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Grau de Química

Treball Final de Grau

Towards asymmetric catalysis by using tripodal pseudopeptidic cages.

Cap a catàlisi asimètrica utilitzant caixes pseudopeptídiques tripodals.

Araceli de Aquino Samper

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Nothing in life is to be feared,
It is only to be understood.
Now is the time to understand more,
So that we may fear less.

Marie Curie

En primer lloc, m'agradaria agrair a l'equip del Ignacio Alfonso que m'acollís i em dongués l'oportunitat de dur a terme el treball amb ells. I en especial, al Ciril per guiar-me durant aquests mesos i estar al meu costat en tot moment. També a la Lucia i al Dani per tota l'ajuda i tots els consells que m'han donat.

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No pot faltar en Zeno, sense ell no hauria descobert aquest projecte que m'ha fet donar el millor de mi.

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REPORT

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1. SUMMARY

The main goal of this project is the synthesis of a supramolecular pseudopeptidic cage that is be able to recognize chloride ions (Figure 1). This recognition will later be proved for asymmetric catalysis on different substrates. Three L-amino acids (phenylalanine) provide it the chiral centres that should allow the cage to act as an asymmetric catalyst. The interest in this kind of catalysis lies in getting enantiomerically enriched compounds, which have high synthetic interest and high value, even though the reagents have affordable prizes.

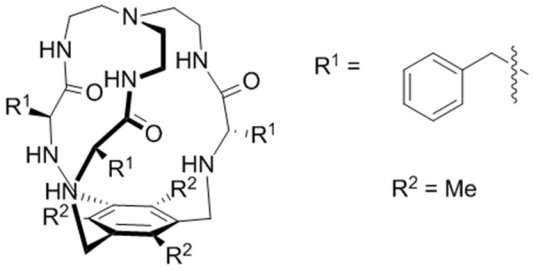


Figure 1. Supramolecular pseudopeptidic cage developed in the group.

In order to get the desired cage (which will be called [1+1] because it is formed by one aromatic electrophile and one tripodal amine) it is needed to use the template effect with the chloride. Thanks to that, the third step of its synthesis (a S_N2 reaction between a triple benzyl bromide and a free triamine) will be promoted in a fully intramolecular way, yielding the desired cage.

Finally, the cage has been characterized by ^{1}H-NMR, ^{1}H-^{1}H (COSY), ^{13}C-^{1}H (HSQC) and by ESI-MS, too. Thanks to these techniques it is possible to identify each proton and with whom it interacts.

In the period available, different substrates were tested to try the asymmetric reactions catalysed with the supramolecular cage. Finally, two reactions were studied: a β-chlorohydrine cyclization to yield epoxides, and a β-chloroacid cyclization to yield β-lactones.

Keywords: supramolecular synthesis, asymmetric catalysis, molecular recognition, pseudopeptides, cages.

2. RESUM

El principal objectiu d'aquest projecte és la síntesis d'una caixa pseudopeptídica supramolecular que és capaç de reconèixer ions clorurs (Figura 1). Aquest reconeixement serà després emprat en catàlisi asimètrica en diferents substrats. Tres L-aminoàcids (fenilalanina) són els centres quirals que haurien de permetre a la caixa funcionar com a catalitzador asimètric. L'interès de la catàlisi radica en aconseguir compostos enantiomèricament enriquits, els quals tenen elevat interès sintètic i econòmic, tot i provenir de reactius assequibles.

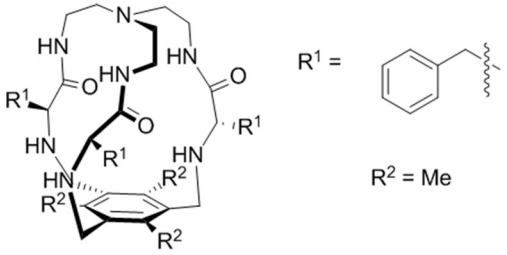


Figura 1. Caixa supramolecular pseudopeptídica desenvolupada pel grup.

Per tal d'aconseguir sintetitzar la caixa desitjada (anomenada [1+1] perquè és fruit de la unió d'un electròfil aromàtic i d'una triamina) cal fer servir l'efecte plantilla que prové un clorur. D'aquesta manera, el tercer pas de la síntesi (reaccions de S_N2 entre l'electròfil de tipus bromur benzílic i l'amina lliure) estarà afavorit de forma completament intramolecular i no pas intermolecular.

La caixa ha estat finalment caracteritzada per RMN de ^1H, ^1H-^1H (COSY) i ^{13}C-^1H (HSQC) i per ESI-MS també. Gràcies a aquestes tècniques podem identificar tots els protons i amb qui interaccionen.

En el període de temps disponible es van fer diverses proves de catàlisi amb substrats adients per intentar emprar-los en reaccions útils amb la caixa supramolecular obtinguda com a catalitzador asimètric. Finalment, dues reaccions van ser estudiades: ciclació de β-clorhidrines i ciclació de β-cloroàcids per tal d'aconseguir β-lactones.

Paraules clau: síntesi supramolecular, catàlisi asimètrica, reconeixement molecular, pseudopèptids, caixes.

3. INTRODUCTION

Supramolecular chemistry is usually described as "chemistry beyond the molecule", bearing on the organized entities of higher complexity that result from the association of two or more chemical species held together by intermolecular forces[1].

The supramolecular chemistry is divided in two main fields: *host-guest chemistry* and *self-assembly chemistry*.

- **Host-guest:** the host has a cavity where one guest can fit and interact with it, so they coordinate. It is characteristic of biological systems (enzyme-substrate association).

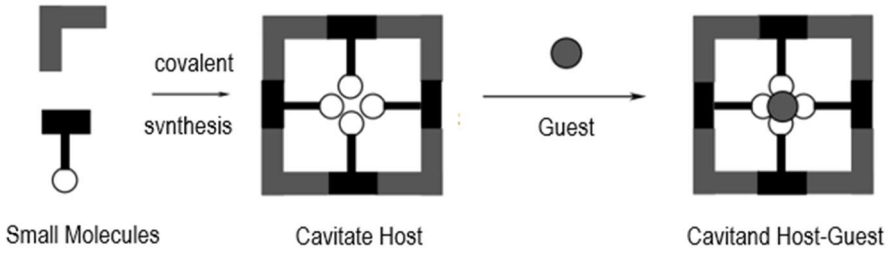


Figure 2. Host-guest chemistry[2].

- **Self-assembly:** two or more small molecules coordinate between them forming a larger molecule by a spontaneous and reversible mechanism. They are complementary between them, so they do not have a concrete role as host or guest.

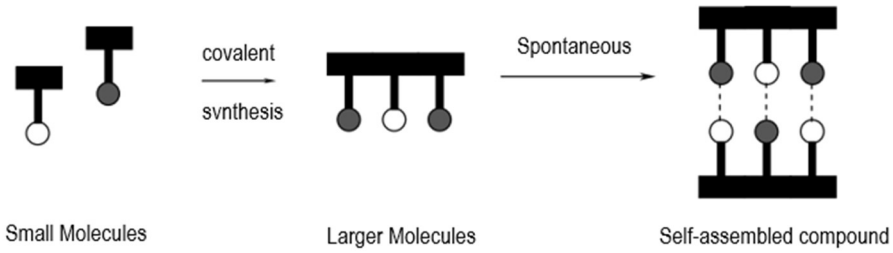


Figure 3. Self-assembly chemistry[2].

The supramolecular product has different characteristics in comparison of its precursors. In addition, its characteristics are not a result of the sum of the previous ones. These new characteristics acquired are innovative and interesting. For example, the amino acid phenylalanine, the tripodal amine (tris(2-aminoethyl)amine) and 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene do not have the recognition function by themselves. In contrast, if they are coupled in a specific way, they form a cage-like structure which is capable of recognise certain type of particles (chloride in the studied case).

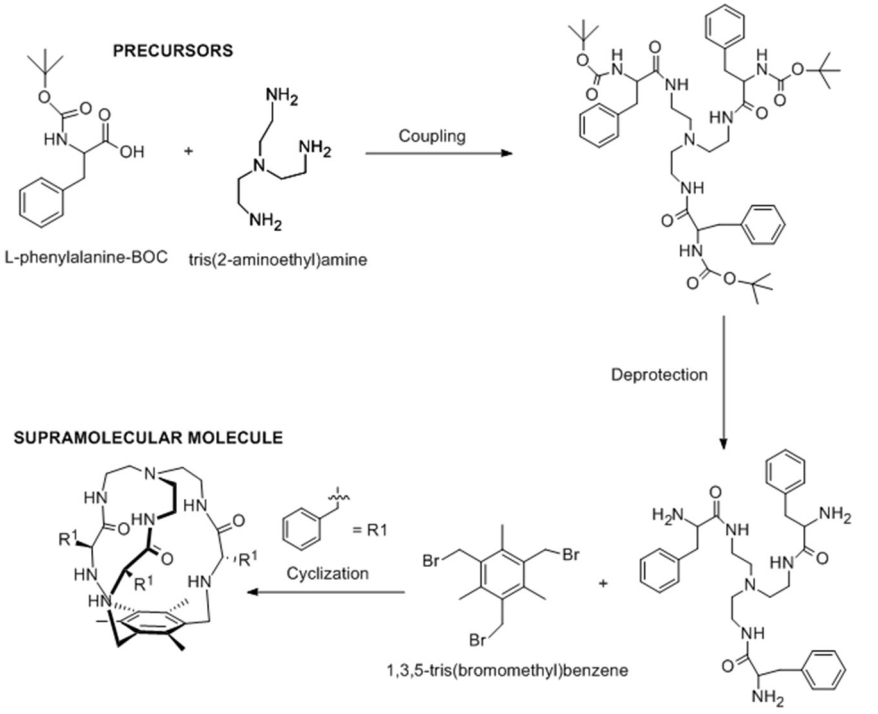


Figure 4. Supramolecular scheme[2].

Usually the supramolecular cages are synthetized by using a metal ion and organic ligands. They have outstanding applications thanks to their unique structure and their internal cavity in: anion recognition, sequestration of hazardous substances, drug delivery, stabilization of reactive reagents and intermediates[3,4,5].

The functionalization or modification of the cavity provides with high selectivity to the cage's structure. The cavity and the intermolecular forces interact with a certain group of compounds by encapsulating them[6].

Many research groups are working on new cages and studying the template effect that allows their formation. By studying the NMR spectra and how the chemical shifts change in comparison of the unreacted reagents, it is possible to determine the host-guest interactions and their effect[7]. In addition, the cage formation reactions can be checked by ESI-MS[8,9] and DOSY (diffusion-ordered spectroscopy)[10].

In this project, cage-like structures (Figure 1) will be synthetized by supramolecular synthesis. This compound has a very organized structure that makes them very stable and gives them the property of encapsulating certain type of ions or molecules in its cavity.

The high stability of the host-guest assemblies is due to the **chelate effect**. This effect is consequence of the presence of chelating ligands or functional groups, which have more than one coordination site with the substrate. Furthermore, chelating or host-guest reactions tend to a higher entropy because cations and anions are strongly solvated, and solvent must be released prior to the assembly. Thus, the entropic effect shifts the equilibrium towards the host-guest system.

For example: $[Cu(NH_3)_4]^{2+} + 2en \rightarrow [Cu(en)_2]^{2+} + 4NH_3$. In this reaction, once the etilendiamine (en) ligand (chelate ligand) is coordinated, the number of molecules in the medium is higher (from 3 to 5 molecules).

In addition, the three-dimensional structure of the supramolecular cage provides it even more stability. This kind of compounds are called **cryptands**.

The capacity of encapsulating specific species makes possible the use of these cages in **recognition processes**. **Supramolecular catalysis** is an important field of the contemporary chemistry. In general, supramolecular catalysts are excellent receptors thanks to their cavities and catalytic sites, so they can selectively bind the substrate[11]. In this work, we will try to show how the cage-like structure is synthetized and how it can encapsulate chloride inside its cavity. Thanks to that interaction between the cage and chloride, we will attempt catalyse reactions of substrates that contain chloride, to forming an enantiomerically enriched product.

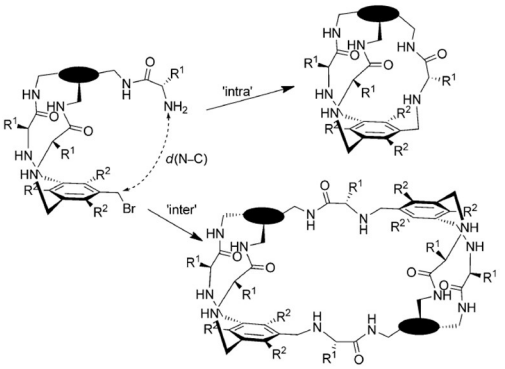
4. OBJECTIVES

The goal of this project is to synthetize a chiral pseudopeptidic supramolecular cage (Figure 1) through a three-step synthesis. In addition, this supramolecular cage will be used to study the selective abstraction of the chloride anion to catalyse some reactions and getting an enantiomerically enriched compound.

The synthesis and study of the substrates for the catalysis' reactions are also goals to achieve during these months, which will require the use of chiral HPLC and planning their synthesis, purification and characterization.

5. SYNTHESIS OF THE PSEUDOPEPTIDIC TRIPODAL CAGE

The key of the synthesis of this compound is the triple S_N2 reaction that takes place between tripodal tris(amido amines) and several 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzenes electrophiles[12].



Scheme 1. Third S_N2 reaction of the cyclization.

The central triamine scaffold determines the success of the cyclization. The orientation of the free NH_2 that react with the Br is determined by the triamine scaffold.

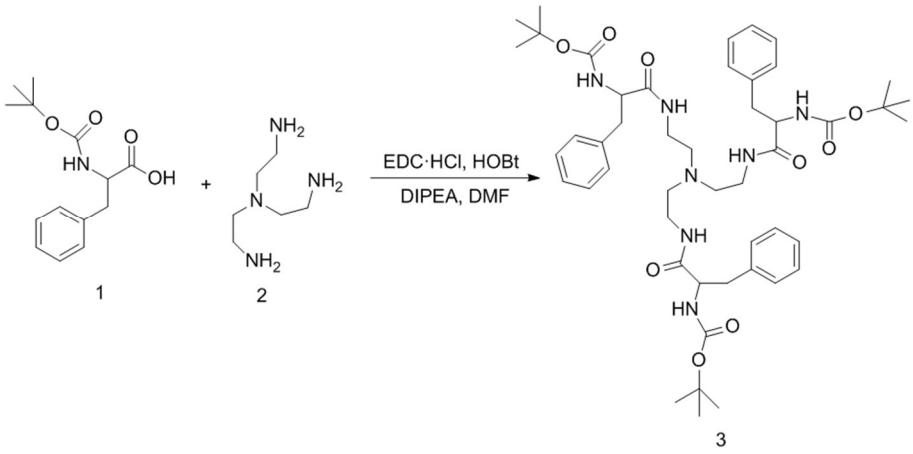
The bonds are established between the amino NH hydrogen atoms and the Br anion of the electrophile, giving as a result a concave conformation with the three amino nitrogen atoms closely[13].

As the group previously observed[12], the nucleophilic substitution is an irreversible reaction, so it is important to have a spatial proximity between the reacting centres that allow them to react by intramolecular reaction besides the intermolecular one.

If the distance between the amino nitrogen and the methylene bromide is bigger, the intermolecular reaction would occur rather than the intramolecular one.

This cage was specifically chosen for the encapsulation of the chloride anion because it was proved that cages derived from phenylalanine (Phe) fitted this anion better[14]. In addition, the same group studied the different binding constants with different R^2 substituents of the aromatic ring from the electrophile and a trend was observed: k(Me)>k(Et)>>k(H). So, the electrophile chosen was the 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene.

5.1. COUPLING REACTION



1 2 3

Scheme 2. Coupling reaction.

This reaction's aim is to couple L-Phenylalanine (1) with a tripodal primary amine (tris(2-aminoethyl)amine) (2) in order to form a protected tripodal molecule (3).

EDC·HCl (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) is an activating agent of carboxylic acids used to couple primary amines to form amides. It deprotonates the carboxylic acid and it binds with the activated molecule.

The HOBt (1-Hydroxybenzotriazole) is useful for synthetizing amides from carboxylic acids of amino acids. It forms an ester with the Aa and urea is synthetized as a sub product.

DIPEA (*N,N*-Diisopropylethylamine) has a nitrogen atom bound to an ethyl group and two bulky isopropyl groups. It is a good base but a poor nucleophile. In addition, DIPEA can neutralize the EDC·HCl so it can be soluble *(Appendix 1)*.

Finally, tren (Tris(2-aminoethyl)amine) (2) reacts with the carbonyl group and the HOBt molecule is recovered.

The obtention of the coupling product (3) was successful, as it is seen by NMR techniques such as ^{1}H-NMR and ^{1}H-^{1}H COSY. The protons named as H_A, H_E and H_B are easy to identify because they have particular shifts, but H_C and H_D protons are not as clear. Thanks to the COSY spectrum it is possible to assign the H_C because H_C protons interact with H_B protons while the H_D protons do not.

Also, the COSY spectrum let us identify which amide is each peak, because the NH_i is the one that interacts with the H_B proton.

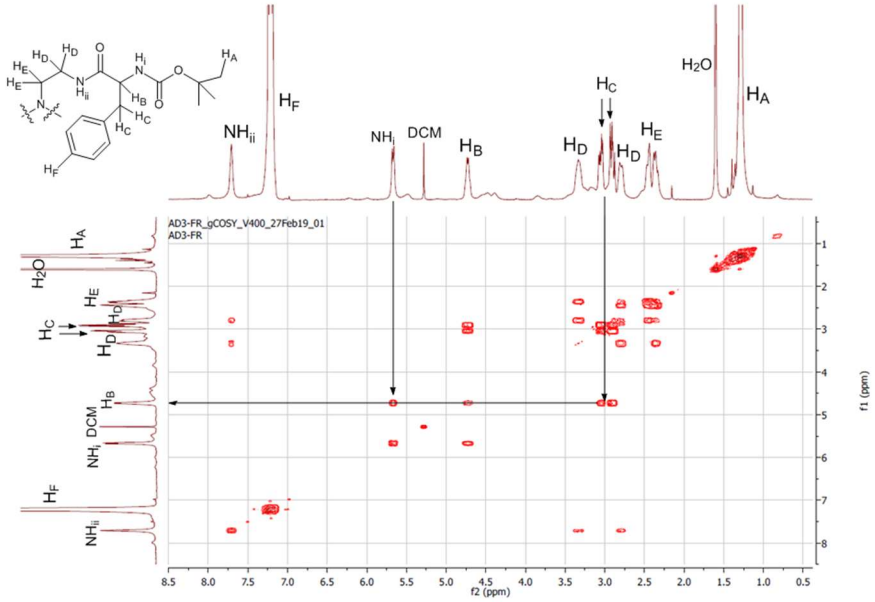
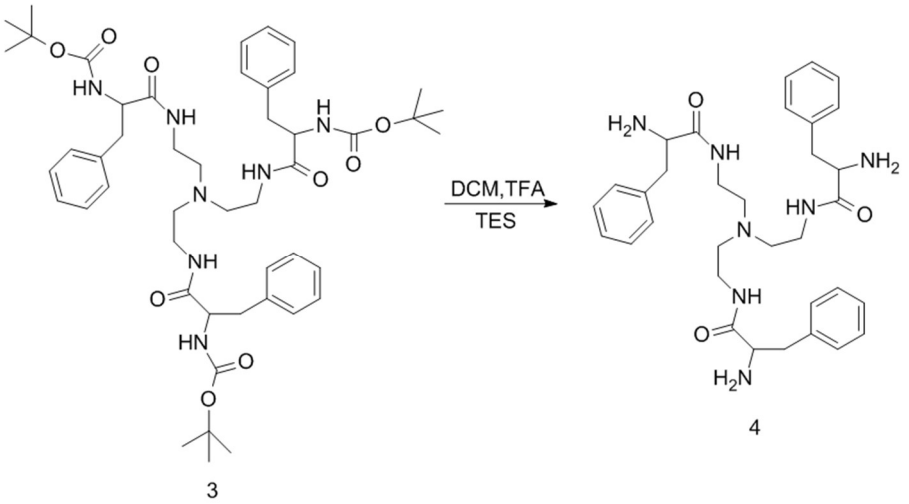


Figure 5. Coupling product's ^{1}H-COSY NMR in $CDCl_3$.

Thanks to the COSY spectrum it is thus possible to assign the H_C and H_D protons properly. It also allows us to differentiate both amides.

5.2. DEPROTECTION REACTION



Scheme 3. Deprotection reaction.

For the deprotection of the molecule (3) TFA (Trifluoroacetic acid) is used. This molecule is a strong carboxylic acid due to the high electronegativity of the trifluoromethyl. TFA can remove the t-BOC (*tert*-butyloxycarbonyl) group.

TES (Triethylsilane) is a mild reducing agent very useful in the removal of t-BOC groups. When it is used, the yields of the reaction increase, the concentration of TFA needed is lower and the reaction time is lower, too. In fact, mild reaction conditions for the deprotection are achieved thanks to this carbocation scavenger. When the amino acid is deprotected the amine is free, but CO_2 and a tert-butyl carbocation are formed. TES (hydride compound) deactivates the carbocation by reducing it and forms another, less-reactive silyl cation *(Appendix 2)*.

The Si-centred cation is less reactive because it is less electronegative than the carbocation, so it stabilizes better the positive charge. In addition, the bulky groups provide steric hindrance.

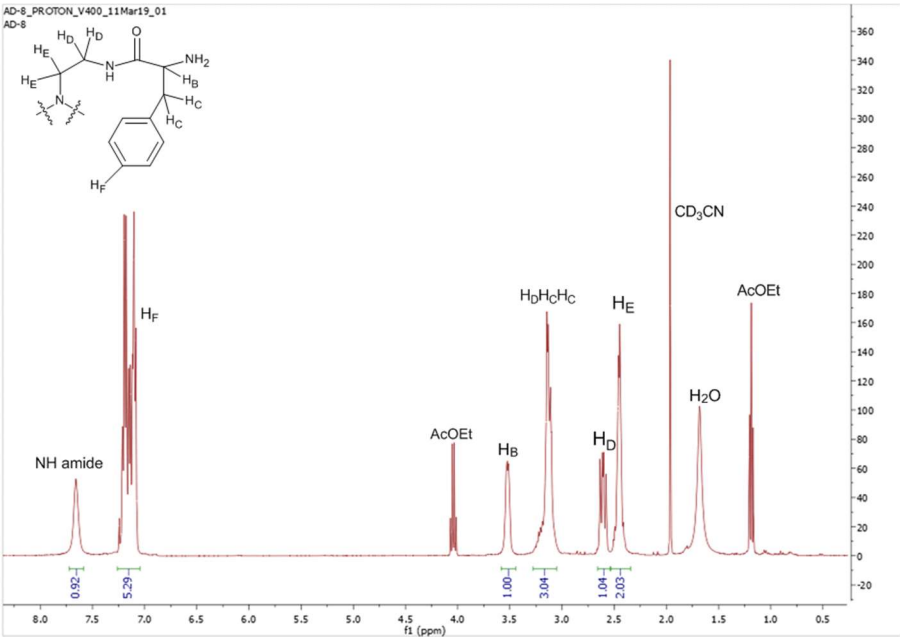
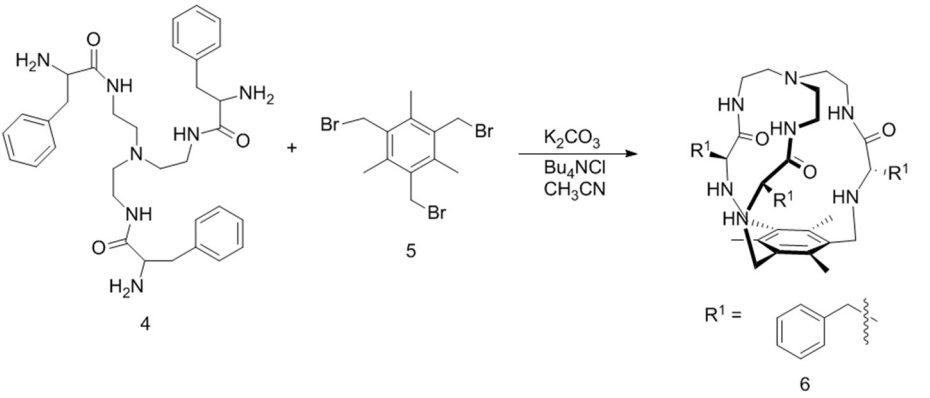


Figure 6. Deprotection product's ^{1}H-NMR spectrum.

The success of the deprotection reaction was checked by ^{1}H-NMR.

5.3. CYCLIZATION REACTION



Scheme 4. Cyclization reaction.

Three S_N2 reactions between the deprotected product (4) and the electrophile (5) should take place in order, so, the third one would determine if the final cage is or not the [1+1] one. The desired product ([1+1] cage) is the one produced by an intramolecular reaction of the last amino nitrogen with the unreacted Br anion, while the bigger cage ([2+2] cage) is produced by the competitive reaction, which is an intermolecular reaction between two intermediates (Scheme 1).

The medium must be basic, that is why K_2CO_3 is used. A S_N2 reaction can only be carried on if the nucleophile (free amine) attacks to an electrophile (bromide compound). If the medium is not basic enough, the amine will be protonated, and as a consequence, it will not have the unpaired electrons available to attack the electrophile *(Appendix 3)*.

NBu_4Cl (Tetrabutylammonium chloride) is used as a template. This compound increases the cyclization yields by non-covalent interactions with the reagents. It fixes the molecule and its orientation in order to promote the intramolecular S_N2 reactions. Without it the [2+2] percentage would be higher.

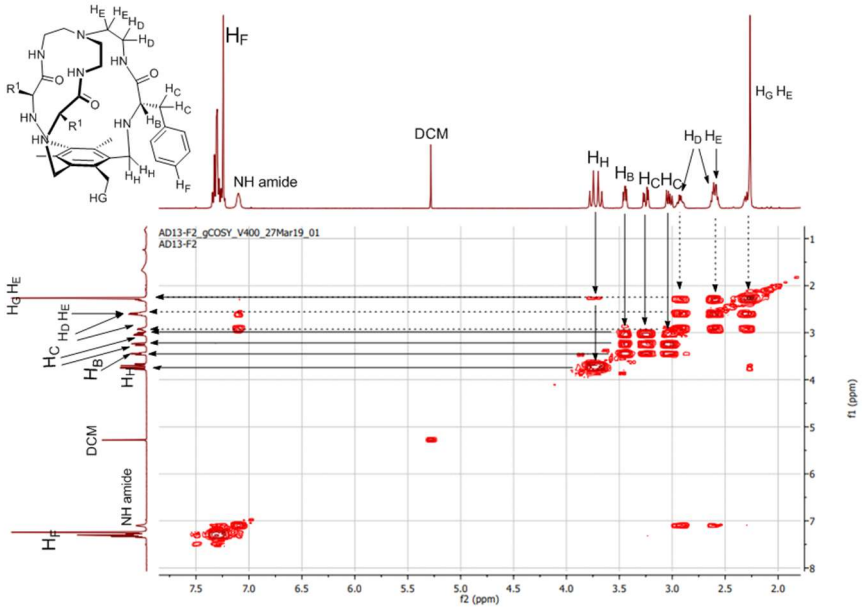


Figure 7. [1+1] cage's COSY.

Thanks to the final cage's COSY spectrum, the protons can be assigned properly. Once the protons are perfectly known, it is possible to identify which carbon corresponds to each peak of the ^{13}C-NMR spectrum thanks to the HSQC spectrum, that combines the ^{1}H-NMR and the ^{13}C-NMR.

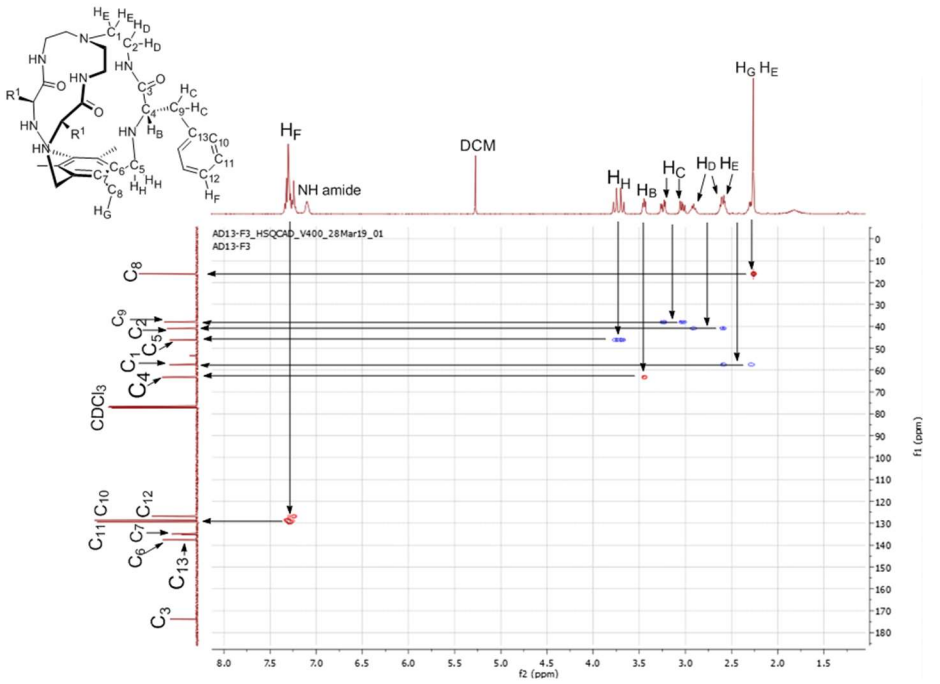


Figure 8. [1+1] cage's HSQC spectrum.

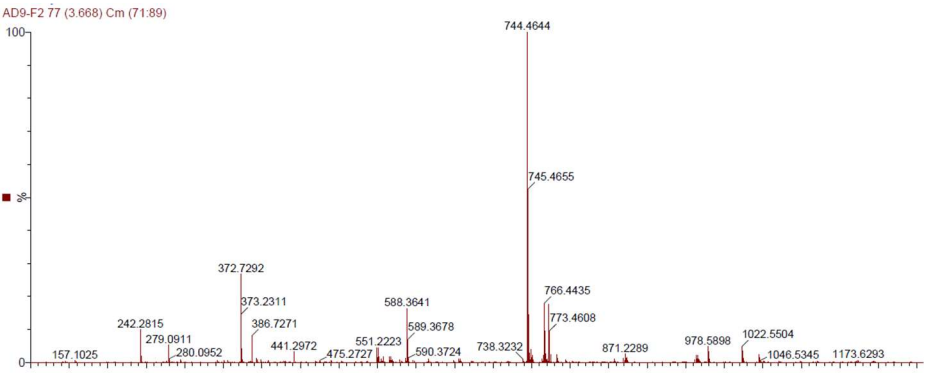


Figure 9. ESI-MS of the cage.

Thanks to the ESI-MS spectrum it is possible to identify the molecular peak (m/z = 744.4644), which belongs to the [1+1] cage. It is necessary to say that the cage does not ionize very well, so the peak should be even bigger. Another peak is also seen at higher m/z (m/z = 1488.9230) which

corresponds to the [2+2] cage. Thanks to the template effect it is possible to promote the intramolecular reactions that lead mainly to the synthesis of the [1+1], but it is not possible to avoid a little percentage of formation of the bigger cage.

A peak corresponding to half the molecular weight of the [1+1] cage (m/z = 372.7292) is also detected. This means an important percentage of the molecule ionized in that way.

The peak with m/z = 588.3641 belongs to a [1+1] cage but with a ramification hydrolysed. That happens when the atmosphere of the reaction is not inert, so it is very important to control this factor to avoid lower yields.

In summary, with all the spectroscopic (NMR) and mass data in hand, we can say that the desired cage has been successfully isolated and characterized.

6. CATALYSIS TESTS

6.1. TESTS WITH β -HALOHYDRINS

Once the cage has been properly synthetized, catalysis' tests can be carried out.

The first idea was to try the kinetic resolution (KR) of racemic β-haloalcohols to form enantioenriched epoxides.

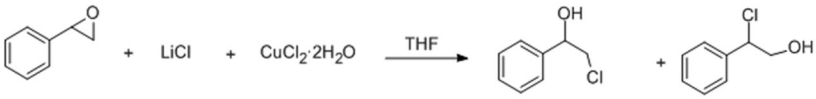
In this kind of reactions (KR) an enantiomer of a racemic compound is transformed stereoselectively to an enantiomerically enriched product[15], whereas the opposite enantiomer of the racemic substrate remains unreacted.

The facile ring opening of epoxides makes them extremely versatile intermediates for organic synthesis[16], which is why this catalysis is so relevant.

Supramolecular catalysis with β-cyclodextrin showed that it is possible to open ring epoxides to halohydrins through regioselective ways[17]. As a consequence, it is reasonable to think that our Phe-cage may be capable of catalysing the transformation of a racemic β-halohydrin to an enantiomerically enriched epoxide.

The interest of this reaction is to get the enantiomerically enriched product, which is an expensive product, from a cheap starting material.

The starting molecule was inexpensive racemic styrene oxide. By using chloride salts in THF, a mixture of two regioisomeric chlorohydrins were obtained, which were separated by flash chromatography and used as substrates in the subsequent catalytic tests.



Scheme 5. Synthesis of both halohydrins.

6.1.1. Catalysis with 2-chloro-1-phenylethanol substrate.

First of all, the substrate was injected in the chiral HPLC (*ChiralPak ID column*, reference in 7.1 section) to know whether the enantiomeric separation was possible.

After 20 minutes, two peaks of the same area were observed in the HPLC's trace with good separation, concluding that the analysis was successful.

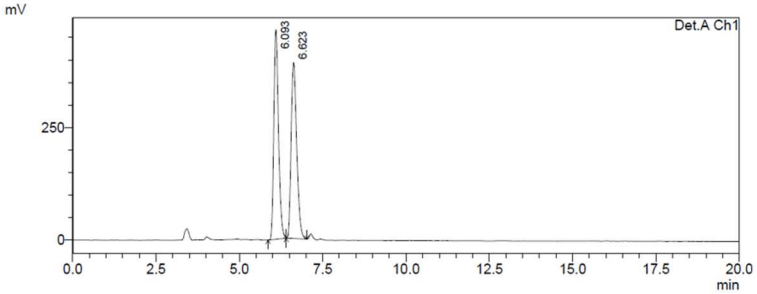


Figure 10. HPLC's diagram of the 2-chloro-1-phenylethanol racemic mixture.

Peak	Ret. Time	Area	Height	Area %	Height %
1	6.093	4550896	462281	**50.031**	54.220
2	6.623	4545246	390327	**49.969**	45.780
Total		9096142	852609	100.000	100.000

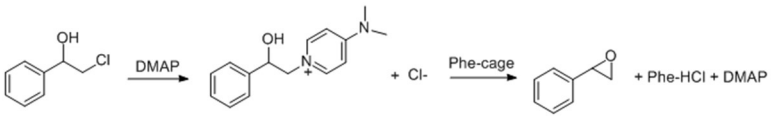
Once the enantiomers were separated by HPLC, we proceeded with the catalysis' test.

The reaction conditions tested were: 1 equivalent of the β-halohydrin, 0.5 eq of the Phe-cage and 270µL of the solvent.

In a first place, different solvents with different polarities were tested such as DCM (dichloromethane), ACN (acetonitrile) and TOL (toluene), to check whether the solvent polarity can affect the reactivity.

The reactions were checked by TLC and ^{1}H-NMR, and none of them worked, so the experiment was repeated at reflux conditions with ACN and TOL (DCM was not tried because of its low boiling point). Again, none of them worked.

Next, the reaction was tried by using 0.5 eq of DMAP (4-dimethylaminopyridine) with DCM or ACN at 35ºC. This molecule forms an intermediate compound that should help the Phe-cage to catch the chloride anion. In fact, it is used as a nucleophile catalyst. Both reactions failed again.



Scheme 6. DMAP's reaction mechanism.

6.1.2. Catalysis with racemic 2-chloro-2-phenylethanol as substrate.

The same HPLC procedure was applied to this molecule to check whether it could be enantiomerically separated as well. As it is shown in Figure 11, the separation with the same chiral column was even better.

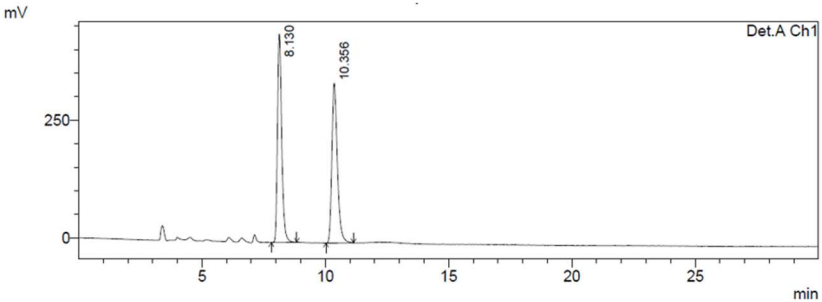


Figure 11. HPLC's diagram of the 2-chloro-2-phenylethanol racemic mixture.

Peak	Ret. Time	Area	Height	Area %	Height %
1	8.130	5235735	441147	**50.026**	56.539
2	10.356	5230397	339109	**49.974**	43.461
Total		10466132	780256	100.000	100.000

The same reaction conditions were tested with this β-chlorohydrine but all of them failed as well. After these deceiving results, these substrates were abandoned and we moved on to more reactive species that could be of synthetic interest as well.

	DCM	ACN	TOL	ACN 110ºC	TOL 110ºC	DCM + DMAP	ACN + DMAP
P1	x	x	x	x	x	x	x
P2	x	x	x	x	x	x	x

Table 1. Catalysis' test for both β-halohydrins.

(P1: 2-chloro-1-phenylethanol; P2: 2-chloro-2-phenylethanol; x: failed reaction)

6.2. TESTS WITH 3-CHLORO-3-(4-FLUOROPHENYL)PROPANOIC ACID

6.2.1. Catalysis of the 3-chloro-3-(4-fluorophenyl)propanoic acid

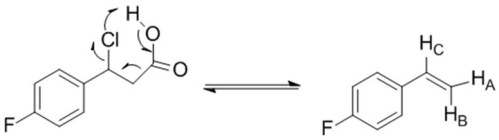
After the catalysis' test of the previous β-chlorohydrines, we proposed an alternative substrate. We proposed that 3-chloro-3-phenylpropanoic acid would be able to react with the catalytic cage to form β-lactones upon chloride abstraction. The reaction should take place easier because the carboxylic acid could protonate the cage and therefore initiate the reaction.

To begin with, we used a similar molecule that was available in the laboratory: 3-(4-fluorophenyl)-propionic acid. In this case, it was very easy to form its 3-chloro derivative through a radical chlorination of the benzylic position using sulfuryl chloride, so the catalysis' test could be done.

In the firsts test 10 mg of Phe-cage (0.5 equivalents) were added to 5.43 mg of the 3-chloro-3-(4-fluorophenyl)propanoic acid (1 eq). Three different vials were prepared with three different solvents. In one of them 270 μL of DCM were added to the reagents. In the other ones TOL and ACN were used.

At room temperature no significant changes were observed, so the vials with TOL and ACN were heated at 80ºC overnight.

Afterwards, the ^{1}H-NMR spectra showed (Figure 12), that the main product detected was actually a styrene compound formed through decarboxylation and elimination of the chloride due to the high temperature (Scheme 8).



Scheme 7. 1-fluoro-4-vinylbenzene's reaction mechanism.

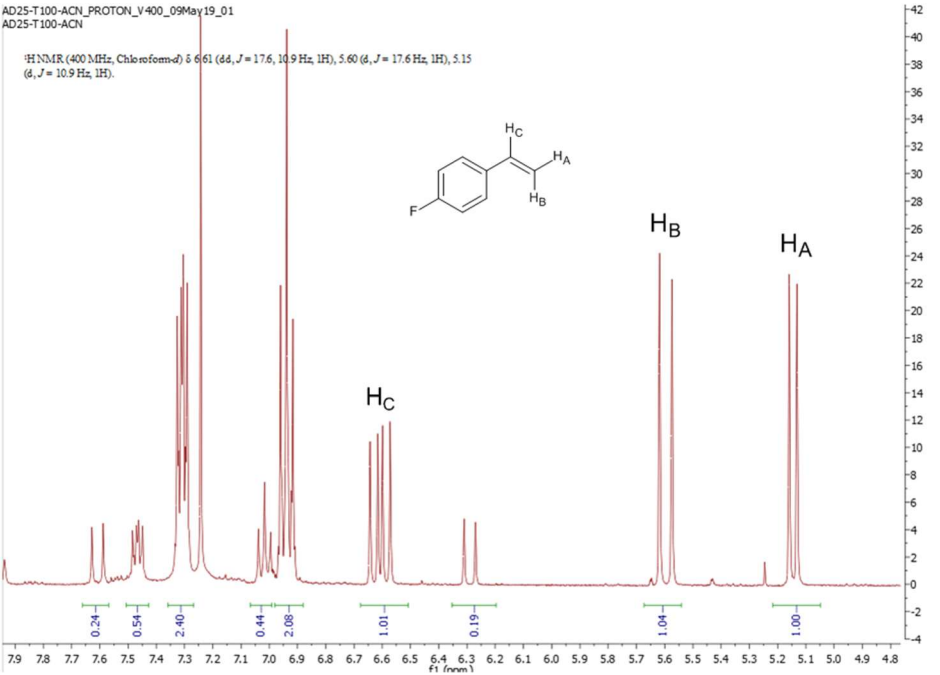


Figure 12. 1-fluoro-4-vinylbenzene's ^{1}H-NMR spectrum.

6.2.2. Lactone's test with silver carbonate

In order to see whether the formation of the desired lactone was possible and as a means of obtaining the racemic product as well, silver carbonate was added to 3-chloro-3-(4-fluorophenyl)propanoic acid under N_2 atmosphere.

40 mg of 3-chloro-3-(4-fluorophenyl)propanoic acid were weighted. The solid was dissolved with 20 mL of dry DCM. After that, 67.3 mg of $AgNO_3$ and 20.9 mg of Na_2CO_3 were added to the solution in order to form silver carbonate *in situ*.

The solution was stirring overnight at room temperature. The next day, the ^{1}H-NMR showed that the lactone compound was present in the solution, in a 1:1 ratio with the starting material.

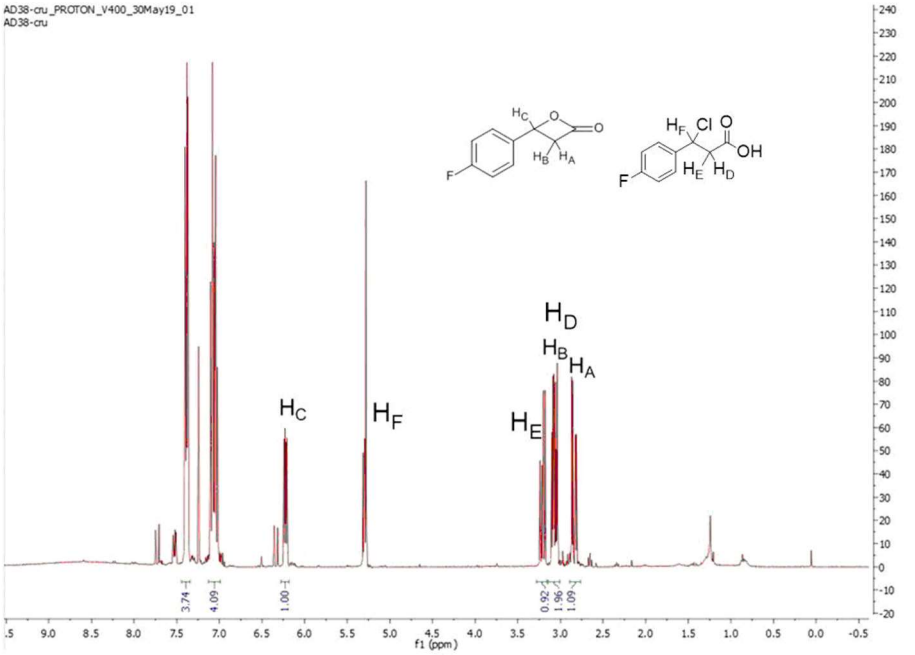


Figure 13. 4-(4-fluorophenyl)oxetan-2-one's ^{1}H-NMR spectrum.

The alpha protons of the lactone compound and the ones that belong to the initial product appear at the same shift approximately, but the H_C proton of the lactone appears at a higher shift than the one that belongs to the non-cycled compound.

7. EXPERIMENTAL SECTION

7.1. MATERIALS AND METHODS

The reagents and solvents were obtained from SigmaAldrich, Iris Biotech and TCI and no further purification has been made to them.

The NMR spectra (^{1}H-NMR and ^{13}C-NMR) were recorded in an *Agilent VNMRS Direct Drive,* with a frequency of 400MHz for ^{1}H-NMR and 101MHz for ^{13}C-NMR. The spectra were analysed with the software *MestReNova*.

The equipment for the mass spectra was the *UPL-ESI-TOF* [Acquity UPLC®BEH C181.7 mm, 2.1x100 mm, LCT Premier Xe, Waters]. (CH3CN + 20 mM HCOOH and H2O + 20 mM HCOOH).

The HPLC was done with an *Auto Sampler SIL-20A HT* and a *Detector SPD-20A* with a chiral column from *Chiral Technologies* called *ChiralPak ID.*

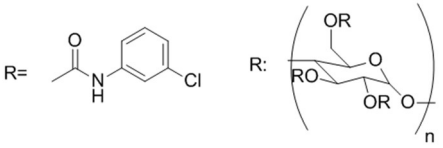


Figure 14. Structure of the chiral column based on an amylose derivative.

7.2. PREPARATION OF PSEUDOPEPTIDIC TRIPODAL CAGE

7.2.1. Coupling reaction

1.122 g of amino acid (BOC-L-Phe-OH) was dissolved in dry DMF (8 mL, the minimum amount needed). Then, 982 mg of EDC·HCl (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride) and 784 mg of HOBt (hydroxybenzotriazole) were added to the previous solution. Finally, 2.68 mL of diisopropylethylamine (DIPEA) was added to the solution. The reaction mixture was stirred under nitrogen at room temperature for 10 minutes to allow the activation of the acid.

All the reagents are commercially available.

Next, 200 µL of tris(2-aminoethyl)amine (Tren) were added using a Hamilton syringe.

The resulting solution was stirred under nitrogen atmosphere at room temperature overnight.

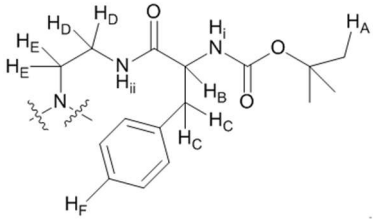
After 22 hours the formation of the product is observed by TLC (DCM:MeOH 95:5).

Work up: DCM (CH_2Cl_2) was added to the reaction crude and it was washed twice with water.

The organic phase was dried over $MgSO_4$ and the solvent was removed under vacuo leaving a solid product.

The resulting solid was purified using a flash column chromatography (DCM:MeOH from 98:2 to 97:3).

^{1}H-NMR (flashed product): ($CDCl_3$, 400MHz) δ (ppm) 1.29 (s, $9H_A$), 2.39 (m, $2H_E$), 2.80 (m, $1H_D$), 2.90 (dd, J=13.6 Hz, 8.9 Hz, $1H_C$), 3.05 (dd, J=13.6 Hz, 6.1 Hz, $1H_C$), 3,34 (m, $1H_D$), 4.74 (q, J=8.8 Hz, $1H_B$), 5.66 (d, J=8.7 Hz, $1NH_i$), 7.20 (m, $5H_F$), 7.70 (s, $1NH_{ii}$).



7.2.2 Deprotection of the peptide

The previous product (0.42 g) was dissolved in 3.64 mL of DCM. Next, 0.8249 g of triethylsilane (TES) and 3.64 mL of TFA (trifluoroacetic acid) were added to the solution.

The solution was stirred at room temperature opened to the air.

After 3 hours no more starting product was observed by TLC (DCM:MeOH 95:5).

Work up: The solvent was evaporated using a N_2 flow. Then, Et_2O was added and a white precipitate was formed. Using a number 3 filter the precipitate was separated from the solution.

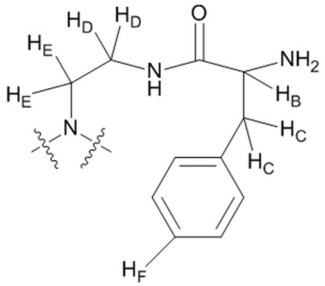
The resulting solid was picked up and NaOH 1M was added until reaching pH 11.

Next, the extraction of the organic phase with AcOEt (Ethylacetate) was done. The aqueous phase was washed 3 times with AcOEt.

The combination of the organic phases was dried with $MgSO_4$ and filtered with cotton.

Finally, the solvent was evaporated under vacuo, so the characterization was possible.

^{1}H-NMR: ($CDCl_3$, 400MHz) δ (ppm) 2.53 (tt, J=13.7 Hz, 7.0 Hz, $2H_E$), 2.64 (dd, J=13.5 Hz, 9.3 Hz, $1H_D$), 3.18 (dd, J=12.8 Hz, 4.7 Hz, $1H_D$, $2H_C$), 3.55 (dd, J=8.9 Hz, 3.7 Hz, $1H_B$), 7.21 (m, $5H_F$), 7.62 (s, 1NH amide).



7.2.3 Cyclization

The previous product (0.250 g) was dissolved in 60 mL of dry ACN (CH_3CN) by a reflux assembly with argon in a balloon, to prevent the hydrolysis of the product. Next, 0.16969 g of electrophile (1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene) were added. Then, 0.0591 g of NBu_4Cl (tetrabuthylamonium chloride) and 1.17567 g of K_2CO_3 (previously dried in the oven) were added to the solution.

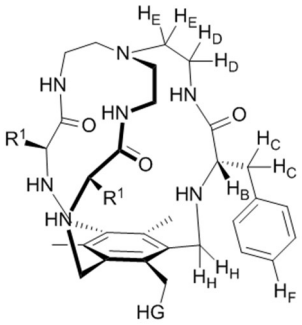
The solution was stirred at 100ºC with reflux overnight.

Work up: The solution was filtrated with a number 3 filter in order to remove the excess of carbonate.

The ACN was removed by evaporation under vacuo.

The resulting oil was purified using flash column chromatography (DCM:MeOH 99:1, 98:2, 97:3).

^{1}H-NMR: ($CDCl_3$, 400MHz) δ (ppm) 2.27 (s, 9H_G, 3H_E), 2.59 (dq, J=10.5 Hz, 5.9 Hz, 3H_E, 3H_D), 2.92 (q, J=6.8 Hz, 3H_D), 3.03 (dd, J=13.8 Hz, 7.3 Hz, 3H_C), 3.24 (dd, J=13.8 Hz, 4.7 Hz, 3H_C), 3.45 (dd, J=7.1 Hz, 5.0 Hz, 3H_B), 3.79 – 3.66 (m, 6H_H), 7.10 (s, 3NH), 7.28 (m, 15H_F).



7.3. SYNTHESIS OF THE CATALYSIS' SUBSTRATE

7.3.1. Synthesis of 2-chloro-1-phenylethanol and 2-chloro-2-phenylethanol

2.14 g of LiCl and 4.3 g of $CuCl_2 \cdot 2H_2O$ were dissolved in 50 mL of dry THF (Tetrahydrofuran) and were stirred under room temperature for 20 minutes.

Next, 1.144 mL of styrene oxide were added to the previous solution and the resulting solution was stirred under N_2 atmosphere overnight.

After 18 hours the progress of the reaction is checked by TLC (Hexane:AcOEt 80:20).

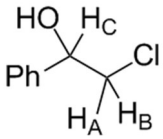
Work up: The reaction was quenched with a phosphate buffer (pH 7.0, 50 mL) and diluted with water.

After that, the extraction with Et_2O was done (5x50mL). Then, the organic phase was washed with water (2x50mL) and dried over $MgSO_4$.

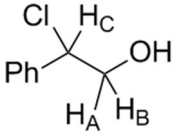
Next, the solvent was evaporated under vacuo.

Finally, both molecules were separated by column chromatography (Hexane:AcOEt from 95:5 to 70:30). Two fractions (determined by TLC and identified by ^{1}H-NMR) were obtained with one compound each one.

^{1}H-NMR (2-chloro-1-phenylethanol): ($CDCl_3$, 400MHz) δ (ppm) 2.63 (d, J=3.2 Hz, 1H (OH)), 3.64 (dd, J=11.2, 8.8 Hz, $1H_A$), 3.74 (dd, J=11.2, 3.4 Hz, $1H_B$), 4.89 (dt, J=8.8, 3.2 Hz, $1H_C$), 7.42-7.28 (m, $5H_{AR}$)



^{1}H-NMR (2-chloro-2-phenylethanol): ($CDCl_3$, 400MHz) δ (ppm) 2.10 (d, J=6.8 Hz, 1 OH), 3.98-3.87 (m, $2H_{A,B}$), 4.98 (dd, J=7.3, 5.8 Hz, $1H_C$), 7.42-7.3 (m, $5H_{AR}$).



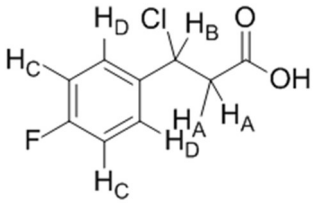
7.3.2. Synthesis of 3-chloro-3-(4-fluorophenyl)propanoic acid

0.5 g of 3-chloro-3-(4-fluorophenyl)propanoic acid and 10 mg of AIBN are weighted and added to the flask. Then, the recipient is purged with N_2 gas to avoid the contact of the oxygen with the solution. Next, 10 mL of benzene and 0.36 mL of SO_2Cl_2 are added with a Hamilton syringe. The solution is left overnight at reflux (100ºC).

Work up: The solvent is evaporated in vacuo. The minimum volume of DCM is added in order to dissolve the solid. Then, the same volume of hexane is added, and the mixture is left stirring for two hours.

After a couple hours the solution is filtered with a number 3 filter.

^{1}H-NMR: ($CDCl_3$, 400MHz) δ (ppm) 3.06 (dd, J=16.4, 6.0 Hz, $1H_A$), 3.21 (dd, J=16.5, 8.8 Hz, $1H_A$), 5.29 (dd, J=8.7, 6.0 Hz, $1H_B$), 7.11-7.00 (m, $2H_C$), 7.43-7.34 (m, $2H_D$).



8. CONCLUSIONS

0 - The phenylalanine [1+1] cage (6) has been synthesised successfully in pure form.

1 - The synthetized cage is not able to catalyse the formation of the epoxide molecule from the halohydrins precursors, as it can be observed in section 6.1.

2 - The phenylalanine cage is not able to catalyse the formation of the lactone molecule from 3-chloro-3-(4-fluorophenyl)propanoic acid, as it can be seen in the ^{1}H-NMR spectrum. At least, not under the conditions that have been tested up to now.

3 - The lactone was synthetized using silver carbonate, so the cyclization is possible.

4 - Further experiments will be carried out on this month in order to figure out whether the cage can catalyse the lactone's formation.

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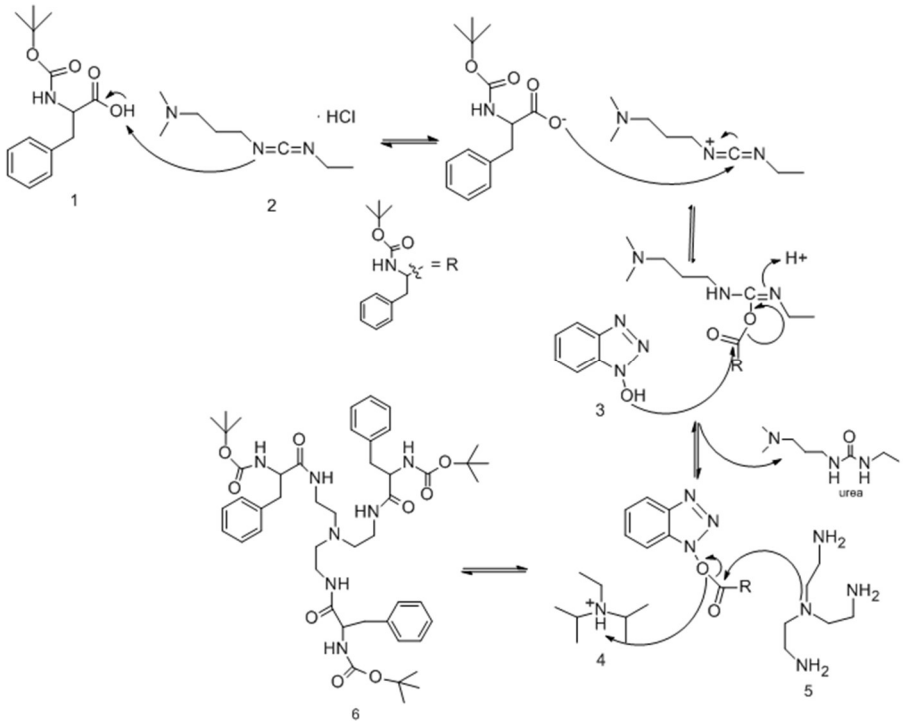
10. ACRONYMS

Aa	Amino acid
COSY	Correlation spectroscopy
D	Doublet
dd	Doublet of doublets
Dq	Doublet of quadruplets
ESI-MS	Electrosocpy ionization-mass sprectroscopy
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum coherence spectroscopy
m	Multiplet
NMR	Nuclear magnetic resonance
q	Quadruplet
S	Singlet
TLC	Thin layer chromatography
tt	Triplet of triplets

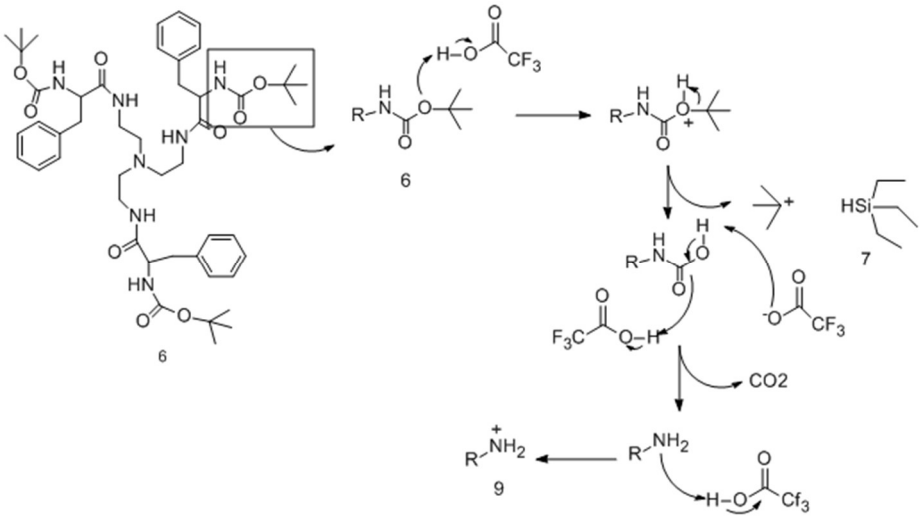
APPENDICES

APPENDIX 1: COUPLING MECHANISM



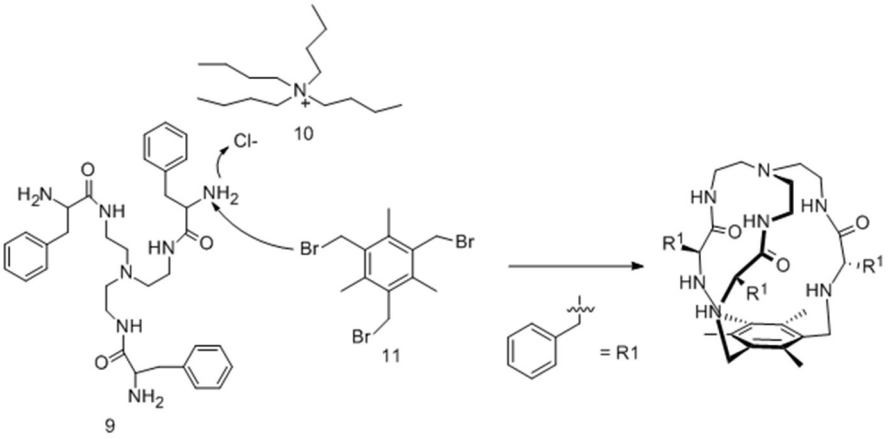
1: L-BOC-Phenilalanine
2: EDC·HCl
3: HOBt
4: DIPEA
5: Tren
6: Coupling product

APPENDIX 2: DEPROTECTION MECHANISM



6: Coupling product
7: Triethylsilane
8: TFA
9: Deprotection product

APPENDIX 3: CYCLIZATION MECHANISM



9: Deprotection product
10: Bu4NCl
11: Electrophile 1,3,5-tris-(bromomethyl)-2,4,6-trimethylbenzene