoate esters are somewhat reluctant substrates. However, all of these compounds are accessible and can be prepared in multi-gram amounts by fermentation.

Ab initio calculations were shown to be in good qualitative agreement with the experimental results for microbial dihydroxylation of *ortho*-substituted halo benzoates. The most pronounced effect was observed in the case of fluorobenzoate **15a**, where TDO oxidation is regioselective. Practically no difference was noted between chloro and bromo derivatives (**15b** and **15c**) in both the theoretical calculations and the experimental results. Preference for the formation of **B** type diol **17d** in the case of the iodo derivative can be partially explained by Boyd's rules. In addition the positive charge (δ+) of iodine in **15d** can influence the orientation of the benzoate during docking to the active site of the enzyme.

The new metabolites will find widespread use in the preparation of optically pure diols not otherwise available from enzymatic oxidation of the corresponding arenes. The short preparation of the intermediate in the kibledone C synthesis lends credence to the above investigation. Although the precise mechanism of the enzymatic dihydroxylation remains unsolved, there is an increase in the power of prediction in investigations of new arene substrates.

Experimental section

Inoculum was obtained from viable cells stored −78 °C in cryovials. They were grown in suitable media as previously described.[11] Substrate was fed in 1 g increments over the course of ~3 h with metabolites being harvested in the usual manner. All non-aqueous reactions were conducted in an argon atmosphere using standard Schlenk techniques for the exclusion of moisture and air. Methylene chloride was distilled from calcium hydride, THF and toluene were dried over sodium/benzophenone. Analytical thin layer chromatography was performed on Silicycle 60 A° 250 mm TLC plates with F-254 indicator. Flash column chromatography was performed using Kieselgel 60 (230–400 mesh). Melting points were recorded on a Hoover Unimelt apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer One FT-IR spectrometer. Optical rotation was measured on a Perkin-Elmer 341 polarimeter at a wavelength of 589 nm. ^1H and ^{13}C spectra were recorded on a 300 MHz and 600 MHz Bruker spectrometer. All chemical shifts are referenced to TMS or residual undeuterated solvent. Data of proton spectra are reported as follows: chemical shift in ppm (multiplicity: singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m)), coupling constants [Hz], integration). Carbon spectra were recorded with complete proton decoupling and the chemical shifts are reported in ppm (δ) relative to solvent resonance as internal standard. Mass spectra and high resolution mass spectra were performed by the analytical division at Brock University.

Computational details and methods

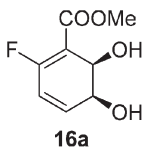
For calculations of molecular structures and energetics DFT theory was used with B3LYP[27] hybrid functional. This is an obvious choice for organic molecules, because it supplies good results and has been used with enough examples that a critical assessment of its performance is available. What is less obvious is the selection of appropriate basis sets, as we need basis set which can describe, with comparable precision, all halogens, including iodine. From relatively few possibilities we choose SBKJC[28] basis set with relativistic pseudopotentials, augmented with 1p and 2d polarization functions.[29] All reported energies correspond to structures, fully optimized at the above level. All calculations were done using GAUSSIAN03 program package.[30]

General procedure for acetonide formation

A catalytic amount of toluenesulfonic acid was added to a stirred solution of diol (2 mmol) and dimethoxypropane (10 mmol) in CH_2Cl_2 (10 mL). The reaction was monitored by TLC on silica gel (1 : 1 EtOAc–hexanes). When all the starting material was consumed, the reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 1.0 M NaOH (5 mL) and dried over anhydrous $MgSO_4$. The filtrate was concentrated by rotary evaporation and further purified by column chromatography on silica gel (1 : 1 EtOAc–hexanes) to afford the acetonide as an oil (80–95% yield).

General procedure for hydrogenation

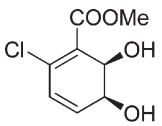
To a solution of acetonide (0.20 mmol) in MeOH (1 mL) was added PtO_2 (catalytic, 10% w/w) and NEt_3 (0.20 mmol). The flask was evacuated and filled with H_2 at atmospheric pressure. After the reaction was judged complete by TLC (8–12 h), the suspension was filtered through Celite; concentrated in the rotary evaporator and purified by column chromatography on silica gel using mixture of hexanes–EtOAc 4 : 1) as eluent. The eluent was concentrated to give an oil (50–61% yield).



16a

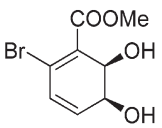
(5*S*,6*R*)-Methyl 2-fluoro-5,6-dihydroxycyclohexa-1,3-dienecarboxylate (16a). mp 74–76 °C (EtOAc); $[\alpha]_D^{20}$ +73.2 (*c* 1.05, MeOH); R_f = 0.15 (1 : 1 hexanes–ethyl acetate); IR (film) 3558,

3025, 1694, 1439, 1401, 1040 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.33 (m, 1H), 5.94 (ddd, J = 10.2, 8.3, 2.6 Hz, 1H), 4.71 (t, J = 6.2 Hz, 1H), 4.55 (m, 1H), 3.83 (s, 3H), 3.17 (brs, 1H), 3.09 (brs, 1H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.0 (d, J = 2.2 Hz), 163.2 (d, J = 281.0 Hz), 143.1 (d, J = 12.1 Hz), 119.6 (d, J = 36.2 Hz), 106.2 (d, J = 2.2 Hz), 69.06 (s), 67.0 (d, J = 6.6 Hz), 52.2 (s) ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ −92.6 (s) ppm; MS (EI) m/z (%): 188 (15), 133 (44), 119 (49), 102 (100), 91 (37), 90 (46), 86 (28), 74 (16), 46 (27); HRMS (EI) calcd for $C_8H_9FO_4$ (M^+): 188.0485; found: 188.0484; MS (FAB) m/z (%): 189 (24) $[M + H]^+$, 188 (16) $[M]^+$, 171 (100), 139 (27), 59 (16); HRMS (FAB) calcd for $C_8H_9FO_4$ (M+): 188.0485; found: 188.0479; Anal. calcd for $C_8H_9FO_4$: C, 51.07; H, 4.82; found: C, 51.18; H, 4.76.



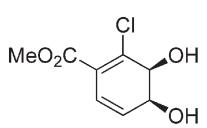
16b

(3*S*,4*S*)-Methyl 2-chloro-3,4-dihydroxycyclohexa-1,5-dienecarboxylate (16b). mp 107–109 °C (pentene–ethyl acetate); $[\alpha]_D^{20}$ = +36.06 (c 1.0, $CHCl_3$); R_f = 0.18 (1 : 1 hexanes–ethyl acetate); IR (KBr) 3398, 3459, 1698, 1317, 1057, 762 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.18 (ddd, J = 10.0, 2.4, 1.2 Hz, 1H), 6.01 (dd, J = 10.0, 2.4 Hz, 1H), 4.64 (ddd, J = 6.0, 4.7, 1.2 Hz, 1H), 4.52 (ddt, J = 8.7, 6.0, 2.4 Hz, 1H), 3.86 (s, 3H), 2.76 (d, J = 9.6 Hz, 1H), 2.60 (d, J = 4.7 Hz, 1H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.32, 138.78, 138.52, 127.50, 123.79, 68.56, 67.72, 52.27 ppm; MS (EI) m/z (%) $[M]^+$: 204 (14), 173 (23), 172 (54), 155 (50), 146 (32), 145 (36), 144 (100), 143 (80), 139 (27), 99 (27), 81 (41), 53 (25), 51 (21); HRMS (EI) calcd for $C_8H_9ClO_4$: 204.0189; found: 204. 0190.



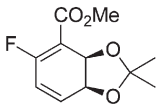
16c

(5*S*,6*R*)-Methyl 2-bromo-5,6-dihydroxycyclohexa-1,3-diene-carboxylate (16c). mp 106–109 °C ($CHCl_3$); $[\alpha]_D^{20}$ = +29.40 (c 1.0, DCM); R_f = 0.18 (1 : 1 hexanes–ethyl acetate); IR (KBr) 3402, 1703, 1437, 1314, 1234, 1048 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.17 (dd, J = 10.0, 2.5 Hz, 1H), 6.04 (ddd, J = 10.0, 2.5, 1.3 Hz, 1H), 4.57 (m, 1H), 4.49 (m, 1H), 3.85 (s, 3H), 3.00 (brd, J = 7.9 Hz, 1H), 2.97 (brs, 1H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.61, 137.54, 129.97, 128.04, 127.31, 68.37, 68.14, 52.28 ppm; MS (EI) m/z (%): 248 (9), 218 (38), 216 (47), 190 (82), 189 (53), 188 (85), 187 (48), 109 (71), 108 (31), 81 (100), 65 (79), 59 (45), 53 (54); HRMS (EI) calcd for $C_8H_9BrO_4$ (M^+): 247.9684; found: 247.9679.



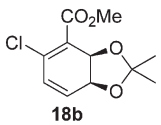
17b

(5*S*,6*R*)-Methyl 2-chloro-5,6-dihydroxycyclohexa-1,3-dienecarboxylate (17b). $[\alpha]_D^{20}$ = +86.6 (c 1.0, $CHCl_3$); R_f = 0.25 (1 : 1 hexanes–ethyl acetate); IR (KBr) 3422, 2959, 1721, 1578, 1444, 1270, 758 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.35 (d, J = 9.8 Hz, 1H), 6.03 (dd, J = 9.8, 3.0 Hz, 1H), 4.44 (m, 1H), 4.31 (d, J = 6.0 Hz, 1H), 3.83 (s, 3H), 2.50 (vbrs, 2H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.77, 140.46, 127.77, 125.09, 124.00, 72.47, 67.36, 52.34 ppm; MS (EI) m/z (%) $[M–H_2O]^+$: 188 (15), 186 (43), 157 (32), 155 (100), 99 (14); HRMS (EI) calcd for $C_8H_9ClO_4$–H_2O $[M - H_2O]^+$: 188.0084; found: 188.0077.



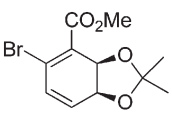
18a

(3aR,7aS)-Methyl 5-fluoro-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (18a). $[\alpha]_D^{20}$ +23.8 (c 1.2, $CHCl_3$); R_f = 0.67 (1 : 1 hexanes–ethyl acetate); IR (film) ν 3020, 2932, 2254, 1706, 1613 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.25 (m, 1H), 5.98 (m, 1H), 5.11 (t, J = 7.5 Hz, 1H), 4.87 (m, 1H), 3.86 (s, 3H), 1.46 (s, 3H), 1.42 (s, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.9, 163.7, 161.9, 138.1, 119.8, 119.5, 106.4, 103.9, 72.0, 70.9, 52.2, 26.8, 25.2, 1.03 ppm; MS (EI) m/z (%): 228 (1.7), 213, (80.0), 197 (11.3), 181 (10.8), 171 (49.8), 139 (42.0), 127 (20.8); HRMS (EI) calcd for $C_{11}H_{13}FO_4$: 228.0798; found: 228.0807.



18b

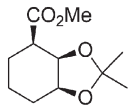
(3aR,7aS)-Methyl 5-chloro-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (18b). $[\alpha]_D^{20}$ +141.4 (c 1.5, CH_2Cl_2); R_f = 0.80 (1 : 1 hexanes–ethyl acetate); IR (film) ν 2989, 2952, 1726, 1644, 1587 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.08 (m, 2H), 5.07 (d, J = 8.1 Hz, 1H), 4.79 (dd, J = 8.1, 2.1 Hz, 1H), 3.86 (s, 3H), 1.43 (s, 3H), 1.40 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 137.0, 132.5, 127.5, 122.1, 106.5, 71.6, 71.0, 52.2, 26.7, 25.3 ppm; MS (EI) m/z (%): 244 (2.3), 231, (16.4), 229 (49.6), 213 (11.3), 197 (14.0), 187 (50.1), 186 (17.9), 157 (17.9), 155 (49.4); HRMS (EI) calcd for $C_{11}H_{13}ClO_4$: 244.0495; found: 244.00502.



18c

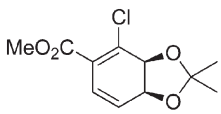
(3aR,7aS)-Methyl-5-bromo-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (18c). $[\alpha]_D^{20}$ +285.8 (c 1.1, CH_2Cl_2); R_f = 0.72 (1 : 1 hexanes–ethyl acetate); IR (film) ν 2988, 2951, 1725, 1639, 1582, 1434 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.17 (d, J = 0.72 Hz, 1H), 5.94 (dd, J = 9.87, 3.36 Hz, 1H), 4.99 (d, J = 8.04 Hz, 1H), 4.75 (m, 1H), 3.82 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 131.3, 129.9, 125.8, 125.7, 106.5,

72.1, 70.6, 52.2, 26.7, 25.2 ppm; MS (EI) *m*/*z* (%): 275 (24), 273 (25), 233 (41), 231 (45), 201 (28), 199 (28), 108 (33); HRMS (EI) calcd for $C_{11}H_{13}BrO_4$: 287.9997; found: 287.9994.



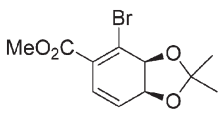
19

(3aR,4R,7aS)-Methyl 2,2-dimethylhexahydrobenzo[*d*][1,3]dioxole-4-carboxylate (19). $[\alpha]_D^{20}$ +10.4 (*c* 1.2, CH_2Cl_2); R_f = 0.29 (6 : 1 hexanes–ethyl acetate); IR (film) *ν* 2987, 2950, 1742 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 4.55 (dd, *J* = 5.4 Hz, *J* = 3.6 Hz, 1H), 4.19 (dt, *J* = 7.8 Hz, *J* = 5.4 Hz, 1H), 3.74 (s, 3H), 2.62 (m, 1H), 1.77 (m, 5H), 1.50 (m, 1H), 1.35 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 172.9, 108.5, 74.1, 73.3, 51.9, 43.0, 27.8, 27.7, 25.8, 20.1, 19.4 ppm; MS (EI) *m*/*z* (%): 199 (100), 157 (17), 139 (10), 125 (58), 97 (29), 79 (59); HRMS (EI) calcd for $C_{11}H_{18}O_4$: 214.1205; found: 214.1201.



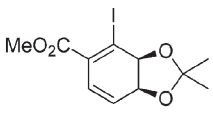
20b

(3aS,7aS)-Methyl 4-chloro-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-5-carboxylate (20b). $[\alpha]_D^{20}$ +92.6 (*c* 1.0, CH_2Cl_2); R_f = 0.80 (1 : 1 hexanes–ethyl acetate); IR (film) *ν* 2990, 2953, 1735, 1654, 1584, 1436, 1374, 1249 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 6.32 (d, *J* = 9.9 Hz, 1H), 5.97 (dd, *J* = 9.9, 3.6 Hz, 1H), 4.74 (dd, *J* = 8.4, 3.6 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 3.81 (s, 3H), 1.41 (s, 3H), 1.39 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 165.0, 137.9, 124.5, 123.8, 123.5, 107.1, 75.8, 71.8, 52.3, 26.7, 25.0 ppm; MS (EI) *m*/*z* (%): 244 (20), 213 (20), 229 (35), 186 (60), 155 (40), 59 (60), 43(100); HRMS (EI) calcd for $C_{11}H_{13}ClO_4$: 244.0495; found: 244.05024.



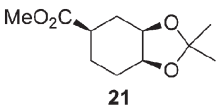
20c

(3aS,7aS)-Methyl 4-bromo-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-5-carboxylate (20c). $[\alpha]_D^{20}$ +94.0 (*c* 0.2, CH_2Cl_2); R_f = 0.72 (1 : 1 hexanes–ethyl acetate); IR (film) *ν* 3436, 2918, 1732, 1435 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 6.29 (d, *J* = 9.6 Hz, 1H), 6.08 (dd, *J* = 9.6, 3.9 Hz, 1H), 4.83 (d, 8.4 Hz, 1H), 4.74 (dd, *J* = 8.4, 3.9 Hz, 1H), 3.87 (s, 3H), 1.47 (s, 3H), 1.27 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 165.7, 129.0, 127.9, 124.5, 123.7, 106.7, 76.8, 71.8, 52.4, 26.8, 25.1 ppm; MS (EI) *m*/*z* (%): 288 (10.6), 233 (56.0), 232 (40.6), 231 (58.8), 230 (36.8), 201 (27.1), 199 (26.6), 189 (11.3), 187 (11.8), 185 (12.5), 183 (14.4); HRMS (EI) calcd for $C_{11}H_{13}BrO_4$: 288.0002; found: 287.9997.



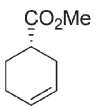
20d

(3aS,7aS)-Methyl 4-iodo-2,2-dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-5-carboxylate (20d). $[\alpha]_D^{20}$ = +32.4 (*c* 3.3, CH_2Cl_2); R_f = 0.35 (4 : 1 hexanes–ethyl acetate); IR (film) 3444, 2987, 2951, 1730, 1372, 1243, 1062 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 6.22 (d, *J* = 9.9 Hz, 1H), 6.10 (dd, *J* = 9.9, 4.2 Hz, 1H), 4.83 (d, *J* = 8.1 Hz, 1H), 4.63 (dd, *J* = 8.1, 4.2 Hz, 1H), 3.85 (s, 3H), 1.45 (s, 3H), 1.43 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 166.2, 133.2, 124.9, 123.8, 107.9, 106.6, 79.6, 71.0, 52.4, 26.8, 25.2 ppm; MS (EI) *m*/*z* (%): 337 (6.7), 336 (41.0), 319 (10.7), 279 (89.8), 278 (97.6), 247 (44.5), 231 (17.8), 220 (12.3), 152 (100.0), 151 (48.4), 127 (13.6); HRMS (EI) calcd for $C_{11}H_{13}IO_4$ (M^+): 335.9864; found: 335.9859.



21

(3aR,5R,7aS)-Methyl 2,2-dimethylhexahydrobenzo[*d*][1,3]dioxole-5-carboxylate (21). $[\alpha]_D^{20}$ +32.8 (*c* 4.4, CH_2Cl_2); R_f = 0.27 (4 : 1 hexanes–ethyl acetate); IR (film) *ν* 3453, 2987, 2952, 1731, 1638, 1436 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 4.17 (m, 1H), 4.10 (m, 1H), 3.70 (s, 3H), 2.25 (m, 2H), 2.10 (m, 1H) 1.77 (m, 4H), 1.60 (m, 2H), 1,52 (s, 3H),1.35 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 175.2, 108.3, 73.7, 72.4, 51.8, 39.4, 31.5, 28.3, 26.3, 26.1, 22.6 ppm; MS (EI) *m*/*z* (%): 199 (100), 125 (13), 97 (30), 78 (52), 69 (11); HRMS (EI) calcd for $C_{11}H_{18}O_4$: 199.0973; found: 199.0970.



22

(S)-Methyl cyclohex-3-enecarboxylate(22)

Preparation of racemic ***22***. A solution of butadiene sulfone (8.0 g, 64.4 mmol), methyl acrylate (3.7 g, 42.8 mmol) and hydroquinone (100 mg, catalytic), in toluene (100 mL) was heated to 110 °C in sealed tube for 2 d. The reaction mixture was allowed to reach room temperature and was concentrated using rotary evaporation of afford a dark brown viscous residue. The residue was chromatographed on silica gel using hexanes as eluent to afford the racemic ester **22** as a colorless oil (4.55 g, 75.6% yield).

Resolution of ester ***22***. A mixture of racemic ester 19 (500 mg, 3.57 mmol) and Porcine Pancreatic Lipase (50 mg) in 0.10 M phosphate buffer (50 mL) was shaken in an orbital shaker at 20 °C. The pH was maintained at 7.0 by the addition of 0.10 M NaOH. At the end of 6.5 h, the enzyme was filtered through a plug of Celite. The filtrate was extracted with EtOAc (3 × 20 mL). The organic extracts were combined and dried over anhydrous Na_2SO_4. The filtrate was concentrated by rotary evaporation and further purified by column chromatography on silica gel (6 : 1 hexanes–EtOAc) to afford ester **22** (148 mg, 29% yield) as a yellowish oil. 89% ee [$\alpha]_D^{20}$ −63.5 (*c* 1.0, $CHCl_3$)) **lit**[19] $[\alpha]_D^{20}$ −82.2 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) *δ* 5.68 (s, 2H), 3.70 (s, 3H), 2.59 (m, 1H), 2.26 (m, 2H), 2.06 (m, 2H), 1.98 (m, 1H) 1.72 (m, 1H) ppm.

*Conversion of **22** to **21**.* A catalytic amount of OsO_4 was added to a stirred solution of ester **19** (100 mg, 0.72 mmol), *N*-methyl morpholine *N*-oxide (82 mg, 0.72 mmol) in acetone–water (2 mL/0.6 mL). The resulting solution was stirred for 1 h at room temperature, and then it was quenched with 15% $NaHSO_3$ solution (1 mL). The reaction mixture was diluted with EtOAc (20 mL) and water (5 mL). The layers were separated and the aqueous layer was extracted further with EtOAc (2 × 10 mL). The combined organic extracts were dried over anhydrous $MgSO_4$. The filtrate was concentrated *via* rotary evaporation and was used as crude in the next step.

To the crude mixture of diol (from the previous step) dissolved in CH_2Cl_2 (10 mL) was added dimethoxypropane (2.0 mL, 16.3 mmol) followed by a catalytic amount of *p*TsOH. The reaction mixture was stirred at room temperature for 30 min. Then it was diluted with 1 N NaOH (2 mL), the two layers were separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 5 mL). The organic layers were combined and dried over anhydrous $MgSO_4$. The filtrate was concentrated by rotary evaporation and further purified by column chromatography on silica gel (4 : 1 hexanes–EtOAc) to afford the desired product **21** (25 mg, 16.3% yield, over two steps) as a yellowish oil. 88% ee $[\alpha]_D^{20}$ −25.1 (*c* 1.0, CH_2Cl_2)) compound **21** from **20b–20d** $[\alpha]_D^{20}$ +32.8 (*c* 4.4, CH_2Cl_2).

Acknowledgements

The authors are grateful to the following agencies for financial support of this work: Natural Sciences and Engineering Research Council of Canada (NSERC) (Idea to Innovation and Discovery Grants); Canada Research Chair Program, Canada Foundation for Innovation (CFI), Research Corporation, TDC Research, Inc., TDC Research Foundation, and Brock University. Thanks are also due to the Ministry of Education (Spain) for fellowships to V.S. (AP2006-03468ESTANCIA-2010) and partial support of Slovak grant agency VEGA (1/0524/11).

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CHAPTER 4

Dauben–Michno oxidative transposition of allylic cyanohydrins Enantiomeric switch of (–)-carvone to (+)-carvone

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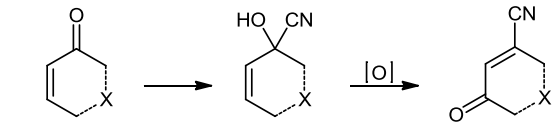
Can. J. Chem. **2011**, *89*, 535-543.

Dauben–Michno oxidative transposition of allylic cyanohydrins
Enantiomeric switch of (–)-carvone to (+)-carvone

Introduction

This chapter is dedicated to an oxidative rearrangement of allylic cyanohydrins, *i.e.* to a specific type of 1,3-oxidative transposition of allylic alcohols (Scheme 1). The 1,3-oxidative transposition of allylic alcohols to α,β-unsaturated carbonyl compounds is a process that does not create or alter the basic carbon skeleton of the substrate but one that may strategically place functionality during the course of a multi-step synthesis.[1]



Acyclic and cyclic enones
X = 0, CH_2, $(CH_2)_2$

Scheme 1 Oxidative rearrangement of allylic cyanohydrins.

This contribution can be considered as a broadening of chromium oxide chemistry in oxidation of organic substrates.[2] To be more exact, the broadening of use of chromyl acetate (CrO_3 in Ac_2O) which is "anhydrous" version of Fieser reagent (CrO_3 in AcOH).[3] Lack of water in the reaction mixture simply permits us to stop the oxidation in the aldehyde state (see Table 1, Entry 4 in the article) and no over-oxidation (to carboxylic acid) is observed.

In the original work of William G. Dauben and Drake M. Michno[4] pyridinium chlorochromate (PCC) was used. Until now this article was referenced more than hundred times, but only tertiary alcohols with electron donating groups (EDG) were employed. The first example with electron withdrawing group EWG is the recent synthesis of Oseltamivir (Tamiflu®) performed in Tomas Hudlicky group (Scheme 2).[5]

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[1] Luzzio, F. A. *Tetrahedron* **2012**, *68*, 5323-5339.
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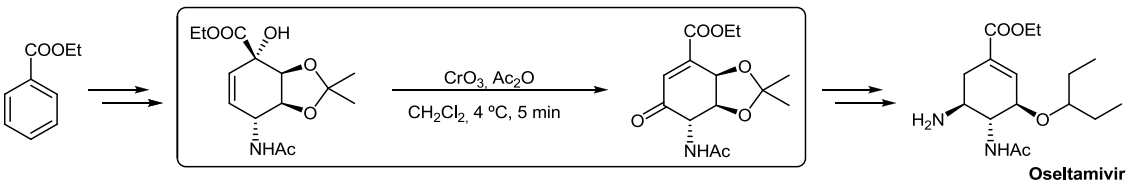


Scheme 2 Hudlicky's oseltamivir synthesis.

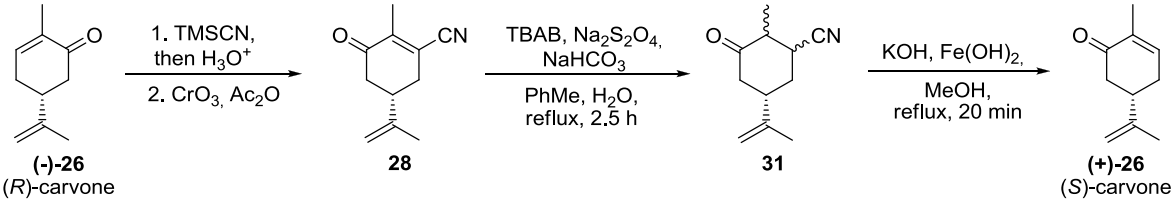
Objectives

1) Explore the scope of the reaction, *i.e.* whether the reaction is general for allylic alcohols substituted with other electron-withdrawing groups.
2) Evaluate the possibility of using of this transposition to generate enantiomers of enones that possess a latent plane of symmetry.

Results

As a starting material for tested cyanohydrins, eight different *α,β*-unsaturated ketones were selected. Ketones were converted to the corresponding cyanohydrins following procedure that uses proline catalysis for the formation of intermediate trimethylsilyl ethers of cyanohydrins. After the acid hydrolysis, free cyanohydrins were obtained in essentially quantitative yield. The Dauben–Michno procedure required the treatment of CrO_3 with acetic anhydride at 80 °C before cooling and addition of cyanohydrin at or below 0 °C. The yields of transposed cyanoenones were in the range of 70% –80%, except for the cases of aromatic substrates (**20**, **24**) where side reaction took place.

Enantiomeric switch of (–)-carvone was performed as a proof of the concept. The enantiomeric switch of carvone was accomplished in two steps *via* a reduction – elimination sequence (Scheme 3). Detailed discussion about formed diastereoisomers **31** can be found in the presented article.



(-)-26
(*R*)-carvone

28

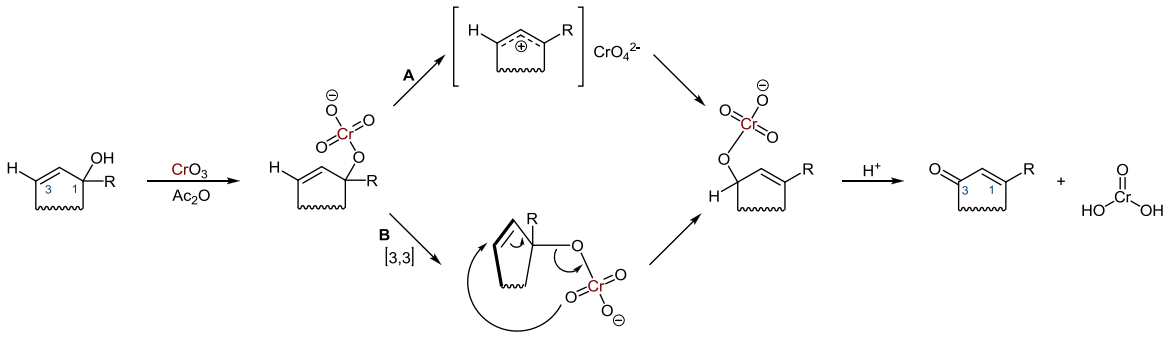
31

(+)-26
(*S*)-carvone

Scheme 3 Enantiomeric switch of (–)-carvone.

Mechanistic considerations

When tertiary allylic alcohols are oxidized with CrO_3 in acid solution, no direct oxidation can take place, but a kind of conjugate oxidation occurs. The first step in Cr(VI) oxidations can take place to give a chromate ester but this intermediate has no proton to lose so it transfers the chromate to the other end of the allylic system (Scheme 4). In terms of mechanism, this transformation may follow one of two pathways in dependence of the acidity of the employed reagents. Acidic reagents (Jones reagent, PCC) may invoke an ionization pathway (path A). Recombination of the carbocation with chromate followed by collapse of the chromate ester follows the normal steps to the product. In cases where more basic reagent (pyridinium dichromate /PDC/, Collins reagent) is employed, path B may be invoked. Hence, the initial chromate ester is formed then followed by [3,3]-sigmatropic rearrangement and further oxidation to the product enone. The final step is normal oxidation in which chromium is reduced from Cr(VI) to Cr(IV).[6]



Scheme 4 Mechanism of oxidative rearrangement of allylic cyanohydrins.

In our case, chromium (VI) oxide / acetic anhydride / dichloromethane (4 ºC / 5 min) protocol is used. This reaction medium is “Jones-like” as it has the potential for a medium to high degree of acidity during the process, *i.e*. both mechanistic pathways are possible. This rationalization can explain observed results in case of (-)-carvol (**35**) oxidation in the attempted Dauben– Michno synthesis of (+)-carvone from (–)-carvol (**35**) (see Figure 7 of the presented article). However, detailed mechanistic study was out of the project scope.

Conclusion

Reliable procedure for the conversion of enones to the corresponding β-cyanoenones with the attendant 1,3-functional transposition of the carbonyl group of the

[6] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*, Oxford University Press, England, **2001**; p 951-952.

original enone was developed. Scope of the reaction was tested on the eight structurally different substrates. Enantiomeric switch of (–)-carvone was performed as a proof of concept experiment.

My contribution to the project

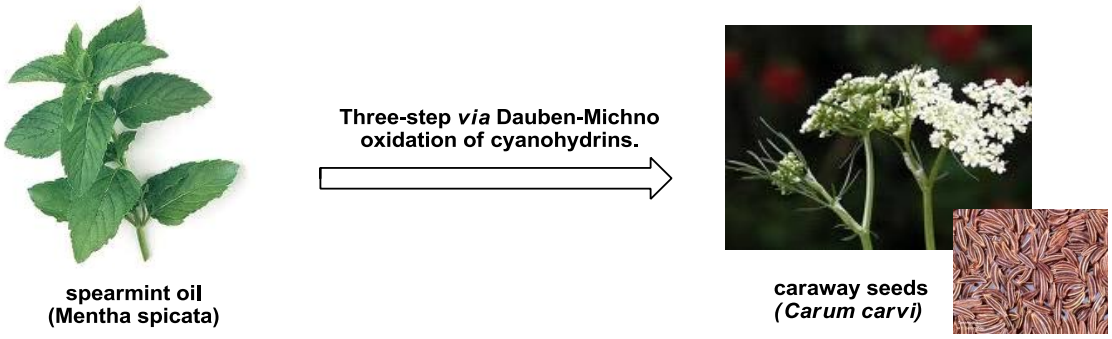
1. Re-check of entrees 1, 2, 3, 4 and 8 (Table 1).
2. Entry 5 (Table 1).
3. Attempt to direct conversion of β-cyanoenone **28** to (+)-carvone (Fig. 2).
4. Enantiomeric switch of (–)-carvone to its enantiomer (Fig. 4).
5. Attempted Dauben–Michno synthesis of (+)-carvone from (–)-carveol (**35**) (Fig. 7).

Obviously, this small project was the spin-off of Hudlicky's chemoenzymatic oseltamivir synthesis. I really enjoy my participation and not only in the laboratory but also during preparation of manuscript. I do hope, that you will enjoy reading the paper published in *Canadian Journal of Chemistry*.

This paper was recently used as an example of 1,3-oxidative transposition of allylic alcohols in organic synthesis by Frederick A . Luzzio in *Tetrahedron Report* (No. 974)[1].

Appendix

(*S*)-(+)- and (*R*)-(-)-carvones are a book example of naturally by occurring enantiomeric compounds with different smell. (*R*)-(–)-carvone is the most abundant compound in the essential oil from several species of mint, particularly spearmint oil (*Mentha spicata*). (*S*)-(+)-carvone is the principal constituent of the oil from caraway seeds (*Carum carvi*).



Alternative way to see the **Scheme 3** Enantiomeric switch of (–)-carvone to (+)-carvone.

AWARD LECTURE / CONFÉRENCE D'HONNEUR

Dauben–Michno oxidative transposition of allylic cyanohydrins — Enantiomeric switch of (–)-carvone to (+)-carvone*

Jason R. Hudlicky, Lukas Werner, Vladislav Semak, Razvan Simionescu, and Tomas Hudlicky

Abstract: Allylic cyanohydrins were subjected to Dauben–Michno oxidation at low temperatures to provide β-cyanoenones in good to excellent yields. The potential of this oxidative transposition as a means of an enantiomeric switch of enones containing a latent plane of symmetry was tested by conversion of (–)-carvone to its enantiomer.

Key words: Dauben–Michno reaction, oxidative 1,3-transposition of allylic alcohols, cyanoenones, enantiomeric switch of carvone.

Résumé : On a soumis des cyanohydrines allyliques à une oxydation de Dauben–Michno, à basse température, pour obtenir des β-cyanoénones avec des rendements allant de bons à excellents. Dans le but d'évaluer le potentiel de cette transposition oxydante comme méthode de réaliser une transposition énantiomère dans les énones contenant un plan de symétrie latent, on a transformer la (–)-carvone en son énantiomère.

Mots-clés : réaction de Dauben–Michno, transposition oxydante 1,3 d'alcools allyliques, cyanoénones, transposition énantiomère de la carvone.

[Traduit par la Rédaction]

Introduction

The Dauben–Michno oxidation constitutes a reliable protocol for oxidative transposition of tertiary allylic alcohols substituted with electron-donating groups.[1,2] The reaction proceeds by a [3,3] sigmatropic rearrangement of the initially formed chromate ester followed by further oxidation of the rearranged chromate ester to the final enone. The process is analogous to the well-known chromium(VI) oxidations of olefins to enones that proceed via an ene reaction of the chromium oxide followed by oxidation. The initially formed chromate ester is either oxidized to an enone or undergoes [3,3] sigmatropic rearrangement prior to the final oxidation. Thus, mixtures of regioisomeric enones are frequently encountered in the oxidation of cyclic olefins.[3,4] Because only one product can arise from the rearrangement of a tertiary allylic alcohol, the Dauben–Michno reaction does not suffer from regiochemical issues. Although well-documented for allylic alcohols flanked by electron-donating groups, this reaction has not been reported for allylic alcohols substituted with esters or nitriles. To our knowledge the first example of such oxidation was the conversion of allylic alcohol **1** to enone **2** during our recently completed synthesis of oseltamivir (**3**) (Tamiflu), Fig. 1.[5]

To establish whether the reaction is general for allylic alcohols substituted with other electron-withdrawing groups we investigated a series of cyanohydrins as means of synthesis of β-cyanoenones. In this paper we report the results of this investigation, along with a procedure for an enantiomeric switch of (–)-carvone to (+)-carvone.

Results and discussion

We began the investigation with a series of cyclic enones (Table 1, entries 1–3) and their conversion to cyanohydrins following a recently published procedure that uses proline catalysis for the formation of the intermediate trimethylsilyl ethers of cyanohydrins.[6] Following the hydrolysis, the free cyanohydrins were obtained in essentially quantitative yield and used without purification in the Dauben–Michno oxidation. The proline-catalyzed protocol is exceedingly efficient and cleanly affords the cyanohydrins, thus obviating any

Received 13 November 2010. Accepted 14 January 2011. Published at www.nrcresearchpress.com/cjc on XX April 2011.

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*Based on the 2010 Bader Award Lecture.

Fig. 1. Dauben–Michno oxidation of α-hydroxybutenoate.

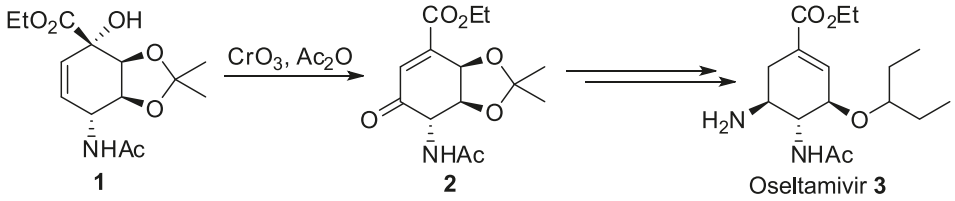
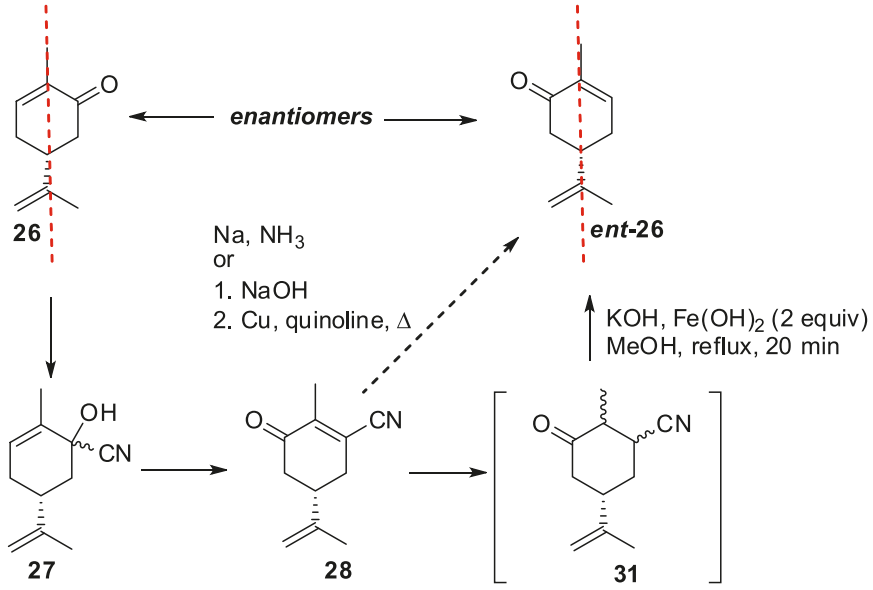


Fig. 2. Proposed enantiomeric switch of (–)-carvone via Dauben–Michno oxidation.



separation issues prior to subsequent reactions. In the case of cyclopentenone, the cyanohydrin was obtained in 60% yield at the expense of the competing 1,4-addition of the cyanide to the enone. Similarly, cyanohydrins were obtained from methylvinyl ketone (Table 1, entry 4), 3-penten-2-one (Table 1, entry 5), 4-phenylbutenone (Table 1, entry 6), acetophenone (Table 1, entry 7), and (–)-carvone (Table 1, entry 8).

The Dauben–Michno procedure required the treatment of CrO_3 with acetic anhydride at 80 °C before cooling and addition of the cyanohydrin at or below 0 °C. The best results were obtained by performing the oxidation below 0 °C to avoid competing reactions at the site of the double bond that may result from the generation of peroxyacetic acid during the reaction. Such competition is absent when the oxidation is performed at –40 °C or below. The yields of transposed cyanoenones were in the range of 70%–80% except in the case of cyclopentenone, where the conversion was excellent and essentially quantitative (vide NMR); however, the volatility of the product contributed to low isolated yields (Table 1).

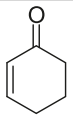
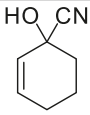
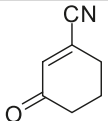
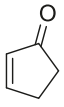
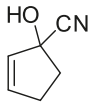
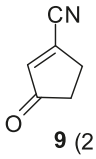
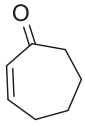
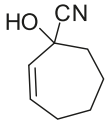
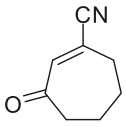
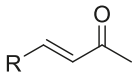
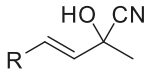
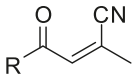
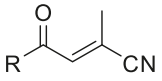
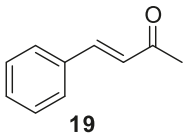
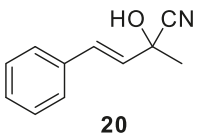
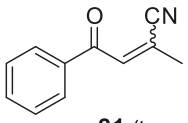
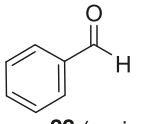
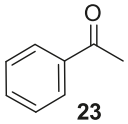
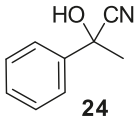
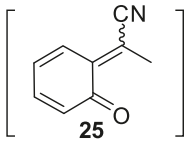
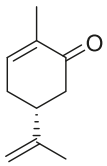
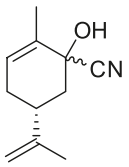
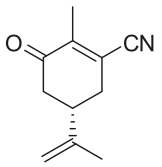
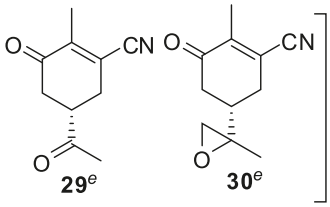
With acyclic enones the results were different; in the case of methylvinyl ketone the corresponding unsaturated aldehyde **15** (a consequence of the oxidation of a primary chromate ester formed by the sigmatropic rearrangement) was isolated as a mixture of *Z*- and *E*-isomers in the ratio of 7:1. (Note: On repetition of these experiments at higher temperatures or on a larger scale only the *Z*-isomer was detected.) The volatility of the product made the accurate determination of yield difficult. It was somewhat surprising that little or no over-oxidation took place whether 1 equiv or up to 3 equiv of the oxidant were used. We did not detect the corresponding acid even after careful repetition of the experiments; however, over-oxidation of an aldehyde to an acid requires the formation of a hydrate, which cannot take place under the conditions of the reaction. If the corresponding acid is desired, a simple dilution of the reaction with water and addition of an additional equivalent of chromium oxide should provide the corresponding carboxylic acid in high yield.

With pent-3-en-2-one (**16**) the corresponding cyanohydrin **17** was isolated in 76% yield and rearranged cleanly to the β-cyanoenone **18** in 84% yield (66% over two steps); only the *Z*-isomer **18a** was observed. In the case of 4-phenylbutenone (**19**), the rearrangement of the corresponding cyanohydrin **20** produced only traces of the transposed enone **21**, and the major product isolated was benzaldehyde (**22**). The formation of benzaldehyde may be rationalized by the oxidative cleavage of the intermediate 1,2-chromate diester formed from the styrene double bond in competition with the Dauben–Michno reaction.

For acetophenone (**23**) and the oxidation of its cyanohydrin **24**, only the formation of an intractable polymer was observed at prolonged reaction times or at higher temperatures. The cyanohydrin derived from acetophenone was still present in the reaction mixture after 16 h at room temperature. We expected the Dauben–Michno to proceed with disruption of aromaticity to provide **25**, in analogy with reported cases of reactivity of this type;[7] however, attempts at trapping intermediates of type **25** with external dienophiles such as maleic anhydride proved unsuccessful.

The oxidative 1,3-transposition could, in principle, be used

Table 1. Dauben–Michno oxidation of cyanohydrins.

Entry	Enone	Cyanohydrin	Product(s) (%)
1	 **4**	 **5**	 **6** (79)[a]
2	 **7**	 **8** (60)[b]	 **9** (25%)[c] (93%)[d]
3	 **10**	 **11**	 **12** (81)[a]
4	 **13** R = H	 **14** R = H (67%)[d]	 (Z:E = 7:1)  **15a** R = H **(Z-)** **15b** R = H **(E-)** (43)[a,c]
5	**16** R = Me	**17** R = Me (78%)	**18a** R = Me (84%)(66%)[a] **18b** R = Me
6	 **19**	 **20**	 **21** (trace)  **22** (major)
7	 **23**	 **24**	 polymer **25**
8	 **26**	 **27**	 **28** (75)[a]  **29**[e] **30**[e]

[a]Isolated yield over two steps.
[b]Yield of 1,2-adduct, 1,4-adduct of cyanide was observed during the cyanohydrin synthesis.
[c]Isolated yield, product is volatile and losses occur on evaporation.
[d]Product content as estimated by NMR.
[e]Formed only in reactions above 0 °C.

to provide enantiomers of homochiral enones that contain a latent plane of symmetry, as shown in Fig. 2 with carvone. To test this idea we applied the Dauben–Michno protocol to carvone with the expectation that further manipulations of the β-cyanoenone **28** would lead to the formation of *ent*-carvone, as depicted in Fig. 2.

The Dauben–Michno oxidation of cyanohydrin **27** derived from carvone provided the corresponding β-cyanoenone **28** in 75% yield. At 0–4 °C, considerable amount of side products, such as the methyl ketone **29** and epoxide **30**, were isolated. The former compound is likely a result of the oxidative cleavage of the 1,2-diol, formed by the addition of CrO_3 to the isopropylidine double bond, whereas the latter is probably formed by epoxidation mediated by peroxyacetic acid generated by in situ oxidation of acetic acid. When the reaction was performed at –40 to –50 °C, only oxidative rearrangement took place, and no side products arising from over-oxidation were observed.

Fig. 3. Synthesis of *ent*-dihydrocarvone from enone **28**.

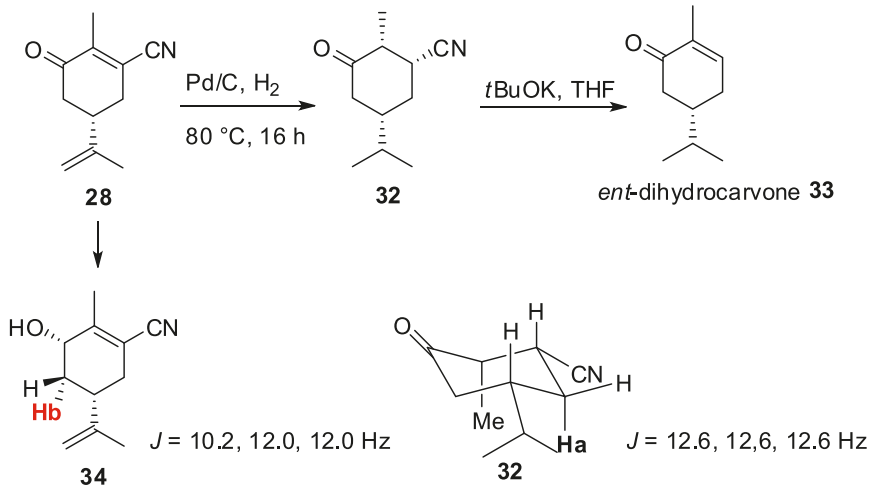


Fig. 4. Enantiomeric switch of (–)-carvone.

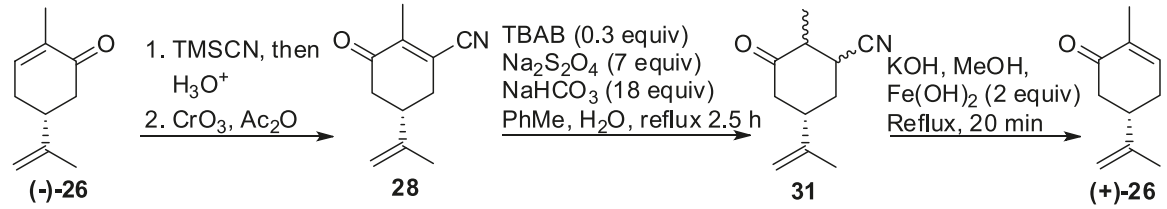
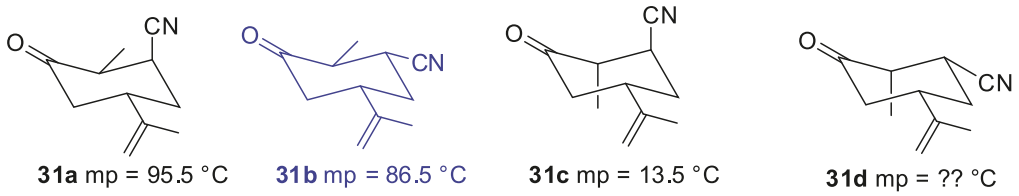


Fig. 5. Djerassi's assignment of diastereomeric dihydrocyanocarvone **31**.



The proof of principle for the enantiomeric switch was demonstrated on a small scale by the conversion of cyanoenone **28** to the fully saturated β-cyano ketone **32** as shown in Fig. 3. Hydrogenation of **28** at 1 atm (1 atm = 101.325 kPa) produced ketone **32** (34% yield), whose structure was assigned by the analysis of coupling constants of H_a, as shown in Fig. 3.

The low yield of the hydrogenation was attributed to the formation of a by-product (2-methyl-3-cyano-5-isopropylphenol) resulting from full aromatization under the conditions of the hydrogenation. Treatment of **32** with *t*-BuOK in THF provided *ent*-dihydrocarvone (**33**). Enone **28** was also reduced under Luche conditions to the allylic alcohol **34** in anticipation of a possible rhodium-catalyzed isomerization[8] ($RhCl_3$, EtOH) to the saturated ketone **31**; however, this isomerization proved unsuccessful. The stereochemistry assignment of **34** was evident from the large coupling constants of the axial H_b, as shown in Fig. 3.

For the direct conversion of cyanoenone **28** to *ent*-carvone (**26**) several methods were investigated, among them hydrolysis to the corresponding acid and thermal decarboxylation,[9] and Birch reduction,[10] as shown in Fig. 2. Neither method proved very efficient in our hands.

The enantiomeric switch of carvone was accomplished in two steps via a reduction–elimination sequence as shown in Fig. 4. The cyanoenone **28** was reduced with sodium dithionite[11] to a mixture of the saturated cyanoketones **31**. All four diastereomers of **31** are known compounds, generated over the years in investigations of various reactions of enones[12–14] that utilized carvone as a convenient starting material. The major product from the dithionite reduction turned out to be the diastereomer **31b** (Fig. 5), as evidenced by melting point as well as by detailed NMR analysis.

In the early 1900s Lapworth[15–17] studied the addition of cyanide to *ent*-carvone and isolated two diastereomers of **31**, one from the reaction of potassium cyanide in ethanol, water, and acetic acid conducted at room temperature, and the other from a reaction performed in refluxing ethanol without acetic acid. (These two compounds would later be identified as **31a** and **31b**, respectively; Fig. 5) When Djerassi et al.[18] repeated Lapworth's experiments in 1963 with (–)-carvone, they found that the physical constants of the two diastereomers matched Lapworth's results. They then studied the equilibration of the two isomers and isolated a third when **31a** was treated with *p*-TsOH in EtOAc at room temperature for 1 week. The third isomer, a much lower melting compound, was later assigned as structure **31c**, Fig. 4.

Fig. 6. Assignment of stereochemistry of **31b** by NMR methods. (A) Resolution enhanced ^{1}H spectrum. (B) Resolution enhanced ^{1}H spectrum selectively decoupled from H7 methyl.

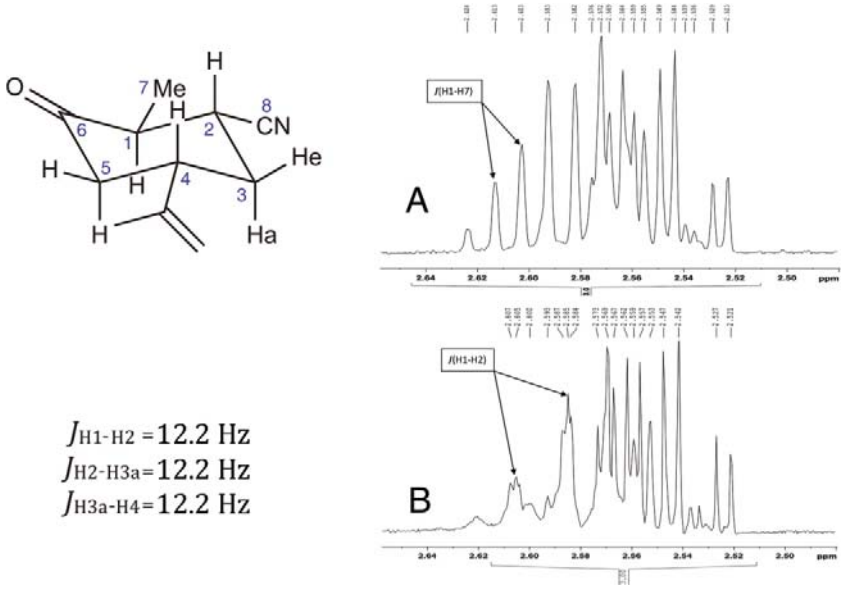
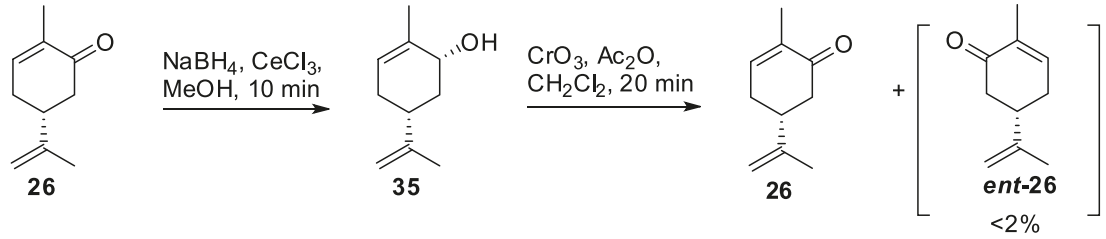


Fig. 7. Attempted Dauben–Michno synthesis of (+)-carvone from (–)-carvol **35**.



Attempted equilibration of **31b** was unsuccessful save for a trace amount of a new compound tentatively assigned as **31d**. Djerassi et al.[18] assigned the stereochemistry of the four diastereomers of **31** by use of detailed conformational analysis and optical rotary dispersion. In addition, they proved by simple yet ingenious experiments that the equilibration of **31a** occurs at C-2 and not at C-3 by conversion of nitriles to benzamides and equilibration of C-2 positions. The stereochemistry of **31b** was evident by matching the melting point with the compound identified by Djerassi et al.[18]

The assignment of **31b** by NMR methods proved to be nontrivial because of overlapping signals of the key protons and required the dispersion field of a 600 MHz spectrometer with advanced methods to separate the overlapping signals. The assignment of stereochemistry is shown in Fig. 6. In the normal proton spectrum, the six protons from the ring are all grouped at 1.9–2.6 ppm with one proton at 2.03 ppm, a multiplet at 2.37 ppm corresponding to three protons, and another multiplet at 2.57 ppm also corresponding to three protons. The proton at 2.03 ppm shows a d–d–d splitting pattern with three equal coupling constants (J = 12.2 Hz). Given the ring structure of cyclohexane there is only one possible case for the assignment of this proton, H3a. It is the only possible case with two large vicinal couplings (axial–axial) and one large geminal coupling. This also establishes that protons H4a and H2a are axial protons, implying that both the CN group and the propylene are in equatorial positions on the ring.

For the assignment of the H1 proton two-dimensional NMR methods had to be employed. From the H–C HSQC and COSY spectra, the locations of the other ring protons were determined. H1, H5, (H2a or H4a) are grouped at 2.6 ppm and H5 and H3e (H4a or H2a) are grouped at 2.37 ppm. H1 is identified from the coupling to the H7 methyl group in the COSY spectrum as being located on the left side of the multiplet, as shown in Fig. 6A. To increase spectral resolution, Gaussian line broadening was employed with a shift of 0.5 and a line-broadening factor of –1 Hz. From the proton spectrum a coupling constant of 6.2 Hz, corresponding to the vicinal coupling of H1 to H7 protons, was determined. This coupling alone would generate a quartet splitting pattern for H1. However, a more complicated pattern is observed for H1, therefore, the coupling H1 to H2 cannot be extracted directly from the proton 1-D NMR spectrum.

A homonuclear proton experiment was employed to measure the H1–H2 vicinal coupling constant. A proton spectrum was acquired selectively decoupling the H7 methyl group protons at 1.30 ppm from H1. As a result the H1 coupling pattern was simplified and reduced to H1–H2 vicinal coupling, as shown in Fig. 6B. By overlapping the normal proton spectrum with the selectively decoupled spectrum, the H1–H2 coupling constant of 12.2 Hz was extracted. This corresponds to an axial position for H1, confirming that the C-1 methyl group occupies an equatorial position. At this point the right side of the 2.57 ppm multiplet can be assigned as

H2a showing the d–d–d pattern expected for H2a, as H4a would generate a d–d–d–d pattern. As this spectral assignment for **31b** would, of course, not have been possible in the 1960s, one must admire the brilliant deductive logic employed by Djerassi et al.[18] in accurately assigning all four diastereomers of **31**.

In preliminary experiments the treatment of **31b** with NaOEt in ethanol provided (+)-carvone, identical in all respects to (–)-carvone except for the sign of optical rotation, thus accomplishing the intended enantiomeric switch. Under these conditions, however, the yields of carvone were low (~35%) at the expense of condensation products. The conditions that employ $Fe(OH)_2$ (shown in Fig. 4) provided the much improved yield of 79% for (+)-carvone.

Finally, we subjected carvol (**35**), obtained by Luche reduction of carvone according to literature protocol,[19] to the Dauben–Michno conditions at –50 °C to investigate the possibility of competition between chromate ester re-oxidation to (–)-carvone versus the Dauben–Michno transposition–oxidation to (+)-carvone (Fig. 7). The rate-determining step in chromium(VI) oxidation of alcohols is the collapse of the chromate ester[20] and not its formation. We speculated that at low temperature, the sigmatropic rearrangement of the initially formed chromate ester required for the transposition may compete with the oxidation even for secondary allylic alcohols.

If the transition state of the [3,3] rearrangement is not symmetrical along the latent plane of symmetry in carvone, then the Dauben–Michno protocol applied to carvol would provide the enantiomer directly. The initial experiment, performed at –55 °C, indeed provided a scalemic mixture of carvone with an optical rotation lower than that of the (–)-enantiomer, indicating that the two processes may indeed compete.

We performed three experiments at different temperatures (–55, –78, and –100 °C) and noted some decrease in the value of rotation for the (–)-enantiomer. However, verification of true *er* by HPLC (Daicel Chiralcel OB-H; flow = 0.6 mL/min (hexane–*i*-PrOH, 95:5; retention time for (*R*)-(–)-carvone = 9.56 min and for (*R*)-(–)-carvone = 10.41 min)[21] provided at best an indication of an ~1%–2% increase in the (+)-enantiomer. Thus, no conclusion can be drawn from these experiments. For now, the three-step high-yielding protocol for the enantiomeric switch via Dauben–Michno oxidation of cyanohydrins should suffice.

Conclusions

We have developed a reliable procedure for the conversion of enones to the corresponding β-cyanoenones with the attendant 1,3-functional transposition of the carbonyl group of the original enone. The possibility of using this transposition to generate enantiomers of enones that possess a latent plane of symmetry was tested and reduced to practice with the enantiomeric switch of (–)-carvone. Future attention should be given to a more detailed study of the Dauben–Michno oxidation of other allylic alcohols, including secondary ones, and the dependence of the outcome of such oxidation on conformational bias and stereochemical features of the substrates.

Experimental section

General procedure for the synthesis of cyanohydrins[6]

To a vial charged with the lithium salt of L-proline (0.1 mmol) was added the enone (1.0 mmol) followed by trimethylsilyl cyanide (1.0 mmol, 133 μL). After the suspension was stirred at room temperature for 12 h, an additional portion of trimethylsilyl cyanide (1.0 mmol, 133 μL) was added, and the reaction mixture was subsequently stirred for another 12 h. Upon completion, THF (2 mL) and 1 mol/L aq HCl (2 mL) were added, and the resulting solution was stirred for 1 h. Following the hydrolysis, the reaction mixture was washed with diethyl ether (30 mL) and water (5 mL), and the aqueous layer was re-extracted with diethyl ether (2 × 15 mL). The combined organic layers were dried over magnesium sulfate with a spatula tip of sodium bicarbonate, then filtered and concentrated under reduced pressure to afford the essentially pure cyanohydrin, which was used without further purification.

General procedure for the Dauben–Michno oxidations[5]

Acetic anhydride (5.3 mmol, 0.5 mL) was added to a vial charged with chromium(VI) oxide (2.5 mmol). The mixture was stirred at 80 °C until complete dissolution (5 min). After cooling to room temperature, the solution was diluted with dichloromethane (2 mL) and added dropwise to a solution of cyanohydrin (1.0 mmol) in dichloromethane (3 mL) at 4 °C. The resulting solution was stirred for 10 min before addition of ethanol (3 mL). The solution was allowed to stir for 30 min at room temperature before concentration under reduced pressure and co-evaporated with toluene. The crude mixture was passed through a column of silica gel using dichloromethane as a solvent to yield β-cyanoenone.

1-Hydroxycyclohex-2-enecarbonitrile (5)[6,22]

^{1}H (300 MHz, $CDCl_3$) δ: 6.03–6.09 (m, 1H), 5.79 (d, 1H, J = 9.9 Hz), 1.79–2.21 (m, 6H).

3-Oxocyclohex-1-enecarbonitrile (6)[23,24]

^{1}H (300 MHz, $CDCl_3$) δ: 6.53 (s, 1H), 2.50–2.59 (m, 4H), 2.10–2.19 (m, 2H).

1-Hydroxycyclopent-2-enecarbonitrile (8)

^{1}H (300 MHz, $CDCl_3$) δ: 6.17 (dt, 1H, J= 2.3, 5.4 Hz), 5.82 (dt, 1H, J = 2.0, 5.4 Hz), 2.14–2.59 (m, 5H). ^{13}C (75 MHz, $CDCl_3$) δ: 138.81, 130.74, 120.88, 67.91, 31.01, 25.52. A by-product of this reaction was the 1,4-adduct, 3-oxocyclopentanecarbonitrile:[25] ^{1}H (300 MHz, $CDCl_3$) δ: 3.15–3.25 (m, 1H), 2.42–2.67 (m, 4H), 2.24–2.36 (m, 2H).

3-Oxocyclopent-1-enecarbonitrile (9)[26]

^{1}H (300 MHz, $CDCl_3$) δ: 6.74 (s, 1H), 2.58–2.91 (m, 2H), 2.53 (t, 2H, J = 4.8 Hz). ^{13}C (75 MHz, $CDCl_3$) δ: 206.24, 143.45, 143.04, 114.81, 34.10, 30.20.

1-Hydroxycyclohept-2-enecarbonitrile (11)

^{1}H (300 MHz, $CDCl_3$) δ: 5.99 (ddd, 1H, J = 6.3, 6.3, 11.4 Hz), 5.68 (ddd, 1H, J = 1.2, 1.2, 11.7 Hz), 3.65 (bs, 1H), 2.19–2.25 (m, 1H), 1.82–2.07 (m, 6H), 1.70–1.75 (m, 1H). ^{13}C (75 MHz, $CDCl_3$) δ: 135.04, 133.16, 120.55, 71.39, 39.28, 27.50, 26.18, 25.21.

3-Oxocyclohept-1-enecarbonitrile (12)[27]

^{1}H (300 MHz, $CDCl_3$) δ: 6.56 (s, 1H), 2.63–2.67 (m, 4H), 1.82–1.93 (m, 4H). ^{13}C (300 MHz, $CDCl_3$) δ: 201.17, 142.81, 127.36, 118.95, 42.96, 32.14, 25.36, 21.14.

2-Hydroxy-2-methylbut-3-enenitrile (14)

^{1}H (300 MHz, $CDCl_3$) δ: 5.95 (dd, 1H, J = 10.2, 17.1 Hz), 5.67 (d, 1H, J = 16.8 Hz), 5.35 (d, 1H, J = 10.2 Hz), 1.67 (s, 3H). ^{13}C (300 MHz, k$CDCl_3$) δ: 137.49, 120.45, 116.77, 68.76, 28.06.

(Z)-2-Methyl-4-oxobut-2-enenitrile (15a) *(major)*

IR (film, cm^{-1}) ν: 2925, 2222, 1688, 1618, 1448, 1381, 1227, 1171, 1095, 1041, 1019, 861, 787, 665. ^{1}H (300 MHz, $CDCl_3$) δ: 10.02 (d, 1H, J = 7.8 Hz), 6.52 (dq, 1H, J = 1.5, 7.8 Hz), 2.25 (d, 3H, J = 1.5 Hz). ^{13}C (300 MHz, $CDCl_3$) δ: 189.62, 141.64, 129.26, 115.41, 21.68. MS (EI+) *m/z* % 94 (M^+ – H): 83 (15), 68 (63), 52 (29), 44 (100).

(E)-2-Methyl-4-oxobut-2-enenitrile (15b) *(minor)*

IR (film, cm^{-1}) ν: 2925, 2222, 1688, 1618, 1448, 1381, 1227, 1171, 1095, 1041, 1019, 861, 787, 665. ^{1}H (300 MHz, $CDCl_3$) δ: 10.07 (d, 1H, J = 7.8 Hz), 6.52 (dq, 1H, J = 1.5, 7.8 Hz), 2.38 (d, 3H, J = 1.5 Hz). ^{13}C (300 MHz, $CDCl_3$) δ: 189.62, 141.64, 129.26, 115.41, 21.68. MS (EI+) *m/z* % 94 (M^+ – H): 83 (15), 68 (63), 52 (29), 44 (100).

(E)-2-Hydroxy-2-methyl-3-pentenenitrile (17)

R_F = 0.76 (hexanes–EtOAc, 5:1). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 6.10 (dq, J = 15.3, 6.6 Hz, 1H), 5.55 (dd, J = 15.4, 1.7 Hz, 1H), 3.08 (brs, 1H), 1.76 (dd, J = 6.6, 1.7 Hz, 3H), 1.64 (s, 3H); –OH signal confirmed by D_2O exchange. ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 130.81, 129.02, 120.80, 68.42, 28.27, 17.26; NMR data are in agreement with those previously reported.[28]

(Z)-2-Methyl-4-oxopent-2-enenitrile (18)

R_F = 0.15 (hexanes–EtOAc, 5:1); mp 37–38 °C (white crystals, pentane–Et_2O). IR (KBr, cm^{-1}) ν: 2359, 1732, 1637, 1385. ^{1}H NMR (600 MHz, $CDCl_3$, ppm) δ: 6.60 (d, J = 1.5 Hz, 1H), 2.39 (s, 3H), 2.16 (d, J = 1.5 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$, ppm) δ: 194.25, 140.10, 119.64, 117.12, 30.06, 22.18. MS (+EI) *m/z* (%): 109 (22), 94 (100), 66 (15), 43 (40). HRMS (+EI) calcd for C_6H_7NO: 109.05276; found: 109.05246.

1-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-enecarbonitrile (27)

^{1}H (300 MHz, $CDCl_3$) δ: 5.70 (dd, 1H, J = 1.2, 3.0 Hz), 4.79 (d, 2H, J = 12.3 Hz), 3.24 (bs, 1H), 2.49–1.90 (m, 5H), 1.86 (s, 3H), 1.74 (s, 3H). ^{13}C (300 MHz, $CDCl_3$) δ: 146.85, 131.76, 128.40, 121.09, 110.18, 70.21, 41.27, 38.96, 30.67, 20.57, 17.01.

β-Cyanocarvone (28)

To a solution of crude cyanohydrin **27** (352 mg, 1.99 mmol) in dichloromethane (3 mL) cooled to –55 °C was added a solution of CrO_3 (499 mg, 4.99 mmol) and Ac_2O (2 mL) in dichloromethane (6 mL). After 30 min the reaction was quenched by the addition of methanol (10 mL) and allowed to warm to room temperature. The mixture was then concentrated and co-evaporated three times with toluene (10 mL). Chromatography of the residue (silica, 20 mL; dichloromethane–hexane, 1:1) afforded enone **28** as a light yellow solid; yield: 260 mg (75%). R_F = 0.6 (dichloromethane–hexane, 1:1). $[\alpha]_D^{20}$ = 82.8 (*c* 1, $CHCl_3$); mp 25–26 °C (neat). IR (KBr, cm^{-1}) ν: 3747, 30.85, 2970, 2925, 2218, 1688, 1648, 1439, 1383, 1318, 1295, 1140, 1108, 1070, 900. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.81 (s, 1H), 4.72 (s, 1H), 2.73–2.53 (m, 3 H), 2.45 (ddq, 1H, J = 3 × 2.4, 12.3, 17.4 Hz), 2.37 (dd, 1H, J= 12.9, 16.2 Hz), 2.00 (dd, 3H, J = 1.8, 2.4 Hz), 1.70 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 196.66, 146.31, 144.74, 124.58, 116.95, 111.86, 42.60, 41.26, 33.07, 20.40, 14.86. MS (+FBA) *m/z* (%): 176 (100). HRMS could not be obtained; the compound proved to be too labile. Structure was confirmed by HRMS after reduction of the keto group.

Oxidation of 27 at elevated temperature

To a solution of crude cyanohydrin **27** (235 mg, 1.33 mmol) in dichloromethane (5 mL) cooled to 0 °C was added a solution of CrO_3 (332 mg, 3.33 mmol) and Ac_2O (0.78 mL) in dichloromethane (2.5 mL). After 20 min at 0 °C a new portion of CrO_3 (332 mg, 3.33 mmol) and Ac_2O (0.78 mL) in dichloromethane (2.5 mL) was added. After an additional 70 min, the reaction was quenched by the addition of ethanol (2 mL) and allowed to warm to room temperature over 45 min. The mixture was then concentrated. Chromatography of the residue (silica, 25 mL; Et_2O–hexane, 1:1) afforded diketone **29** (yield: 17.6 mg), epoxide **30** (yield: 73.2 mg), and a mixed fraction (yield: 61 mg).

5-Acetyl-2-methyl-3-oxocyclohex-1-enecarbonitrile (29)

$[\alpha]_D^{20}$ = 25.1 (*c* 1, $CHCl_3$). IR (film, cm^{-1}) ν: 3022, 2964, 2220, 1685, 1416, 1358, 1314, 1265, 1216, 1104, 752, 668, 629. ^{1}H (600 MHz, $CDCl_3$) δ: 3.23 (dddd, 1H, J = 4.2, 7.2, 10.5, 10.5 Hz), 2.82–2.78 (m, 3H), 2.65 (dd, 1H, J = 11.4, 16.8 Hz), 2.25 (s, 3H), 2.10 (s, 3H). ^{13}C (600 MHz, $CDCl_3$) δ: 205.98, 194.49, 146.66, 122.89, 116.56, 46.69, 38.99, 29.71, 27.88, 14.97. MS (EI+) *m/z* % 177 (M^+): 135 (45), 107 (6), 79 (7), 43 (100), HRMS calcd for $C_{10}H_{11}NO_2$: 177.1998; found: 177.0790.

2-Methyl-5-(2-methyloxiran-2-yl)-3-oxocyclohex-1-enecarbonitrile (30)

$[\alpha]_D^{20}$ = 57.5 (*c* 1, $CHCl_3$). IR (film, cm^{-1}) ν: 2927, 2218, 1686, 1436, 1386, 1313, 1140, 1070, 894, 820, 682, 622. ^{1}H (300 MHz, $CDCl_3$) δ: 2.70 (dd, 1H, J = 4.1, 14.2 Hz), 2.66–2.57 (m, 3H), 2.50–2.43 (m, 1H), 2.33–2.26 (m, 1H), 2.26–2.14 (m, 1H), 2.04 (s, 3H), 1.32 (d, 3H, J = 8.4 Hz). ^{13}C (300 MHz, $CDCl_3$) δ: 195.81, 146.45, 124.28, 116.78, 57.39, 52.64, 40.13, 39.41, 29.85, 18.82, 14.87. MS (EI+) *m/z* % 176 (M^+ – CH_3): 148 (51), 134 (100), 122 (53), 58 (81), 43 (96). HRMS calcd for $C_{11}H_{13}NO_2$: 191.2264; found: 176.0712.

(1R,2R,5S)-2-Methyl-3-oxo-5-(prop-1-en-2-yl) cyclohexanecarbonitrile (32)

A solution of enone **28** (0.1g, 0.57 mmol) with Pd/C (20 mg, 10%) in ethanol (3 mL, 95%) was hydrogenated in

an H_2 atmosphere at 80 °C for 16 h. The reaction mixture was then filtered through Celite and concentrated. Chromatography (silica, 8 mL; dichloromethane–hexane, 1:1) of crude product afforded 35 mg (34%) of fully saturated ketone **32** as a white solid. The remaining mass balance consisted of fully aromatized material identified as 2-methyl-3-cyano-5-isopropylphenol. R_F = 0.5 (dichloromethane–hexane, 1:1). $[\alpha]_D^{20}$ = –49.26 (*c* 1, $CHCl_3$); mp 77 °C (hexane). IR (KBr, cm^{-1}) *v*: 3412, 2979, 2959, 2938, 2916, 2874, 2241, 1713, 1477, 1469, 1448, 1429, 1381, 1369, 1346, 1313, 1245, 1231, 1218, 1147, 1076, 870, 628. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.58–2.44 (m, 3 H), 2.27 (dddd, 1H, *J* = 2.4, 2.7, 2.7, 13.5 Hz), 2.14 (dd, 1H, *J* =13.2, 12.9 Hz), 1.81 (ddd, 1H, *J* = 12.6, 12.6, 12.6 Hz), 1.63 (dd, 1H, *J* = 12.6, 14.1 Hz), 1.61 (m, 1H), 1.25 (d, 3H, *J* = 6.0 Hz), 0.93 (d, 3H, *J* = 6.6 Hz), 0.92 (d, 3H, *J* = 5.4 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 207.54, 120.28, 46.35, 44.53, 43.71, 36.13, 32.78, 32.36, 19.28, 19.06, 12.71. MS (+EI) *m/z* (%): 179 (100). HRMS (+EI) calcd for $C_{11}H_{17}N_1O_1$: 179.13101; found: 179.13136.

(3S,5S)-3-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-1-enecarbonitrile (34)

To the cooled (4 °C) solution of enone **28** (50 mg, 29 mmol) in methanol (2 mL) was added $CeCl_3{\cdot}7H_2O$ (106 mg, 29 mmol). After 5 min of stirring, $NaBH_4$ (11 mg, 29 mmol) was added portionwise. After an additional 10 min stirring, the reaction was quenched by the addition of a citric acid solution (0.5 mL, 5% *w*/*w*) and concentrated. The residue was dissolved in dichloromethane (25 mL) and washed with citric acid (5 mL, 5% *w*/*w*). The aqueous layer was re-extracted with additional dichloromethane (5 mL), the combined organic layers were dried over $MgSO_4$, and then concentrated to yield essentially pure product. Chromatography (silica, 5 mL, dichloromethane) yielded 48 mg (95%) of the allylic alcohol **34** as a colourless oil. R_F = 0.2 (dichloromethane). $[\alpha]_D^{20}$ = 40.42 (*c* 1, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 3446, 3084, 2926, 2859, 2215, 1646, 1441, 1378, 1288, 1261, 1071, 1056, 1025, 895. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.81 (s, 1H), 4.76 (s, 1H), 4.24 (m, 1H), 2.36–2.13 (m, 5 H), 2.11 (s, 3H), 1.75 (s, 3H), 1.55 (ddd, 1H, *J* = 10.2, 12.0, 12.0 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 154.69, 146.75, 118.34, 110.52, 108.08, 70.25, 39.28, 36.62, 32.71, 20.43, 18.33. MS (+EI) *m/z* (%): 177 (3), 159 (100), 144 (33), 134 (59). HRMS (+EI) calcd for $C_{11}H_{15}N_1O_1$: 177.11536; found: 177.11525. HRMS (+EI) calcd for $C_{11}H_{13}N_1$: 159.10480; found: 159.10496.

Reduction of 28 to 31a, 31b, and 31c

To a round-bottomed flask containing water (5 mL) and tetrabutylammonium bromide (100 mg, 0.3 mmol) was added a solution of enone **28** (175 mg, 1.0 mmol) in toluene (5 mL). The emulsion was degassed in an ultrasonic bath for 20 min. A condenser was attached, and the system was flushed with nitrogen. Sodium bicarbonate (1.52 g, 18 mmol) was added, and the reaction mixture was heated to reflux. Sodium dithionite (7 × 200 mg, 85%, 6.95 mmol) was added in seven portions over 2 h. After an additional 30 min of stirring at reflux, the reaction mixture was allowed to cool to room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and water (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with 0.5 N HCl (5 mL), then water (5 mL), and dried over magnesium sulfate. After filtration and evaporation of the solvent, the crude mixture was purified via flash column chromatography (EtOAc in hexanes, 10%–25%) to yield 145 mg (82%) of the mixture consisting of three of the four isomers of cyanoketone **31**. This mixture was suitable for use in the elimination to (+)-carvone.

Identification of isomers 31a, 31b, and 31c

To a round-bottomed flask containing water (60 mL), sodium bicarbonate (4.31 g, 51.30 mmol), and tetrabutylammonium bromide (275 mg, 0.855 mmol) was added a solution of enone **28** (500 mg, 2.85 mmol) in toluene (60 mL). The reaction mixture was heated to reflux, and sodium dithionite (6 × 875 mg, 4.28 mmol) was added in six portions over 3 h. After an additional 1 h of stirring at reflux, the reaction mixture was allowed to cool to room temperature. The organic layer was separated from the aqueous layer and then dried over magnesium sulfate. After filtration and evaporation of solvent, the crude mixture was purified via flash column chromatography (hexane – diethyl ether, 7:1) to provide 75 mg (15%) of saturated ketone **31b** as a white solid. Further separation provided the remaining isomers **31a** and **31c**.

(1S,2S,5S)-2-Methyl-3-oxo-5-(prop-1-en-2-yl) cyclohexanecarbonitrile (31a)

R_F = 0.17 (hexanes–EtOAc, 5:1). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 4.86 (s, 1H), 4.80 (s, 1H), 3.34 (dt, *J* = 6.0, 3.7 Hz, 1H), 2.86–2.72 (m, 1H), 2.66–2.47 (m, 2H), 2.36–2.18 (m, 2H), 2.05–1.86 (m, 1H), 1.77 (s, 3H), 1.25 (d, *J* = 6.6 Hz, 3H).

^{1}H NMR data are in agreement with the literature.[14]

(1R,2S,5S)-2-Methyl-3-oxo-5-(prop-1-en-2-yl) cyclohexanecarbonitrile (31b)

White solid. R_F = 0.42 (hexane – ethyl acetate, 5:1). $[\alpha]_D^{20}$ = –56 (*c* 1, $CHCl_3$). mp 82–84 °C (first crystallization: hexanes – diethyl ether; second crystallization: mp 85–86 °C (lit.[18] mp 86.5). IR ($CHCl_3$, cm^{-1}) *v*: 2244, 1719, 1648, 1452, 901. ^{1}H NMR ($CDCl_3$, 600 MHz, ppm) δ: 4.85 (s, 1H), 4.78 (s, 1H), 2.61–2.52 (m, 3 H), 2.40–2.36 (m, 3H), 2.01 (ddd, 1H, *J* = 10.9, 11.9, 12.4 Hz), 1.75 (s, 3H), 1.28 (d, 3H, *J* = 6.4 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz, ppm) δ: 206.66, 145.27, 119.98, 111.30, 46.28, 44.14, 36.00, 34.25, 20.23, 12.67. MS (+EI) *m/z* (%): 177 (12), 95 (55), 82 (54), 68 (100), 67 (74), 41 (41). HRMS (+EI) calcd for $C_{11}H_{15}NO$: 177.11536; found: 177.11509.

(1S,2R,5S)-2-Methyl-3-oxo-5-(prop-1-en-2-yl) cyclohexanecarbonitrile (31c)

Viscous oil; assignment is tentative (**31c** has a mp of 13 °C). IR (KBr, cm^{-1}) *v*: 2359, 1650, 1385, 1088. ^{1}H NMR (600 MHz, $CDCl_3$, ppm) δ: 4.87 (s, 1H), 4.79 (s, 1H), 3.14 (td, *J* = 5.1, 2.5 Hz, 1H), 2.64 (t, *J* = 12.8 Hz, 1H), 2.40 (dt, *J* = 12.8, 2.3 Hz, 1H), 1.87–1.80 (m, 1H), 1.78 (s, 1H), 1.61–1.49 (m, 1H), 1.45 (d, *J* = 6.8 Hz, 1H). MS (+EI) *m/z* (%): 177 (27), 95 (72), 82 (52), 69 (29), 68 (100), 67 (70), 41 (42).; HRMS (+EI) calcd for $C_{11}H_{15}NO$: 177.11536; found: 177.11498.

ent-Carvone

The mixture of saturated ketones **31a**, **31b**, and **31c** obtained (445 mg, 2.5 mmol) was dissolved in MeOH (15 mL). The solution was degassed in an ultrasonic bath for 5 min (to avoid oxidation of Fe(II) by air). A condenser was attached, and $FeSO_4 \cdot 7H_2O$ (1.39 g, 5.0 mmol) was added. KOH (2.25 g, 35 mmol, 10 + 4 equiv) was added to a vigorously stirred suspension to generate $Fe(OH)_2$. The reaction flask was placed in a preheated oil bath, and the mixture heated at reflux for 20 min. The resulting dark black mixture was quickly cooled to room temperature and diluted with EtOAc (100 mL) and water (10 mL). The organic layer was washed with 0.2 N HCl (3 × 10 mL) and dried over magnesium sulfate. After filtration and evaporation of solvent, the crude product was filtered through a short pad of silica gel (eluted with 10% EtOAc in hexanes) to yield *S*-(+)-carvone (296 mg, 79%).

S-(+)-Carvone

$[\alpha]_D^{19} = +54.2$ (*c* 1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 6.82–6.65 (m, 1H), 4.80 (s, 1H), 4.75 (s, 1H), 2.78–2.51 (m, 2H), 2.51–2.37 (m, 1H), 2.37–2.19 (m, 2H), 1.85–1.76 (m, 3H), 1.75 (s, 3H). (Commercially available (+)-carvone is 96% optically pure; (–)-carvone is 98% optically pure).

Acknowledgements

The authors are grateful to the following agencies for financial support of this work: Natural Sciences and Engineering Research Council of Canada (NSERC) (Idea to Innovation and Discovery Grants); Canada Research Chair Program; Canada Foundation for Innovation (CFI); Research Corporation; Noramco, Inc.; TDC Research, Inc.; TDC Research Foundation; and Brock University, St. Catharines, Ontario. Thanks are also due to the Ministry of Education (Spain) for fellowships to V.S. (AP2006-03468ESTANCIA-2010). The authors thank K. Ridolfo for preliminary experiments performed as part of his fourth-year Honours Thesis, Spring 2010.

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CONCLUSIONES

CONCLUSIONES

Capítulo 1

Se ha desarrollado una síntesis enantioselectiva eficiente para acceder a quinolizidinas 1*S*-etil-4-sustituidas sin emplear grupos protectores. Estos compuestos tienen un potencial interés biológico, ya que compuestos con estructuras análogas han mostrado actividad como bloqueadores de receptores nicotínicos. El (*S*)-fenilglicinol es la fuente de quiralidad que define los estereocentros en dos reacciones estereocontroladas clave, una α-amidoalquilación y una adición organometálica. La metodología desarrollada permite acceder a quinolizidinas 1*S*-etil-4-sustituidas epiméricas en la posición angular C-9a. La reacción final en la secuencia sintética implica una adición de reactivos organometálicos que trascurre con diferente estereoselectividad dependiendo de la estereoquímica en el carbono angular. Esta diferencia de comportamiento se racionaliza mediante estudios de cálculo teórico. La conclusión es que la adición del ión hidruro a las sales de iminio intermedias se produce bajo control estereoelectrónico.

La secuencia sintética desarrollada para preparar quinolizidinas 1*S*-etil-4-sustituidas implica en uno de sus pasos la eliminación del inductor quiral, el fragmento de *N*-(2-hidroxi-1-fenil)etilo. En este contexto, se ha desarrollado un método barato, de fácil manipulación y benigno con el medio ambiente que utiliza aire e hidróxido sódico como reactivos para producir la desbencilación.

Capítulo 2

Se ha desarrollado un método barato para producir triheptanoina de elevada calidad en cantidades considerables. Este procedimiento implica que se utiliza un leve exceso de ácido heptanoico, no se utilizan metales en el proceso, únicamente se utilizan disolventes y catalizadores que respetan el medioambiente y no se necesita un proceso de purificación que implique cromatografía en columna. Posteriormente, esta triheptanoina se ha formulado galénicamente para poder ser administrada a roedores como dieta cetogénica y hacer los estudios farmacológicos correspondientes.

Capítulo 3

Se han identificado siete nuevos metabolitos en la dihidroxilación de *o*-halobenzoatos catalizada por tolueno dioxigenasa. En general, la conversión de los ésteres benzoicos en dioles trascurre con menor rendimiento que la de los halobenceno derivados. Los compuestos se han preparado en escala multigramo por fermentación. Además, se han llevado a cabo estudios teóricos que explican los resultados obtenidos en la dihidroxilación microbiológica de halobenzoatos *o*-sustituidos. Utilizando la metodología desarrollada se ha accedido a un intermedio clave en la síntesis de la Kibledona C.

Capítulo 4.

Se ha descrito un procedimiento eficaz para la conversión de enonas en las correspondientes β-cianoenonas que va acompañada de una trasposición 1,3-funcional del grupo carbonilo de la enona original. La metodología desarrollada se ha testado en la conversión de la (-)-carvona en su enantiómero.

SUPPORTING MATERIAL

CHAPTER 1 – PART A

SUPPORTING MATERIAL

Enantioselective, Protecting Group-Free Synthesis of 1*S*-Ethyl-4-Substituted Quinolizidines

Amat, M.; Semak, V.; Escolano, C.; Molins, E.; Bosch, J.
Org. Biomol. Chem. **2012**, *10*, 6866-6875.

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Enantioselective Protecting Group-Free Synthesis of 1*S*-Ethyl-4-Substituted Quinolizidines

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1 Materials and Methods

General methods

NMR spectra were recorded in $CDCl_3$ at 300 or 400 MHz (^1H) and 75.4 or 100.6 MHz (^{13}C), and chemical shifts are reported in δ values downfield from TMS or relative to residual chloroform (7.26 ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, multiplicity, coupling constant (J) in hertz (Hz), integrated intensity. Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; br s, broad signal, app, apparent. Evaporation of solvents was accomplished with a rotatory evaporator. Melting points were determined in a capillary tube and are uncorrected. Thin-layer chromatography was done on SiO_2 (silica gel 60 F_{254}), and the spots were located by UV, 1% aqueous $KMnO_4$ or iodoplatinate (for tertiary amines). Chromatography refers to flash column chromatography and was carried out on SiO_2 (silica gel 60, SDS, 230-400 mesh) or Al_2O_3 (Aluminium oxide 90 active basic, Merck). Mass spectra were recorded on a LTQ spectrometer using electrospray (ES^+) ionization techniques.

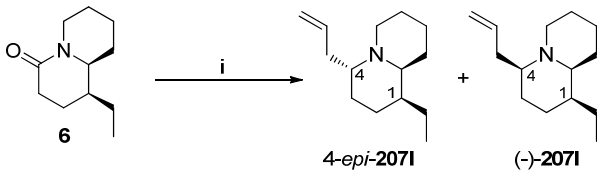
Materials

Analytical grade solvents were used. Solvents have been dried by distillation from CaH_2 prior to use. THF was dried by distillation from sodium with benzophenone as dryness indicator prior to use. Commercially available anhydrous cerium(Ill) chloride was activated according to modified Dimitrov's procedure.[1]

Procedure for activation of $CeCl_3$: commercially available anhydrous cerium(Ill) chloride [CAS Number: 7790-86-5] (Sigma-Aldrich or Alfa Aesar) was placed in a Schlenk flask equipped with stirring bar and connected to vacuum pumping unit (6 Torr). Content was heated to 90 °C (external temperature) overnight. Without breaking the vacuum the high vacuum oil pump (< 1 Torr) was connected and flask was heated to 100 °C (1 h), 110 °C (1 h), 120 °C (1 h), 130 °C (1 h) and 140 °C (4 h). $CeCl_3$ was stored in a Schlenk flask under argon atmosphere.

[1] V. Dimitrov, K. Kostova and M. Genov, *Tetrahedron Lett.*, 1996, **37**, 6787-6790.

2 Experimental Details - Screening of Reducing Agents



Scheme 1 Reagents and conditions: (i) (a) $CeCl_3$ (2.0-4.0 equiv), allylmagnesium bromide (4.0-8.0 equiv), THF, rt, 18 h; (b1) $NaBH_3CN$ (6.0 equiv), THF, AcOH, -78 °C, 60 min, d.r. = 66:34; (b2) $NaBH(AcO)_3$ (2.0 equiv), THF, AcOH, -78 °C to rt, 2 h, d.r. = 77:23; (b3) $NaBH_4$ (1.25 equiv), MeOH, AcOH, -78 °C, 30 min, 58%, d.r. = 97:3; (b4) DIBAL-H (6.0 equiv), THF, PhMe, -78 °C, 60 min, d.r. = 96:4.

Table 1 Diastereoseletivity of iminium salt reduction.

Entry	Reducing agent	Conditions	d.r. 4-*epi*-**207I** : (-)-**207I**
b1*	$NaBH_3CN$	THF, AcOH, -78 °C, 60 min	66 : 34
b2*	$NaBH(AcO)_3$	THF, AcOH, -78 °C to RT, 120 min	77 : 23
b3	$NaBH_4$	THF, MeOH, AcOH, -78 °C, 30 min	97 : 3
b4*	DiBAl-H	THF, PhMe, -78 °C, 75 min	96 : 4

* A 10% of diallylated product was also isolated.

Experimental procedure

Entry b1

Anhydrous $CeCl_3$ (0.82 g, 3.32 mmol) was added to a solution of **6** (150 mg, 0.83 mmol) in anhyd THF (5 mL). The resulting suspension was vigorously stirred at rt for 1 h. AllylMgBr in Et_2O (6.70 mL, 6.70 mmol, 1.0 M solution) was added dropwise over 30 min and the mixture was stirred for additional 18 h. MeOH was added to quench the reaction and the mixture was cooled at -78 °C. $NaBH_3CN$ (320 mg, 4.97 mmol) and AcOH (0.5 mL) were added and the mixture was stirred at -78 °C for 60 min. The mixture was concentrated and the residue was partitioned between Et_2O and 1N HCl solution. Then, the acidic aqueous phase was washed with Et_2O and basified with 4N NaOH solution (pH=12-14). The resulting suspension was centrifuged (800 *g* for 30 min at rt) and the supernatant was extracted with CH_2Cl_2. The combined organic extracts were dried, filtered, and concentrated under reduced pressure to give 203 mg of yellowish oily solid, which was analyzed by GC-MS, ^{13}C and ^{1}H NMR.

Entry b2

Anhydrous $CeCl_3$ (1.48 g, 6.0 mmol) was added to a solution of **6** (272 mg, 1.50 mmol) in anhyd THF (7 mL). The resulting suspension was vigorously stirred at rt for 1 h. AllylMgBr in Et_2O (12.0 mL, 12.0 mmol, 1.0 M solution) was added dropwise over 30 min and the mixture was stirred for additional 18 h. MeOH was added to quench the reaction and the mixture was cooled at -78 °C. $NaBH(AcO)_3$ (635 mg, 3.00 mmol) and AcOH (0.25 mL) were added. The reaction mixture start to be a dens suspension and the magnetic stirring was inefficient. The reaction mixture was allowed to reach RT during 2 h. At ca. -40 °C the stirring start to be efficient. The mixture was concentrated and the residue was dissolved in water and basified by addition of solid Na_2CO_3 (2.5 g). The resulting fine white aqueous solution was extracted with CH_2Cl_2. The combined organic extracts were washed with water, dried over Na_2SO_4 and concentrated under reduced pressure to give brownish oil (0.35 g) which was analyzed by GC-MS, ^{13}C and ^{1}H NMR.

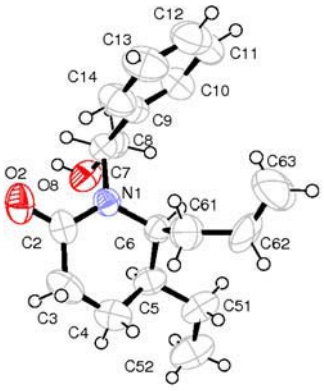
Entry b3 – see Experimental Section

Entry b4

Anhydrous $CeCl_3$ (0.82 g, 3.32 mmol) was added to a solution of **6** (150 mg, 0.83 mmol) in anhyd THF (5 mL). The resulting suspension was vigorously stirred at rt for 1 h. AllylMgBr in Et_2O (6.70 mL, 6.70 mmol, 1.0 M solution) was added dropwise over 30 min and the mixture was stirred for additional 18 h. The reaction mixture was cooled (-78 °C) and DIBAL-H (3.0 mL, 5.1 mmol, 1.7 M solution in toluene) was added dropwise over 30 minutes by syringe pump. The reaction mixture was stirred for additional 45 minutes and quenched by addition of saturated solution of Rochele's salt at -78 °C. The mixture was filtered under reduce pressure with aid of EtOAc and water. The aqueous phase was extracted with EtOAc, the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give 120 mg of brownish oil, which was analyzed by GC-MS, ^{13}C and ^{1}H NMR.

3 X-Ray Crystallography

(5*S*,6*S*)-6-Allyl-5-ethyl-1-[(1*S*)-2-hydroxy-1-phenylethyl]-2-piperidone, (2)



Note:
Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	*d(D-H)*	*d(H..A)*	*<DHA*	*d(D..A)*	*A*
O8-H8	*0.820*	*1.909*	*171.18*	*2.722*	*O2 [x-1/2, -y+1/2, z]*

Table 2 Crystal data and structure refinement for **2**.

Identification code	Jb87	
Empirical formula	C18 H25 N O2	
Formula weight	287.39	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/a	
Unit cell dimensions	a = 7.938(3) Å	= 90°.
	b = 23.823(4) Å	= 104.96(3)°.
	c = 9.277(3) Å	= 90°.
Volume	1694.9(9) $Å^3$	
Z	4	
Density (calculated)	1.126 Mg/m^3	
Absorption coefficient	0.073 mm^{-1}	
F(000)	624	
Crystal size	0.36 x 0.24 x 0.15 mm^3	
Theta range for data collection	1.71 to 26.97°.	
Index ranges	-10<=h<=9, 0<=k<=30, 0<=l<=11	
Reflections collected	7949	
Independent reflections	3691 [R(int) = 0.0471]	
Completeness to theta = 26.97°	100.0 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3691 / 30 / 210	
Goodness-of-fit on F^2	0.888	
Final R indices [I>2sigma(I)]	R1 = 0.0528, wR2 = 0.1158	
R indices (all data)	R1 = 0.1709, wR2 = 0.1479	
Largest diff. peak and hole	0.149 and -0.114 $e.Å^{-3}$	

Table 3 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for **2**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(2)	5781(2)	2512(1)	3440(2)	80(1)
O(8)	2238(2)	1460(1)	3330(2)	71(1)
N(1)	5458(2)	1630(1)	2535(2)	52(1)
C(2)	6287(3)	2017(1)	3503(3)	59(1)
C(3)	7840(3)	1839(1)	4734(3)	78(1)
C(4)	8207(4)	1228(1)	5004(3)	83(1)
C(5)	6792(3)	847(1)	4106(3)	64(1)
C(6)	6193(3)	1066(1)	2517(3)	56(1)
C(7)	3761(3)	1767(1)	1496(3)	54(1)
C(8)	2312(3)	1416(1)	1836(3)	61(1)
C(9)	3863(3)	1758(1)	-114(3)	58(1)
C(10)	3014(4)	1377(1)	-1139(3)	74(1)
C(11)	3149(4)	1391(2)	-2610(4)	90(1)
C(12)	4161(5)	1785(2)	-3028(4)	93(1)
C(13)	5020(4)	2174(2)	-2031(4)	94(1)
C(14)	4853(4)	2157(1)	-580(3)	78(1)
C(51)	7321(4)	231(1)	4207(3)	84(1)
C(52)	7614(4)	-14(2)	5765(4)	110(1)
C(61)	7618(4)	1056(1)	1649(3)	81(1)
C(62)*	7875(14)	544(4)	885(10)	101(3)
C(63)*	7310(20)	495(7)	-547(10)	216(9)
C(62D)*	7221(16)	662(7)	434(16)	181(8)
C(63D)*	8240(18)	286(4)	188(16)	171(5)

* half site occupancy

Table 4 Bond lengths [Å] and angles [°] for **2**.

O(2)-C(2)	1.243(3)
O(8)-C(8)	1.406(3)
N(1)-C(2)	1.335(3)
N(1)-C(6)	1.467(3)
N(1)-C(7)	1.477(3)
C(2)-C(3)	1.508(4)
C(3)-C(4)	1.495(4)
C(4)-C(5)	1.515(4)
C(5)-C(6)	1.519(3)
C(5)-C(51)	1.523(4)
C(6)-C(61)	1.549(3)
C(7)-C(9)	1.516(3)
C(7)-C(8)	1.519(3)
C(9)-C(10)	1.361(3)
C(9)-C(14)	1.372(3)
C(10)-C(11)	1.397(4)
C(11)-C(12)	1.356(4)
C(12)-C(13)	1.361(4)
C(13)-C(14)	1.387(4)
C(51)-C(52)	1.520(4)
C(61)-C(62D)	1.439(8)
C(61)-C(62)	1.451(7)
C(62)-C(63)	1.293(8)
C(62D)-C(63D)	1.266(8)
C(2)-N(1)-C(6)	120.7(2)
C(2)-N(1)-C(7)	119.7(2)
C(6)-N(1)-C(7)	119.57(19)
O(2)-C(2)-N(1)	121.8(3)
O(2)-C(2)-C(3)	119.5(3)
N(1)-C(2)-C(3)	118.7(3)
C(4)-C(3)-C(2)	119.1(2)
C(3)-C(4)-C(5)	114.0(2)
C(4)-C(5)-C(6)	109.3(2)
C(4)-C(5)-C(51)	112.9(2)
C(6)-C(5)-C(51)	113.7(2)
N(1)-C(6)-C(5)	109.20(19)
N(1)-C(6)-C(61)	111.4(2)
C(5)-C(6)-C(61)	114.3(2)
N(1)-C(7)-C(9)	111.59(19)
N(1)-C(7)-C(8)	110.78(19)
C(9)-C(7)-C(8)	115.7(2)
O(8)-C(8)-C(7)	113.0(2)
C(10)-C(9)-C(14)	117.5(3)
C(10)-C(9)-C(7)	123.7(2)
C(14)-C(9)-C(7)	118.8(2)
C(9)-C(10)-C(11)	121.2(3)
C(12)-C(11)-C(10)	119.8(3)
C(11)-C(12)-C(13)	120.5(3)
C(12)-C(13)-C(14)	118.8(3)
C(9)-C(14)-C(13)	122.2(3)
C(52)-C(51)-C(5)	113.6(3)
C(62D)-C(61)-C(62)	25.6(6)
C(62D)-C(61)-C(6)	112.0(7)
C(62)-C(61)-C(6)	118.7(5)
C(63)-C(62)-C(61)	121.5(9)
C(63D)-C(62D)-C(61)	125.6(11)

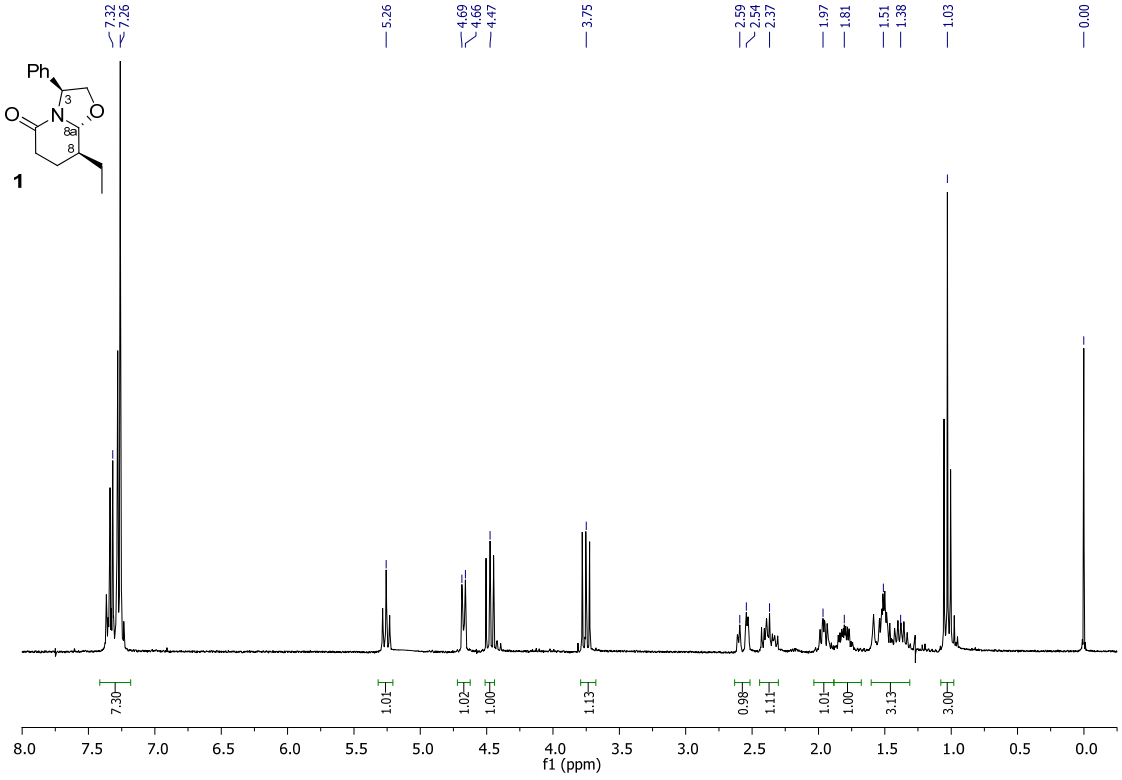
Table 5 Anisotropic displacement parameters ($Å^2 x\ 10^3$)for **2**. The anisotropic displacement factor exponent takes the form: $-2\ ^2[\ h^2a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

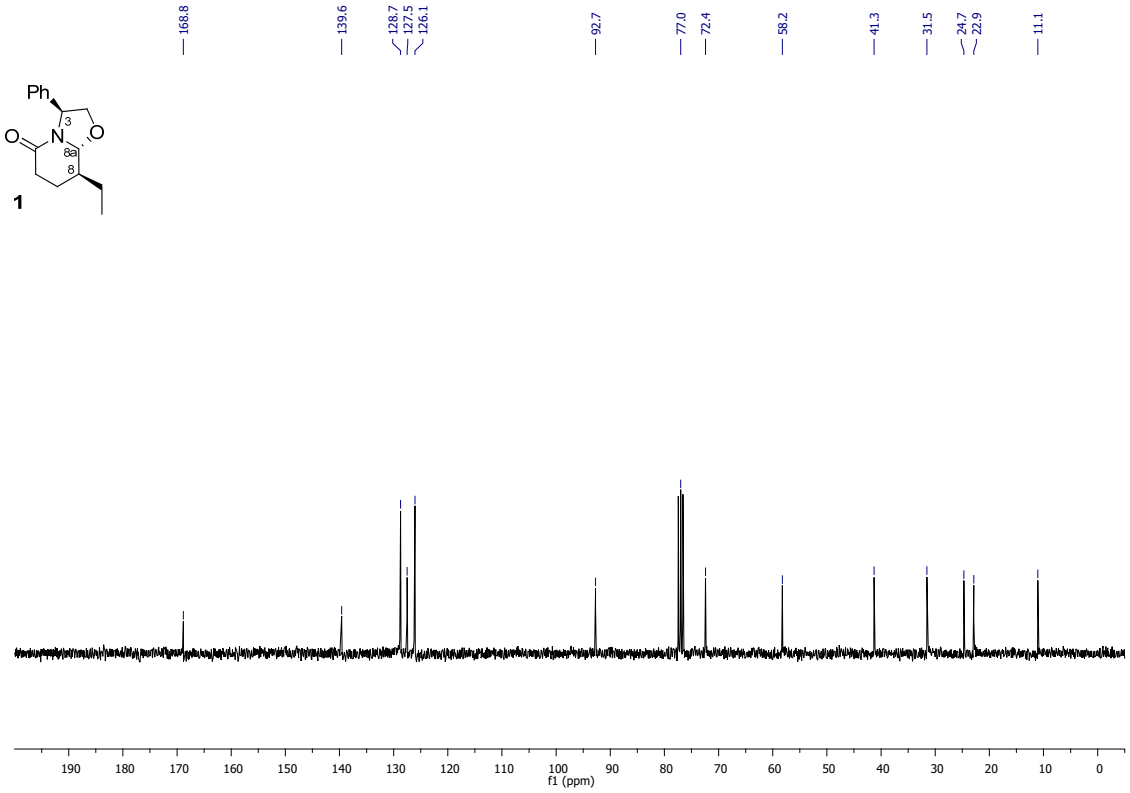
	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(2)	83(1)	59(1)	100(2)	-21(1)	30(1)	-16(1)
O(8)	76(1)	70(1)	76(1)	13(1)	35(1)	12(1)
N(1)	50(1)	53(1)	53(1)	-4(1)	13(1)	-4(1)
C(2)	61(2)	59(2)	64(2)	-11(2)	29(1)	-13(2)
C(3)	70(2)	99(3)	63(2)	-8(2)	12(2)	-30(2)
C(4)	77(2)	94(2)	73(2)	5(2)	7(2)	-4(2)
C(5)	50(1)	71(2)	71(2)	10(2)	15(1)	3(1)
C(6)	52(1)	53(2)	61(2)	-4(1)	14(1)	1(1)
C(7)	53(2)	52(2)	57(2)	2(1)	14(1)	2(1)
C(8)	52(2)	59(2)	72(2)	0(1)	15(1)	4(1)
C(9)	56(2)	59(2)	55(2)	1(1)	9(1)	1(1)
C(10)	76(2)	81(2)	64(2)	-5(2)	15(2)	-9(2)
C(11)	90(2)	100(3)	70(2)	-18(2)	1(2)	-3(2)
C(12)	91(2)	121(3)	64(2)	4(2)	16(2)	10(2)
C(13)	98(2)	117(3)	67(2)	20(2)	23(2)	-17(2)
C(14)	90(2)	83(2)	59(2)	6(2)	16(2)	-19(2)
C(51)	69(2)	81(2)	104(3)	24(2)	24(2)	16(2)
C(52)	100(3)	110(3)	123(3)	47(2)	33(2)	22(2)
C(61)	80(2)	95(2)	75(2)	-4(2)	33(2)	11(2)
C(62)	107(8)	110(7)	103(8)	7(6)	57(6)	69(6)
C(63)	202(13)	330(20)	92(9)	-85(11)	-9(8)	141(12)
C(62D)	99(8)	304(19)	127(11)	-81(12)	3(7)	96(9)
C(63D)	209(14)	152(11)	179(12)	-30(10)	97(11)	22(10)

4 Copies of the ^{1}H and ^{13}C NMR spectra.

^{1}H NMR and ^{13}C NMR spectra of

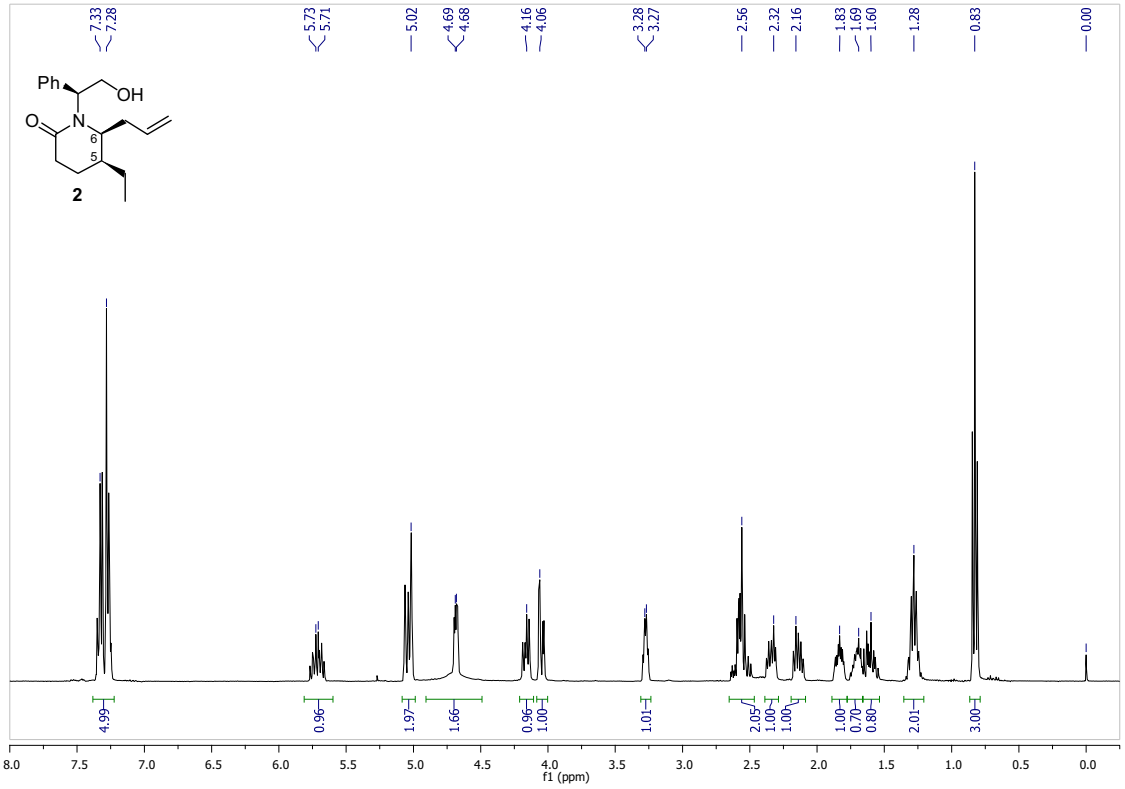
(3*S*, 8*S*, 8a*R*)-8-Ethyl-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo[3,2-a]pyridine, (**1**).

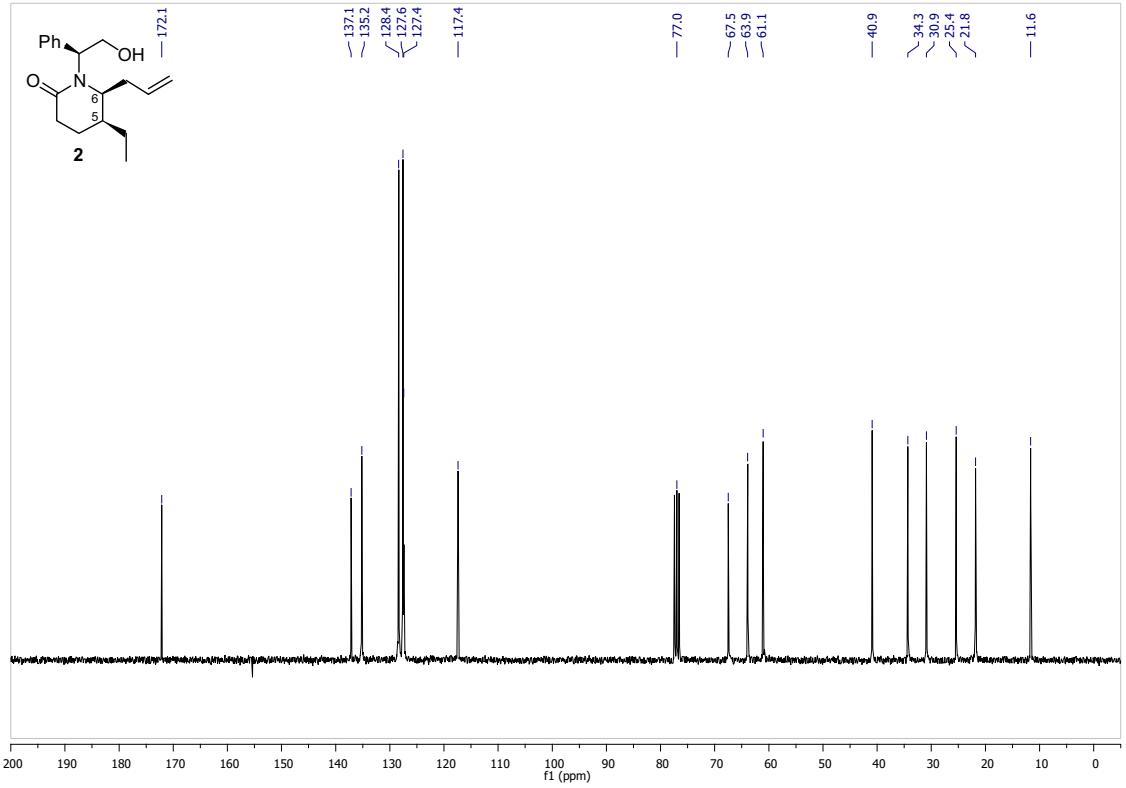




^{1}H NMR and ^{13}C NMR spectra of

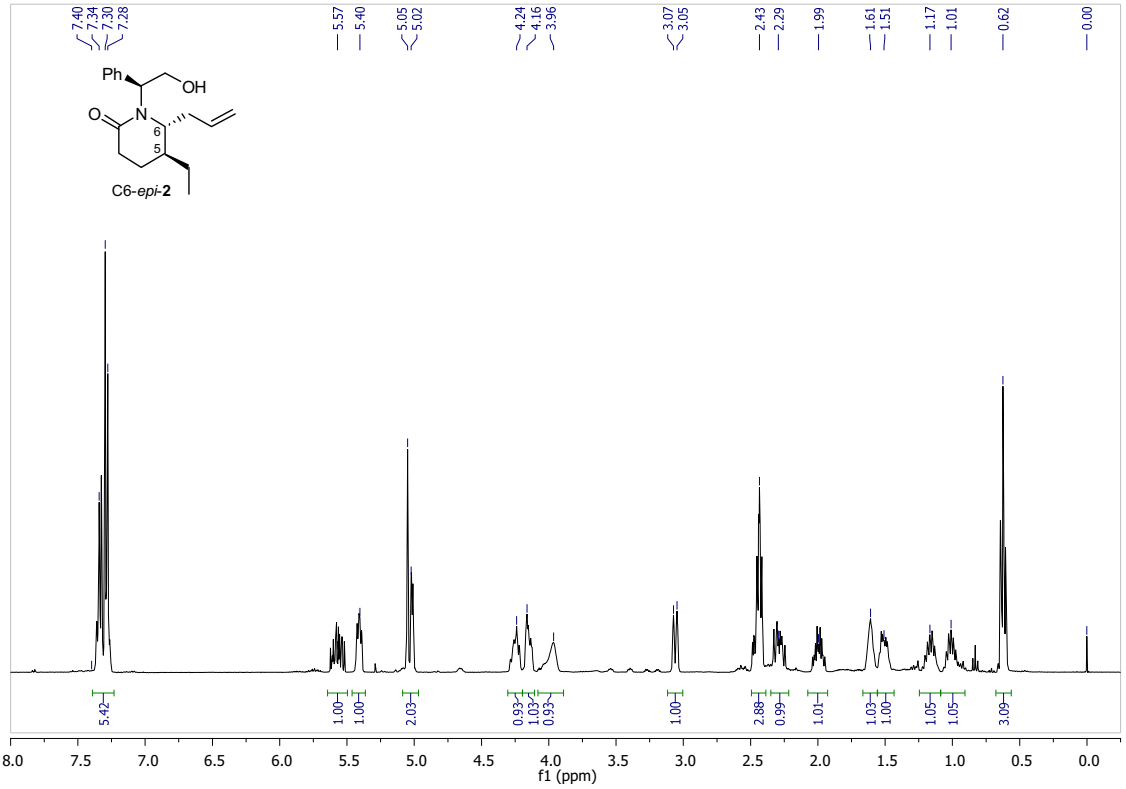
(5*S*,6*S*)-6-Allyl-5-ethyl-1-[(1*S*)-2-hydroxy-1-phenylethyl]-2-piperidone, (**2**).

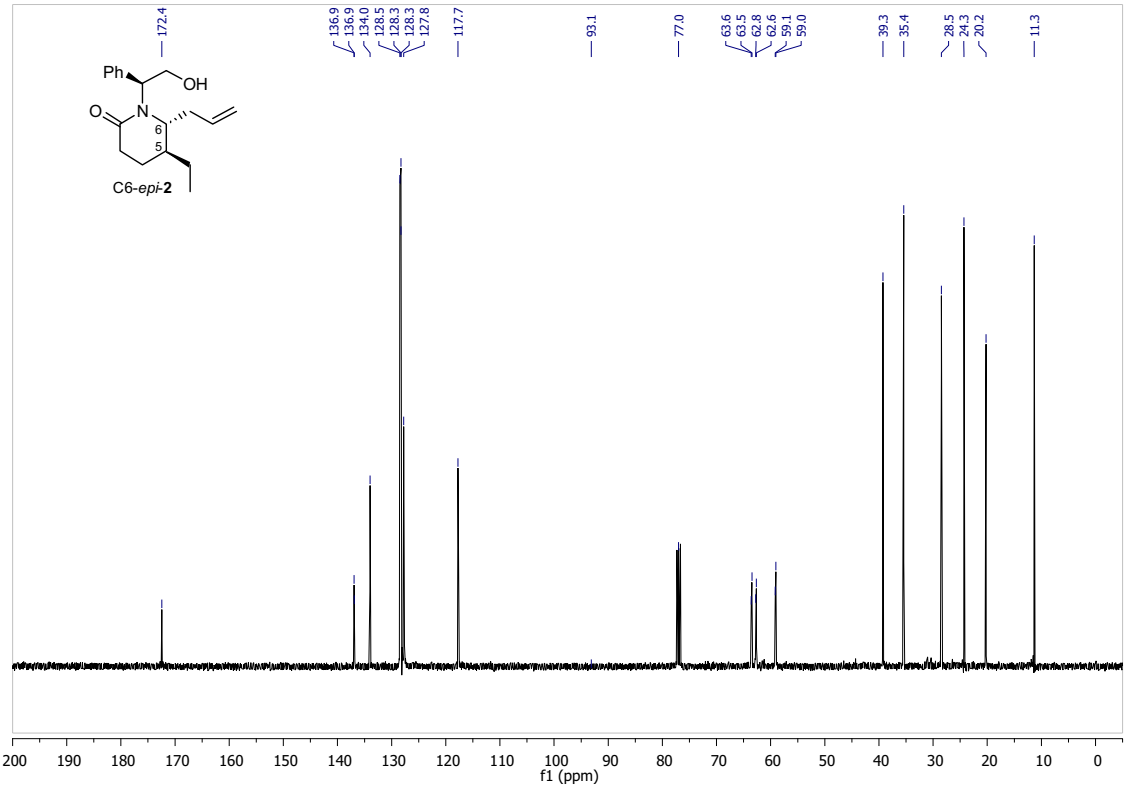




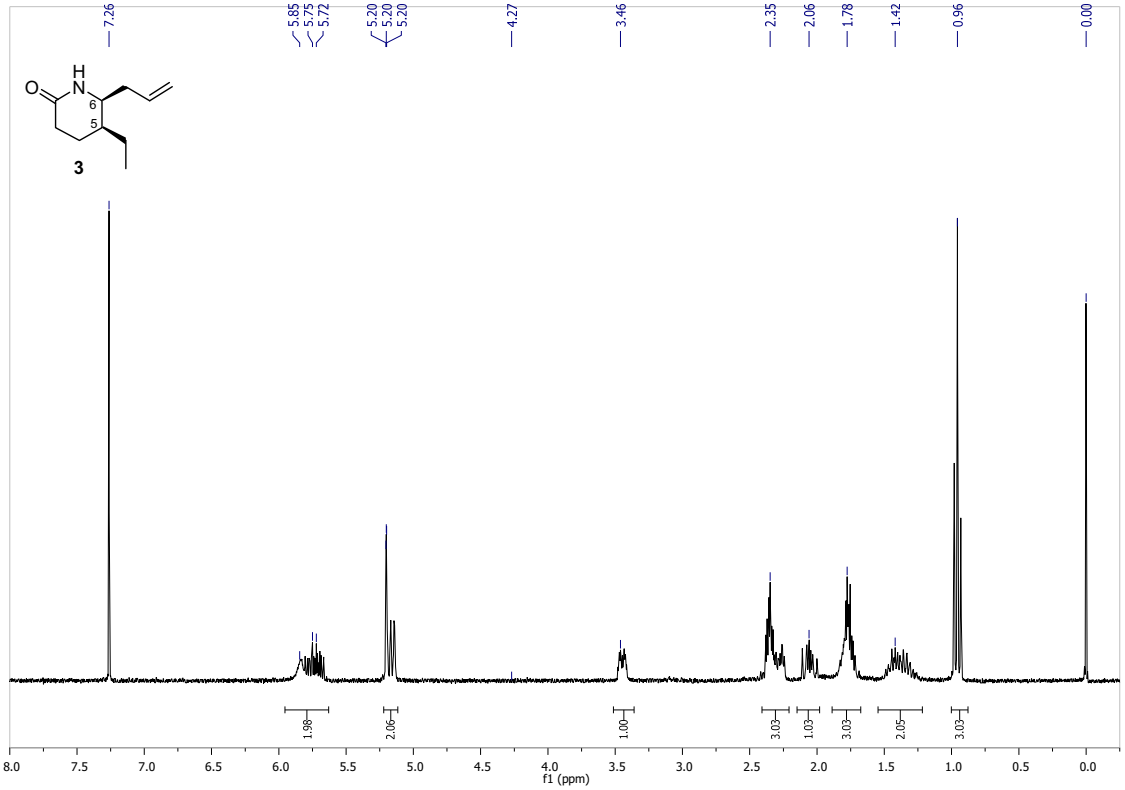
^{1}H NMR and ^{13}C NMR spectra of

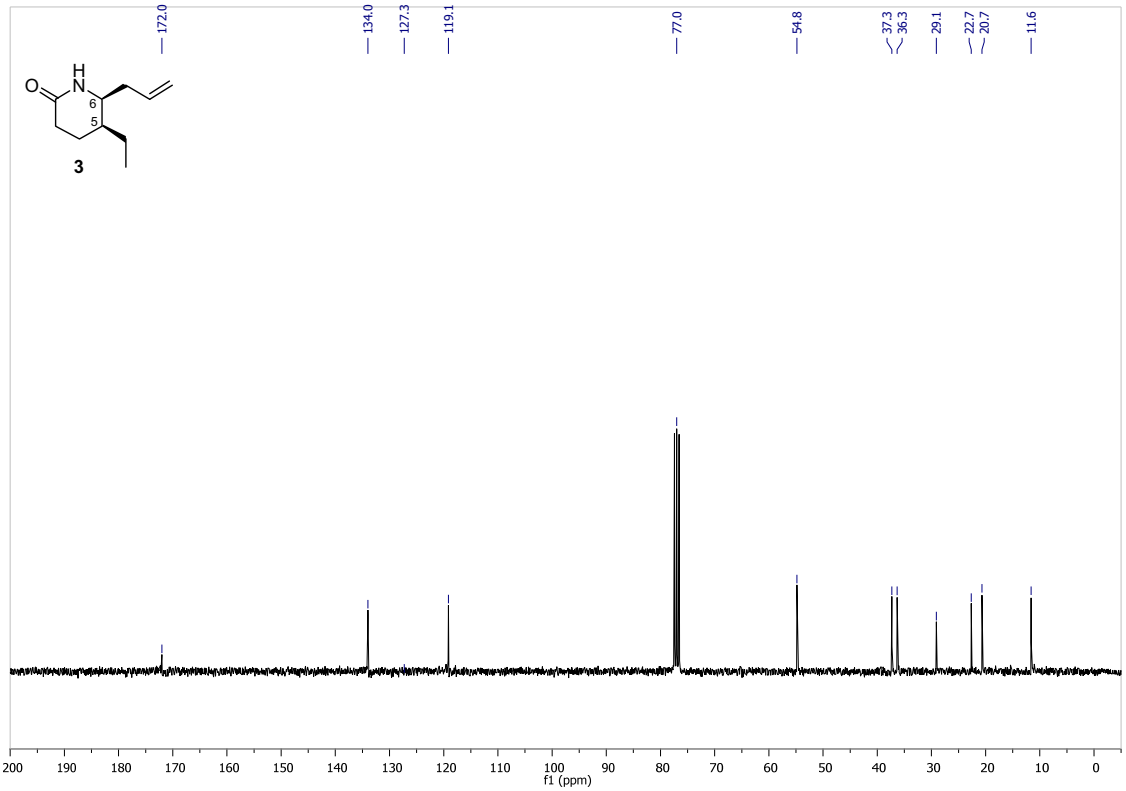
(5*S*,6*R*)-6-Allyl-5-ethyl-1-[(1*S*)-2-hydroxy-1-phenylethyl]-2-piperidone, (6-*epi*-**2**).



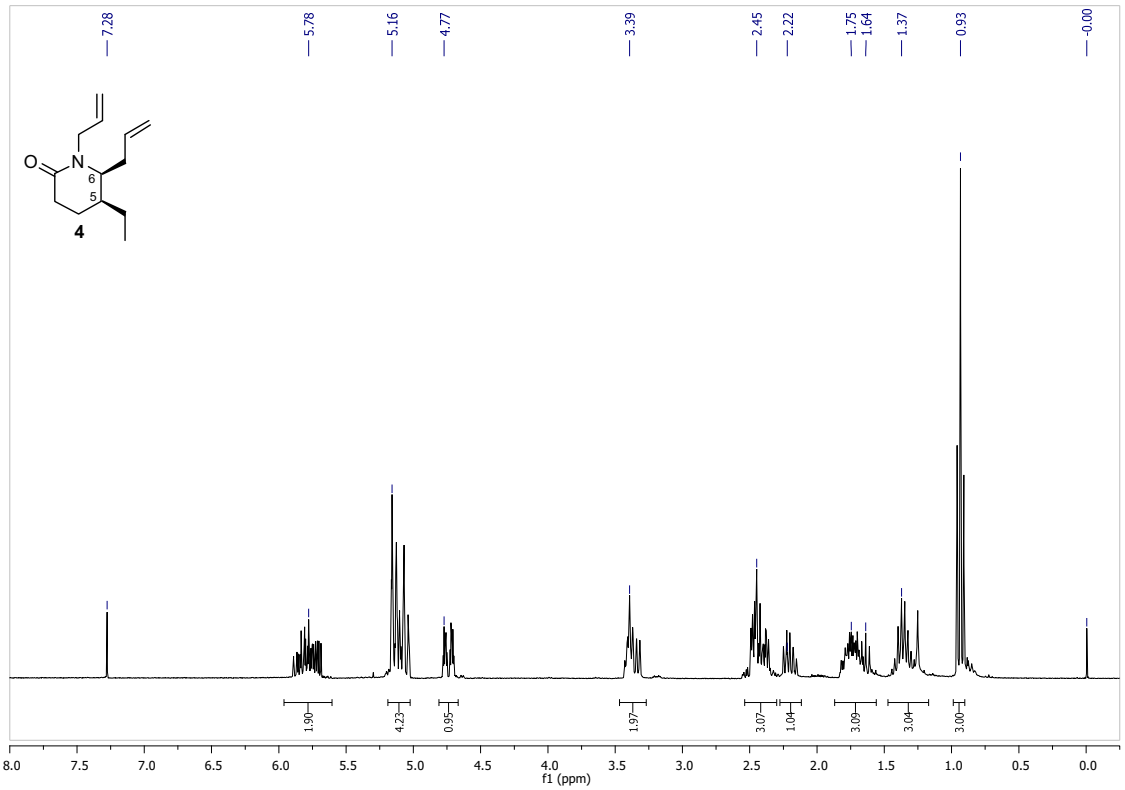


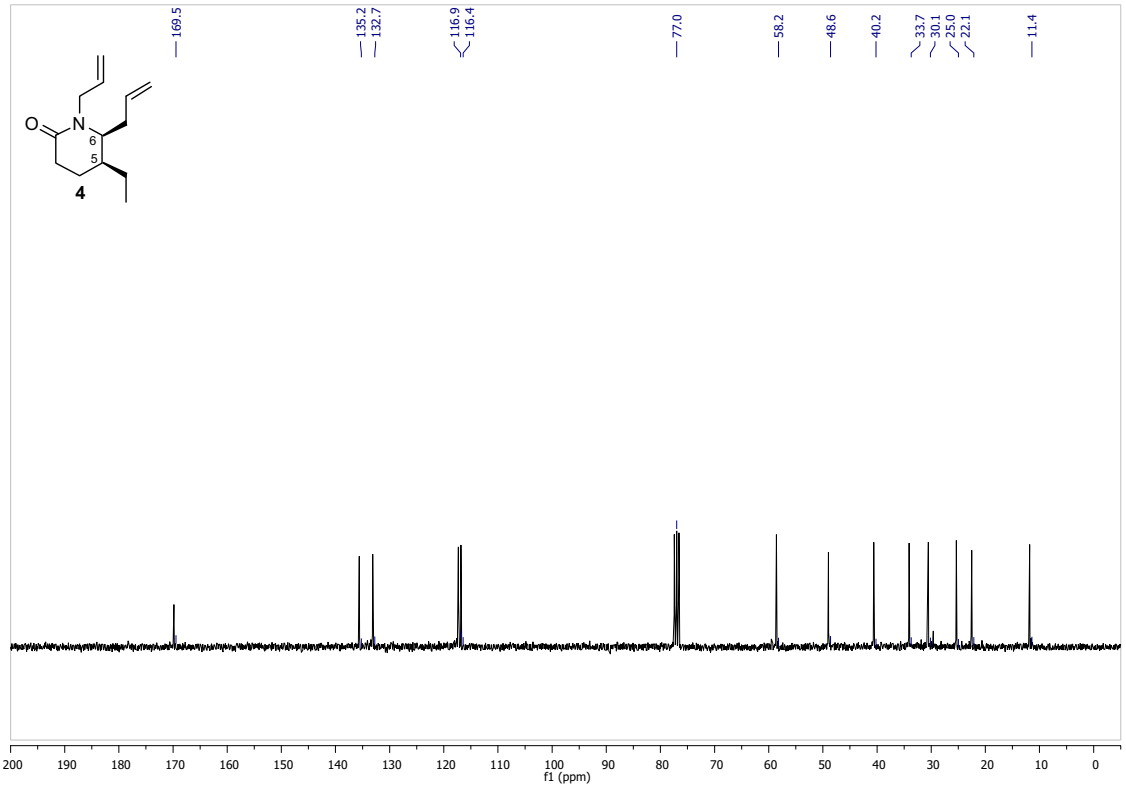
^{1}H NMR and ^{13}C NMR spectra of (5*S*,6*S*)-6-Allyl-5-ethyl-2-piperidone, (**3**).





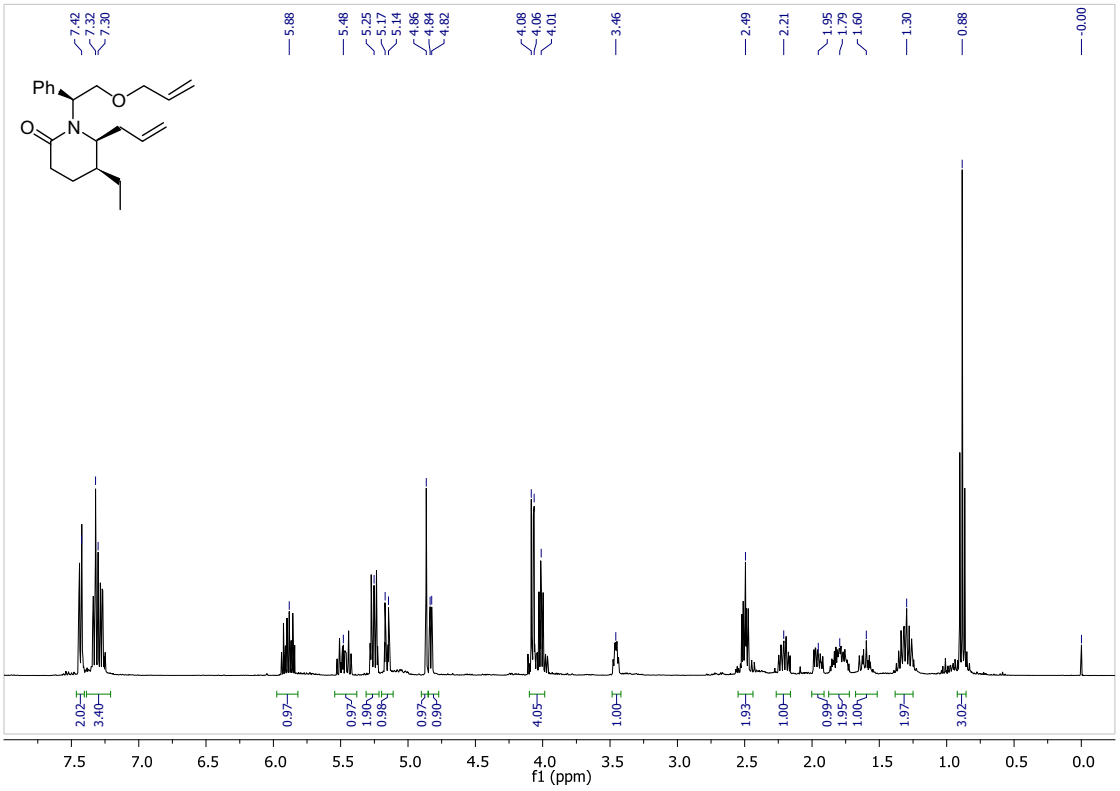
^{1}H NMR and ^{13}C NMR spectra of (5*S*,6*S*)-1,6-Diallyl-5-ethyl-2-piperidone, (**4**).

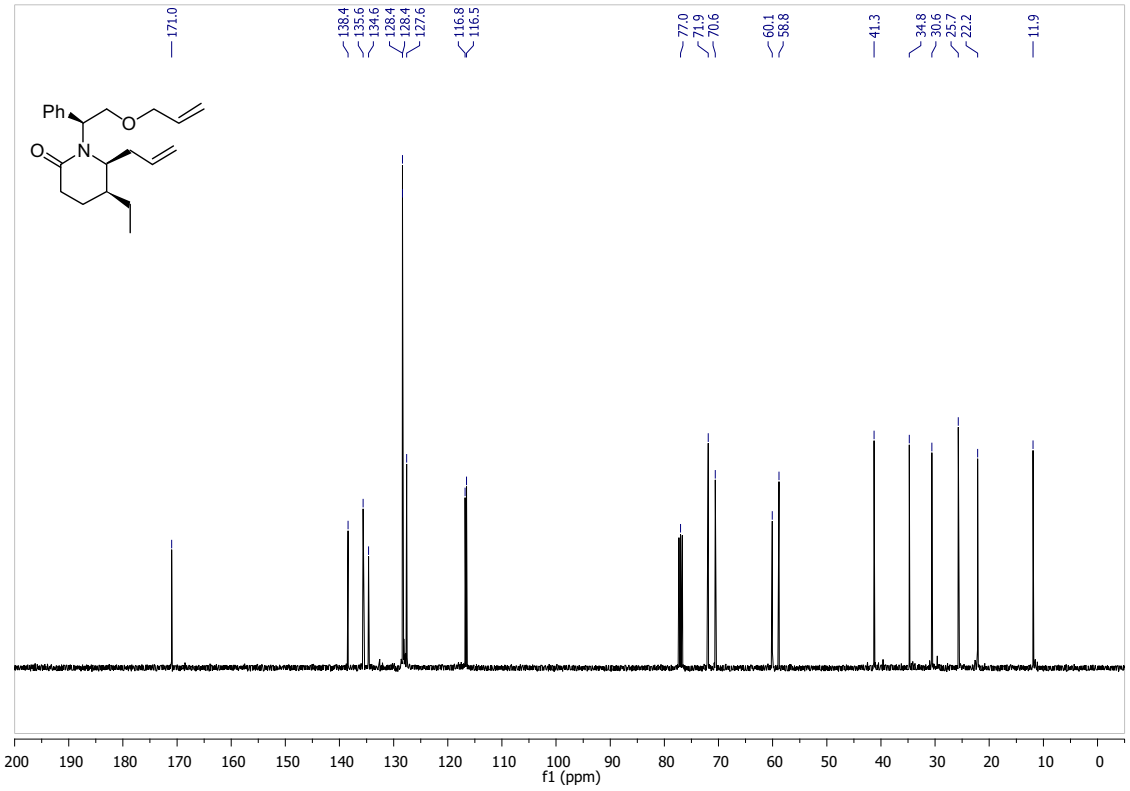




^{1}H NMR and ^{13}C NMR spectra of

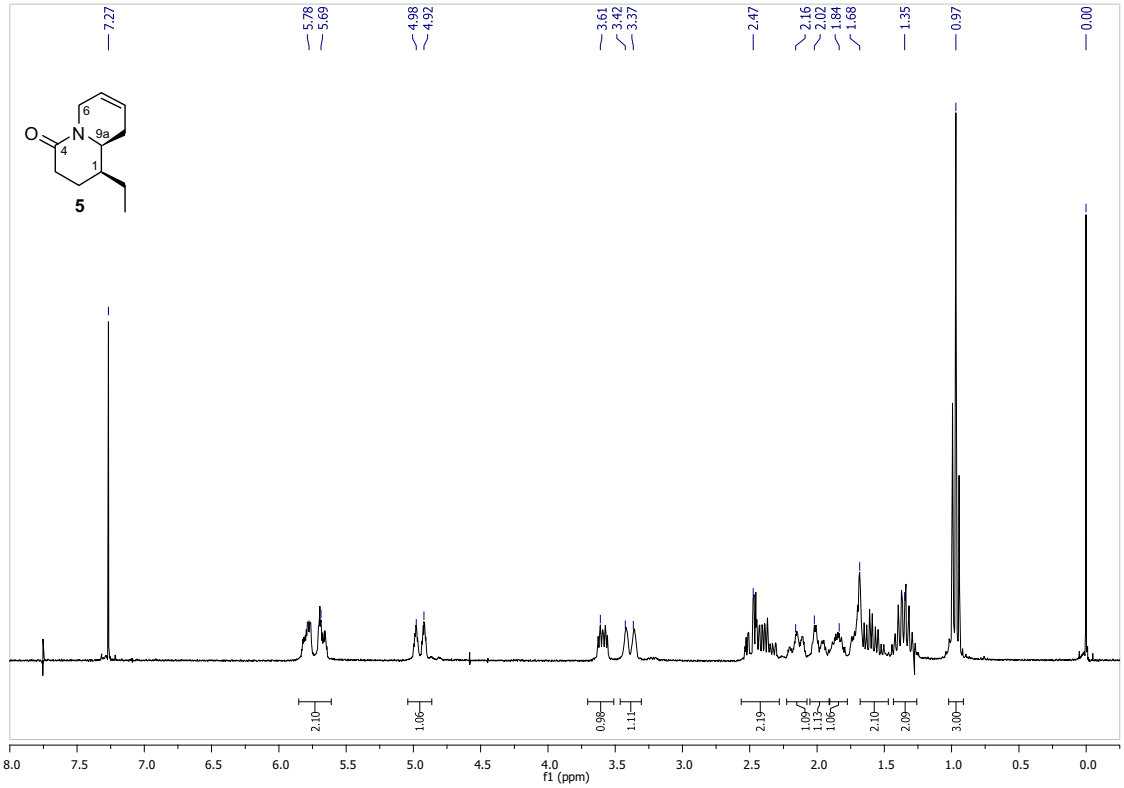
(5S,6S)-6-allyl-1-[(S)-2-(allyloxy)-1-phenylethyl)]-5-ethylpiperidin-2-one.

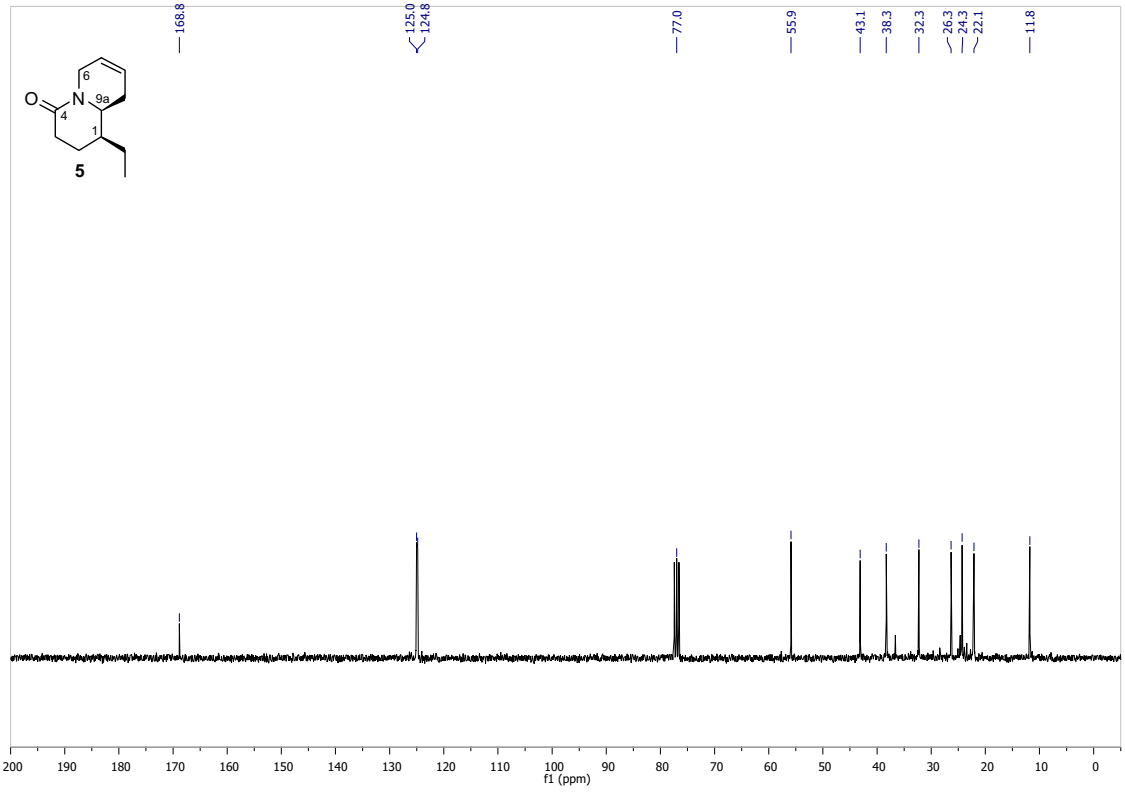




^{1}H NMR and ^{13}C NMR spectra of

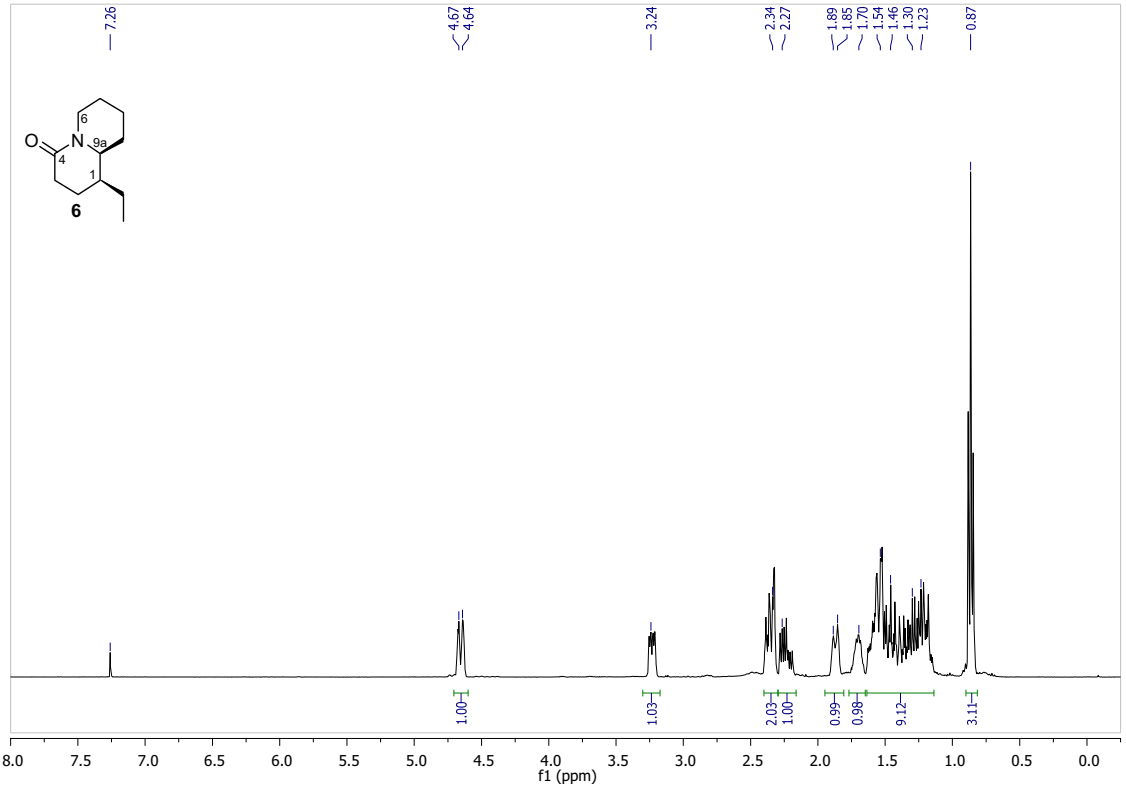
(1*S*,9a*S*)-1-Ethyl-4-oxo-1,2,3,6,9,9a-hexahydro-4*H*-quinolizine, (**5**).

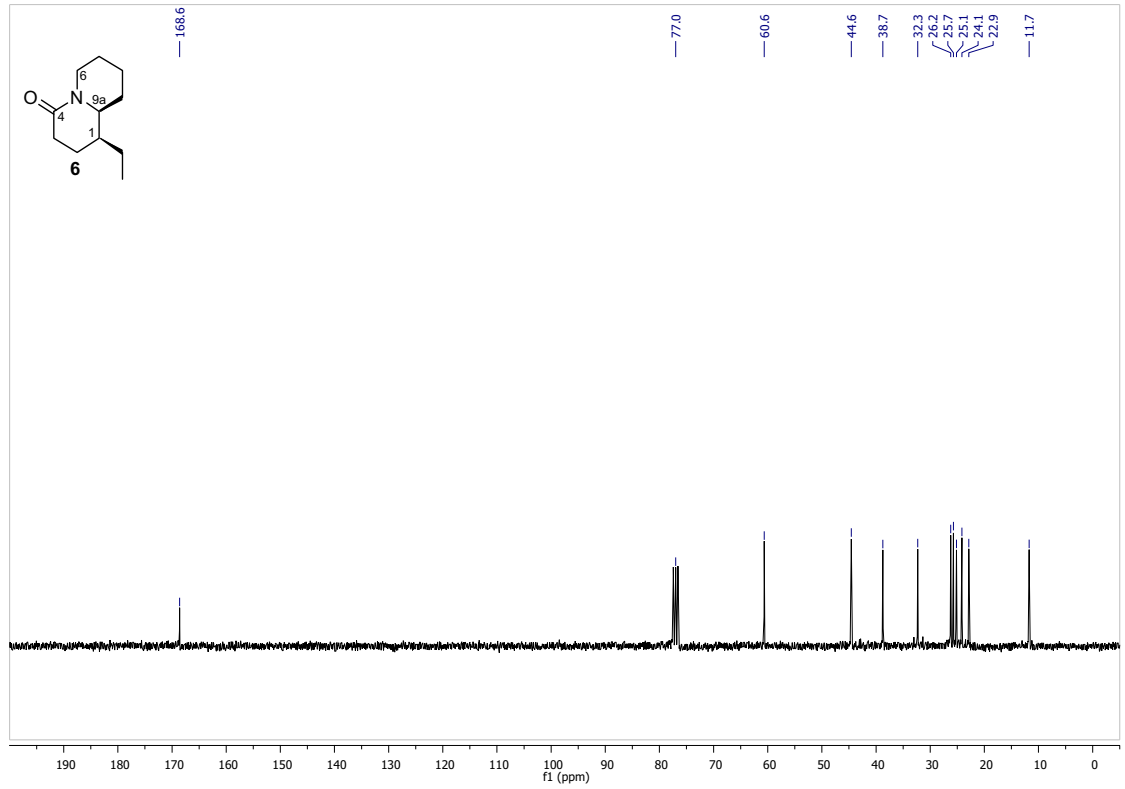




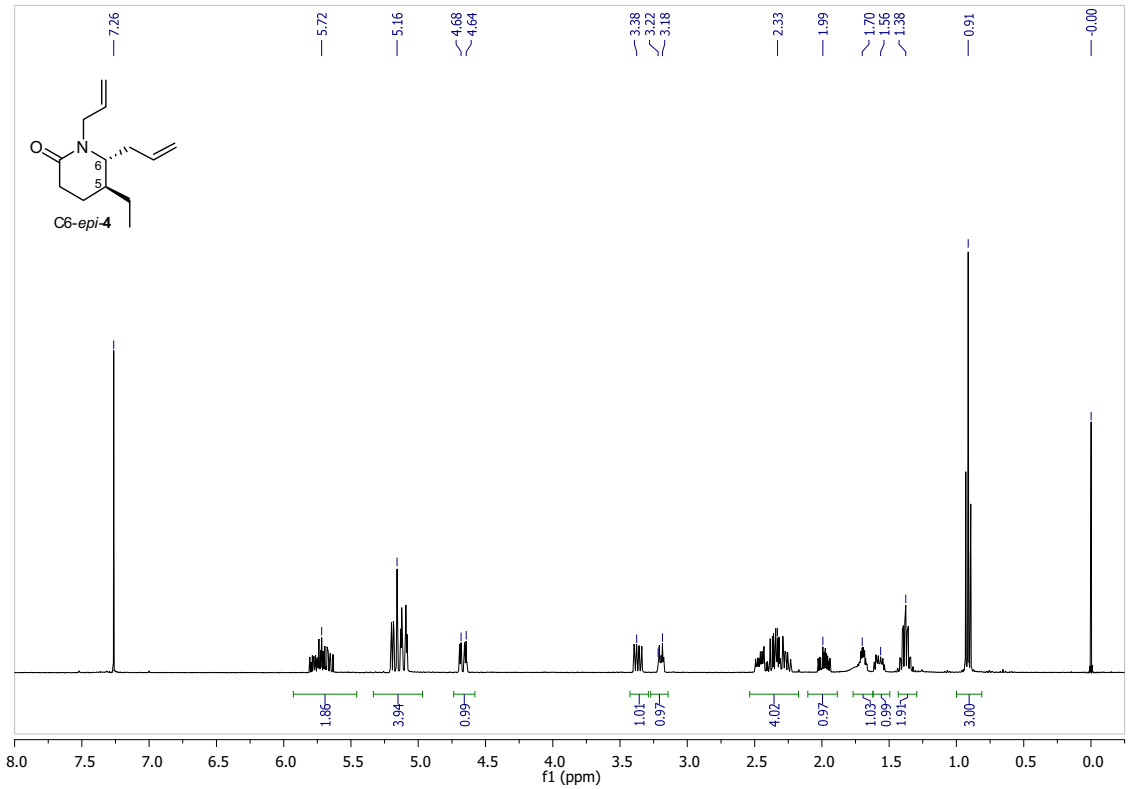
^{1}H NMR and ^{13}C NMR spectra of

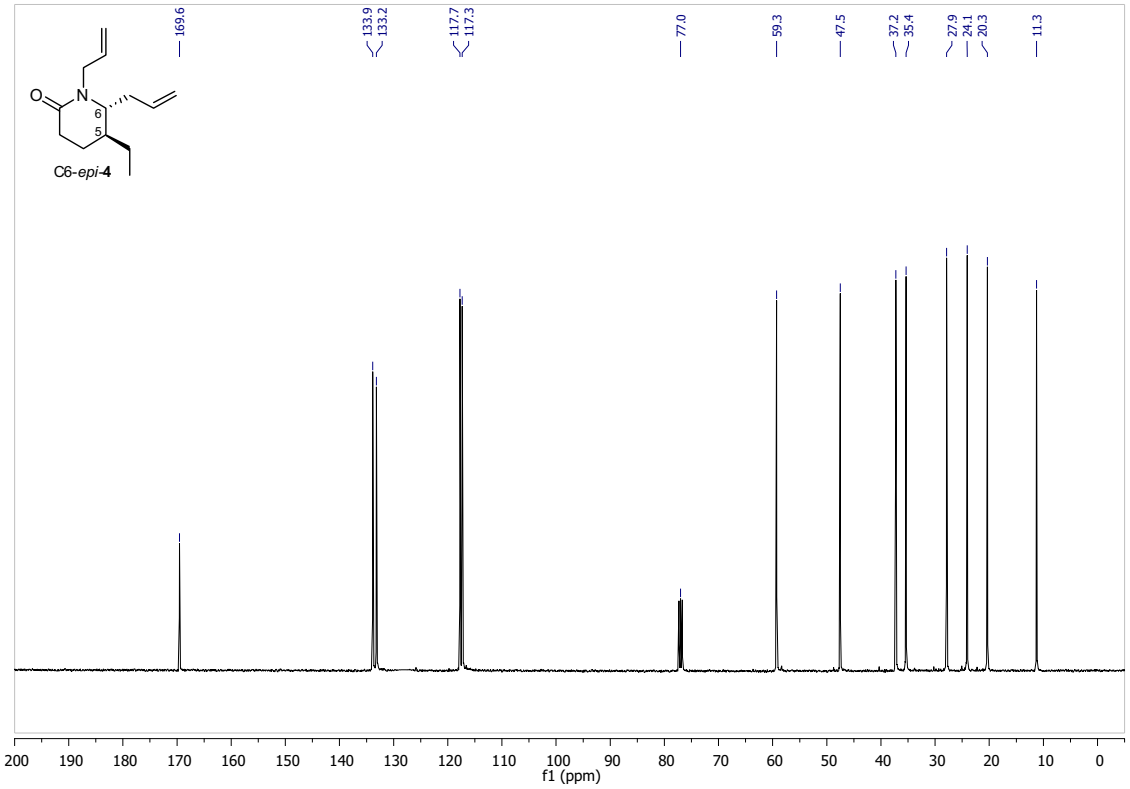
(1*S*,9a*S*)-1-Ethyl-4-oxo-1,2,3,6,7,8,9,9a-octahydro-4*H*-quinolizine, (**6**).





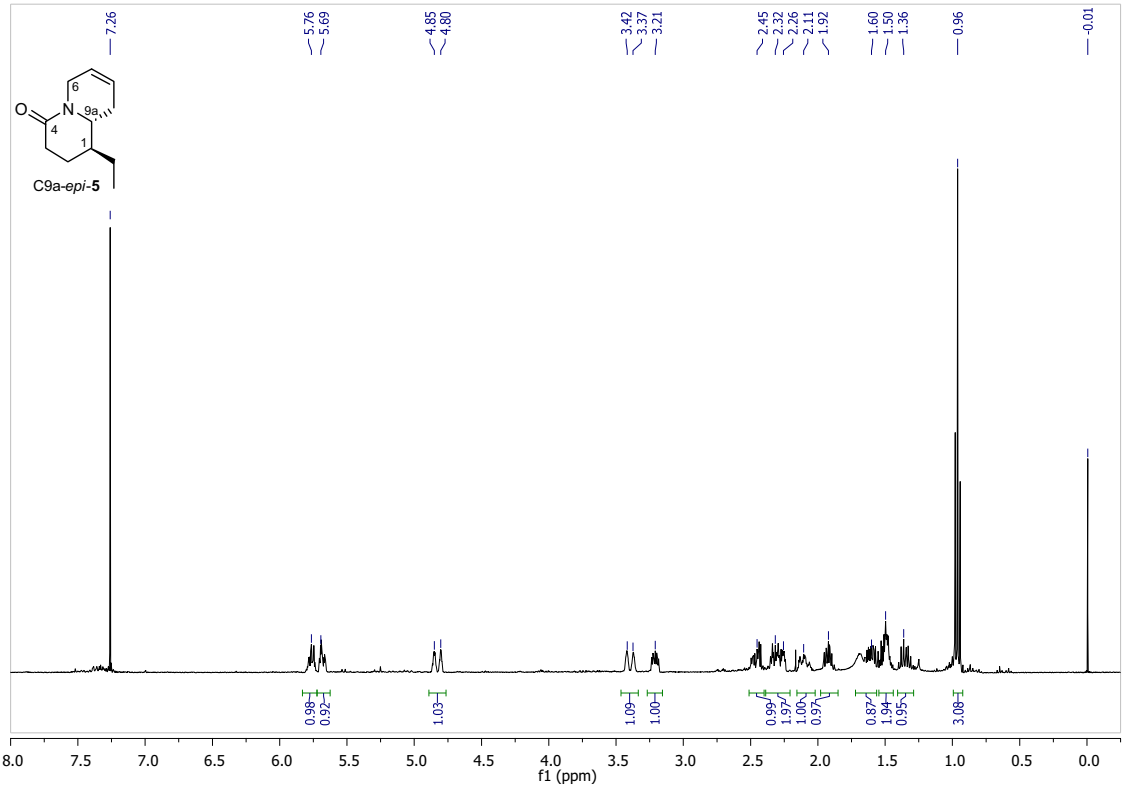
^{1}H NMR and ^{13}C NMR spectra of (5*S*,6*R*)-1,6-Diallyl-5-ethyl-2-piperidone, (6-*epi*-**4**).

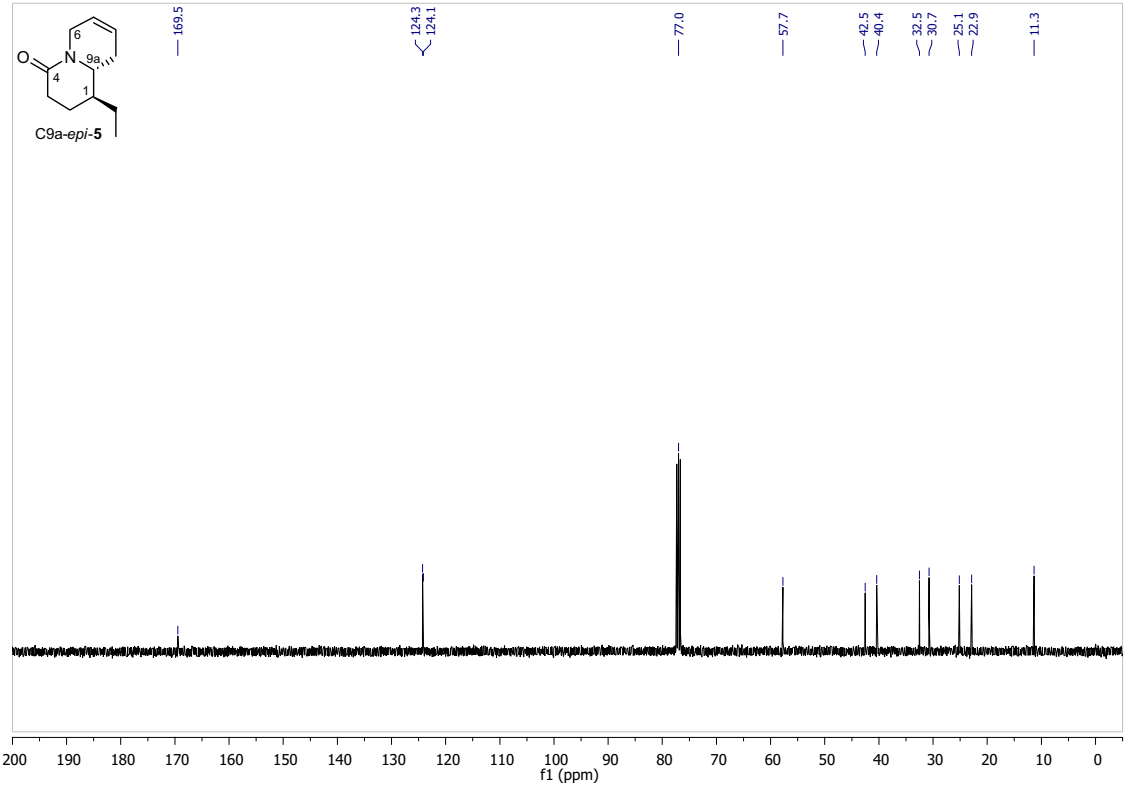




^{1}H NMR and ^{13}C NMR spectra of

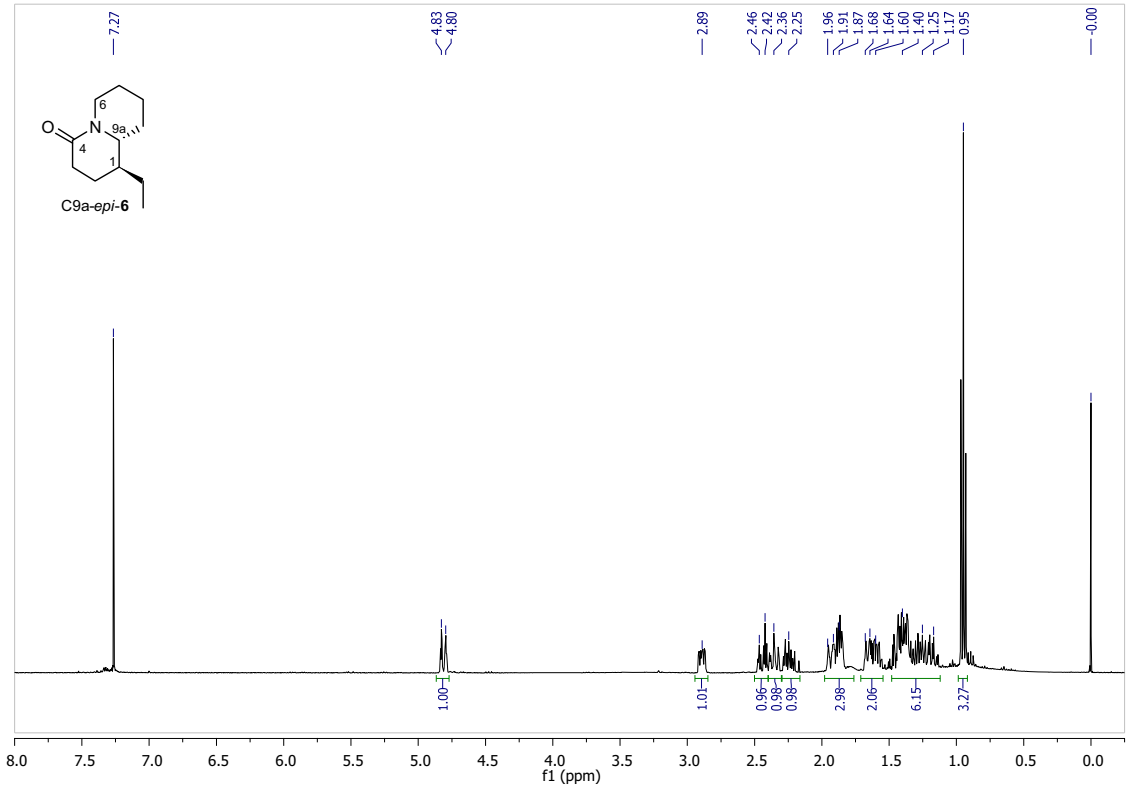
(1*S*,9a*R*)-1-Ethyl-4-oxo-1,2,3,6,9,9a-hexahydro-4*H*-quinolizine, (9a-*epi*-**5**).

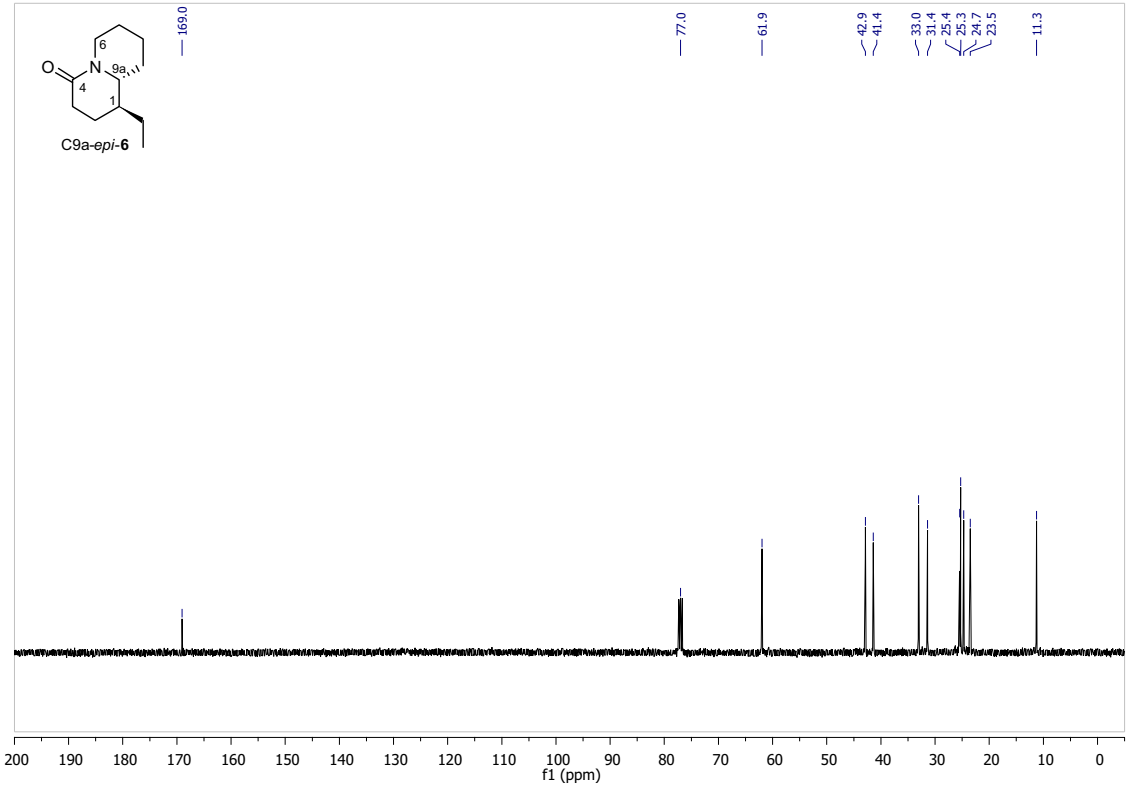




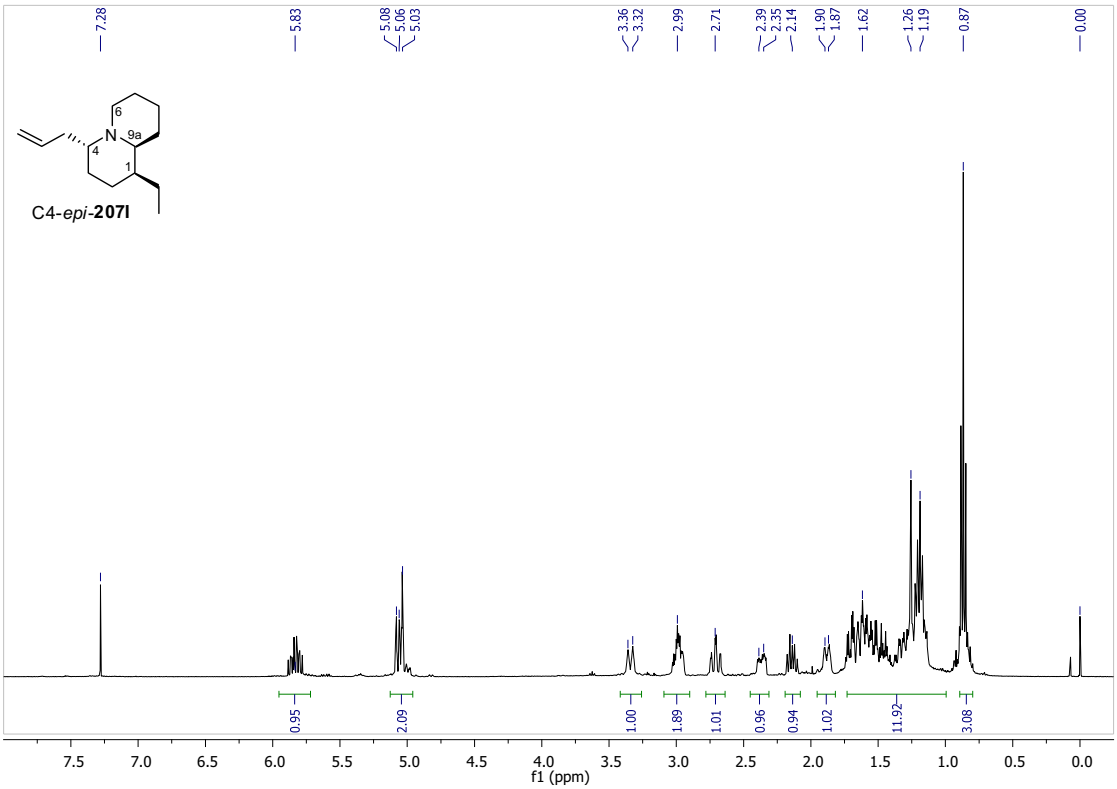
^{1}H NMR and ^{13}C NMR spectra of

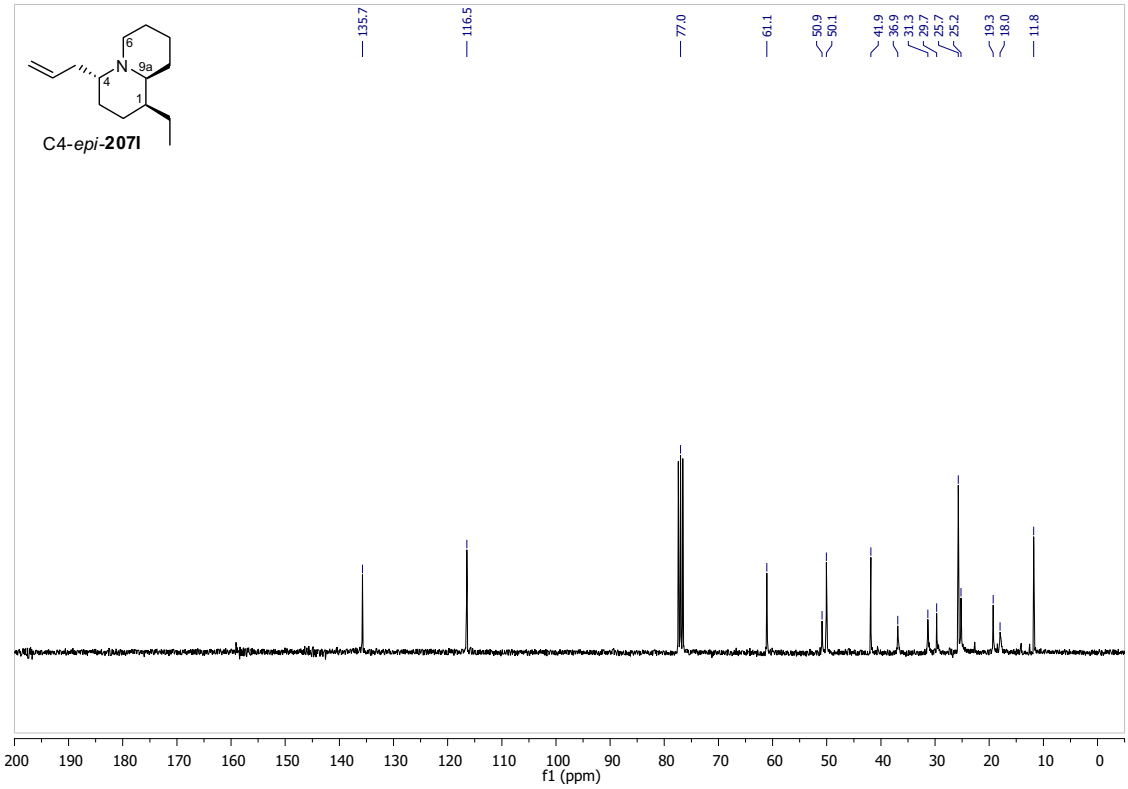
(1*S*,9a*R*)-1-Ethyl-4-oxo-1,2,3,6,7,8,9,9a-octahydro-4*H*-quinolizine, (9a-*epi*-**6**).





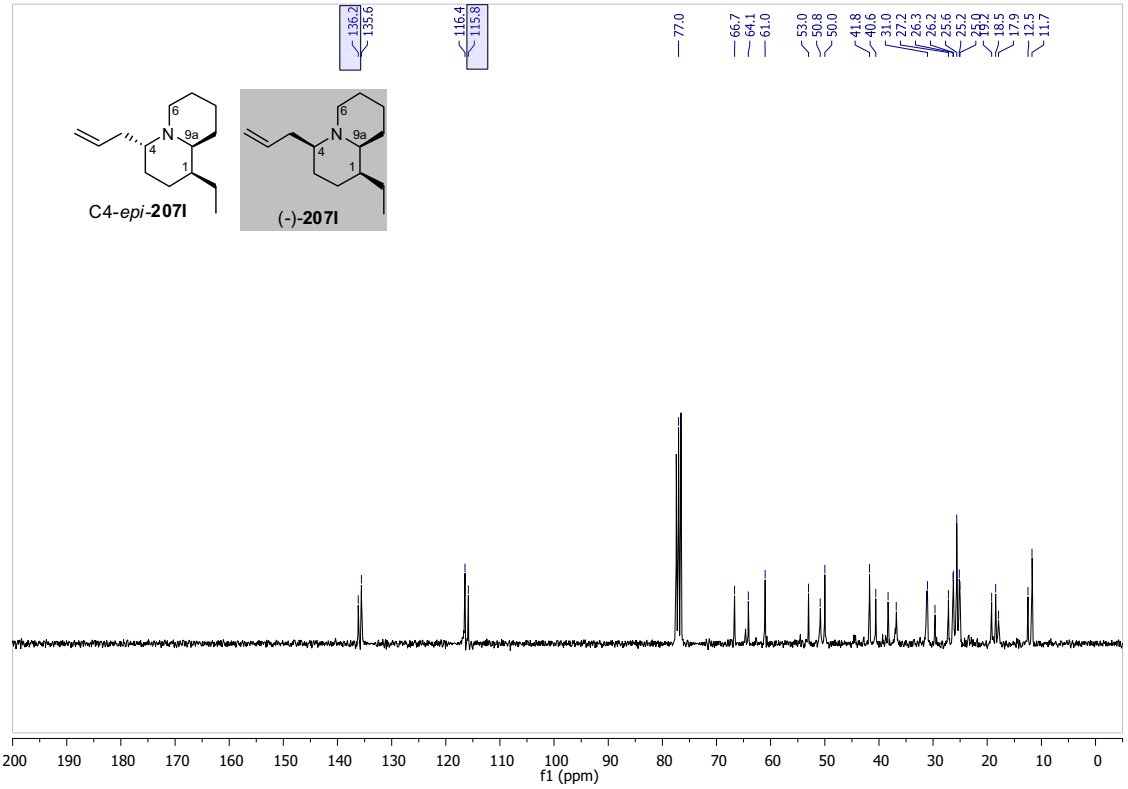
^{1}H NMR and ^{13}C NMR spectra of (1*S*,4*R*,9a*S*)-4-Allyl-1-ethylquinolizidine, (4-*epi*-**207l**).

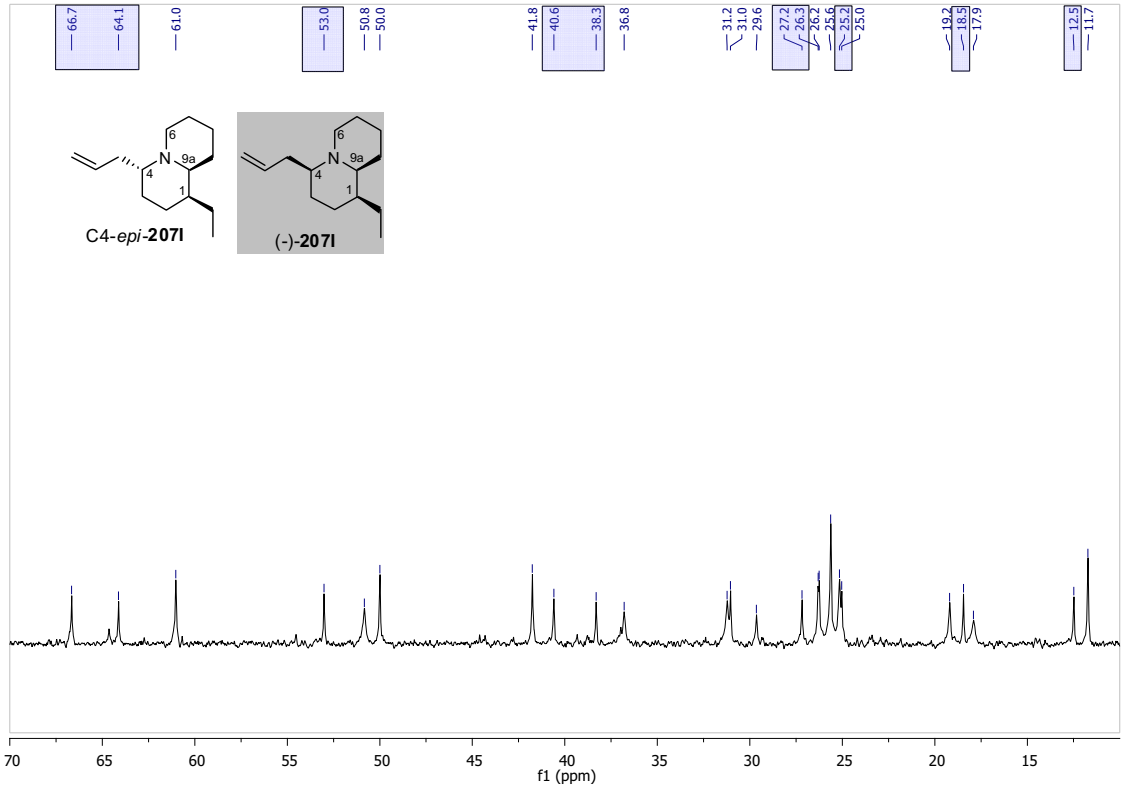




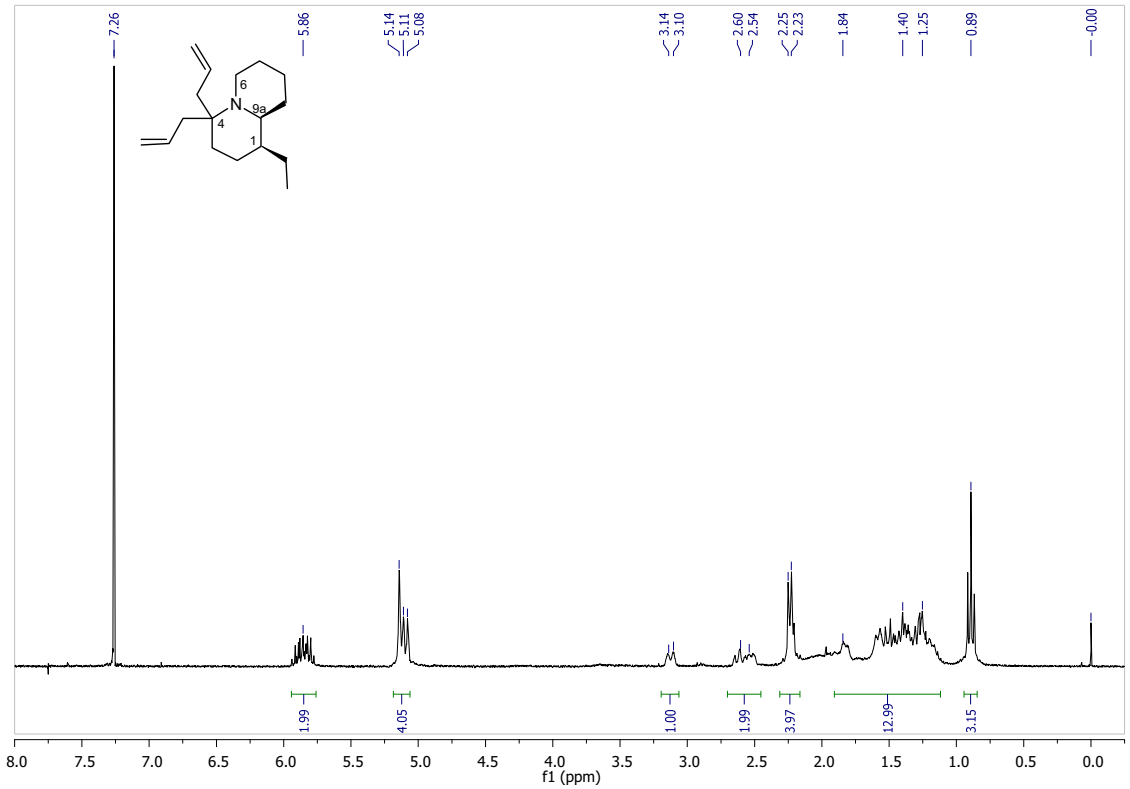
^{13}C NMR spectra of (1*S*,4*S*,9a*S*)-4-allyl-1-ethylquinolizidine, (-)-**207l**;

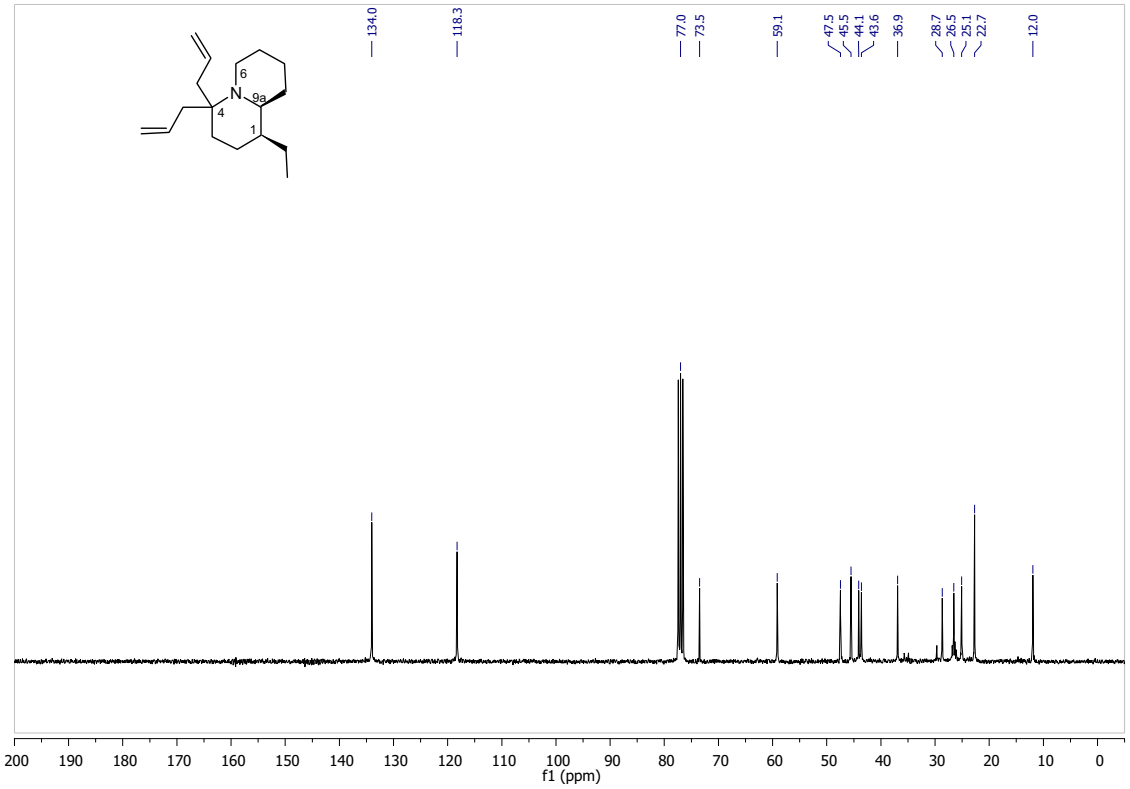
(mixture of 4-*epi*-**207l** and (-)-**207l**).





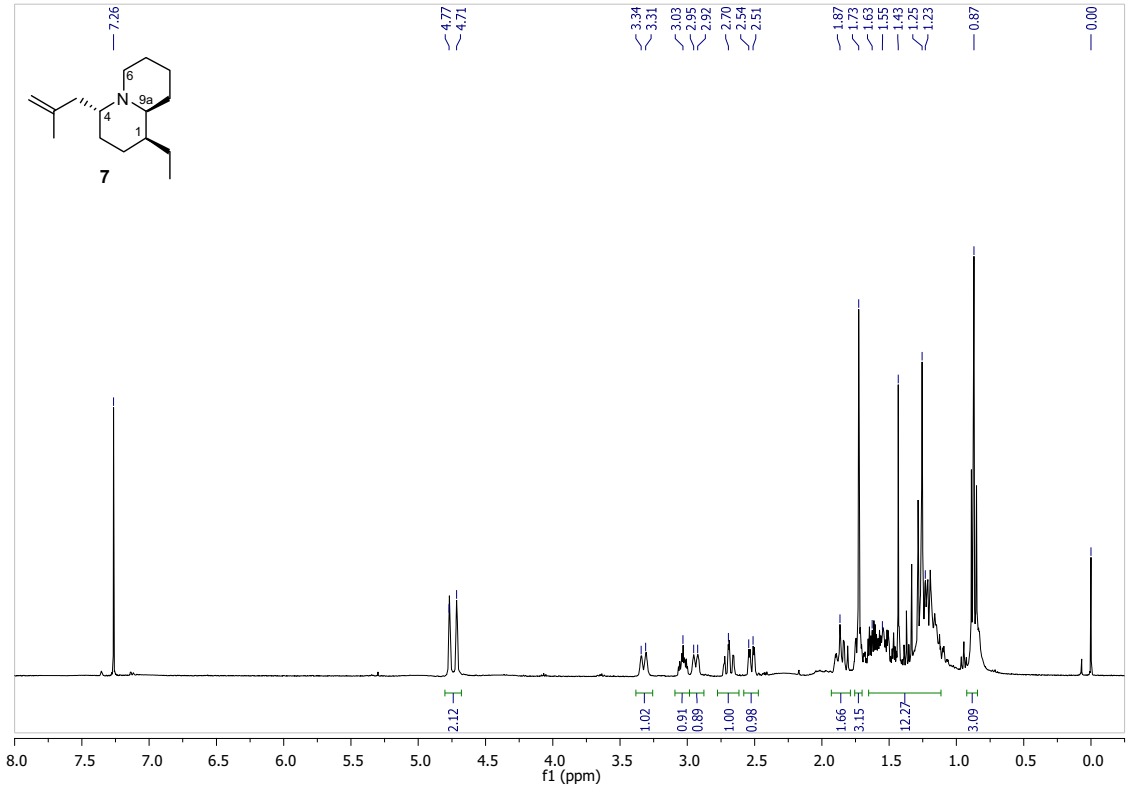
^{1}H NMR and ^{13}C NMR spectra of (1*S*,9a*S*)-4,4-diallyl-1-ethylquinolizidine.

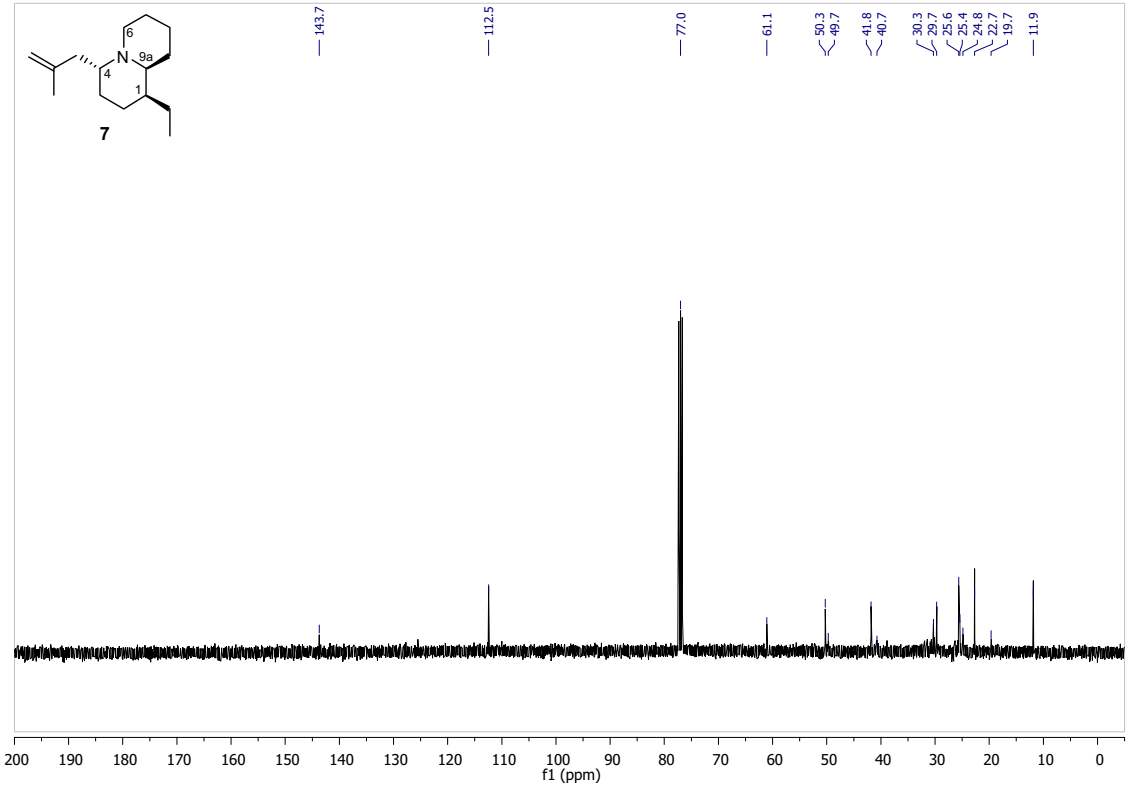




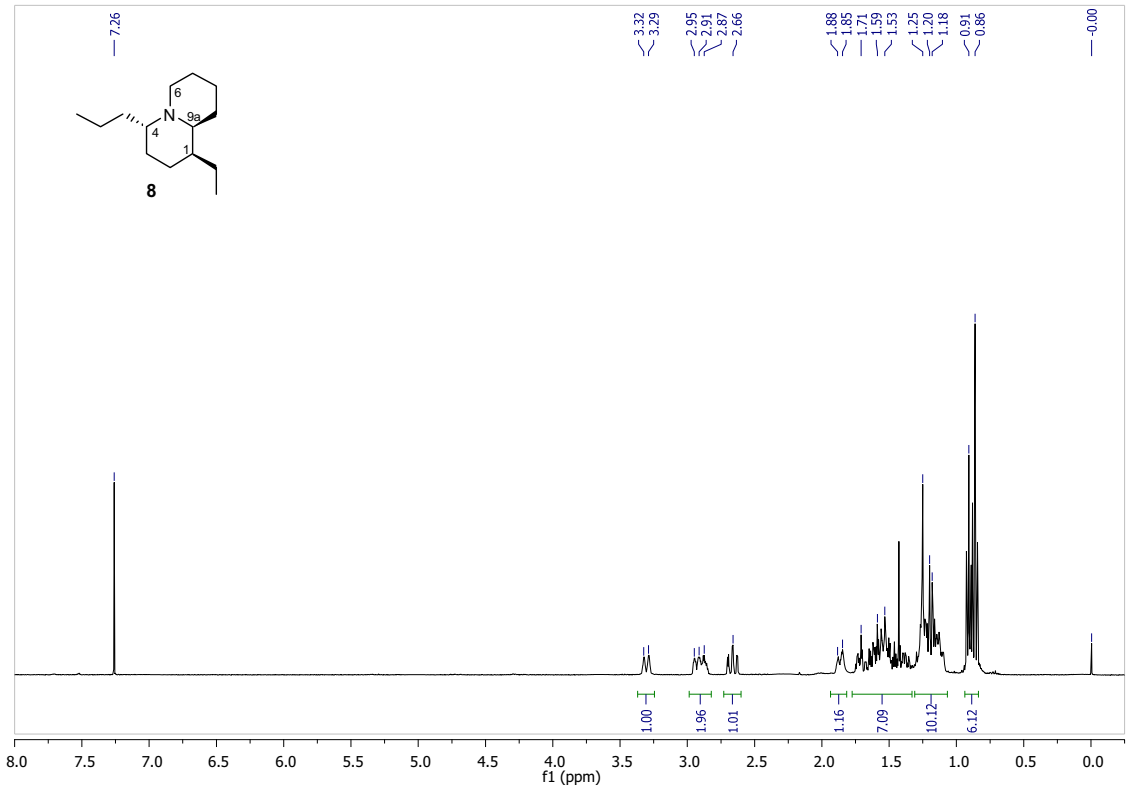
^{1}H NMR and ^{13}C NMR spectra of

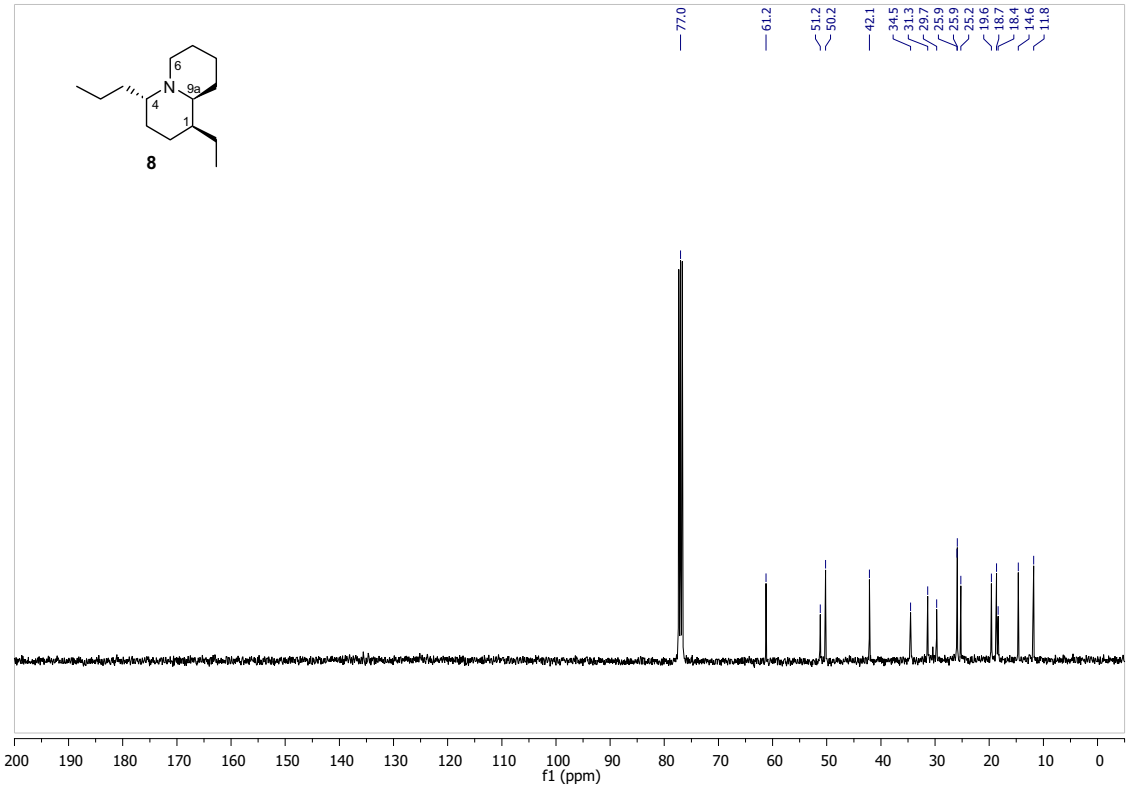
(1*S*,4*R*,9a*S*)-1-Ethyl-4-(2-methylallyl)quinolizidine, (**7**).



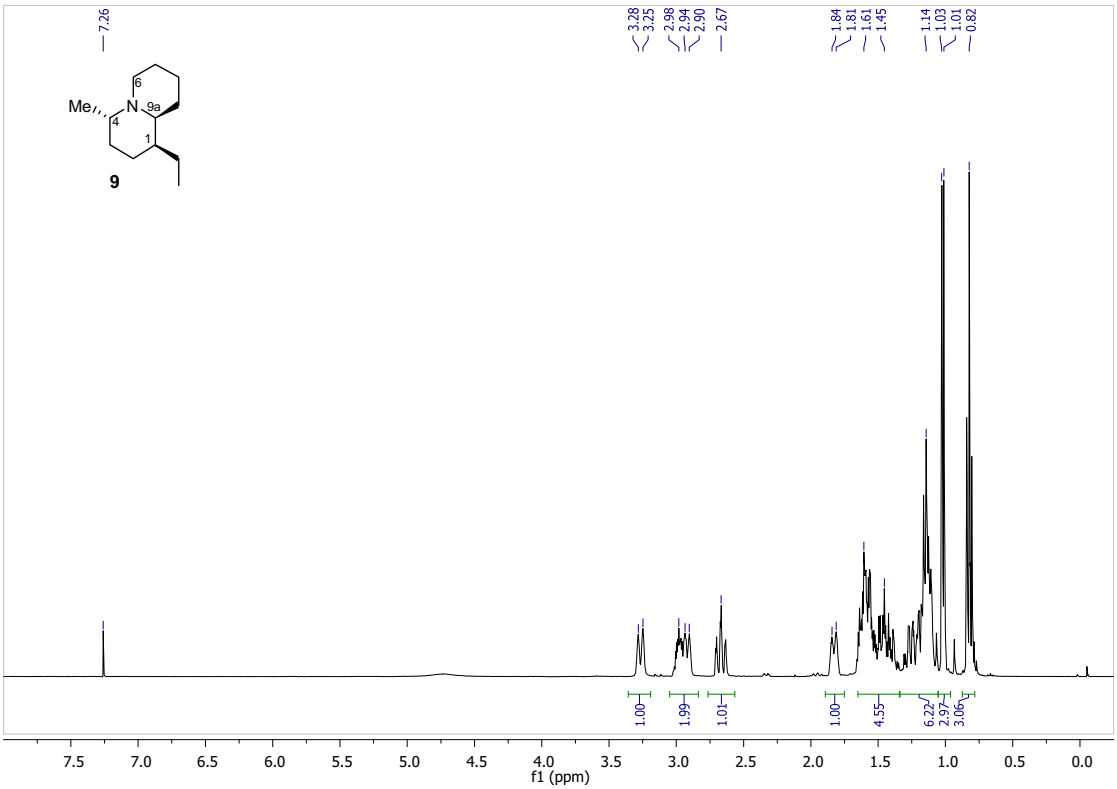


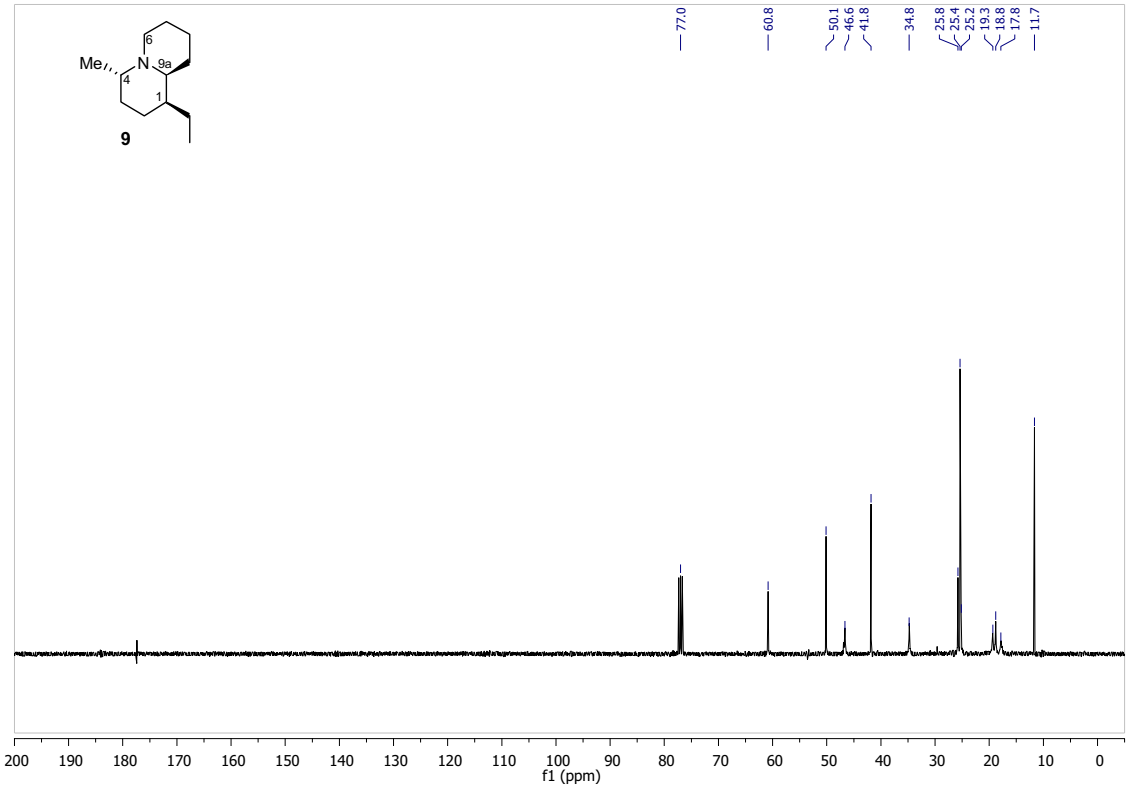
^{1}H NMR and ^{13}C NMR (50 °C) spectra of (1*S*,4*S*,9a*S*)-1-Ethyl-4-propylquinolizidine, (**8**).



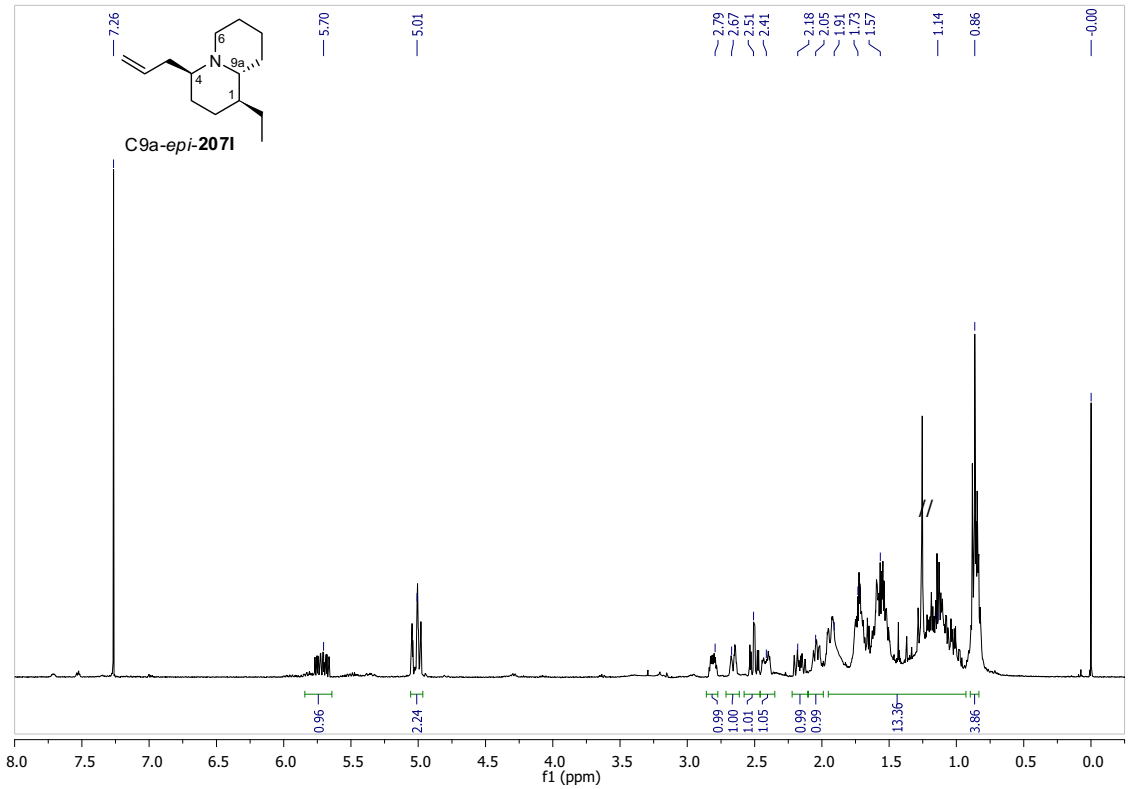


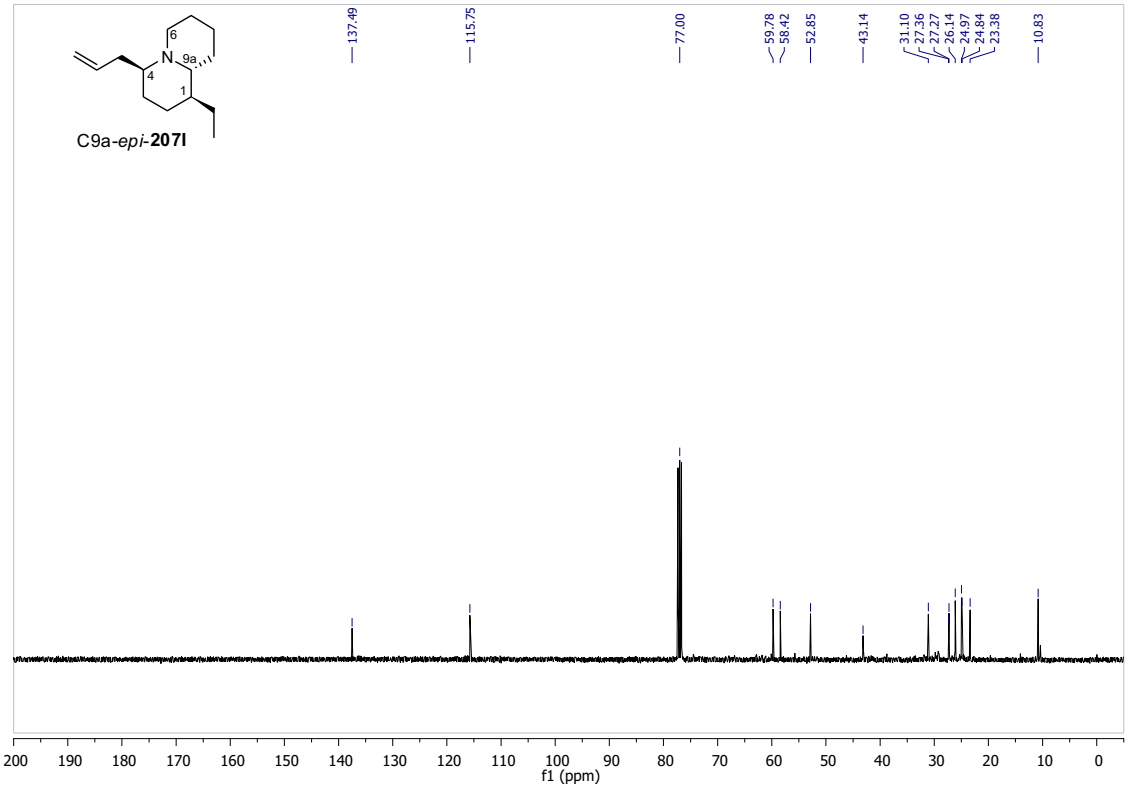
^{1}H NMR and ^{13}C NMR spectra of (1*S*,4*S*,9a*S*)-1-Ethyl-4-methylquinolizidine, (**9**).





^{1}H NMR and ^{13}C NMR spectra of (1*S*,4*S*,9a*R*)-4-allyl-1-ethylquinolizidine, (9a-*epi*-**207l**).





NOTE: NOE experiments upon compound 9a-*epi*-**207l** were carried out to confirm that irradiation of peak at 2.81 ppm assigned to H-4 did not have any interaction with H-9a, indicating that both protons are in different faces of the bicyclic system.

5 Copies of the Infrared Spectra of the selected 1S-Ethyl-4-Substituted Quinolizidines.

Absence of Bohlmann band (2800-2700 cm^{-1} region) in the prepared C4-subtitued quinolizidines (4-*epi*-**207I**, 9a-*epi*-**207I**, **7**, **8**, **9**) indicate relative *trans* H_4-H_{9a} configuration.[2] In contrast, in (-)-**207I** significant Bohlamn bands shows that hydrogen at C-4 and C-9a are *cis*.[3] The bands in the region ≥ 2850 cm^{-1} are assigned to the normal CH_2 symmetric vibrations, common to both H_4-H_{9a} *cis*/*trans* ring fusion.

Table 6 Significant IR data (cm^{-1}) in Bohlmann region for quinolizidines.

Entry	(-)-207I*		4-*epi*-**207I**	9a-*epi*-**207I**	**7**	**8**	**9**
1	2941	(2929)	2957	2958	2956	2958	2959
2	2880	(2862)	2928	2928	2926	2928	2928
3	2789	(2786)	2856	2856	2855	2858	2858
4		(2757)					

* According to ref. 3a. Values in brackets are according to ref. 3b.

[2] T. A. Crabb, R. F. Newton and D. Jackson, *Chem. Rev.*, 1971, **71**, 109-126.
[3] (a) N. Toyooka, K. Tanaka, T. Momose, J. W. Daly and H. M. Garraffo, *Tetrahedron*, 1997, **53**, 9553-9574.
(b) N. Toyooka and H. Nemoto, *Tetrahedron Lett*., 2003, **44**, 569-570.

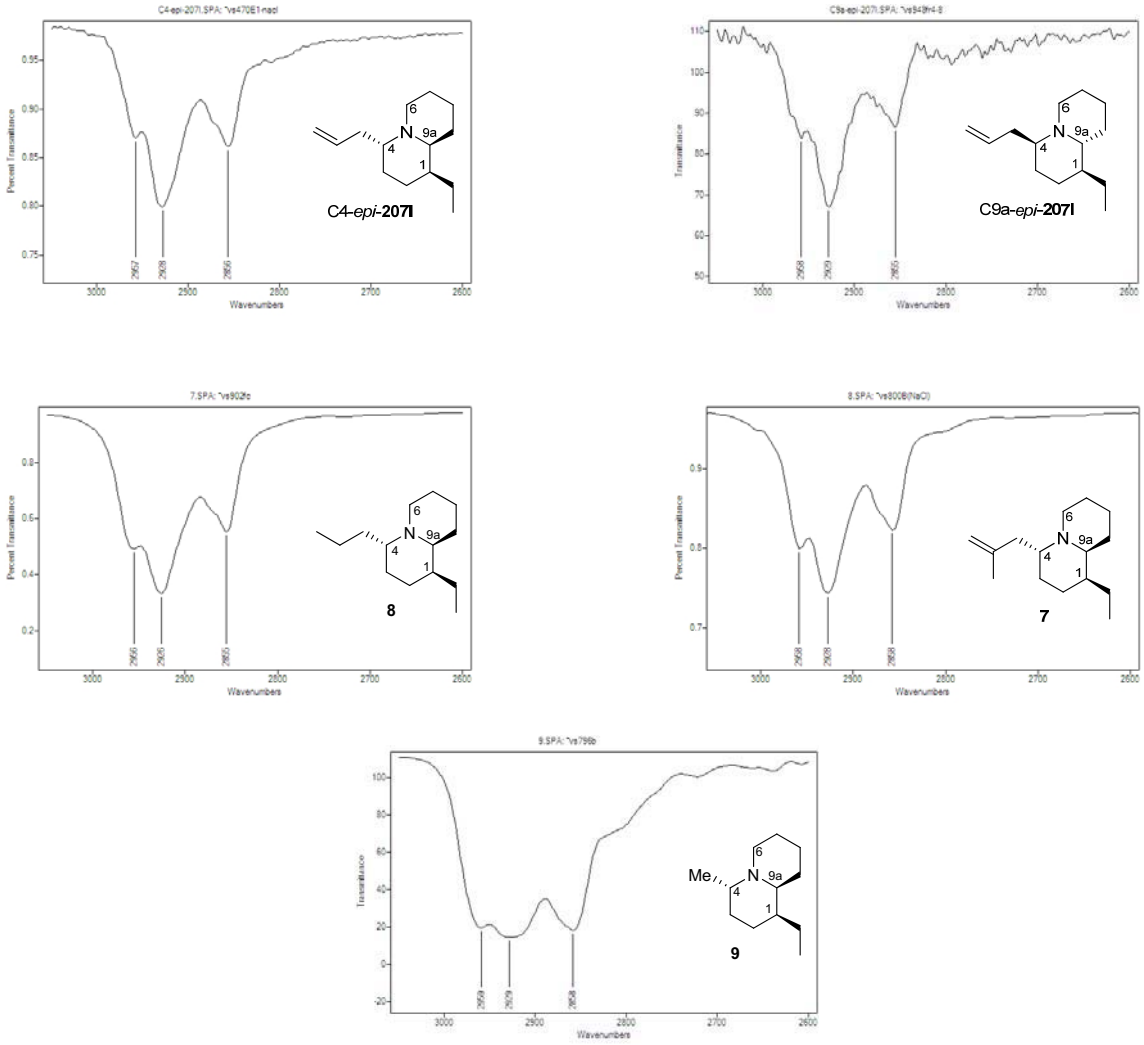
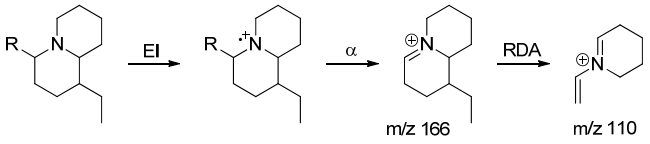


Figure 1 FTIR spectra (region 3050-2600 cm^{-1}) of 1*S*-Ethyl-4-Substituted Quinolizidines: 4-*epi*-**207I**, 9a-*epi*-**207I**, **7**, **8**, **9**.

6 Copies of the Mass Spectra of Quinolizidines.

Mass spectrometry is a useful tool that provides molecular structural information for the characterization of natural products.[4] In the case of the prepared 1,4-disubstituted quinolizidines, the same fragmentation pattern was observed in the EI-MS. A fragment from the α cleavage at the C-C bond next to the nitrogen (*m*/*z* 166) was the major peak. The second most abundant peak *m*/*z* 110 arose from a Retro Diels-Alder (RDA) reaction (Scheme 2).



Scheme 2 Proposed fragmentation pathway for 4-substituted 2-ethylquinolizidines.

[4] H. M. Garraffo, T. F. Spande, T. H. Jones and J. W. Daly, *Rapid Commun. Mass Spectrom.*, 1999, **13**, 1553-1563.

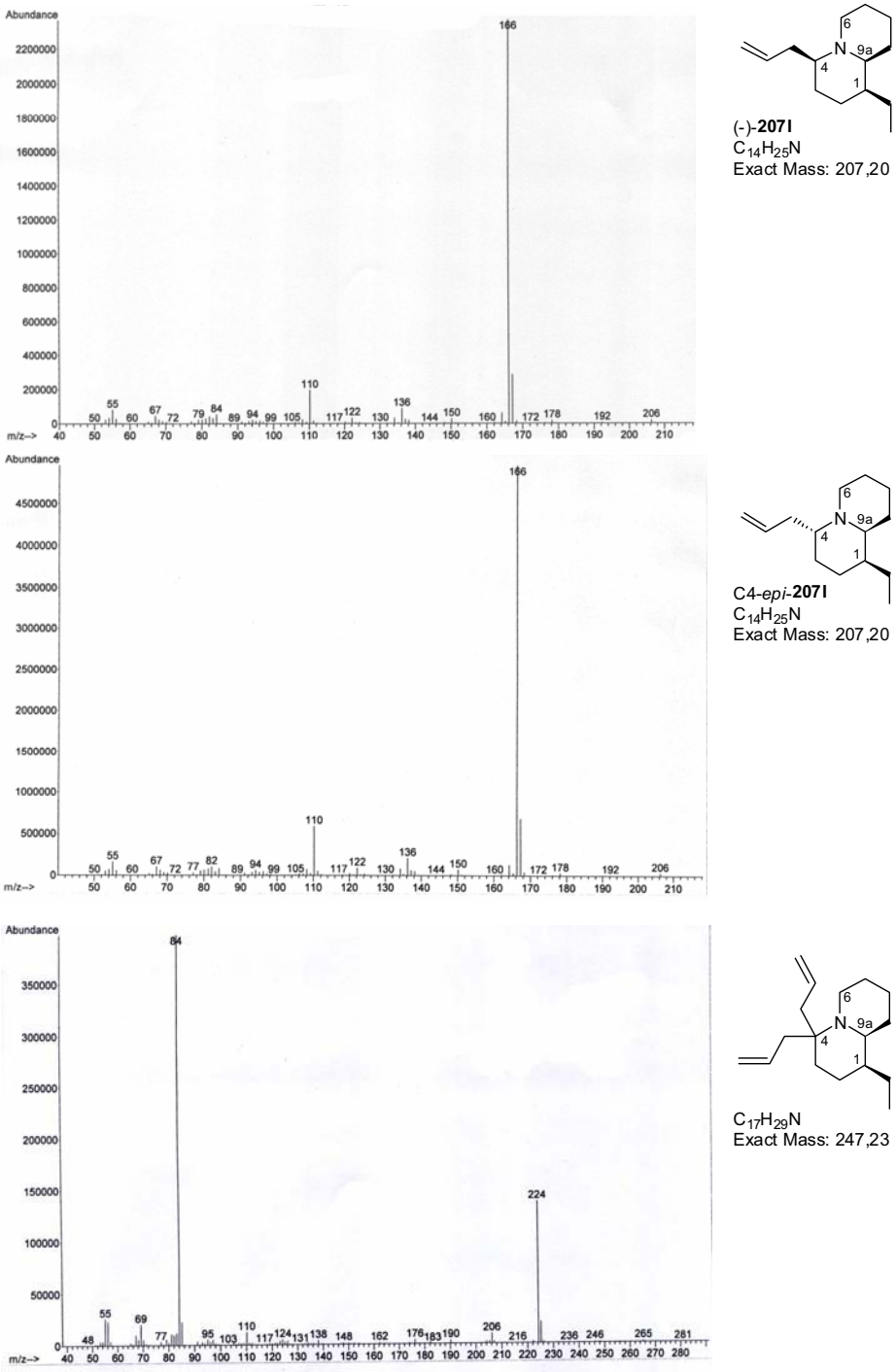


Figure 2 Mass spectra of Quinolizidines: (-)-**207I**, 4-*epi*-**207I** and (1*S*,9a*S*)-4,4-diallyl-1-ethylquinolizidine.

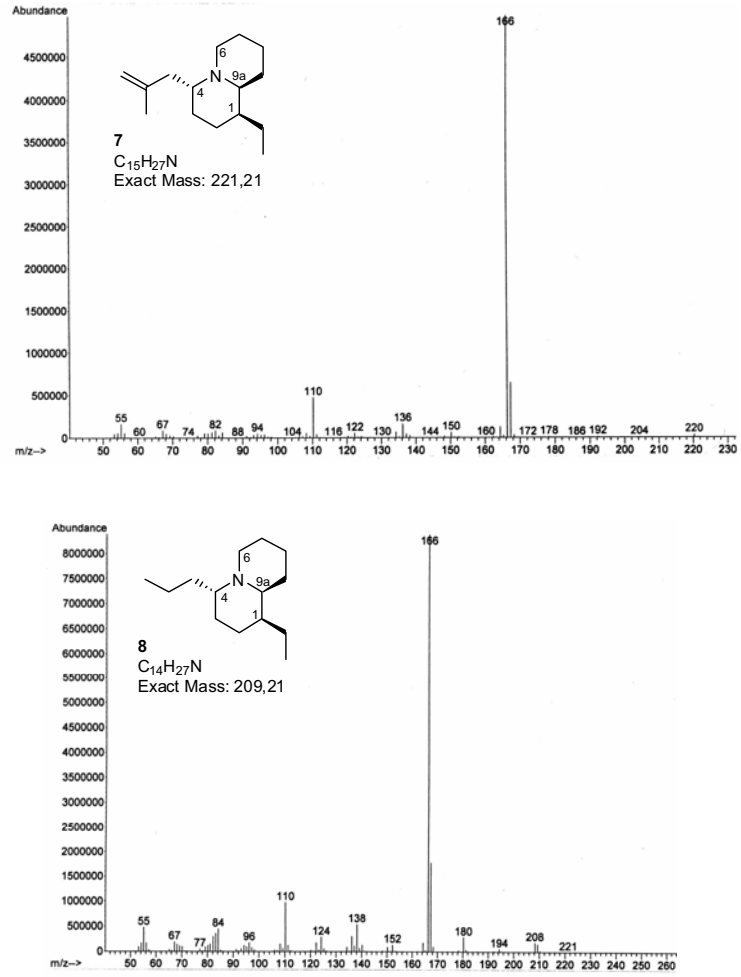


Figure 3 Mass spectra of Quinolizidines: **7** and **8**.

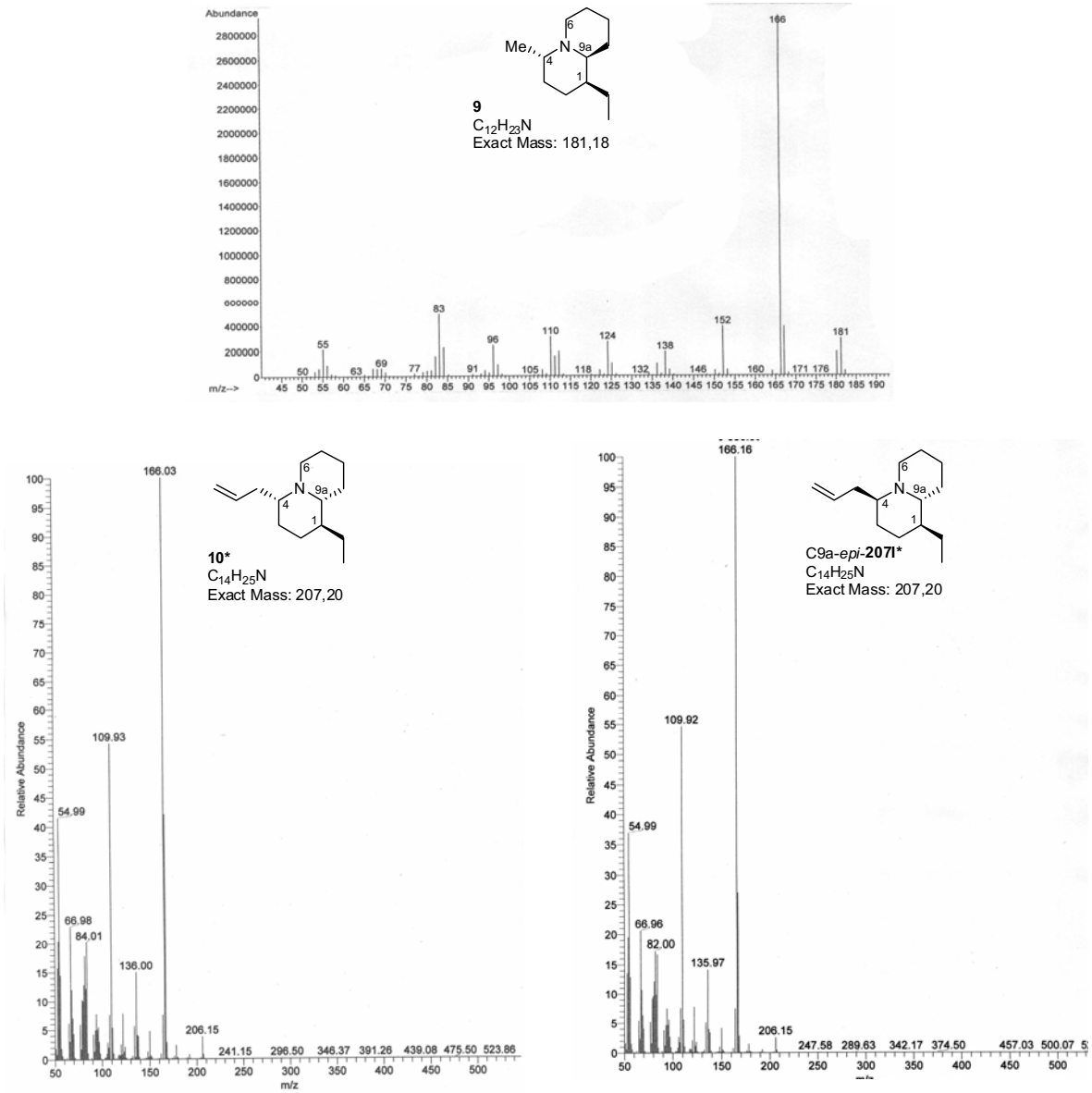


Figure 4 Mass spectra of quinolizidines: **9**, **10**, C9a-*epi*-**207l**.

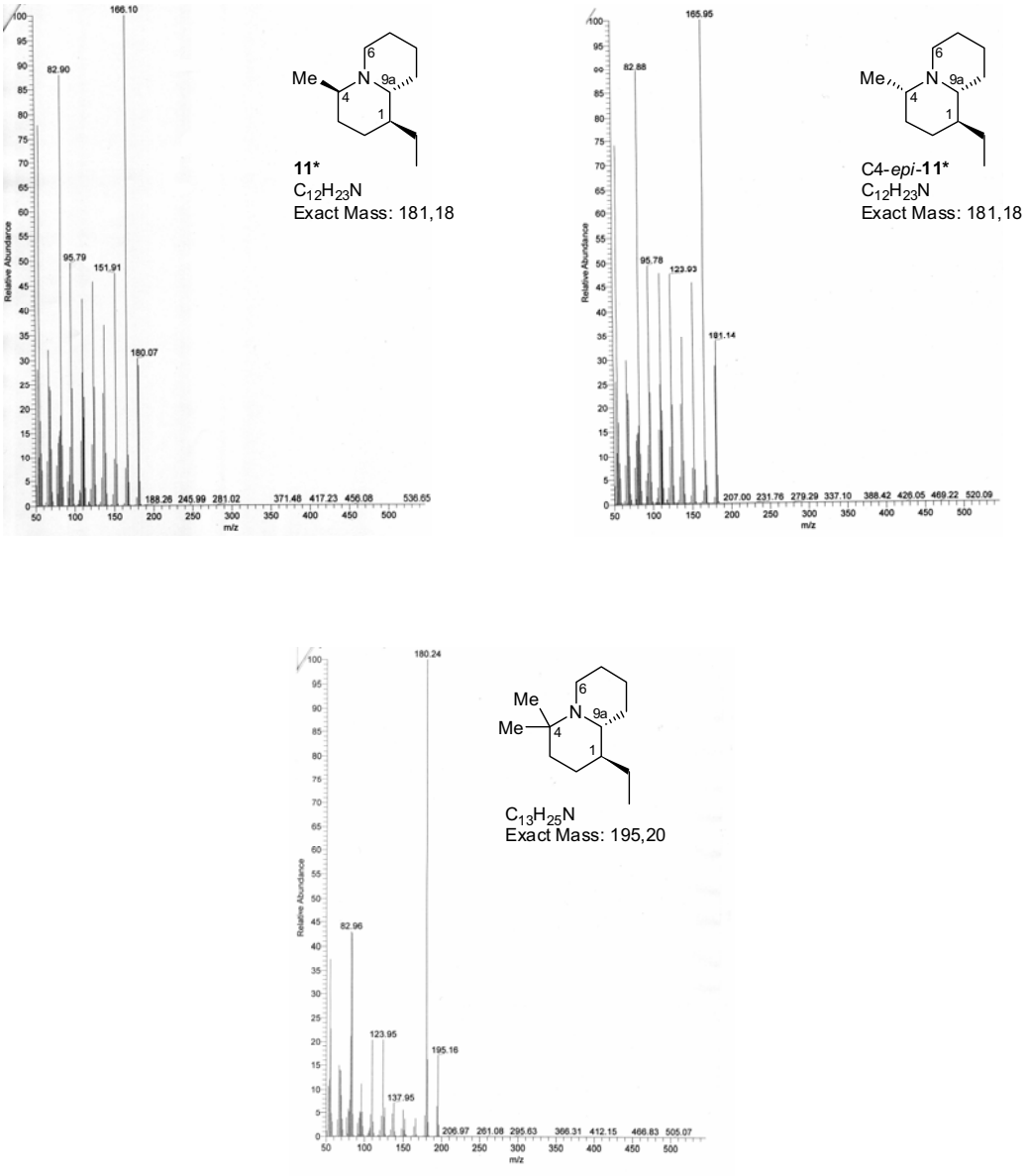
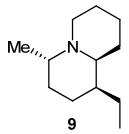


Figure 5 Mass spectra of quinolizidines: **11**, 9a-*epi*-**11** and dimethylated quinolizidine.

7 Computational details

(1*S*,4*S*,9a*S*)-1-Ethyl-4-methylquinolizidine, (9)



9

B3LYP/6-31G(d): -525.886909309 hartrees

Imaginary Frequencies: none found

Zero-point correction= 0.339034 (Hartree/Particle)

Thermal correction to Energy= 0.351868

Thermal correction to Enthalpy= 0.352813

Thermal correction to Gibbs Free Energy= 0.301077

Sum of electronic and zero-point Energies= -525.547875

Sum of electronic and thermal Energies= -525.535040

Sum of electronic and thermal Enthalpies= -525.534096

Sum of electronic and thermal Free Energies= -525.585831

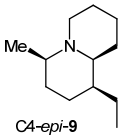
Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-0.126166	-1.862305	0.597229
2	6	0	-1.570142	-2.367846	0.544398
3	6	0	-2.277537	-1.720897	-0.648073
4	6	0	-2.193351	-0.201070	-0.520702
5	7	0	-0.811754	0.284863	-0.450215
6	6	0	-0.017488	-0.325973	0.634713
7	6	0	-0.765648	1.765380	-0.499075
8	6	0	1.468025	0.133441	0.562935
9	6	0	0.693532	2.243402	-0.568980
10	6	0	1.525501	1.671287	0.582968

11	6	0	2.254399	-0.440920	-0.649980
12	6	0	3.186418	-1.609845	-0.303393
13	6	0	-1.541951	2.493775	0.624278
14	1	0	0.396557	-2.237612	-0.289925
15	1	0	0.397313	-2.265365	1.473815
16	1	0	-1.588089	-3.462566	0.471788
17	1	0	-2.099485	-2.101902	1.471355
18	1	0	-3.331518	-2.022401	-0.697806
19	1	0	-1.798875	-2.038978	-1.583701
20	1	0	-2.782879	0.092601	0.370089
21	1	0	-2.669367	0.281541	-1.384385
22	1	0	-0.403279	-0.002249	1.624460
23	1	0	-1.250336	2.039591	-1.447059
24	1	0	1.944759	-0.235393	1.483061
25	1	0	0.717674	3.340242	-0.560279
26	1	0	1.123869	1.922138	-1.525182
27	1	0	1.146274	2.045745	1.543426
28	1	0	2.567317	2.009097	0.509010
29	1	0	2.868396	0.358013	-1.086643
30	1	0	1.552879	-0.734822	-1.439469
31	1	0	3.930083	-1.308405	0.445157
32	1	0	3.731320	-1.956357	-1.189784
33	1	0	2.640121	-2.466761	0.105335
34	1	0	-1.171514	2.241099	1.623147
35	1	0	-1.441917	3.578143	0.498601
36	1	0	-2.610583	2.259979	0.598593

(1*S*,4*R*,9a*S*)-1-Ethyl-4-methylquinolizidine, (4-*epi*-9)

(Experimentaly not observed epimer.)



C4-*epi*-**9**

B3LYP/6-31G(d): -525.890121360 hartrees

Imaginary Frequencies: none found

Zero-point correction= 0.338864 (Hartree/Particle)

Thermal correction to Energy= 0.351725

Thermal correction to Enthalpy= 0.352669

Thermal correction to Gibbs Free Energy= 0.300655

Sum of electronic and zero-point Energies= -525.551258

Sum of electronic and thermal Energies= -525.538396

Sum of electronic and thermal Enthalpies= -525.537452

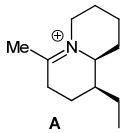
Sum of electronic and thermal Free Energies= -525.589466

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	0.011323	-1.988962	0.360407
2	6	0	-1.410602	-2.546128	0.436115
3	6	0	-2.306684	-1.712180	-0.477924
4	6	0	-2.210760	-0.231195	-0.112192
5	7	0	-0.838278	0.297970	-0.172879
6	6	0	0.080490	-0.486802	0.686947
7	6	0	-0.832137	1.740678	0.172644
8	6	0	1.537079	0.037068	0.594563
9	6	0	0.592066	2.315278	0.143497
10	6	0	1.553055	1.512871	1.017968

11	6	0	2.197335	-0.170071	-0.797595
12	6	0	3.197923	-1.331930	-0.853169
13	6	0	-1.721585	2.565554	-0.770768
14	1	0	0.397293	-2.157059	-0.652476
15	1	0	0.676951	-2.521344	1.051888
16	1	0	-1.426728	-3.605480	0.150613
17	1	0	-1.782493	-2.488468	1.469642
18	1	0	-3.355211	-2.028413	-0.407943
19	1	0	-1.995430	-1.845394	-1.522737
20	1	0	-2.833350	0.340064	-0.803979
21	1	0	-2.641424	-0.085370	0.902008
22	1	0	-0.231049	-0.371288	1.748909
23	1	0	-1.217996	1.866403	1.207724
24	1	0	2.113856	-0.535634	1.335575
25	1	0	0.550836	3.359931	0.476630
26	1	0	0.947372	2.328160	-0.895628
27	1	0	1.243590	1.596858	2.069985
28	1	0	2.570472	1.920397	0.954228
29	1	0	2.730209	0.747391	-1.081254
30	1	0	1.417892	-0.303152	-1.556899
31	1	0	4.016646	-1.179105	-0.138555
32	1	0	3.642875	-1.423024	-1.851301
33	1	0	2.727822	-2.291228	-0.611024
34	1	0	-1.484422	2.331126	-1.815103
35	1	0	-1.533775	3.632850	-0.609626
36	1	0	-2.791003	2.400900	-0.613484

Iminium salt intermediate, (AI)



A

B3LYP/6-31G(d): -525.086549975 hartrees

Imaginary Frequencies: none found

Zero-point correction= 0.328440 (Hartree/Particle)

Thermal correction to Energy= 0.341480

Thermal correction to Enthalpy= 0.342424

Thermal correction to Gibbs Free Energy= 0.289986

Sum of electronic and zero-point Energies= -524.758110

Sum of electronic and thermal Energies= -524.745070

Sum of electronic and thermal Enthalpies= -524.744126

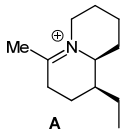
Sum of electronic and thermal Free Energies= -524.796564

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-0.321394	2.151018	0.513053
2	6	0	-1.437432	1.154447	0.824442
3	6	0	-1.511782	0.102524	-0.287096
4	6	0	-0.168830	-0.643338	-0.387199
5	7	0	1.000722	0.313846	-0.420457
6	6	0	0.942803	1.553585	-0.034232
7	6	0	0.138081	-1.706233	0.687147
8	6	0	1.462247	-2.428532	0.393194
9	6	0	2.617681	-1.439004	0.200105
10	6	0	2.262219	-0.359629	-0.833815
11	6	0	2.138332	2.463584	-0.111563

12	6	0	-2.699460	-0.871647	-0.149531
13	6	0	-4.057207	-0.220837	-0.439604
14	1	0	-0.039821	2.741914	1.394827
15	1	0	-0.649956	2.896551	-0.228814
16	1	0	-1.254284	0.673515	1.792917
17	1	0	-2.383505	1.693986	0.917076
18	1	0	-1.638393	0.636933	-1.241479
19	1	0	-0.128216	-1.136082	-1.366529
20	1	0	-0.682980	-2.428178	0.702294
21	1	0	0.170011	-1.233771	1.677078
22	1	0	1.697247	-3.122162	1.206706
23	1	0	1.348380	-3.037457	-0.513630
24	1	0	3.522522	-1.952638	-0.141830
25	1	0	2.867333	-0.951091	1.151288
26	1	0	2.076831	-0.802504	-1.817840
27	1	0	3.057069	0.375717	-0.937255
28	1	0	1.841207	3.485988	0.128253
29	1	0	2.591986	2.460883	-1.107557
30	1	0	2.907416	2.160676	0.609552
31	1	0	-2.553777	-1.710144	-0.844125
32	1	0	-2.707243	-1.303125	0.859404
33	1	0	-4.091169	0.195938	-1.452839
34	1	0	-4.857917	-0.962060	-0.359154
35	1	0	-4.289348	0.584560	0.265452

Iminium salt intermediate, (AII)



A

B3LYP/6-31G(d): -525.081623367 hartrees

Imaginary Frequencies: none found

Zero-point correction= 0.328660 (Hartree/Particle)

Thermal correction to Energy= 0.341540

Thermal correction to Enthalpy= 0.342484

Thermal correction to Gibbs Free Energy= 0.290273

Sum of electronic and zero-point Energies= -524.752963

Sum of electronic and thermal Energies= -524.740083

Sum of electronic and thermal Enthalpies= -524.739139

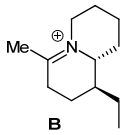
Sum of electronic and thermal Free Energies= -524.791350

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	1.298752	1.779791	0.152925
2	6	0	1.833209	0.718959	1.110879
3	6	0	1.374889	-0.668566	0.644575
4	6	0	-0.171612	-0.709456	0.694709
5	7	0	-0.825061	0.572028	0.197784
6	6	0	-0.173201	1.656151	-0.108941
7	6	0	-0.786270	-1.912415	-0.046590
8	6	0	-2.312690	-1.971249	0.043986
9	6	0	-2.903277	-0.671713	-0.503762
10	6	0	-2.319654	0.529884	0.237811
11	6	0	-0.855014	2.869166	-0.679255

12	6	0	1.992993	-1.091540	-0.710731
13	6	0	3.526207	-1.038210	-0.737371
14	1	0	1.817224	1.757375	-0.817275
15	1	0	1.462720	2.795425	0.536375
16	1	0	2.921990	0.781734	1.166828
17	1	0	1.455781	0.917464	2.121854
18	1	0	1.699996	-1.405817	1.390262
19	1	0	-0.480278	-0.758496	1.747559
20	1	0	-0.334585	-2.814170	0.382178
21	1	0	-0.491041	-1.878652	-1.100848
22	1	0	-2.685286	-2.830503	-0.522341
23	1	0	-2.629198	-2.116051	1.085791
24	1	0	-3.991260	-0.641083	-0.381019
25	1	0	-2.698439	-0.579014	-1.577955
26	1	0	-2.573765	0.471217	1.303872
27	1	0	-2.712248	1.469442	-0.142813
28	1	0	-0.104369	3.543019	-1.097861
29	1	0	-1.385021	3.419380	0.109461
30	1	0	-1.574992	2.620833	-1.463130
31	1	0	1.686319	-2.119491	-0.927493
32	1	0	1.590256	-0.481781	-1.532264
33	1	0	3.958373	-1.600909	0.098342
34	1	0	3.901879	-1.483315	-1.663593
35	1	0	3.915380	-0.015616	-0.689825

Iminium salt intermediate, (BI)



B3LYP/6-31G(d): -525.087173137 hartrees

Imaginary Frequencies: none found

Zero-point correction= 0.328224 (Hartree/Particle)

Thermal correction to Energy= 0.341336

Thermal correction to Enthalpy= 0.342280

Thermal correction to Gibbs Free Energy= 0.289409

Sum of electronic and zero-point Energies= -524.758950

Sum of electronic and thermal Energies= -524.745838

Sum of electronic and thermal Enthalpies= -524.744893

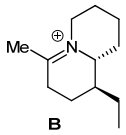
Sum of electronic and thermal Free Energies= -524.797764

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-0.405506	2.218664	-0.396883
2	6	0	-1.579368	1.470170	0.226993
3	6	0	-1.517670	-0.011866	-0.150245
4	6	0	-0.164536	-0.628608	0.263584
5	7	0	0.994746	0.347604	0.282521
6	6	0	0.921714	1.590115	-0.091731
7	6	0	0.241661	-1.804460	-0.650470
8	6	0	1.549224	-2.481998	-0.227130
9	6	0	2.677506	-1.450924	-0.127613
10	6	0	2.255544	-0.283560	0.768809
11	6	0	2.134990	2.470675	-0.194405

12	6	0	-2.675292	-0.829873	0.464400
13	6	0	-4.052724	-0.493860	-0.118074
14	1	0	-0.357011	3.260506	-0.057176
15	1	0	-0.503406	2.276541	-1.492667
16	1	0	-1.551841	1.584541	1.318843
17	1	0	-2.516454	1.916048	-0.115538
18	1	0	-1.592643	-0.081612	-1.245806
19	1	0	-0.228560	-0.979593	1.301879
20	1	0	0.327608	-1.427095	-1.678312
21	1	0	-0.581878	-2.525147	-0.647946
22	1	0	1.414451	-2.976317	0.744303
23	1	0	1.812145	-3.265404	-0.944902
24	1	0	2.940344	-1.069731	-1.122819
25	1	0	3.585337	-1.893219	0.296320
26	1	0	3.030005	0.475465	0.852661
27	1	0	2.031665	-0.641950	1.780330
28	1	0	1.893922	3.351503	-0.793650
29	1	0	2.992350	1.966412	-0.646229
30	1	0	2.432466	2.826050	0.801475
31	1	0	-2.680571	-0.670714	1.551910
32	1	0	-2.483765	-1.899491	0.313367
33	1	0	-4.075121	-0.649954	-1.202873
34	1	0	-4.817861	-1.138661	0.324595
35	1	0	-4.347659	0.541745	0.081174

Iminium salt intermediate, (BII)



B3LYP/6-31G(d): -525.085837633 hartrees

Imaginary Frequencies: none found

Zero-point correction= 0.328390 (Hartree/Particle)

Thermal correction to Energy= 0.341393

Thermal correction to Enthalpy= 0.342338

Thermal correction to Gibbs Free Energy= 0.289828

Sum of electronic and zero-point Energies= -524.757448

Sum of electronic and thermal Energies= -524.744444

Sum of electronic and thermal Enthalpies= -524.743500

Sum of electronic and thermal Free Energies= -524.796010

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	1.081793	1.568520	0.858670
2	6	0	1.548919	0.199785	1.351590
3	6	0	1.375834	-0.855541	0.249551
4	6	0	-0.105571	-0.920569	-0.161687
5	7	0	-0.719781	0.446794	-0.354580
6	6	0	-0.200751	1.556471	0.079965
7	6	0	-1.026442	-1.720949	0.780404
8	6	0	-2.457124	-1.810663	0.229238
9	6	0	-3.016039	-0.422900	-0.109565
10	6	0	-2.053643	0.365368	-1.011324
11	6	0	-0.851543	2.889414	-0.170795

12	6	0	2.297358	-0.647586	-0.975033
13	6	0	3.794900	-0.699638	-0.653195
14	1	0	1.831353	2.047796	0.209676
15	1	0	0.945114	2.272001	1.690278
16	1	0	2.590515	0.262023	1.675684
17	1	0	0.967541	-0.085920	2.235706
18	1	0	1.614774	-1.838304	0.675276
19	1	0	-0.171119	-1.372021	-1.159349
20	1	0	-1.025993	-1.259003	1.775934
21	1	0	-0.600070	-2.723567	0.892824
22	1	0	-2.458743	-2.434979	-0.674294
23	1	0	-3.108238	-2.308942	0.954441
24	1	0	-3.195456	0.152927	0.807688
25	1	0	-3.977339	-0.502495	-0.628362
26	1	0	-2.425843	1.366609	-1.216255
27	1	0	-1.903603	-0.145827	-1.967880
28	1	0	-0.179694	3.691852	0.139222
29	1	0	-1.777483	2.986899	0.409508
30	1	0	-1.099396	3.035363	-1.226629
31	1	0	2.065896	-1.433960	-1.705276
32	1	0	2.061686	0.301334	-1.478275
33	1	0	4.059125	-1.634544	-0.145674
34	1	0	4.383213	-0.645771	-1.574200
35	1	0	4.116079	0.130709	-0.014902

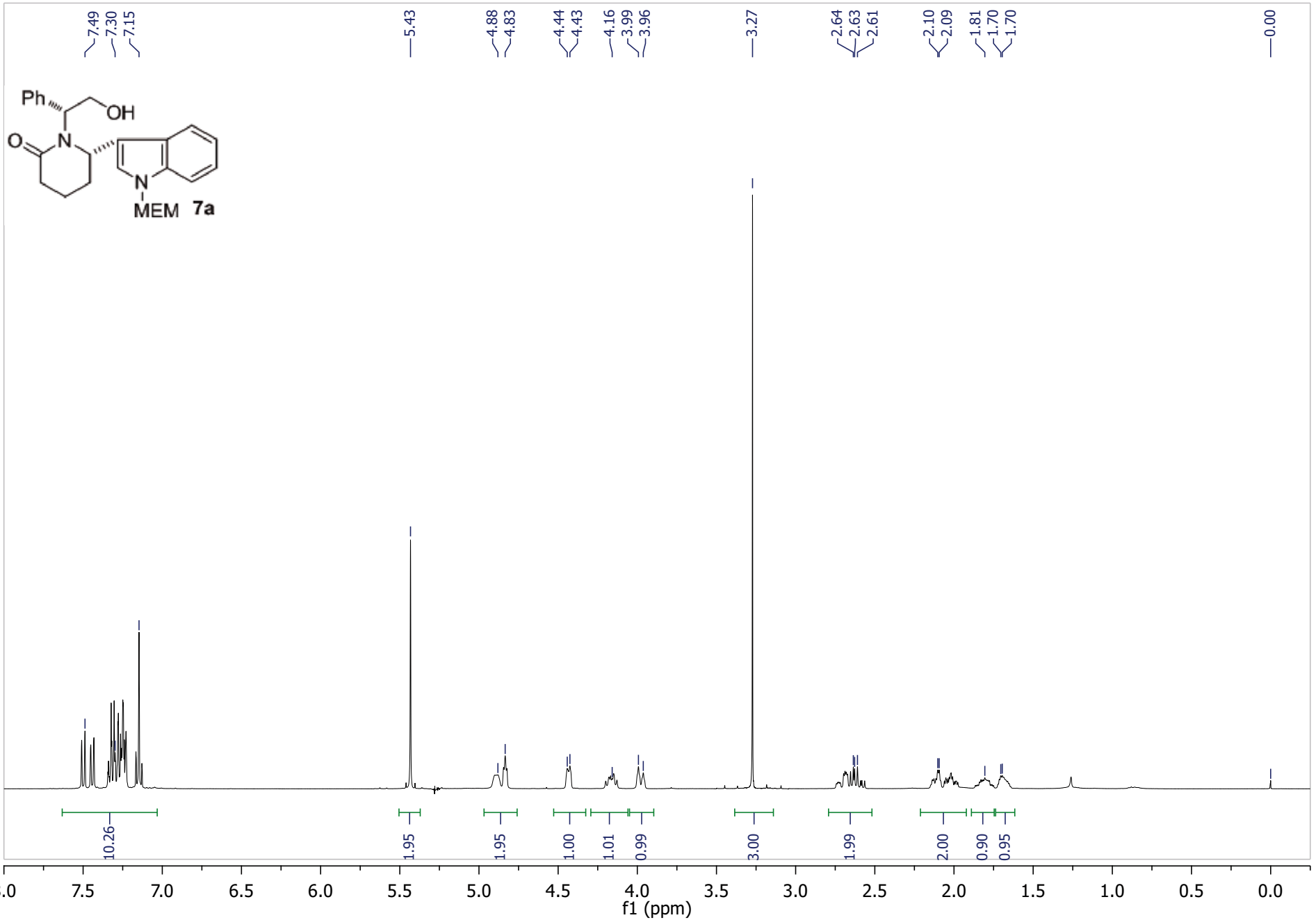
CHAPTER 1 – PART B

SUPPORTING MATERIAL

A practical procedure for the removal of the phenylethanol moiety from phenylglycinol-derived lactams

V. Semak; C, Escolano; C. Arróniz; J. Bosch; M. Amat

Tetrahedron: Asymmetry **2010**, *21*, 2542-2549.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1.70
1.70
0.00
Ph
OH
O
N
N
MEM
7a
10.26
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1.95
1.00
1.01
0.99
3.00
1.99
2.00
0.90
0.95
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7.0
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6.0
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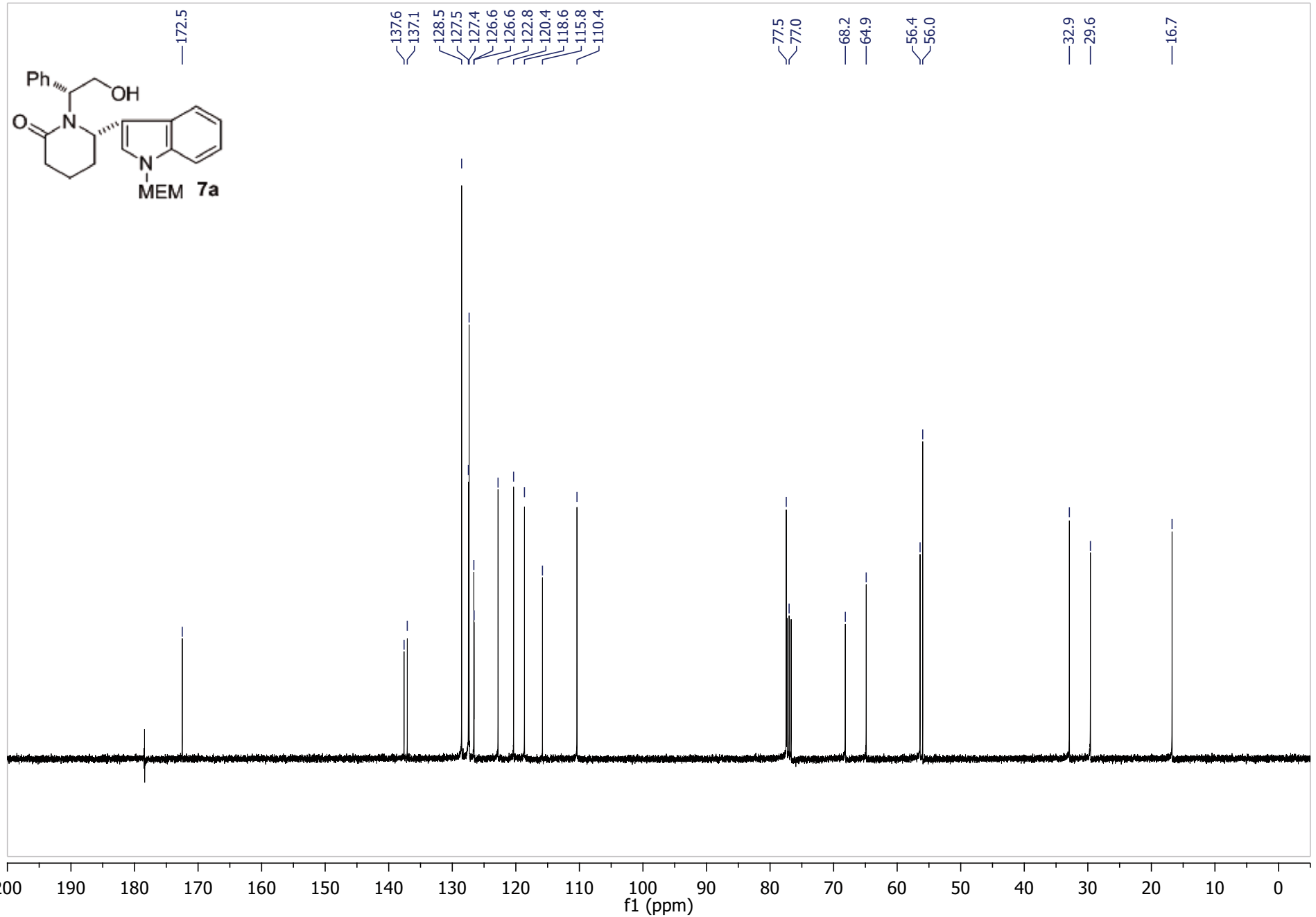
f1 (ppm)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126.6
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77.5
77.0
68.2
64.9
56.4
56.0
32.9
29.6
16.7
Ph
OH
O
N
N
MEM
7a
200
190
180
170
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90
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50
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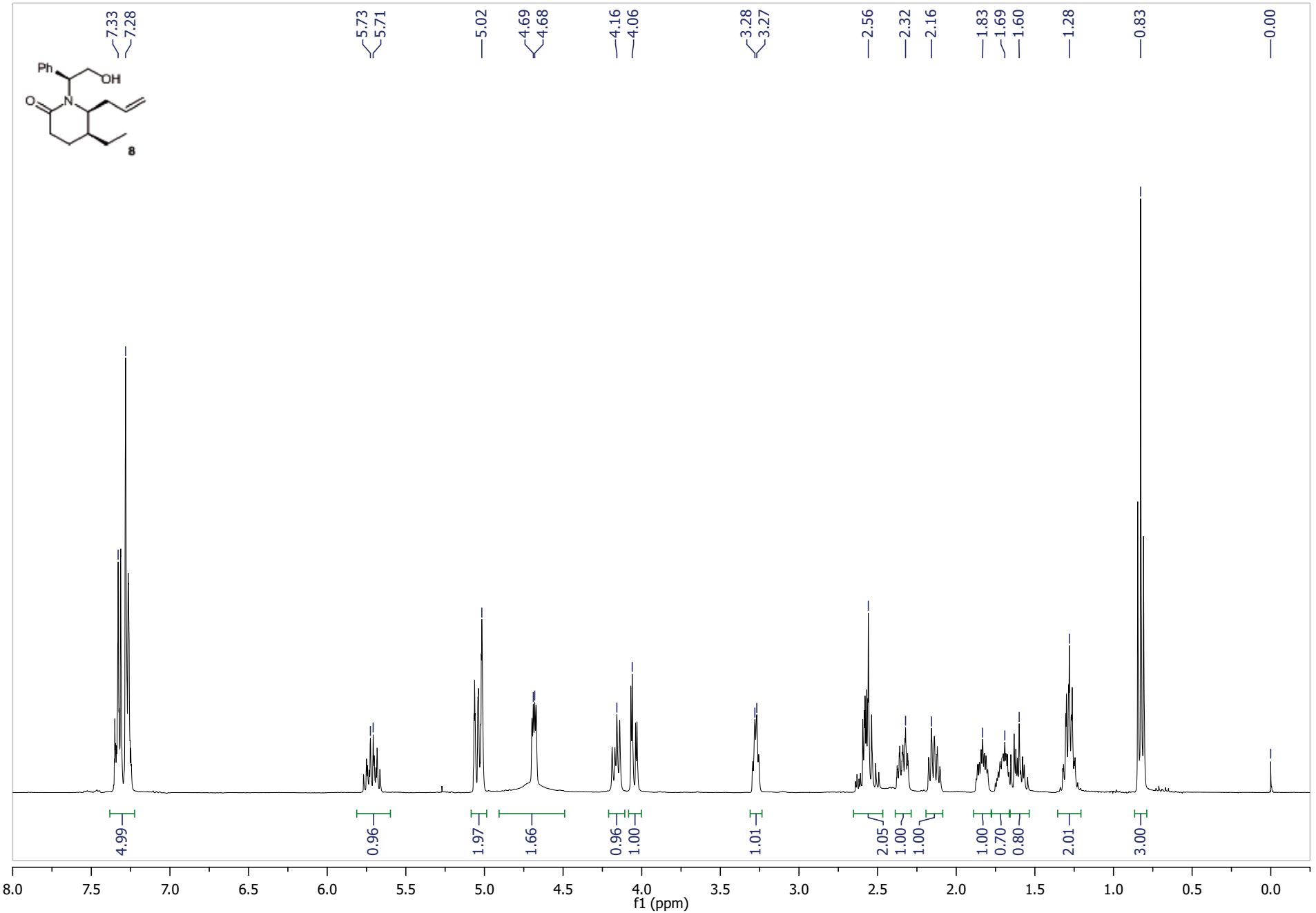
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2.16
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1.69
1.60
1.28
0.83
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Ph
OH
O
N
8
4.99
0.96
1.97
1.66
0.96
1.00
1.01
2.05
1.00
1.00
1.00
0.70
0.80
2.01
3.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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3.0
2.5
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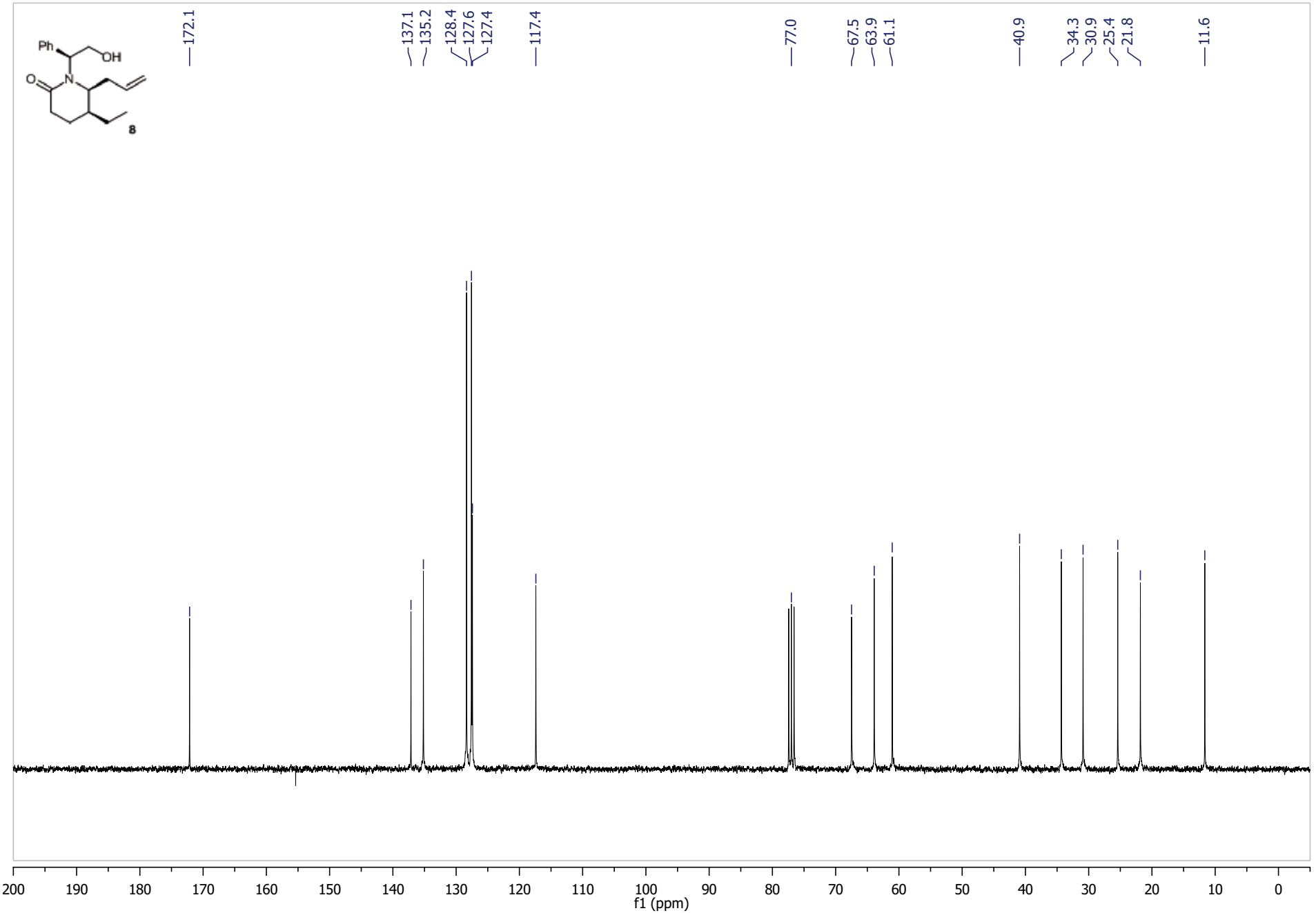
0.0
f1 (ppm)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127.6
127.4
117.4
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63.9
61.1
40.9
34.3
30.9
25.4
21.8
11.6
200
190
180
170
160
150
140
130
120
110
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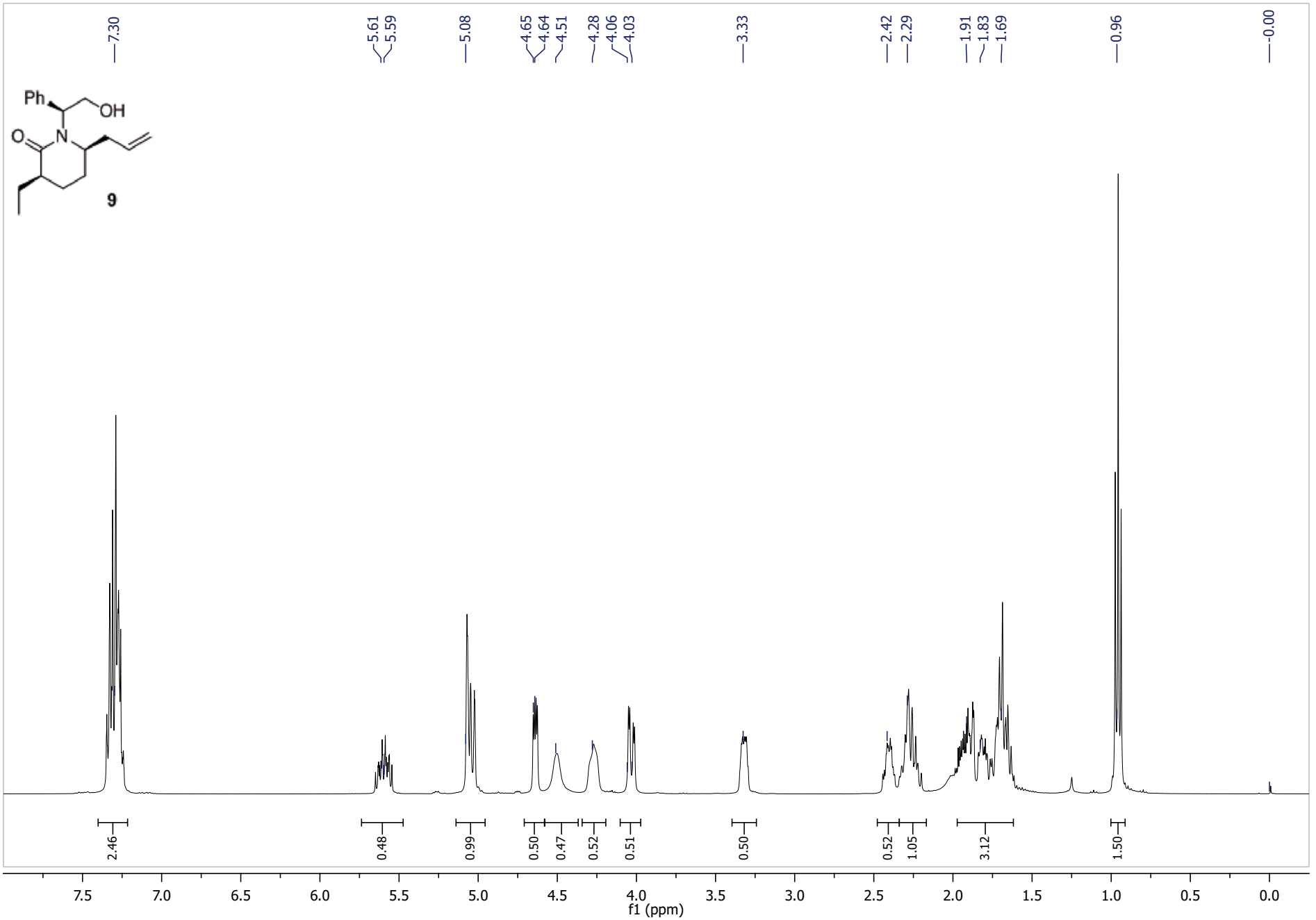
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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4.28
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4.03
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2.29
1.91
1.83
1.69
0.96
-0.00
Ph
OH
O
N
9
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0.48
0.99
0.50
0.47
0.52
0.51
0.50
0.52
1.05
3.12
1.50
7.5
7.0
6.5
6.0
5.5
5.0
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1.0
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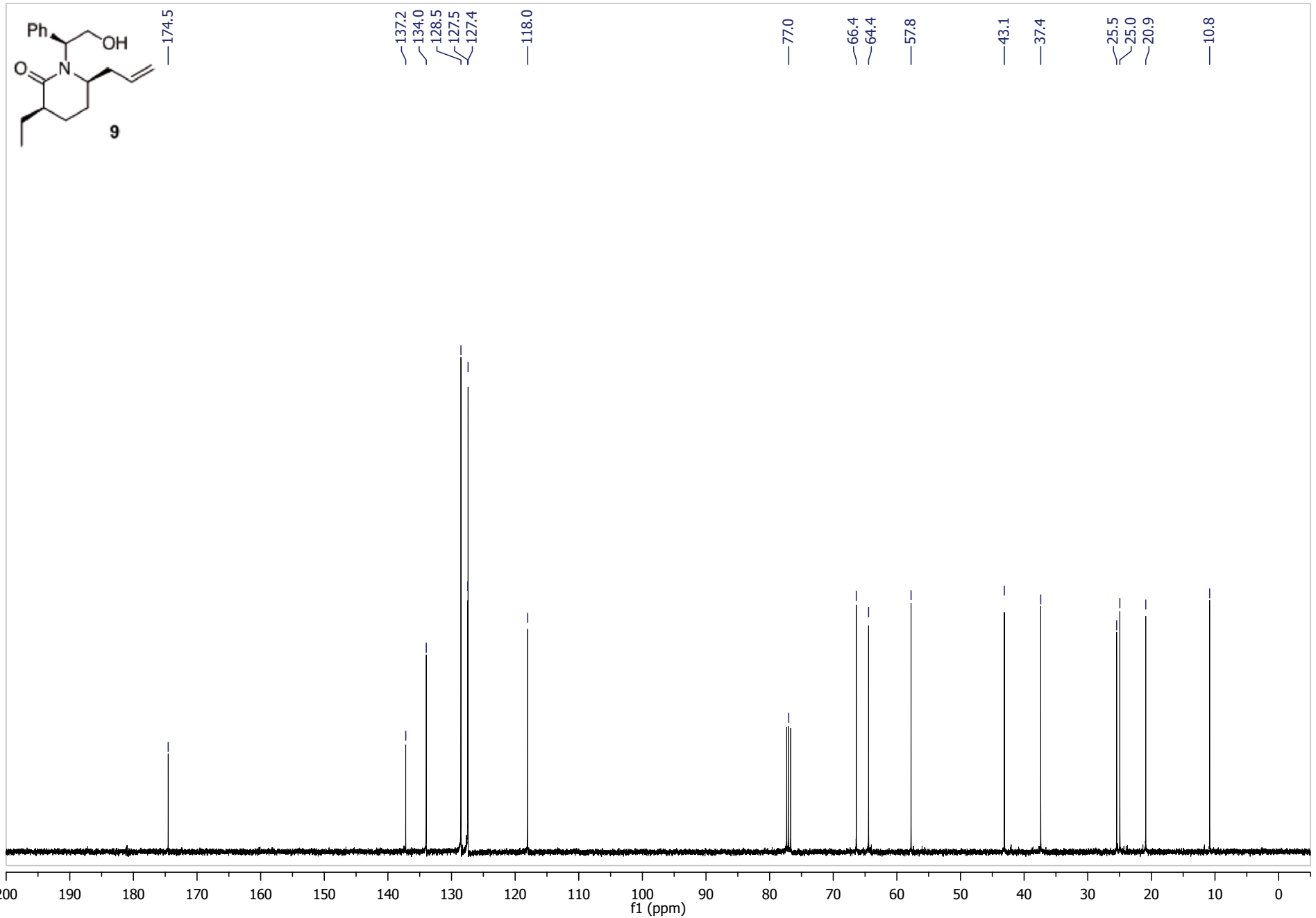
0.0
f1 (ppm)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77.0
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64.4
57.8
43.1
37.4
25.5
25.0
20.9
10.8
200
190
180
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90
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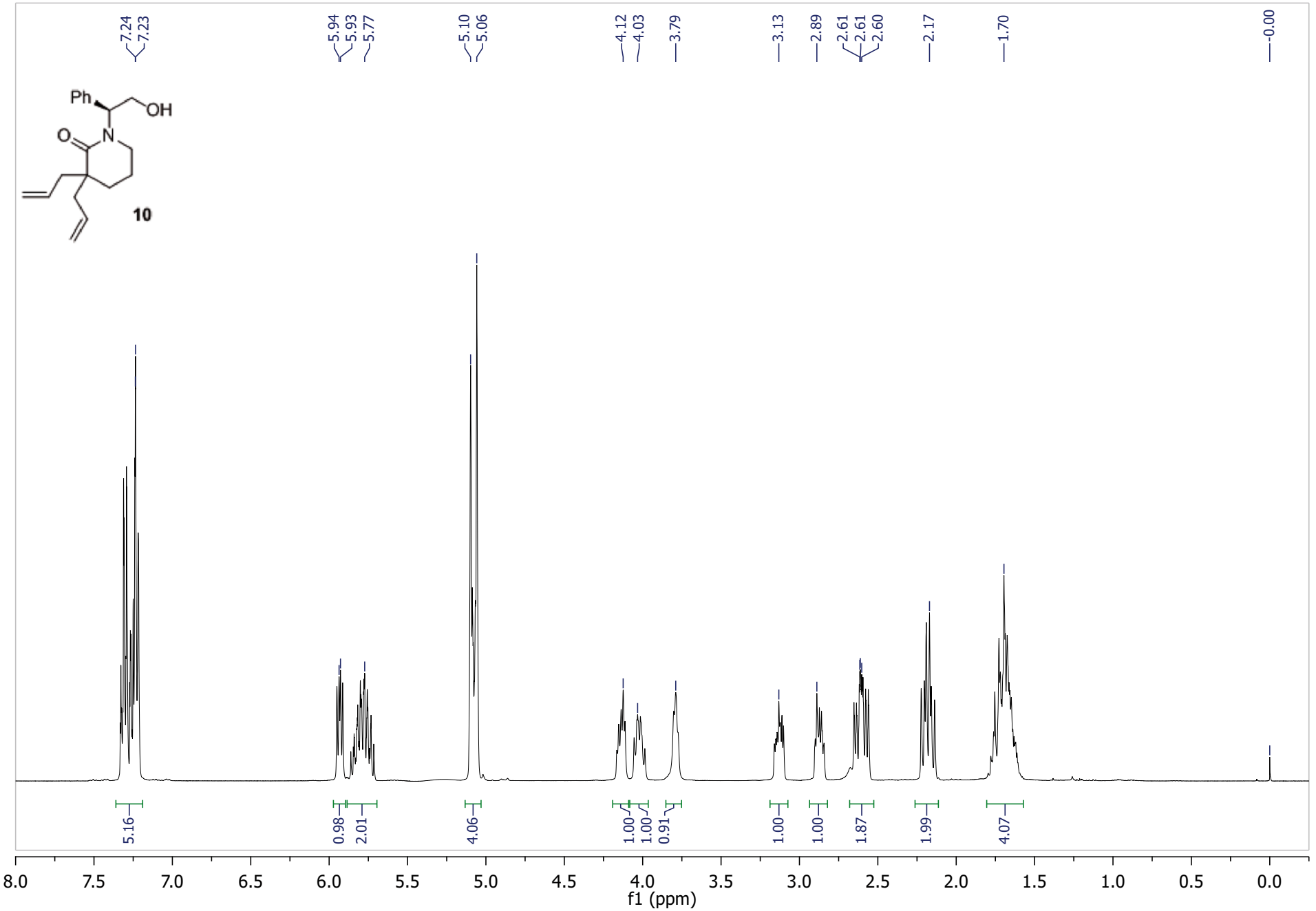
f1 (ppm)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2.61
2.60
2.17
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Ph
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O
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1.00
1.00
1.87
1.99
4.07
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7.5
7.0
6.5
6.0
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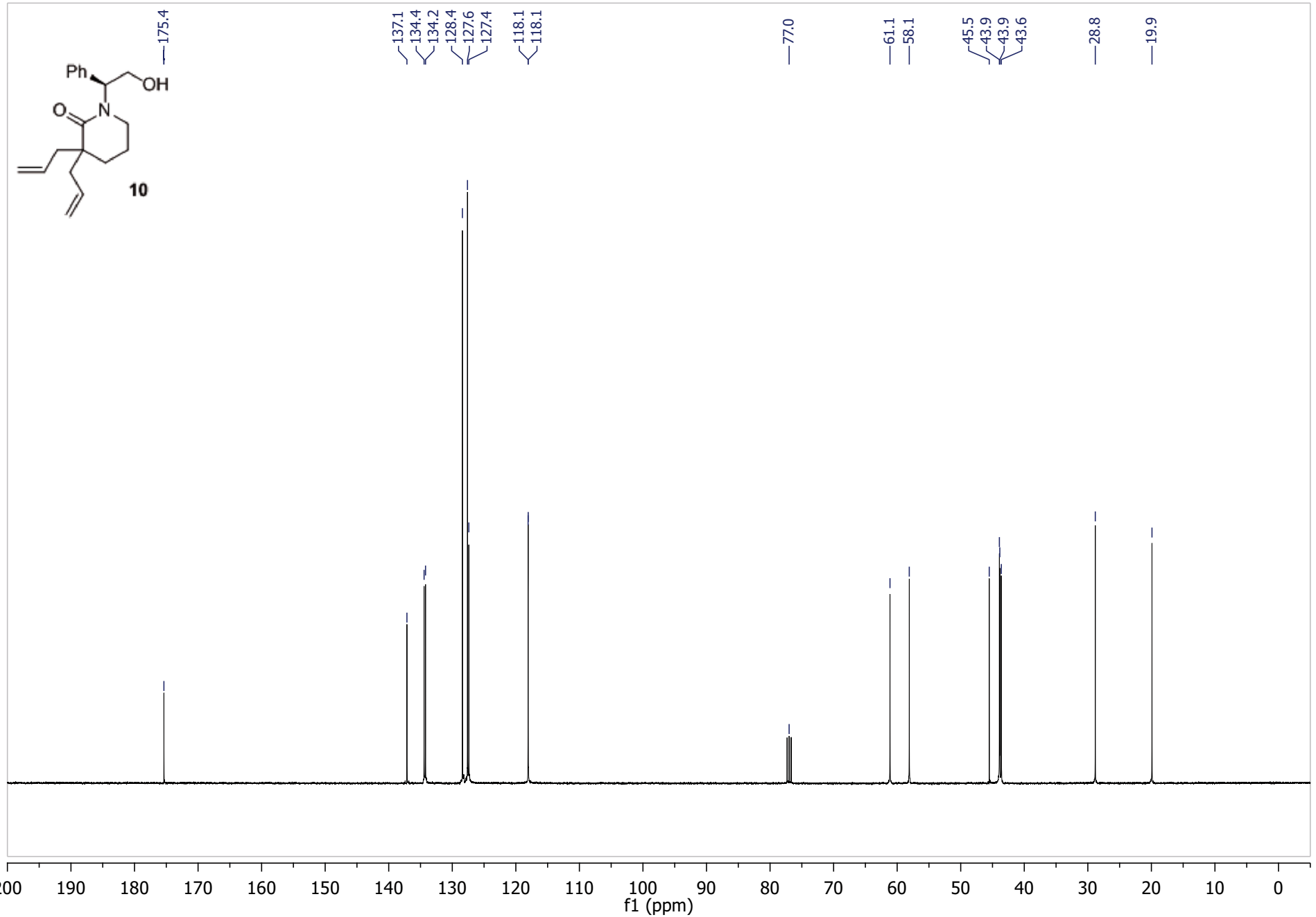
f1 (ppm)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127.4
118.1
118.1
77.0
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58.1
45.5
43.9
43.9
43.6
28.8
19.9
Ph
OH
O
N
10
200
190
180
170
160
150
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130
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50
40
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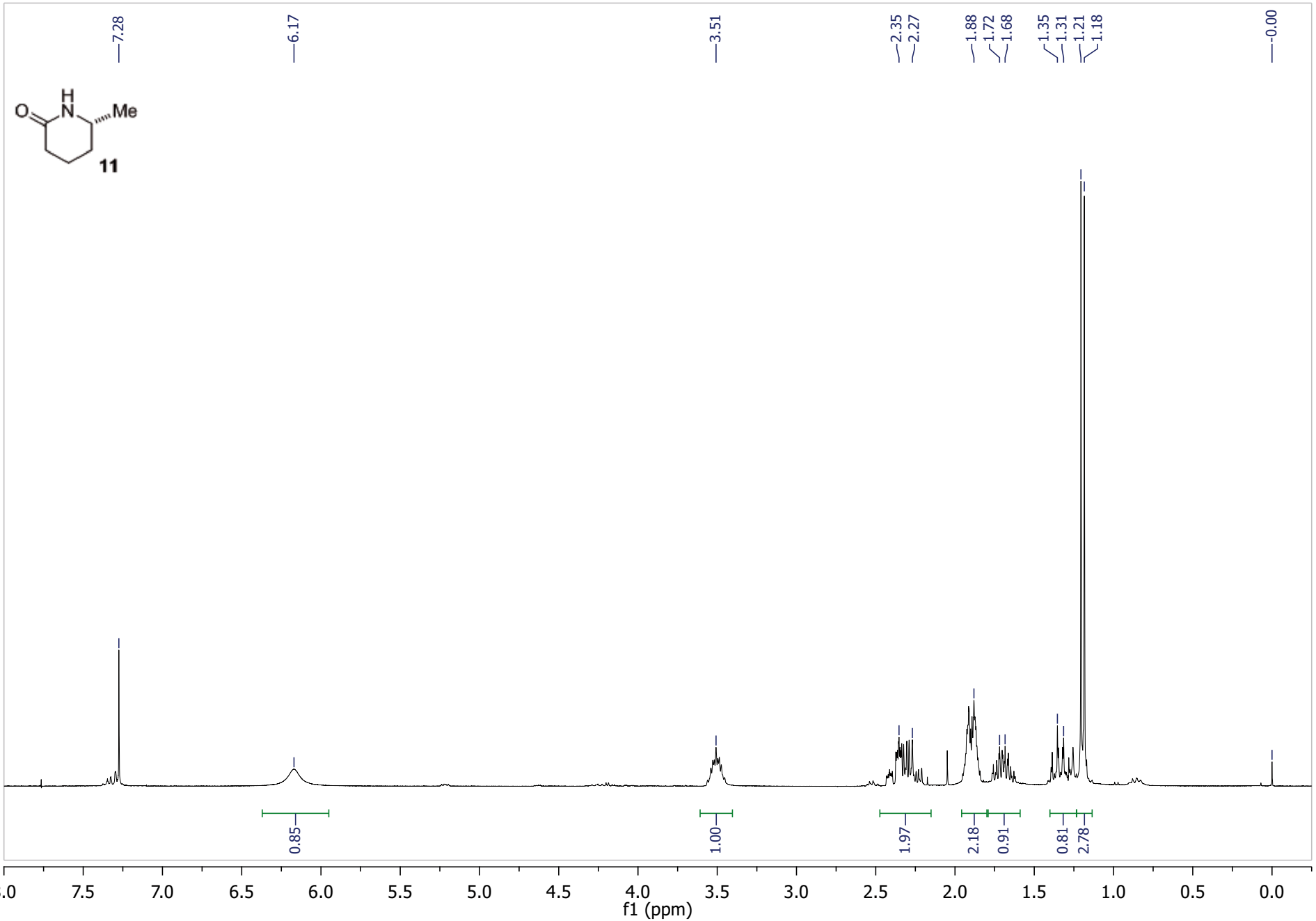
f1 (ppm)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1.68
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1.31
1.21
1.18
0.00
H
N
O
Me
11
0.85
1.00
1.97
2.18
0.91
0.81
2.78
8.0
7.5
7.0
6.5
6.0
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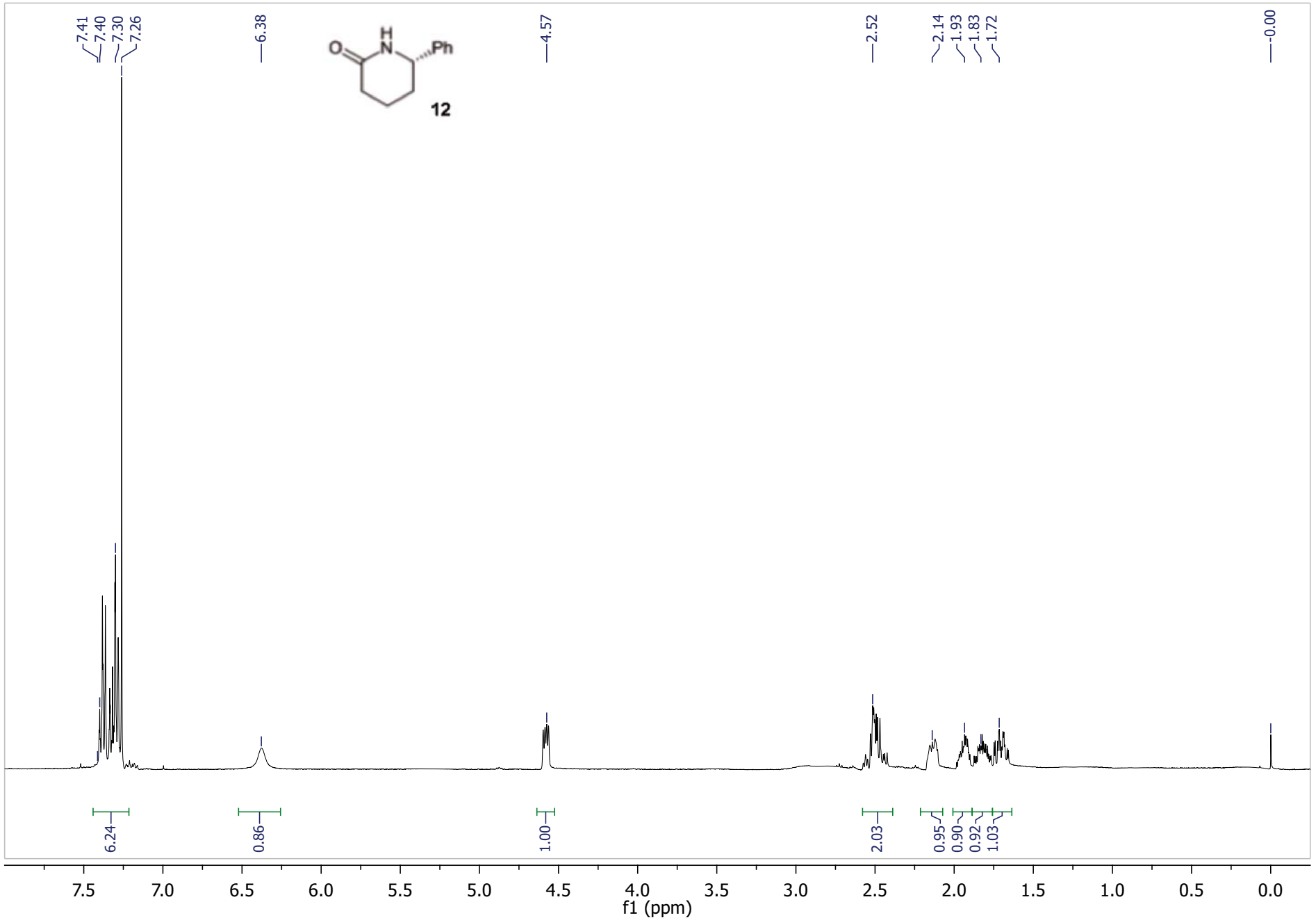
f1 (ppm)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7.26
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2.14
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1.83
1.72
0.00
O
H
N
Ph
12
6.24
0.86
1.00
2.03
0.95
0.90
0.92
1.03
7.5
7.0
6.5
6.0
5.5
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3.0
2.5
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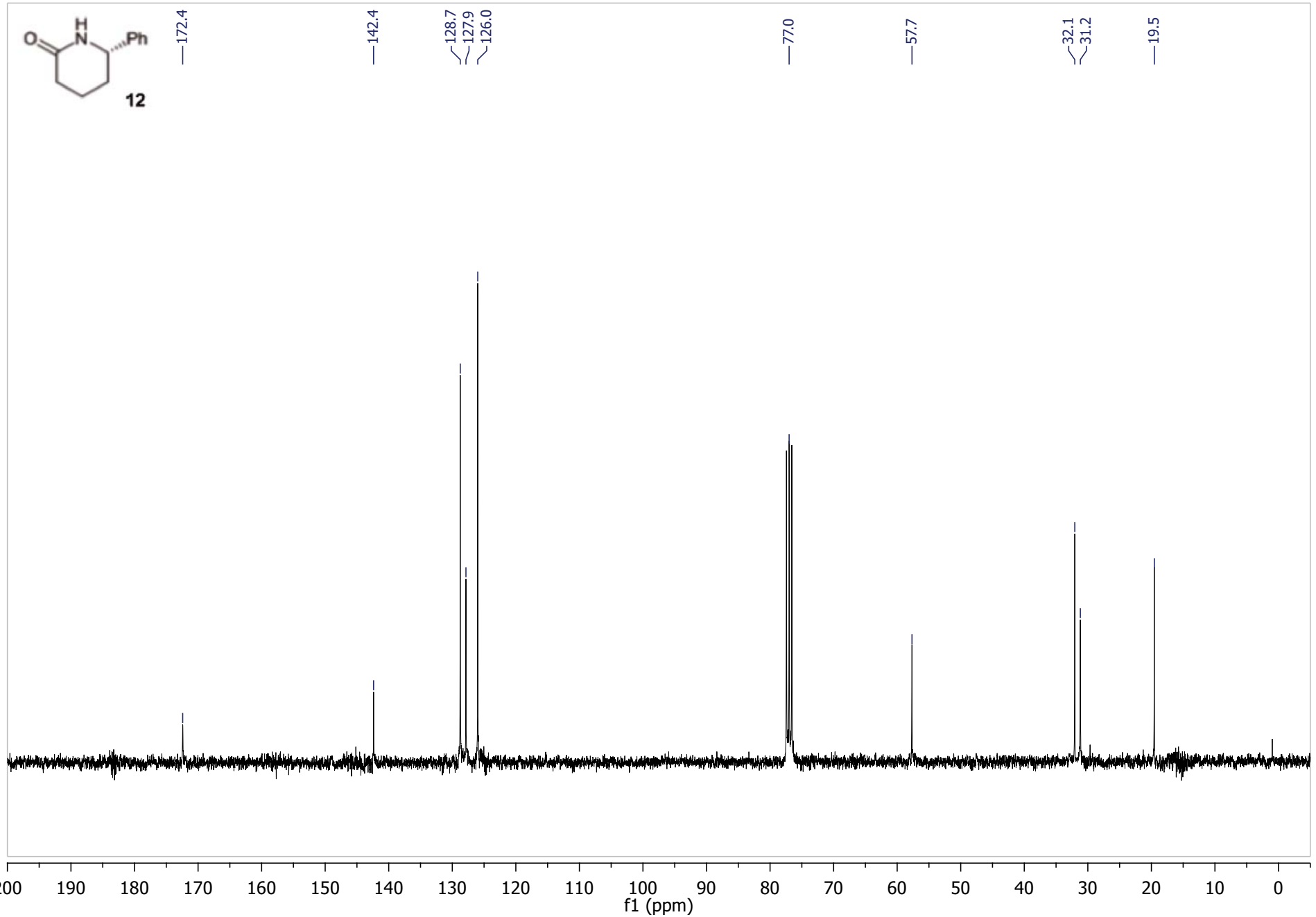
f1 (ppm)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N
Ph
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32.1
31.2
19.5
200
190
180
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120
110
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70
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10
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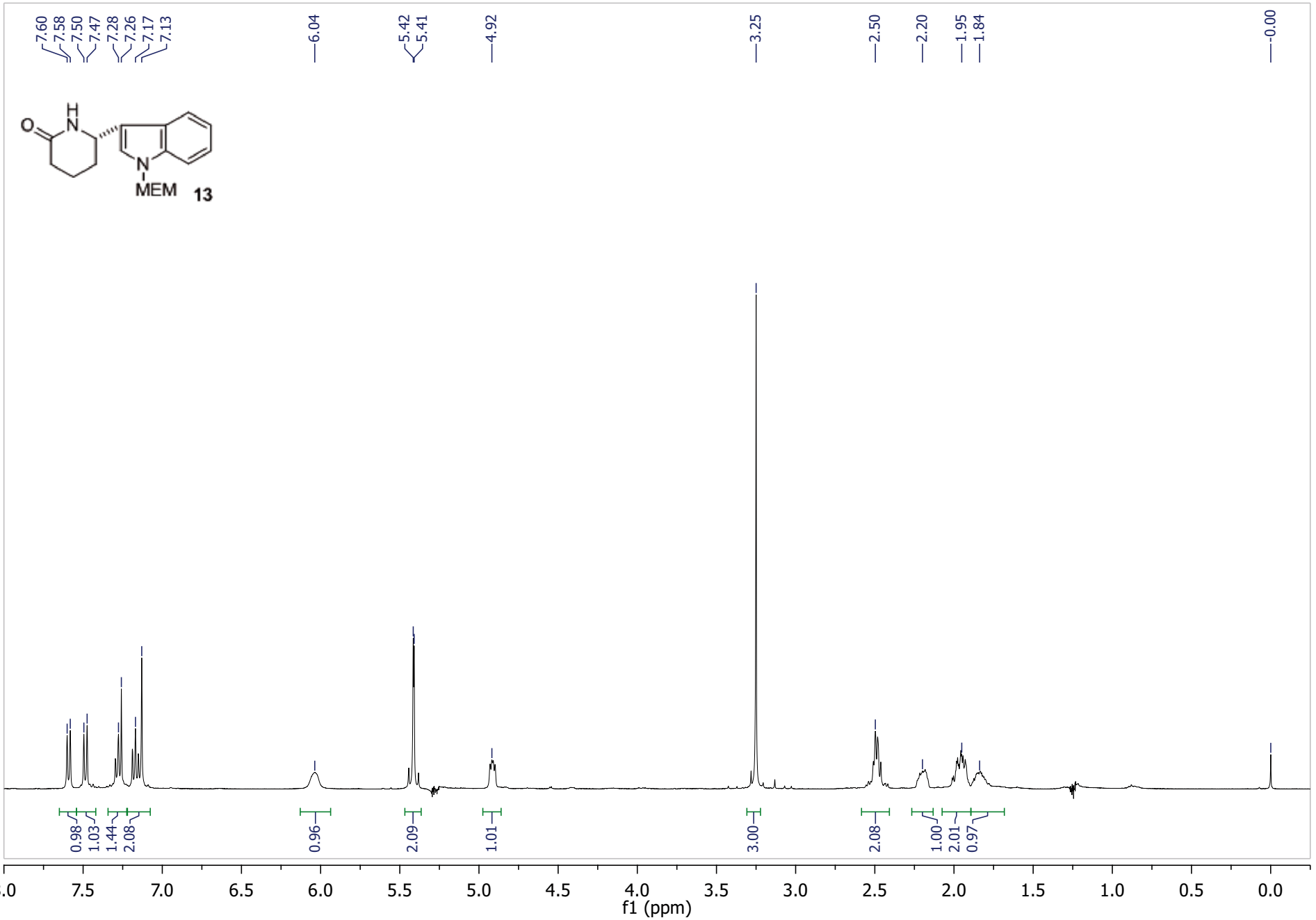
f1 (ppm)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4.92
3.25
2.50
2.20
1.95
1.84
0.00
O
H
N
N
MEM
13
0.98
1.03
1.44
2.08
0.96
2.09
1.01
3.00
2.08
1.00
2.01
0.97
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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3.5
3.0
2.5
2.0
1.5
1.0
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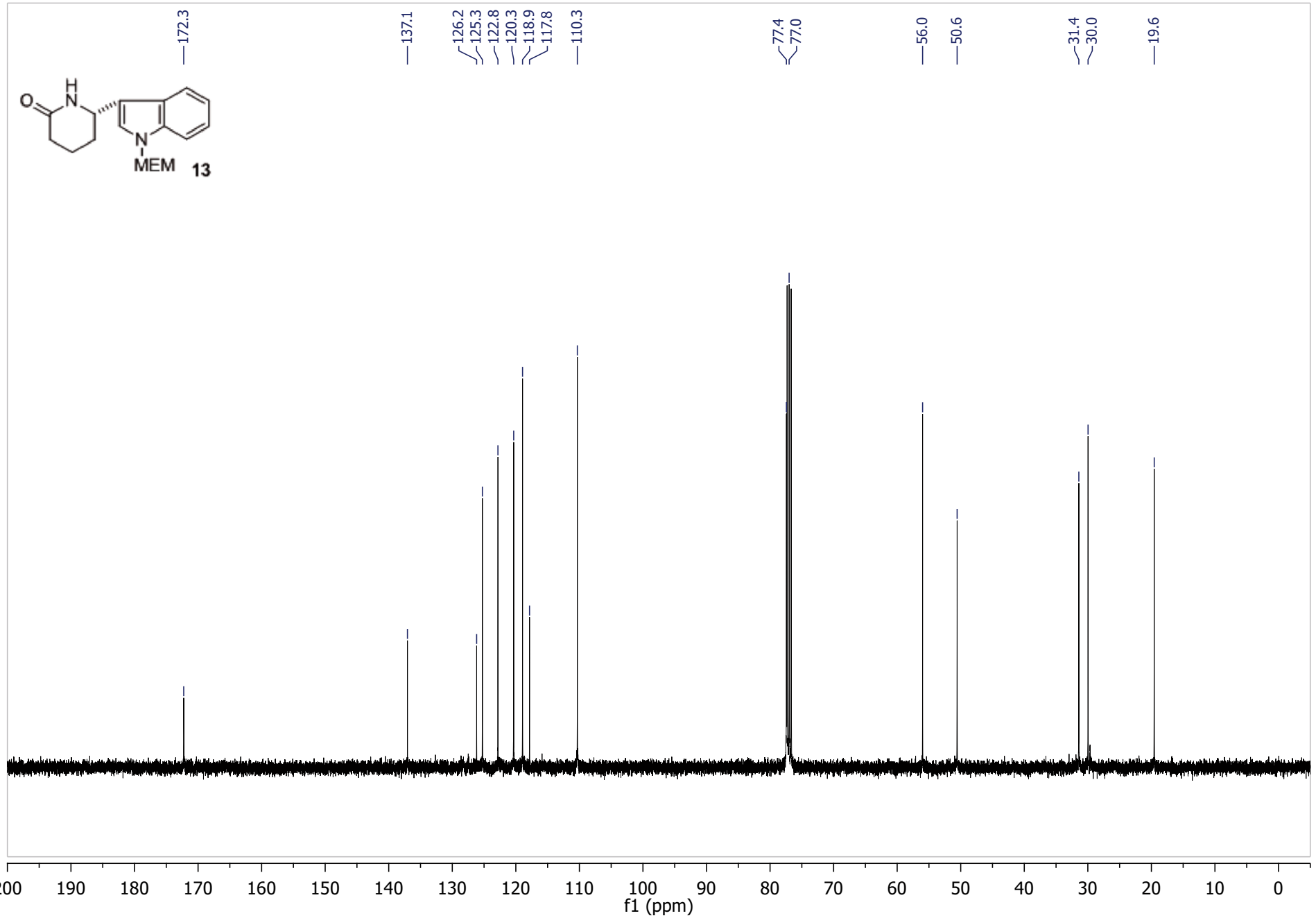
0.0
f1 (ppm)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122.8
120.3
118.9
117.8
110.3
77.4
77.0
56.0
50.6
31.4
30.0
19.6
MEM
13
200
190
180
170
160
150
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130
120
110
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70
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50
40
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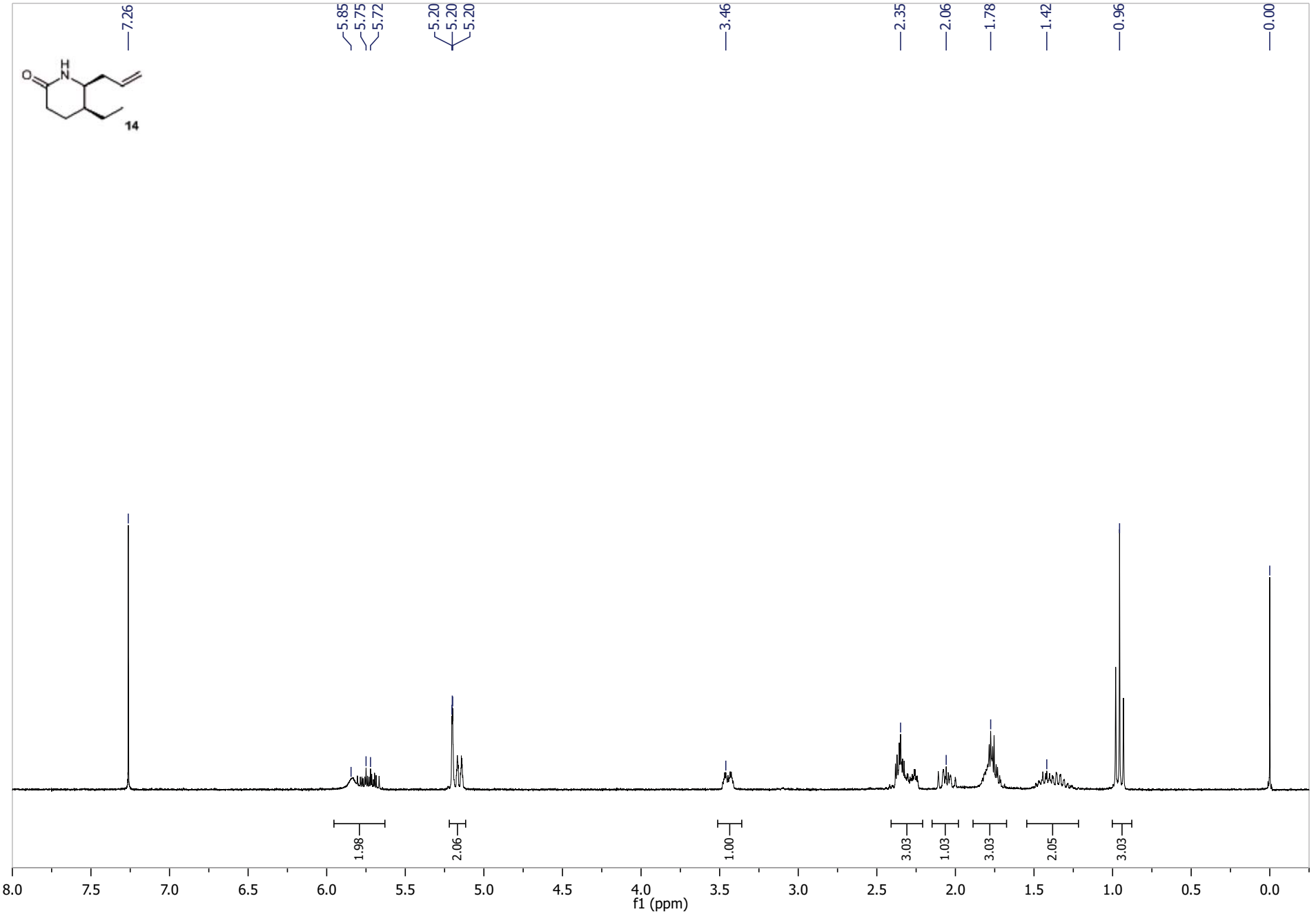
20
10
0
f1 (ppm)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5.72
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14
1.98
2.06
1.00
3.03
1.03
3.03
2.05
3.03
8.0
7.5
7.0
6.5
6.0
5.5
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4.5
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3.5
3.0
2.5
2.0
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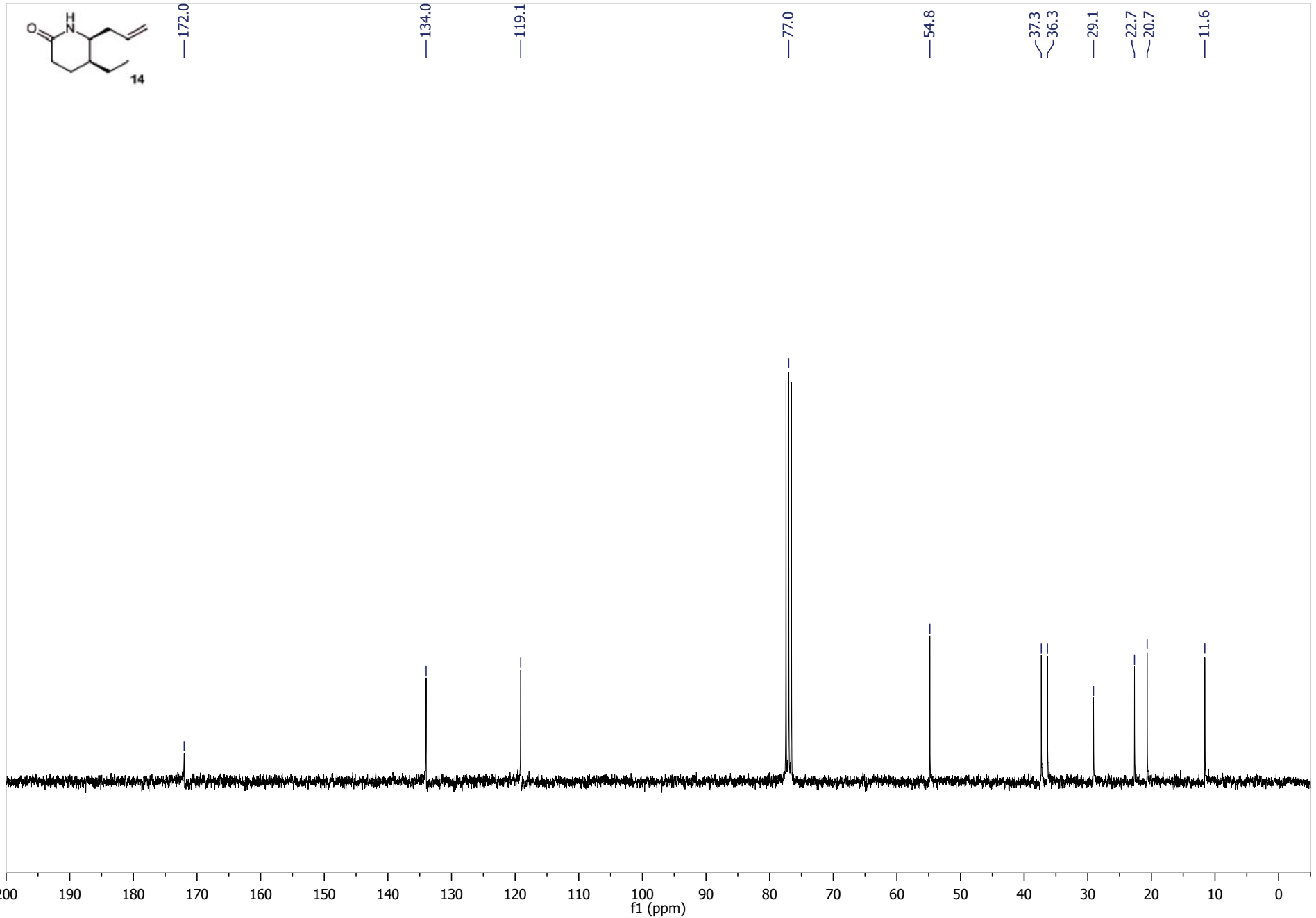
1.0
0.5
0.0
f1 (ppm)


O
H
N
14
172.0
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77.0
54.8
37.3
36.3
29.1
22.7
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11.6
200
190
180
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130
120
110
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90
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70
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50
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10
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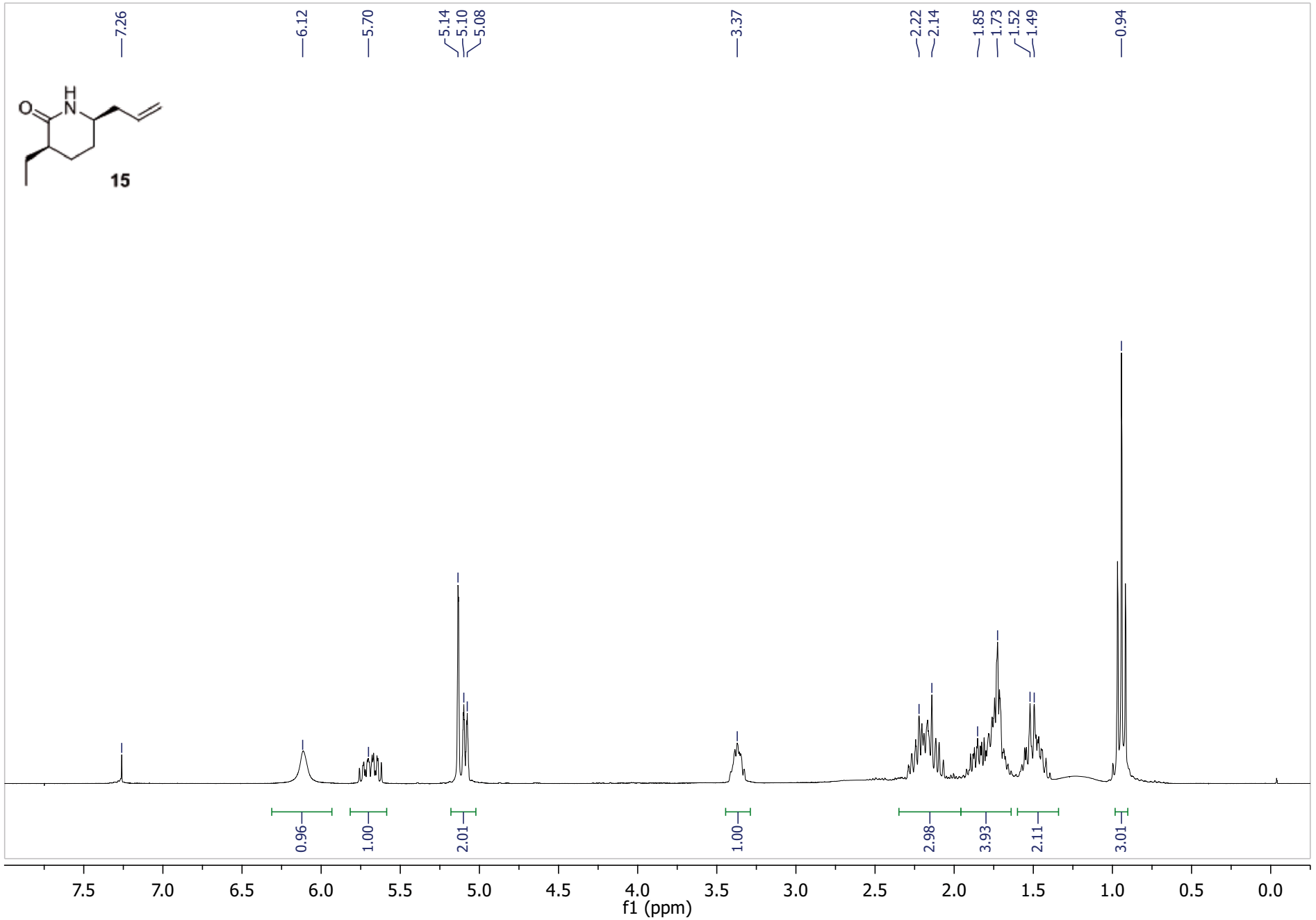
f1 (ppm)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1.73
1.52
1.49
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O
H
N
15
0.96
1.00
2.01
1.00
2.98
3.93
2.11
3.01
7.5
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6.5
6.0
5.5
5.0
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3.5
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1.0
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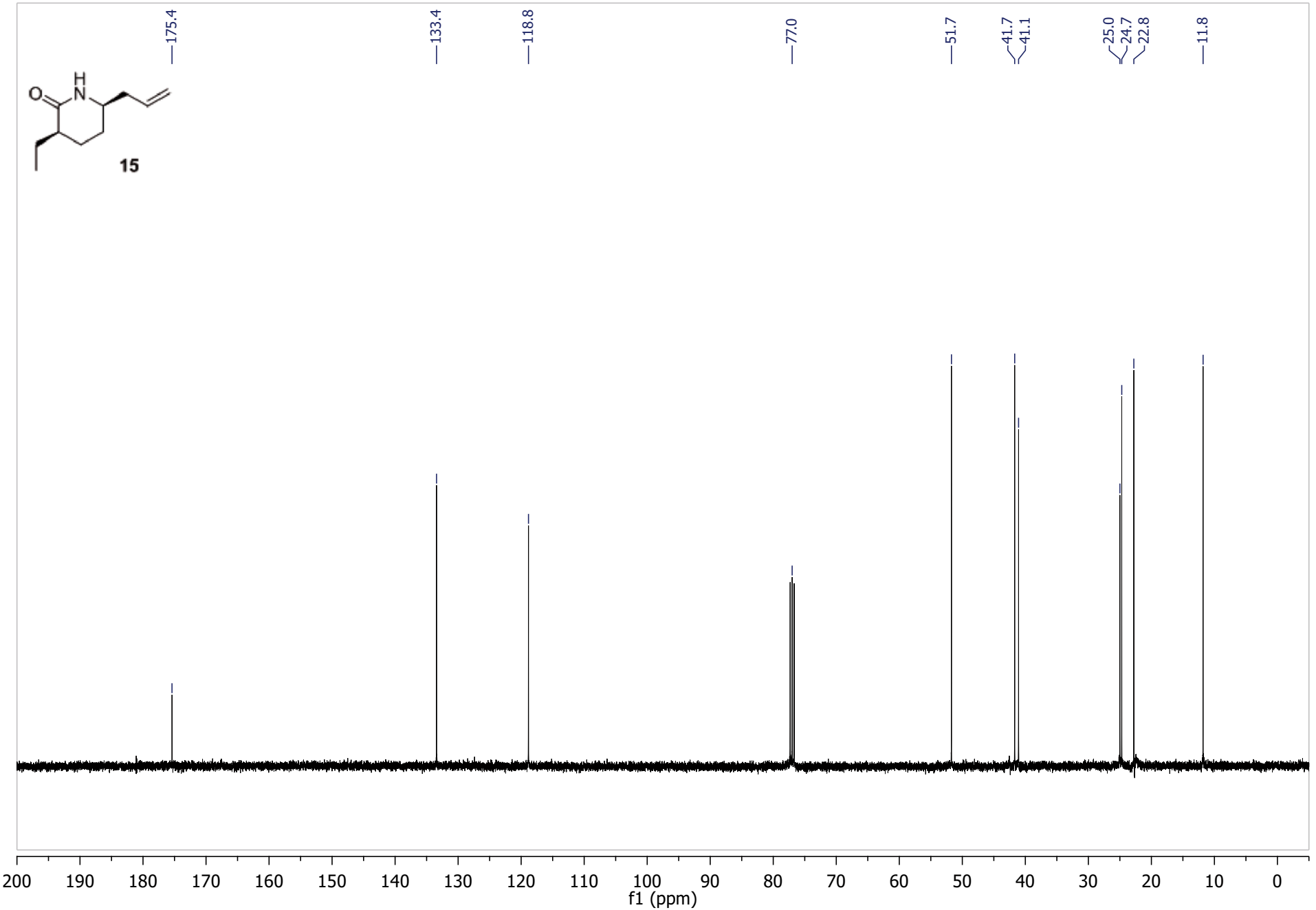
f1 (ppm)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118.8
77.0
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41.7
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25.0
24.7
22.8
11.8
O
H
N
15
200
190
180
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130
120
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100
90
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50
40
30
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10
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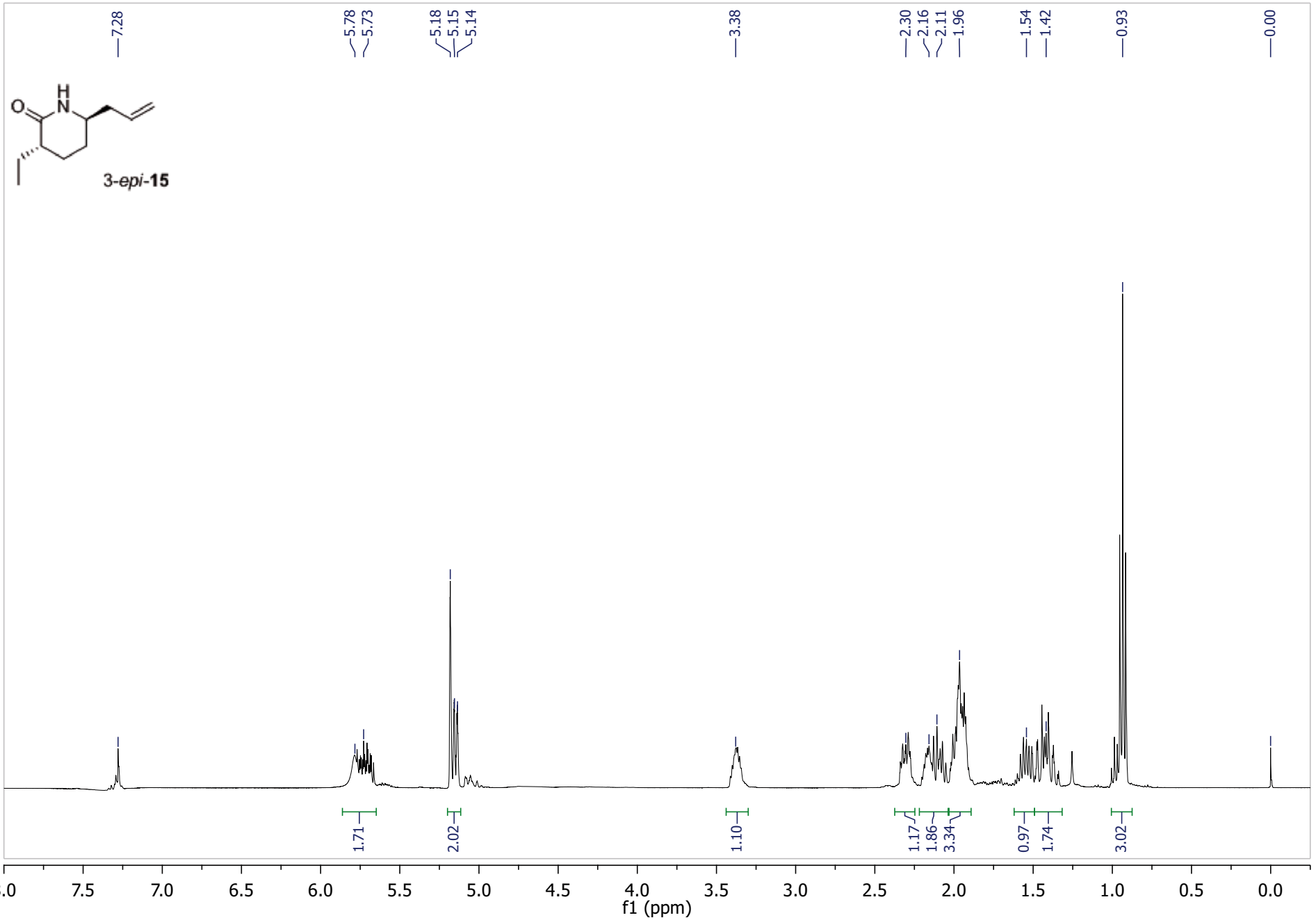
f1 (ppm)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1.42
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2.02
1.10
1.17
1.86
3.34
0.97
1.74
3.02
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6.5
6.0
5.5
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1.0
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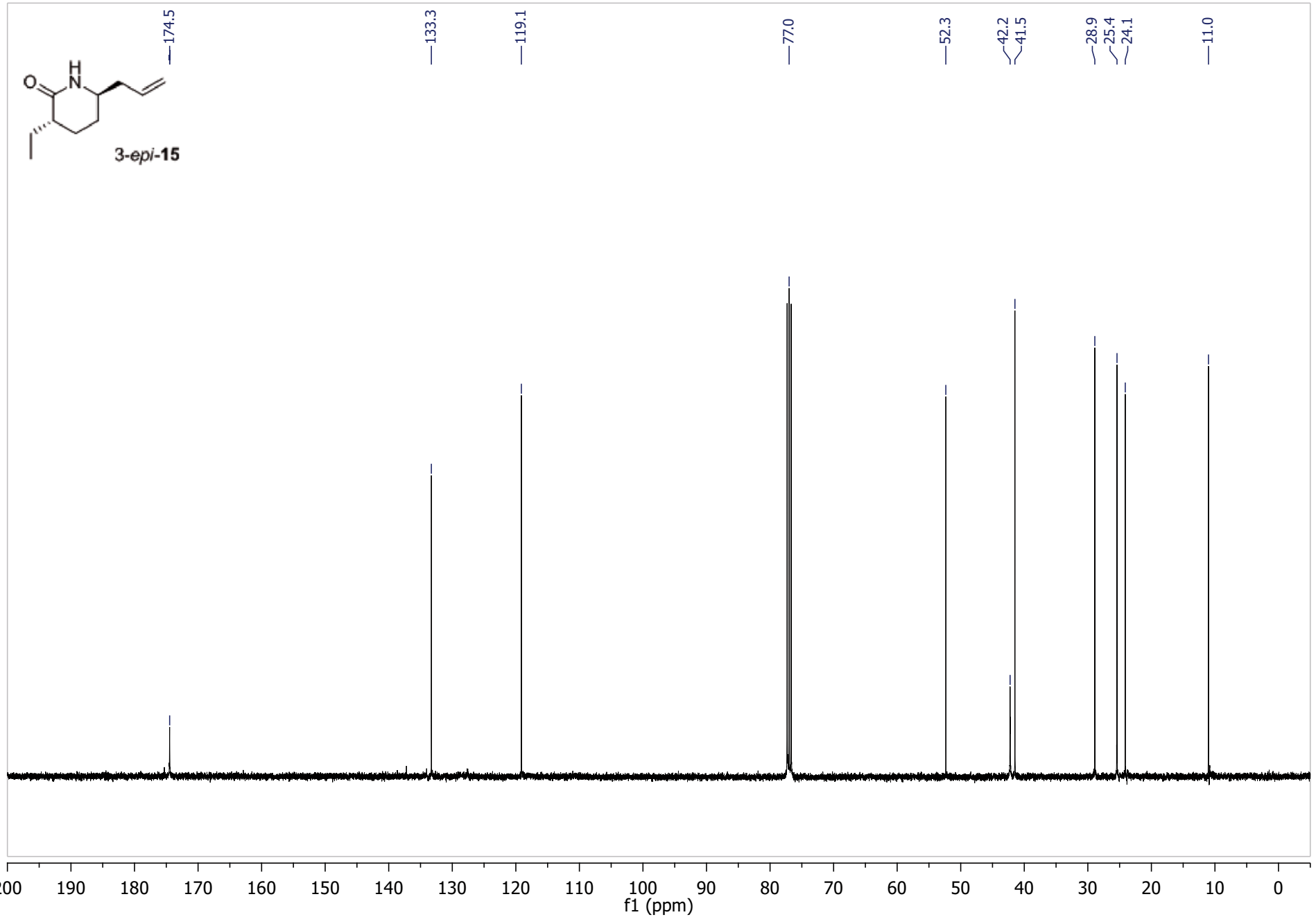
f1 (ppm)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28.9
25.4
24.1
11.0
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H
N
3-epi-15
200
190
180
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130
120
110
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70
60
50
40
30
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10
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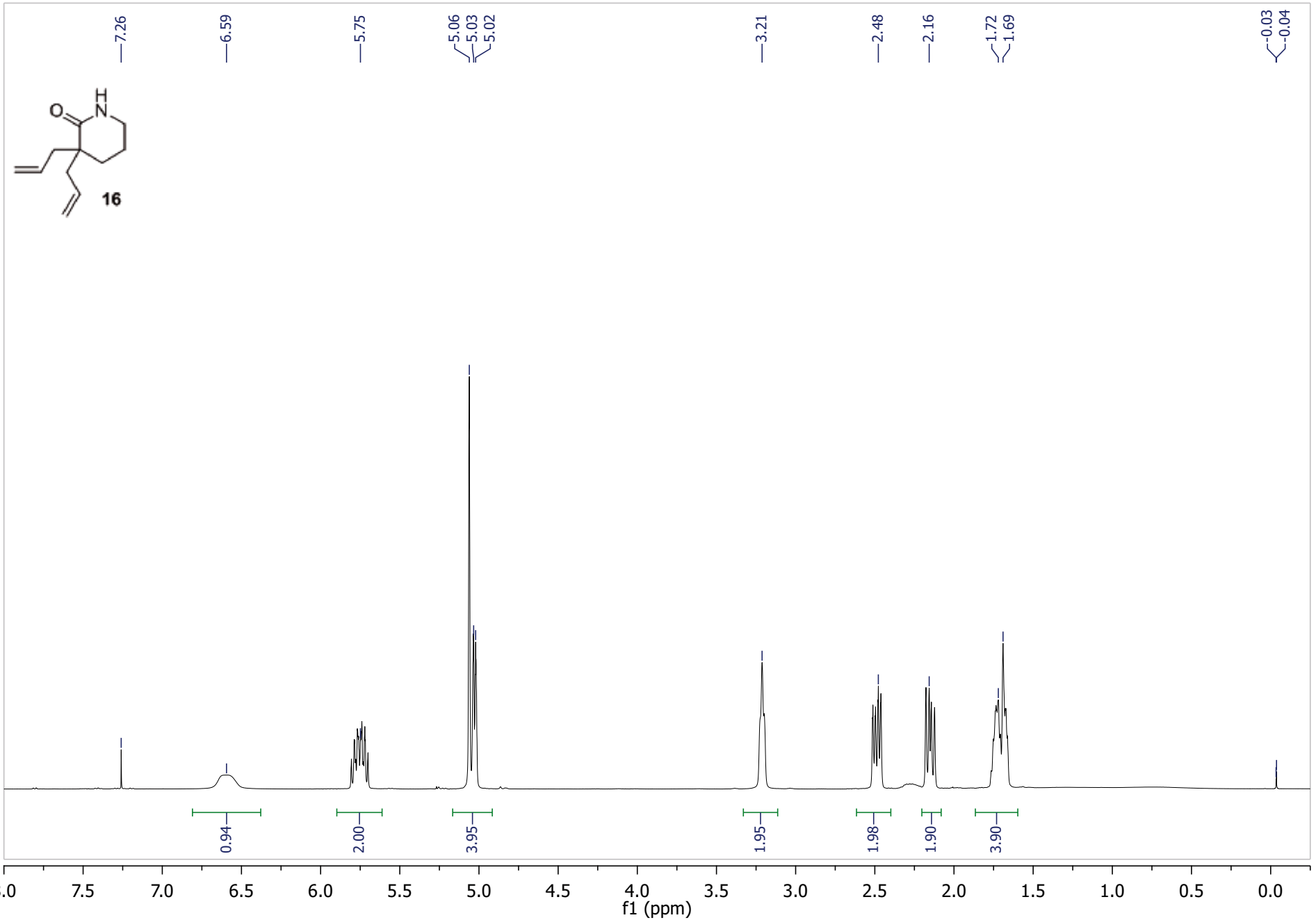
f1 (ppm)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-0.03
-0.04
O
H
N
16
0.94
2.00
3.95
1.95
1.98
1.90
3.90
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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3.5
3.0
2.5
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1.0
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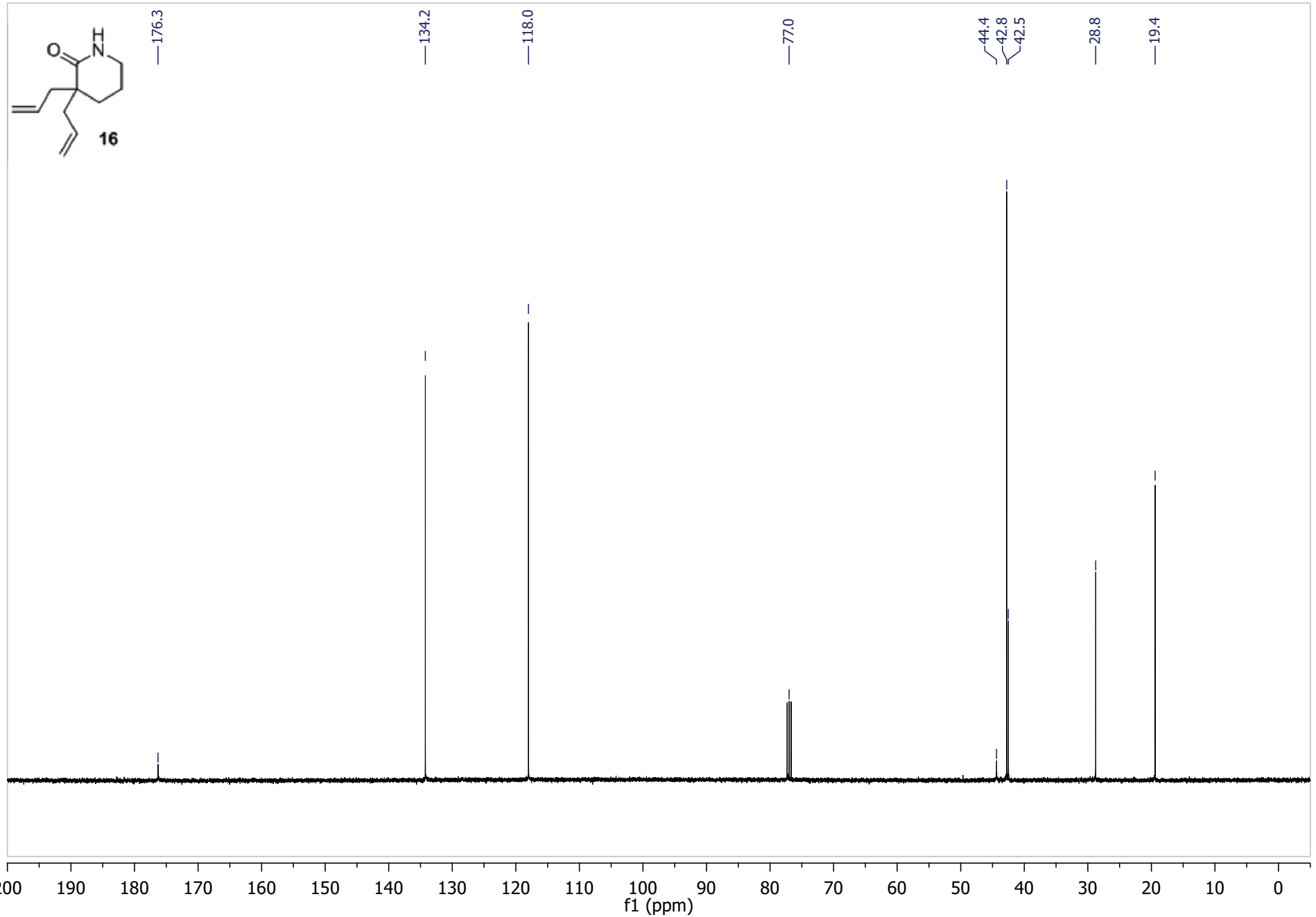
f1 (ppm)


O
H
N
16
176.3
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77.0
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42.5
28.8
19.4
200
190
180
170
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150
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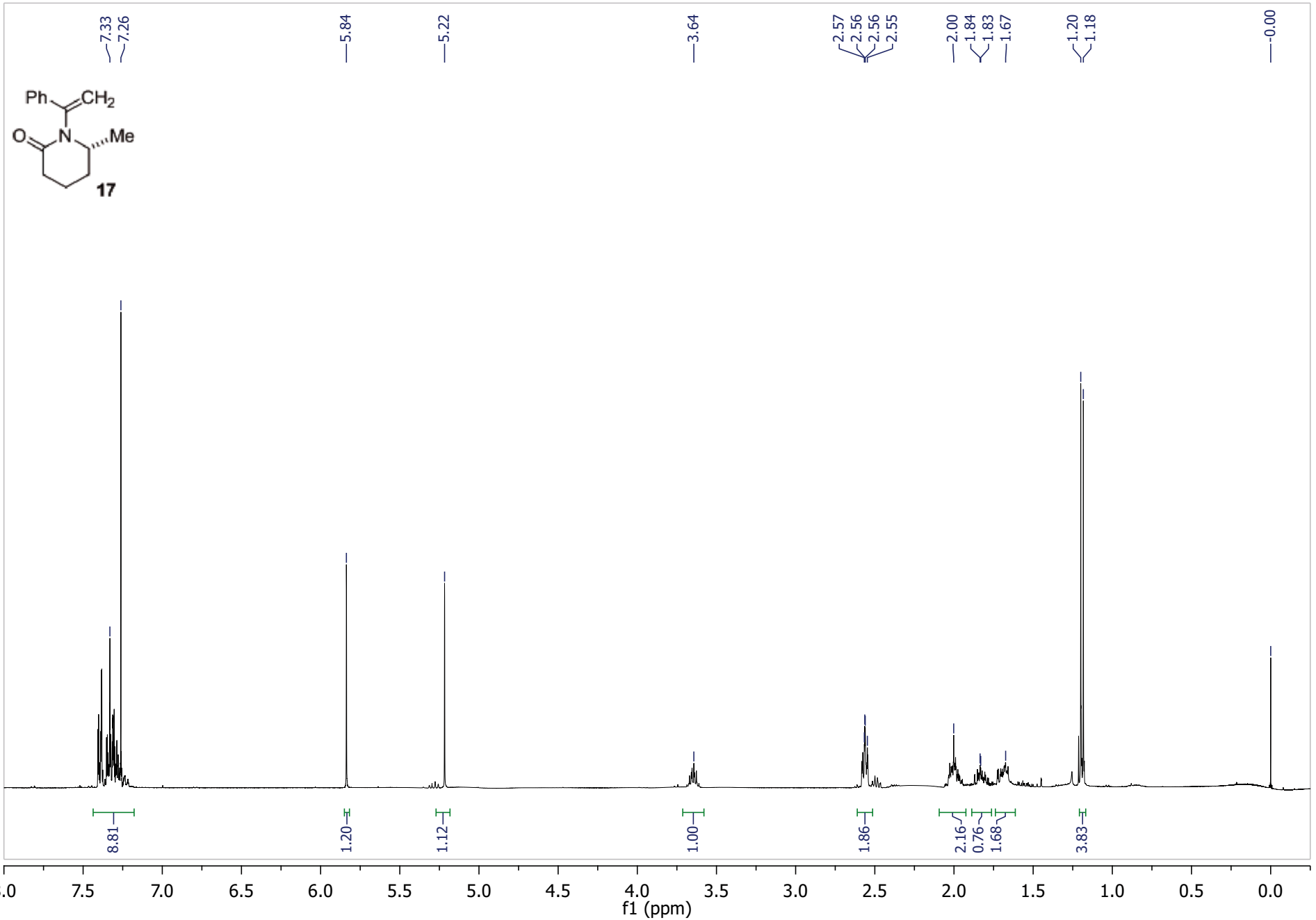
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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1.67
1.20
1.18
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Ph
CH2
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N
Me
17
8.81
1.20
1.12
1.00
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2.16
0.76
1.68
3.83
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6.5
6.0
5.5
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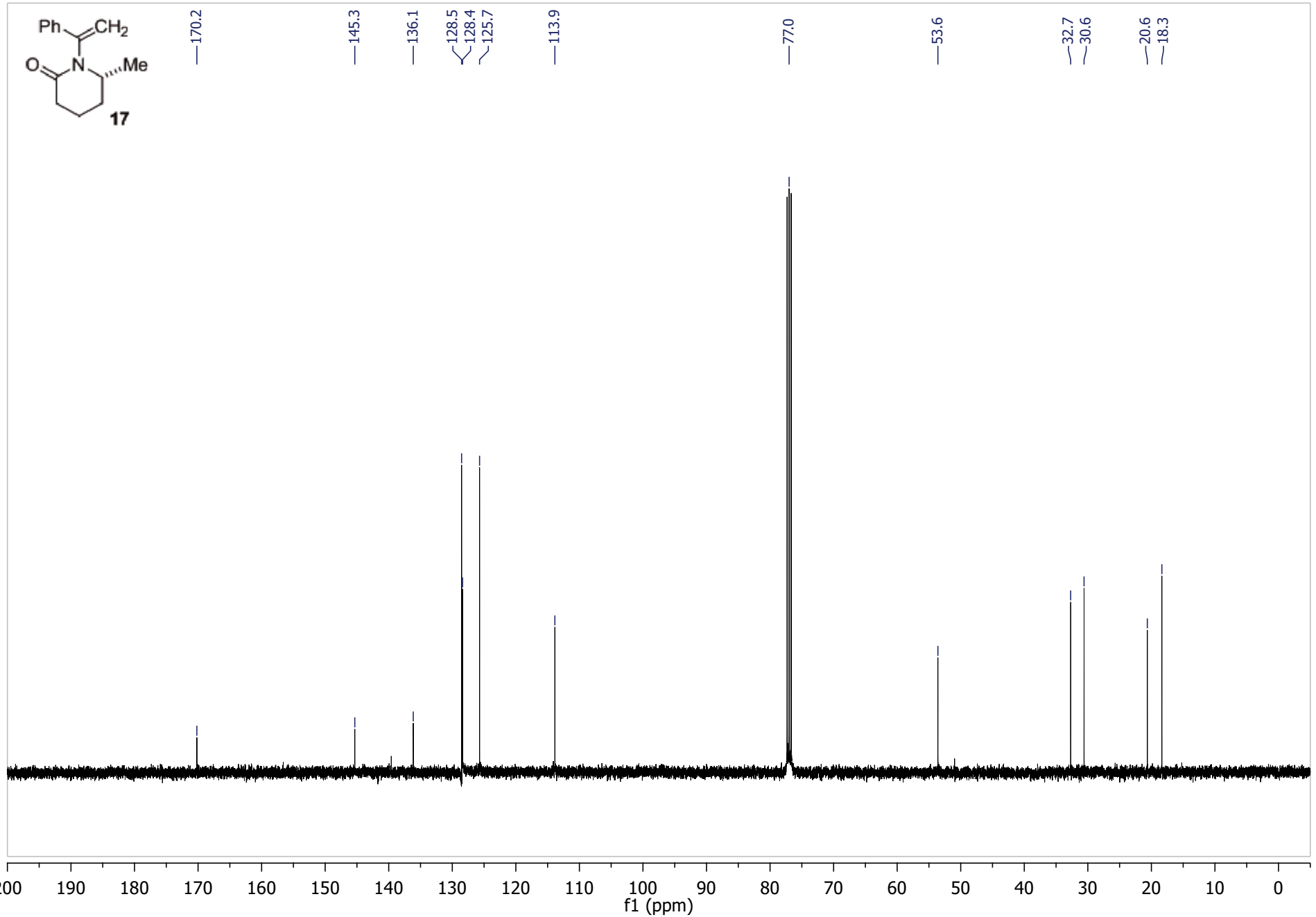
0.0
f1 (ppm)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Me
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125.7
113.9
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32.7
30.6
20.6
18.3
200
190
180
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110
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50
40
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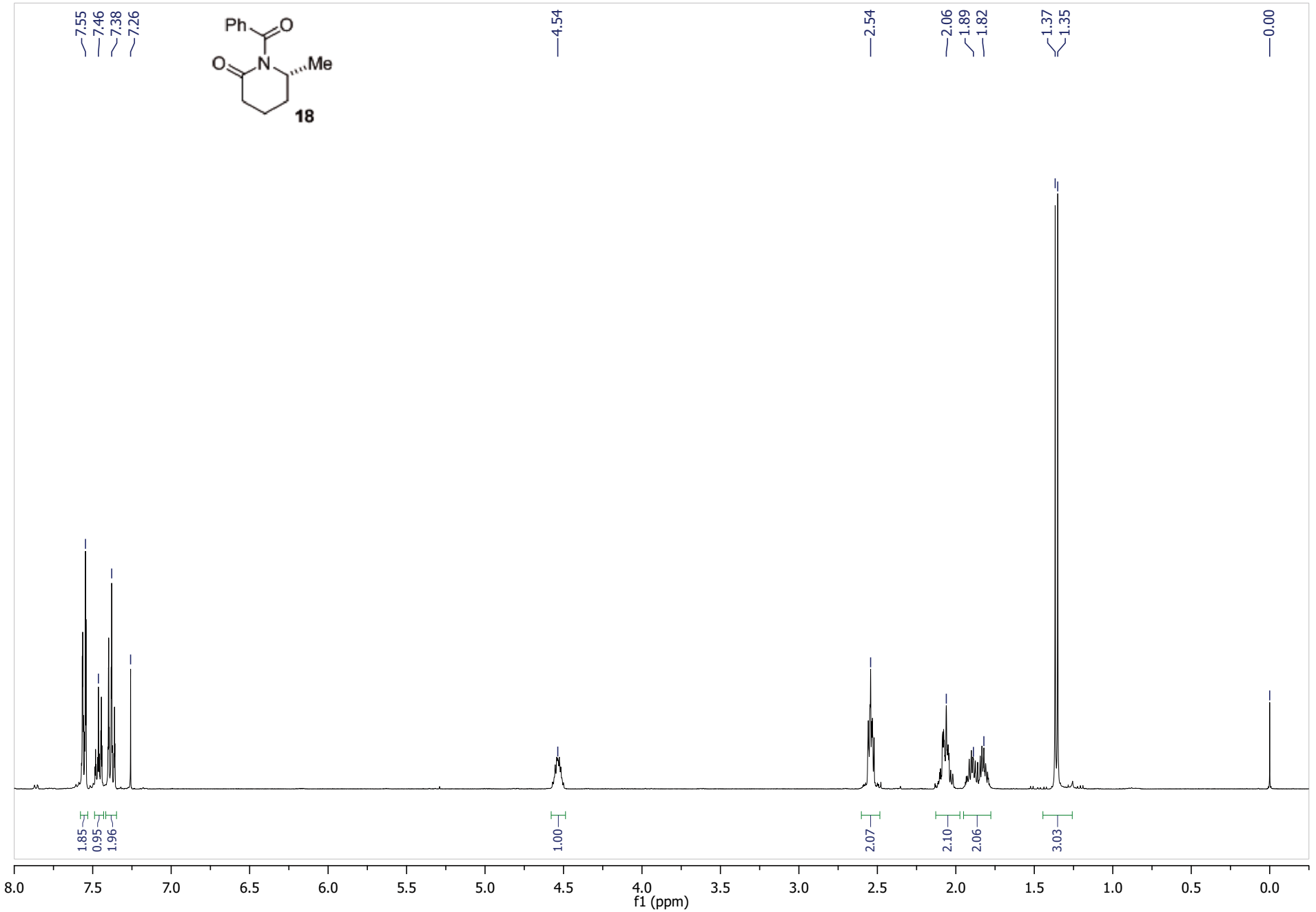
f1 (ppm)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O
N
Me
18
1.85
0.95
1.96
1.00
2.07
2.10
2.06
3.03
8.0
7.5
7.0
6.5
6.0
5.5
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4.5
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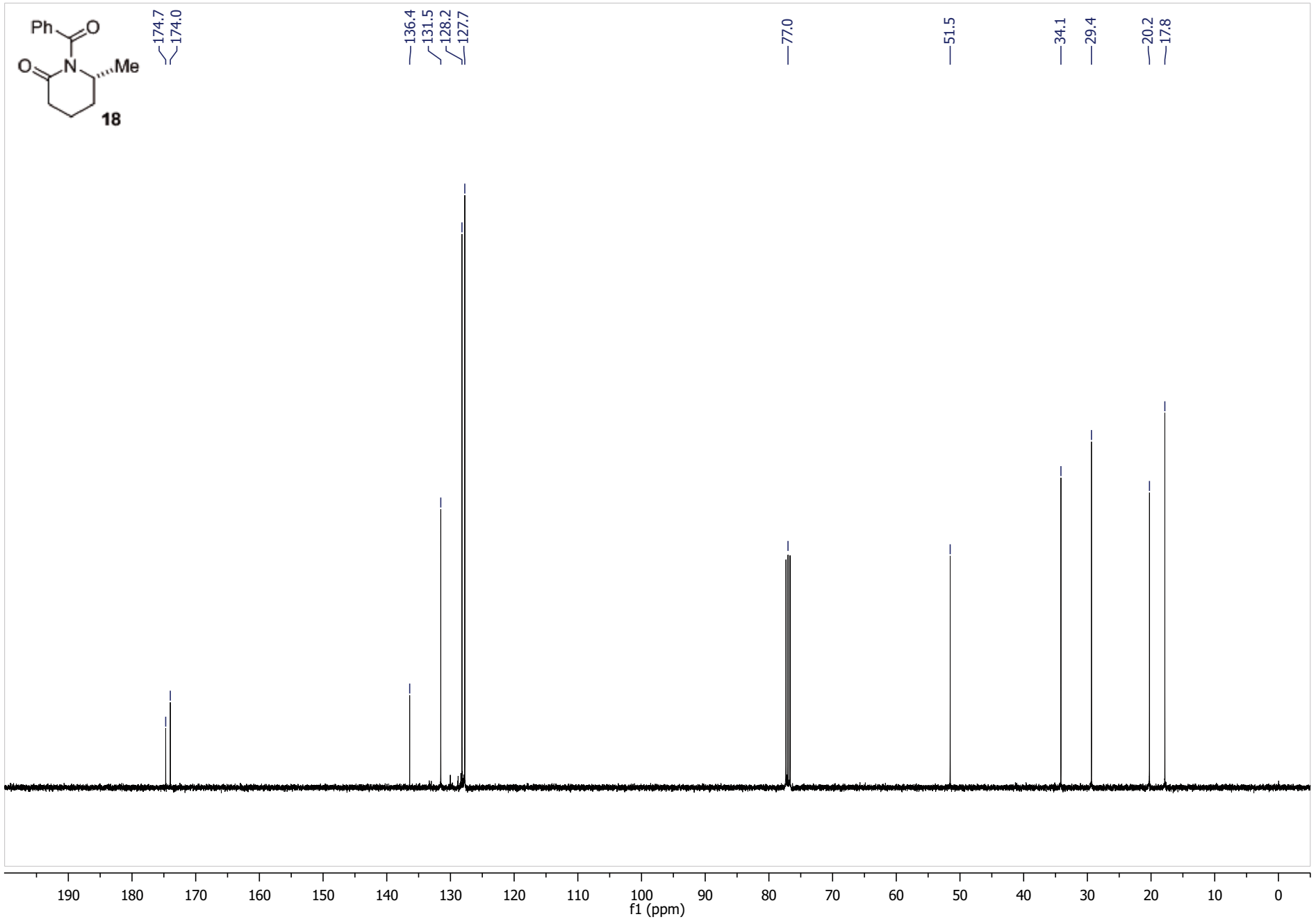
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Ph
O
O
N
Me
18
174.7
174.0
136.4
131.5
128.2
127.7
77.0
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34.1
29.4
20.2
17.8
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

CHAPTER 2

SUPPORTING MATERIAL

Synthesis of triheptanoin and formulation as a solid diet for rodents

Semak, V.; Semakova, J.; Halbaut, L.; Asó, E.; Ferrer, I.; Calpena, A.; Escolano, C.; Perales, J. C. *Eur. J. Lipid Sci. Technol.* **2012**, *114*, 889-895.

DOI: 10.1002/ejlt.201100425

GALENIC PART

Spreading capacity – measurement.

A sketch in vertical cross-section of the overall apparatus is shown in Fig. 1. This device includes two stainless coaxial cylinders (a, b) and a crosslinked transparent plastic cover (c). The outer cylinder (a), 37 mm high and 50 mm diameter has a round plate top (d), 3 mm high and 90 mm diameter, provided with a lever (e) intended to turn right or left this element. The bottom of the outer cylinder presents a difference in level of 2 mm between the two half parts and its central zone forms a cavity (175.8 mm^2 base) through which the inner cylinder (b) with a diameter and a height according to the size of the cavity of the outer cylinder is introduced.

This inner cylinder is in fixed position on the central part of the round support (f), 40 mm high and 100 mm diameter. On the support, there is a round top screw (g), 2 mm high, intended to raise the outer cylinder 2 mm when the lever is turned 180°.

The basic steps of the operative method are:

① Introduce the inner cylinder into the cavity of the outer cylinder. In this "Stand Position", the tops of both cylinders are on the same level.

② Turn 180 º the lever (e). The assembly is now in "Filling Position": a cavity appears (2 mm depth and 175.8 mm^2 base) in the central part of the device. Fill the cavity with sample and level carefully,

③ Place the transparent cover (c) upon the top (d) of the outer cylinder and then place the lower selected weight (200 g in this case) on the centre of the cover. The assembly is now in "Start Position".

④ Turn 180 º the lever in the same direction as before. The assembly is now in "Measure Position": the sample is situated between the cover and the plate, squashed by the weight. After 1 minute from the weight location, remove the weight and measure the two perpendicular diameters of the sample spreading circle (D_1 and D_2). Calculate the corresponding spreading area (E) in mm^2 from the mean diameter (D): $E = (\pi \cdot D^2)/4$.

Clean the apparatus and repeat the assay with two new sample and express the results as mean and standard deviation of three measurements (in this case at room temperature 23 ± 1 ºC).

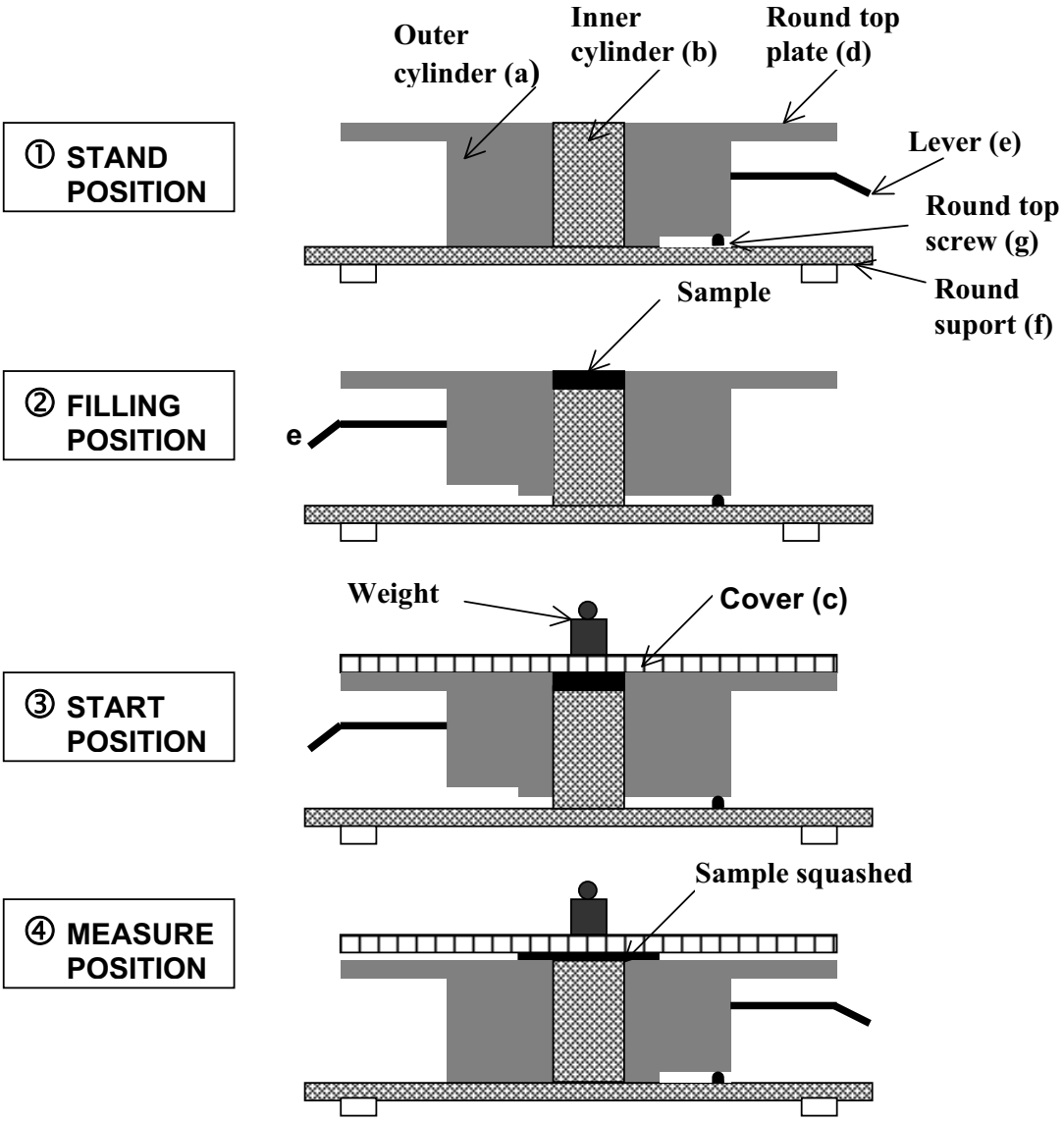
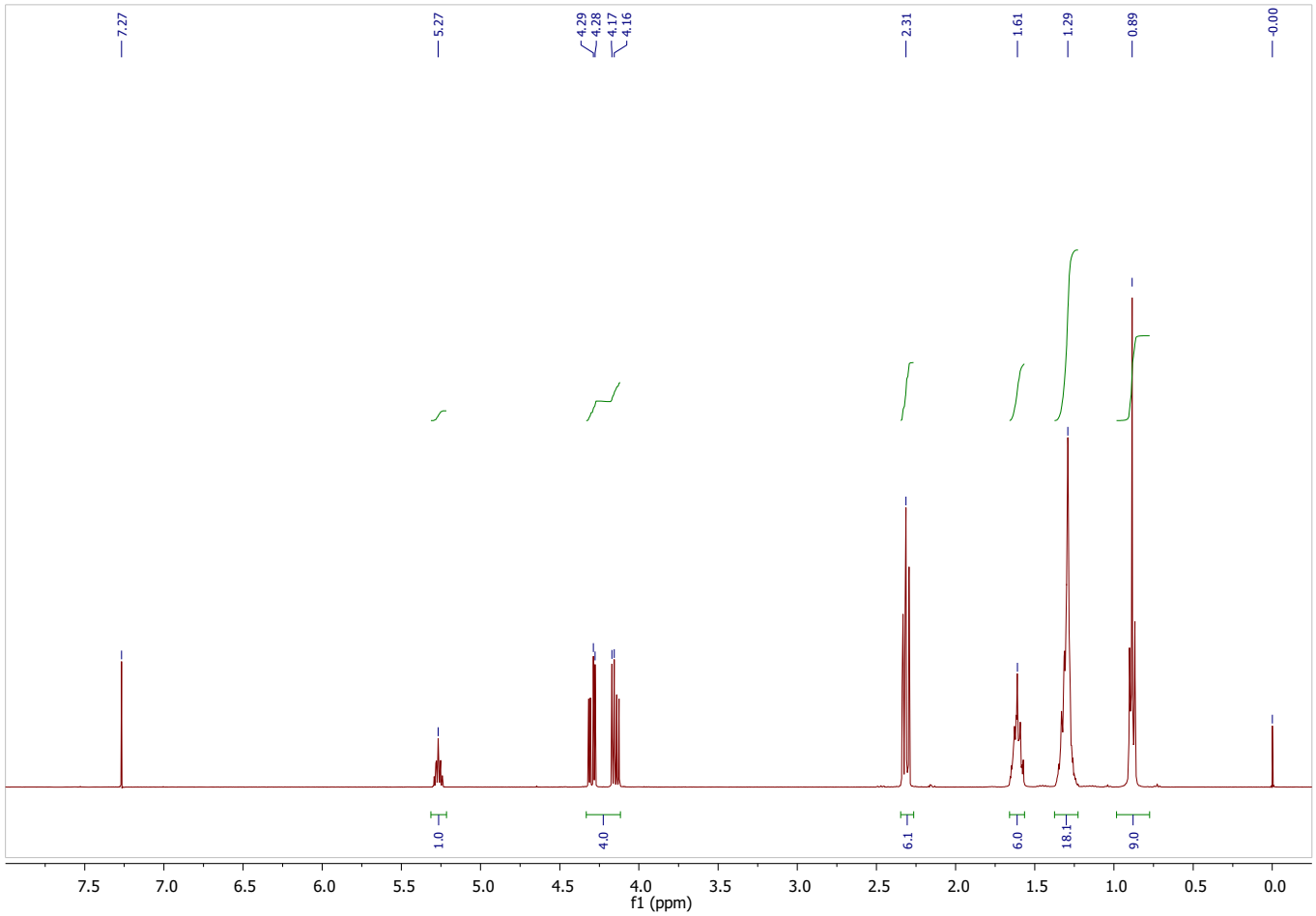


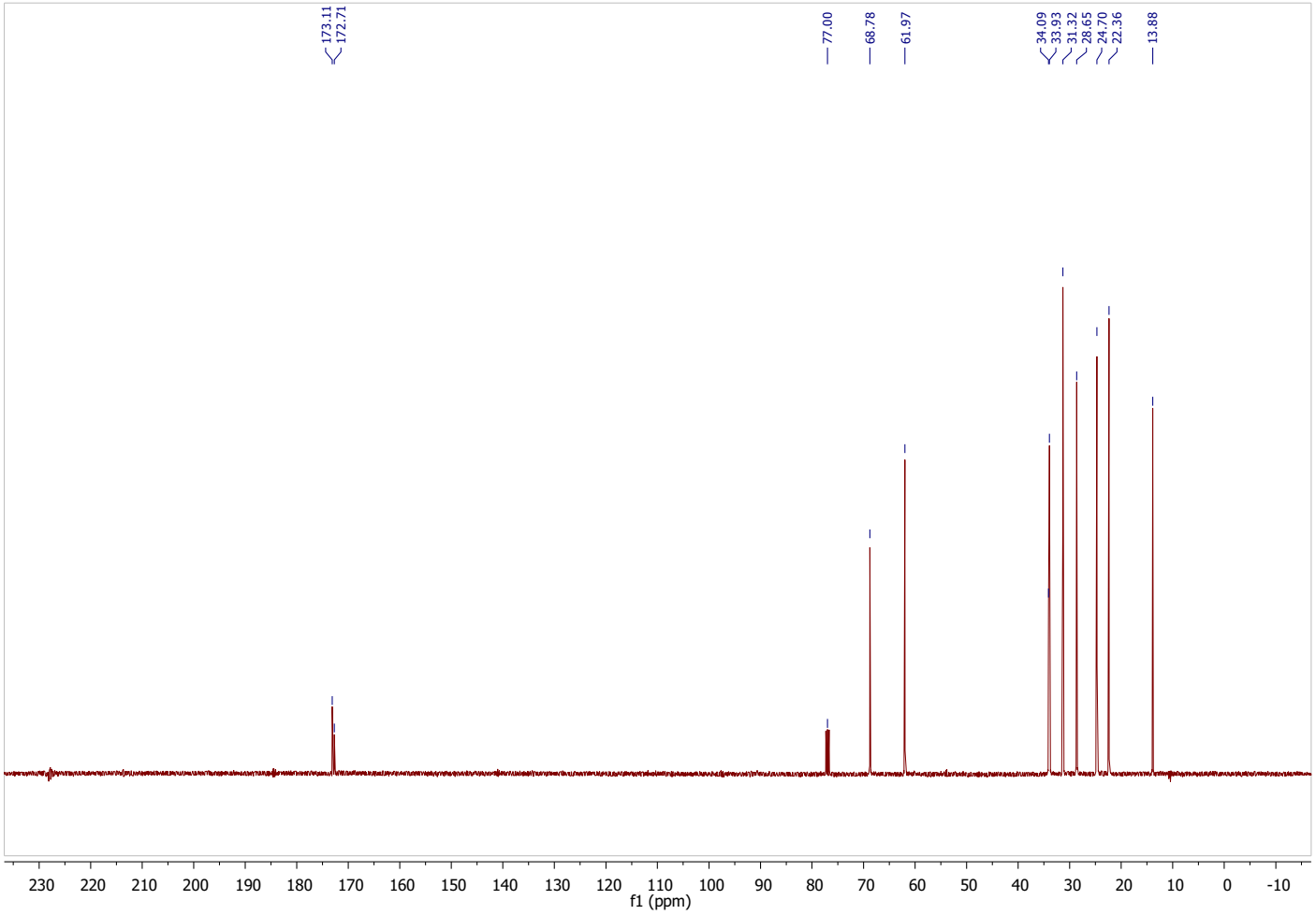
Figure 1: Sketch in vertical cross-section of the apparatus for spreading capacity determination.

CHEMICAL PART: Analytical data of triheptanoin:

Copy of ^{1}H-NMR spectrum (400 MHz, $CDCl_3$)



Copy of ^{13}C-NMR spectrum (100.6 MHz, $CDCl_3$)



Copy of GC-MS spectrum

Figure 2: Gas Chromatography-Mass Spectrometry (GC-MS) spectrum of synthesized triheptanoin shows only one peak at 17.7 min.

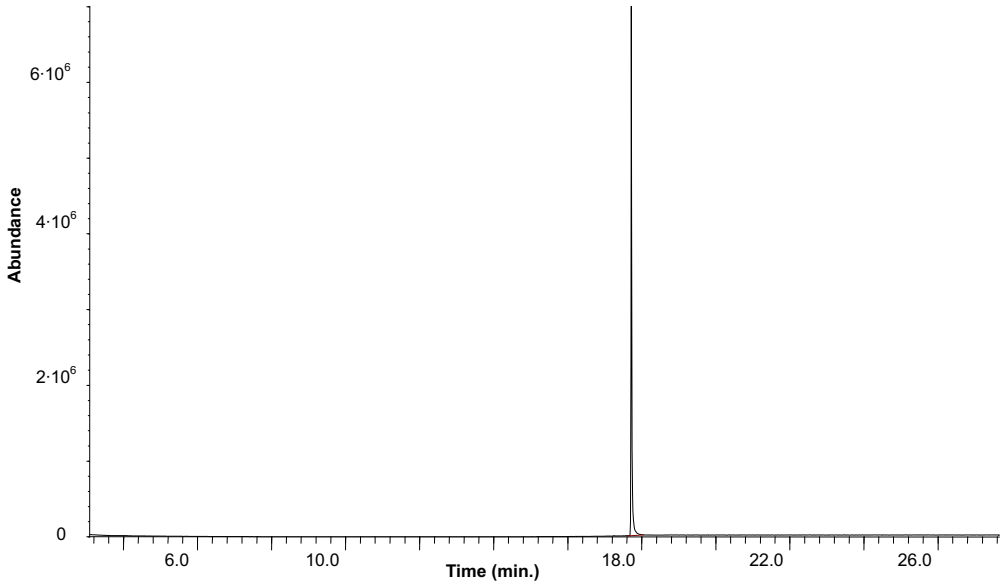
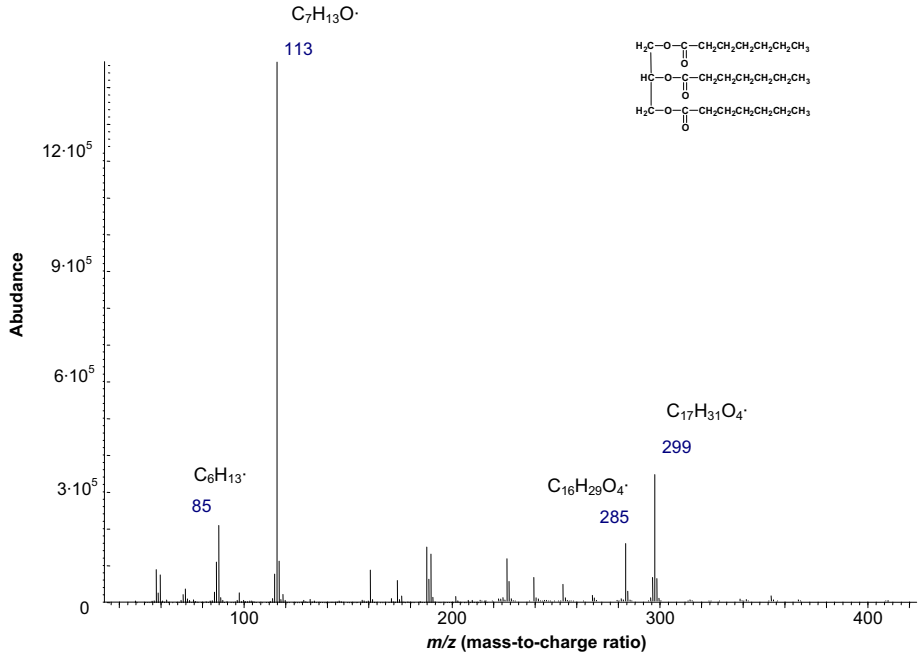


Figure 3: Mass spectrum analysis – Chemical fragmentation of peak (17.7 min) clearly shows only fragments of triglyceride structure.



CHAPTER 3

SUPPORTING MATERIAL

Toluene dioxygenase (TDO) mediated oxidation of halogen-substituted benzoate esters

Semak, V.; Metcalf, T. A.; Endoma-Arias, M. A. A.; Mach, P.; Hudlicky, T.
Org. Biomol. Chem. **2012**, *10*, 4407-4416.

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Toluene dioxygenase-mediated oxidation of halogen-substituted benzoate esters

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[b] Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynská dolina, 842 15 Bratislava, Slovakia

[To whom correspondence regarding calculations should be addressed]

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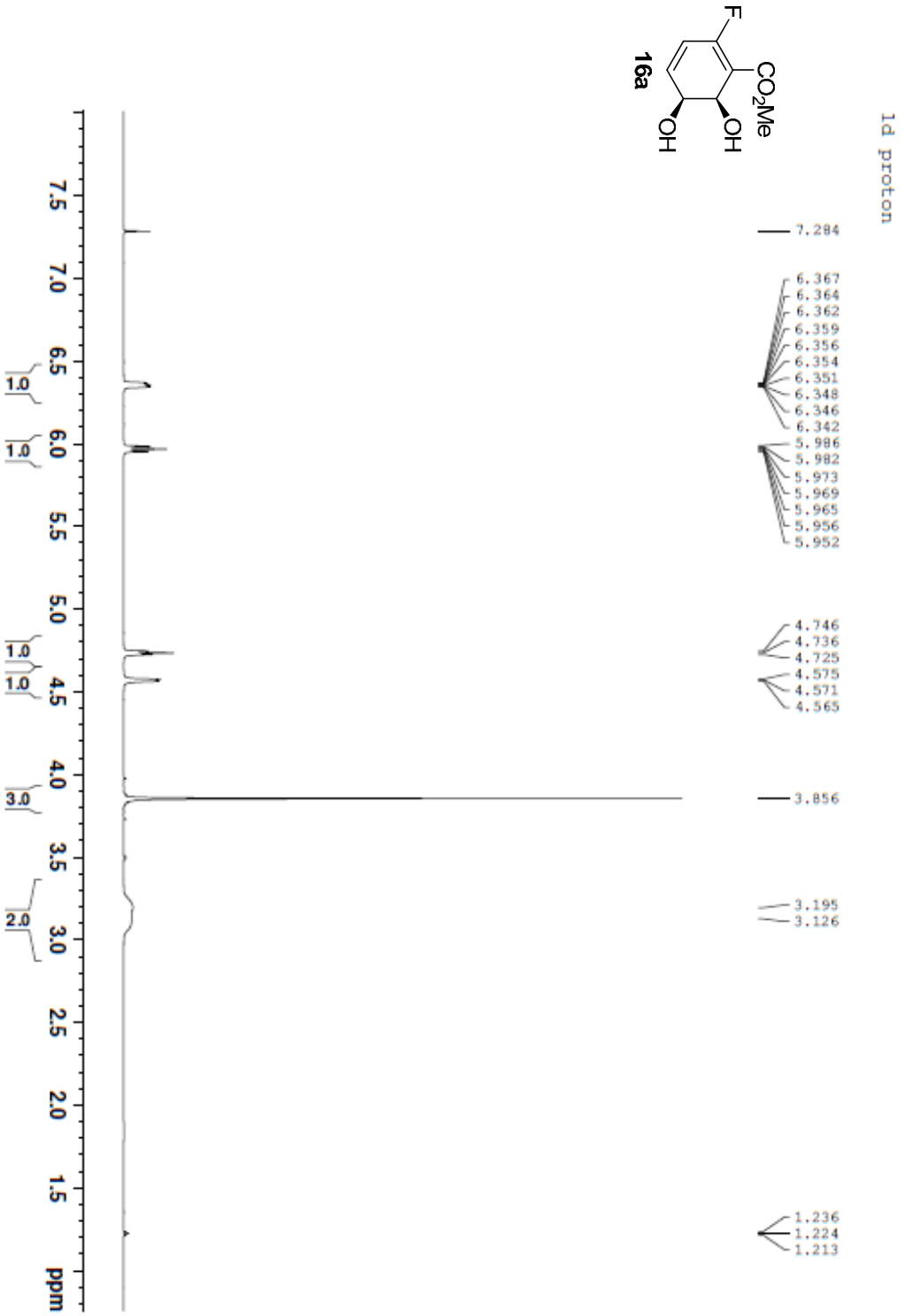
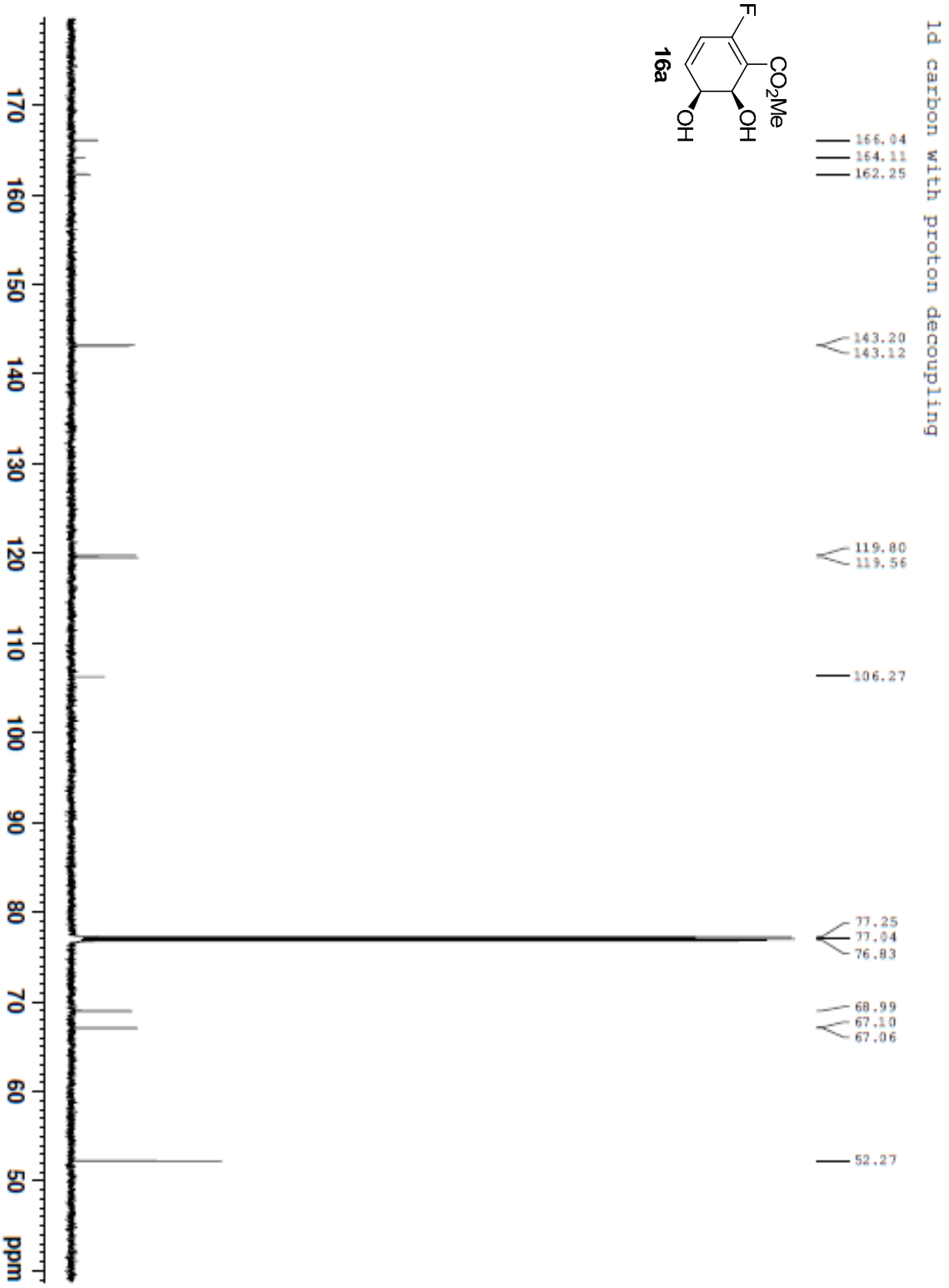
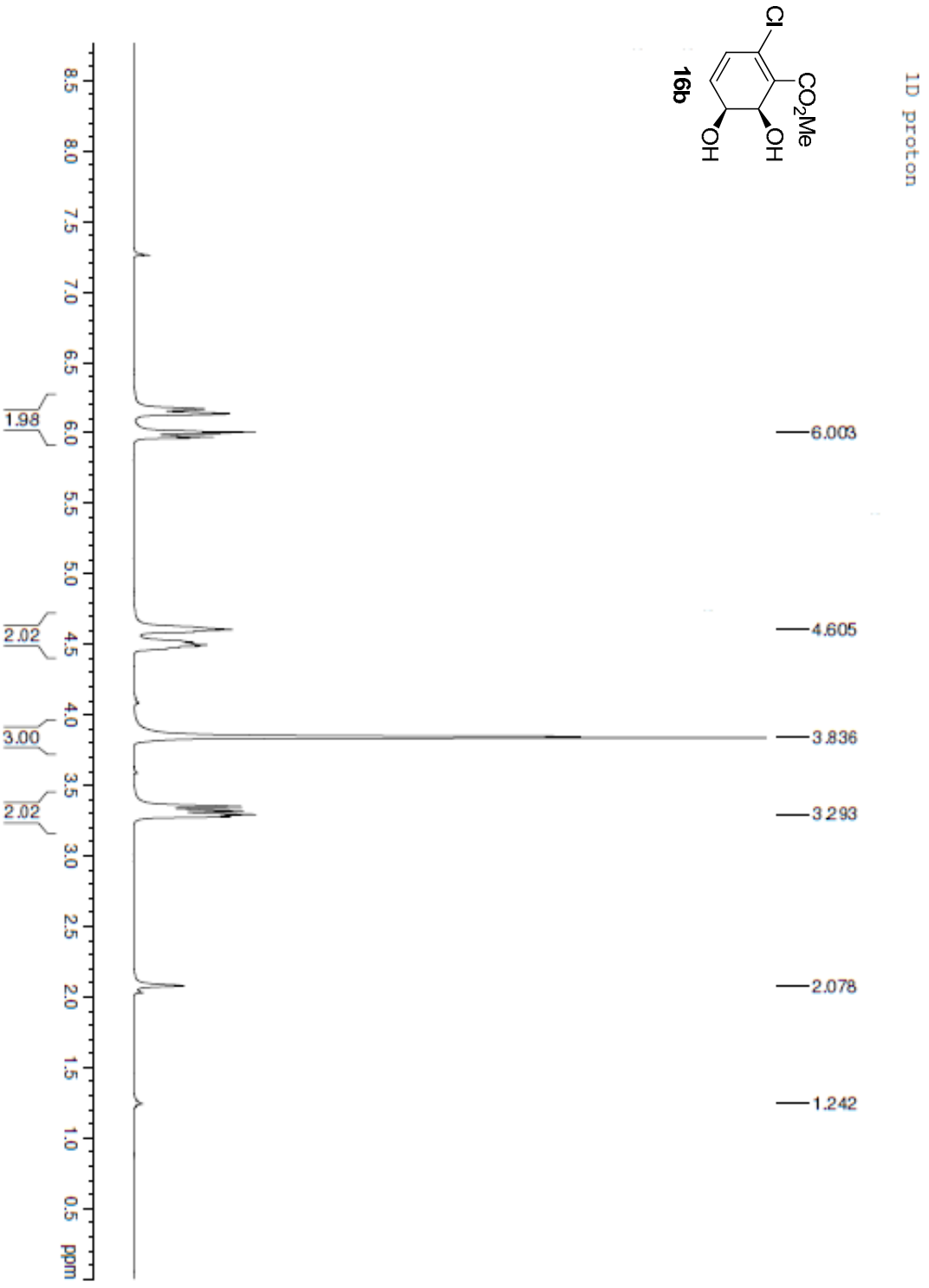
Copies of ^{1}H and ^{13}C spectra	S2-S25



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2.078
1.242
8.5
8.0
7.5
7.0
6.5
6.0
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4.5
4.0
3.5
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2.5
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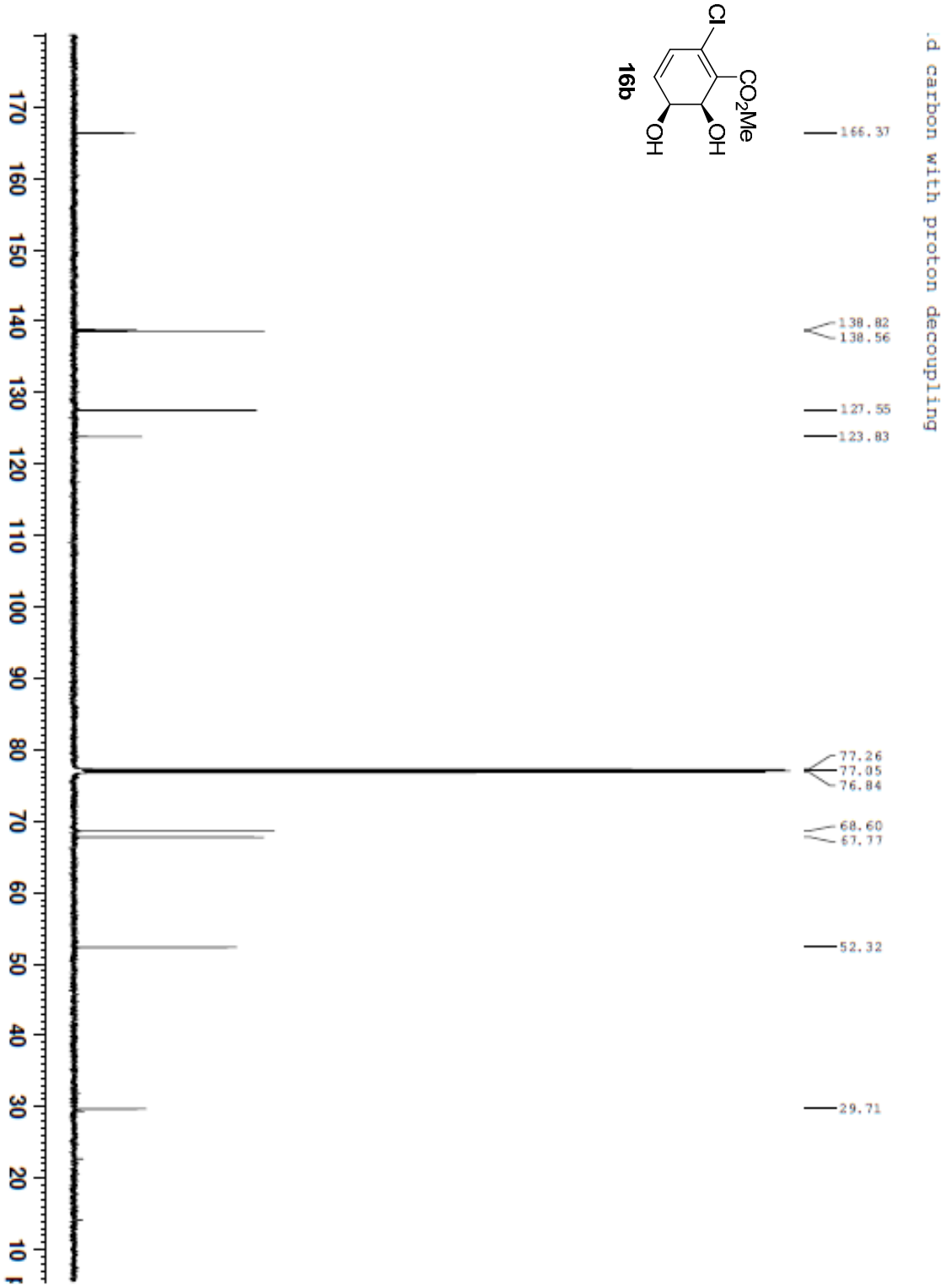
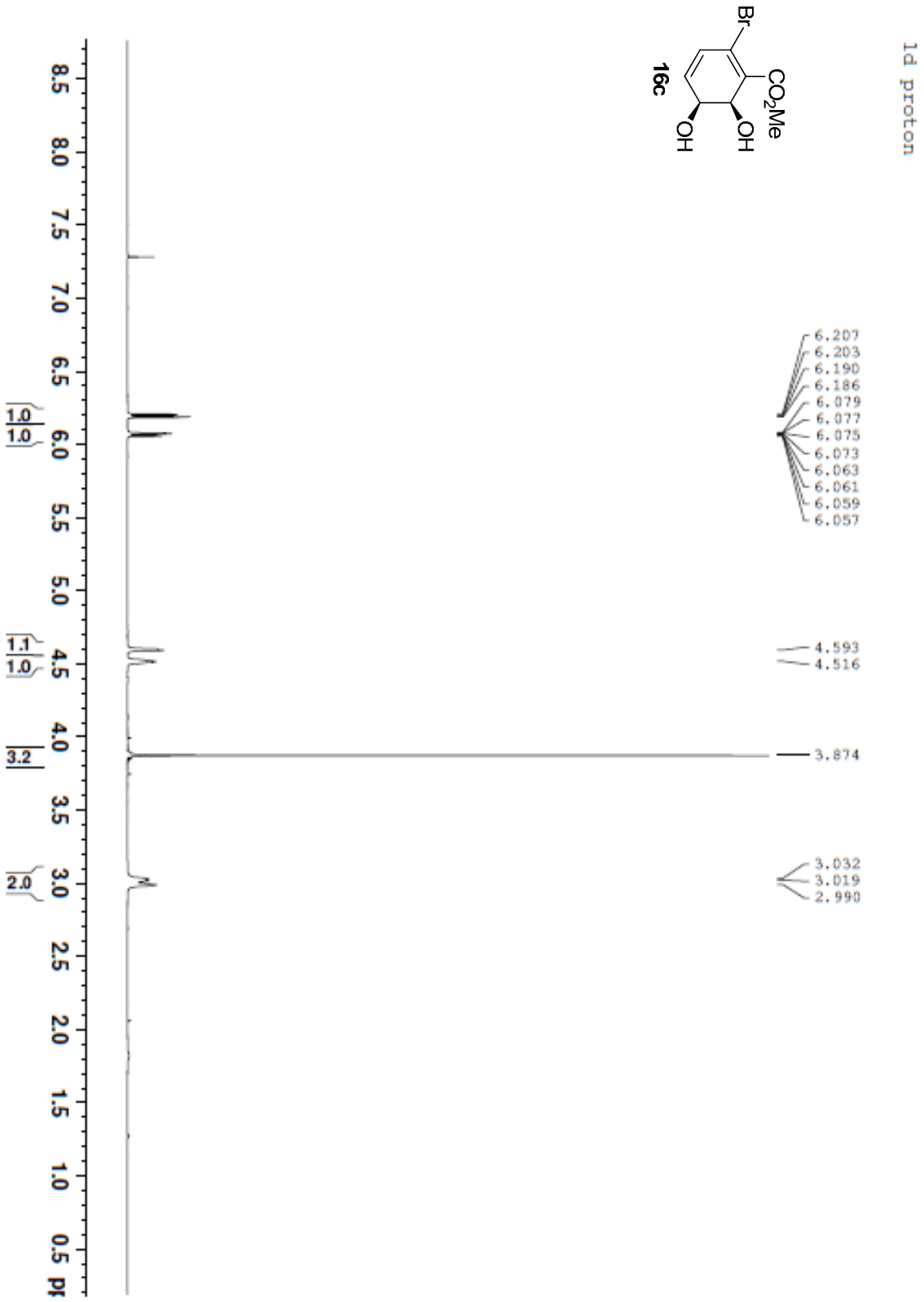
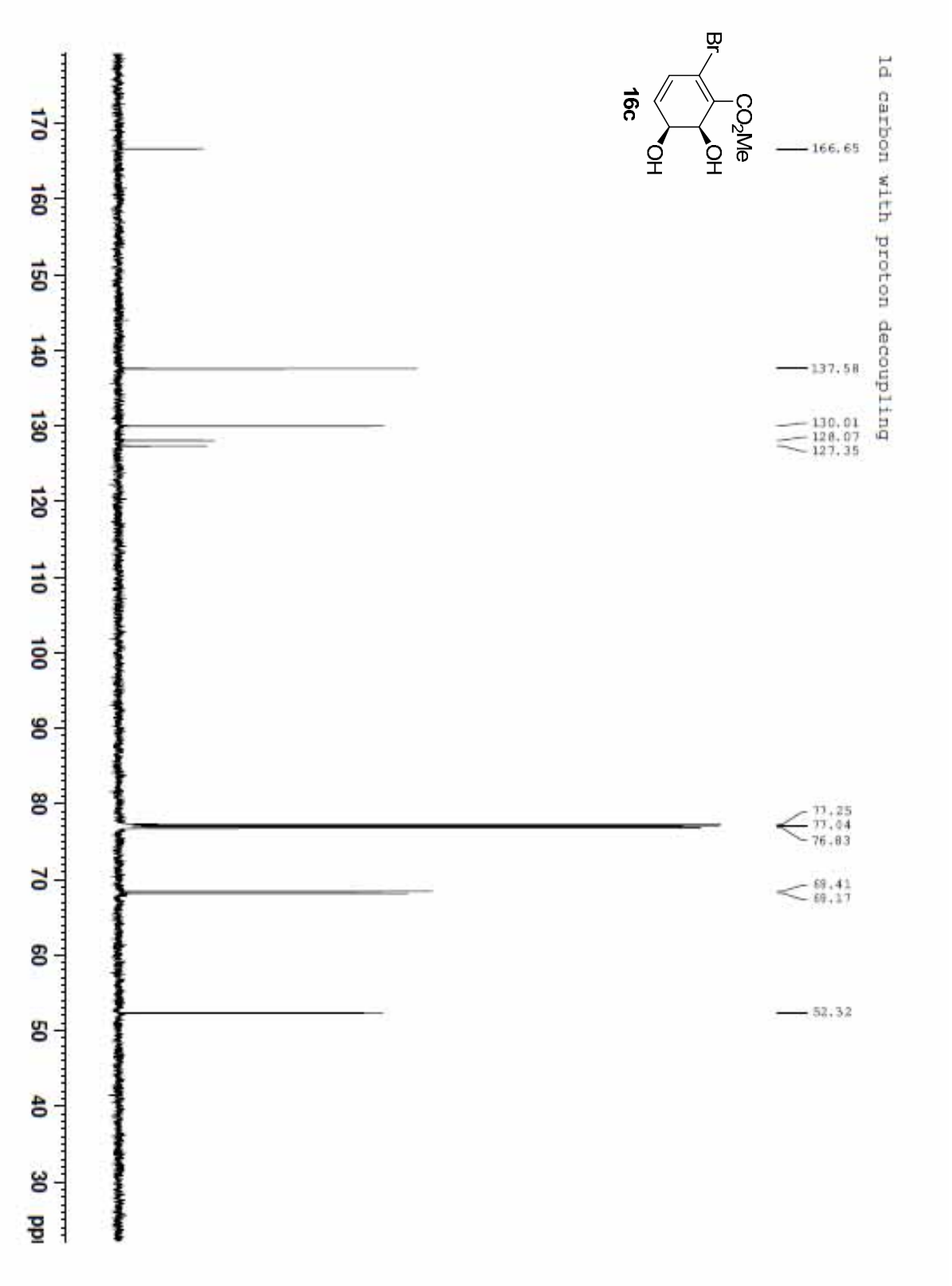
1.0
0.5
ppm






1d carbon with proton decoupling
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CO2Me
OH
OH
16c
166.65
137.58
130.01
128.07
127.35
77.25
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76.83
69.41
69.17
52.32
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160
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120
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100
90
80
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60
50
40
30
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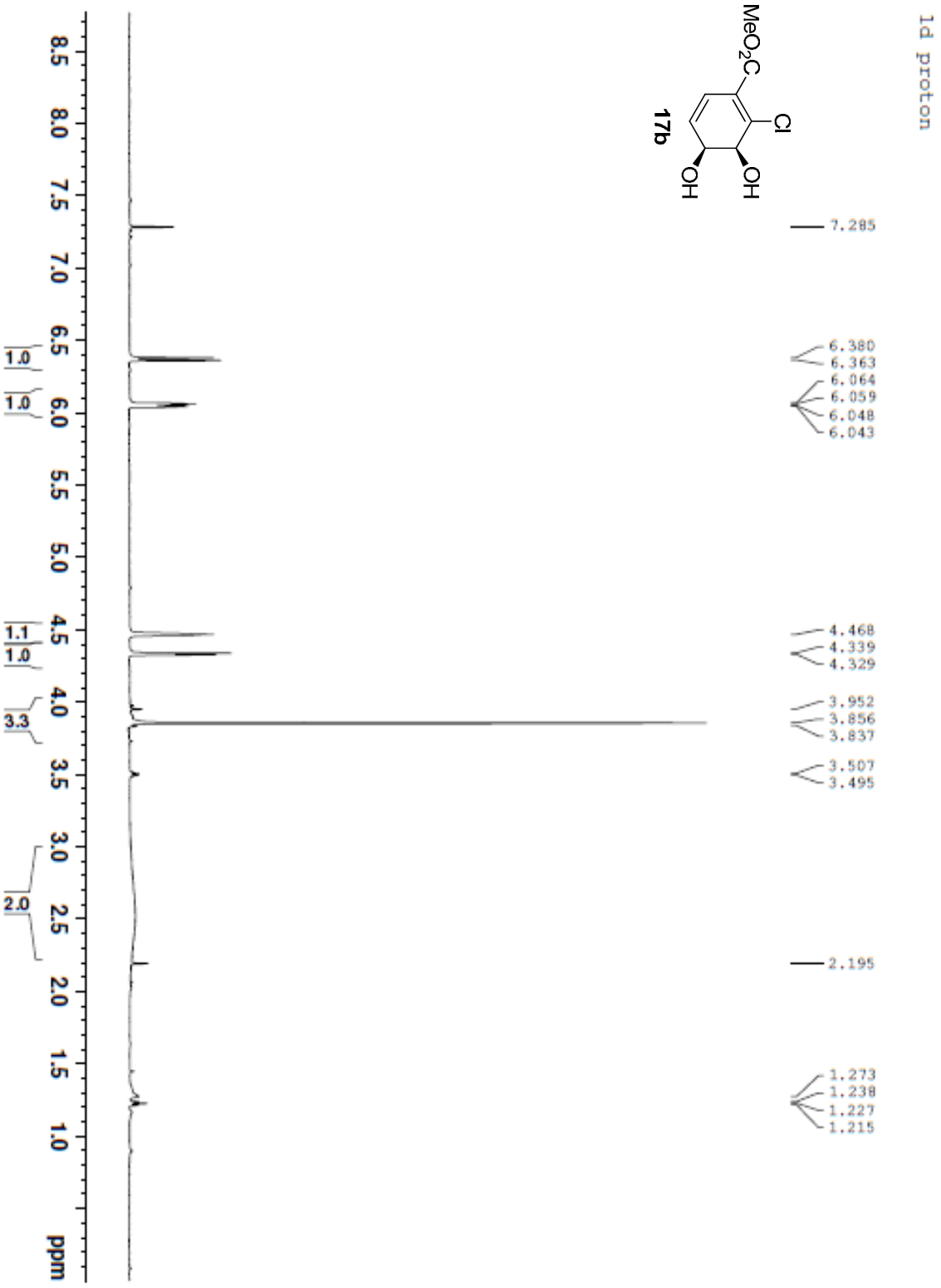

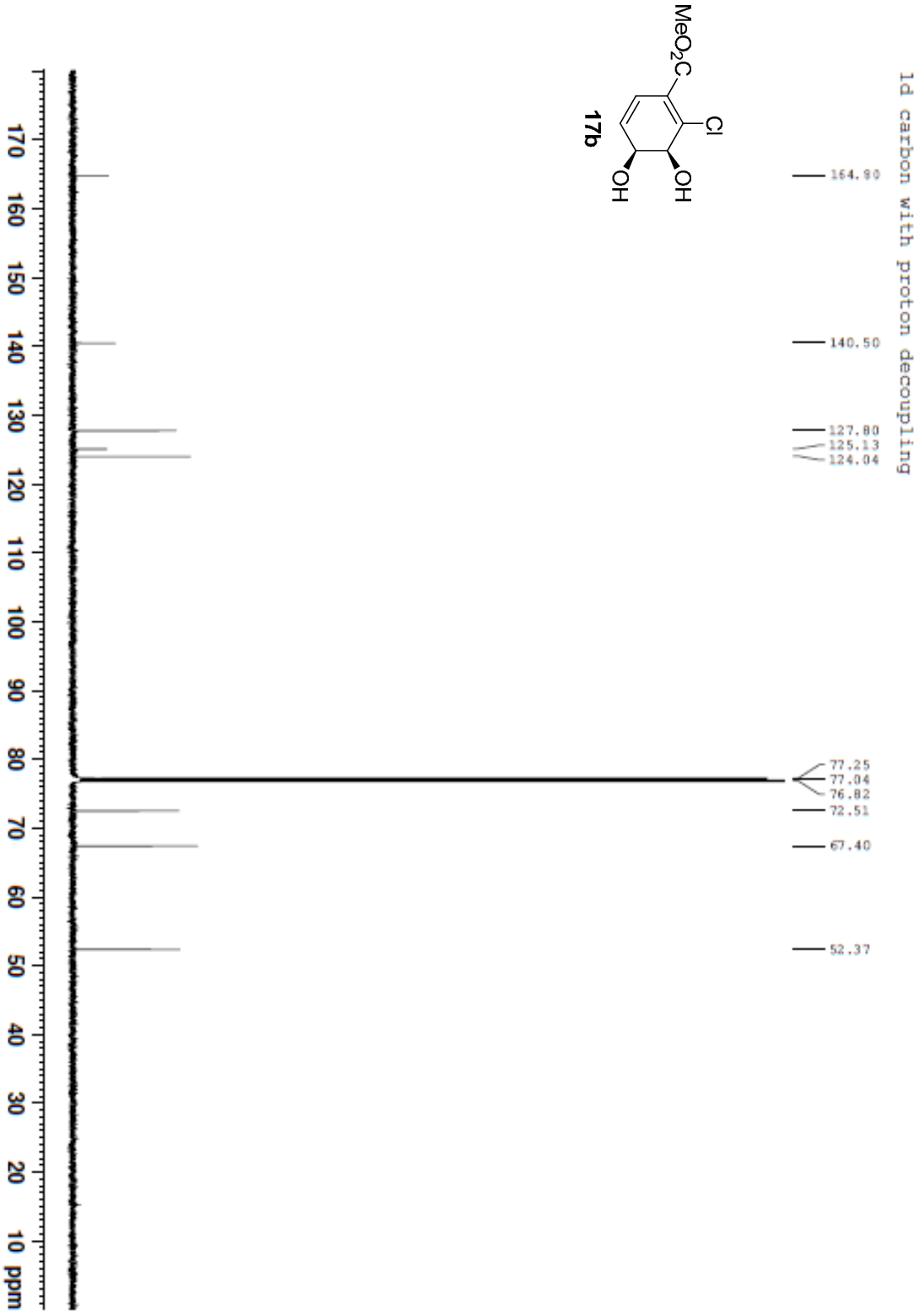

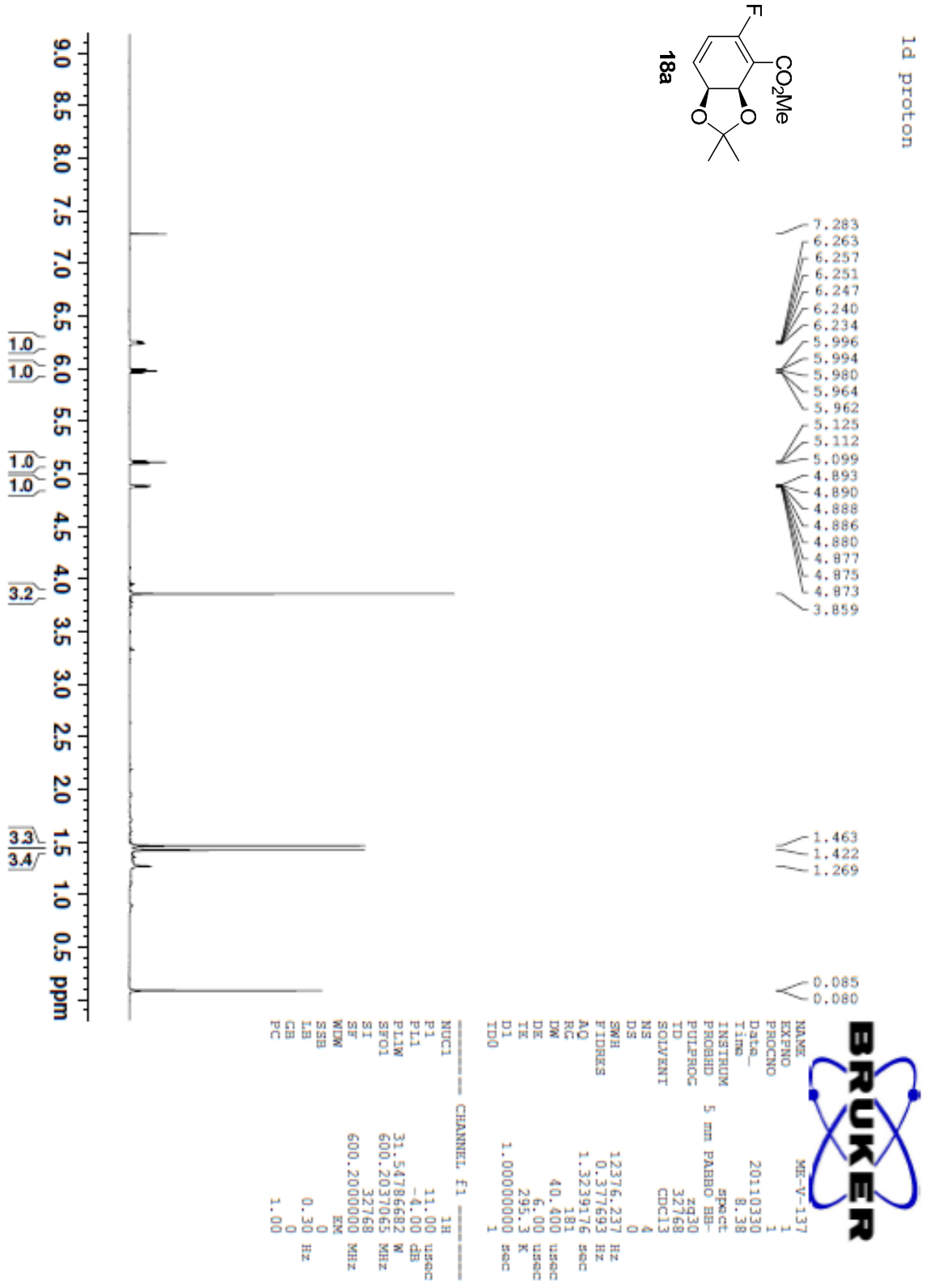
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6.263
6.257
6.251
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6.240
6.234
5.996
5.994
5.980
5.964
5.962
5.125
5.112
5.099
4.893
4.890
4.888
4.886
4.880
4.877
4.875
4.873
3.859
1.463
1.422
1.269
0.085
0.080
1.0
1.0
1.0
1.0
3.2
3.3
3.4
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
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FIDRES 0.377693 Hz
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RG 181
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DE 6.00 usec
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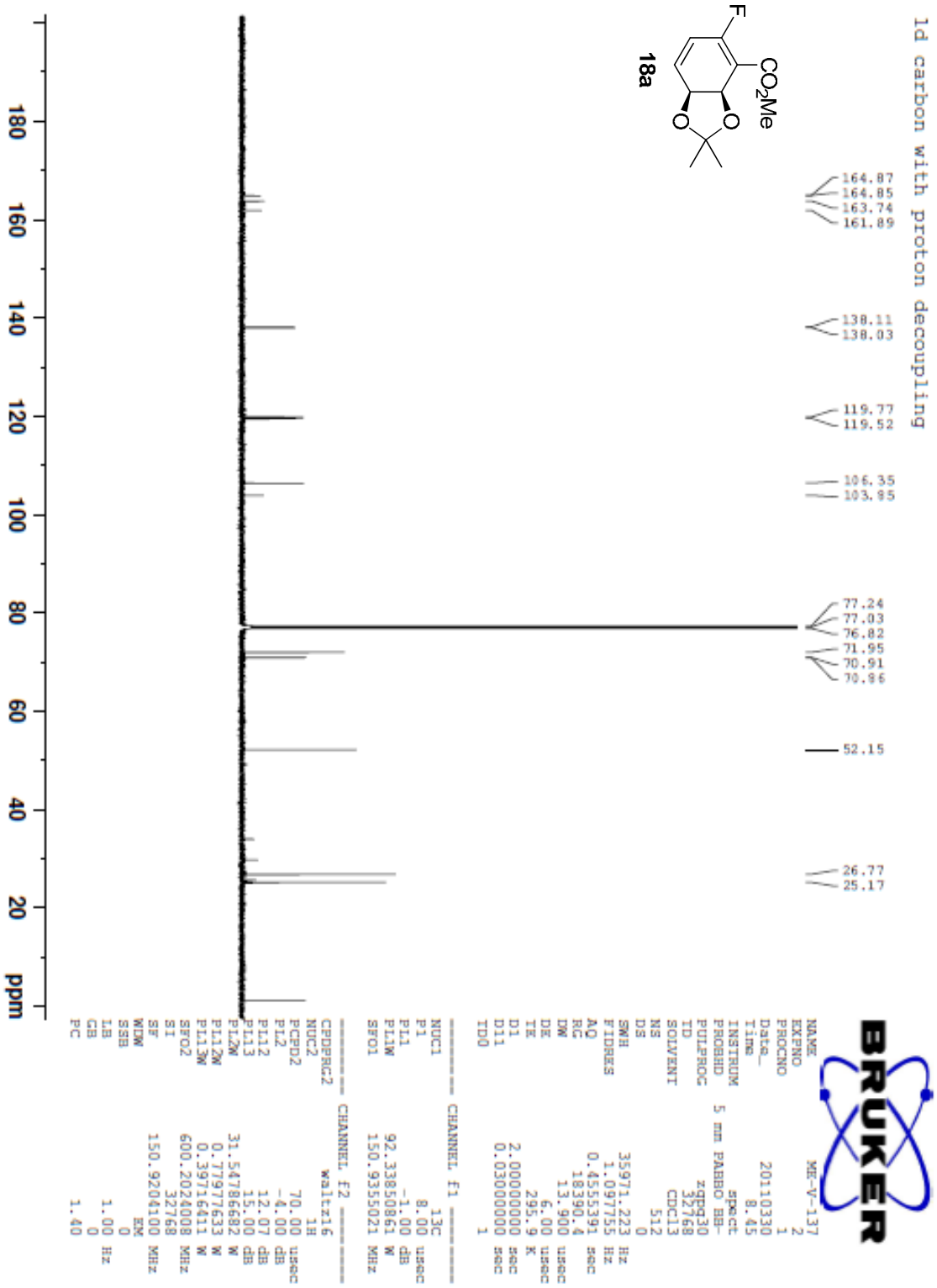
1d carbon with proton decoupling
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CO2Me
O
O
O
164.87
164.85
163.74
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138.03
119.77
119.52
106.35
103.95
77.24
77.03
76.82
71.95
70.91
70.86
52.15
26.77
25.17
180
160
140
120
100
80
60
40
20
ppm
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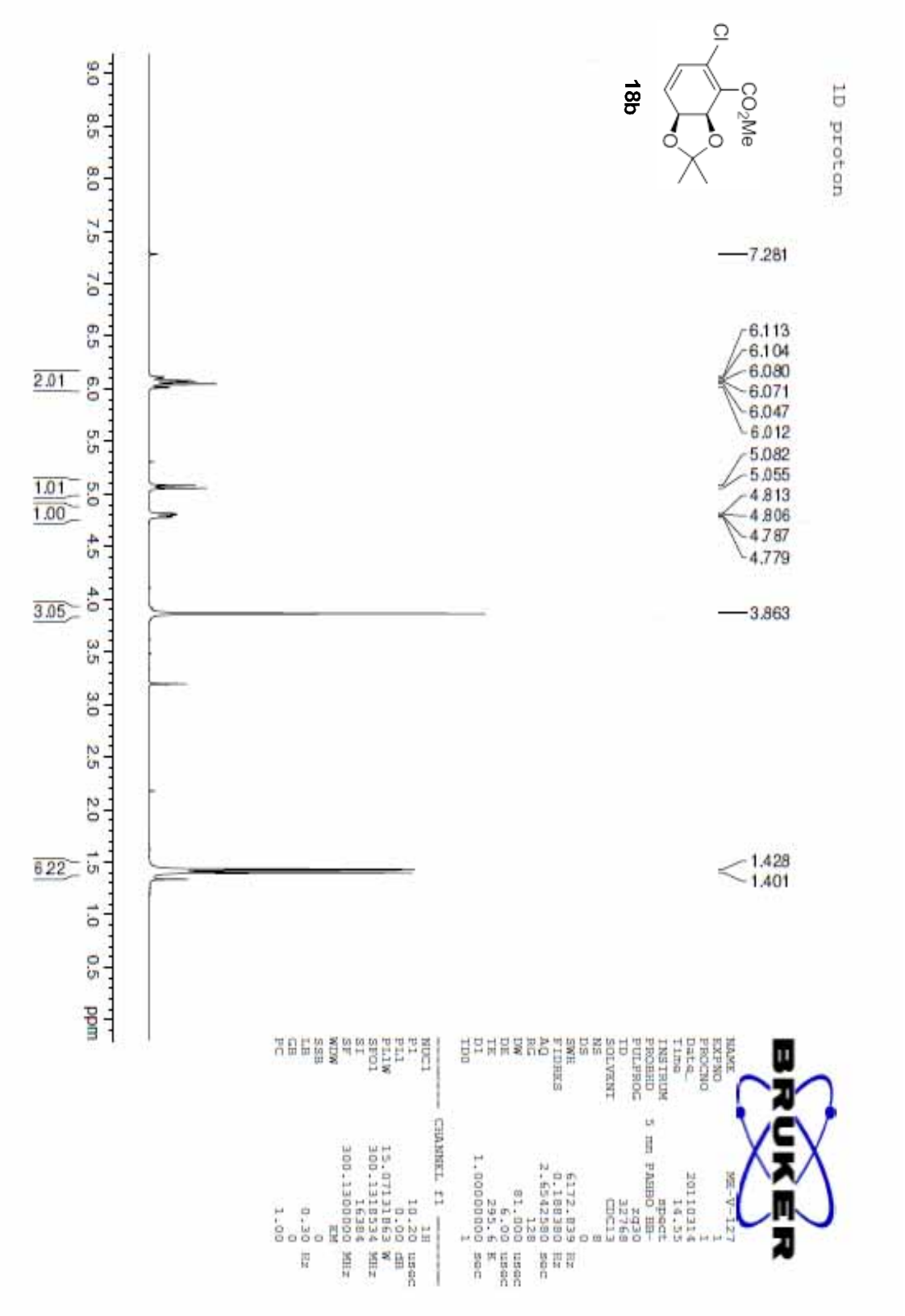
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6.080
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5.055
4.813
4.806
4.787
4.779
3.863
1.428
1.401
2.01
1.01
1.00
3.05
6.22
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
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DE 6.00 usec
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D1 1.00000000 sec
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PL1 0.00 dB
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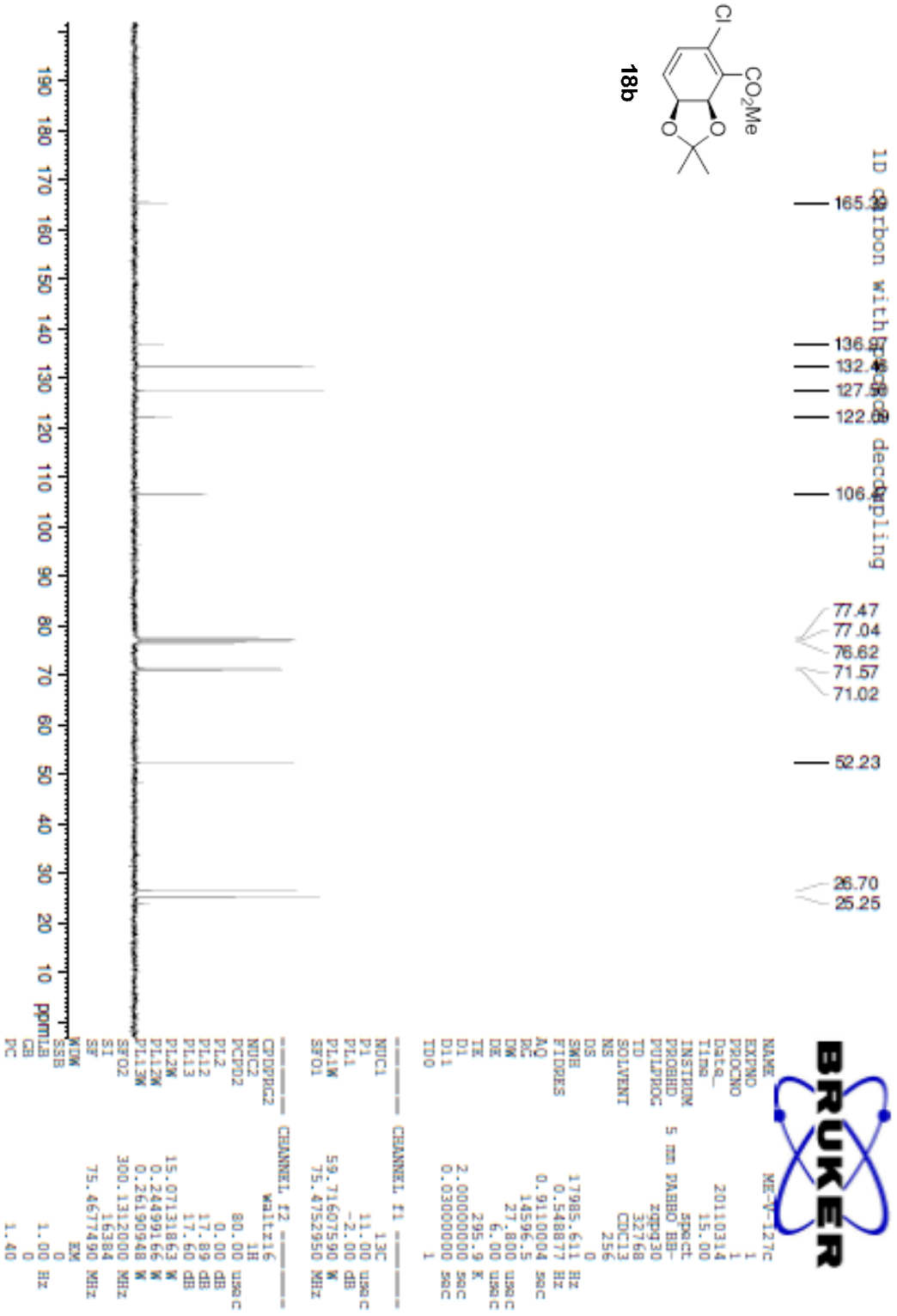
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Cl
CO2Me
1D Carbon with proton decoupling
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136.84
132.46
127.58
122.69
106.47
77.47
77.04
76.62
71.57
71.02
52.23
26.70
25.25
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Time 15.00
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PULPROG zgpg30
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SOLVENT CDCl3
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FIDRES 0.548877 Hz
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RG 14596.5
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D11 0.03000000 sec
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PL1 -2.00 dB
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SFO1 75.4752950 MHz
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PCPD2 80.00 usec
PL2 0.00 dB
PL12 17.89 dB
PL13 17.60 dB
PL2W 15.07131863 W
PL12W 0.24499166 W
PL13W 0.26190948 W
SFO2 300.1312000 MHz
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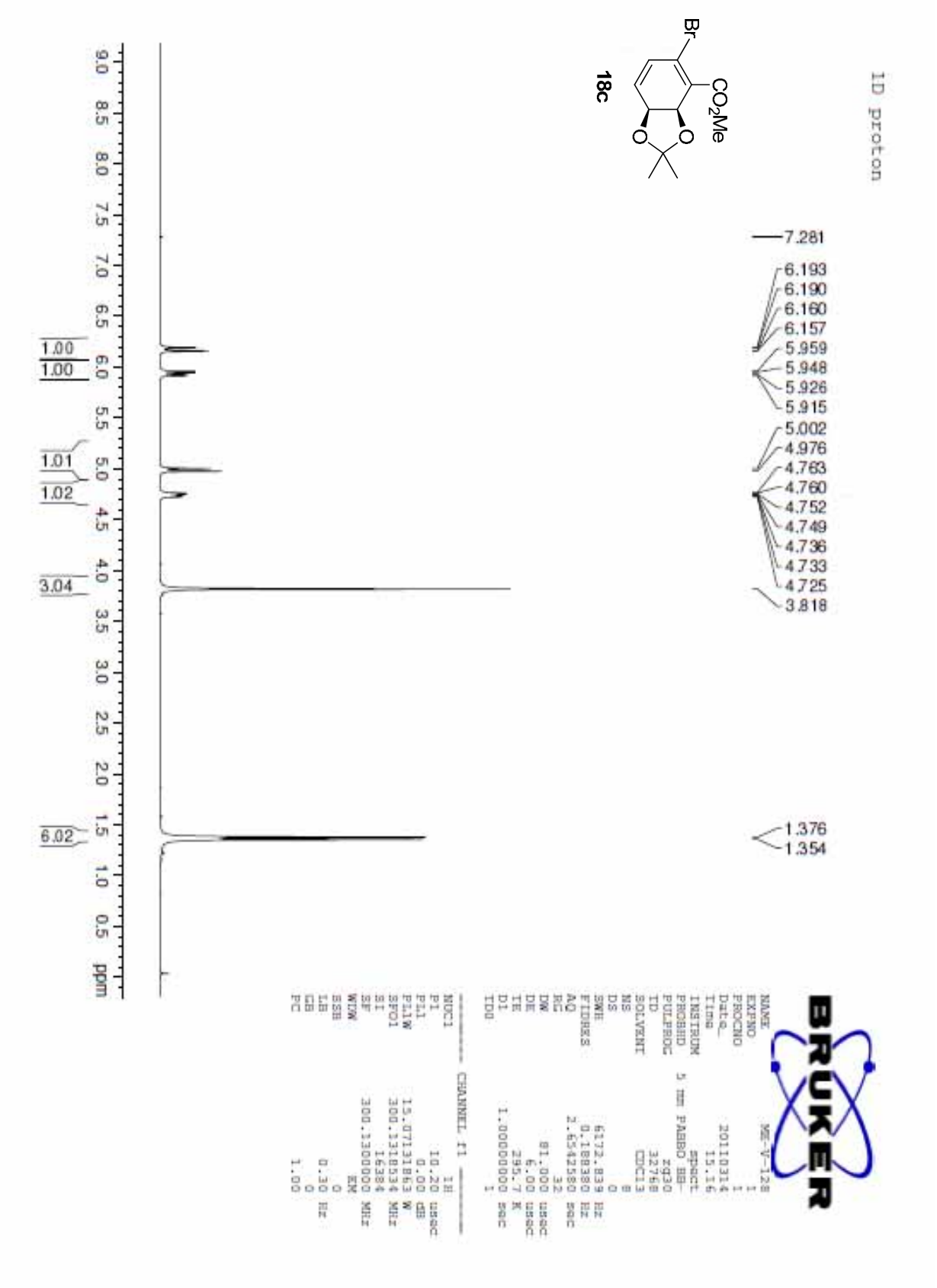

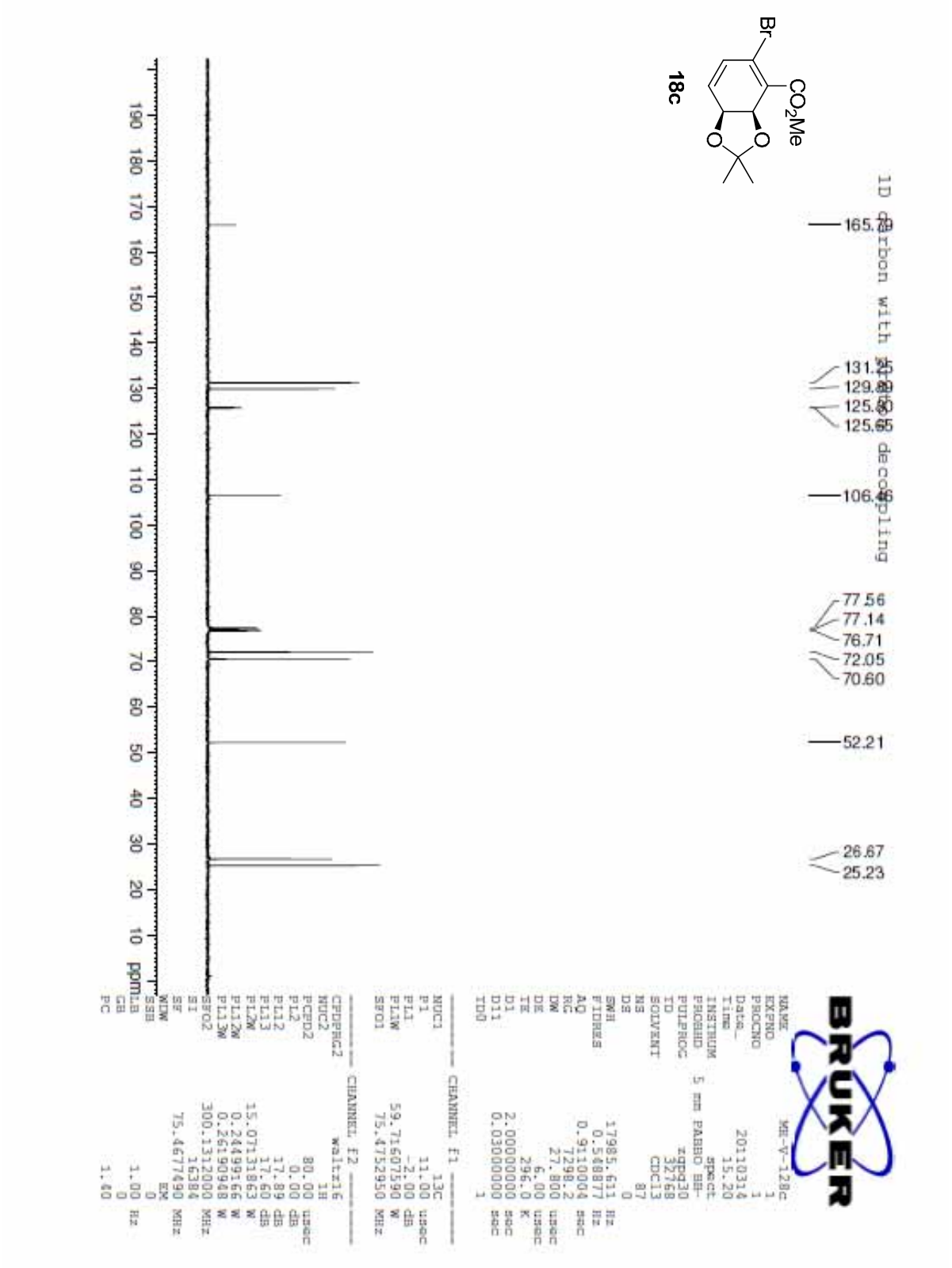
18c
Br
CO2Me
1D carbon with proton decoupling
165.89
131.21
129.88
125.45
125.05
106.46
77.56
77.14
76.71
72.05
70.60
52.21
26.67
25.23
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PROCNO 1
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Time 15.20
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PULPROG zgpg30
TD 32768
SOLVENT CDCl3
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FIDRES 0.548877 Hz
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RG 7298.2
DW 27.800 usec
DE 6.00 usec
TE 296.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13c
P1 11.00 usec
PL1 -2.00 dB
PL1W 59.71607590 W
SFO1 75.4752950 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 17.89 dB
PL13 17.60 dB
PL2W 15.07131863 W
PL12W 0.24499166 W
PL13W 0.26190968 W
SFO2 300.1312000 MHz
SI 16384
SF 75.4677490 MHz
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SSB 0
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GB 0
PC 1.40
ppm
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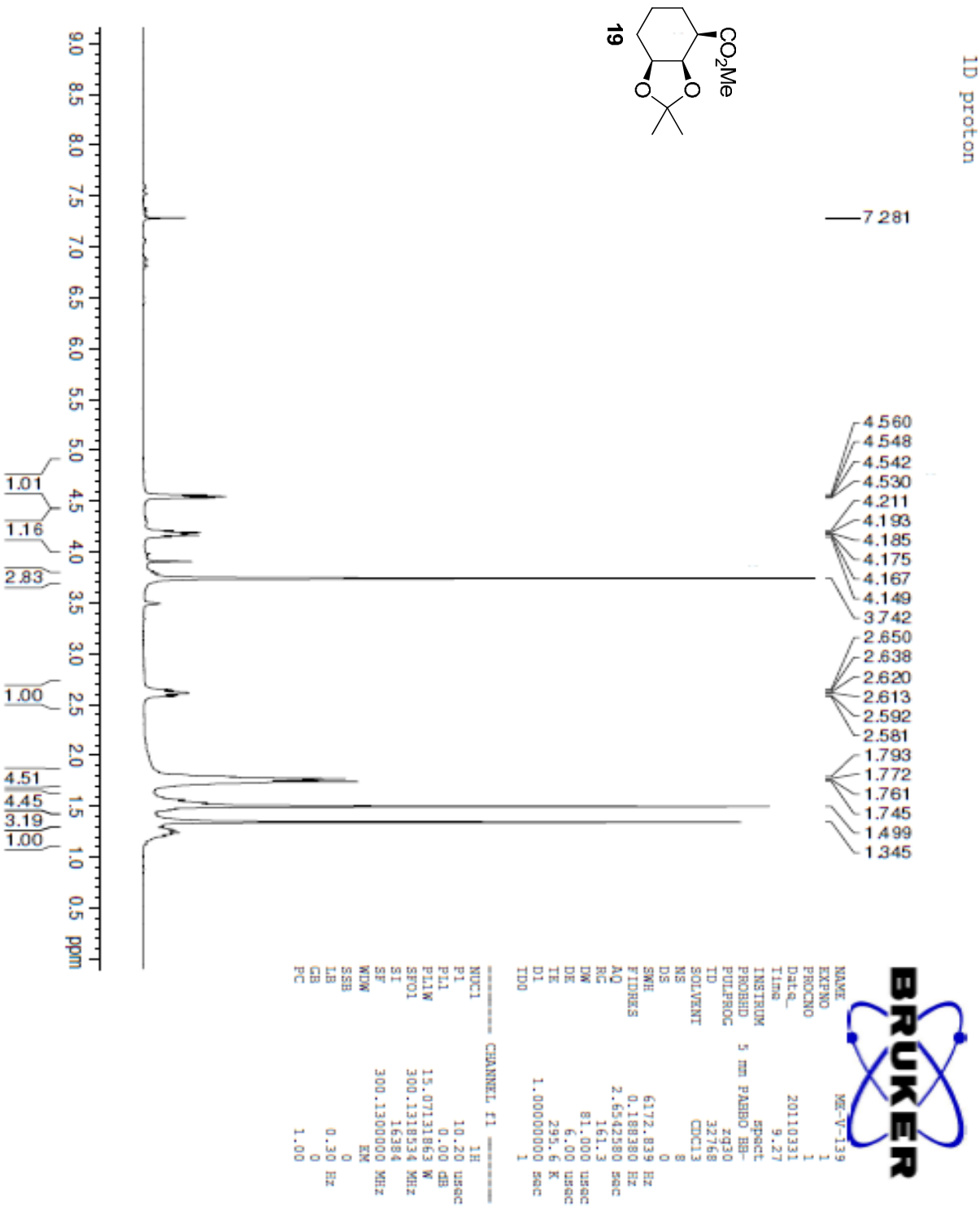
19
CO2Me
1D proton
7.281
4.560
4.548
4.542
4.530
4.211
4.193
4.185
4.175
4.167
4.149
3.742
2.650
2.638
2.620
2.613
2.592
2.581
1.793
1.772
1.761
1.745
1.499
1.345
1.01
1.16
2.83
1.00
4.51
4.45
3.19
1.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
NAME ME-V-139
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PROCNO 1
Date_ 20110331
Time 9.27
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
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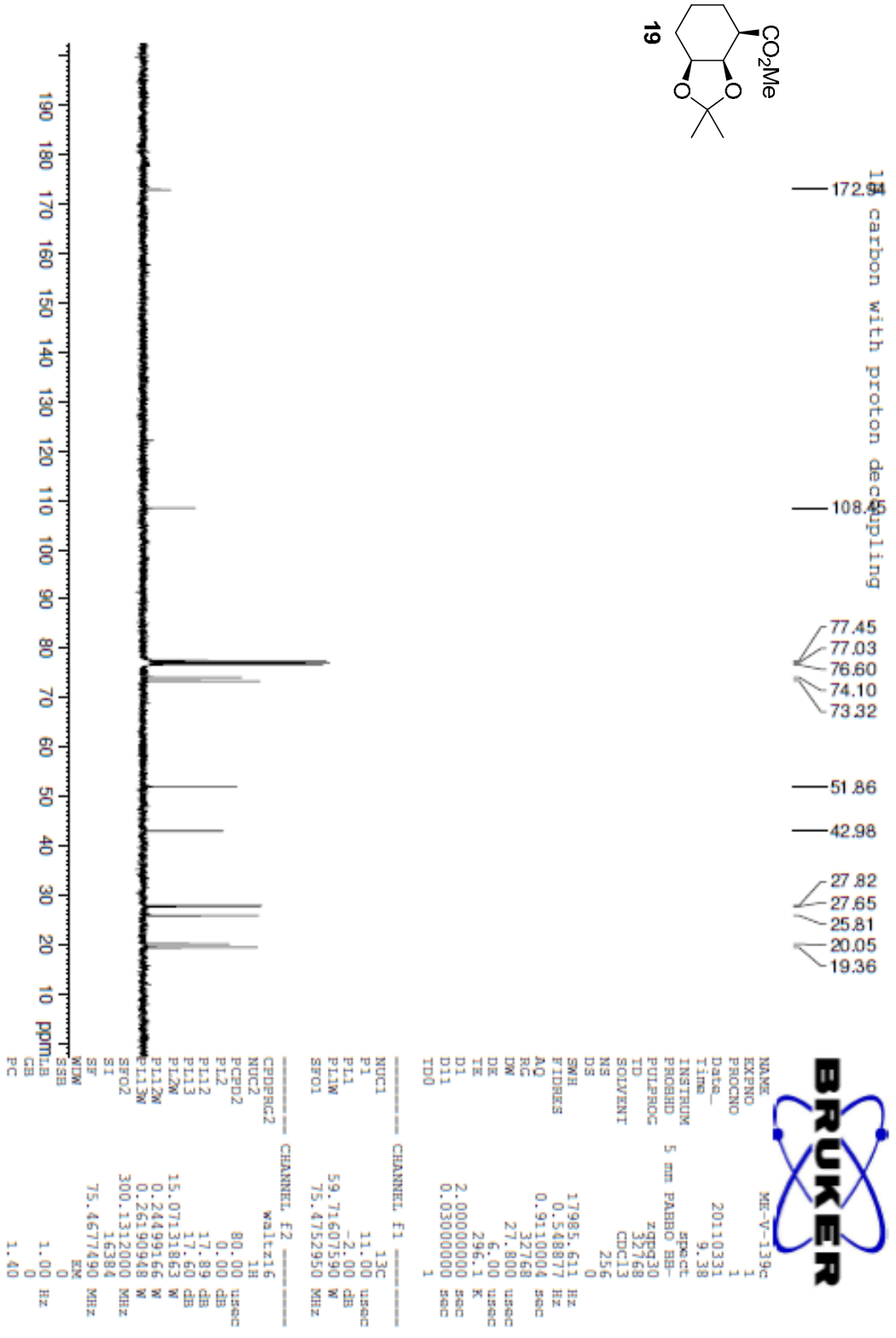
FIDRES 0.188380 Hz
AQ 2.6542580 sec
RG 161.3
DW 81.000 usec
DE 6.00 usec
TE 295.6 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 10.20 usec
PL1 0.00 dB
PL1W 15.07131863 W
SFO1 300.1318534 MHz
SI 16384
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


19
CO2Me
13C carbon with proton decoupling
172.94
108.45
77.45
77.03
76.60
74.10
73.32
51.86
42.98
27.82
27.65
25.81
20.05
19.36
BRUKER
NAME ME-V-139c
EXPNO 1
PROCNO 1
Date_ 20110331
Time 9.38
INSTRUM spect
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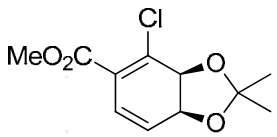
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 256
DS 0
SWH 17985.611 Hz
FIDRES 0.548877 Hz
AQ 0.9110004 sec
RG 32768
DW 27.800 usec
DE 6.00 usec
TE 296.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 11.00 usec
PL1 -2.00 dB
PL1W 59.71607590 W
SFO1 75.4752950 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 17.89 dB
PL13 17.60 dB
PL2W 15.07131863 W
PL12W 0.24499166 W
PL13W 0.26190948 W
SFO2 300.1312000 MHz
SI 16384
SF 75.4677490 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

1D proton



20b

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PULPROG	zg30	
TD	32768	
SOLVENT	CDCl3	
NS	8	
DS	0	
SWH	6172.839	Hz
FIDRES	0.188380	Hz
AQ	2.6542580	sec
RG	90.5	
DW	81.000	usec
DE	6.00	usec
TE	673.2	K
D1	1.00000000	sec
TD0	1	
======== CHANNEL f1 ========		
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P1	12.00	usec
PL1	-3.00	dB
PL1W	30.07123375	W
SFO1	300.1318534	MHz
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LB	0.30	Hz
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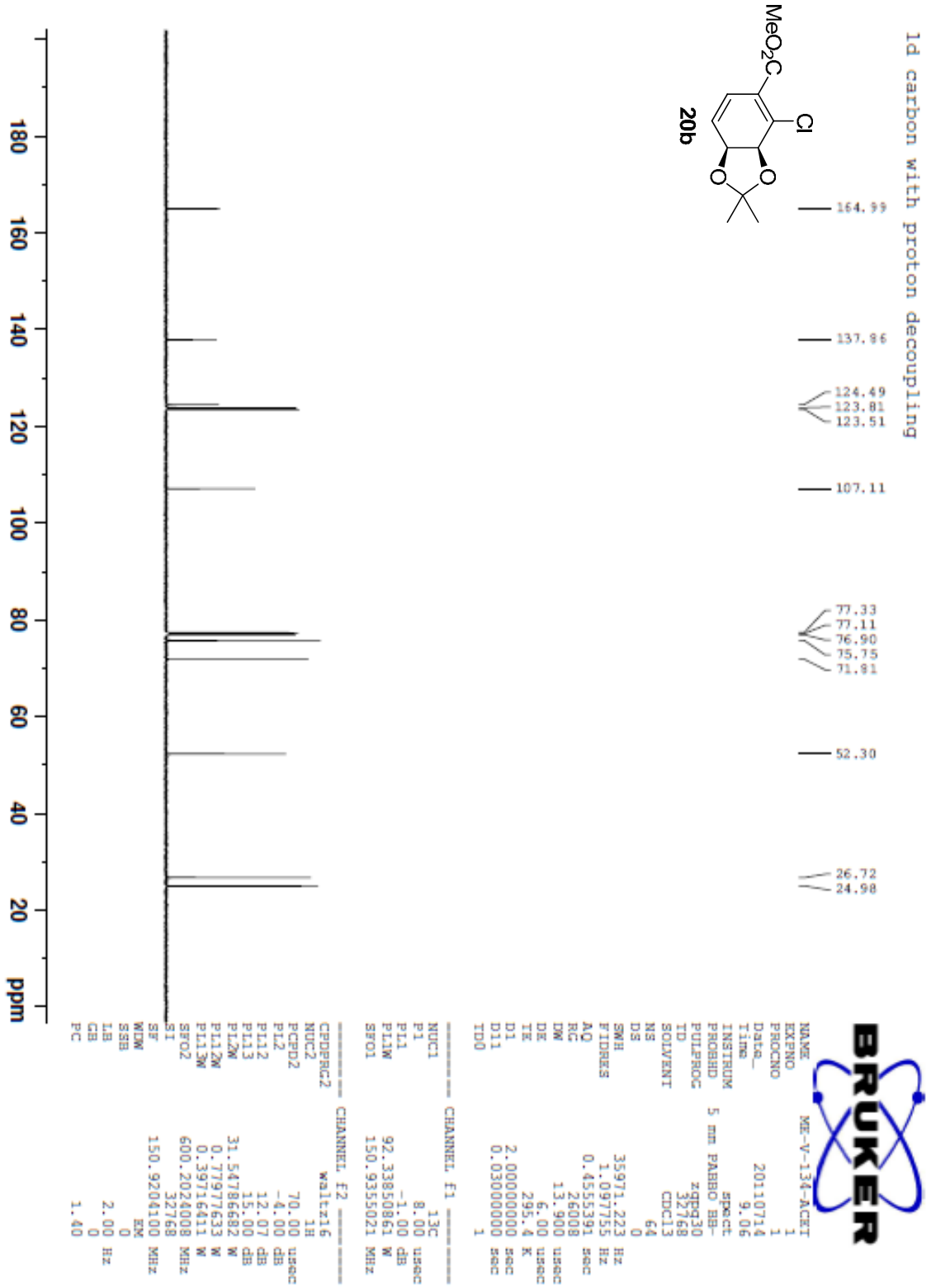
PC	1.00	


1d carbon with proton decoupling
20b
MeO2C
Cl
164.99
137.96
124.49
123.81
123.51
107.11
77.33
77.11
76.90
75.75
71.91
52.30
26.72
24.98
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Time 9.06
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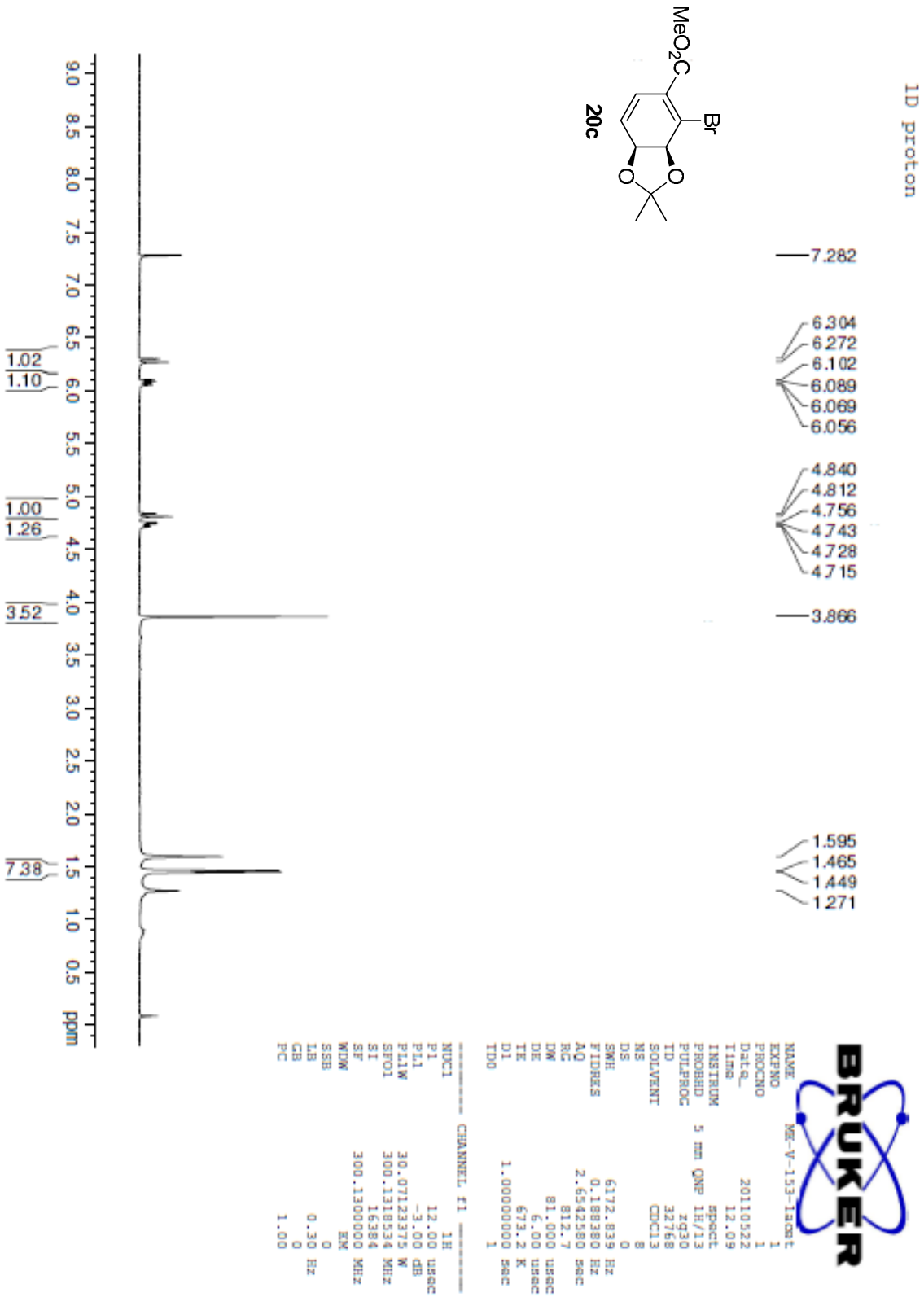
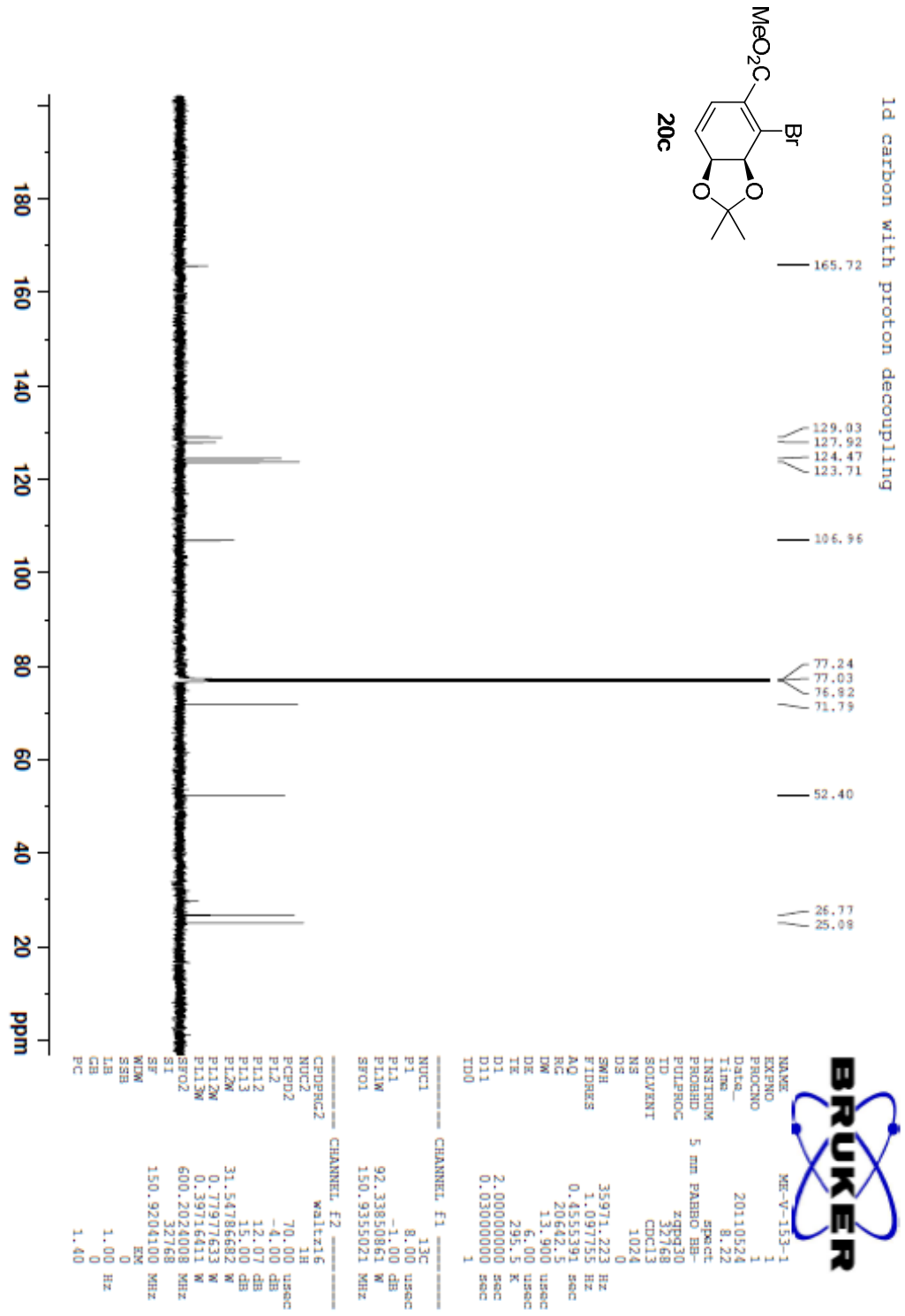
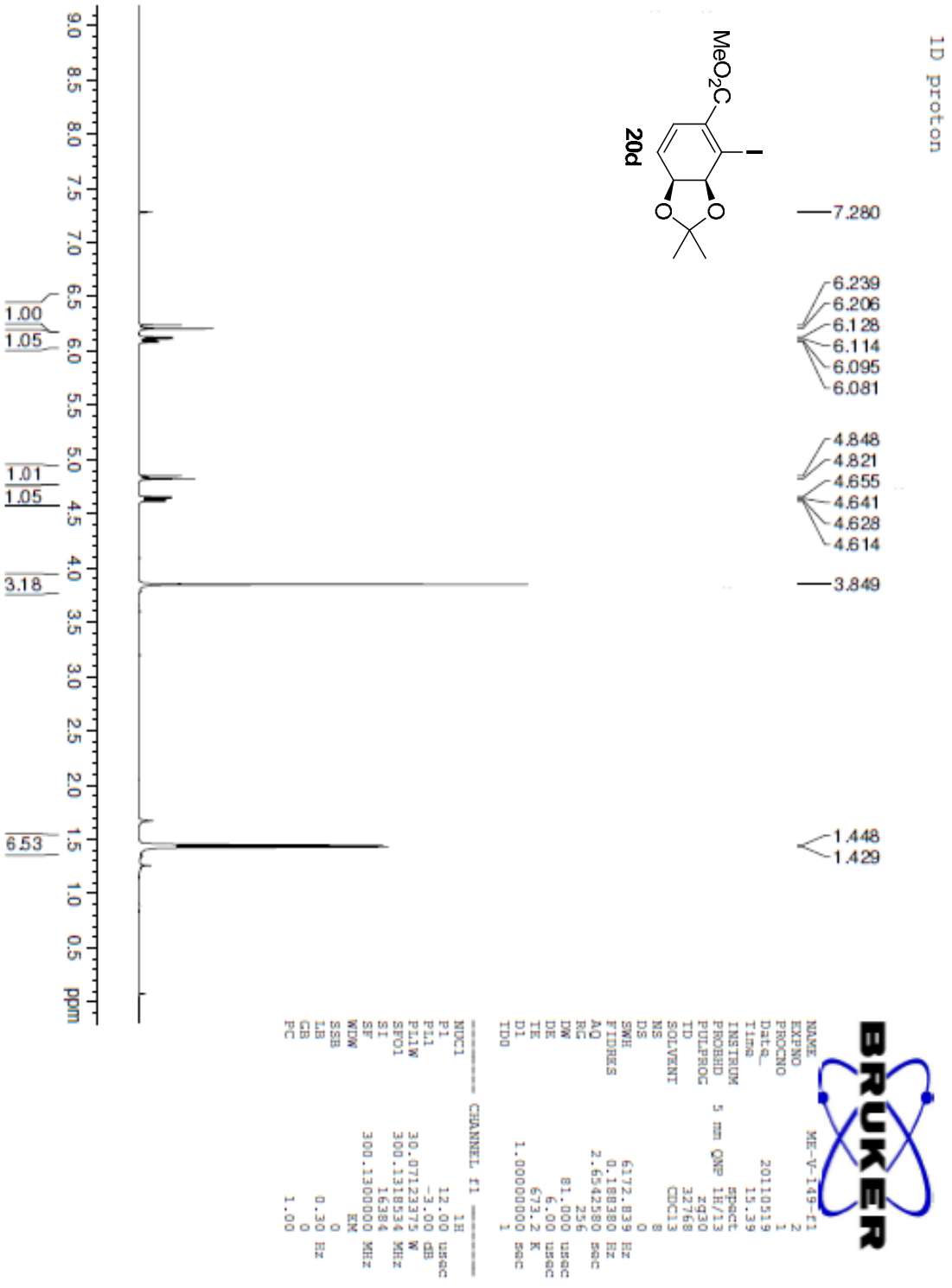
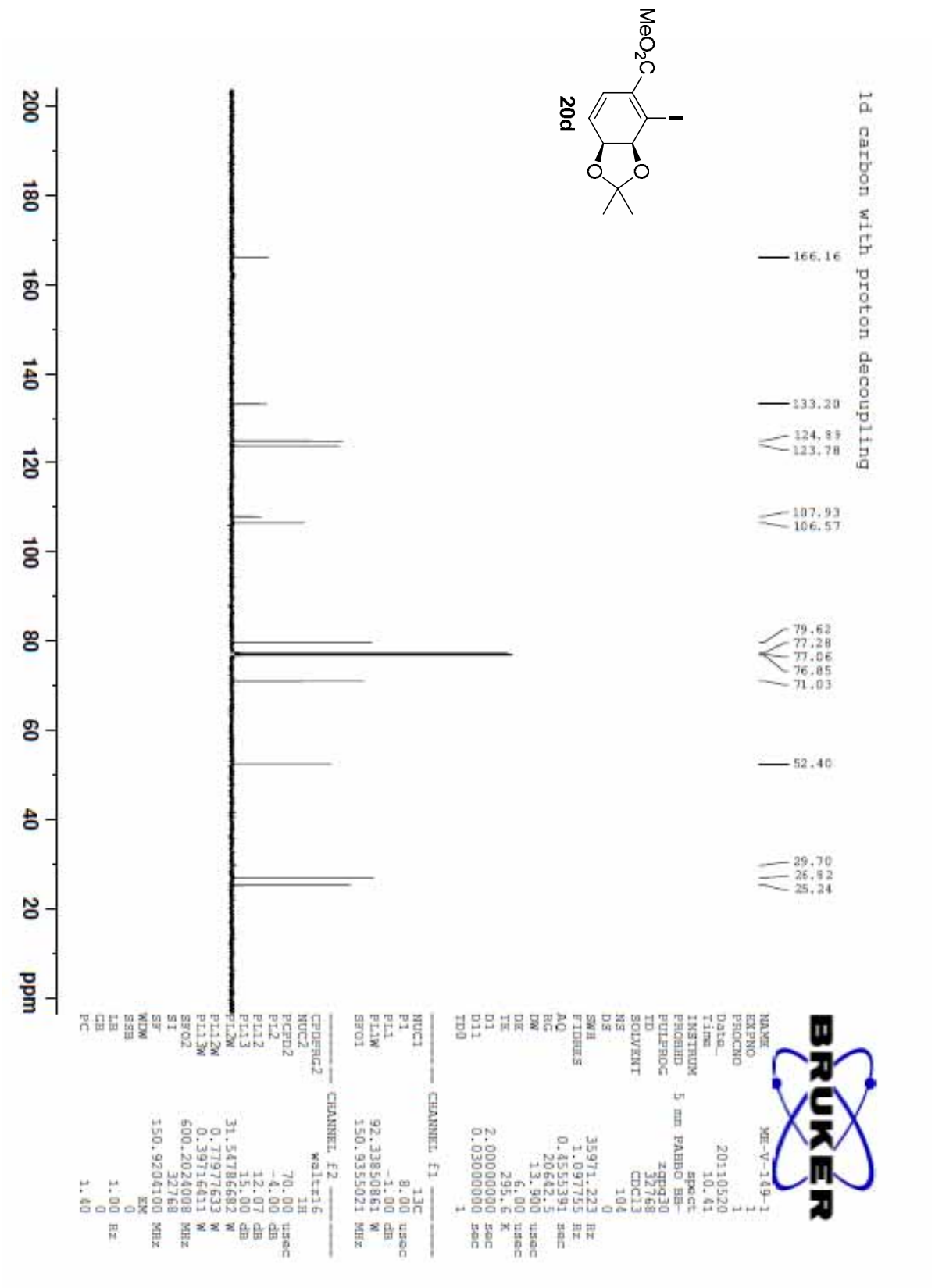
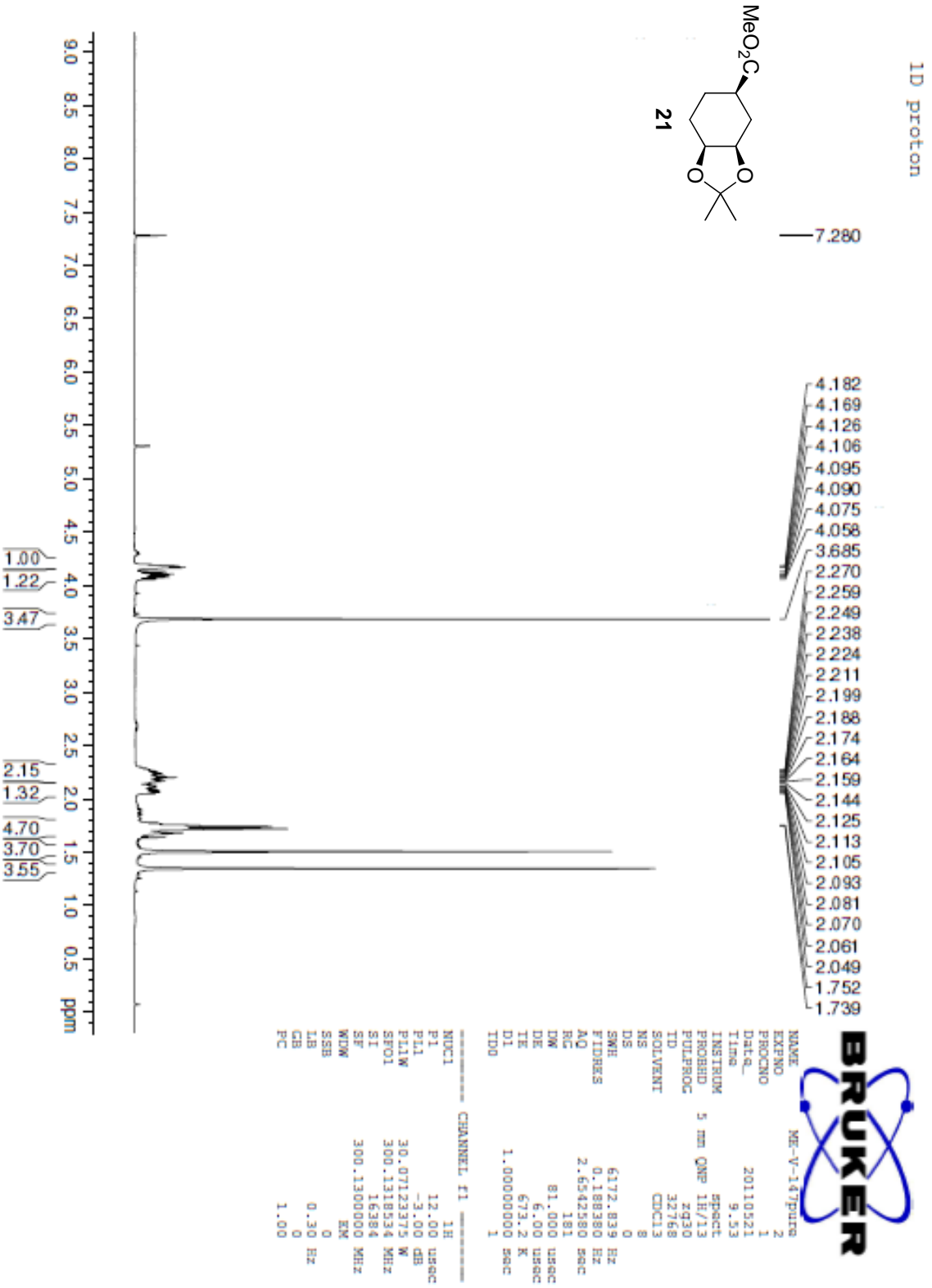
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 64
DS 0
SWH 35971.223 Hz
FIDRES 1.097755 Hz
AQ 0.4555391 sec
RG 26008
DW 13.900 usec
DE 6.00 usec
TE 295.4 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 8.00 usec
PL1 -1.00 dB
PL1W 92.33850861 W
SFO1 150.9355021 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -4.00 dB
PL12 12.07 dB
PL13 15.00 dB
PL2W 31.54786682 W
PL12W 0.77977633 W
PL13W 0.39716411 W
SFO2 600.2024008 MHz
SI 32768
SF 150.9204100 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.40
180
160
140
120
100
80
60
40
20
ppm

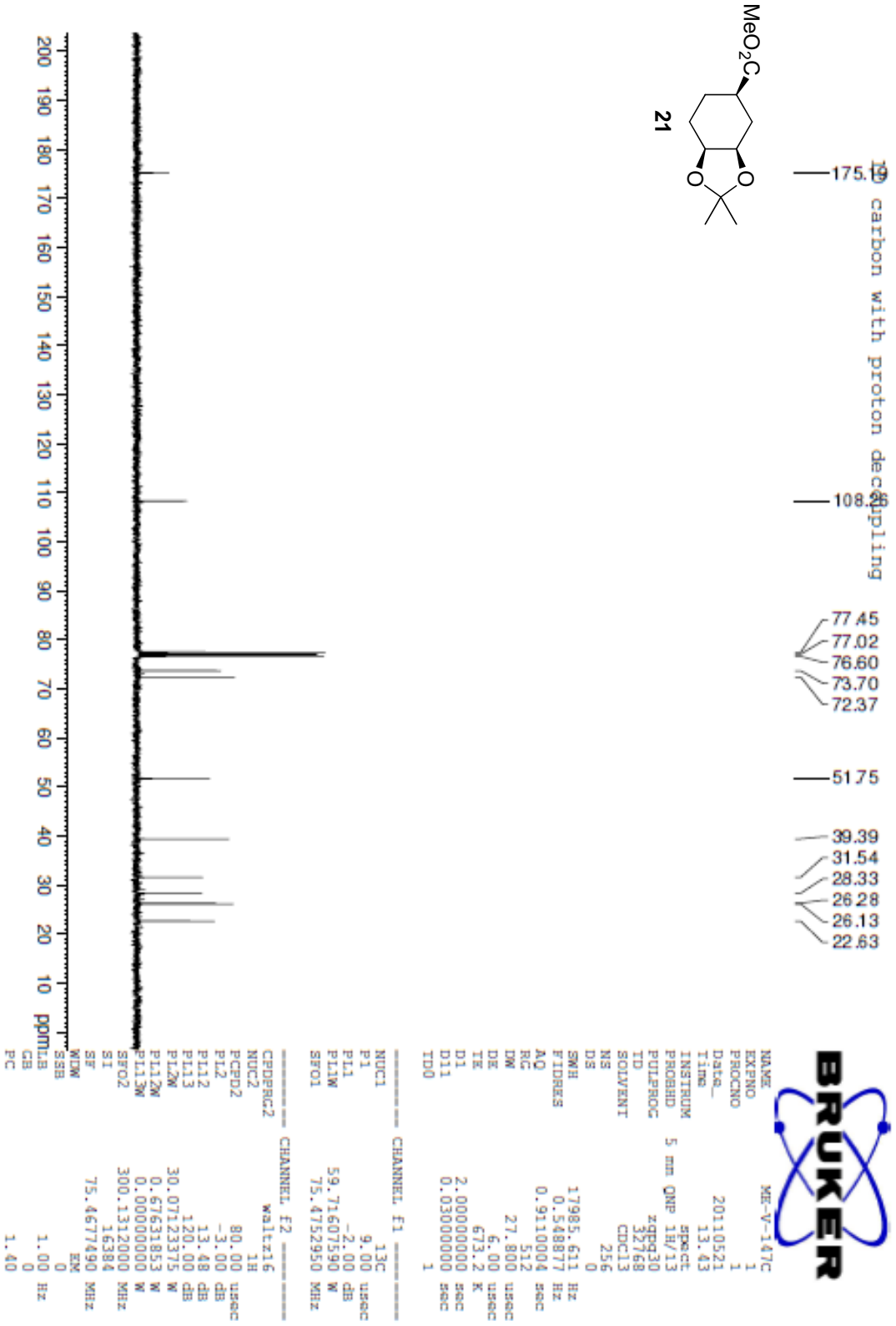

1D proton
20c
MeO2C
Br
7.282
6.304
6.272
6.102
6.089
6.069
6.056
4.840
4.812
4.756
4.743
4.728
4.715
3.866
1.595
1.465
1.449
1.271
1.02
1.10
1.00
1.26
3.52
7.38
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
NAME ME-V-153-1aopt
EXPNO 1
PROCNO 1
Date_ 20110522
Time 12.09
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6542580 sec
RG 812.7
DW 81.000 usec
DE 6.00 usec
TE 673.2 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 12.00 usec
PL1 -3.00 dB
PL1W 30.07123375 W
SFO1 300.1318534 MHz
SI 16384
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


1d carbon with proton decoupling
20c
MeO2C
Br
165.72
129.03
127.92
124.47
123.71
106.96
77.24
77.03
76.82
71.79
52.40
26.77
25.09
ppm
180
160
140
120
100
80
60
40
20
BRUKER


1D proton
20d
MeO2C
I
O
O
7.280
6.239
6.206
6.128
6.114
6.095
6.081
4.848
4.821
4.655
4.641
4.628
4.614
3.849
1.448
1.429
1.00
1.05
1.01
1.05
3.18
6.53
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
BRUKER
NAME ME-V-149-f1
EXPNO 2
PROCNO 1
Date_ 20110519
Time 15.39
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6542580 sec
RG 256
DW 81.000 usec
DE 6.00 usec
TE 673.2 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
PL1 -3.00 dB
PL1W 30.07123375 W
SFO1 300.1318534 MHz
SI 16384
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


1d carbon with proton decoupling
20d
MeO2C
I
O
O
166.16
133.20
124.89
123.78
107.93
106.57
79.62
77.28
77.06
76.85
71.03
52.40
29.70
26.92
25.24
BRUKER
NAME ME-V-169-1
EXPNO 1
PROCNO 1
Date_ 20110520
Time 10.41
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 104
DS 0
SWH 35971.223 Hz
FIDRES 1.097755 Hz
AQ 0.4555391 sec
RG 20642.5
DW 13.900 usec
DE 6.00 usec
TE 295.6 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 8.00 usec
PL1 -1.00 dB
PL1W 92.33850861 W
SFO1 150.9355021 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -4.00 dB
PL12 12.07 dB
PL13 15.00 dB
PL2W 31.54786682 W
PL12W 0.77977633 W
PL13W 0.39716411 W
SFO2 600.2024008 MHz
SI 32768
SF 150.9204100 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200
180
160
140
120
100
80
60
40
20
ppm


1D proton
MeO2C
21
7.280
4.182
4.169
4.126
4.106
4.095
4.090
4.075
4.058
3.685
2.270
2.259
2.249
2.238
2.224
2.211
2.199
2.188
2.174
2.164
2.159
2.144
2.125
2.113
2.105
2.093
2.081
2.070
2.061
2.049
1.752
1.739
1.00
1.22
3.47
2.15
1.32
4.70
3.70
3.55
ppm
BRUKER


MeO2C
21
175.19
108.26
77.45
77.02
76.60
73.70
72.37
51.75
39.39
31.54
28.33
26.28
26.13
22.63
13C carbon with proton decoupling
BRUKER
NAME ME-V-147C
EXPNO 1
PROCNO 1
Date_ 20110521
Time 13.43
INSTRUM spect
PROBHD 5 mm CNP 1H/13
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 256
DS 0
SWH 17985.611 Hz
FIDRES 0.548877 Hz
AQ 0.9110004 sec
RG 512
DW 27.800 usec
DE 6.00 usec
TE 673.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 9.00 usec
PL1 -2.00 dB
PL1W 59.71607590 W
SFO1 75.4752950 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 13.48 dB
PL13 120.00 dB
PL2W 30.07123375 W
PL12W 0.67631853 W
PL13W 0.00000000 W
SFO2 300.1312000 MHz
SI 16384
SF 75.4677490 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

ACKNOWLEDGEMENTS & EPILOGUE

ACKNOWLEDGEMENTS

No tenía miedo a las dificultades.
Lo que le asustaba era la obligación de tener que escoger un camino.
Escoger un camino significaba abandonar otros.

El Alquimista
Paulo Coelho

I would like to give my sincere thanks to supervisor of my doctoral thesis **Dr. Carmen Escolano**, for her professional leadership, valuable recommendations, attitude and last but not least, for her immense patience.

I thank **Prof. Joan Bosch** and **Prof. Mercedes Amat** for their help in solution of theoretical & practical scientific problems. I would like to thank those three people, for being so important in my professional life, as well as for their help with any personal problem.

I thank **Prof. Tomáš Hudlický** for the great possibility to form part of his research group in summer 2010.

I am deeply grateful to Dr. Pilar Franco and Dr. David Pubill for their help in the project of quinolizidine alkaloids derivatives; namely for the experimental efforts to separate (-)-207I and 4-*epi*-207I by HPLC chromatography and preliminary biological evaluation of synthesised products.

I express my gratitude to all members of the M. Amat / J. Bosch group. Especially, I appreciate Carlos Arróniz, Viviane Elias Khalil, Oscar Lozano, Arantxa Gómez-Esqué and Laura Navío for their kind help I needed, when I joined the group and embarked on a UB journey.

I appreciate Ben Bradshaw, Gorka Etxebarria-Jardí, Alejandro Cordero and all members of "*students seminars*" for their kind attitude to my questions related to chemistry and helpful discussions regarding my research and my scientific view.

I thank Dr. Pavel Mach for his help with theoretical calculations related to autoxidation of 1,2-amino alcohols and biotransformations.

My gratitude belongs to the technical stuff, especially to Ana Linares (NMR) and Asunción Marín (GC-MS); these usually "omitted" persons, which are so important in our daily work.

I express my appreciation to all members of Laboratory of Organic Chemistry and colleagues from all laboratories A-B-C-D for their understanding and willingness to help.

EPILOGUE

Philosophical instruments are an endless fund of knowledge…
… while nature herself is the agent that shows the result.

Joseph Priestley (1767)

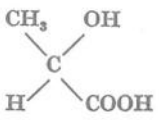
I would like to conclude this thesis with one short look back. In 1924 Cambridge University Press published a book titled "*Matter and Change: An Introduction to Physical and Chemical Science*" written by William Cecil Dampier Whetham.[1] One chapter is dedicated to "*Physical Isomers*" and we can read there a claim: *"If a compound containing an asymmetric carbon atom is made in the laboratory, the product is always optically inactive…"*. Today this statement is totally disproved; however in 1924 it was true. We will see how preparative organic chemistry will change and progress in next decades. I am sure, that we will see really a great show.

And therefore, I would like to ask the (future) Reader to be tolerant to our "premature claims".

[1] Cambridge University Press is gratefully acknowledged for reprint permission of the pages 156-159 from William Cecil Dampier Whetham: *Matter and Change: An Introduction to Physical and Chemical Science* (1924).

Reprint of the page 158 (Chapter VI, Physical Isomers) from William Cecil Dampier Whetham: *Matter and Change: An Introduction to Physical and Chemical Science*, Cambridge University Press (1924).

carbon atom is directly united with four different atoms or groups of atoms. Thus lactic acid, present in sour milk, has the structural formula in which each valency of the carbon atom is satisfied differently. As long as we represent this molecule by a formula written on one plane, no variation in it is possible, but there is no reason to suppose that the atoms in molecules are arranged in a plane—we have to picture them in three-dimensional space. Let us suppose that the carbon atom lies at the centre of a regular tetrahedron, connected with the other atoms at the four corners as suggested in the sketch of a solid model shown in Fig. 66. If we inter-



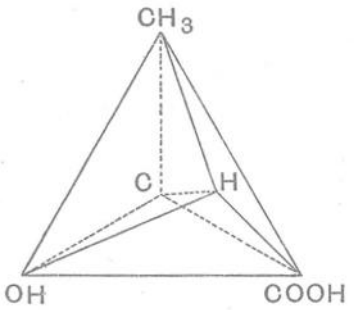


Fig. 66.

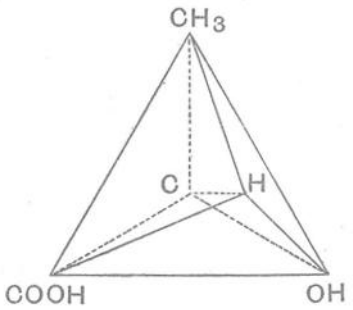


Fig. 67.

change the OH and COOH groups, as in Fig. 67, we get a picture of a different molecule, the two being related to each other as are an object and its image in a mirror. Thus in this theory it is supposed that the structure of the crystals reflects the arrangement of the molecule.

When more than one asymmetric carbon atom are present, more than two alternatives are possible. By these considerations many complex phenomena have been elucidated.

If a compound containing an asymmetric carbon atom is made in the laboratory, the product is always optically inactive, and consists, like racemic acid, of a mixture in equal quantities of the two optically active isomers. But substances occurring in nature often show optical activity, and, though chemically identical, optical isomers may be physiologically