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Supramolecular Cages for the Recognition and Transmission of Chirality

Gàbies supramoleculars per al Reconeixement i Transmissió de la Quiralitat

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"Jugar al fútbol es sencillo, pero jugar un fútbol sencillo es la cosa más difícil que hay"

"Johan Cruyff"

A tot el personal del laboratori 309 del CSIC per resoldre'm tots els dubtes que m'han sorgit durant la realització del treball: l'Ignacio, el Willson, la Mònica, el Dani, l'Anna, el Jordi i sobretot el meu tutor, en Ciril, per donar-me suport en tot moment, i transmetre'm la calma necessària per seguir endavant quan hi havia complicacions.

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REPORT

IDENTIFICATION AND REFLECTION ON THE SUSTAINABLE DEVELOPMENT GOALS (SDG)

The CSIC has an agenda entitled 'White Book of Challenges for 2050', which in several volumes describes the challenges that the institution will face in the coming years. These include the goals set by the United Nations in its 2030 agenda. The CSIC volumes explore multiple areas linked to the 5Ps: People, Prosperity, Planet, Peace, and Partnerships.

One of the objectives of scientific research is to improve people's quality of life, and climate change plays an important role in this. That is why CSIC has been promoting global change by using historical data and previous knowledge to enable scientists to develop models that facilitate the prediction and mitigation of the climate effect.

The synthesis of the supramolecular cage itself and the characterisation of the cage using spectroscopic techniques do not match with any of the 17 Sustainable Development Goals (SDG). But on the other hand, the results of the work do. Relating it to the 5Ps, it can be related to Prosperity, as supramolecular cages may have very interesting applications in the future in molecular sensing. For example, in the field of catalysis, they can improve the efficiency of reactions. Also in molecular sensors, they can capture certain small molecules and detect if there is a specific molecule in a medium. Also in drug delivery, it is expected that cages could be used as a transport medium. In medical applications, supramolecular cages could be of significant value.

CONTENTS

1. SUMMARY

When a molecule cannot be superimposed with its mirror image, it is said to be chiral. This property is key in nature, as it is intrinsically present: for example, in nature there are D-sugars and, alternatively, L-amino acids. Chirality therefore plays a very important role in chemistry, as the properties of a compound can vary depending on its form.

In the context of a molecule, chirality is most commonly defined by the presence of an asymmetric carbon, i.e. a carbon that has four different substituents. In contrast, when talking about supramolecular chemistry, chirality refers to the distribution of the overall structure in an asymmetric manner, for example, like the secondary or tertiary structure of proteins.

In this work, using dynamic covalent chemistry (DCC), a chiral supramolecular cage (due to the presence of an asymmetric carbon) is synthesised. The aim is that a metal coordinates inside it and can recognise the chiral environment and coordinate in a specific way (Λ or Δ) to result in a supramolecular structure with a defined chirality. As a result, chirality will be conveyed from asymmetric carbon to supramolecular chirality.

As ligands for the cage synthesis a tripodal tris(ethylamino)amine (Tren) coupled to L-Phenylalanine (Tren-L-Phe) has been used. Tren-L-Phe, which provides the chirality of the asymmetric carbon and acts as an encapsulant. As a second ligand, a bypiridine derivative with carbaldehyde groups as substituents has been used, which allow coupling with the amines of the Tren-L-Phe via an imine bond. Moreover, the bipyridine can coordinate to a metal using the lone electron pairs of the nitrogen atoms.

To determine if the supramolecular cage has formed, whether with a coordinated metal inside or not, mainly ^{1}H NMR and circular dichroism measurements have been used. Using these techniques, it has been observed that by controlling the chirality of the asymmetric carbon, it is possible to control the way in which a metal coordinates inside the cage, confirming that there is chirality induction.

Keywords: Chirality, Covalent Dynamic Chemistry, Supramolecular Cage, Chirality induction…

2. RESUM

Quan una molècula no pot ser superposada amb la seva imatge especular, se li diu que és quiral. Aquesta propietat és clau en la naturalesa, ja que hi està present de manera intrínseca: Per exemple, a la naturalesa hi ha els D-sucres o, per altra banda, els L-aminoàcids. La quiralitat, per tant, té un gran paper en la química, pel fet que depenent de quina forma del compost estigui present, les propietats d'aquest poden variar.

En el context d'una molècula, el més habitual és definir la quiralitat observant si hi ha la presència d'un carboni asimètric, és a dir, un carboni que té quatre substituents diferents. En canvi, quan es parla de química supramolecular, la quiralitat fa referència a la distribució general de l'estructura d'una manera asimètrica, per exemple, en el cas de l'estructura secundària i terciària de les proteïnes.

En aquest treball, mitjançant química dinàmica covalent, s'ha sintetitzat una gàbia supramolecular quiral (per la presència d'un carboni asimètric). L'objectiu és que un metall es coordini en seu l'interior, sigui capaç de reconèixer aquest entorn quiral, i coordinar-se d'una manera específica (Λ o Δ) per donar com a resultat una estructura supramolecular amb una quiralitat definida. D'aquesta manera es produeix la inducció de quiralitat de carboni asimètric a quiralitat supramolecular.

Com a lligands per la síntesi de la gàbia s'han utilitzat la tris(etilamina)amina (Tren) acoblada a la L-Fenilalanina formant la Tren-L-Fenilalanina (Tren-L-Phe), que aporta la quiralitat de carboni asimètric i actua com a encapsulant. També un derivat de la bipiridina amb grups carbaldehid com a substituents, que permeten l'acoblament amb les amines de la Tren-L-Fenilalanina mitjançant un enllaç imina. A més, les bipiridines permeten la coordinació amb un metall mitjançant els parells d'electrons lliures dels nitrògens.

Per tal de determinar si la gàbia supramolecular s'ha format, ja sigui amb un metall coordinat en el seu interior o no, s'ha utilitzat principalment ^{1}H RMN i s'han fet mesures de dicroisme circular. Mitjançant aquestes tècniques, s'ha pogut observar que controlant la quiralitat de carboni

asimètric, es pot controlar la manera en que un metall es coordina a l'interior de la gàbia, confirmant així que hi ha inducció de la quiralitat supramolecular.

Paraules clau: Quiralitat, Química Dinàmica Covalent, Gàbia supramolecular, Inducció de Quiralitat

3. INTRODUCTION

Supramolecular chemistry is a relatively new branch of chemistry which has had a major impact since the end of the last century. This branch of chemistry, in contrast to traditional chemistry which studies covalent systems, focuses on how molecules organise and work collectively using also non-covalent bonds like hydrogen bonds, van der Waals forces, coordination bonds, electrostatic forces and others, to form the larger structures known as supramolecular structures.

3.1 DYNAMIC COVALENT CHEMISTRY

Dynamic covalent chemistry (DCC) is very useful in the context of supramolecular chemistry as it allows the formation of large superstructures in a relatively simple way. It is based on the formation of large structures through the formation of reversible covalent bonds under thermodynamic control. The fact that the bonds formed are reversible allows the different reactants to adapt to the conditions in which they find themselves or to respond to certain external inputs by generating a response. This factor is of great importance in the structures known as rotaxanes and catenans. [1]

One of the most interesting bonds in dynamic covalent chemistry and the one that has been used in of this work is the imine bond (C=N).

3.1.1 Dynamic Imine Chemistry (DIC)

Imine bonds formation, which consist of the reaction of a primary amine and an aldehyde, has the great advantage that it is a thermodynamically controlled reaction. The possible competitive kinetic subproducts or intermediates are transformed into the more energetically stable product simply by the passage of time. [2]

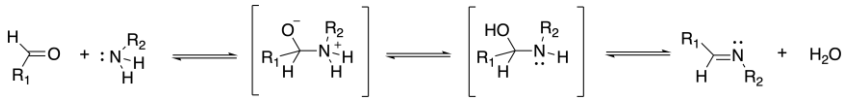


Figure 1. *Scheme of imine bond formation*

Imine bond formation is one of the many reactions that define DCC, which is widely used in the construction of molecules and large structures. [3] As they are reversible reactions, the products can be corrected if the expected products are not obtained by displacing the equilibrium to the reactants. This is a great advantage compared to other reactions because simply by adding the reactants in solution the reaction takes place. As the amine and aldehyde come into contact, they react. The imine bond has the special characteristic that it is strong and labile. This is surprising because usually, covalent bonds are strong and therefore inert, because they have a high bonding energy, but in this case, it can be easily hydrolysed. Thanks to the fact that the bond is labile, the imines can form and break until the thermodynamic product is obtained. [4]

Different operations can be performed to promote the formation of the thermodynamic product. A good option is to modify the starting compounds to increase the degree of preorganization to make the reaction more efficient towards the thermodynamic product. Non-covalent interactions are often used to do this type of operation.

An example of this is to use a metal as a template to preorganize the ligands so that they can react efficiently. Depending on the final structure that you want to obtain, one metal or another will be used. It is very important to consider the preferential geometry in which the metal is coordinated. If there are 6 anchoring points, an octahedrally coordinated metal will be used, but if there are only 4 anchoring points, the metal to be used must be coordinated tetrahedrally.

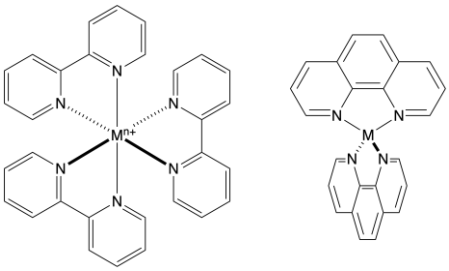


Figure 2. *Octahedral (left) and tetrahedral (right) coordination of a transition metal*

In Figure 2 right, it can be seen how the metal coordinates in a tetrahedral way and orders the bipyridines around it. A typical metal that can act in this way is Cu^{+}, which coordinates tetrahedrally when there are bipyridines in its proximity. On the other hand, in figure 2 left can be seen how a metal coordinates octahedrally with three bipyridines in a particular arrangement. In this case, Fe^{2+} or Co^{3+} can be used, because both can coordinate octahedrally. [5]

3.2 Supramolecular Tridimensionality Structures

In supramolecular chemistry there are many three-dimensional structures which, as discussed in the previous section, can be synthesised using DCC and specifically DIC. Some examples are rotaxanes or catenans, which are structures that can produce molecular machines. Molecular machines are a type of structure where the relative position of the components can vary directionally in function of an external input. The machines move in a particular direction when a stimulus or a reaction is applied modifying their structure. The current aim is transforming this movement into external work that can be applied at a macroscopic level. [6]

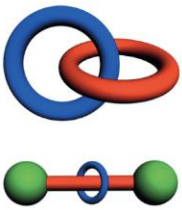


Figure 3. *Representation of rotaxanes (bottom) and catenans (top). Figure extracted from* [6].

Another example of a three-dimensional supramolecular structure are the helicates. These types of structures are formed by organic chains with coordination points in their structure, such as bipyridines. These organic chains are coordinated to transition metals, resulting in helicoidal structures. These structures have chirality due to the rotation of the organic chains along the helical axis. Depending on the direction of rotation, the enantiomer P is obtained if the rotation is to the right and the enantiomer D is obtained if the rotation is to the left. These structures are of great interest as they are very similar to biological DNA recognition structures and therefore, helicates allow us to understand what kind of interactions take place between these structures and DNA and the role of chirality, metal charge and size. [7]

As seen in the previous examples, supramolecular structures are of great interest and have great potential for study. In this work, the three-dimensional structures which are studied are supramolecular cages.

3.2.1 Supramolecular Cages

Supramolecular cages are three-dimensional structures which have a cavity inside them. In this cavity, different chemical entities can be bonded depending on the characteristics of the supramolecular cage and the atoms that form it (size, charge, coordinating atoms, etc.). Cages with nitrogen containing groups typically can bind transition metals. Similarly, when oxygen containing groups are present in the structure, different types of compounds can be encapsulated, such as alkaline or alkaline earth compounds, which are highly oxophilic, i.e., they have a high affinity for oxygen.

Also, to encapsulate anions it is common to use amino groups (R-N-H···X·) as they can stabilise the negative charge by means of hydrogen bonds. [8] In any case, a very important factor is the size of the cavity and the size of the compound to be encapsulated. If the structure is very rigid, the size of the cavity will be very specific and therefore only compounds with a very similar size (peak selectivity) can be captured. If the structure is more flexible, the selectivity for compounds will not be as specific and therefore compounds with small differences in size can be encapsulated (plate selectivity).

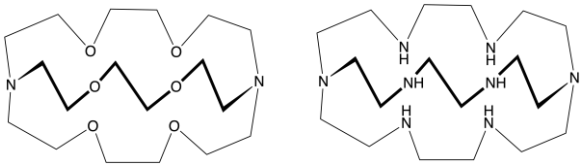


Figure 4. *Cryptand (left) and azacryptand (right)*

Figure 4 shows two structures, a cryptand and an azacryptand. The cryptand consists of a three-dimensional structure with oxygen in the organic chains and therefore can encapsulate alkalis and alkaline earths. K^+ is very similar in size to cryptane and therefore can be encapsulated inside it, but Na^+, can no longer be encapsulated in the same way, as K^+ and Na^+ vary in size (peak selectivity). On the other hand, azacryptand has amines with H in its chains and is therefore able to stabilise anions inside it. [9]

Supramolecular cages have different applications mainly in the field of catalysis, drug delivery and molecular sensors. The operation of these cages in the different fields of application is as follows:

In the field of catalysis, the cage captures the agent to be reacted and places it in such a way that it can react properly, thus improving the efficiency of the reaction. An example of catalysis is as follows: to perform a $KMnO_4$ oxidation, [2,2,2]-cryptand can be added to the reaction medium, which as mentioned is very specific for K^+. K^+ is captured and the free permanganate anion can perform its oxidative function more efficiently [10]. In the field of drug delivery [11] , the aim of supramolecular cages is to improve the drug delivery capacity and its transport within the body due to the strong interaction of the drug with the cage. This prevents uncontrolled drug release.

In the case of molecular sensors, cages are very useful because they allow greater control of the analyte, as the affinity is increased by the cage.

But in this work, the main question is how supramolecular cages can be used to study chirality and to see how the asymmetric chirality of carbon is transmitted when a metal complex is formed in the cage. First, an introduction of the necessary concepts of chirality in molecules and chirality in supramolecular cages will be done.

3.3 CHIRALITY IN SUPRAMOLECULAR STRUCTURES

When a molecule cannot be superimposed with its mirror image, it is said to be chiral. Chirality has great importance because it is present in biological systems. For example, in nature, the main components of proteins and enzymes are L-amino acids, and on the other hand, D-sugars are the main components of DNA or RNA.

The most common method for detecting chirality in a molecular system is to observe if there are asymmetric carbons, i.e. sp^3 carbons that are connected to four different substituents. Even if there is no asymmetric carbon, a molecule can be chiral if it has a plane ring which has two substituents that are attached to one face of the ring, making different the top and bottom of the ring and therefore a chiral molecule. This chirality is known as planar chirality. If the molecule has an axis around which a set of substituents is held in a spacial disposition which is not superimposable on its mirror image, this gives axial chirality. [12]

To define asymmetric carbons, the Cahn-Ingold-Prelog nomenclature is used, where the substituents are prioritised according to atomic number, and the molecule is then oriented in such a way that the group with the lowest priority is at the back. If the order of substituents turns to the right it is called a chiral centre R. If it turns to the left it is S. For chiral axes the notation aR and aS is used. To define which type of axis there is, one must look at the molecule from the side. First, the order of priority of the close substituents is assigned and then the priority is assigned to distant substituents. Then observe which way you have to turn to go from 2 to 3. If you go to the right, there is an axis aR and if you go to the left there is an aR. Finally, to determine what kind of chirality there is in a plane, names are given to the atoms. The x, y, and z are assigned as shown in the figure, where z is the first atom that is not in the plane. Then, from x, two substituents are assigned, where “a” is the one with the highest atomic number. Once this is done, the x-y bond is projected and the direction in which it has to turn to go from ‘a’ to ‘z’ is observed. If it turns to the left, there is a pS chiral plane and if it turns to the right there is a pR chiral plane.

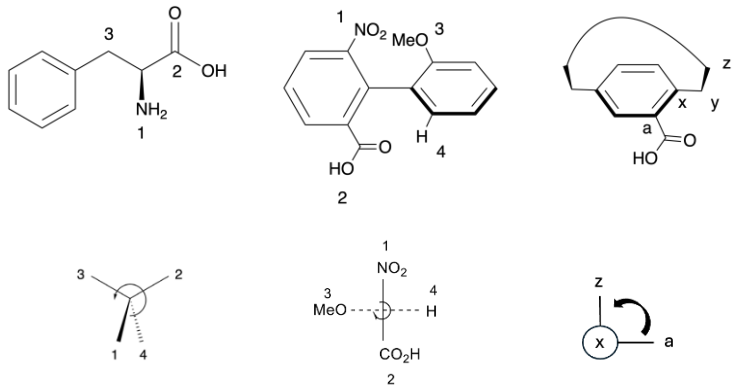


Figure 5. *Examples and representations of molecular chirality*

Supramolecular chemistry, as discussed previously, is based on noncovalent interactions and therefore its chirality is caused by how the components of supramolecular structures are disposed asymmetrically by noncovalent interactions. The chiral nature of the components plays an important role in determining the shape of the superstructures. A supramolecular structure can be chiral even if its components do not have any element of molecular chirality such as an asymmetric carbon, an axis, or a chiral plane, because the chirality of supramolecular structures depends on the arrangement of the elements of the structure, but this case is not of much interest, because the result is usually a racemic mixture. Therefore, the systems of interest are those in which the components that form the supramolecular structure have a certain chirality and there is induced chirality in the process of formation of the 3D structure. [13]

3.3.1 Induced Chirality

Induced chirality refers to supramolecular systems where an achiral component interacts with a chiral environment producing the induction of chirality, resulting in a supramolecular structure with chirality.

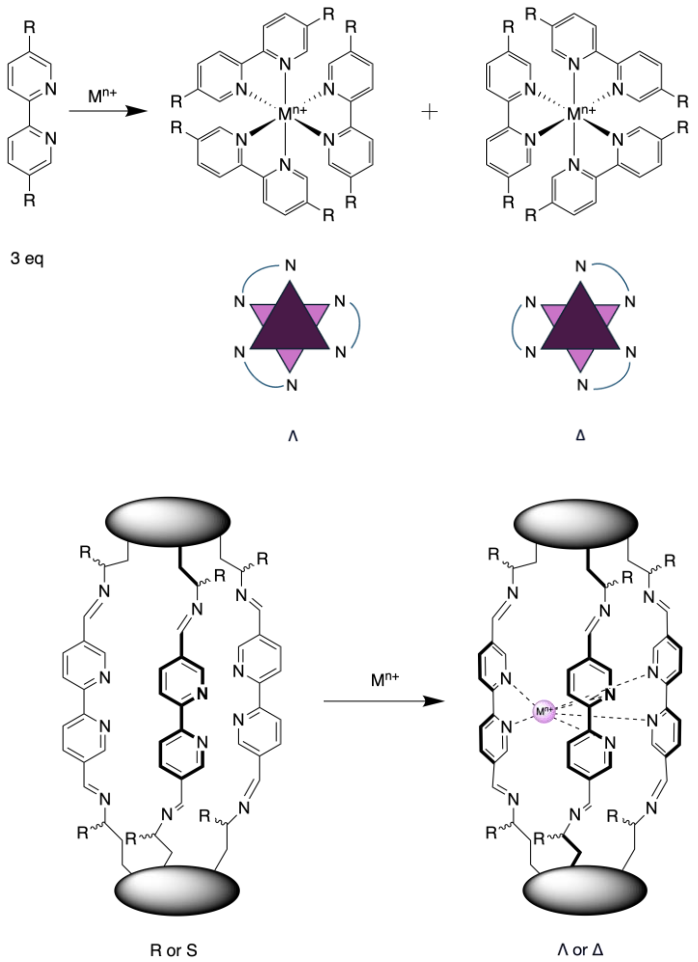


Figure 6. *Coordination of a metal in an achiral environment (top) and coordination of a metal in a chiral environment to give induced chirality (bottom).*

This concept of induced chirality can be understood from figure 6. A metal which coordinates octahedrally can do it in two different ways when 3 equivalents of a bidentate ligand are added, giving two enantiomers: the Λ and the Δ. The way to differentiate them is to look across the C3-axis and see which way the ligands turn. If they turn to the right, it is Δ and if they turn to the left, it is Λ. When the ligands that coordinates to the metal are achiral, a racemic mixture of the two enantiomers is obtained (top).

On the other hand, if a metal coordinates octahedrally with bidentate ligands with a defined chirality, the metal may coordinate in only one way, resulting in either a Δ or a Λ. This can be seen in Figure 6 (bottom), which is a model of a supramolecular cage formed by imine bonds coupling bipyridines with chiral tripodal amines. The fact that the tripodal ligand has asymmetric carbon chirality causes the metal to coordinate in a chiral environment, resulting in the induction of octahedral chirality. The result is a supramolecular structure where the bipyridines coordinate with the metal in a specific asymmetric way (Δ or Λ). If an encapsulant with defined chirality (R or S) is used and a specific metal coordination is obtained, using the encapsulant with opposite chirality results in the enantiomer of the supramolecular structure. [14]

This is a very interesting concept because by controlling the chirality of the asymmetric carbon you can control how a metal is coordinated. This allows a degree of control over chirality, which is very important because it is quite common in nature.

3.3.2 Characterization of Supramolecular Chirality

The most common method for determining chirality in supramolecular structures is circular dichroism (CD), which is a spectroscopic technique based on the application of circularly polarised electromagnetic radiation and detecting a response that is generated due to the interaction between the molecule and the radiation. The difference in absorption of the light polarised to the left versus to the right is measured. If this phenomenon occurs, it indicates that the molecule under analysis has optical activity. To observe this phenomenon, it is necessary to have chromophore groups in the system, otherwise circular dichroism will not be observed.

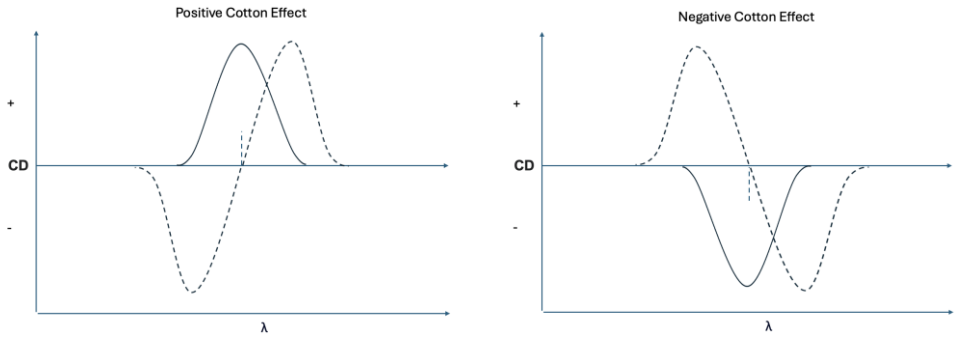


Figure 7. *Graphs of circular dichroism and the Cotton effect*

When the circular dichroism is measured, spectra with different shapes can be obtained.

Usually, peaks and valleys are observed. This is known as the Cotton effect, which corresponds to the change of circular dichroism in the vicinity of an absorption band. The Cotton effect is positive when there is a peak, and negative when there is a valley (Figure 7 solid line).

On the other hand, a second type of peak can be obtained which corresponds to the dashed lines in figure 7. These peaks, which change their sign of circular dichroism at the absorption maximum, appear if two or more chromophore groups are chirally oriented between them.

With circular dichroism spectra and the Cotton effect, chiral properties of molecular and supramolecular structures can be determined, as they help to understand how systems are organised in space and the interactions that are present in molecular systems. [14]

4. OBJECTIVES

Considering the concepts previously explained in the introduction, the aim of this work is the synthesis of a supramolecular cage to study the recognition of a chiral environment and to see how a chiral three-dimensional supramolecular structure is obtained when a metal is coordinated inside the cage.

For the synthesis, two types of molecules will be used: The bidentate ligands that interact with the metal, and the molecule that closes the system and has the asymmetric carbon chirality.

A bipyridine derivative carbaldehyde groups is used for coordination with the metal. Carbaldehyde groups will be used to make an imine bond with the encapsulant and close the system.

As the encapsulant molecule, a derivative of Tren coupled to L-Phenylalanine through an amide bond will be used. This molecule is a tripodal ligand which, starting from a nitrogen atom, has 3 equivalent branches which can bind with the aldehyde from the bipyridine to close the cage by making an imine bond. In this molecule there is an asymmetric carbon coming from L-Phenylalanine that provides the necessary chiral information to induce the chirality when the metal is coordinated.

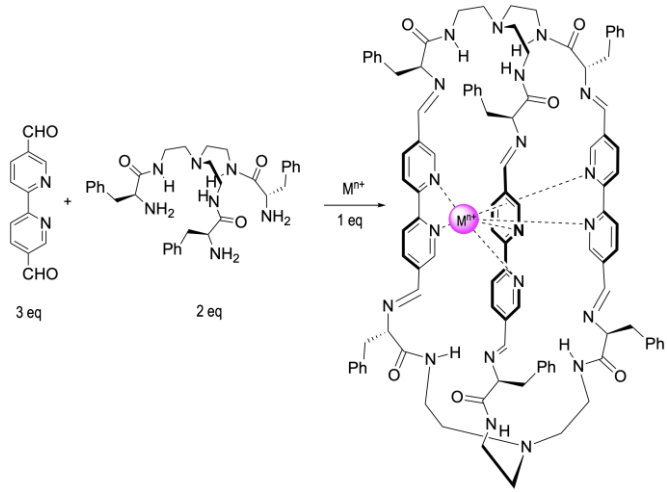


Figure 8. *Formation of a chiral complexed cage*

The aims of the work are summarised below in a descending order of their complexity.

- The first objective is to obtain the supramolecular cage without the coordinated metal. This is done to obtain information about the cage and to make comparison with a coordinated metal cage.

- Determine under which conditions a metal can be coordinated in a supramolecular cage. At what concentration, which solvents can be used, and which metal can be complexed on the supramolecular structure.

- The most important aim of the work is to determine that chirality is being transmitted when the metal is coordinated in the cage and that by using encapsulants with Tren-L-Phenylalanine, which has a defined chirality, only one coordination environment is obtained: Δ or Λ.

- Once this has been done, determine if using Tren-D-Phenylalanine gives the enantiomer from the previous cage.

5. RESULTS AND DISCUSSION

As indicated in the previous section, the first aim is the synthesis of the supramolecular cage. As mentioned in the theoretical framework, a good way to synthesise these types of compounds is by means of an amine and an aldehyde to obtain the imine and to get the three-dimensional cage. Therefore, there are two molecules to synthesise: The one that will complex octahedrally with the metal that is the [2,2'-bipyridine]-5,5'-dicarbaldehyde, and the molecule that acts as an encapsulant and makes the system cyclic by closing the complex witch is the Tren-L-phenylalanine.

Once the two molecules necessary to form the cage will have been synthesized, the cage will be assembled with and without metal to observe the differences between the two compounds and to see if the asymmetric carbon chirality is transmitted to the metal coordination.

5.1 SYNTHESIS OF REACTION BLOCKS

5.1.1 Synthesis of [2,2'-bipyridine]-5,5'-dicarbaldehyde

The synthesis process of [2,2'-bipyridine]-5,5'-dicarbaldehyde mainly consists of oxidation and reduction reactions to obtain the final aldehyde. All the reagents are easy to access, and the reaction conditions are easy to carry out. This synthesis block has been one of the most time consuming, because although the reactions are easy to do, it has been done several times to obtain a good amount of final product and to be able to make several tests to obtain the supramolecular cage and to find the optimal conditions.

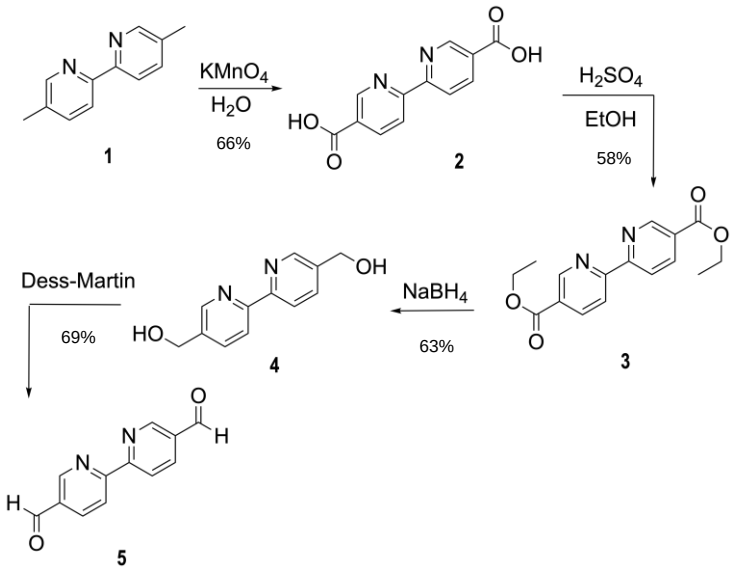


Figure 9. *Synthesis of [2,2'-bipyridine]-5,5'-dicarbaldehyde*

The first reaction consists in the oxidation of 5,5'-dimethyl-2,2'-bipyridine (1) using $KMnO_4$, obtaining carboxylic acid as product (2). After obtaining the acid, it is important to freeze-dry it to extract all the water that may remain in the product. The acid is added to ethanol with catalytic amounts of sulphuric acid to make the Fischer esterification and obtain diethyl [2,2'-bipyridine]-5,5'-dicarboxylate (3). The next step is to reduce the ester to alcohol. To do this, $NaBH_4$ is added to an ethanol solution with the ester and [2,2'-bipyridine]-5,5'-diyldimethanol (4) is obtained. Finally, the Dess-Martin reagent is used to oxidise the alcohol to aldehyde and obtain the [2,2'-bipyridine]-5,5'-dicarbaldehyde (5). The acid and ester do not require to be purified. The alcohol and aldehyde must be purified by chromatographic column. All products are obtained quantitatively. This procedure has been extracted from the following article. [15]

These oxidation and reduction processes are done in this way because it is easier to obtain the alcohol and then oxidise it to aldehyde than to reduce the carboxylic acid partially to aldehyde, as this is difficult to control it.

5.1.2 Synthesis of Tren-L-Phenylalanine

The second synthesis block consists of obtaining the Tren-L-Phenylalanine, which will react with the aldehyde to obtain the final cage.

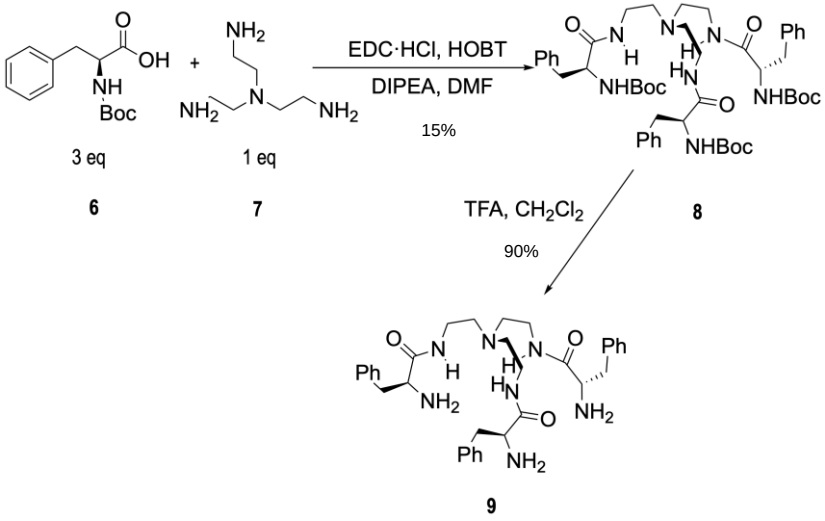


Figure 10. *Synthesis of Tren-L-Phenylalanine*

Initially, 3 equivalents of Boc protected L-Phenylalanine are reacted with 1 equivalent of Tren. In the reaction medium there is EDC·HCl, which is a carboxyimide that is used as an activating agent and allows coupling between the carboxylic acid and the primary amine to obtain the amide. The advantage of using this coupling agent is that a salt is generated as a by-product and can be extracted by liquid-liquid extractions from the reaction medium. Hydroxybenzotriazole (HOBt) is used to make an active ester, which reacts more efficiently with the primary amine to give the amide. N-N-diisopropylethylamine (DIPEA) is used as a base to activate the carboxylic acid.

As a product of the first reaction, Tren-L-phenylalanine (8) is obtained with the amino groups protected with Boc. The yield of the coupling reaction between Tren and L-Phenylalanine has been low, but it is known that this reaction works well.

Tren-L-Phenylalanine must then be deprotected from the Boc groups. To do this, trifluoroacetic acid (TFA) in dichloromethane (DCM) in a 2:1 ratio is used.

Once the reaction blocks have been obtained, the supramolecular cages can be synthesised.

5.2 FORMATION OF NON-COMPLEXED CAGE

Cage formation consists of the reaction between 3 equivalents of bipyridine dialdehyde and 2 equivalents of Tren-L-phenylalanine.

To determine the conditions at which the supramolecular cage was formed, different reaction tests were done.

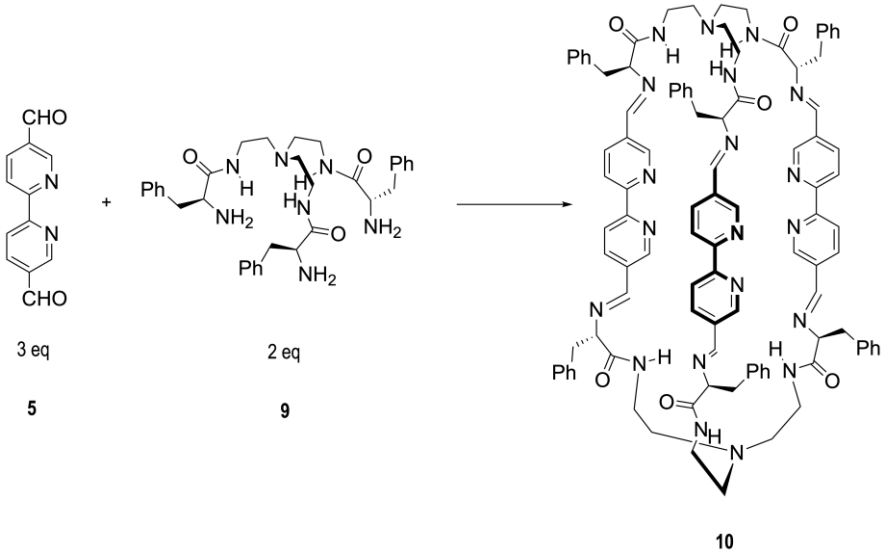


Figure 11. *Synthesis of non-complexed Cage*

5.2.1 Reaction Tests

To carry out the reaction, different solutions were prepared at a concentration of 10 mM of the two reagents. Subsequently, 300 microliters of aldehyde and 200 microliters of encapsulant were added. The main problem in this reaction is the weighing of the Tren-L-Phenylalanine to prepare the solution because it is a very viscous solid and it is difficult to weigh an exact amount. This can be problematic to obtain the supramolecular cage because if the reagents are not well weighed, they will not be in the necessary proportion to obtain the cage. That is why a control was done by ^{1}H NMR to check that the product was the desired one.

5.2.2 Control of Cage Reaction with ^{1}H NMR

To check that the cage was being obtained, proton RMN of the product were made. The fact that the imine covalent bond is chemically dynamic allows the reaction system to be modified and, depending on the conditions, the most stable product will be obtained, because all species are in equilibrium. If there is an excess of aldehyde, more Tren-L-Phenylalanine can be added to form the supramolecular cage completely and avoid a mixture of products.

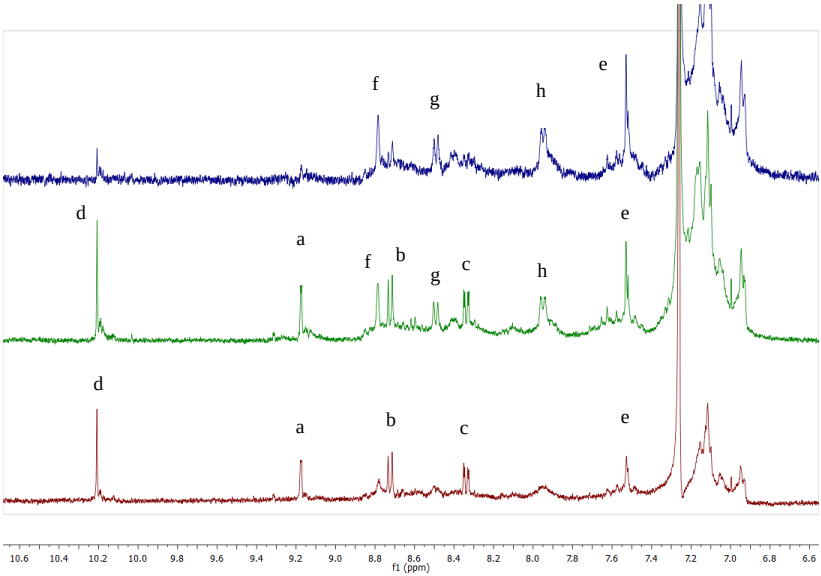


Figure 12. *Evolution of cage formation by ^{1}H NMR spectra from an excess of bipyridine dicabraldehyde (red spectrum), through an intermediate situation (green spectrum) to the formation of the cage (blue spectrum).*

The red spectrum corresponds to the first product obtained. The characteristic aromatic peaks of bipyridine (a,b,c) and the aldehyde peak (d), which is the most shifted, can be observed. At a shift of 7.5 ppm the imine peak (e) can be seen. This indicates that some coupling has occurred between the aldehyde and the amine but there is still an excess of aldehyde. There is the characteristic peak at 10.2 ppm and in the aromatic region only the bipyridine peaks with aldehyde as a substituent are clearly observed.

The green spectrum is the product of the red spectrum, but with 100 microlitres (10 mM) of additional Tren-L-Phenylalanine. It can be seen how in the aromatic zone the peaks of the red

spectrum appear, but there are 3 new peaks (f, g, h). This indicates that the coupling is taking place, as the imine has different substituent characteristics than the aldehyde and therefore the aromatic peaks appear at a different shift. The fact that the aromatic peaks of bipyridine (a, b, c) with aldehyde and the peak (d) at 10.2 ppm are still observed means that an additional 100 microlitres (10 mM) of Tren-L-Phenylalanine must be added to shift the equilibrium to cage formation.

In the blue spectrum it can already be seen that the peaks that were initially present in the red spectrum (a, b, c, d) have disappeared and there are only the aromatic peaks that appear in the green spectrum (f, g, h) and which correspond to the peaks of the bipyridine with C=N as a substituent. The aldehyde peak has also practically disappeared and therefore, there is only the supramolecular cage.

The overall spectrum in which all the proton peaks in the cage are observed is as follows:

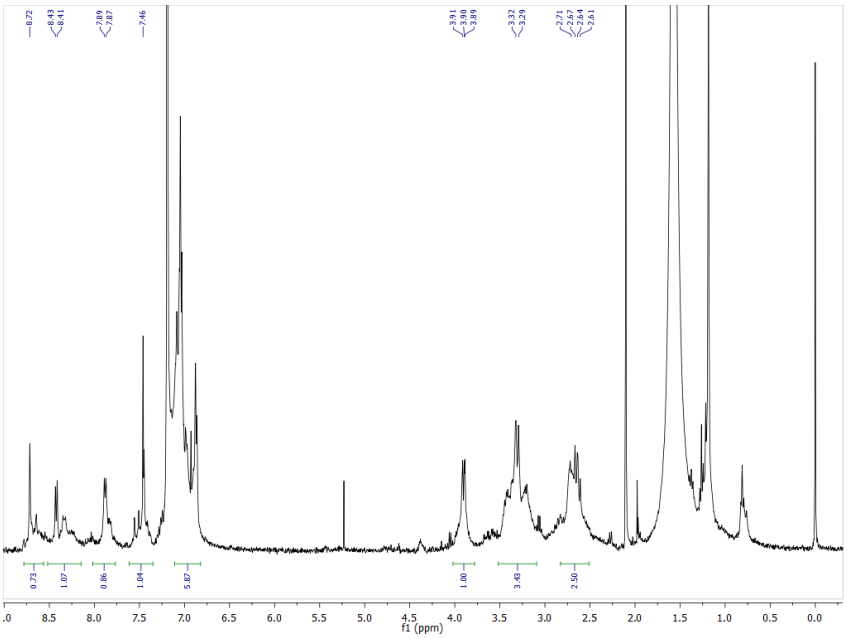


Figure 13. *^{1}H NMR spectra of the non-complexed cage (10)*

Aromatic benzene peaks of Tren-L-Phenylalanine appear below 7.26 ppm which is the peak of deuterated chloroform ($CDCl_3$). They are not very well defined because the cage is not a rigid structure, and this causes constant changes in the environment. The same happens with the protons of Tren-L-Phenylalanine which are not aromatic and appear between 2.5 ppm and 4.5 ppm. The peaks are not very well defined and that is why to determine that the cage has been formed it is observed how the aromatic peaks of the bipyridine vary when the cage is complexed and if there is the characteristic peak of the imine.

5.3 Formation of Complexed Cage

The reaction consists in the addition of 3 equivalents of dialdehyde bipyridine (5), 2 equivalents of Tren-L-Phenylalanine (9) and 1 equivalent of a transition metal salt.

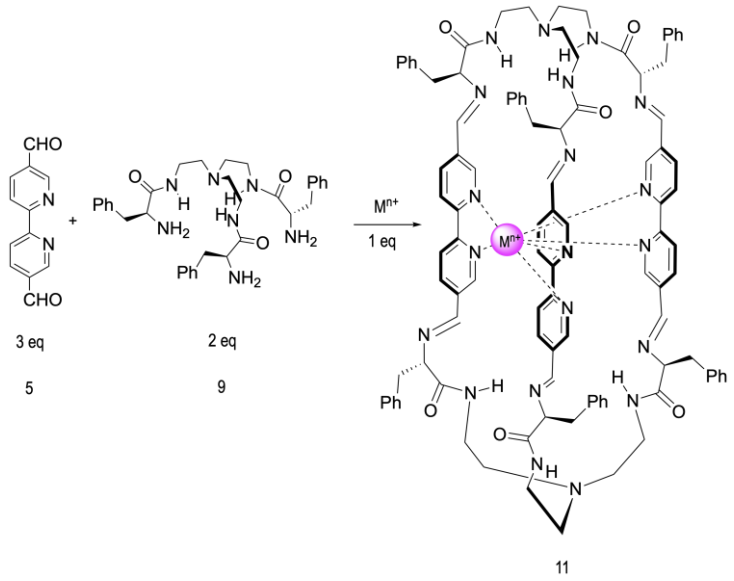


Figure 14. *Synthesis of complexed cage*

For the formation of the supramolecular cage with a complexed metal inside, different reaction conditions like solvent and concentration of the components were tested. In addition, the metal to be used must be octahedrally coordinated and must be dissolved in a solvent in which Tren-L-Phenylalanine and bipyridine dicarbaldehyde are also well dissolved.

5.3.1 Solubility Test

Fe^{2+} and Co^{3+} both coordinate octahedrally, so different tests were tried with different salts of these metals. First, solubility tests were done to find the best compound to obtain the cage.

Solubility Test	Compounds	Solvents		
		CDCl3	CD3CN	MeOD
1	$Fe(BF_4)_2 \cdot 6H_2O$	Yes	Yes	-
2	$Fe(C_2O_4) \cdot 2H_2O$	No	No	No
3	$[Co(NH_3)]Cl_3$	No	No	No
4	$Co(acac)_3$	Yes	No	-

Table 1. *Table of solubility of Fe^{2+} and Co^{3+} metal compounds in different organic solvents*

Solubility tests showed that $Fe(BF_4)_2 \cdot 6H_2O$ and $Co(acac)_3$ are the compounds that were soluble in the solvents of interest. We decided to make the supramolecular cage with Fe^{2+}, as it was also soluble in CD_3CN.

5.3.2 Reaction Tests

To make the reaction, first the three equivalents of bipyridine dicarbaldehyde and one equivalent of the metal-containing compound are added. A colour change is observed due to the coordination of the metal with the bipyridines. After half an hour, Tren-L-Phenylalanine is added.

The main problem with the formation of the supramolecular cage with the metal is that the polymerisation reaction is in competition with the macrocyclization that is favoured at very low concentrations [16]. Different tests were carried out to see under which conditions and at which concentrations macrocyclization with the coordinated metal is obtained.

	TESTS WITH $Fe(BF_4)_2 \cdot 6H_2O$						
	Tren-L-Phenylalanine		**Bipyridine Dicarbaldehyde**		**$Fe(BF_4)_2 \cdot 6H_2O$**		**Complexed Cage**
	C (mM)	D	C (mM)	D	C (mM)	D	
1	3,33	$CDCl_3$	5	$CDCl_3$	1,66	CD_3CN	X
2	1,33	$CDCl_3$	2	$CDCl_3$	6,66	CD_3CN	X
3	1,66	$CDCl_3$	2,5	$CDCl_3$	0,83	$CDCl_3$	X
4	5,8	$CDCl_3$	3,87	CD_3CN	1,93	CD_3CN	X - ✓
5	5,8	$CDCl_3$	3,87	CD_3CN	1,93	D_2O	X
6	**1,5**	**$CDCl_3$**	**2,25**	**CD_3CN**	**0,75**	**CD_3CN**	✓

a) C = Concentration, D=Dissolvent
b) All solvents are deuterated to do the ^1H NMR of each reaction product.

Table 2. Conditions of complexed cage formation with Fe^{2+} as metal

The first attempt to form the supramolecular cage with complexed metal was made with $CDCl_3$ as a solvent for Tren-L-Phenylalanine and bipyridine dicarbaldehyde. The product was a blue precipitate that when it was filtered, the colour was lost. This indicates that the product is not in solution and that the polymer was probably obtained. The ^1H NMR result was a spectrum similar to the spectrum of figure 13, indicating that the cage was formed, but not with the complexed metal.

Looking at the results of reaction 1, experiment 2 and 3 were done at the same time. In experiment 2, an excess of $Fe(BF_4)_2 \cdot 6H_2O$ was added by mistake. In this case, no precipitate was formed, which was surprising because the molar ratios were not correct. Observing the ^1H NMR it was confirmed that the result was not as expected, but the fact that no precipitate was formed gave the information that the concentrations of Tren-L-Phenylalanine and Dialdehyde Bipyridine had to be similar at 1.33 mM and 2 mM respectively.

In experiment 3, the difference is that in the addition of $Fe(BF_4)_2 \cdot 6H_2O$, which is initially dissolved in CD_3CN, more volume of $CDCl_3$ was added. Then, Bipyridine and Tren-L-Phenylalanine were added, both dissolved in $CDCl_3$. The result was, again, a blue precipitate as in the case of reaction 1. This indicated that $CDCl_3$ was not a good solvent to obtain the cage.

Knowing that chloroform was not a good option, bipyridine dicarbaldehyde was dissolved in CD_3CN thereafter. Experiments 4 and 5 were carried out simultaneously.

In experiment 4, at the beginning, the result of the reaction was not the complexed cage, although no precipitate was observed. When diluting with more acetonitrile by adding it directly to the round-bottomed flask and doing a ^1H NMR spectrum, it was observed that the cage had formed. The problem was that I did not know exactly how much solvent had been added.

On the other hand, in experiment 5, dissolving $Fe(BF_4)_2 \cdot 6H_2O$ in D_2O, the complexed cage was not obtained.

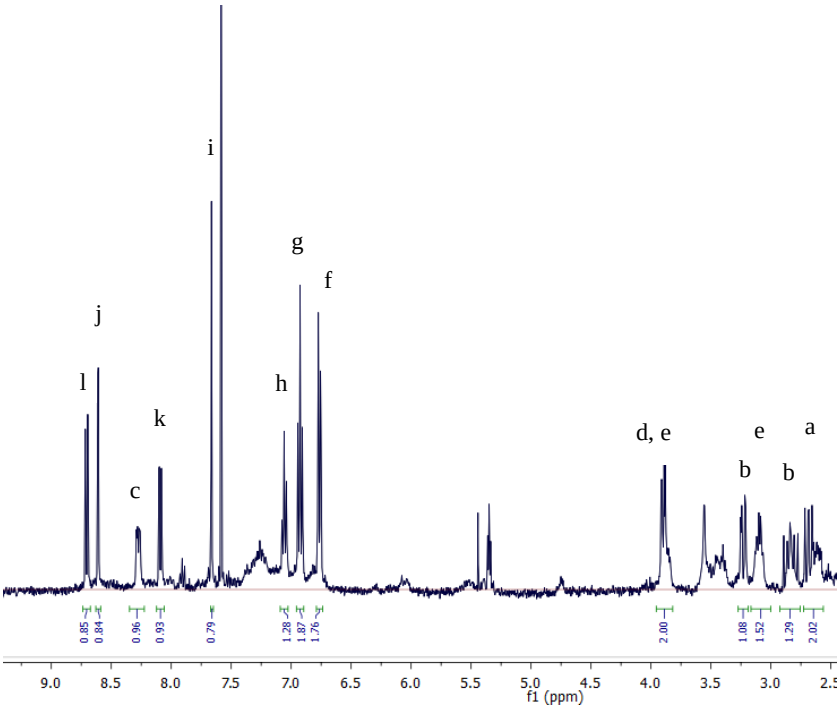
Finally, knowing that CD_3CN was the best solvent to obtain the complexed cage, and that the conditions had to be very dilute, experiment 6 was performed, obtaining the supramolecular complexed cage as a result. ^1H NMR confirmed that the cage had been formed.

5.3.3 Control of Complexed Cage Formation with ^1H NMR

The main difference between the spectrum of the complexed cage and the cage without metal is that all the peaks are better defined, because when the metal is coordinated, it makes the structure more rigid and therefore the environment is the same all the time. This makes the spectrum look much more defined, with clearer, narrower peaks

This effect can be observed especially in the aromatic peaks (f, g, h) of Tren-L-Phenylalanine, which are observed at a shift between 6.5 ppm and 7.3 ppm. The multiplicity of the peaks can be seen clearly and the corresponding 2:2:1 integration. The protons a, b, d, and e can also be seen more clearly at a shift of 2.5-4 ppm. Previously in the spectrum of the figure 11, these peaks were not correctly observed.

Finally, the bipyridine (j,k,l) and imine (i) peaks still appear at a similar displacement to the spectrum of the metal-free cage.



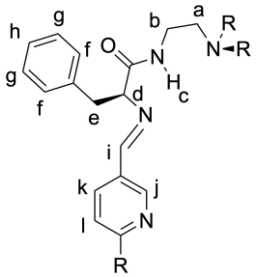


Figure 15. *^{1}H NMR spectra of the Fe^{2+} complexed cage (11)*

5.4 CIRCULAR DICHROISM MEASUREMENT

Once the cage without metal and the complexed cage were obtained, different dissolutions of each cage were prepared to measure circular dichroism. As explained in the introduction, circular dichroism is manifested in the absorption bands of the optically active species.

In principle, the metal-free cage is optically active due to the asymmetric carbons of Tren-L-Phenylalanine. On the other hand, the complexed cage, in addition to the chirality of the asymmetric carbons of the encapsulant, must have chirality due to metal coordination. In principle, the chirality of the asymmetric carbons should lead to metal coordination giving the Δ or Λ, making the cage optically active.

Compounds		Concentration (mM)
1	Tren-L-Phenylalanine - Bipyridine Dicarbaldehyde	0,12 0,024
2	Tren-L-Phenylalanine - Fe^{2+}- Bipyridine Dicarbaldehyde	0,12 0,024
3	Tren-D-Phenylalanine - Bipyridine Dicarbaldehyde	0,12 0,024
4	Tren-D-Phenylalanine - Fe^{2+}- Bipyridine Dicarbaldehyde	0,12 0,024

Table 3. Solutions for measuring circular dichroism

5.4.1 Circular Dichroism of non-Complexed Cages

The first CD measurements were made of the supramolecular cages without metal to observe the spectra obtained and later, when measuring the supramolecular cages with metal, to observe the differences and obtain conclusions. Figure 14 shows the comparison of the circular dichroism spectra of a cage formed with Tren-L-Phenylalanine and Tren-D-Phenylalanine. It is necessary to comment that the Tren-D-Phenylalanine was previously synthesised by the research group in which this work has been carried out, allowing me to make the cage with Tren-D-Phenylalanine and to be able to make the corresponding comparisons.

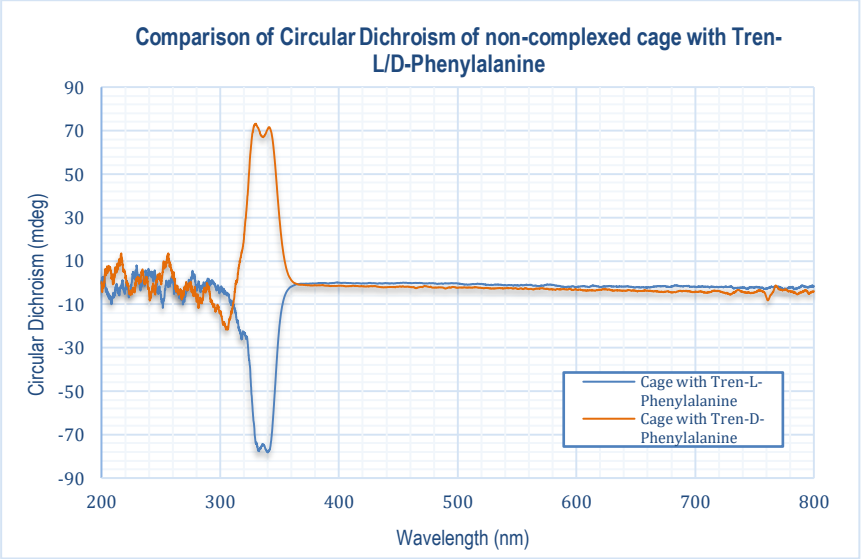


Figure 16. *Comparison of Circular Dichroism of non-complexed cage with Tren-L/D-Phenylalanine*

As can be seen in the graph, opposite spectra are obtained, indicating that the supramolecular cages are enantiomers, which agrees with expectations. Circular dichroism is observed at 330 nm, which is the wavelength at which bipyridines, the chromophore group, absorb [17]. In these spectra, there are no bisignal plots, indicating that the chromophore groups are not chirally ordered.

5.4.2 Circular Dichroism of Complexed Cages

Once the results of the circular dichroism of the cages without metal were obtained, the circular dichroism of the complexed cages was measured, and the following graphs were obtained.

There are important variations in the graphs obtained which give important information and confirm that the metal is coordinated in the cage.

Apart from the band at 330 nm due to the absorption of the bipyridines, a signal at 576 nm can now also be observed. This new band appears due to the charge transfers that take place when Fe^{2+} coordinates with the bipyridines, indicating that the complexed supramolecular cage is forming. [18]

Another key factor that indicates the formation of the complexed cage is that the signals are bisgnated bands. As discussed in the introduction, these band forms appear when the chromophore groups are arranged in a chiral manner. This indicates that the bipyridines are coordinating with the Fe^{2+} and this causes them to orient themselves in a specific way giving chirality to the superstructure.

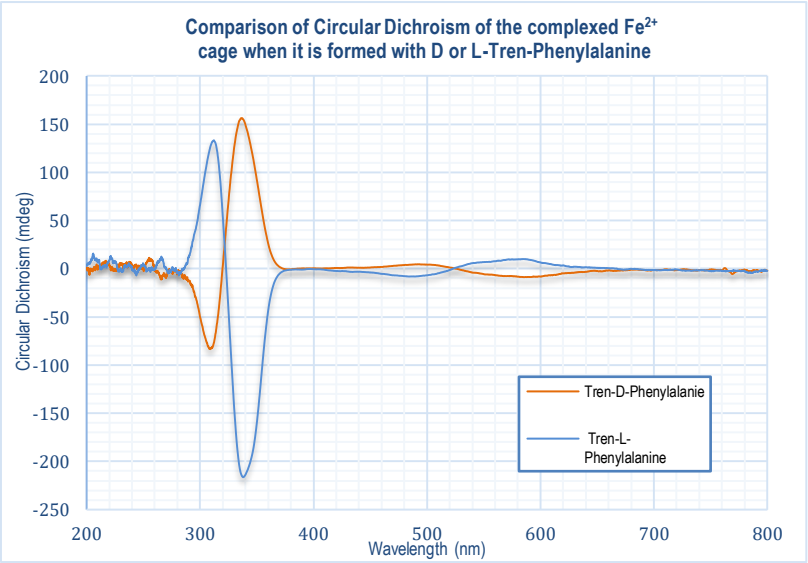


Figure 17. *Comparison of Circular Dichroism of the complexed Fe^{2+} cage when it is formed with D or L-Tren-Phenylalanine*

Another key factor observed is that the graphs obtained for the cage synthesised with Tren-L-Phenylalanine and Tren-D-phenylalanine are completely opposite in form. This confirms that the asymmetric carbon chirality is being induced properly and that the cages obtained are enantiomeric. This allows us to affirm that when an encapsulant with a defined stereochemistry is used, a single enantiomer cage is obtained, and not a diastereorecemic mixture.

To determine which type of coordination is obtained when the L or D encapsulant is used, the sign of the circular dichroism band of the bipyridine must be observed, because it is the one that is oriented around the metal. Following the assignment made in the reference [19], when Tren-D-Phenylalanine is used, the sign of the bipyridine band is positive and therefore the coordination is Λ. When Tren-L-phenylalanine is used, the sign of the band is negative, and the coordination is Δ. Therefore, only metal-cages with (D, Λ) or (L, Δ) stereochemistry are formed since the chirality at the metal complex is dictated by the stereochemistry of the chiral tripodal ligand.

6. EXPERIMENTAL SECTION

6.1. MATERIALS AND METHODS

6.1.1. Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H NMR and ^{1}H-^{1}H COSY were recorded on a Brucker 400 MHz spectrometer. The chemical shifts (δ) are in ppm and the coupling constants (*J*) in Hz. The solvent peaks have been used to reference the spectra. The multiplicity of the peaks is described as singlet (s), doublet (d), triplet (t), quadruplet (q), multiplet (m) and doublet of doublets (dd). To obtain the spectra, between 2 mg and 10 mg, depending on each sample, were dissolved in about 1 ml of deuterated solvent. The spectras were analysed with MestreNova and are displayed on Appendix 1.

6.1.2. Column Chromatography

The purifications of the products have been done with silica gel (SiO_2) as stationary phase and different mixtures of organic solvents as mobile phase. In each case that a column chromatography has been made, the type of eluent used is indicated.

6.1.3. Thin Layer Chromatography (TLC)

TLC was carried out on aluminium-supported sheets with a silica layer. In some cases, $KMNO_4$ was added to the silica support to observe the product marks. The TLCs were visualised with UV-light (254 nm).

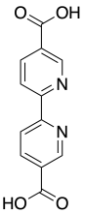
6.1.4 Circular Dichroism

Circular dichroism measurements were done on an instrument Jasco J-1500 CD spectrometer. To do the measurement correctly, first a blank measurement of the solvent was made in each case and then the sample was measured.

6.2 PREPARATION OF [2,2'-BIPYRIDINE]-5,5'-DICARBALDEHYDE (5)

6.2.1 Synthesis of [2,2'-bipyridine]-5,5'-dicarboxylic acid (2)

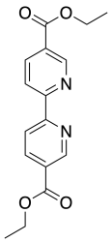
In a 250 ml round-bottomed flask, 3 g of 5,5'-dimethyl-2,2'-bipyridine (16.28 mmol, 1 equivalent) was added. Subsequently, 12.86 g $KMnO_4$ (81.4 mmol, 5 equivalents) and 185 ml H_2O were added as solvent. The reaction was refluxed at about 120ºC for 24 hours. After this, the reaction was cooled to 0°C and concentrated HCl was added until pH=3. The precipitate was filtered and freeze-dried for 48 hours to remove water from the solid. A total of 2.61 g of product was obtained (10.68 mmol, 66% yield).



White solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 13.55 (s,1H), 9.20 (d, *J* = 2.2 Hz, 1H), 8.58 (d, *J* = 8.1 1H), 8.45 (dd, J = 8.3, 2.1 Hz, 1H) ppm.

6.2.2 Synthesis of diethyl [2,2'-bipyridine]-5,5'-dicarboxylate (3)

The 2.61g of acid obtained in the previous section (10.68 mmol, 1 equivalent) were added in a 250 ml round-bottomed flask. 150 ml of 98 % EtOH and 4 ml of concentrated H_2SO_4 were added as catalyst. It was refluxed for about 5 hours and subsequently cooled in an ice bath. NaOH 4 M was added until basic pH to neutralise the previously added acid. Once precipitated, the solid was filtered and freeze-dried for 24 hours. 1.85 grams of product (6.16 mmol, 58 % yield) was obtained.



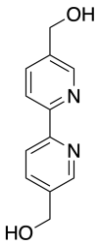
White solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 9.30 (dd, *J* = 2.1, 0.9 Hz, 1H), 8.58 (dd, *J* = 8.3, 0.9 Hz, 1H), 8.44 (dd, *J* = 8.3, 2.2 Hz, 1H), 4.45 (q, *J* = 7.1 Hz, 2H), 1.44 (t, *J* = 7.1 Hz, 3H) ppm.

6.2.3 Synthesis of diethyl [2,2'-bipyridine]-5,5'-diyldimethanol (4)

1 g of diethyl [2,2'-bipyridine]-5,5'-dicarboxylate (3.32 mmol, 1 equivalent) was added to a 250 ml round-bottomed flask with 1.25 g of $NaBH_4$ (33.2 mmol, 10 equivalents). A total of 200 ml of 98 % ethanol was added and left to reflux for 24 hours. After this, 4 M NH_4Cl is added to stop the reaction and the ethanol is rotavaporated, leaving the water from the NH_4Cl solution in the round-bottomed flask. Subsequently, a liquid-liquid extraction was made with ethyl acetate (3·100 ml). The combined organic extracts were dried with $MgSO_4$, filtered, and the solvent removed in the rotavapor.

The crude product was purified by column chromatography on silica gel using DCM/MeOH mixtures as the mobile phase, with methanol containing a small percentage of NH_3, which is used to deactivate the stationary phase. The mixtures of the mobile phase vary from 100:0 DCM/MeOH to 90:10 respectively. The crude product was prepared for purification by dissolving it in DCM and adding some silica gel. Upon removal of the solvent in vacuo, the silica-adsorbed crude was transferred to the column. The mobile phase is passed through the column and the different compounds are separated according to their affinity to the stationary phase. The mobile phase is collected in test tubes and TLC is performed to check if the collected fractions contain the product.

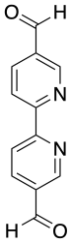
As stationary phase of the TLC, silica gel plates are used, and as the mobile phase, a mixture of DCM/MeOH 90:10 is used. Two fractions were obtained and the tubes containing the pure compounds were put together and rotavaporated. A 0.45 g product (2.08 mmol, 63% yield) was obtained.



White solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.64 (s, 1H), 8.29 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.92 (dd, *J*=8,1 1H), 4.72 (s, 2H) ppm.

6.2.4 Synthesis of [2,2'-bipyridine]-5,5'-dicarbaldehyde (5)

A 0.4 g (1.85 mmol, 1 equivalent) obtained from compound **4** was added to a 100 ml round-bottomed flask. Then 25 ml of DCM was added, and 3.31 g of Dess-Martin periodane (7.76 mmol, 4.2 equivalents) was added to oxidise the alcohol to aldehyde. The reaction was heated to reflux (about 50°C) and left to react for 3 hours. After the reaction, liquid-liquid extractions must be made to clean the product. A larger quantity (50 ml) of Et_2O than the previously applied DCM is added to the round-bottomed flask so that when extractions with aqueous solutions are made, the organic phase remains on top. The organic solution is then added to a 250 ml separating funnel washed with a 20 % solution of $Na_2S_2O_3$ (2x30 ml) and saturated $NaHCO_3$ (2x30 ml). The organic solution was dried with Na_2SO_4, filtered, and concentrated to dryness in the rotavapor obtaining 0.27 g of product (1.27 mmol, 69% yield).



White solid. ^{1}H NMR (400 MHz, Chloroforml-d4) δ 10.21 (s, 1H), 9.17 (dd, *J* = 2.1, 0.8 Hz, 1H), 8.72 (dd, *J*=8,2 1H), 8.34 (dd, *J*=8.2, 2.1 Hz,1H) ppm.

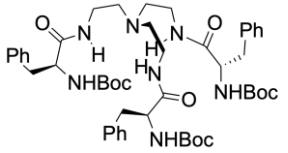
6.3 Preparation of Tren-L-Phenylalanine (9)

6.3.1 Synthesis of Protected Tren-L-Phenylalanine (8)

876 mg (3.3 mmol, 3.3 equivalents) of Boc L-Phenylalanine was added to a 10 ml round-bottomed flask. Then 5 ml of DMF, 750 mg of EDC·HCl (4 mmol, 4 equivalents), 610 mg of HOBt (4 mmol, 4 equivalents) and 2.1 ml of DIPEA (1.55 g, 12 mmol, 12 equivalents) were added. After 10 minutes, 150 µL of Tren (146 mg, 1 mmol, 1 equivalent) was also added. After 24 hours of reaction, the mixture was transferred to a separation funnel and washed with $NaHCO_3$ (3x10ml) and LiBr (1x10ml). Then, the organic phase was dried with Na_2SO4, filtered, and concentrated to dryness in vacuo.

The crude product was purified by column chromatography with SiO_2 as stationary phase and DCM/MeOH mixtures of 100:0 to 97:3 respectively (100 ml for each mixture) as mobile phase.

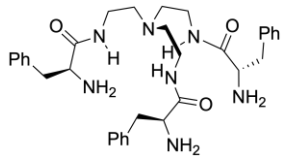
For TLC, the silica gel plate was used as stationary phase and a DCM/MeOH mixture 95:5 as mobile phase. TLC was developed using UV light (254 nm) and $KMnO_4$. Tubes containing the product were collected and rotavaporated, obtaining 150 mg of product (0.168 mmol, 15% yield).



Viscous Yellow solid.^{1}H NMR (400 MHz, Chloroform-d) δ 7.73 (s, 1H), 7.33 – 7.07 (m, 7H), 5.70 (d, *J* = 8.8 Hz, 1H), 4.75 (q, *J* = 8.4 Hz, 1H), 3.44 – 3.25 (m, 1H), 3.15 – 2.70 (m, 3H), 2.42 (ddd, *J* = 27.2, 7.4, 3.6 Hz, 1H) ppm.

6.3.2 Deprotection of Tren-L-Phenylalanine

To the 150 mg (0.168 mmol, 1 equivalent) obtained from product **8**, 4 ml of DCM and 2 ml of TFA were added. The reaction was left stirring for 3 hours. Afterwards, liquids were removed with a stream of N_2 (g). A liquid-liquid extraction was then carried out with NaOH 1M and AcOEt, keeping the product of interest in the organic phase. The organic solution was concentrated in the rotavapor obtaining 83 mg of solid. (0.16 mmol, 90% yield)



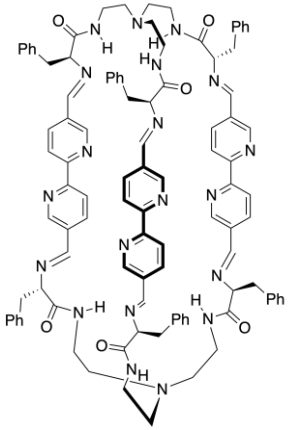
Viscous Yellow Solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.60 (s, 1H), 3.58 (dd, *J* = 9.2, 4.1 Hz, 1H), 3.2 (m, 3H), 2.65 (dd, *J* =13.6, 9.3 Hz, 1H), 3.54 (dt, *J* =7.9, 5.7 Hz, 2H) ppm.

6.4 FORMATION OF SUPRAMOLECULAR CAGES

6.4.1 Synthesis of Non-Complexed Cages (10)

To synthesise the supramolecular cage, 10 mM solutions of Tren-L-Phenylalanine and Dialdehyde Bipyridine were prepared using $CDCl_3$ and CD_3CN as solvents respectively. Then, 200 microlitres of Tren-L-Phenylalanine (0.002 mmols, 2 equivalents) and 300 microlitres of Dialdehyde Bipyridine (0.003 mmol, 3 equivalents) were added to obtain 0.001 mmol of

supramolecular cage (1 equivalent). The yield was not determined as the supramolecular cage was kept in solution to perform ^{1}H NMR and circular dichroism.

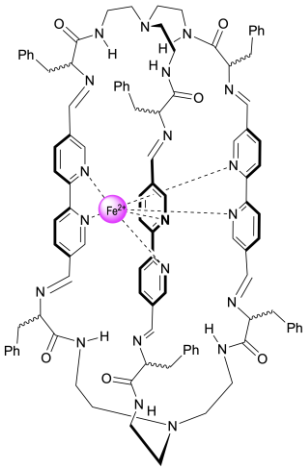


^{1}H NMR (400 MHz, Chloroform-d) δ 8.72 (s, 1H), 8.42 (d, J = 8.1 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.46 (s, 1H), 6.75-7.2 (m, 6H), 3.85 (dd, J = 10.3, 3.3 Hz, 1H), 3.45 - 3.1 (m, 3H), 2.65 (m, 3H) ppm.

6.4.2 Synthesis of Complexed Cages (11)

To synthesise the supramolecular cage with metal, 4.77 mg of bipyridine dicarbaldehyde (0.022 mmol, 3 equivalents) were weighed into a 25 ml round bottomed flask with a magnetic core. Subsequently, 8.5 ml of CD_3CN was added to dissolve the bipyridine and 2.4 mg of $Fe(BF_4)_2 \cdot 6H_20$ ($7.49 \cdot 10^{-3}$ mmol, 1 equivalent) was added. On adding the Fe^{2+} compound, the colour of the solution changed to blue because of the metal coordination with the bipyridines. After 30 minutes, 1.5 ml of the 10 mM Tren-L-Phenylalanine solution (0.015 mmol, 2 equivalents) was added. This is left for about 24 h with stirring and the colour changes to violet. This is an indication that the Tren-L-Phenylalanine has coupled with the aldehydes of the bipyridines. The yield was not determined as the cage was kept in solution for ^{1}H NMR and circular dichroism measurements.

The same procedure was followed to obtain the cage with Tren-D-Phenylalanine.



Violet solution. ^{1}H NMR (400 MHz, Acetonitrile-d3) δ 8.71 (d, *J* = 8.2 Hz, 1H), 8.61 (d, *J* = 1.6 Hz, 1H), 8.27 (d, *J* = 7.5 Hz, 1H), 8.09 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.7 (s,1H), 7.06 (t, *J* = 7.3 Hz, 1H), 6.93 (t, *J* = 7.6 Hz, 2H), 6.76 (d, *J* = 7.5 Hz, 2H), 3.90 (dd, *J* = 10.3, 3.3 Hz, 2H), 3.23 (dd, *J* = 13.3, 3.1 Hz, 1H), 3.09 (m, *J* = 5.9 Hz, 1H), 2.95 – 2.76 (m, 1H), 2.72 (m, 2H) ppm.

6.5 CIRCULAR DICHROISM MEASUREMENTS

Circular dichroism measurements have been made of the supramolecular cage without metal with the two types of encapsulant, and the cages with metal with the two types of encapsulant.

Dissolutions of concentrations 0.12 mM and 0.024 mM of each compound were prepared. It must be noted that the concentrations are approximate because the dilutions have been made from the reaction solution itself, considering a yield of 100%. The results of the measurements can be seen in Appendix 2.

7. CONCLUSIONS

The conclusions of the work are the next ones:

- By means of dynamic covalent chemistry and more specifically, the formation of the imine bond, supramolecular cages as described in this work can be synthesised.

- To obtain the cage with coordinated Fe^{2+}, it is important that the concentration is around 0.75 mM so that the bipyridines can coordinate properly to the metal and the encapsulant can react correctly, otherwise, polymeric by-products can be obtained.

- It can be affirmed that, having the asymmetric carbon chirality defined, chirality can be induced and cause a metal to coordinate in a stereoselective way.

- By using the compound of opposite chirality in the encapsulant, the enantiomeric cage can be obtained.

- Using ^1H NMR, a large change in the spectrum can be observed when the metal is coordinated or not, as the coordination of the metal causes the structure to be much more rigid and therefore the spectrum to be much more defined.

- By making circular dichroism measurements it can be determined that enantiomeric cages are obtained depending on the chirality of the asymmetric carbon present in the cage.

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9. ACRONYMS

- ^{1}H-^{1}H COSY: Homonuclear correlation spectroscopy
- 5P: People, Planet, Prosperity, Peace, and Partnerships
- AcOEt: Ethyl Acetate
- Boc: Tert-Butyloxycarbonyl
- CD: Circular Dichroism
- Co: Cobalt
- Cu: Copper
- DCC: Dynamic Covalent Chemistry
- DCM: Dichloromethane
- DIC: Dynamic Imine Chemistry
- DIPEA: N-N-diisopropylethylamine
- DMF: Dimethylformamide
- DNA: Deoxyribonucleic acid
- EDC·HCl: (3-Dimethylamino-propyl)-ethyl-carbodiimide Hydrochloride
- Et_2O: Diethyl ether
- EtOH: Ethanol
- Fe: Iron
- HOBt: Hydroxybenzotriazole
- *J*: Coupling constants
- K: Potassium
- M^{n+}: Metallic Ion
- NMR: Nuclear Magnetic Resonance
- RNA: Ribonucleic acid

- SDG: Sustainable Development Goals
- TFA: Trifluoroacetic acid
- Tren: Tris(ethylamino)amine
- Tren-L-Phe: Tren-L-Phenylalanine
- acac: Acetylacetonate
- d: Doublet
- dd: Doublet of doublets
- m: Multiplet
- q: Quadruplet
- s: Singlet
- t: Triplet
- δ: Chemical shifts

APPENDICES

APPENDIX 1: ^{1}H NMR AND ^{1}H-^{1}H COSY SPECTRA

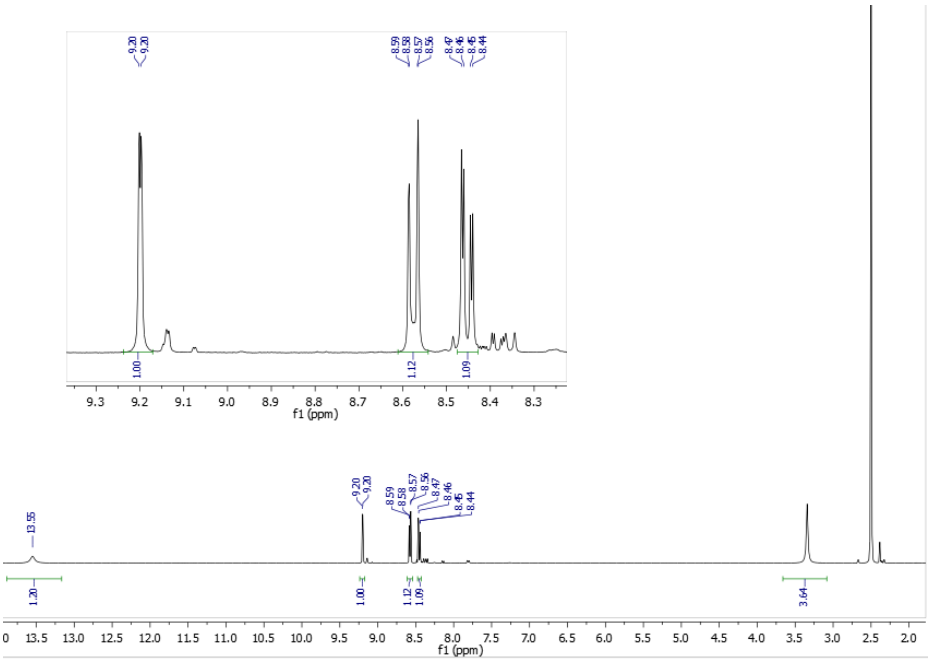


Figure 1: *^{1}H NMR spectra of [2,2'-bipyridine]-5,5'-dicarboxylic acid*

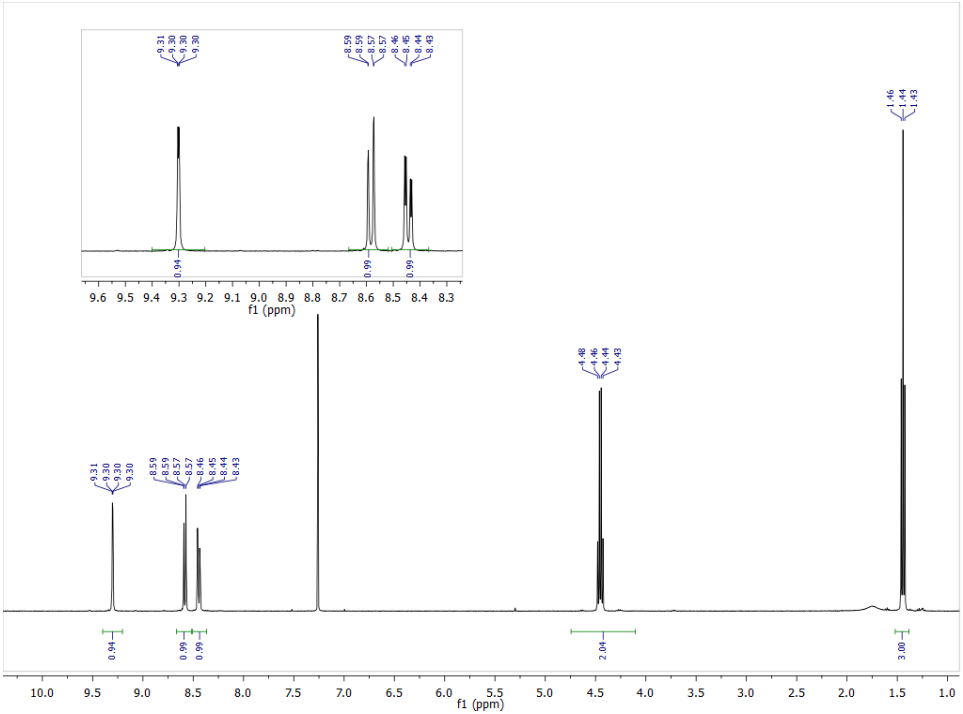


Figure 2: *^{1}H NMR spectra of diethyl [2,2'-bipyridine]-5,5'-dicarboxylate*

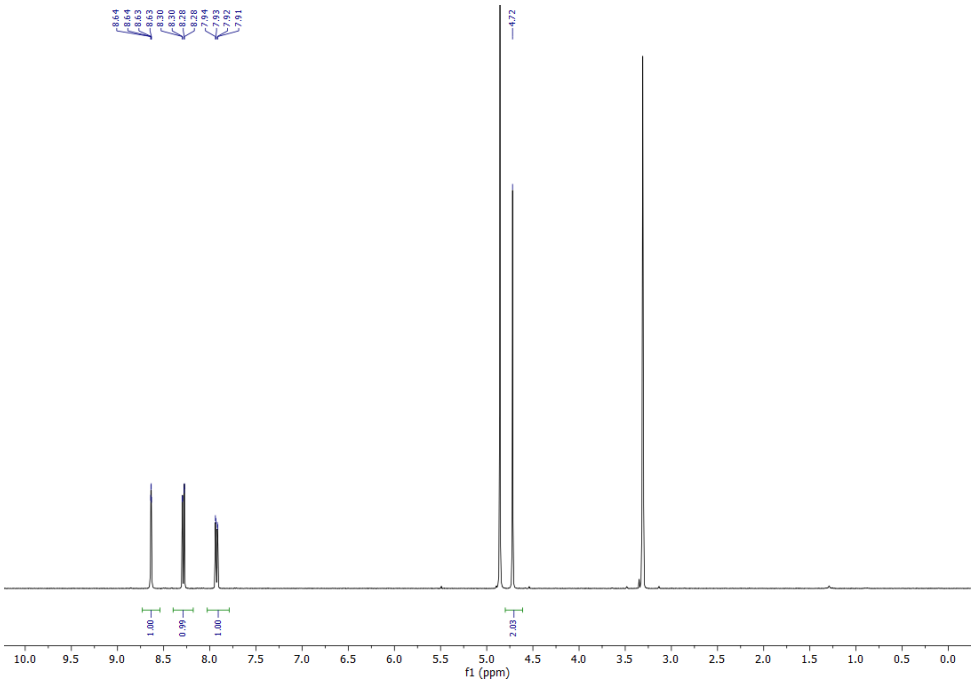


Figure 3: *^{1}H NMR spectra diethyl [2,2'-bipyridine]-5,5'-diyldimethanol*

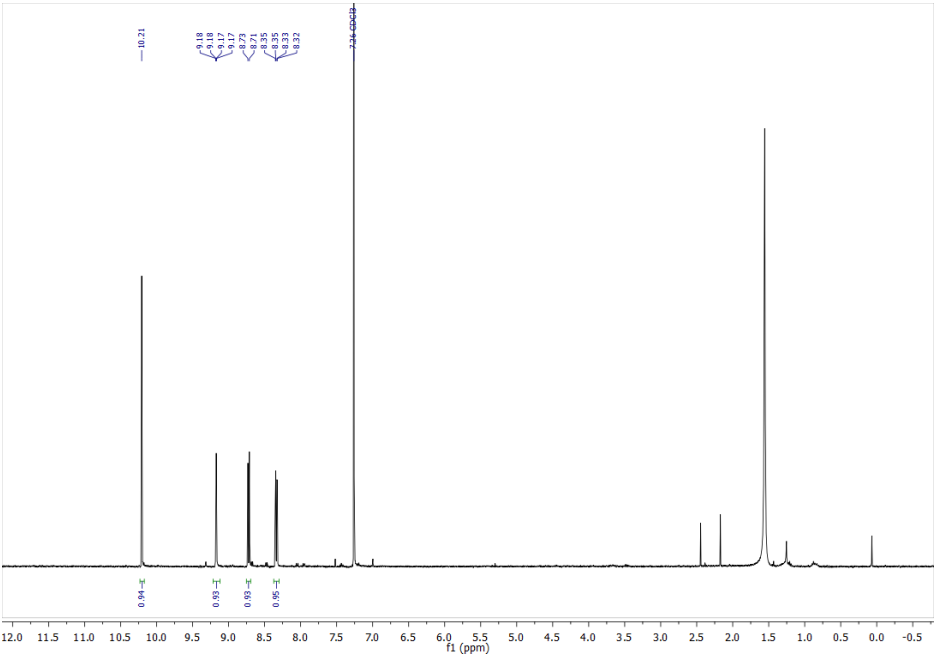


Figure 4: *^{1}H NMR spectra of [2,2'-bipyridine]-5,5'-dicarbaldehyde*

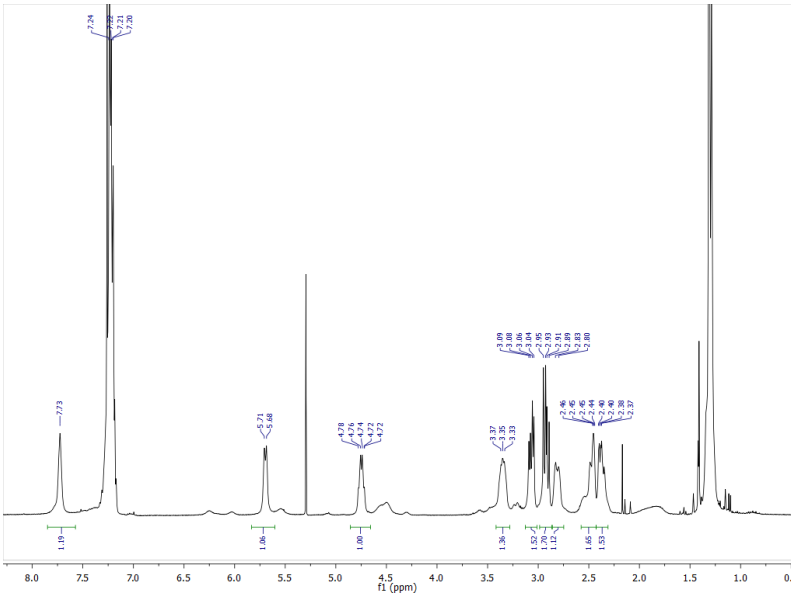


Figure 5: *[1]H NMR spectra of Protected Tren-L-Phenylalanine*

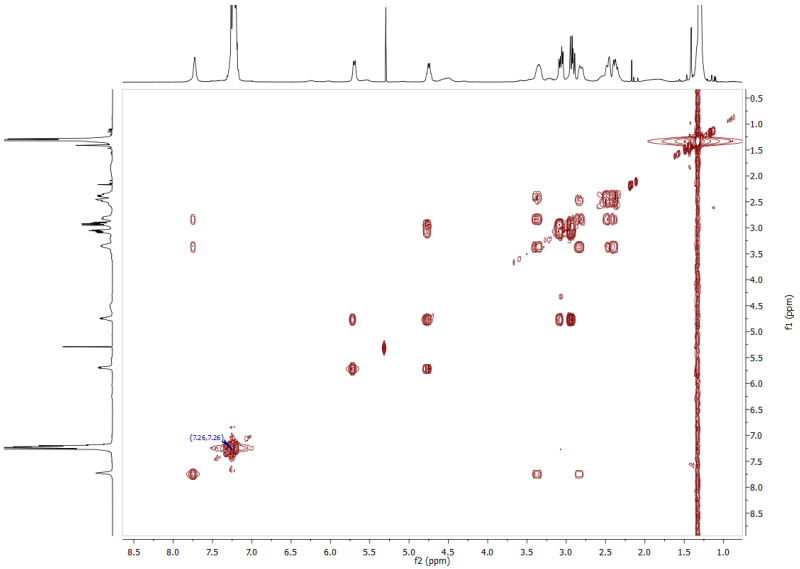


Figure 6: *[1]H-[1]H COSY NMR spectra of Protected Tren-L-Phenylalanine*

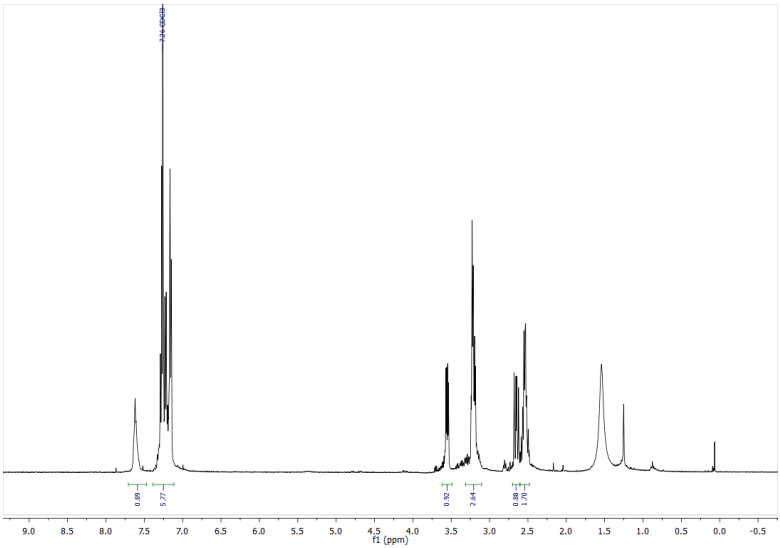


Figure 7: *^{1}H NMR spectra of Tren-L-Phenylalanine*

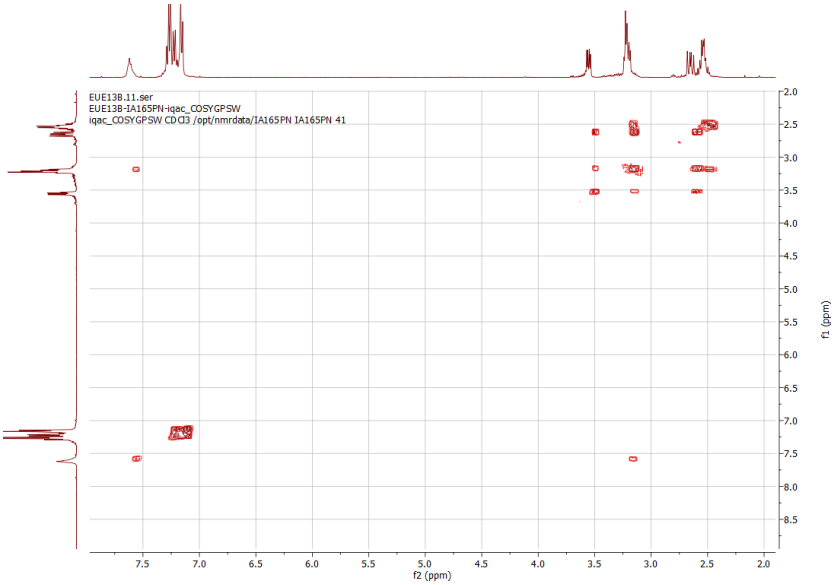


Figure 8: *^{1}H-^{1}H COSY NMR spectra of Tren-L-Phenylalanine*

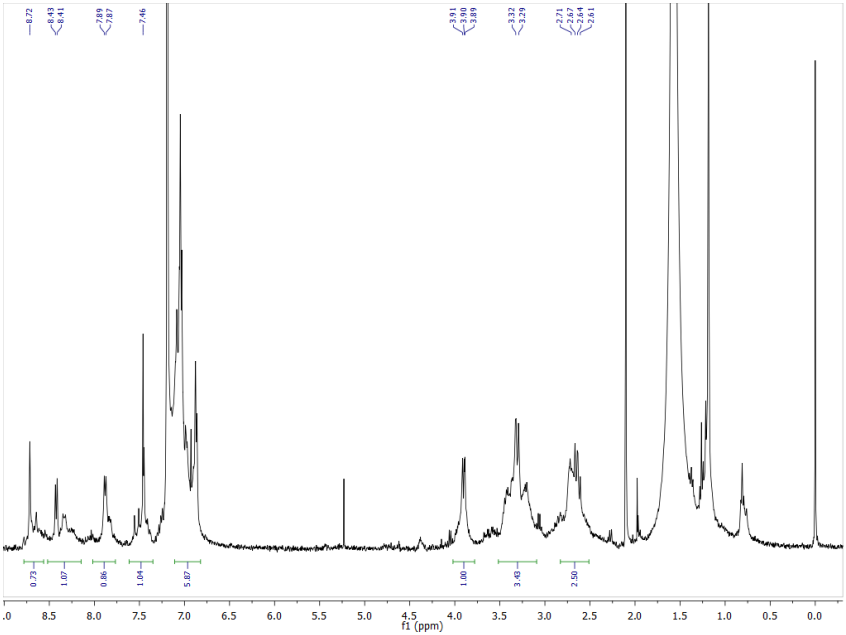


Figure 9: *^{1}H NMR spectra of non-Complexed Cage*

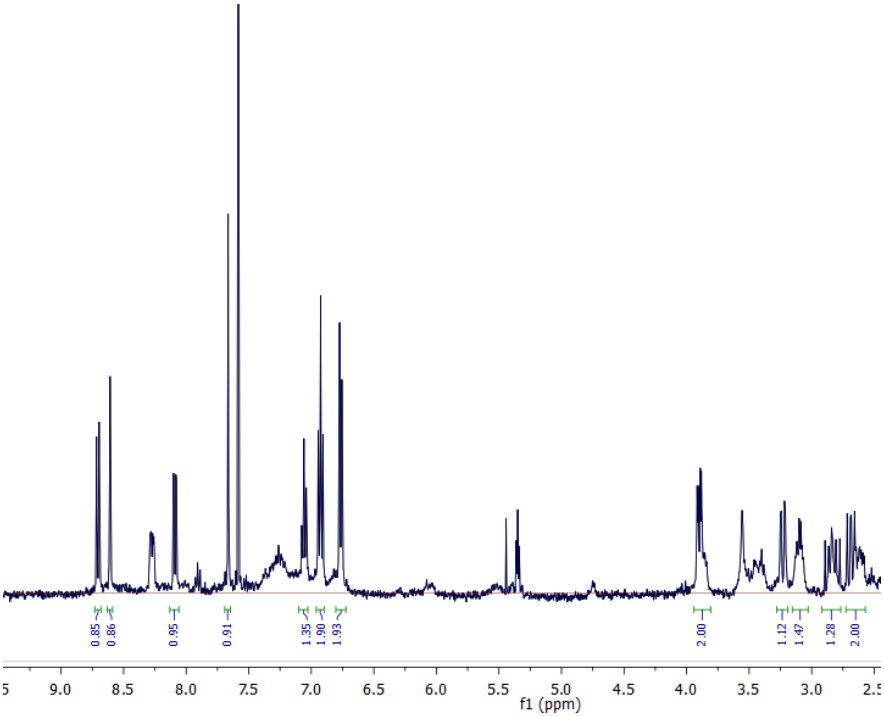
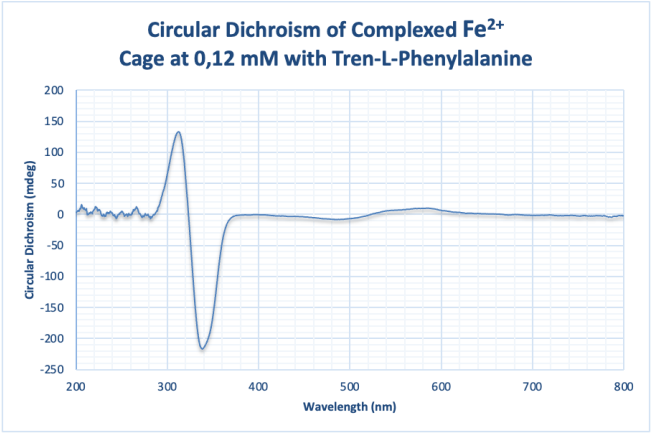
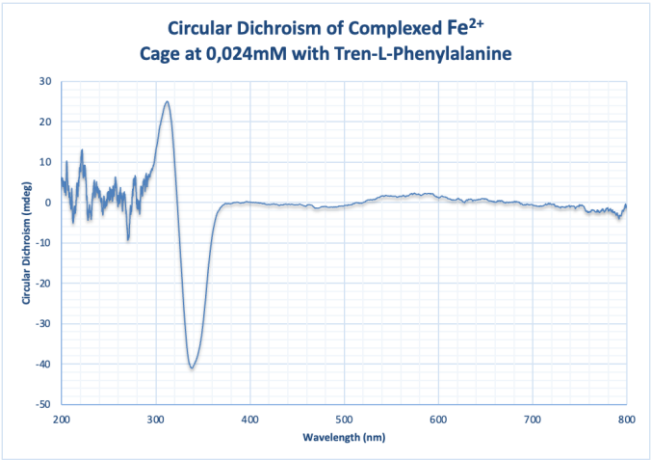


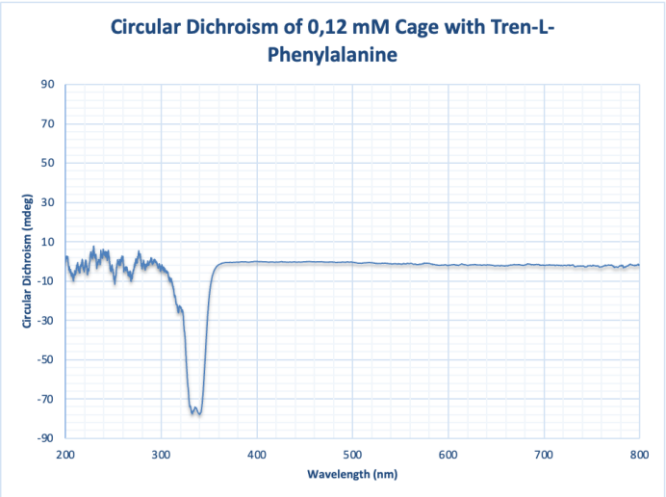
Figure 10: *^{1}H NMR of Complexed Fe^{2+} Cage*

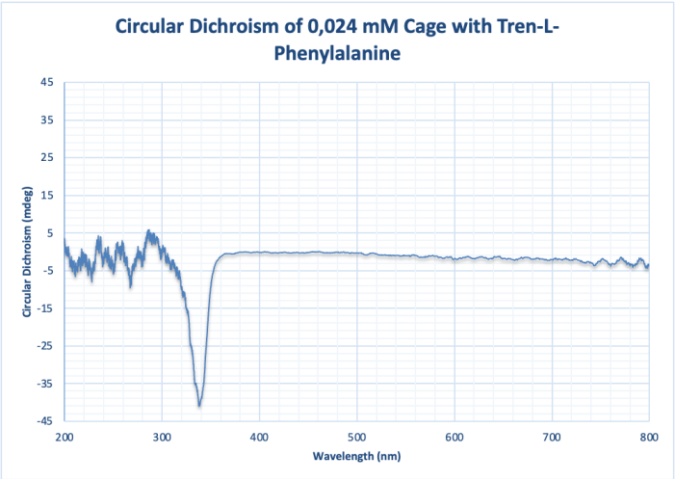
APPENDIX 2: CIRCULAR DICHROISM SPECTRA



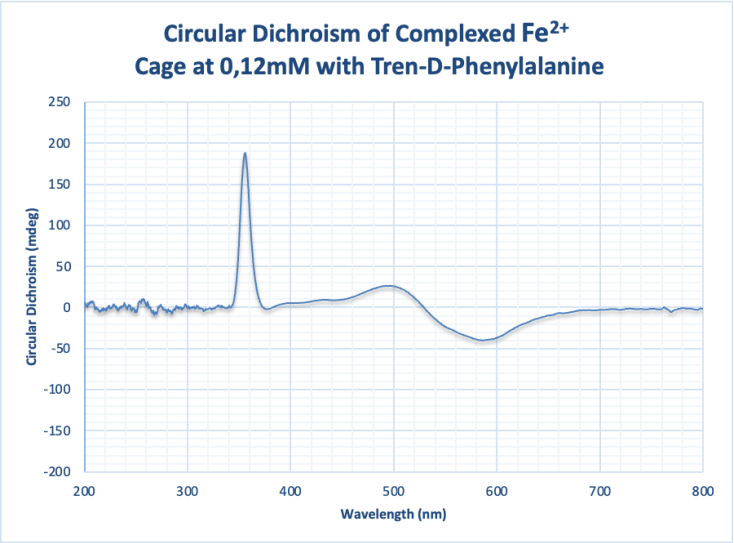


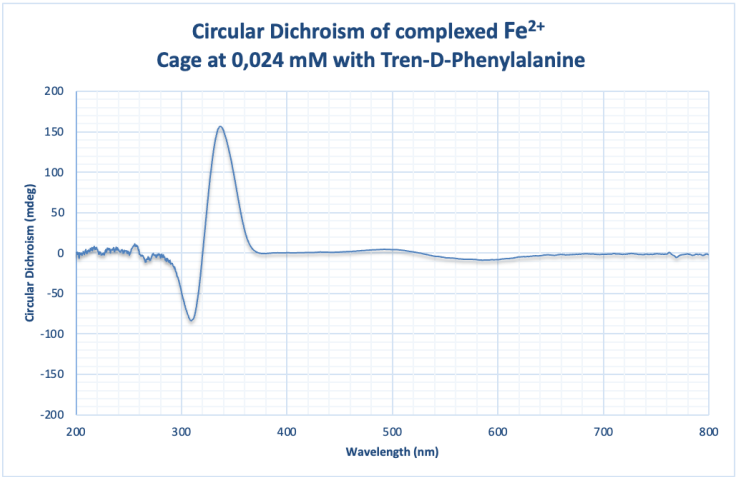
Figures 1. *Circular Dichroism of Complexed Fe^{2+} Cage at 0,12 mM and 0,024 mM with Tren-L-Phenylalanine*



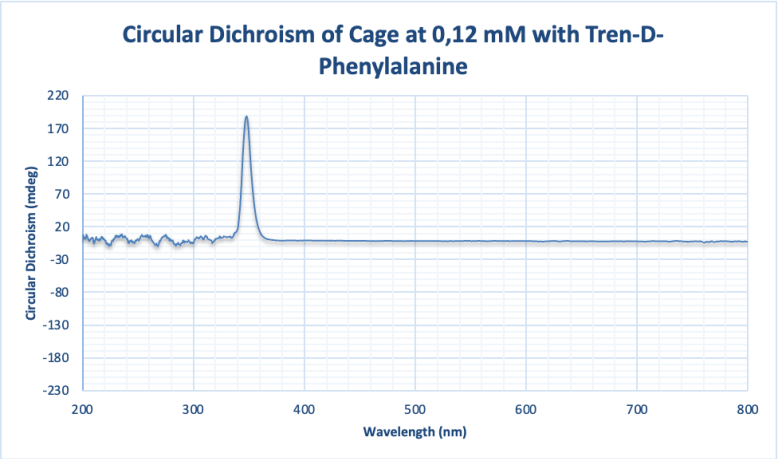


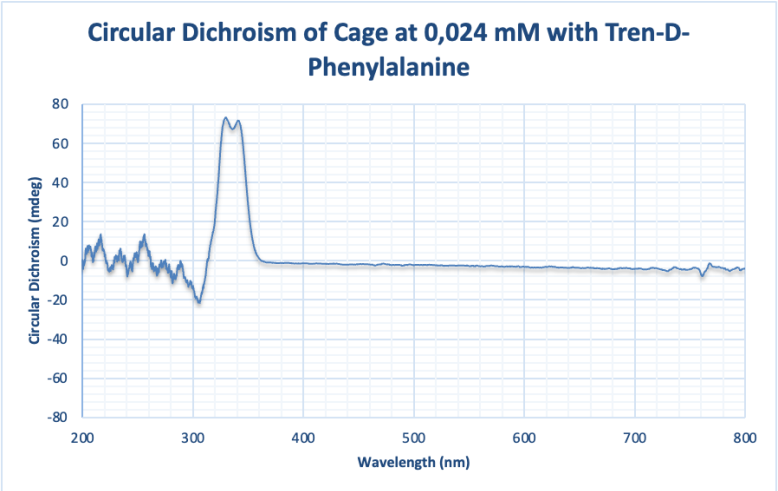
Figures 2. *Circular Dichroism of non-Complexed Cage at 0,12 mM and 0,024 mM with Tren-L-Phenylalanine*





Figures 3. *Circular Dichroism of Complexed Fe^{2+} Cage at 0,012 mM and 0,024 mM with Tren-D-Phenylalanine*





Figures 4. *Circular Dichroism of Complexed Fe2+ Cage at 0,12 mM and 0,024 mM with Tren-D-Phenylalanine*