Escuela superior de Tecnología y Ciencias Experimentales

Departamento de Química Inorgánica y Orgánica

Group of Organic Molecular Nanomaterials with Biomedical Application

LIGHT RESPONSIVE LOW MOLECULAR WEIGHT GELS FROM AN AZOBENZENE DERIVATIVE

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BACHELOR'S DEGREE RESEARCH PROJECT

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CERTIFICAN

Que el trabajo fin de grado con el título **LIGHT RESPONSIVE LOW MOLECULAR WEIGHT GELS FROM AN AZOBENZENE DERIVATIVE** ha sido realizado por Jose Juan Andreu Olaria bajo su dirección, en el grupo Organic Molecular Nanomaterials with Biomedical Applications del Departamento de Química Inorgánica y Orgánica de la Universitat Jaume I de Castellón de la Plana.

Lo que certificamos a los efectos oportunos en Castellón de la Plana a 18 de julio de 2019.

Fdo. Dr. Juan F. Miravet Celades

Fdo. Dr. César A. Angulo Pachón

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“Lo que nos hace grandes es el hecho de saber lo pequeños que somos”

Abbreviations

Azo	4-aminoazobenzene
Cbz	Benzyloxycarbonyl
Boc	tert-butoxycarbonyl
BocValOH	tert-butoxycarbonyl-L-Valine acid
$(CO)_2Cl_2$	Oxalylchloride
COSY	2D correlation spectroscopy
CbzGlyOH	((benzyloxy)carbonil)glycine
DIPEA	N,N-Diisopropylethylamine
DMF	N,N'-Dimethylformamide
EDCI	Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et_3N	Triethylamine
Gly	Glycine
HMBC	Heteronuclear multiple bond correlation
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
LMWG	Low molecular weight gels
LMWG's	Low molecular weight gelators
MGC	Minimum Gelator Concentration
NMR	Nuclear Magnetic Resonance
Oxyma	Ethyl (hydroxyimino)cyanoacetate
rt	Room temperature
SEM	Scanning Electron Microscopy
Suc	Succinic acid
TEM	Transmission Electron Microscopy

THF	Tetrahydrofuran
UV/Vis	Ultraviolet/Visible
Val	Valine
Wt	weight

Index

Introduction

Introduction

1.1 World of Gels

1.1.1 What are gels?

Gels are soft solids that are commonly found in the daily life of any person, but their shape, touch and physical conditions incite to think that they are peculiar compounds. We can find them in form of gelatine, toothpaste, medicines, cosmetics, etc.

All of them show that can not be defined as a solids or a viscous liquid and that is why their flexibility, malleability, storage capacity and more properties are being studied in many fields, such as medicine, new materials, pharmacology, food engineering or electronics.[1]

The beginning of study of gels can be dated back to the end of the 19th century, in the 1860s. However, despite all the progress made up to the drafting of this project, even today it is difficult to find a true definition of gel that encompasses all the varieties that this entails. In 1926, Ph. D. Dorothy Jordon Lloyd correctly summarised the complexity of the study of gels by making the following statements:[2]

- The gel is one which is easier to recognise than to define.

- Only one rule seems to hold for all gels, and is that they must be built up from two components, one of which is a liquid at the temperature under consideration, and the other which, the gelling substance proper, often spoken of as the gelator, is a solid. The gel itself has the mechanical proprieties of a solid, i.e. it can maintain its form under the stress of its own weight, and under any mechanical stress it shows the phenomenon of strain.

[1] Sangeetha, N. M.; Maitra, U. *Chem. Soc. Rev.* **2005**, *34,* 821.
[2] Lloyd, D. J. *Colloid Chem*. **1926**, *1*, 767.

From then on, more definitions were made, but none could surpass Lloyd's until 1974, when Flory proposed a new definition able to summarize gels nature and at the same time, be easy to understand:[3]

- ❖ Gels can be defined as continuous structures with macroscopic dimensions that are permanent in time, at least, until the end of the experiments, and that their main behaviour is the solid-like state, deforming itself due to its elastic body.

However, increasing research activity in recent years in this field and the possibility of classifying them in several subclasses, has given rise to numerous and various explanations of what is a gel.

Thus, it is possible to find definitions from the most elaborate: "gels are viscoelastic structures formed by a three-dimensional cross linked network and solvent, which is the major component; the solid appearance of the gel is the result of occlusion and adhesion of the liquid on the surface of the solid matrix 3D; the formation of this matrix is the result of cross-linking formed from the union of molecules through interactions polymeric fibers physical or chemical",[4] to the simplest: "if it looks gelatin and is capable of maintaining the shape under stress of its own weight, that is, does not fall, this is a gel".[5]

Therefore, no matter how long we study gels and how many times we change its definition, because the general idea remains:

- ✓ Gel is a structure where the gelator is able to form a 3D network which is able to encapsulate the solvent strong enough to resist its own weight stress.

[3] Flory, P. J. *Faraday Discuss.* **1974**, *57*, 7.
[4] Sangeetha, N. M.; Maitra, U. *Chem. Soc. Rev.* **2005**, *34,* 821.
[5] Estroff, L. A.; Hamilton, A. D. *Chem. Rev.* **2004**, *104,* 1201.

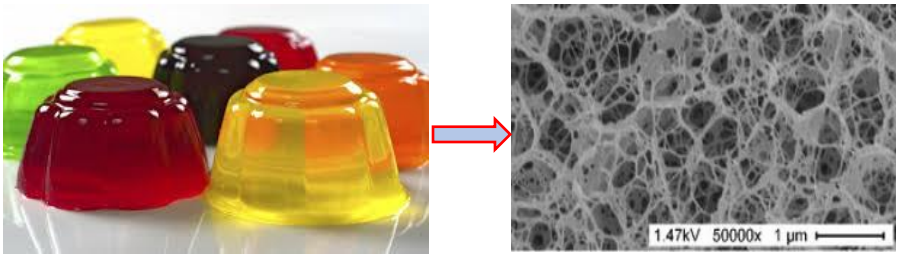


***Figure 1-1**. Jelly and its real structure shown by SEM microscopy.*

1.1.2 Types of gels

Research in gels field has progressed a lot, and because of that, gels classification has become bigger, going from a somewhat confusing world to today, where gels have a long way to go in daily life and it is already possible to foresee what kind of molecules may or may not form gels, simply by making slight variations in their structure.

Gels can be classified in different ways depending on factors such as the type of solvent they hold, the nature from which they come, the type of structures that form the gel or simply the interactions between molecules that hold the fibers together.

If a classification is made at the source level, natural gels (produced in nature) or artificial gels (from the laboratory) can be defined.

For the solvent they store, organic gels (organic solvents) or aqueous gels (formed by water) can be described. In this part of the classification, it is interesting to note that both organic gels and hydrogels can be treated to give rise to those known as Aerogels or Xerogels. The difference between them is that in Xerogel the solvent has been eliminated by evaporation (vacuum oven, for example) and the Aerogel, once the solvent has been eliminated, has been replaced by air.

Another classification that was not taken into account until the middle of the twentieth century was based on its constitution. Supramolecular gels can be defined when these are constituted by molecules of low molecular weight and

macromolecular or polymeric gels, when these are constituted by monomers of great atomic weight.

Finally, it should be pointed out that the interactions that unite gels also vary, being these very important factors to take into account when gels are obtained. Supramolecular gels are constituted by non-covalent forces such as hydrogen bonds, π-π staking or van der Waals forces. These gels are known as physical gels. On the other hand, macromolecular gels may be made up of these non-covalent forces, but mainly they are made up of covalent interactions, receiving the name of chemical gels.[4]

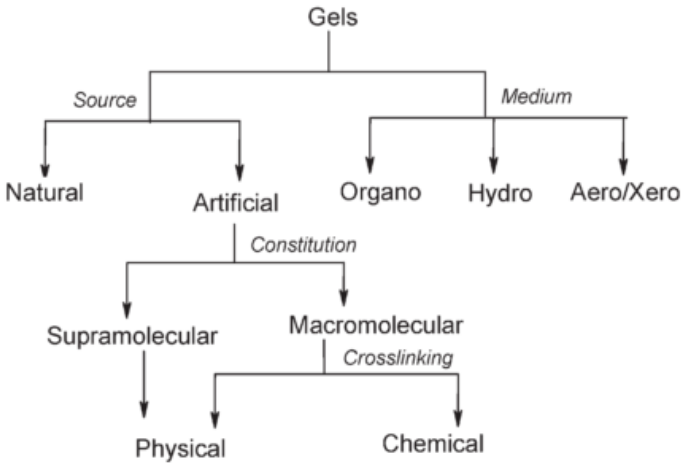


Figure 1-2. *Gels classification.*

1.1.3 Low molecular weight gels

Low molecular weight gels (LMWG) or supramolecular gels are those that are formed by low weight organic molecules, known as low molecular weight gelators (LMWG's), typically smaller than 3000 Da,[6] however it can vary depending on the author consulted.

[6] Abdallah, D. J.; Weiss, R. G. *Adv. Mater.* **2000**, *12*, 1237; b) Terech, P.; Weiss, R. G. *Chem. Rev.* **1997**, *97,* 3133; c) Smith, D. K. *Chem. Soc. Rev*. **2009**, *38*, 684; d) Banerjee, S.; Das, R. K.; Maitra, U. *J. Chem*. *Mat.* **2009**, *19,* 6649.

The main feature of supramolecular gels, apart from molecular weight is the forces that allow them to form fibers. The union of the gelling molecules is due to non-covalent forces such as hydrogen bond, van del Waals forces, solvation forces, *π-π* stacking and donor-acceptor forces. This has allowed some authors to name this kind of gels as "supramolecular polymers" because as well as chemical polymers, they are constituted by monomer units which are assembled by directional interactions, fact that makes them reversible.[7] The fact of being able to be in gel-state and later dissolved or in solid-state just by external stimuli such as pH, light or temperature (physical or chemical stimuli) is an advantage in front of covalent-gels, because they can be destroyed and formed again so many times.[8]

With respect of the molecules that usually form LMWG, molecules with certain functional groups are more capable of forming gels. For example, molecules capable of forming hydrogen bond such as urea's and amides, carboxylic acids or even carbohydrates and alcohols are quite good gelling agents. Other good gelators would be molecules with aromatic rings, because they allow the *π-π* stacking, or long alkyl chains, which can form gels by van der Waals interactions.

Finally, other interesting feature of gels is the minimum amount of gelling molecules that are needed to form gel in a determined solvent. This parameter is named minimum gelator concentration (m.g.c.). It is important to define it because every molecule can vary its m.g.c in every solvent.

Typically, commercial gels do not contain a concentration higher than 1.0 wt%. A higher concentration may limit their commercial application. The most common is that a good LMWG has a concentration between 0.1-1.0 wt%. A supergelator is defined as a class of gelants that are capable of forming gel with a minimum gelator concentration below of 0.1 wt%.[9]

[7] Lehn, J. M. *Science* **1993**, *260*, 5115.
[8] Segarra-Maset, M. D.; Nebot, V .J.; Miravet, J. F.; Escuder, B. *Chem.Soc. Rev*. **2013**, *42,* 7086.
[9] Zinic, M.; Vogtle, F. Fages, F. *Top. Curr. Chem*. **2005**, *256*, 39.

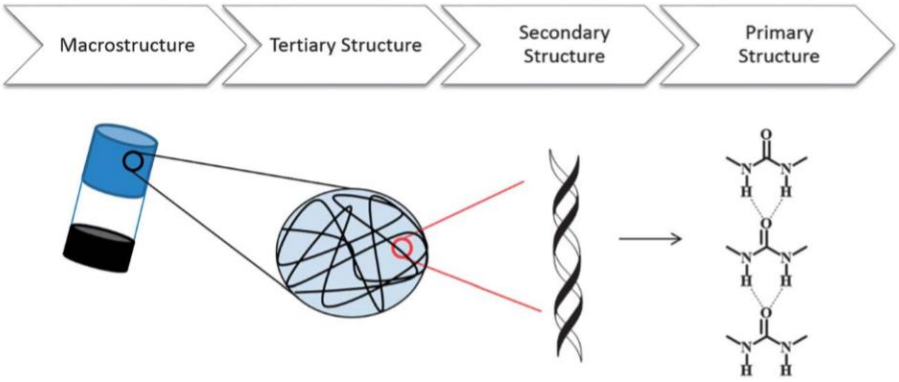


***Figure 1-3**. Primary, secondary, tertiary structures and macrostructure of a self-assembled physical network. Hydrogen bonding between individual urea molecules represents the primary structure. Under certain conditions e.g. change in temperature, pH, light stimuli, etc.[8] the gelator will self-assemble via non-covalent interactions into an intertwined helical structure (structure proposed for the example), leading to form the gel at a macroscopic level.[10]*

1.2. Azobenzene group

As it has been said above, physical stimuli can be used to form or undo gels. More specifically, light stimuli can be of great interest in gels applications if azobenzene molecule is in the structure.

The study of the light effects is highly interesting since it can induce conformational changes in photo-active molecules, which can imply changes in shape and these in turn, imply changes at macromolecular level.

The most ubiquitous natural molecule for reversible shape change is the rhodopsin–retinal protein system that enables vision, and this is perhaps the quintessential reversible photo-switch. Here, the small retinal molecule embedded in a cage of rhodopsin helices isomerizes from a *cis*- geometry to a *trans*- geometry around a C=C double bond with the absorption of just a single photon. The modest shape change of only a few angstroms is quickly amplified, and sets off a cascade of larger and larger shape and chemical modifications, eventually culminating in an electrical signal to the brain of a vision event

[10] Skilling, K.J.; Citossi, F.; Bradshaw, T.D.; Ashford, M.; Kellan, B.; Marlow, M. *Soft Matter* **2014**, *10*, 237.

A synthetic analog of this photo-switching effect can be found in the azobenzene molecule.

Azobenzene is a molecule which is formed by an azo group (-N=N-) and two aromatic rings. Although it was described for the first time in 1834,[11] it was not until 1937 that G. S. Hartley published a study of the influence of light on the configuration of N=N double bonds.[12] Nowadays, because of its great response to light stimuli, its effects on intelligent materials (such as gels), are still being studied.

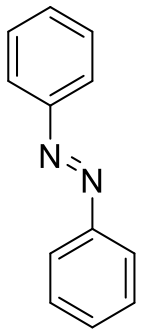


***Figure 1-4**. Azobenzene molecule.*

1.2.1 Structure and isomers

Due to the N=N double bond, like in a compound with C=C, azobenzene has two geometric isomers (*trans*-/*cis*-).

Isomer *trans*- is considered to be thermally stable, and on the contrary, *cis*- form is regarded as a meta-stable form. It is because of this isomerization that azobenzene is considered as a photochromic compound. Both isomers (*Z/E*) have different absorption bands, so they present different coloration.

[11] Mitscherlich, E. *Ann. Pharm*. **1834**, *12*, 311.
[12] Hartley, G. S. *Nature* **1937**, *140*, 281.

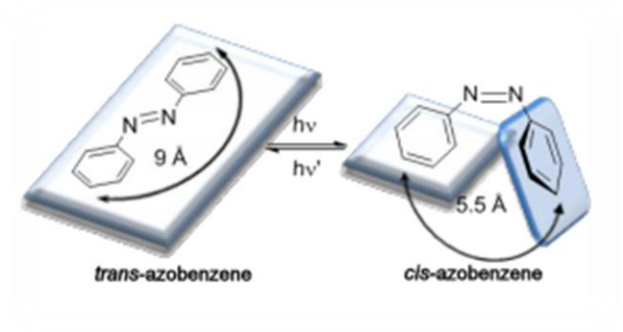


***Figure 1-5**. Photoisomerization of azobenzene.*[13]

Trans-azobenzene can quickly turn into *cis-azobenzene* just by irradiation of UV light (320-380 nm). In the same way, *cis*-azobenzene can return to its original *trans-state* by irradiation of 400-460 nm light. Apart from light stimuli, *trans-azobenzene* can also be obtained by heating or just by spontaneous conversion due to its more stable configuration.[13]

The maximum peak absorbance of *trans-isomer* (320-380 nm) is associated with the transition $\pi \rightarrow \pi^*$ ($S_2 \leftarrow S_0$) and *cis-isomer* (400-460 nm) to $n \rightarrow \pi$ ($S_1 \leftarrow S_0$).[13]

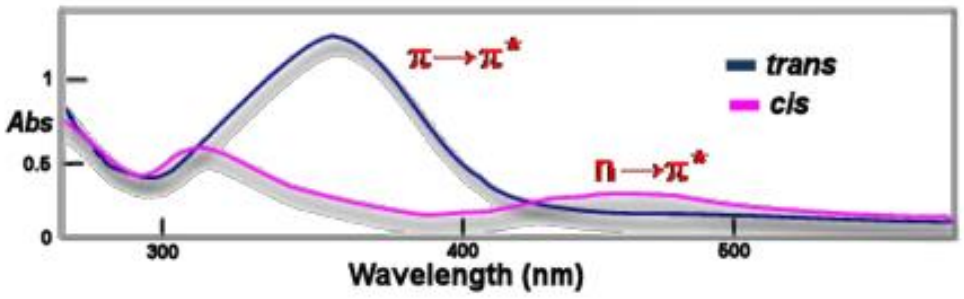


***Figure 1-6**.Example of irradiation effect on azobenzene molecule. Trans-azobenzene and cis-azobenzene typical spectra.*[10]

[13] Merino, E.; Ribagorda, M. *J. Org. Chem*. **2012**, *8*, 1071.

1.2.2 Azobenzene classification

Azobenzene family can be divided into three classes, according to Rau:[14]

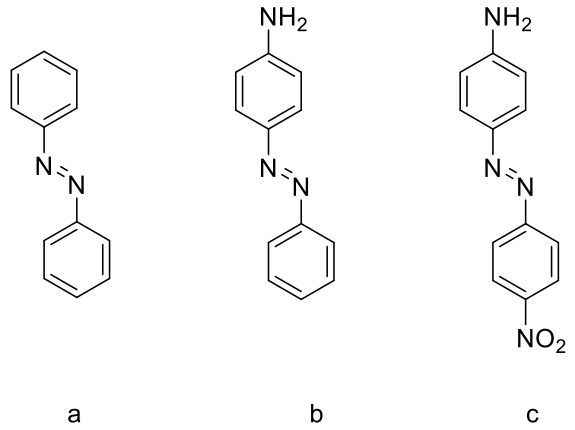


Figure 1-7. *Examples of azobenzene chromofores: a)azobenzene class; b)aminoazobenzene class; c) pseudo-stilbene class.*

The figure shown above describes azobenzene-type molecules which are: unsubstituted azobenzene (a); aminoazobenzene-type (b), that are ortho or para-substituted with an electron-donating group; pseudo-stilbenes (c), which are substituted at the 4 and 4' positions with an electron donating and an electron-withdrawing group.

According to Rau's classification, not only the structure vary but also does their spectra:[13]

a) *Azobenzene type*: The $\pi \rightarrow \pi^*$ band is very intense in the UV region, and there is one $n \rightarrow \pi^*$ weaker in the visible (yellow colour).

b) *Aminoazobenzene type*: The $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ bands are very close of collapsing in the UV–vis region (orange color).

c) *Pseudo-stilbene type*: The absorption band corresponding with $\pi \rightarrow \pi^*$ *trans*ition is shifted to red, changing the appearance order concerning the band $n \rightarrow \pi^*$.

[14] Rau, H. *Photoisomerization of Azobenzenes,* ed. J. Rebek, CRC Press, Boca Raton FL, **1990**.

1.3 Combined application

In recent years, research related to LMWG's has begun to attract great interest, due to their physical and morphological properties. As their properties and the way in which they behave have started to be understood, their applications have gone from low-cost bulk applications such as personal care products and lubricants to others uses of high-tech applications, such as nanoelectronics, environmental detection or controlled drug release.

Combining the current applications of LMWG's with the properties presented by the azobenzene group, the combination of both could be used for the controlled release of drugs using light as a stimulus.

1.3.1 Drug delivery

There are many methods of drug delivery, however, in the recent years LMWG's have presented new properties that make them compounds so interesting as new carriers for therapeutic agents.

Currently, there are three strategies for drug delivery using LMWG's:

1. The compound may be physically trapped within an inert matrix of gelators (defined as a "scaffold") and then released from the gel by diffusion or during the degradation of the gel.

2. The therapeutic agent can be conjugated covalently with a functional group, creating a prodrug with amphiphilic characteristics, this will self-assemble and slowly degrade with enzymes.

3. A functionalized linker can be conjugated covalently with a therapeutic agent, after the enzymatic cleavage of part of the linker and the amphiphilic prodrug can self-assemble.[10]

Considering the methods of drugs delivery and the properties that the isomerization of azobenzene can bring to LMWG's, the combination of this could provide that strategy 1 together with the appropriate light stimuli, facilitate the release of drugs, even in places or conditions currently unattainable.

Below, in Figure 1-8 the combination of strategy 1 and light stimuli is exposed.

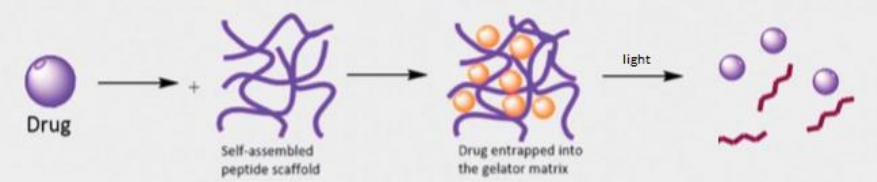


***Figure 1-8**. LMWG's combined with azobenzene properties to release drugs.*

Objectives

Objectives

General objective

- Develop and characterize a molecule capable of forming supramolecular gels that are light responsive because of the presence of an azobenzene unit.

Specific objectives

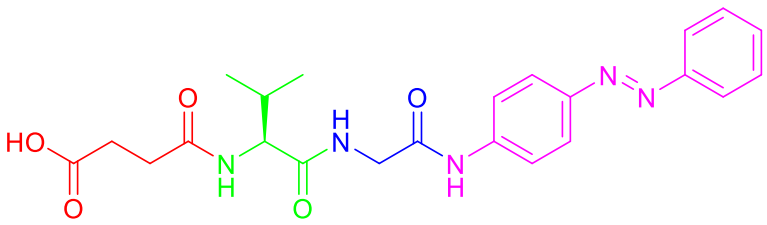


***Figure 2-1**. Molecular structure of SucValGlyAzo.*

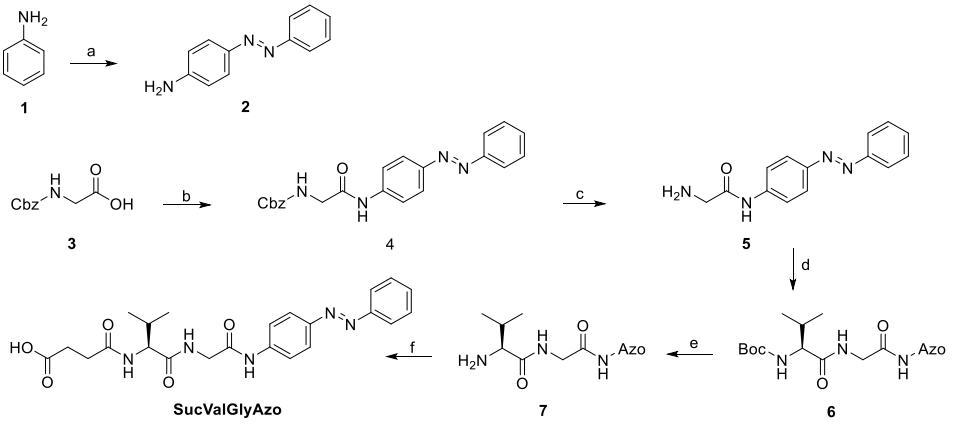
- Synthesize and characterize the molecule SucValGlyAzo.
- Determinate the m.g.c. in organic and aqueous media.
- Evaluate the acid-base constants of SucValGlyAzo to assess the range of pH where the molecule is neutral and prone to aggregation.
- Study effects on SucValGlyAzo of UV light irradiation and Vis light recovery using UV/Vis spectroscopy and NMR spectrometry.

Results and Discussion

Results and Discussion

3.1 Synthesis of SucValGlyAzo

Previously to this research project, the research group had synthesized similar molecules which are considered to be good gelling agents. Because of this, the target molecule has been synthesized following a commonly used synthetic route by the research group.



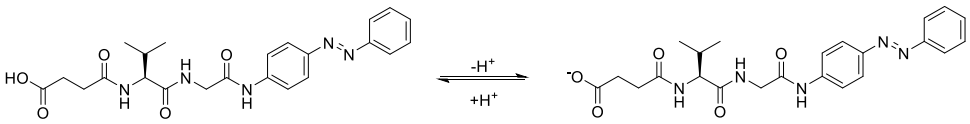
Scheme 3-1. *Global synthesis of SucValGlyAzo: a) $NaNO_2$, HCl, NH_3, H_2O, 3h , 72% b) CH_2Cl_2, 4-aminoazobenzene, DMF, $(CO)_2Cl_2$, Et_3N, 16h, 83%; c) HBr, 16h; d) BocValOH, DIPEA, EDCI, Oxyma, 16h, 82%; e)$CHCl_3$, TFA, 16h, 90%%; f) THF, Na_2CO_3, succinic anhydride, 16h, 85%.*

Scheme 3-1 shows the general process of synthesis of the desired molecule, SucValGlyAzo. The preparation is divided into six main steps (more details in the Experimental Section). The first step was the obtainment of the azobenzene molecule (4-aminoazobenzene) by coupling two aniline molecules. Step b was the activation of Carbobenzyloxy-Glycine with oxalyl chloride ($(CO)_2Cl_2$) and dimethylformamide (DMF) to form the corresponding acyl chloride. The acyl chloride was reacted *in situ* with 4-aminoazobenzene. The amine 5 was obtained by deprotection of the Cbz group with concentrated hydrogen bromide acid in acetic acid. Step d consists in the coupling of Boc-L-Valine with molecule 5. After that, the protecting group Boc was removed using trifluoroacetic acid (TFA). Finally, SucValGlyAzo was obtained by reaction with succinic anhydride.

3.2 Acid-base and aggregation properties

To analyse the properties of the SucValGlyAzo, its acid-base constant and minimum gelator concentration were determined.

3.2.1 Determination of acididy constant



Scheme 3-2. *Acid-base equilibrium in SucValGlyAzo.*

Determining the acid-base constant of the compound is very important because it indicates at which pH values the molecule is in its unprotonated state, which is a fact that influences directly in the gelling process.

The clearest example is in the aqueous medium where the ionized molecule will be soluble in water, preventing gelation. On the other hand, if the molecule is in its neutral form, the solubility decreases, allowing for intermolecular interactions and forming fibers.

Potentiometric titrations were carried out by addition of 0.1 M HCl to the gelator dissolved in NaOH 0.05 M. Data analysis made with the program HYPERQUAD, which fits the experimental data to calculated ones iteratively, giving the following value as the pK_a:

$$pK_a = 6.13 \pm 0.03$$

In order to compare with other carboxylic acids, some acidity constants are provided in Table 3-1.

***Table 3-1**. Some interesting acid constants.*

Compund	pK_{a1}	pK_{a2}
Acetic acid	4.757	-
Propanoic acid	4.874	-
Butanoic acid	4.819	-
Pentanoic acid	4.843	-
Succinic acid	4.207	5.636

As can be seen, none of them is close to the obtained value. The explanation for the decrease of the acidity of our compound is based on its aggregation capabilities. The pK_a value englobes both, the deprotonation and aggregation process. Namely, for this compound deprotonation is less favourable that for other common carboxylic acids because it is thermodynamically penalized with aggregates disassembly.

Apart from knowing the pK_a of the molecule, with the data we have it is also possible to obtain the speciation diagram, as it is shown below in Figure 3-1.

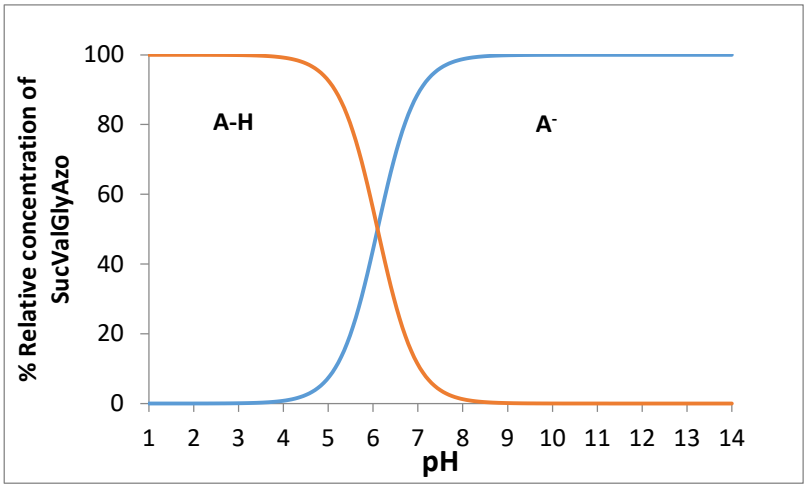


***Figure 3-1.** Speciation diagram of SucValGlyAzo.*

3.2.2 Determination of the minimum gelator concentration

The efficacy of a gelator depends not only on the molecule but also on the solvent used. This parameter is known as minimum gelator concentrations (m.g.c.), and it defines the minimum amount required to form a gel in the solvent.

For LMWG, the m.g.c. tends to be quite interesting, from a practical point of view, if it is lower than 1% wt. Usually, the m.g.c. of bulk gels that are commercial or are being studied range from 0.1% to 1% wt. The gels that are below 0.1% are known as supergelators.[9] However, a gelator can be defined as supergelator but in another solvent may not form even a gel.

To determine if the aggregation obtained could be named as a gel, we used the inverted tube method, because it provides a fast test to identify the formation of gel.

There are different methods to obtain a gel,[8] but Table 2 collects the results which presented the best gel formation.

***Table 3-2**. Features of different SucValGlyAzo gels.*

SOLVENT	*m.g.c. (mg/mL)*	*% WT*	*Obtention method*
Toluene	5	0.5	Heat-Cooling. Cooling at room temperature
CH_3CN	20	2	Heat-Cooling. Cooling at room temperature
CH_2Cl_2	10	1	Heat-Cooling. Cooling by fridge
$CHCl_3$	5	0.5	Heat-Cooling. Cooling by sonicating
EtOAc	20	2	Heat-Cooling. Cooling by sonicating
Acetone	-	-	-
Water	0.5	0.05	pH change. NaOH 0.01 M and D-(+)-Gluconic acid δ-lactone

Thus, the three solvents with the lowest m.g.c. were determined to continue the research. Those solvents were toluene, chloroform water. They are shown below in Figure 3-2.

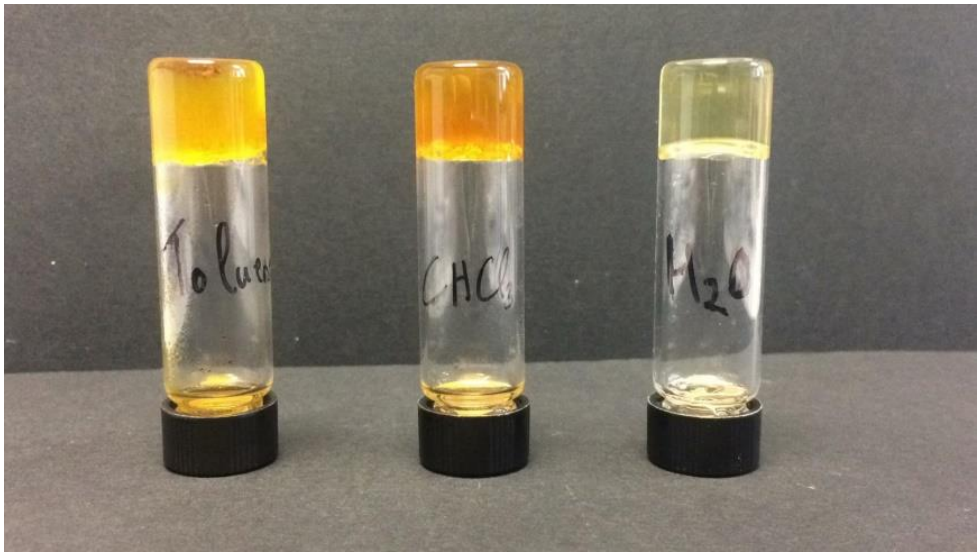
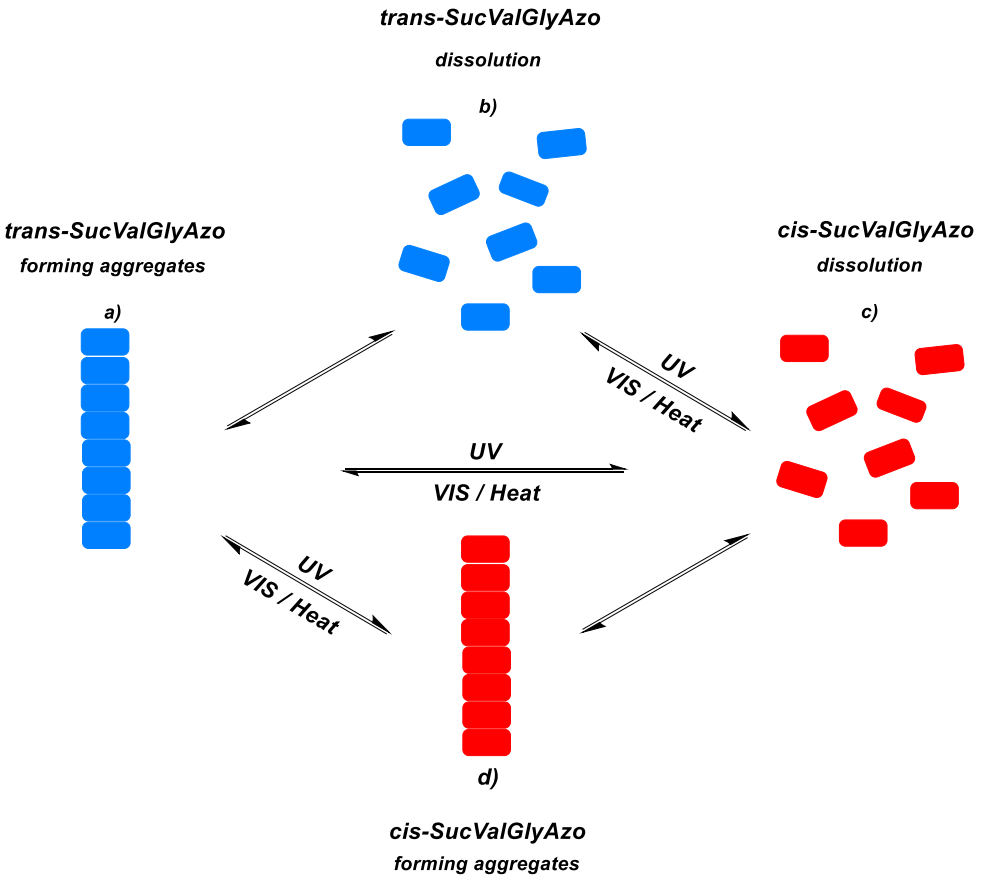


Figure 3-2. *From left to right: toluene gel, chloroform gel and hydrogel.*

It should be noted that the toluene gel is not homogeneous. Although we tried to obtain a homogeneous gel repeatedly, it always remained some solid without dissolving, and if the m.g.c. was decreased, the gel was not reproducible. This behaviour can be explained according to the solubility of the compound. It seemed that SucValGlyAzo was not fully soluble in hot toluene, but it was in chloroform, for example.

3.3 Light stimuli response

Below, in Scheme 3-3 are exposed all possible equilibria in LMWG's containing azobenzene group.



Scheme 3-3. *Proposed equilibria of LMWG's containing azobenzene group.*

In the previous scheme are shown all possible states of SucValGlyAzo and which stimuli can make it to vary from one state to another.

It is important to enhance that all fibers or aggregates that form a network present an equilibria between the aggregates and free molecules. Depending on different factors, such as temperature, pH, or even the solvent and molecules

structure, the equilibria will be more displaced to fibers or free molecules.[15] This case occurs between *state a* and *state b* and *state d* and *state c.*

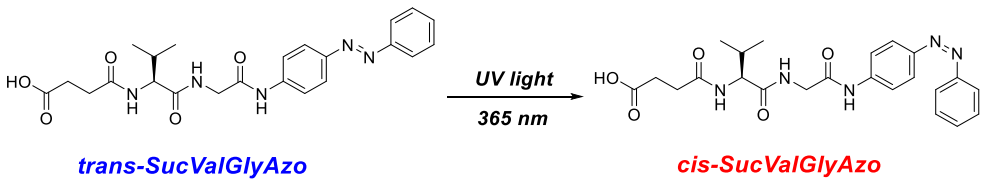
Equilibrium between *state b* and *state c* depends on light stimuli because, as explained in introduction, all molecules containing azobenzene group are able to isomerize with UV light stimuli and recover *trans-state* with VIS light stimuli or heat. Equilibrium between *state a* and *state c* is basically the same, just starting from aggregates and ending in dissolution. The main reason for this state transition would be isomerization, since it causes the disunion of the fibrillary network due to molecules prefer to be dissolved rather than maintain the structure in its *cis-form*.

Finally, equilibrium between *state a* and *state d* also can be produced by UV/Vis light and heat. The only difference is that instead of dissolving the molecules when they are isomerized, they prefer to maintain the structure because of their weak of solubility.

The main aim of this study was to check in which condition all those equilibria could happen.

3.3.1 UV light stimuli

The first step of the research was to carry out experiments with UV light. Previously has been explained that azobenzene compounds isomerize if they are irradiated with 320-380 nm light.[13] For this reason, the aim of this study was to check if that light stimuli produced any change on SucValGlyAzo molecule. To carry it out, a LED of 365 nm was used.



Scheme 3-4.*UV light stimuli isomerization effect on SucValGlyAzo.*

[15] Escuder, B.; Llusar, M; Miravet, J. F. *J.Org.Chem.* **2006**, *71*, 7747.

A) Spectroscopic response

The clearest effect of UV irradiation on molecules containing azo group is a variation in their absorbance spectrum, as shown in Figure 1-6. It is for this reason that spectroscopic studies were realized.
Ultraviolet spectroscopy is a technique that makes use of Lambert-Beer's law, according to which, the observed absorbance is the product of the quotient between the molar extinction coefficient, the length of the light passage in the irradiated cell and the concentration of the studied compound. Since the values of b and c do not vary, the variation in absorbance will be due to the term ε.

$$A = \varepsilon \cdot b \cdot c$$

Solutions of the compound were prepared in toluene, chloroform, and basic water. Basic water solution was used because the studied molecule is hydrophobic, so to have it dissolved in an aqueous medium, it must be basic.

The concentration used was the same in the three solvents assayed. A concentration was chosen that did not saturate the spectrophotometer and that avoided aggregation. The solutions concentration was 0.025 mg/mL or 0.055 mM.

Results of irradiation are shown in Figure 3-4. Only spectra in chloroform are shown, but basic water and toluene also suffered the same effect.

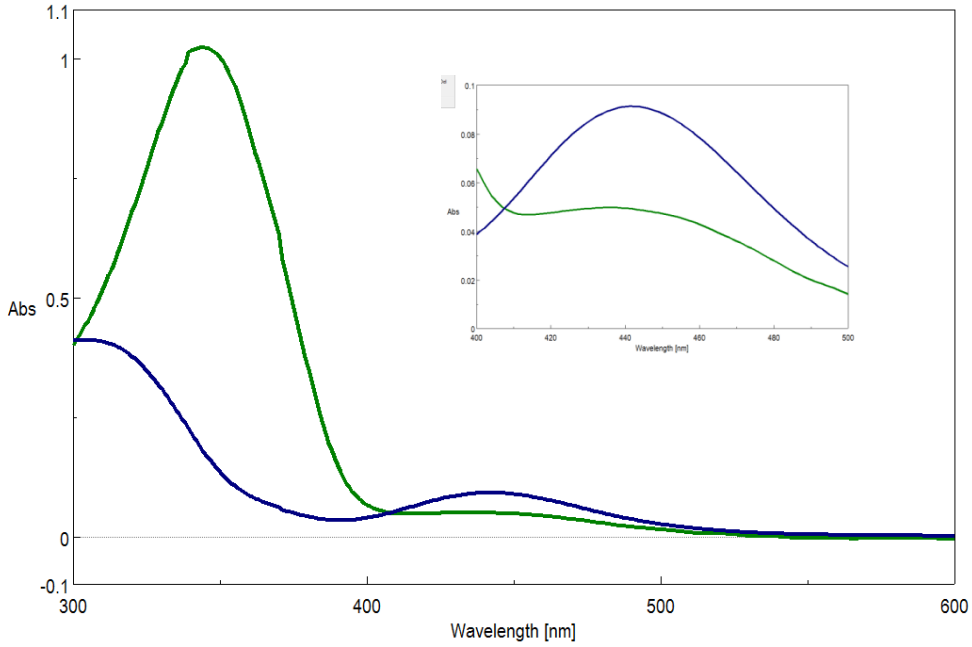


Figure 3-4. *SucValGlyAzo in $CHCl_3$ at 0.055mM. Evolution of the compound during irradiation. Green line represents the freshly prepared compound, and the blue line shows the result after 365 nm irradiation for 2h.*

Apart from simple irradiation for 2 hours, a minimum irradiation time study was also carried out.

From the spectrum shown above and the others obtained in the lab, several things could be concluded:

- As predicted in bibliography,[10,13,14] all non-irradiated spectra show two differentiated zones: The first one is a high peak which is associated with the *trans-isomer* (350 nm approx.) and the second is a small one which is related with the *cis-isomer* (440 nm approx.).
- The shift of the spectra in different solvents was not big enough to associate it with a bathochromic (larger wavelengths) or hypochromic (shorter wavelengths) effect. The observed variation is ±5 nm.
- All irradiated samples, regardless of the used solvent, showed a significant fall in *trans-peak(π→π*)* and an increase in *cis-peak (n→π)* as predicted.

- In dissolution, all tested solvents showed that just 10 minutes of irradiation was enough to reach the maximum isomerization.
- It is proved that SucValGlyAzo is a photochromic compound due to its colour changes once it is irradiated.

Below in Figure 3-5, samples before and after irradiation are showed, proving that SucValGlyAzo is a photochromic compound.

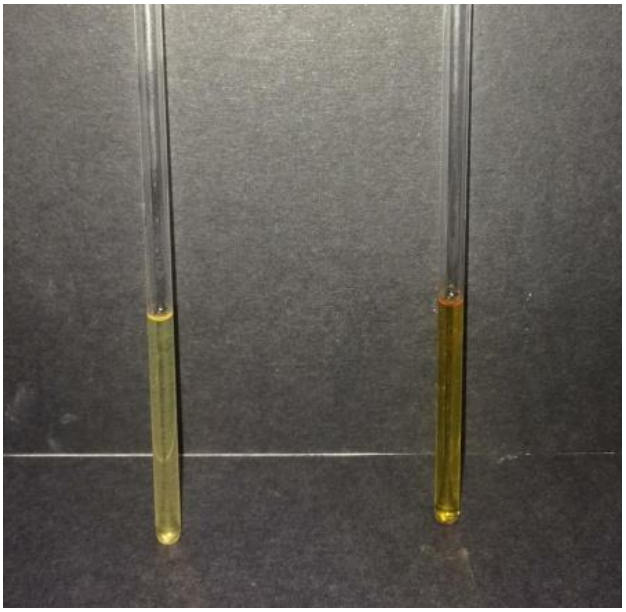


***Figure 3-5**. NMR tubes before (left) and after 60 minutes irradiation (right).*

- **NMR test**

NMR studies were carried out to see if the isomerization detected by UV-Vis spectroscopy could be observed with this technique. The study was carried out with chloroform and basic water, being the results similar.

Differences between non-irradiated and irradiated samples are shown in Figure 3-5:

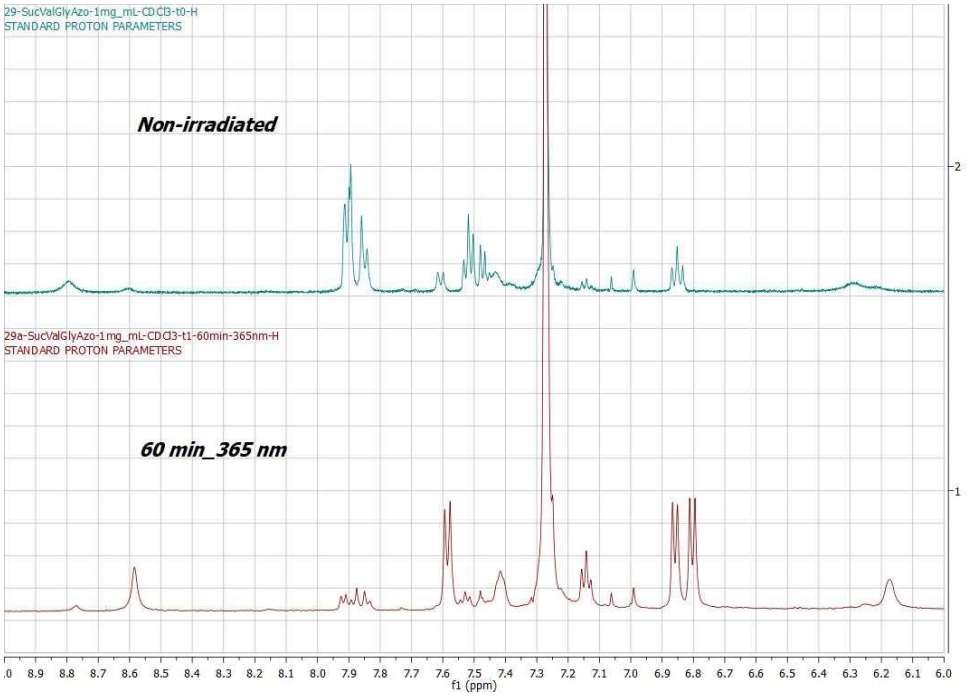


Figure 3-6. *NMR of SucValGlyAzo in $CDCl_3$ at 1mg/mL. Blue spectrum represents the non-irradiated sample and red spectrum the nmr tube irradiated 60 minutes.*

As can be appreciated in Figure 3-5, both spectra show several differences, proving that isomerization took place. It can be noted that some small peaks (associated with *cis-isomer*) of the blue spectrum have increased, and others (associated with *trans-isomer*) have decreased or even disappeared.

Going back to Scheme 3-3, it can be confirmed that equilibrium between state b and *state c* always occurs if molecules are irradiated with UV light.

B) Macroscopic response

Previously, isomerization of SucValGlyAzo in solution has been demonstrated, so this study aimed to check whether this isomerization observed at microscopic level could lead to significant changes at the macroscopic level, such as the breakage of gels.

The gels were irradiated directly with the 365 nm LED for 2 hours.

To be sure that the observed effect was due to the irradiation of UV light, all tests were performed by duplicate plus other gel acting as a control that was protected from irradiation. All the changes observed and the proposed explanations are reported below:

- **Toluene gel**

The toluene gel did not undergo any apparent change, as its consistency seemed to remain intact as well as the colour. This effect indicates that the isomerization *cis-gelator* is able to form stable fibrillar aggregates in this solvent

Based on Scheme 3-3, equilibrium from *state a* to *d* can be supposed to happened.

- **Hydrogel**

The hydrogel did undergo a change in its tonality, going from a very weak yellowish translucent tone to a more intense yellow. As for its consistency, nothing seemed to had changed. It can be explained in this way:

As observed in Figure 3-5 and solution experiments, it is proved that the change in tonality is due to isomerization of the molecules, so it can be considered that the fibers suffered the isomerization. But in the same way that toluene gel, fibers remained intact and were not destroyed. As explained before, hydrogel was obtained by pH-change because the molecule is too hydrophobic in its acid form. Indeed, the molecule is probably too hydrophobic that despite assuming a structural change in the fiber, it is thermodynamic favourable to maintain its gel form rather than to dissolve in water.

In Figure 3-6 is shown the mentioned tonality change.

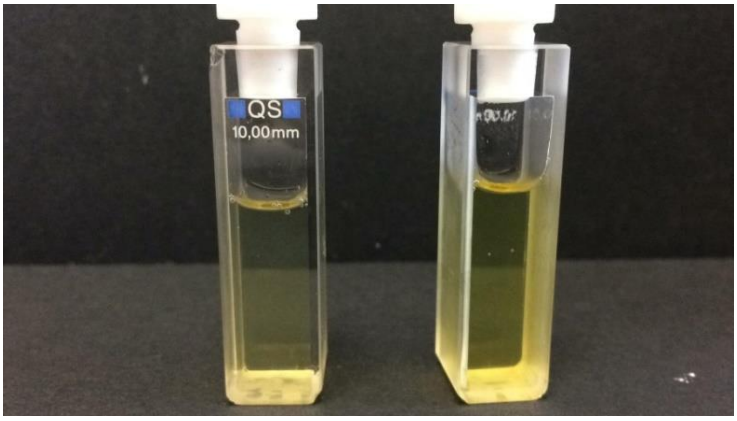


Figure 3-7. *Hydrogel before irradiation (left) and after 2 hours irradiation (right).*

In order to get more information about what occurred in hydrogel when it was irradiated, an NMR study was performed.

It was found that any soluble species were observed before and after irradiation, indicating in both cases the formation of fibers.

Applying this information to Scheme 3-3, it can be confirmed that equilibrium between *state a* and *state d* occurs in hydrogel. Equilibria between *state a* and *b* and *state d* and *c* can be also supposed but heavily displaced to fibers.

- **Chloroform gel**

The $CHCl_3$ gel did undergo a radical change at the macroscopic level. As the irradiation took place, the gel was gradually dissolved. On the other hand, the part that remained in gel form was darkening its tonality, which seemed to indicate that the molecules that had not been isomerized remained in the gel.

That effect can be explained in this way:

As the radiation reached the fibers, they were being destroyed due to the isomerization of the compound, which was gradually dissolving.

Paying attention to scheme 3-3, this is a clear case in which equilibrium from *state a* to state *c* can occurs, going from the *trans-gel state* to *cis-dissolution state.*

In Figure 3-8 is shown the evolving of chloroform gels.

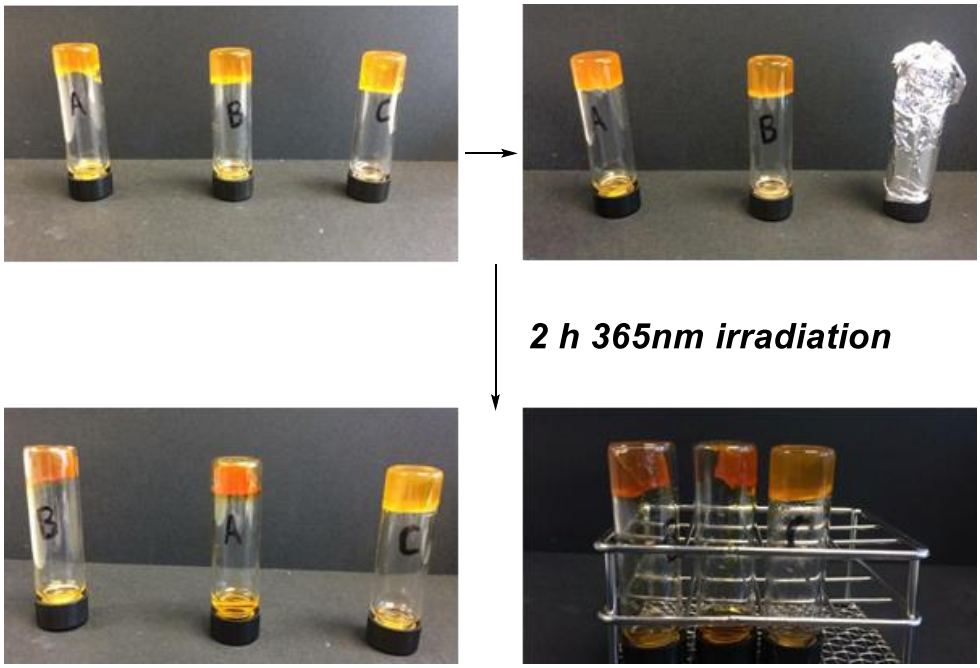
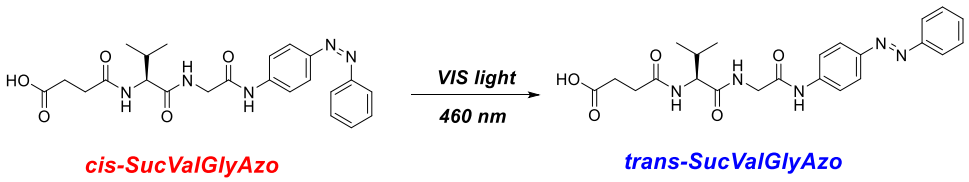


Figure 3-8. *Evolving of chloroform gels. Up-pictures show gels before irradiation and down-pictures after 2 hours of 365 nm irradiation from different perspectives.*

3.3.2 Vis light recovery study

In the same way that in the previous section the main objective was to study the effect of UV light stimuli on gels, it was proposed to study the inverse effect. This section aimed to investigate whether it was possible to recover the gel in its original form, or the previous state to irradiation using Vis light stimuli.

Previously it has been explained that to isomerize *trans-isomer* to *cis-isomer* a 400-460 nm light is needed. Because of that, a 460 nm LED was used.



Scheme 3-5. *VIS light stimuli isomerization effect on SucValGlyAzo.*

A) Spectroscopic response

Although it had already been demonstrated that with 10 minutes of irradiation the isomerization was maximum, the same study of minimum isomerization time was carried out to be sure. However, since Figure 3-4 corresponds to the sample irradiated for 2 hours at 365 nm, Figure 3-9 shows the recovery of the compound after 2 hours of irradiation 460 nm. As before, all solvents showed the same behaviour.

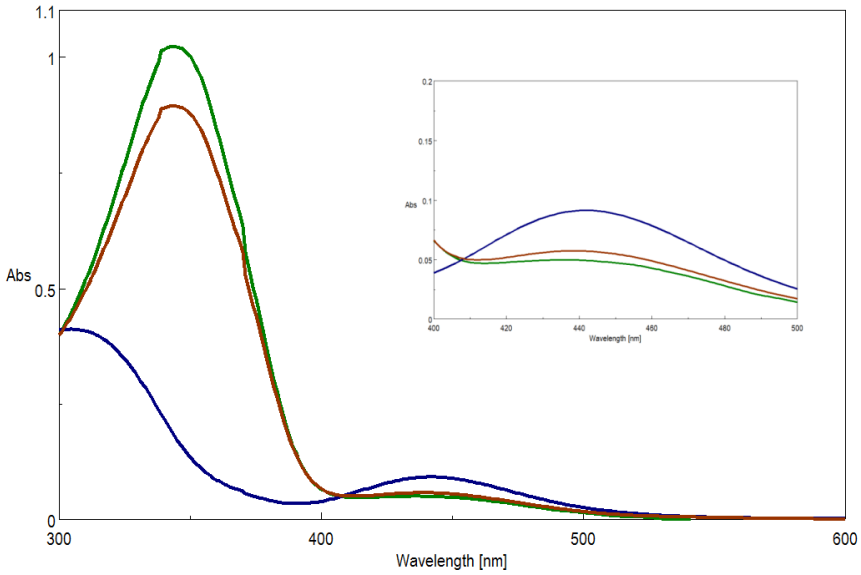


Figure 3-9. *SucValGlyAzo in $CHCl_3$ at 0.055mM. Evolution of the compound during irradiation. Green line represents the freshly prepared compound, blue line shows the result after 365 nm irradiation for 2h and brown line the results after 2 h of 460 nm irradiation.*

From the figure shown above and other spectra in the laboratory, several conclusions could be made:

- As it was expected, 460 nm light produced the increase of trans-peak and decrease of cis-peak, proving the *cis→trans* isomerization.
- As in UV irradiation, 10 minutes is time enough to achieve maximum isomerization.
- There is a *cis-trans* equilibrium. Despite having been irradiated during the same period, and having been left to rest for 24 hours, both, *trans* and *cis* peaks do not reach their original values, which is associated with a *cis-trans* equilibrium.
  It seems that the energy barrier separating *cis-isomer* and *trans-isomer* (in dissolution) is hardly existent producing an equilibrium. It should be remembered that the initial state was prepared by heating the sample, and should be the excess of thermal energy that causes that all its molecules pass to the most thermodynamically stable state, the *trans-isomer*. However, hydrogel also presents the same effect and it was not prepared by heating, so this effect can be associated to the prevalence of *trans-state* in the ionized state previous to gel formation.

Those studies were useful to confirm, according to Scheme 3-3, the equilibrium between *state b* and *state c.*

- **NMR test**

An NMR study was made to look for some more evidence that could reaffirm the behaviour observed by optical spectroscopy, The study, as well as in Figure 3-9, was made on the NMR tube sample previously exposed to UV radiation. Figure 3-10 shows the observed changes.

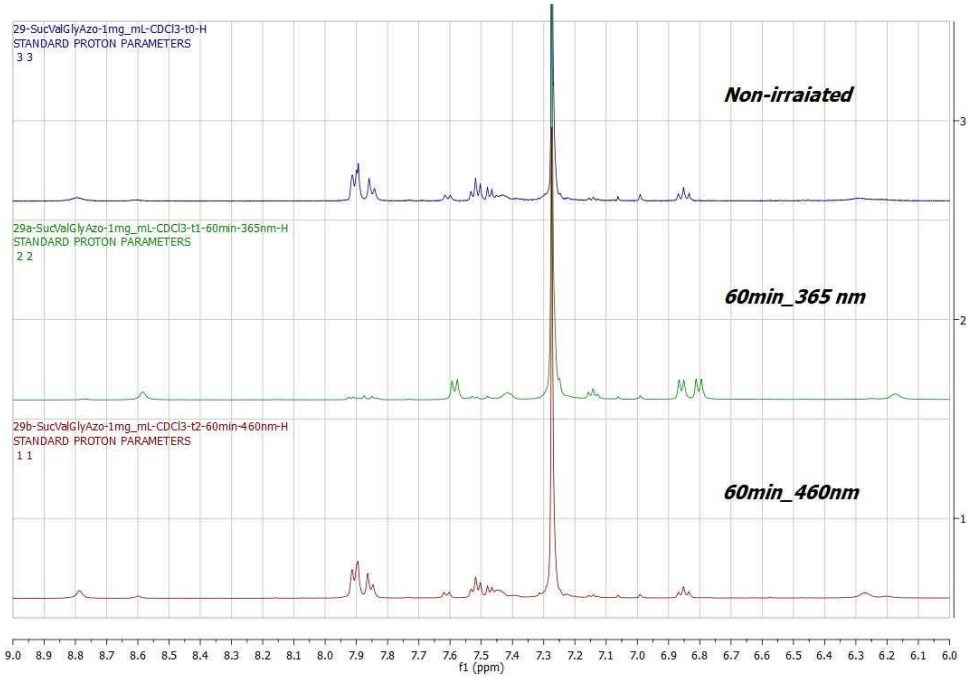


Figure 3-10. *Evolution of ^{1}H NMR of SucValGlyAzo after 1 hour 365 nm irradiation and 1 hour 460 nm recovery.*

From those spectra it can be appreciated, as expected, that the spectrum of the recovered sample looks almost equal to the one of the initial sample.

B) Macroscopic response

To study the macroscopic response to visible light, gels were broken both, mechanically and by the effect of UV. However, total reconstruction of gels could not be achieved. Gels that were broken mechanically did not show any change. Since UV light only affected hydrogels and chloroform gels, they were the only that showed some slight differences.

Chloroform solutions presented the formation of new aggregates upon Vis-light radiation but a self- restoring gel was not recovered.

On the other hand the *cis-isomerized* hydrogel lost some colour intensity upon Vis light irradiation, indicating that isomerization took place, but not recovering fully its original colour.

As well as in microscopic response, these results also indicated that equilibrium between *trans-cis* states can be reversible.

3.4 Gel reset

Other recovery study that was carried out was what we called “gel reset”. In the introduction has been explained how non-covalent forces constitute supramolecular gels and, because of that, they can be reset by the application of heat.

Toluene and chloroform gels were broken by mechanical stirring and UV irradiation and heated as in its formation. The process was repeated up to three times, and gels did not seem to show any inconvenience in its reformation. Therefore, it can be concluded that the application of heat can reform gels.

3.5 Thixotropic study

A gel is considered to be thixotropic when after its mechanical breakage, it is capable without energy or any other type of catalytic source of recovering its previous structure.

In order to determinate if the obtained gels were thixotropic, they were broken and left in darkness a week, however any change was observed, so they can be considered non-thixotropic.

3.6 TEM study

It was proposed to perform a study on the electronic transmission microscope (TEM) to gain insight into the fibrillary structure of SucValGlyAzo. In the following images for xerogels from different solvents are shown.

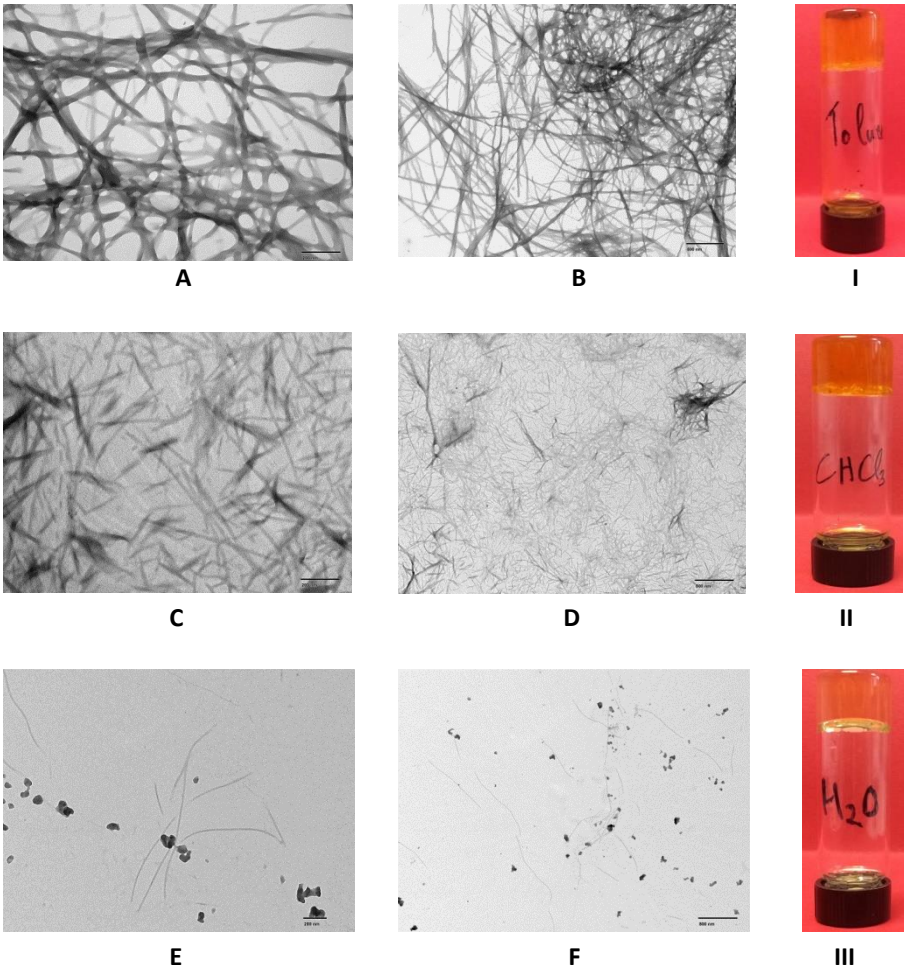


Figure 3-11. *TEM images of toluene gel (A, B), chloroform gel (C,D) and hydrogel (E, F). I, II, III shows gels before preparing TEM samples.*

As can be seen, each figure is constituted by two images with different magnification and a third one showing the real gel. Left images show bar scales of 200 nm and right images of 800 nm, showing all of them an entanglement of self-assembled fibrillary objects observed commonly in molecular gels. Numbered images show the appearance of the gel before xerogel formation

On the other hand, the general trend that relates the size and the length of the fibers with the opacity of the gel is also fulfilled. Chloroform gel exhibited the longest and broadest fibers, forming the most opaque gel. Also, toluene gel showed show long fibers, but not so thick, explaining why it was more translucent. Finally, the hydrogel, whose concentration was ten times lower, showed very small and narrow fibers, rationalizing its translucent, almost transparent characteristics. Also, toluene pictures show quite curious fibers, since they look like if they present chirality. For being sure of this, more studies of nanoparticles and gels in TEM should be performed.

Conclusions

Conclusions

- ✓ SucValGlyAzo was obtained, purified and characterized successfully with a global yield of 41%.

- ✓ Potentiometric titrations of SucValGlyAzo revealed that this molecule has its pK_a value on 6.13±0.03

- ✓ Minimum gelator concentration (m.g.c.) of SucValGlyAzo was determined in several solvents by inverted tube method. The smallest m.g.c. obtained were in toluene, chloroform and water, revealing this last solvent that SucValGlyAzo can be considered as supergellator on it.

- ✓ SucValGlyAzo light responsiveness was tested successfully in dissolution.

- ✓ The minimum time in dissolution to reach maximum isomerization was down to 10 minutes.

- ✓ SucValGlyAzo was proved to disassemble in chloroform gel in front of UV irradiation.

- ✓ The hydrogel was proved to suffer isomerization in front of UV/Vis light stimuli, however due to its hydrophobic behavior, fibers could not be broken.

Future Work

- ✓ Study of the formation of nanoparticles and its behaviour in front of light stimuli.

Experimental Section

Experimental Section

5.1 General methods

$^{1}H/^{13}C$ NMR spectra were recorded on a Varian Unity of 500 MHz and 400 MHz in the indicated solvent at 30 ºC. Signals of the deuterated solvent (DMSO-*d6* or $CDCl_3$) were taken as the reference in DMSO-*d6*, the singlet at 2.50 and the quadruplet centered at 39.52 ppm for ^{1}H and ^{13}C NMR, respectively, and the reference in $CDCl_3$, the singlet at 7.26 and singlet at 77.16 ppm for ^{1}H and ^{13}C NMR. ^{1}H and ^{13}C signals were assigned with the aid of 2D methods (COSY, HSQC and HMBC). Reactions which required an inert atmosphere were carried out under N_2. Commercially available reagents were used as received. In the characterization of the spectra the abbreviations s, d, t, q, p, m, br, dd which means singlet, doublet, triplet, quadruplet, quintet, multiplet, broad and doublet of doublets.

Mass spectra were run by the electro-spray mode (ESMS). Masses spectra were recorded at Mass Spectrometry triple Quadrupole Q-TOF Premier (Waters) with simultaneous Electrospray and APCI Probe.

5.2 UV/VIS spectroscopy

The UV/Vis absorption measurements of each sample were recorded in a JASCO V-630 spectrophotometer. The measurements were carried out using 1400µL SUPRAISAL quartz cells with 10 mm light path from Hellma Analytics. The spectra were recorded between 300 and 600 nm, with a bandwidth of 1.0 nm, 400 nm/min and medium response at 25 ºC. Chloroform, toluene and water spectra were used as baseline.

Before measurements, chloroform and toluene samples were heated until total dissolution of compound. All measurements were carried out at 0.025 mg/mL or 0.055 mM to avoid saturations, as well as aggregates.

5.3 UV/VIS light irradiation

All irradiations were carried out by duplicated plus other sample acting as control (protected with aluminum layer). All samples were placed around 10 cm away from LED.

5.3.1 UV light

Samples were irradiated with two 365 nm UV LEDs with 1200 mW flux output at 2.7 W power dissipation and 4.4 mm x 4.4 mm of foot print (LZ1- 00UV00).

Macromolecular studies were irradiated up to 2 hours to avoid overheating of LED. Minimum isomerization time study was carried out irradiating 10 minutes and later analyzing the spectrum until three of them were equal.

5.3.2 VIS light

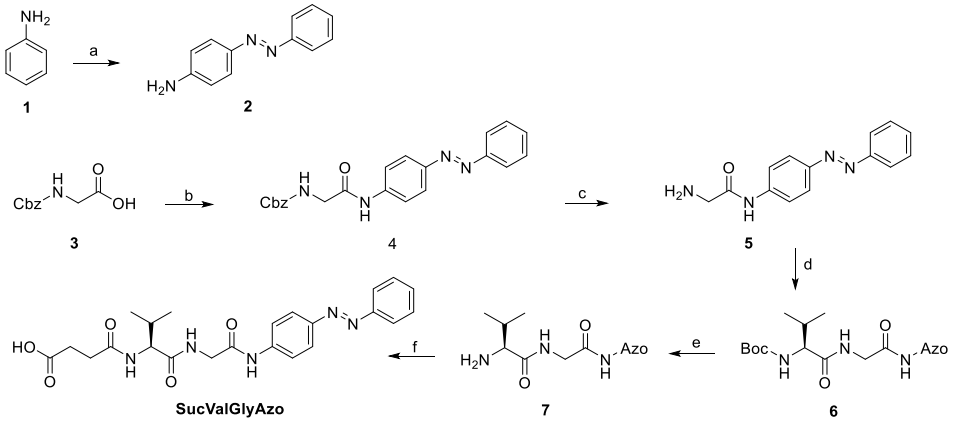
Samples were irradiated with three High Luminous Efficacy Blue 457 nm LED Emitter, 4.4 mm x 4.4 mm of foot print and up to 1.5 A drive current (LZ1-00B202).

Macromolecular studies were irradiated up to 2 hours to avoid overheating of LED. Minimum isomerization time study was carried out irradiating 10 minutes and later analyzing the spectrum until three of them were equal.

5.4 Transmission Electron Microscopy (TEM).

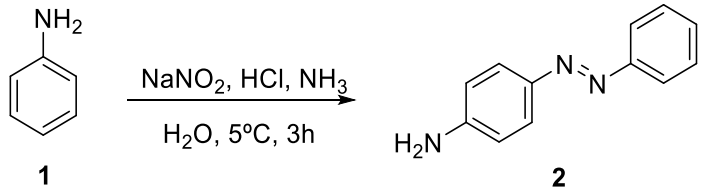
Transmission electron micrographs were obtained using a JEOL 2100 microscope with a thermionic gun LaB6 200 kV equipped with a Gatan Orius high resolution CCD camera. TEM samples were prepared over a Formvar/Carbon film on 200 mesh copper grids. Fresh gels were applied directly onto the grid and the expelled solvent was carefully removed by capillary action with paper. The grids were immediately stained with one drop of 1% aqueous phosphotungstic acid for 2 min and the liquid was subsequently removed by capillary action.

5.5 Experimental procedure for synthesis of SucValGlyAzo



Scheme 5-1. *Global synthesis of SucValGlyAzo: a) $NaNO_2$, HCl, NH_3, H_2O, 3h , 72% b) CH_2Cl_2, 4-aminoazobenzene, DMF, $(CO)_2Cl_2$, Et_3N, 16h, 83%; c) HBr, 16h; d) BocValOH, DIPEA, EDCI, Oxyma, 16h, 82%; e)$CHCl_3$, TFA, 16h, 90%; f) THF, Na_2CO_3, succinic anhydride, 16h, 85%.*

5.5.1 Obtention of 4-aminoazobenzene



Aniline (0.92 g, 10 mmol) was added dropwise to a solution of concentrated HCl (37%, 3 mL) in deionized water (30 mL).The mixture was stirred in an ice bath to keep the reaction temperature at 0ºC. Then a water solution (5 mL) of sodium nitrite (0.70 g, 10.1 mmol) was added slowly for 10 min. The mixture was stirred at 0ºC for further 60 min. A yellow transparent diazonium salt solution was obtained. A coupling solution was prepared as follows: aniline (0.93 g, 10.1 mmol) and HCl (1 N, 10 mL) was dissolved in 30 mL of water under vigorous stirring at 0ºC. Then the diazonium salt solution was added dropwise to the coupling

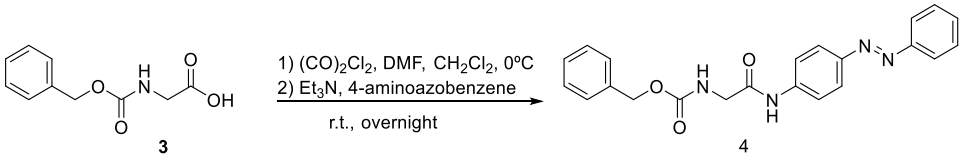
solution with the temperature of 0º C. The system was kept being stirred at 5º C for 3 h. Then the final solution was added slowly to 30 mL of NH_3 (1 N) and an orange precipitate of the azo-compound formed. The precipitate was filtered and washed with water containing a little amount of sodium hydrogen carbonate (pH≈8). The precipitate was collected by filtration, washed with deionized water three times, and dried under vacuum.

4-aminoazobenzene (Compound 2): Orange crystals were obtained (yield 72%);

^{1}H NMR and ^{13}C NMR spectra were consistent with those described in the literature.[16]

HR ESMS: *m/z*: calcd for $C_{12}H_{11}N_3$: 198.1031; found: 198.1034 *[M+H]*$^{+}$.

5.5.2 Coupling of CbzGlyOH with 4-aminoazobenzene



Commercially available CbzGlyOH (2.34 g, 11 mmol, 1.09) was dissolved in anhydrous dichloromethane (37 mL) in a two-neck flask. Then $(CO)_2Cl_2$ (5.6 mL; 11.2 mmol, 1.098 eq) and DMF (90 µL, 8.0 µL/mmol $(CO)_2Cl_2$) were added dropwise. The mixture was cooled and stirred at 0ºC and N_2 atmosphere for 2 hours. After that, ice bath was removed and later, the coupling azobenzene solution : 4-aminoazobenzene (2.0g, 10.2 mmol, 1 eq) in anhydrous dichloromethane (100 mL), was added dropwise. Et_3N (1.64 mL, 11 mmol, 1.098 eq) was added with syringe. Reaction was left overnight under N_2 conditions. Then, the solvent is dried under vacuum. A dark red paste (crude) was obtained and purified by acid (HCl, 1M, 200 mL) and basic wash (NaOH, 0.1M, 200 mL). Finally, molecule 4 was washed with H_2O until neutral pH and left to the vacuum oven at 55ºC overnight.

[16] Schoenberger, M; Trauner, D. *Angew. Chem.* **2014** *53*, 3329 ;

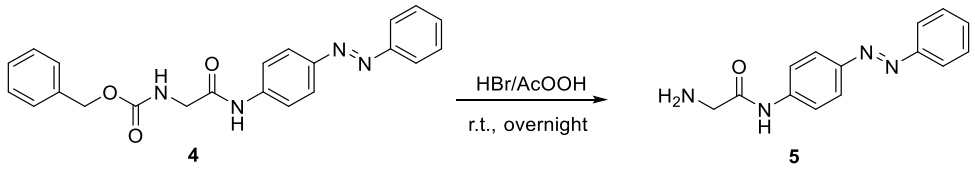
CbzGlyAzo (molecule 4): The compound was obtained with a yield of 83% as an orange solid.

^{1}H NMR (500 MHz, DMSO-*d6*): δ 10.29 (s, 1H), 7.93-7.80 (m, 4H), 7.63 – 7.47 (m, 6H), 7.46 – 7.21 (m, 5H), 5.07 (s, 2H), 3.88 (d, J = 5.9 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-*d6*): δ 168.5, 156.6 (C=O), 152.0, 147.5, 142.0, 137.0 (C), 131.0, 129.4 (x2), 128.3 (x2), 127.8, 127.7 (x2), 123.7 (x2), 122.3 (x2), 119.3 (x2) (CH), 65.5, 44.2 (CH_2).

HR ESMS: *m/z*: calcd for $C_{22}H_{20}N_4O_3$: 389.1613; found: 389.1614 $[M+H]^+$.

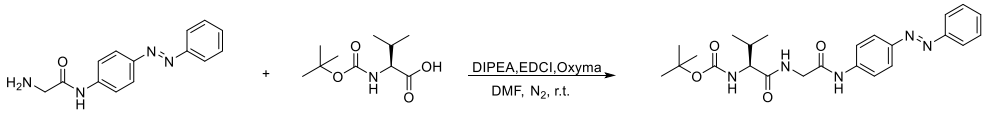
5.5.3 Deprotection of Cbz amine protecting group



4 5

A dissolution of HBr 33% in acetic acid (28 mL, 3.3 mL/ mmol Cbz) was added to a solid CbzGlyAzo (3.22 g, 8.29 mmol). The reaction was stirred under N_2 atmosphere and 0ºC during 30 minutes. After that, the ice bath was removed and the mix was stirred overnight at room temperature. After this time, diethyl ether was added dropwise and the formation of a red precipitate was observed. The redish solid obtained was filtered off under vacuum and the residue was dissolved in water (100 mL); then pellets of sodium hydroxide were added at 0 ºC until observe the formation of a orange precipitate at pH = 12. The residue was washed with water (300 mL). The compound 5 was dried under reduced pressure at 50 ºC overnight and used directly in the next step, in the form of orange solid.

5.5.4 Aminoacid coupling between BocValOH and HGlyAzo



5 6

Amine 5 (1.45 g, 5.70 mmol, 1 eq), DIPEA (1.19 mL, 6.84 mmol, 1.2 eq) and BocValOH (1.24 g, 5.70 mmol, 1 eq), were dissolved in anhydrous DMF (150 mL) at r.t. in a two-neck flask. EDCI (1.19 g, 6.21 mmol, 1.09 eq.) and Oxyma (0.91 g, 6.213 mmol, 1.09 eq, CAS:3849-21-6) were added and the reaction was stirred at room temperature overnight. After this time, water was added and the formation of an orange precipitate was observed. The solid obtained was filtered under vacuum and the residue was washed with acid (HCl, 1M, 200 mL) and basic (NaOH, 0.1M, 200 mL). Finally, it was washed with H_2O (400 mL) until neutral pH and dried under reduced pressure at 50ºC overnight.

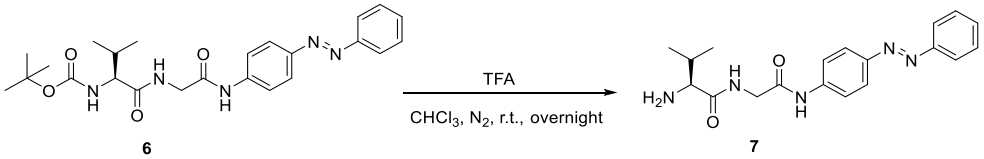
BocValGlyAzo (molecule 6): The product was obtained with 82% of yield as an orange-brown solid.

^{1}H NMR (400 MHz, DMSO-*d6*) Signals for the major *E* isomer: *δ* 10.20 (s, 1H), 8.29 (t, *J*= 4.8 Hz, 1H), 7.95 – 7.80 (m, 6H), 7.64 – 7.49 (m, 3H), 3.96 (m, 1H), 3.85 (m, 2H), 1.97 (m, 1H), 1.40 (s,1H), 0.92– 0.88(dd, J_1= 7.6 Hz, J_2= 6.4 Hz, 3H). Amine 17 not visible.

^{13}C NMR (101 MHz, DMSO-*d6*) Signals for the major *E* isomer: *δ* 172.2, 168.3, 155.9 (C=O), 152.2, 147.8, 142.0 (C), 131.2, 129.6 (x2), 123.9 (x2), 122.5 (x2), 119.5 (x2) (CH), 78.4 (C), 60.1 (CH), 43.0 (CH_2), 30.4 (CH), 28.4 (x3), 19.4, 18.39 (CH_3).

HR ESMS: *m/z*: calcd for $C_{24}H_{31}N_5O_4$: 454.2454; found: 454.2447 $[M+H]^+$.

5.5.5 Deprotection of the Boc amine protecting group



6 7

A solution of the *tert*-butyl ester compound (1.83 g, 4.04 mmol, 1 eq) in a mixture of TFA (8.1 mL, 2 mL/ mmol Boc), dichloromethane (40 mL, 10 mL/mmol Boc), which were added dropwise, was stirred under N_2 atmosphere at room temperature overnight. Later, chloroform and TFA were evaporated under

reduced pressure and the residue (dark oil liquid) was dissolved again with NaOH 0.2 M (200 mL) and extracted three times with chloroform (800 mL in total). The organic phase was concentrated until dryness and left under vacuum conditions for 1 hour.

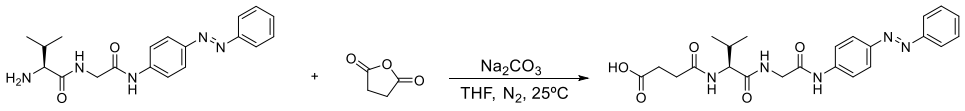
HValGlyAzo (compound 7): It was obtained as an orange solid, with a yield of 90%.

^{1}H NMR (500 MHz, DMSO-*d6*) Signals for the major *E* isomer: *δ* 10.33 (s, 1H), 8.24 (s, 1H), 7.92 – 7.78 (m, 6H), 7.60 – 7.48 (m, 3H) 3.97 (m, 2H), 3.06 (d, *J*= 5.0 Hz, 1H), 1.97 (m, 1H), 0.93 (d, *J*= 7.0 Hz, 6H). Amine proton 17 not visible.

^{13}C NMR (126 MHz DMSO-*d6)*) Signals for the major *E* isomer: δ 175.1, 168.3 (C=O), 152.5, 147.6, 142.0 (C), 131.0, 129.4 (x2), 123.7 (x2), 122.3 (x2), 119.2 (x2), 59.9 (CH), 42.7 (CH_2), 31.4 (CH), 19.6 (CH_3) (x2).

HR ESMS: *m/z*: calcd for $C_{19}H_{23}N_5O_2$: 354.1930; found: 354.1926 *[M+H]$^+$*.

5.5.6 Coupling of succinic acid to amino-valine group



7 SucValGlyAzo

A solution of compound 7 (1.42 g, 4.04 mmol, 1 eq.) in THF (120 mL, 30 mL/mmol HValGlyAzo) was treated at r.t. under N_2 with solid Na_2CO_3 (1.50 g,14.14 mmol, 3.5 eq.). The mixture was stirred overnight after the addition of a dissolution of commercial available succinic anyhidride (808 mg, 8.08 mmol, 2 eq.) in THF (60 mL) at room temperature. After this time, the solution was concentrated under reduced pressure and the crude residue was dissolved in water (100 mL); then hydrochloric acid concentrate was added dropwise at 0 ºC until observe the formation of an orange precipitate at pH=2. The white solid obtained was filtered under vacuum, and the residue was washed with water (300 mL). The compound was dried under reduced pressure at 50 ºC overnight.

SucValGlyAzo (target molecule): The desired molecule was obtained as an orange-brown solid with a yield of 85%.

^{1}H NMR (400 MHz, DMSO-*d6*): Signals for the major *E* isomer: δ 12.07 (s, 1H), 10.11 (s, 1H), 8.36 (s, 1H), 8.02 (d, *J*= 7.2 Hz, 1H), 7.95 – 7.82 (m, 6H) , 7.62 – 7.53 (m, 3H), 4.14 (t, *J*= 6.4 Hz, 1H), 3.94 (d, *J*= 4.4 Hz, 2H), 2.49 (s, 4H), 2.03 (m, 1H), 0.92 (d, *J*= 6.0 Hz, 6H).

^{13}C NMR (101 MHz, DMSO-*d6*): Signals for the major *E* isomer: δ 173.9, 171.8, 171.7, 168.2 (C=O), 152.0, 147.6, 141.7 (C), 131.0, 129.4 (x2), 123.7 (x2), 122.3 (x2), 119.3 (x2), 58.5 (CH), 42.8 (CH_3), 30.0 (CH), 29.9 , 29.2 (CH_2), 19.2 (CH_3)(x2).

HR ESMS: *m/z*: calcd for $C_{23}H_{27}N_5O_5$: 452.1934; found: 452.1937 *[M-H$^+$]$^-$*.

5.6 Experimental procedure for determination of pK_a

Potentiometric titrations to determine acid−base constants were carried out at 298 K by triplicate.

In a typical experiment 7 mL of basic solution of the corresponding SucValGlyAzo (20 mg) and NaOH (0.05M) were titrated with normalized dissolution HCl 0.1 N commercially available and vigorous stirring.

The acid was added with an NE-300 “Just Infusion” TM Syringe Pump (0,08 mL/min-inside diameter 14.57 mm) using a SGE Analytical Science syringe 10 mL which had connected a needle of stainless steel cono. Luer. Look 0,7mm x 300mm. The pH was monitored every 10 s (in a S220 Seven Compact pHmeter, Mettler Toledo).

Thermodynamic constants for the species in solution could be calculated with HYPERQUAD using titration data previous to the experimental aggregation onset.

To assess the solubility product of the acids derivatives, titration was stopped when a solid precipitate was observed. Then the solubility product was calculated iteratively with HYSS2009, adjusting its value to fit the calculated and experimental pH.

The results of titrations are shown below in Figure 5-1.

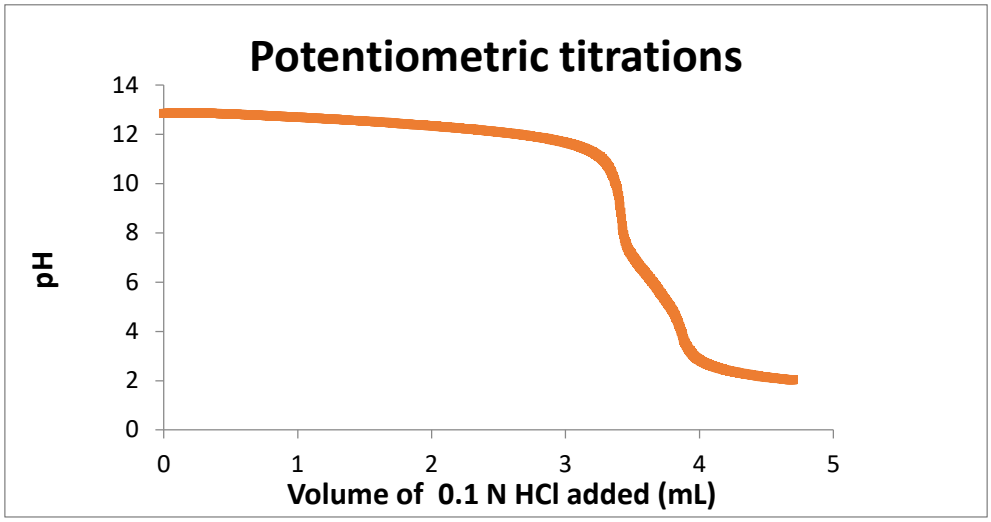


Figure 5-1. Potentiometric titrations of SucValGlyAzo.

It's important to enhance the fact that in the picture shown above, there are two main falls; the first one (from pH≈12 to pH≈7) corresponds to the neutralization of NaOH by HCl, being the second one the one that focus the chemical interest (from pH≈5 to pH≈2.5).

5.7 Experimental method for determination of m.g.c.

All determinations were done by triplicate.

5.7.1 Heat-cooling method

In a typical experiment, 10mg of SucValGlyAzo and 2 mL of solvent were introduced into a cylindrical screw-capped glass vial (8 mL, diameter =1.5 cm). The system was heated up with heat air to 300ºC with an air pistol. Once the solid was totally dissolved, it was cooled. All types of gels were cooled with leaving them at room temperature (maximum 2 hours), leaving them in the fridge for 30 minutes and introducing them into a sonicating bath at 25ºC for 15 minutes.

Once the gel was formed, the gel checking was done by inverted vial test.

5.7.2 pH-change method

In a typical experiment, 10 mg SucValGlyAzo were dissolved in 2 mL of NaOH 0.01M (1 eq.) in a clip vial. Once it was totally dissolved, it was introduced into a cylindrical screw-capped glass vial (8 mL, diameter= 1.5 cm), where there were 14.2 mg of D-*(+)-Gluconic acid δ-lactone* (4 eq.). The solution was sonicated for 5 minutes and left 4 hours.

Once the gel was formed, the gel checking was done by inverted vial test.

ANNEX

Annex

6.1 NMR Spectra

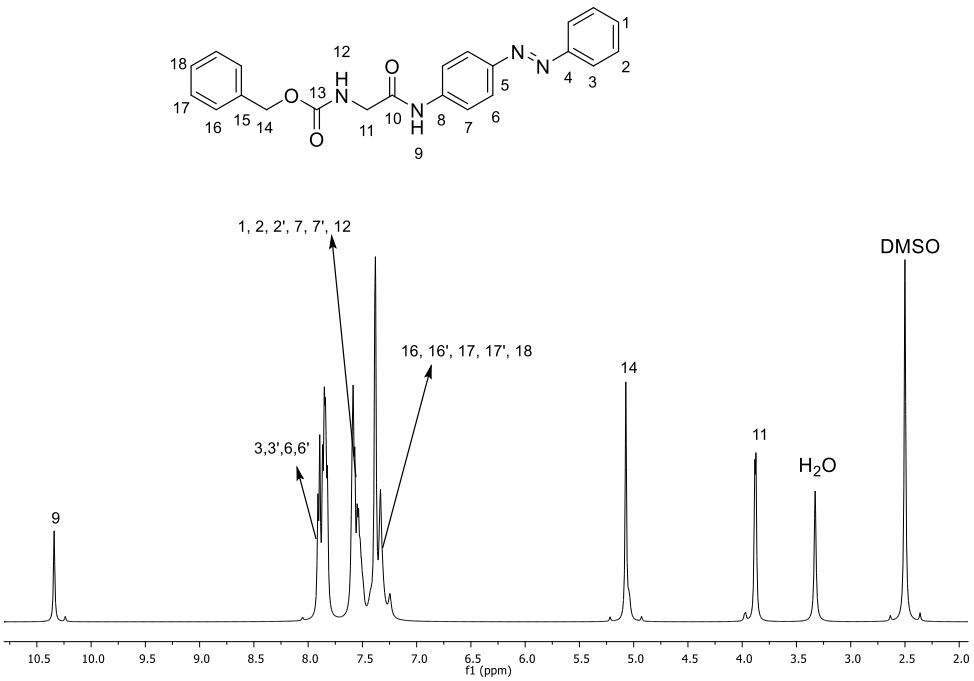


Figure 6-1. *^{1}H NMR of product 4.*

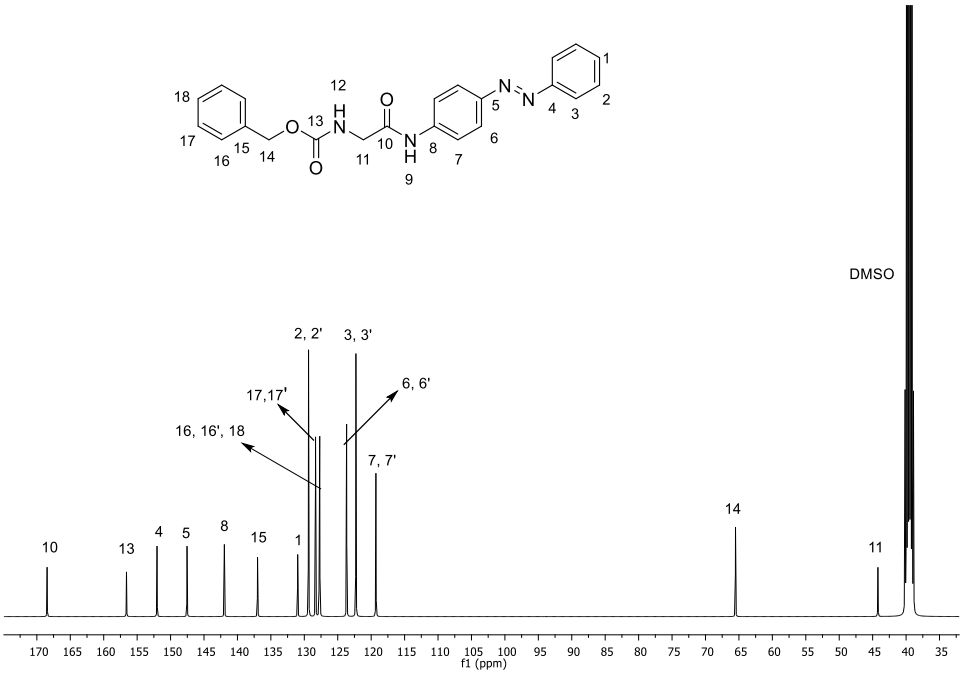


Figure 6-2. *^{13}C NMR of product 4.*

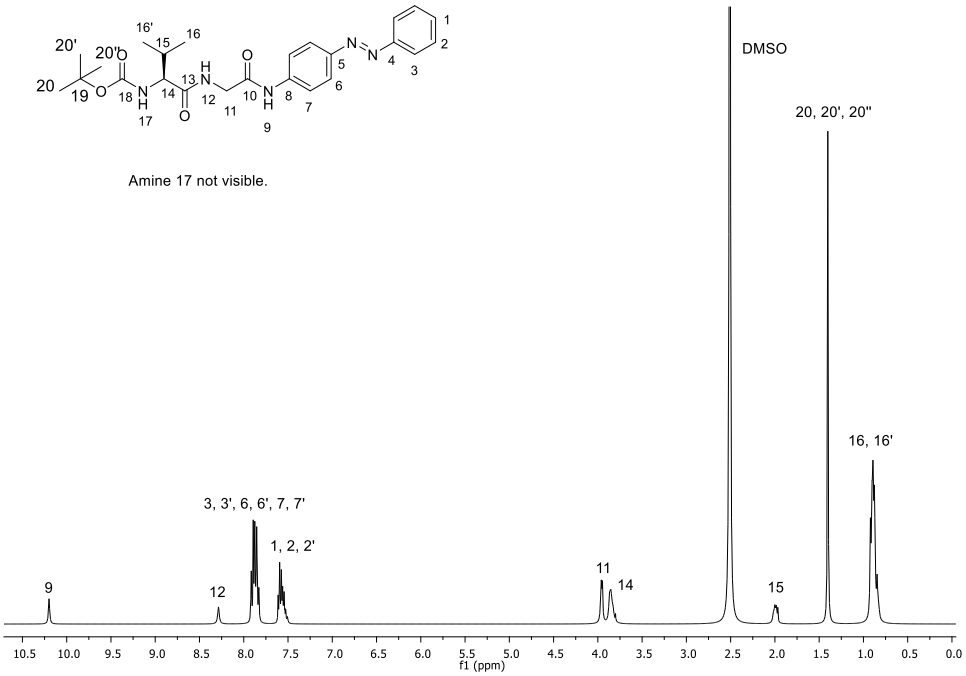


Figure 6-3. *^{1}H NMR of molecule 6.*

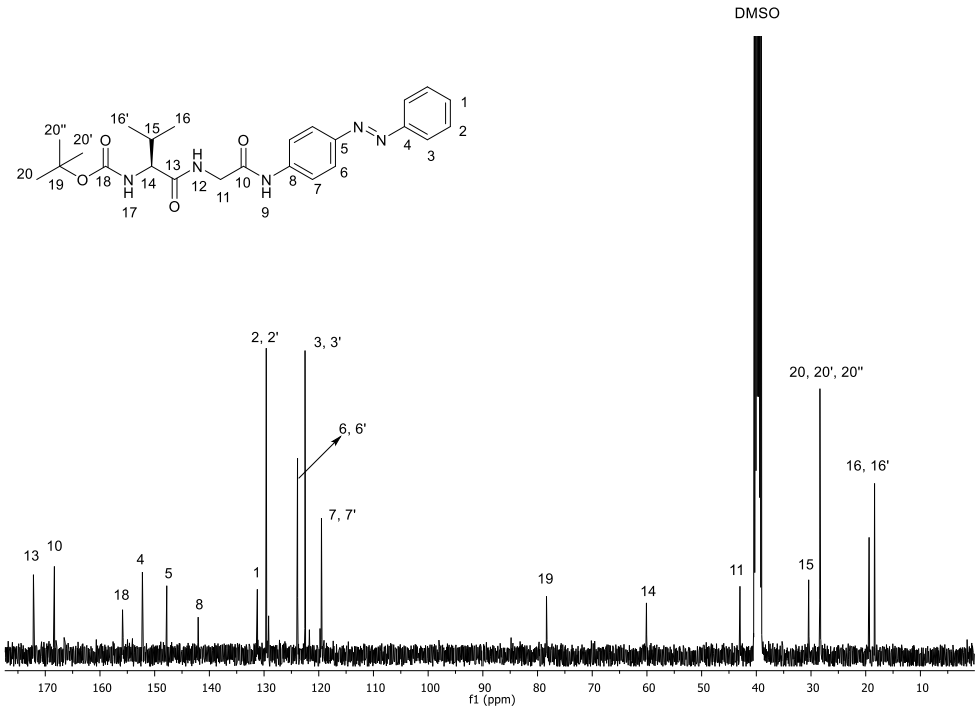


***Figure 6-4**. ^{13}C NMR of molecule 6.*

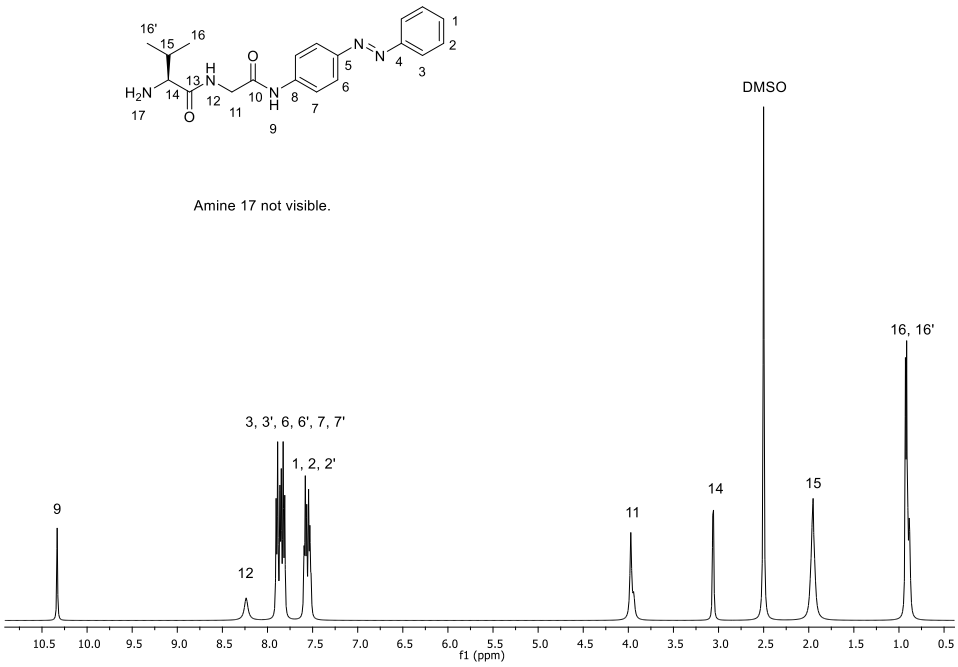


Figure 6-5. *^{1}H NMR of product 7.*

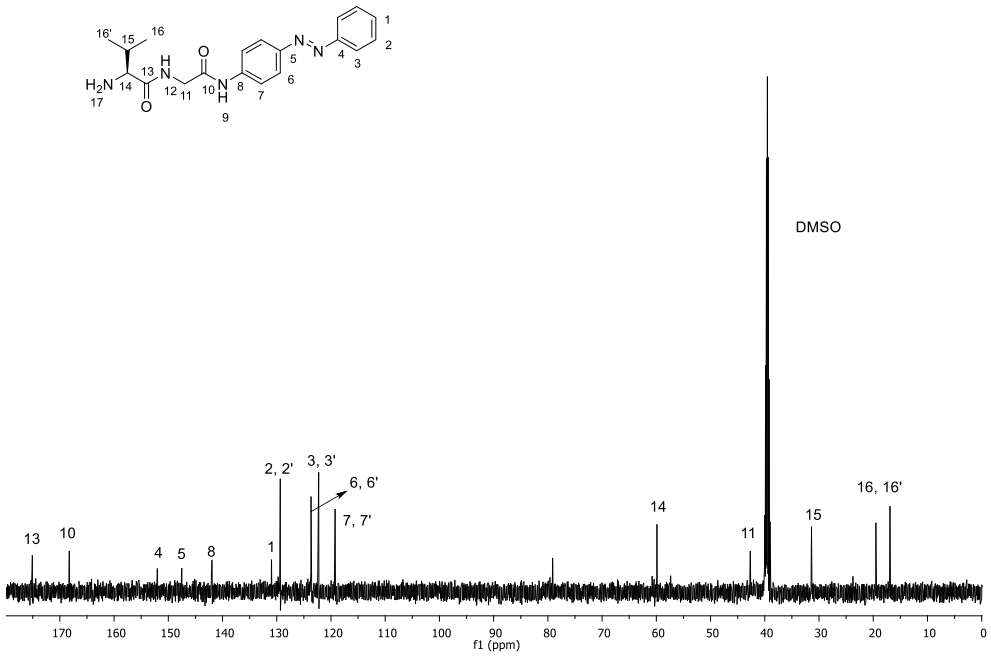


Figure 6-6.-. *^{13}C NMR of molecule 7.*

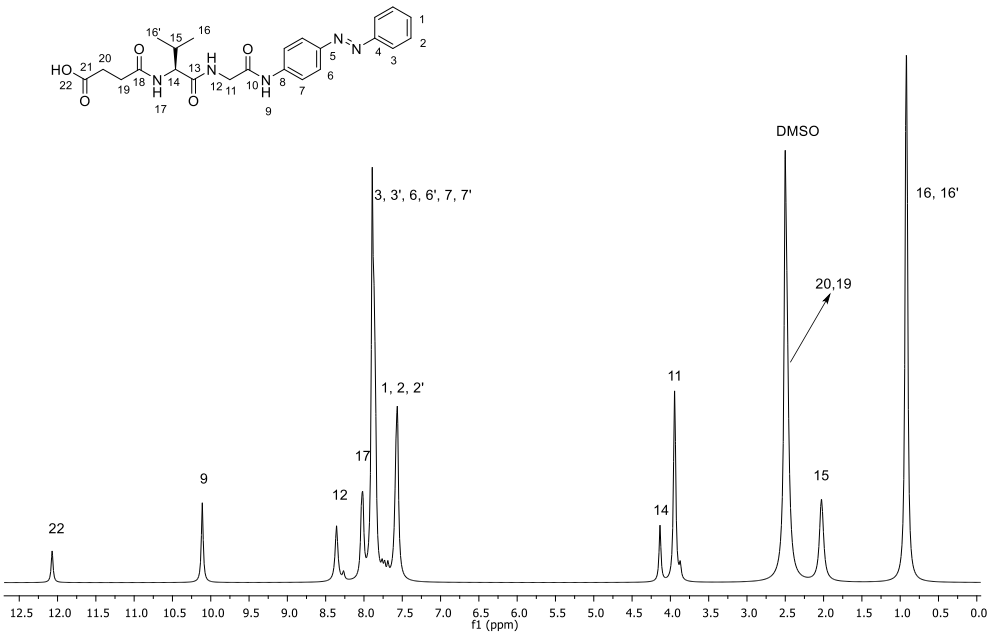


***Figure 6-7**. ^{1}H NMR of SucValGlyAzo*

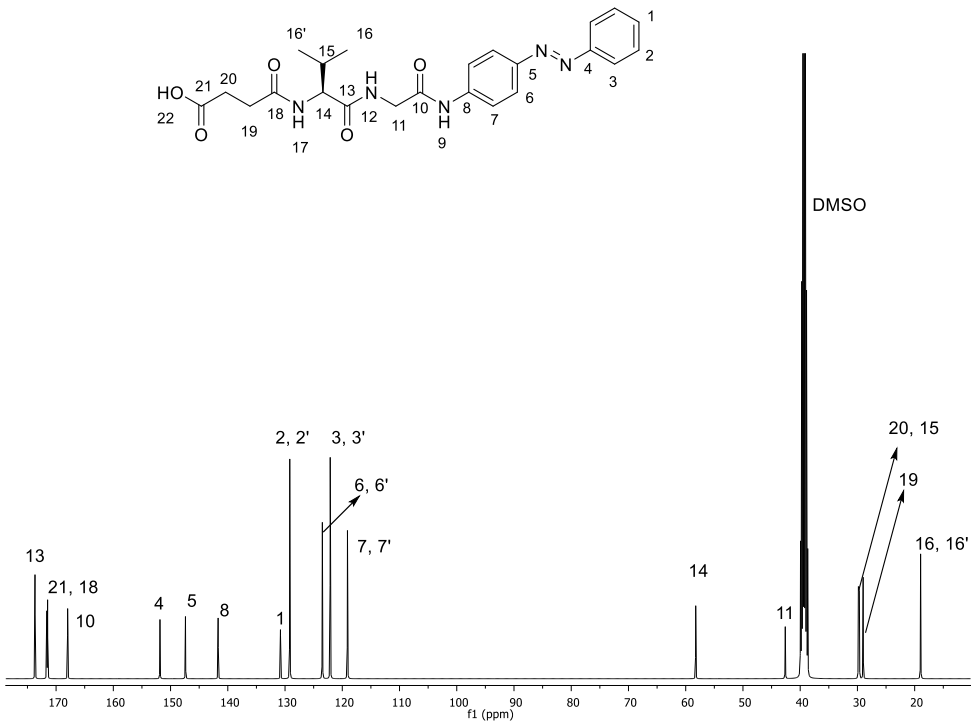


***Figure 6-8**. ^{13}C NMR of SucValGlyAzo.*