Escuela superior de Tecnología y Ciencias Experimentales
Departamento de Química Inorgánica y Orgánica
Grupo de Química Supramolecular

SYNTHESIS AND HYDROLYTIC STABILITY OF SUCCINIC ACID DERIVED GELATORS

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CHEMISTRY DEGREE RESEARCH PROJECT

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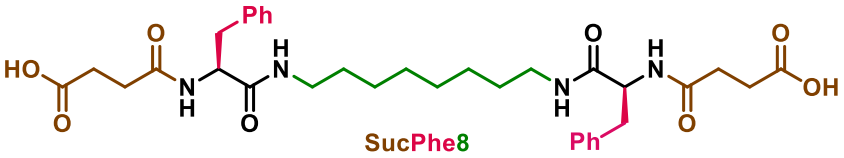
Abbreviations

LMWGs	Low molecular weight gelators
Cbz	Benzyloxycarbonyl
SPPS	Solid Phase Peptide Synthesis
NMR	Nuclear magnetic resonance
Mgc	Minimum gelator concentration
DCC	*N,N*-dicyclohexylcarbodiimide
DCU	*N,N*'-Dicyclohexylurea
DMSO	Dimethyl sulfoxide
THF	Tetrahydrofuran
Suc	Succinic acid radical
Glu	Glutaric acid radical
Phe	Phenylalanine radical
Ile	Isoleucine radical
Val	Valine radical
Ala	Alanine radical
Hx	Hexyl radical
Cy	Ciclohexyl radical
Oct	Octyl radical

Synthesized and studied gelators



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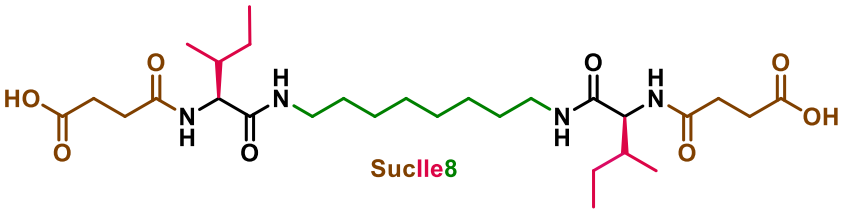

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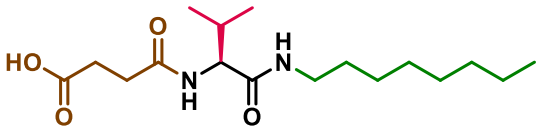

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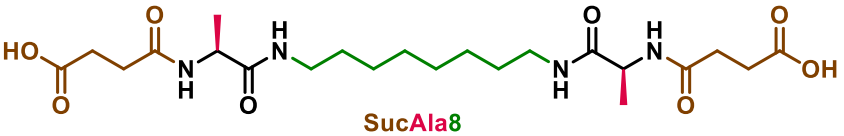

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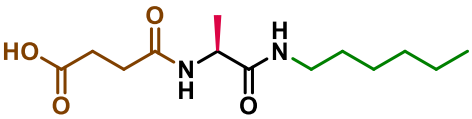

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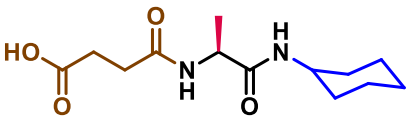

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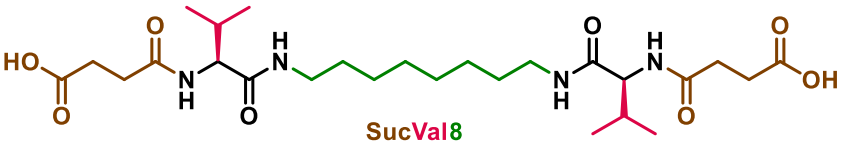

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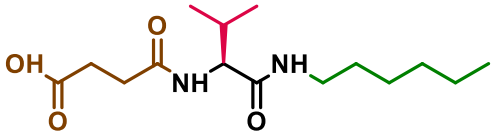

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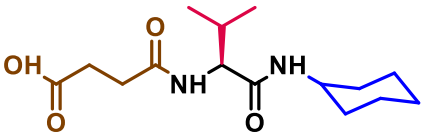

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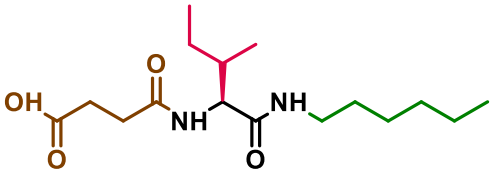

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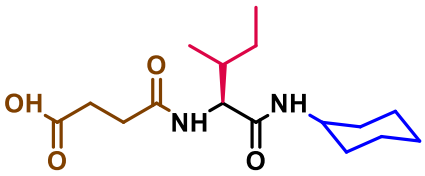

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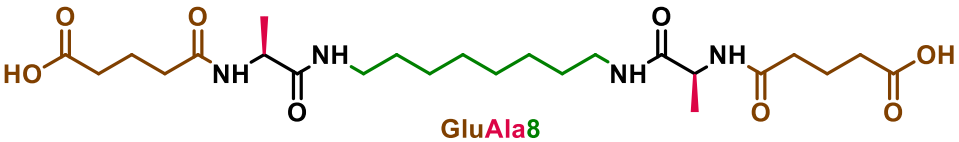

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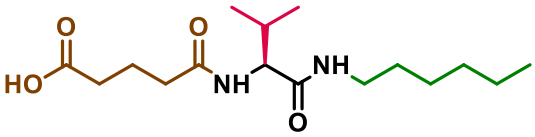
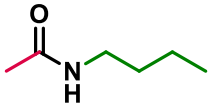


GluValHx



N-butylacetamide

El Dr. Juan Felipe Miravet Celades, Profesor Titular del Departamento de Química Inorgánica y Orgánica de la Universitat Jaume I de Castellón de la Plana, y el Dr. César Augusto Angulo Pachón, Investigador de la Universitat Jaume I,

CERTIFICAN

Que el trabajo fin de grado con el título **SYNTHESIS AND HYDROLYTIC STABILITY OF SUCCINIC ACID DERIVED GELATORS** ha sido realizado por Diego Navarro Barreda bajo su dirección, en el grupo de Química Supramolecular 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 21 de julio de 2016.

Fdo. Dr. Juan F. Miravet Celades

Fdo. Dr. César A. Angulo Pachón

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A very satisfying experience, both academic and personal, as well as the first contact with the world of research.

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Introduction

1 Chapter: Introduction

1.1 What are gels?

Gels pervade our daily life in a variety of forms such as agents in cosmetics and pharmaceutical formulations, as well as cleaning products or food.

Figure 1-1. Examples of common gels.

A very wide range of materials have been recognised since the 1860's to form gels, often with quite different characteristics. But, due to its variety and complexity of the diverse systems it is not easy to write an absolute definition of gel. Dorothy Jordon Lloyd wrote in 1926 that "the gel state is easier to recognise than to define".[1]

The reason lies in the fact that there is usually not a minimum of physical parameters that allow one to write a clear-cut definition, however the designation of a substance as a gel is only a visual observation (if no flow is observed, the solution is said to have become a gel).

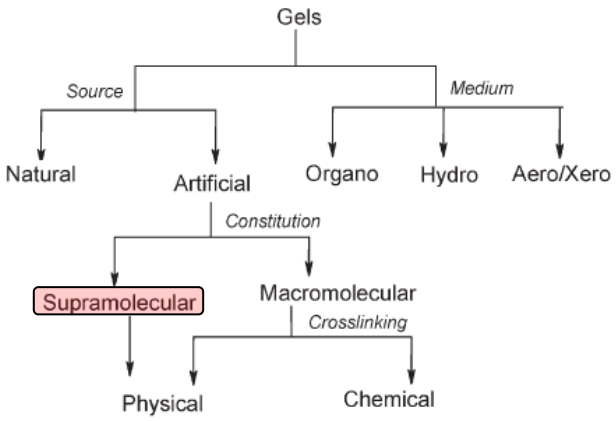
In 1974, Flory defined a gel as a two-component colloidal dispersion with two distinct features:

- Continuity of its microscopic structure with macroscopic dimensions that is permanent on the time scale of an analytical experiment.
- Permanence of its rheological properties, similar to that of solid-like materials.[2]

[1] Weiss, R. G. "The past, present, and future of molecular gels. What is the status of the field, and where is it going?", *J. Am. Chem. Soc.*, **2014,** *136*, 7519-7530.
[2] Terech, P.; Weiss, R. G. "Low Molecular Mass Gelators of Organic Liquids and the Properties of Their Gels", *Chem. Rev.*, **1997**, *97*, 3133-3160.

Generally, gels are predominantly liquid in composition. Typically, 99% by weight of the gel is a solvent while the remaining 1% is the gelator. And the solid-like appearance of a gel is a result of the entrapment and adhesion of the solvent in the large surface area solid 3D matrix formed by the gelator.

Gels can be classified in different ways depending on their source, medium, constitution or type of interaction that creates their 3D network (scheme 1-1).[3]



Scheme 1-1. Classification of gels.

1.2 Supramolecular gels.

Supramolecular gels are a type of physical gels that are formed by small organic compounds with a molecular weight of less than 2000 Da. These small organic compounds are called low molecular weight gelators (LMWGs). Examples of them are included in figure 1-2.[4]

As a physical gel, the network is held together by weak noncovalent interactions like hydrogen bonding, π-π stacking, donor-acceptor interactions, metal coordination, host-guest interaction, solvophobic forces (specially for gels in water) and Van der Waals interactions.

[3] Sangeetha, N. M.; Maitra, U. "Supramolecular gels: Functions and uses", *Chem. Soc. Rev.*, **2005,** *34*, 821-836.
[4] Escuder, B. (Ed.); Miravet J. F. (Ed.), (**2014**). "*Functional Molecular Gel".* Universitat Jaume I, Spain: The Royal Society of Chemistry.

These weak noncovalent interactions are easy to break, hence such gels are reversible and can be readily transformed to a fluid on changing environmental conditions such as temperature, pH, ionic strength, light, redox agents, and electronic and magnetic fields. Due to this thermoreversible nature, dynamic behaviour and chemical sensitivity (also known as smart materials), along with the high biodegradability and biocompatibility or their easy control of functionalization and composition, these supramolecular gels are excellent candidates for fields such as regenerative medicine, tissue engineering, drug delivery of therapeutic agents or optoelectronic applications and catalysis.[3]

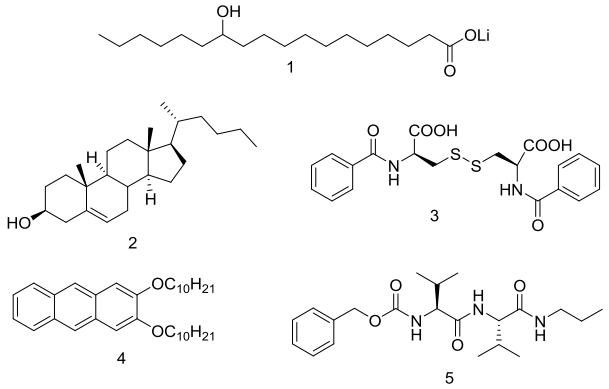


Figure 1-2. Structure of various molecules known to act as LMWGs.

Supramolecular gels are usually prepared by heating the gelator in an appropriate solvent and cooling the resulting isotropic supersaturated solution to room temperature. When the hot solution is cooled, the molecules start to condense and three situations are possible:

(1) A highly ordered aggregation giving rise to crystals i.e., crystallization.

(2) A random aggregation resulting in an amorphous precipitate.

(3) An aggregation process intermediate between these two, yielding a gel.[3]

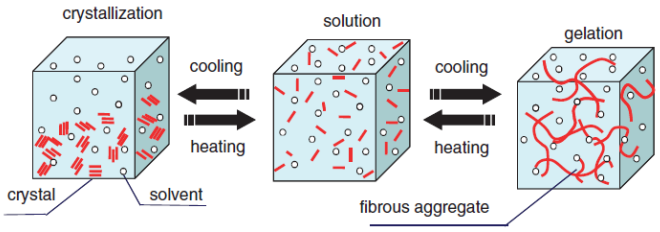
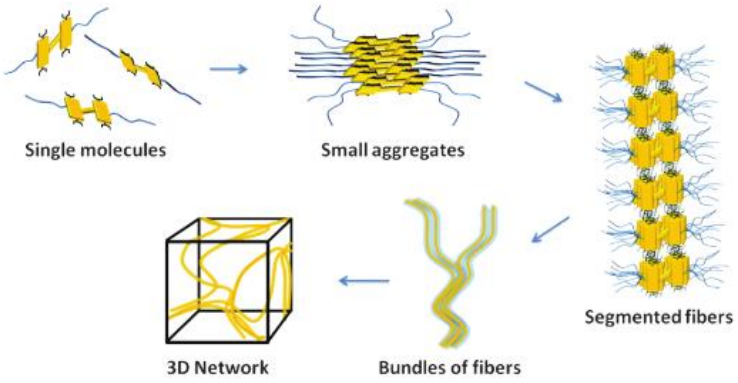


Figure 1-3. Crystallization vs. gelation of low-molecular-weight compounds.[5]

The gelation process generally, occurs with a low concentration of gelator: less than 50 g/l. At first, gelator molecules are self-assembled during the cooling process, producing fibrous assemblies (of typically 5-100 nm). Then, these fibrous assemblies form a three-dimensional network structure, and gelation occurs by trapping solvent in the networks. This process prevents the flow of solvent under gravity and the mass appears like a solid.



Scheme 1-2. Representation of the hierarchical self-assembly of molecular gels.[6]

Incidentally, the discovery of such molecules has been largely serendipitous (typically from a failed crystallization attempt!). However, with theknowledge gained on the aggregation of gelator molecules during the past decade, gelators have been designed by the incorporation of structural features (for instance, H-

[5] Hanabusa, K.; Suzuki, M. "Development of low-molecular-weight gelators and polymer-based gelators", *Polym. J.*, **2004,** *46,* 776-782.
[6] "Supramolecular Gel Based on a Perylene Diimide Dye: Multiple Stimuli Responsiveness, Robustness, and Photofunction", *J. Am. Chem. Soc.* **2009**, *131*, 14365-14373.

bonding motifs such as amide, urea and alcohol) that are known to promote one-dimensional aggregation.

1.3 The amide bond. *The Peptide Bond.*

Among all LMWGs used for the design of molecular gels, those derived from amino acids or peptides are especially attractive due to their inherent biocompatibility and chemical diversity.[7] Amide groups are present in such gelators (see compound 4, in figure 1-2) and as described later, this work is focused on their hydrolytic stability.

The amide moiety is a fundamental unit in biological chemistry, particularly in chemistry of proteins. Proteins are formed by a large number of amino acids, and the amide bond between two amino acids is called *Peptide Bond*. In nature, the formation of these bonds is carried out in the ribosome where there are enzymes that catalyse the ordered and successive formation of peptide bonds between different amino acids in the process of protein synthesis.

The formation of peptide bonds can also be carried out artificially, what is known as *Peptide Synthesis*. The history of peptide synthesis started with Emil Fisher, who obtained glycyl-glycine dipeptide in 1901. The first synthesized polypeptides were homopolymers because there were problems due to the possibility of unintended reactions when different amino acids were combined. This problem was solved, in 1932, by Max Bergmann and Leonidas Zervas, who synthesized the first protecting group: benzyloxycarbonyl (Cbz). Thenceforth, the protecting groups were indispensable in synthesis of peptides.[8]

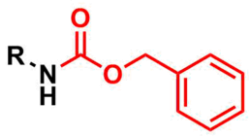


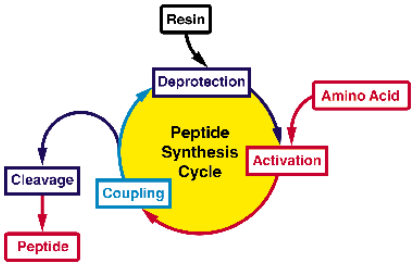
Figure 1-4. The benzyloxycarbonyl group (Cbz).

[7] Berdugo, C. Ph. D. Thesis "Fundamental studies and applications of molecular gels formed by peptide derivatives". **2015**, Universitat Jaume I, Castellón de la Plana.

[8] (a)Handbook of Reagents for Organic Synthesis: Activating Agents and Protecting Groups; Pearson, A. J., Roush, W. R., Eds.; Wiley: New York, 1999. (b) *Chem. Rev.,* **2009,** *109,* 2455-2504.

The classical method that scientists used, when they discovered how to generate peptides in vitro, is called *Liquid Phase Peptide Synthesis.* It is still commonly used for large-scale synthesis. This method is slow and labor-intensive, because the product has to be manually removed from the reaction solution after each step.

However, in the sixties, Bruce Merrifield introduced *Solid Phase Peptide Synthesis* (SPPS) and the strategy of peptide synthesis dramatically changed and simplified the tedious and demanding steps of purifications. In this method, instead of C-terminal protection with a chemical group, the C-terminal of the first amino acid is coupled to an activated solid support, such as polystyrene or polyacrylamide, providing a rapid method to separate the growing peptide product from the different reaction mixtures during synthesis.



Scheme 1-3. Solid Phase Peptide Synthesis.

In addition, the amide bond may be broken by hydrolysis under appropriate conditions. A typical chemical procedure for hydrolysing amides is a 10h reflux in HCl 8M. Even with the strongest acids or bases in high concentrations, prolonged heating is necessary. However, this remarkable resistance to hydrolysis is a key feature of the stability of complex protein structures (at neutral pH and 25°C, the hydrolysis of a deactivated peptide bond has a half-life on 500 years). But, proteins, that have fulfilled their goals, must be biodegraded to the synthesis of new proteins. Proteins ingested in the diet must be broken into smaller peptides for absorption in the intestine. The enzymes whose function is to break these proteins, via a hydrolysis reaction, are called **proteases** or **peptidases**. For example, the Chymotrypsin.[9,10]

[9] Blow, D. M. "Structure and Mechanism of Chymotrypsin", **1976**, *9*, 145-152.
[10] Fastrez, J.; Fersht, A. R. "Mechanism of Chymotrypsin Structure, Reactivity, and Nonproductive Binding Relationships", *Biochemistry*, **1973**, VOL. 12, Nº 6.

The design of artificial enzymes capable of cleaving peptide bonds of proteins as proteases, has been the focus of intense effort for a number of years as a model for the study of hydrolysis of amides.[11] Moreover, because of this extreme unreactivity, kinetic and mechanistic studies have been done almost exclusively with amides that are variously activated by substituents,[12] by ring strain,[13] by proximate functional groups or by metal complexes.[14]

For example, in 1998, Menger and Ladika reported that the pyrrolidyl amide of Kemp's triacid undergoes intramolecular hydrolysis. This hydrolysis reaction depended on the relative geometric disposition, between the amide group and the carboxylic acids, which forces the compounds to react. The hydrolysis could be explained on the basis of a pathway involving intramolecular nucleophilic displacement by the terminal carboxylic acid group on the amide function, that is to say, by "proximate" functional group.[15, 16]

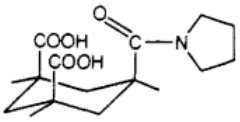


Figure 1-5. Pyrrolidyl amide of Kemp's triacid.

[11] Kahne, D.; Still, W. C. "Hydrolysis of a Peptide Bond in Neutral Water", *J. Am. Chem. Soc.*, ***1988**, Vol. 110,* Nº 22.

[12] Cunningham, B. A.; Schmir, G. L. "Hydroxyl Group Participation in Amide Hydrolysis. The Influence of Catalysts on the Partitioning of aTetrahedral Intermediate", *Journal of the American Chemical Society,* **1967.**

[13] Lopez, X.; Mujika, J. I.; Blackburn,G. M.; Karplus, M. *J. Phys. Chem. A*, **2003**, *107*, 2304-2315.

[14] Zhu, L.; Kostic, N. M. *Inorg. Chem*., **1992**, *31*, 3994-4001.

[15] Menger, F. M.; Ladika, M. "Fast Hydrolysis of an Aliphatic Amide at Neutral pH and Ambient Temperature. A Peptidase Model", *J. Am. Chem. Soc.,* **1988,** *110,* 6794-6796.

[16] Curran, T. P.; Borysenko, C. W.; Abelleira, S. M.; Messier, R. J. *J. Org. Chem.,* **1994,** *59,* 3522-3529.

Objectives

2 Chapter: Objectives

Previously, the research group synthesized a family of compounds derived from succinic acid. Unexpectedly, the warming of one of them resulted in the appearance of unknown peaks in ^{1}H-NMR spectra. It was proposed that a possible hydrolysis could take place.

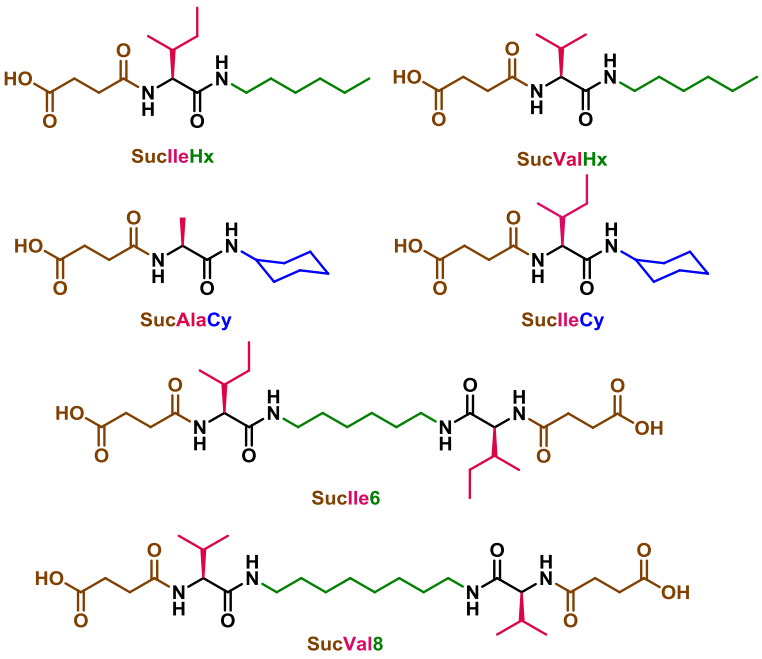


Figure 2-1. Structures of the compounds derived from succinic acid previously synthetized.

The main objective of this work is to determine whether the compounds actually break when they are subjected to heating.

The specific objectives are:

- Synthesize and characterize succinic acid derived gelators.

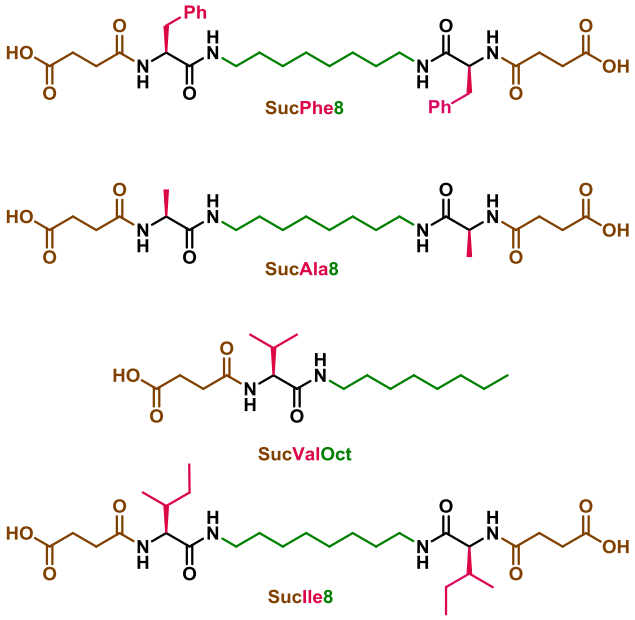


Figure 2-2. Succinic acid derivatives synthesized in the present project.

- Study the hydrolytic stability of these compounds upon heating at 80 ºC during 24 hours.

Results and Discussion

3 Chapter: Results and Discussion

3.1 Synthesis of amino-acid derivatives.

Previously the research group synthesized a family of compounds derived from succinic acid; some of them are showed in Figure 3-1.

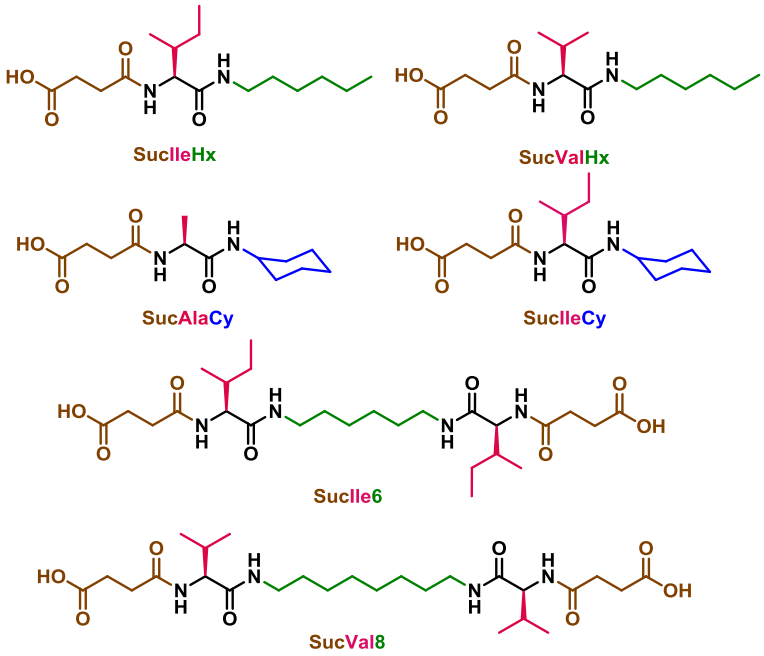
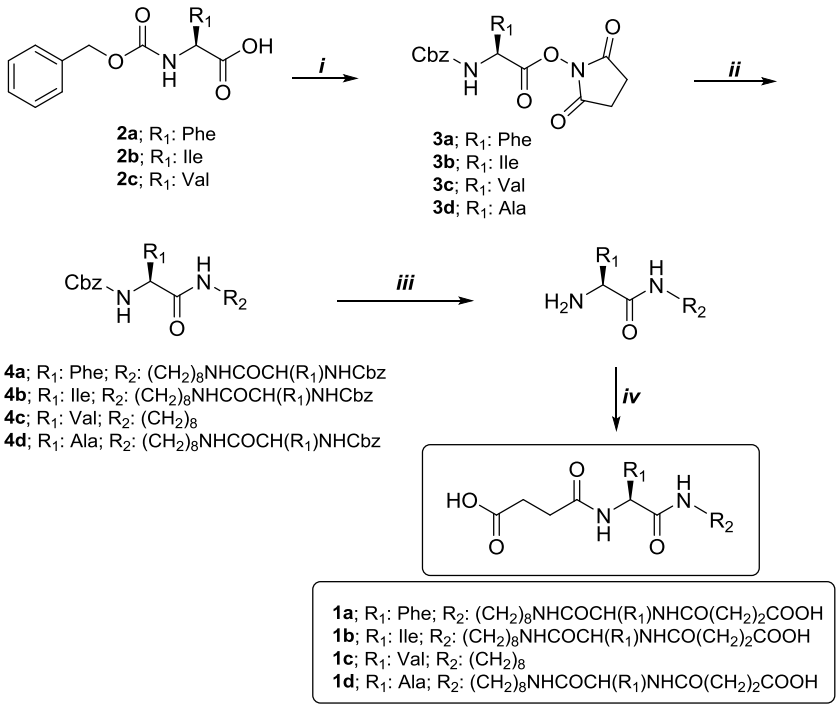


Figure 3-1. Examples of succinic acid derived compounds.

Among them, SucVal8 presented an unexpected NMR spectrum when it was heated during gelation assays, and it didn't become a gel in conditions in which the gel formation was expected. Because of that, it was decided to study in more detail this fact and to synthesize other succinic acid derived compounds to study how the amino acid or the alkyl chain (or both) affects to the stability of this type of compounds.

All the compounds have been synthesized following a commonly used synthetic route by the research group. Scheme 3-1 shows the steps and reagents which were used to prepare these compounds.



Scheme 3-1. Reagents and conditions: i) *DCC*, *N*-hydroxysuccinimide, THF, 1h 30min, 73-84%; ii) 1,8-diamino octane or octylamine, THF, 5h, 90-96%; iii) Pd/C, H_2, MeOH, 1.5-7.5 h, 96-99%; iv) Succinic anhydride, K_2CO_3, THF, 16h, 53-91%.

The first step consists of an activation of 2a-c to form activated acids 3a-c (3d was obtained from commercial suppliers with purity higher than 98 %) as reported previously.[17] The activated acids were coupled with *1,8-diaminooctane or octylamine* to form the compounds 4a-d. Then, the removal of the Cbz group was performed by hydrogenolysis using Pd/C as catalyst, obtaining the amines 5a-d. Finally, the amines and *succinic anhydride* were mixed under mild basic conditions,[18] producing the succinic derivatives 1a-d. Figure 3-2 shows the new succinic acid derivatives prepared for the studies carried out in the present project.

[17] Fontanillo, M.; Angulo-Pachón, C. A. *J. Colloid Interv. Scl.,* **2003**, *412*, 65-71.
[18] Detsi, A.; Micha-Screttas, M.; Igglessi-Markopoulou, O. *J. Chem. Soc., Perkin Trans*. *1*, **1998**, 2443-2450.

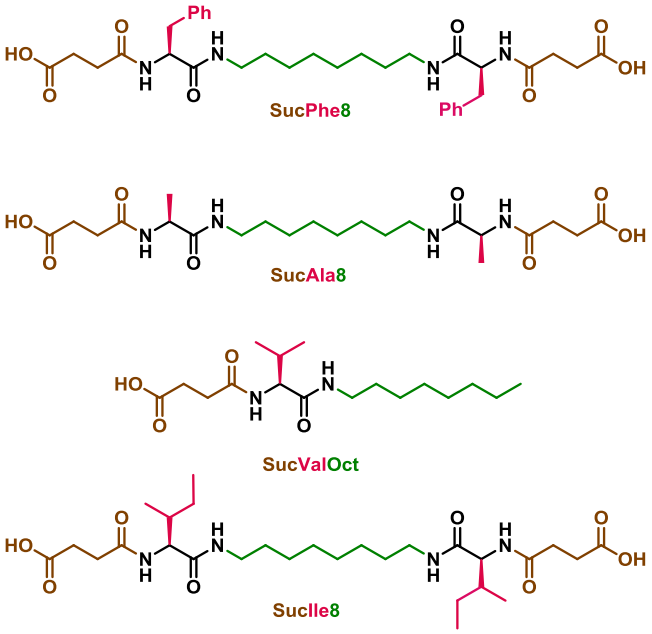


Figure 3-2. Succinic acid derivatives synthesized in the present project.

3.2 Hydrolytic study of succinic acid derivatives.

In order to know whether the succinic acid derivatives undergo hydrolysis when they are subjected to heating, the samples were prepared by ***the method of changing pH,*** which is a method widely used for the preparation of gels.

Taking advantage of the fact that the molecules have an acid group, the first step is to dissolve the product with a basic solution (forming a carboxylate group which is soluble in aqueous media), and the resulting isotropic solution is treated with the amount needed of acid solution to neutralize the base added previously.

When the acid solution is added, the product is protonated again, and three situations are possible:

i. The product forms a gel.
ii. The product aggregates, but not form a gel.
iii. The product remains completely in solution.

These three situations are produced depending on whether the product is in a concentration higher or lower than ***minimum gelator concentration (mgc).***

Minimum gelator concentration (mgc) is the minor amount required of a product to form a gel in a solvent. It is considered that a gelator is better when it has a smaller *mgc* value. A gelator whose *mgc* is higher than 30 mM, it is considered a "bad gelator".[19]

Hence, if the concentration is higher than *mgc*, the situation *i* will be produced, if the concentration is lower than *mgc*, the situation *ii* will be take place, and if the gelator is soluble in the solvent, it will be produced the situation *iii*. Also, it may happen that a compound, despite forming aggregates, does not become gel whatever its concentration (resulting in situation *ii*).

3.2.1 Hydrolytic studies of "mono" acid succinic derivates.

Hydrolytic studies were performed by heating at 80 ºC for 24 h the following compounds: SucValOct (synthesized in this project), SucValCy, SucValHx, SucAlaCy, SucAlaHx, SucIleCy and SucIleHx (provided by the research group).

The obtained spectra were the following:

[19] Nebot Carda J. V. *Ph. D*. Thesis. "Design, study and applications of supramolecular hydrogels based on amino acids", **2012**, Universitat Jaume I, Castellón de la Plana.

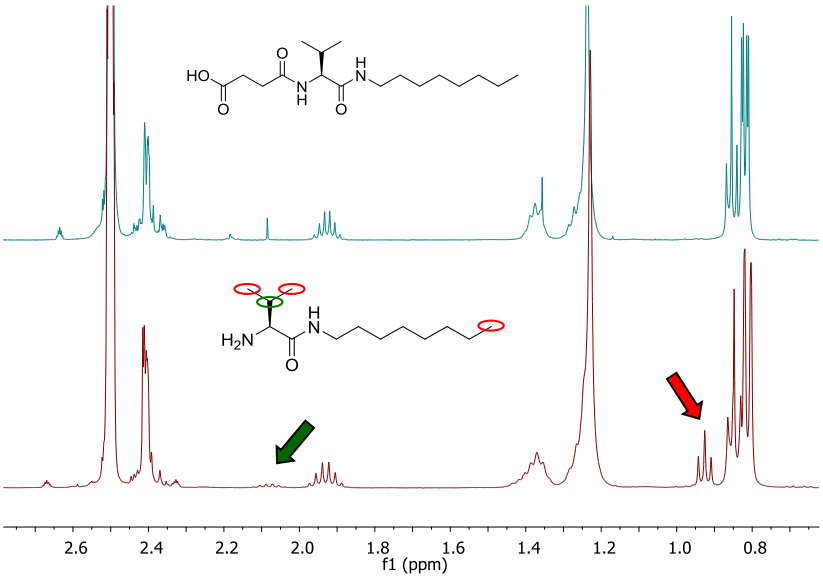


Figure 3-3. Comparison of ^{1}H-NMR spectra of pure SucValOct (up) and SucValOct after the heating step (down).

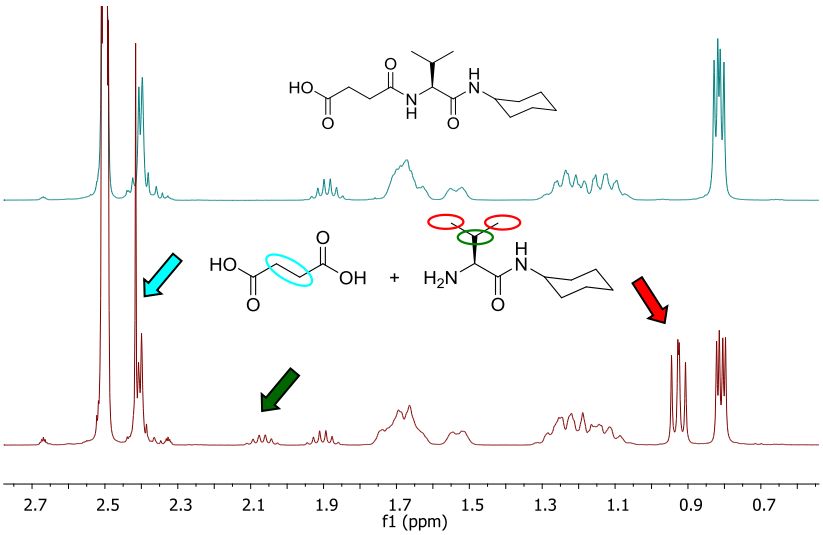


Figure 3-4. Comparison of ^{1}H-NMR spectra of pure SucValCy (up) and SucValCy after the heating step (down).

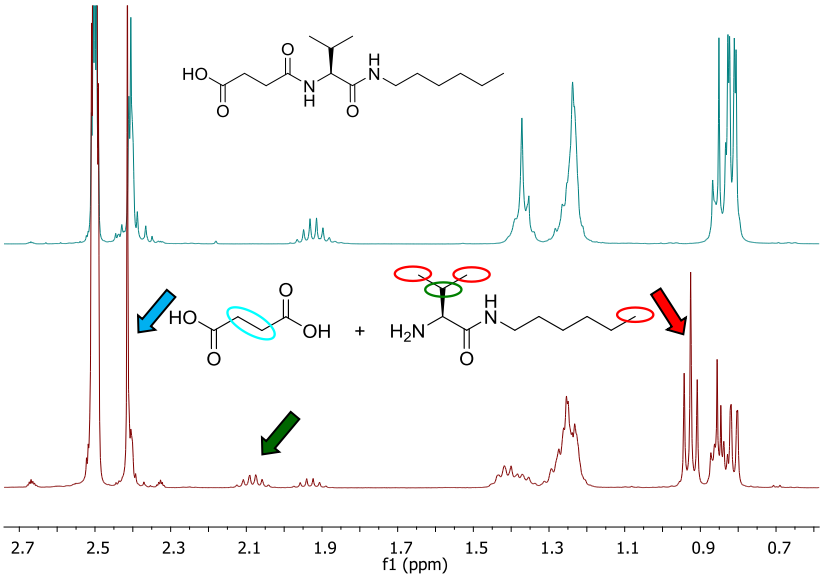


Figure 3-5. Comparison of ^{1}H-NMR spectra of pure SucValHx (up) and SucValHx after the heating step (down).

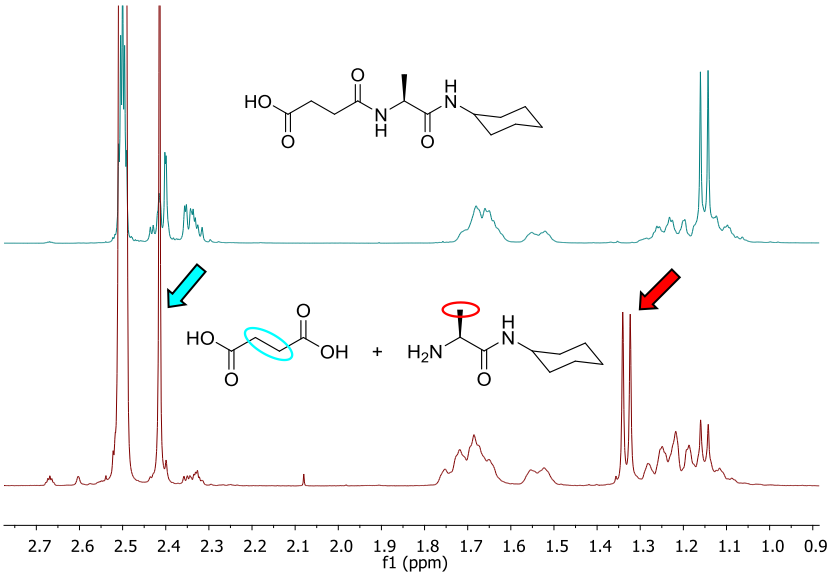


Figure 3-6. Comparison of ^{1}H-NMR spectra of pure SucAlaCy (up) and SucAlaCy after the heating step (down).

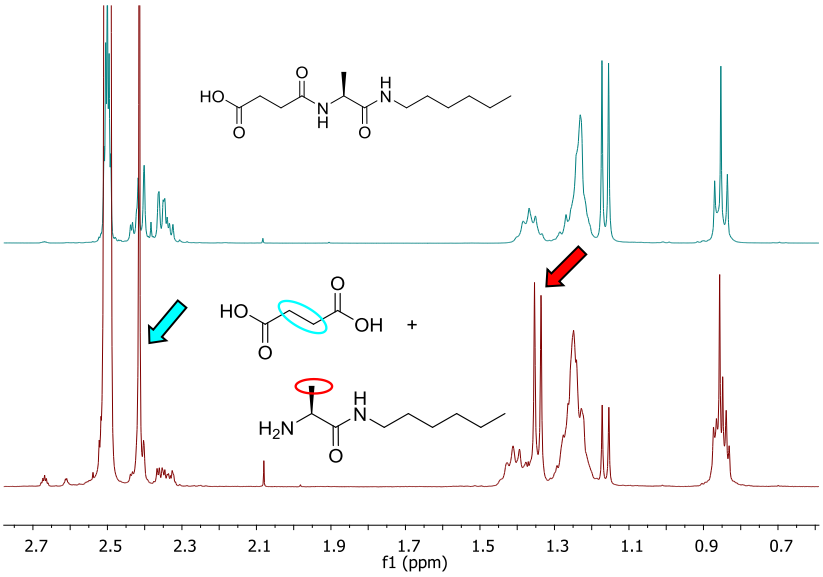


Figure 3-7. Comparison of ^{1}H-NMR spectra of pure SucAlaHx (up) and SucAlaHx after the heating step (down).

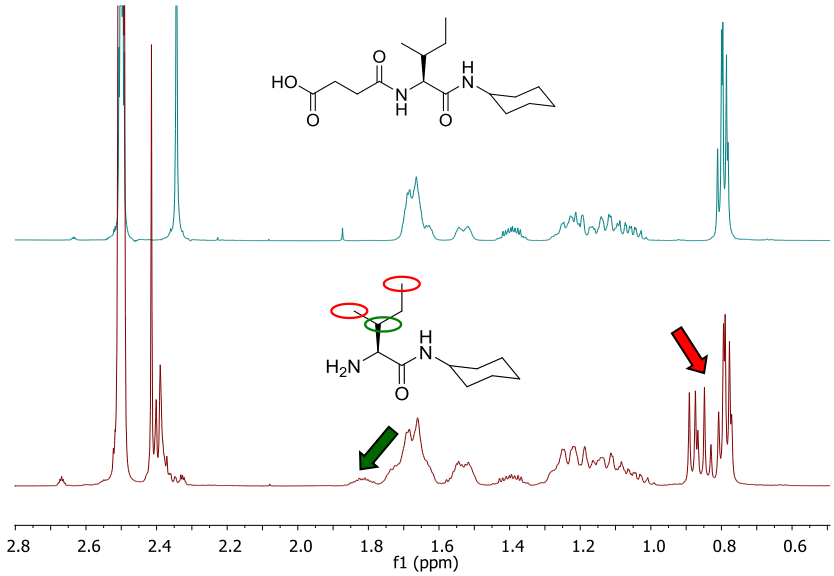


Figure 3-8. Comparison of ^{1}H-NMR spectra of pure SucIleCy (up) and SucIleCy after the heating step (down).

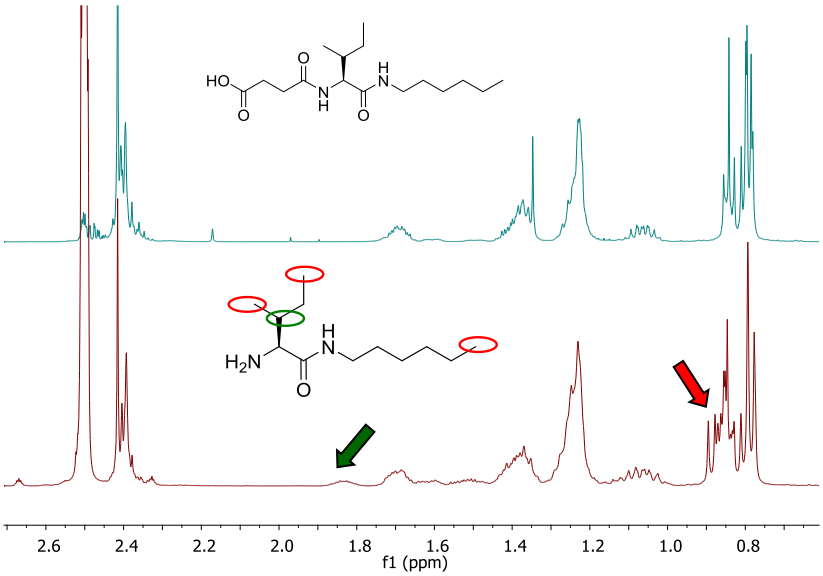


Figure 3-9. Comparison of ^{1}H-NMR spectra of pure SucIleHx (up) and SucIleHx after the heating step (down).

In all of them, the appearance of new peaks is observed. Probably, these new peaks correspond to the hydrolysed compound. The most significant peaks and from which it can be stated that the compounds break down, are those corresponding to the methyl of the amino acid andaliphatic chain (red arrow) or are those corresponding to the proton of carbon isopropyl (green arrow). Moreover, a signal corresponding to succinic acid (at 2.4 ppm approx., blue arrow) confirms the breakage of the initial compound.

This experiment confirms that when the compounds are to 80 ºC during 24 hours a hydrolytic decomposition occurs, producing the starting products (succinic acid and the corresponding amine).

The next table includes the state of the compounds (situation i, ii or iii) before heating step, and all percentages of hydrolysis reaction.

Table 3-1. States and percentages of hydrolysis reaction of “mono” acid succinic derivatives.

Compound [9mM]	Appearance (Situation)	Hydrolysis (%)*
SucValOct	Gel (i)	14
SucValCy	Solution (iii)	47
SucValHx	Solution (iii)	64
SucAlaCy	Solution (iii)	82
SucAlaHx	Solution (iii)	73
SucIleCy	Aggregates (ii)	41
SucIleHx	Aggregates (ii)	31

(*) The percentages of hydrolysis were calculated by integration of the signals of ^{1}H-NMR spectra.

In view of the results, it may be stated that there is some relationship between the level of aggregation, and the rate of hydrolysis of the molecule. The compounds which were in solution before heating have higher percentage of hydrolysis (above 45%) than those compounds that formed aggregates (below 45%). And SucValOct, which formed gel, is hydrolize only in a percentage of 14%. A more detailed study of the influence of aggregation in the percentage of hydrolysis will be performed in paragraph 3.2.4.

3.2.2 Hydrolytic studies of "double" acid succinic derivatives.

These studies were performed for compounds: SucAla8, SucIle8, SucPhe8 (synthesized in this project) and SucVal8 (provided by the research group). The first aim is to check whether the "double" compounds also undergo hydrolysis when are heated or not. And the second is to make a comparison between SucVal8 ("double acid succinic derivate) and SucValOct ("mono" succinic acid derivate).

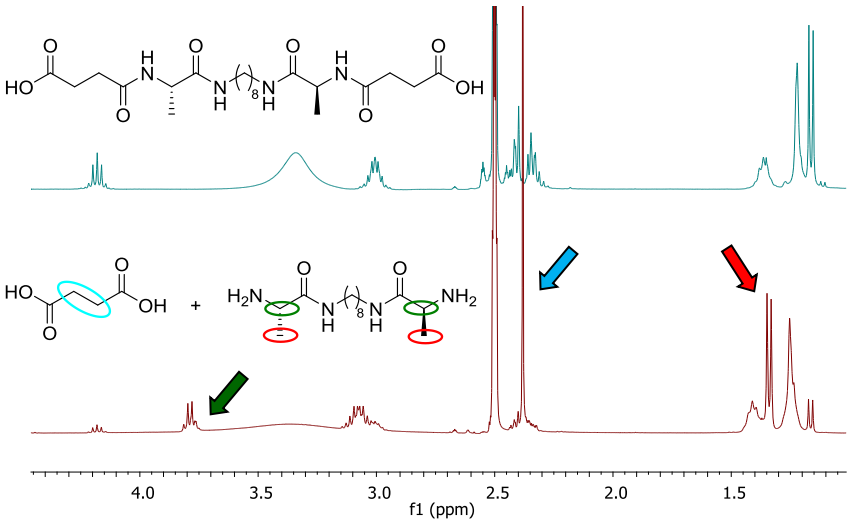


Figure 3-10. Comparison of ^{1}H-NMR spectra of pure SucAla8 (up) and SucAla8 after the heating step (down).

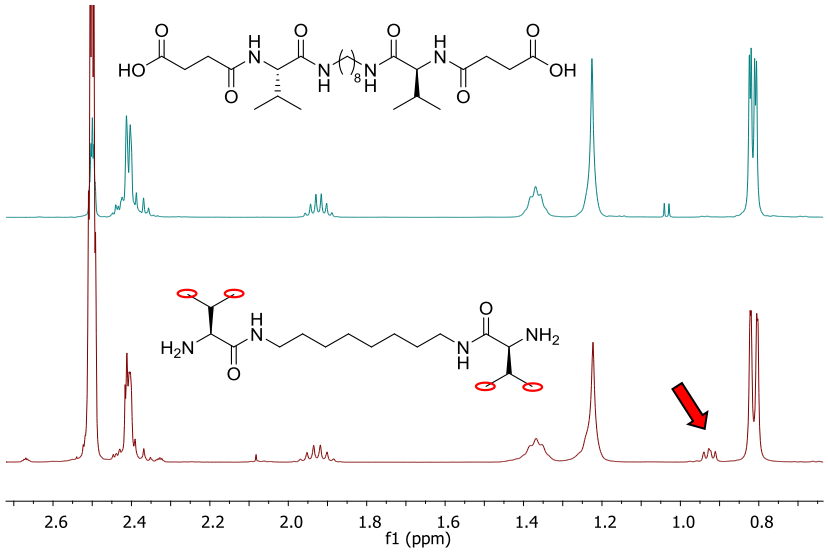


Figure 3-11. Comparison of ^{1}H-NMR spectra of pure SucVal8 (up) and SucVal8 after the heating step (down).

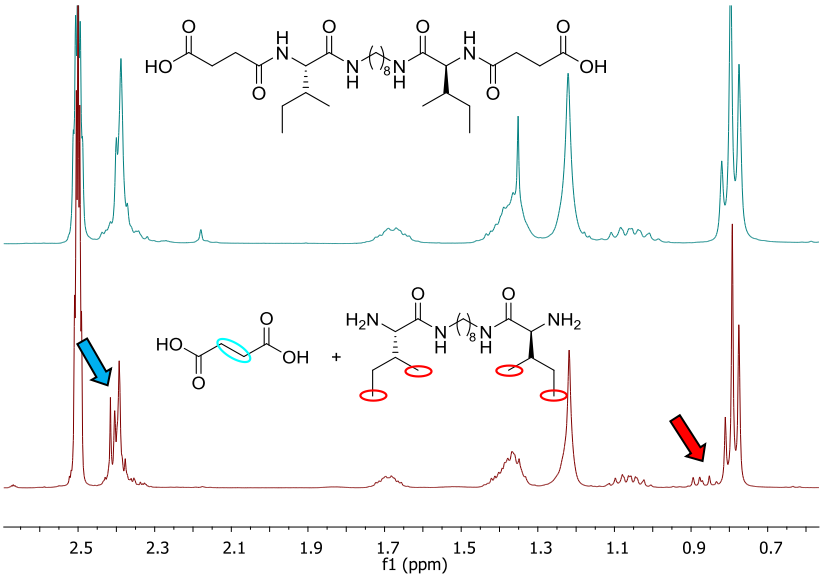


Figure 3-12. Comparison of ^{1}H-NMR spectra of pure SucIle8 (up) and SucIle8 after the heating step (down).

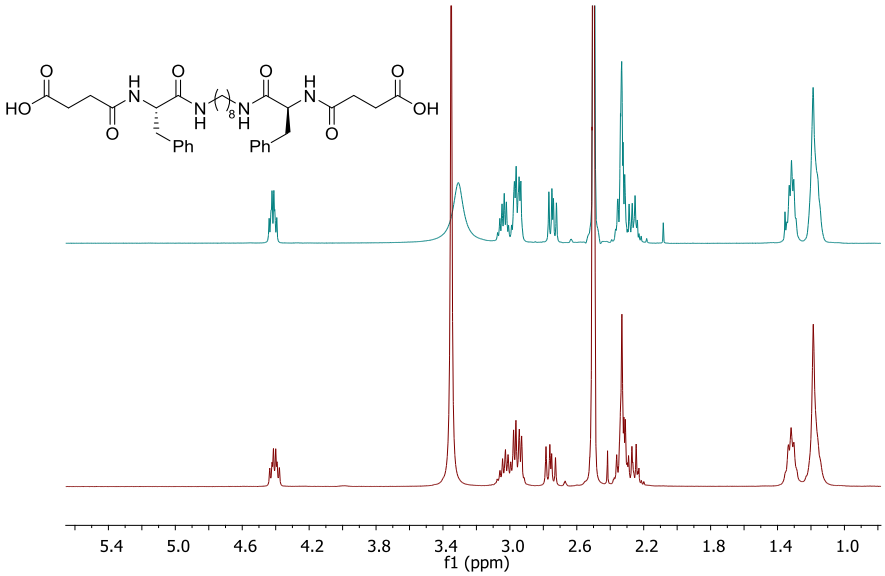


Figure 3-13. Comparison of ^{1}H-NMR spectra of pure SucPhe8 (up) and SucPhe8 after the heating step (down).

All percentages of hydrolysis reaction of these compounds are shown in the following table:

Table 3-2. Hydrolysis reaction's percentages of "double" acid succinic derivatives and appearance before heating.

Compound [9mM]	Appearance (Situation)	Hydrolysis (%)*
SucAla8	Solution (iii)	81
SucVal8	Almost gel (ii)	10
SucIle8	Gel	4
SucPhe8	Aggregates	0

(*) The percentages of hydrolysis were calculated by integration of the signals of ^{1}H-NMR spectra.

Like in "mono acid succinic derivatives", the appearance of new peaks is observed (red and blue arrows) and the existence of new chiral protons (is clearly seen in SucAla8, yellow arrow) ensure that the compounds undergo hydrolysis. In view of these results, it seems that there is some relationship between the level of solubility of the molecule and the rate of hydrolysis of it. SucAla8, which is very soluble in water, have the highest percentage of hydrolysis. However, SucPhe8, which is practically insoluble in water, apparently is not reacting at all. And finally, SucVal8 and SucIle8, which are more soluble in water than SucPhe8, do react, although, they have low percentages of degradation most likely because they form aggregates that decrease the rate of hydrolysis (see paragraph 3.2.4)

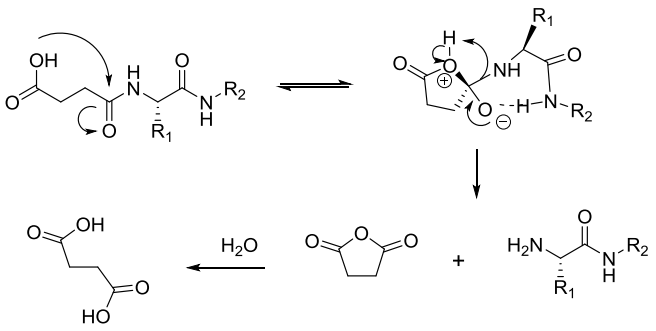
Otherwise, if we compare the percentage of hydrolysis of SucValOct and SucVal8, we can say that there are not significant differences between "mono" and "double" succinic acid derivates. The small differences would be only due to the difference in solubility of them in water.

Table 3-3. Comparison of the percentage of hydrolysis of SucValOct and SucVal8

Compound [9mM]	Hydrolysis (%)*
SucValOct	14
SucVal8	10

3.2.3 Study of neighbouring group effect.

It is proposed that the hydrolysis reaction takes place through an intramolecular reaction. It could be explained on the basis of a pathway involving intramolecular nucleophilic attack by the terminal carboxyl group on the amide function. That lability of the succinic acid group in these substrates could be explained by considering that the tetrahedral intermediate formed upon nucleophilic attack of the anion carboxylate to the carbamate carbonyl is stabilised by intramolecular H-bonding with the vicinal amide group, as shown in Scheme 5.[20]



Scheme 3-2. Proposed mechanism for the hydrolysis of the succinic group.

Computational studies were carried out by Chem3D program and the possible behaviour of the molecules, when they are in solution, using molecular mechanics with MM2 parametrization was studied. The results agree with the proposed mechanism. As illustrated in Figure 3-14, formation of H-bond between the amide carbonyl and the vicinal amide group stabilises the tetrahedral intermediate and this H-bond is feasible according to the calculations. Moreover, a second hydrogen bond between the carbonyl of the amide and the hydrogen OH unit of the carboxylic acid appears. This latter hydrogen bond keeps the acid group close to the carbonyl group and this effect is likely accelerating the nucleophilic addition to the amide. Thus, the amide carbonyl group is doubly activated. On the one hand, by the stabilization that the neighbouring amide group gives, and on the other hand by the hydrogen bond between the carbonyl group and the acid group, forming a ring of seven atoms in both cases.

[20] Tena-Solsona, M.; Angulo-Pachón, C. A; Escuder, B.; Miravet, J. F. "Mechanistic Insight into the Lability of the Benzyloxycarbonyl (Z) Group in *N*-Protected Peptides under Mild Basic Conditions", *Eur. J. Org. Chem.*, **2014**, 3372-3378.

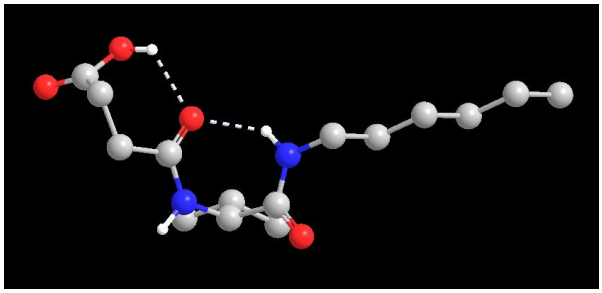


Figure 3-14. Energy minimized molecular mechanics model for the SucValHx in solution.

However, this situation is not predicted for glutaric acid derivatives. This acid has one additional methylene in its structure, then the hydrogen bond between the acid group and the carbamate carbonyl group is not so favourable, because a ring of eight atoms would be formed. In conclusion, the hydrolysis reaction would not be accelerated for glutaric acid derivatives.

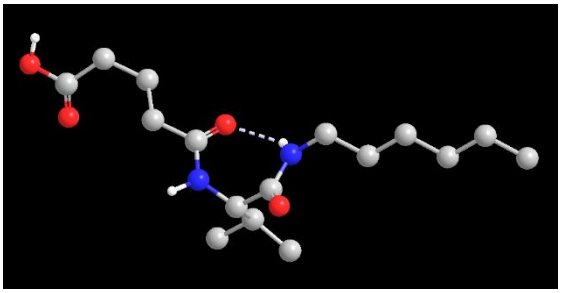


Figure 3-15. Energy minimized molecular mechanics model for the GluValHx in solution.

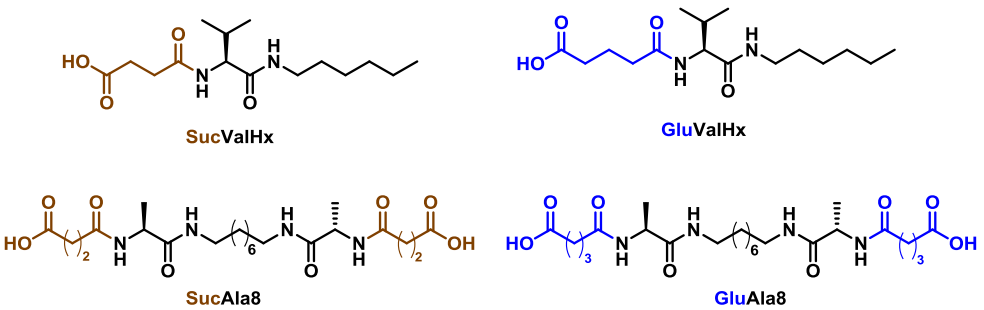


Figure 3-16. Structures of the derived succinic acid and glutaric acid.

In order to demonstrate that glutaric acid derivatives do not undergo hydrolysis, two tests are performed: GluValHx ("mono" acid derivative) and GluAla8 ("double" acid derivative). The results were the following:

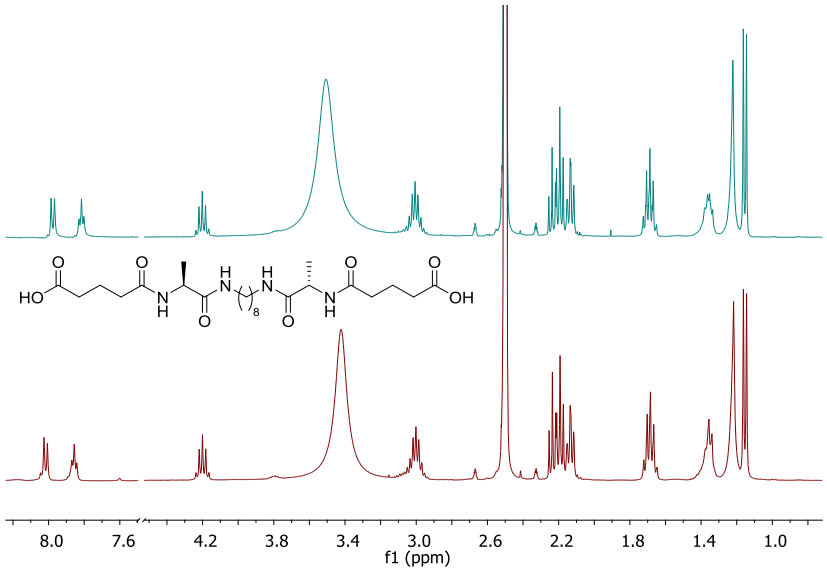


Figure 3-17. Comparison of ^{1}H-NMR spectra of pure GluAla8 (up) and GluAla8 after the heating step (down).

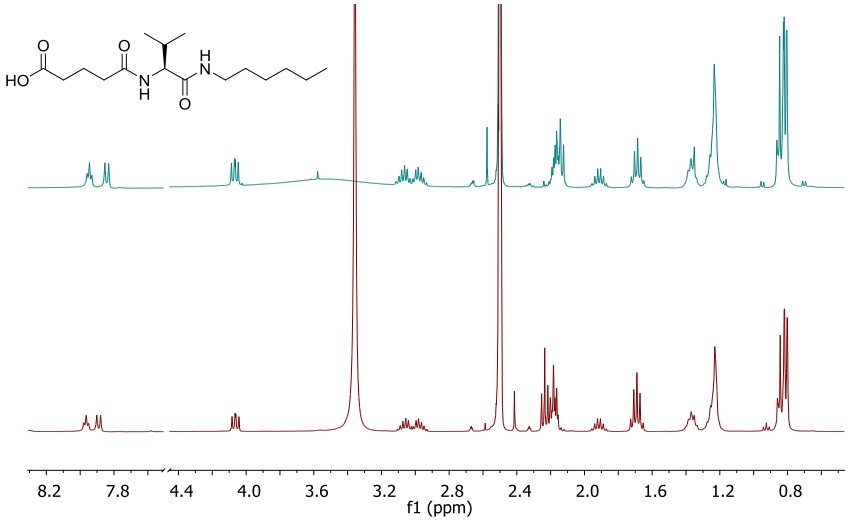


Figure 3-18. Comparison of ^{1}H-NMR spectra of pure GluValHx (up) and GluValHx after the heating step (down).

Indeed, it is observed that these products apparently do not suffer any reaction. Although it is true that a small peak is observed at 0.9 ppm in GluValHx, if we compare this peak with the peak of SucValHx (Figure 3-5), it can be said that the reaction rate is negligible in this case.

3.2.4 Study of aggregation effect on hydrolysis reaction.

Once it is demonstrated that when the molecules are in dissolution, is where show a higher percentage of hydrolysis, we decided carry a study to know of whether the state of aggregation affected in the percentage of hydrolysis reaction.

This study only carried out for the compounds: SucValOct,SucVal8, SucIle8 and SucPhe8. The results were the followings:

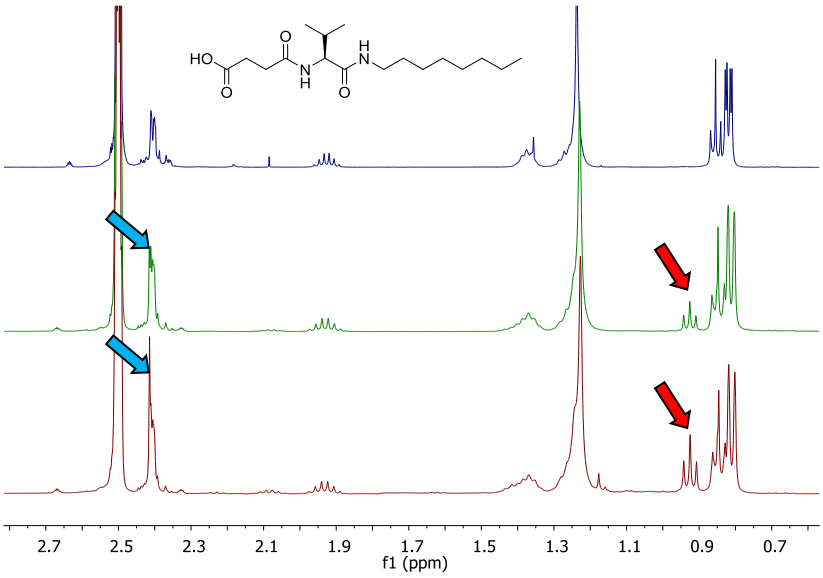


Figure 3-19. Comparison of ^{1}H-NMR spectra of pure SucValOct (up) and aggregated SucValOct (centre) and SucValOct into solution (down) after the heating step.

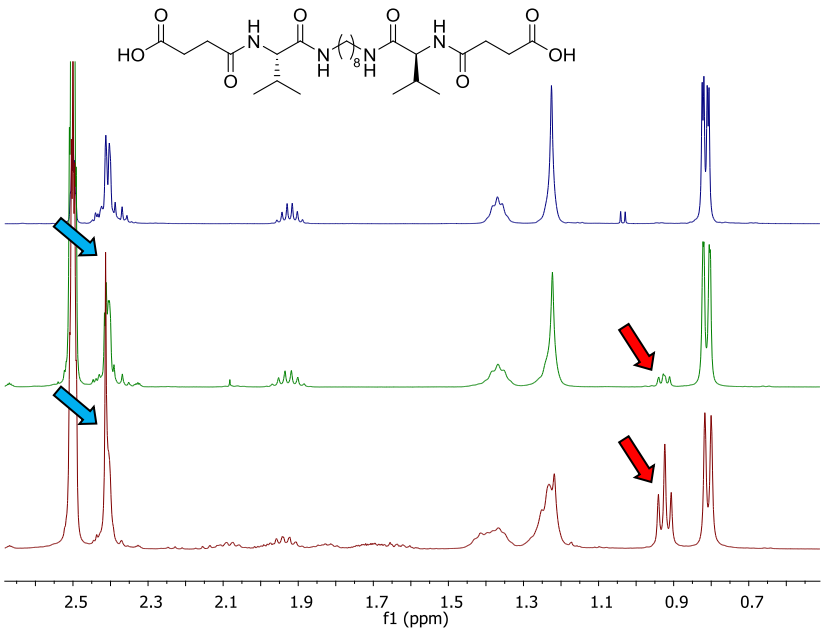


Figure 3-20. Comparison of ^{1}H-NMR spectra of pure SucVal8 (up) and aggregated SucVal8 (centre) and SucVal8 into solution (down) after the heating step.

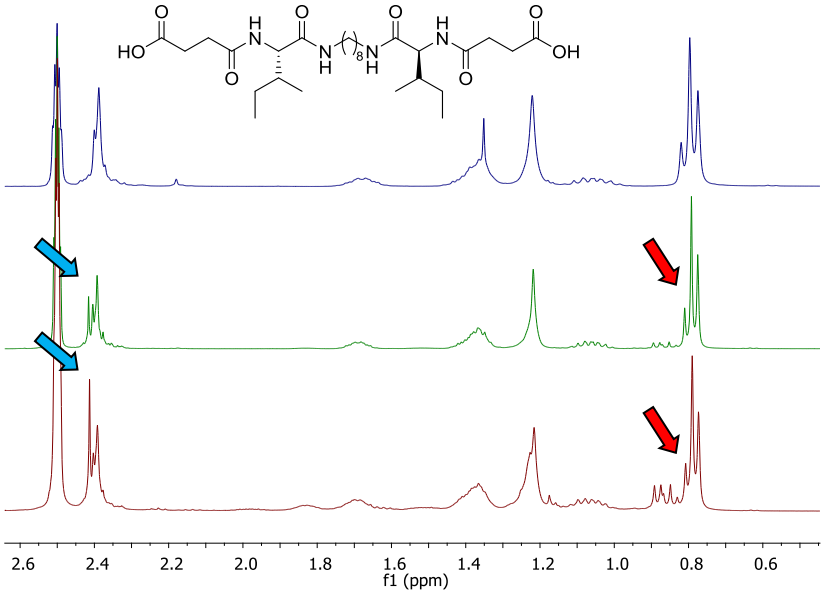


Figure 3-21. Comparison of ^{1}H-NMR spectra of pure SucIle8 (up) and aggregated SucIle8 (centre) and SucIle8 into solution (down) after the heating step.

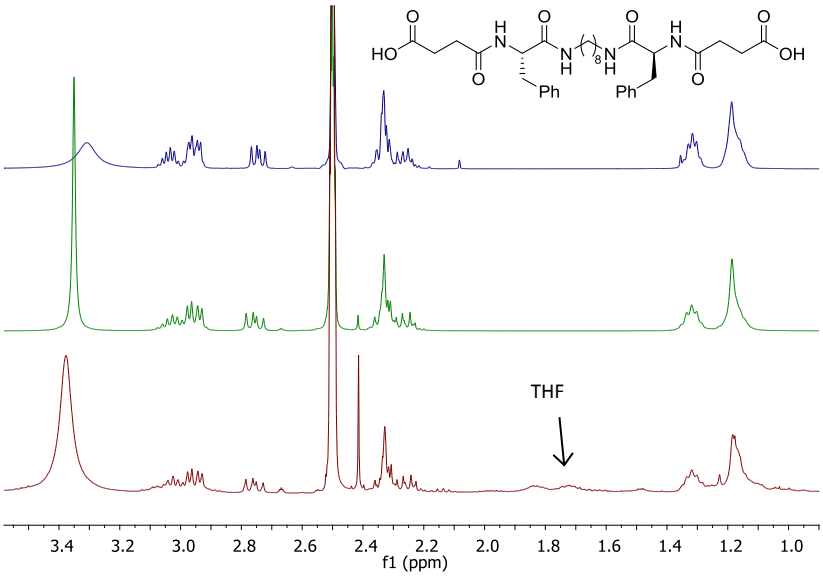


Figure 3-22. Comparison of ^{1}H-NMR spectra of pure SucPhe8 (up) and aggregated SucPhe8 (Middle) and SucPhe8 into solution (down) after the heating step.

All percentages of hydrolysis reaction of these compounds are shown in the following table:

Table 3-4. Comparison of hydrolysis reaction percentages between gel state and solution state.

Compound	State	Hydrolysis (%)
SucValOct	Gel [9mM]	14
	Solution [1.3mM]	26
SucVal8	Aggregation [9mM]	10
	Solution [1.4mM]	41
SucIle8	Gel [9mM]	4
	Solution [1.3mM]	17
SucPhe8	Aggregation [9mM]	0
	Solution [0.5mM]	0

(*) The percentages of hydrolysis were calculated by integration of the signals of the spectra.

In view of the results, it can be said that there is a relationship between the level of aggregation, and the rate of hydrolysis of it. For SucValOct, the percentage of hydrolysis in solution is, approximately, twice higher than the percentage in gel state. And SucVal8 and SucIle8 the percentage in solution is, approximately, four times higher than the percentage in gel state.

The explanation for this phenomenon may be the following:

The molecules of these compounds, as a physical gels, are self-assembled by hydrogen bonding (along with others solvophobic forces and Van der Waals interactions), producing small aggregates (Figure 3-23).

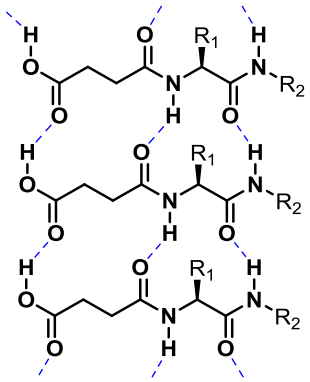


Figure 3-23. Representation of the aggregation of the gelator molecules.

Then, these small aggregates form fibrous assemblies which produce the three-dimensional network structure. At that time, molecules have no free rotation or "freedom", because they are immobilized due to assembly. As a result of this, the molecule cannot adopt the appropriate geometry that allows it to perform the intramolecular reaction.

But despite being gel, the molecules have a certain percentage of reaction. This is because there are always single molecules that do not form part of the fibers, remaining in solution. Moreover, the molecules do not remain static in the structure, there is a dynamic interchange of molecules into and out of the structure, keeping the chemical equilibrium. Hence, it can be predicted that molecules that undergo hydrolysis are those that remain in solution. As there are molecules that are breaking, other molecules leave the network structure to keep

the equilibrium, and that leads to rupture of the gel due to the decrease of the gelator concentration.

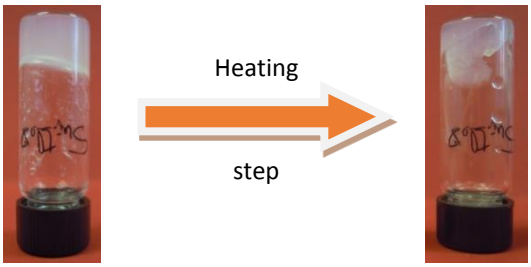


Figure 3-24. Comparison between 8 before (left) and after the heating step (right)

This is consistent with the results obtained for the SucPhe8. This is so insoluble, that practically all molecules are assembled, and hence no reaction takes place. However, in the cases of SucVal8, SucIle8 and SucValOct, which are more soluble, some degree of hydrolysis is observed.

3.2.5 Hydrolytic studies of *N*-butylacetamide.

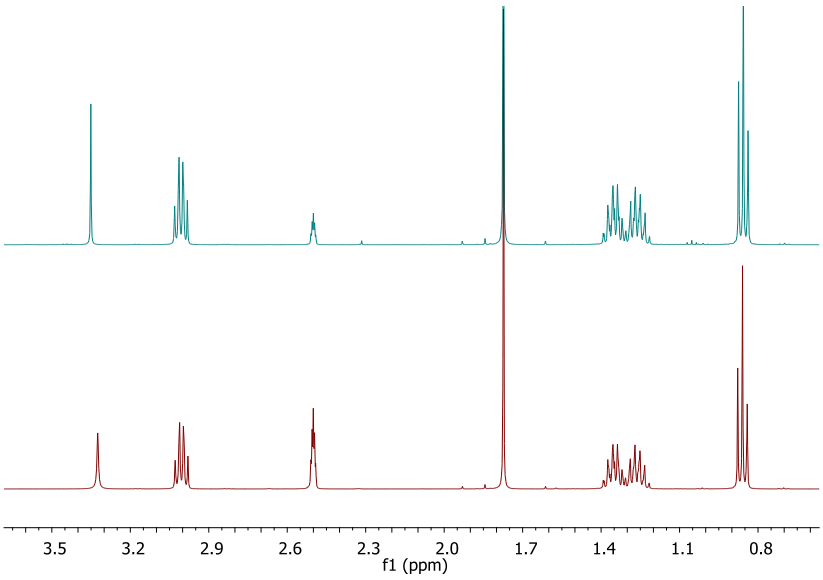


Figure 3-25. Comparison of ^{1}H-NMR spectra of pure *N*-butylacetamide (up) and *N*-butylacetamide after the heating step (down).

The study of this amide was conducted in order to demonstrate that a "classical amide" (without the existence of neighboring groups or conformations that force it to react) does not suffer hydrolysis reaction under these conditions. Indeed, in view of the ^{1}H-NMR spectra obtained, it can be said that the molecule remains intact after heating, without suffering any breakage.

Conclusions

4 Chapter: Conclusions

- Different gelators derived from succinic acid were successfully prepared and characterized.

- The mono amide of succinic acid present in the studied compounds is found to be hydrolysed under unusually mild conditions. Therefore, care should be taken when preparing gels from this family of compounds under thermal conditions.

- The observed hydrolysis acceleration should be ascribed to a proximity effect which favors the nucleophilic attack of the carboxylic acid moiety to the neighbor amide group.

- Hydrolysis is thought to take place mainly in solution, being the self-assembled species much less reactive.

- The vicinal amide of the amino acid is likely contributing to the hydrolysis by means of hydrogen bonding to the succinamide carbonyl, affording acid catalysis.

- The use of a larger diacid such as glutaric acid in the preparation of the gelators precludes the observed hydrolysis to a great extent and therefore that new family of compounds is more appropriate for gel preparation.

Experimental Section

5 Chapter: Experimental Section

5.1 General methods.

Unless otherwise noted, all starting materials were obtained from commercial suppliers with purity higher than 98 % and were used without further purification.

All compound synthesised were dried in a vacuum oven at 60 °C to constant weight (except for the free amines which are dried under vacuum pump at room temperature). Reactions which required an inert atmosphere were carried out under N_2.

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 500, Bruker 400 or Bruker 300 in the indicated solvent at 30 ºC. Chemical shifts are expressed in parts per million (δ) using residual solvent protons or solvent carbon as internal standard (^{1}H δ 2.50 ppm for DMSO-d_6, ^{13}C δ 39.52 ppm for DMSO-d_6). Coupling constants, *J*, are reported in Hertz (Hz), and splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad).

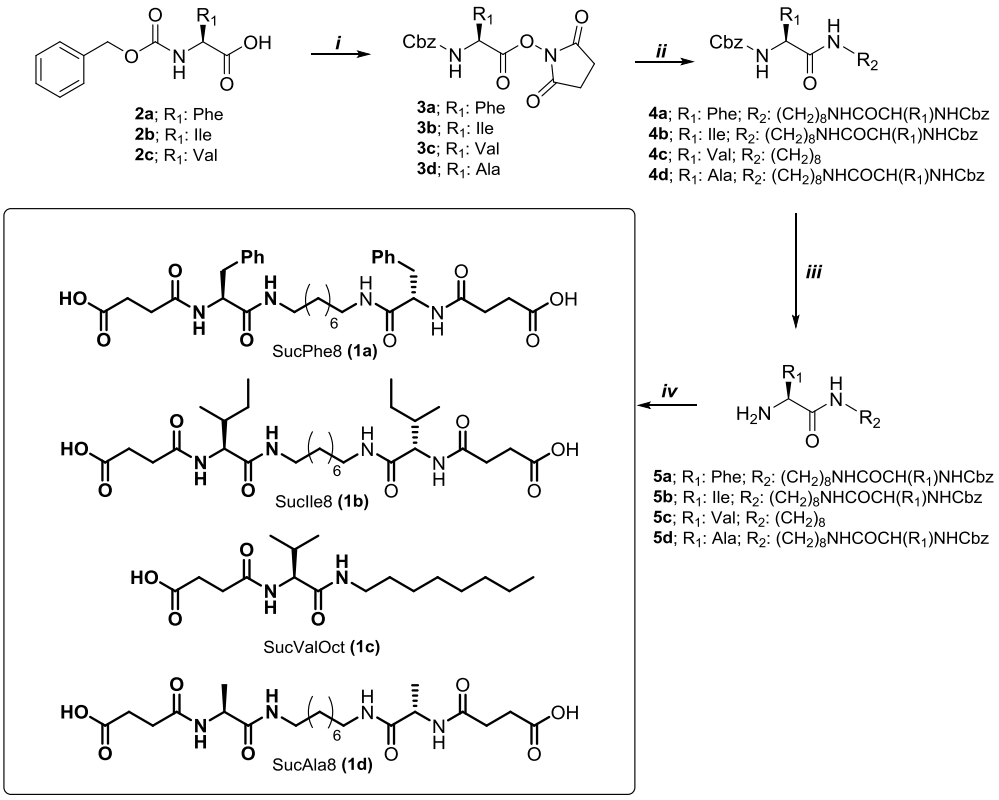
High resolution electrospray ionization (ESI) mass spectra were obtained on a Mass Spectrometry Triple Quadrupole Q-TOF Premier (Waters) with simultaneous Electrospray and APCI Probe.

5.2 General procedure of the hydrolytic reactions.

In a screw vial, the corresponding amount of succinic acid derivative is dissolved in 1.0 mL of basic solution (NaOH 0.05 M); then, 100 μL HCl 0.5 M were added to neutralize the base (final pH around 5). The final concentration was 9 mM for mono and double succinic derivate. For studies of aggregation effect on hydrolysis reaction the final concentrations were 1.4 mM (solution) and 9 mM (gel or aggregate).

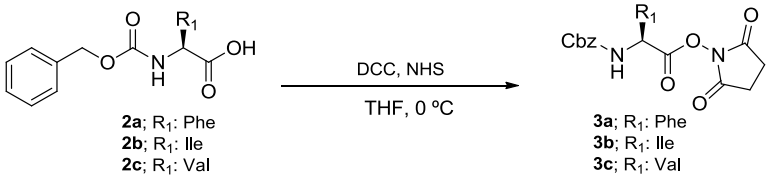
The vials were put a thermostat bath to 80 ºC during 24 hours. Finally, the solvent was evaporated under air flow and the sample was taken directly with deuterated DMSO to be analysed by NMR.

5.3 Experimental procedure.



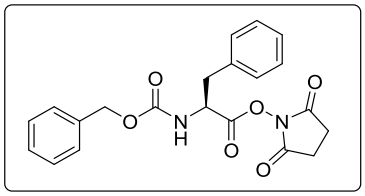
Scheme 5-1. Reagents and conditions: i) *DCC*, *N*-hydroxysuccinimide, THF, 1 h 30 min, 73-4%; ii) 1,8-diaminooctane or octylamine, THF, 5 h, 90-96%; iii) Pd/C, H_2, MeOH, 1.5-7.5 h, 96-99.9%; iv) Succinic anhydride, K_2CO_3, THF, 16 h, 53-91 %.

5.3.1 Activation of amino acids 2a-c to form activated acids 3a-c.



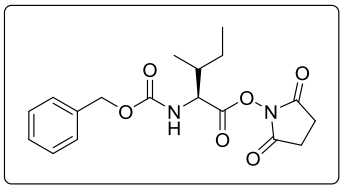
N-Cbz-L-amino acid (5g, 1.0 eq.) and *N*-hydroxysuccinimide (1.0 eq.) were dissolved in dry THF (5 mL/mmol). Once a clear solution had been obtained, *N,N'*-dicyclohexylcarbodiimide (DCC) (1.01 eq.) in anhydrous THF (2.5 mL/mmol) was added dropwise under N_2 atmosphere at 0 °C with a dropping funnel. The resulting solution was further stirred for 1.5h. Elapsed time, the volume of the solution was reduced by rotary evaporator and the mixture was allowed to stand into refrigerator for 16h. The *N,N'*-dicyclohexylurea formed was filtered off under vacuum and the filtrate was concentrated to dryness. The crude product was purified by crystallization in 2-propanol to furnish the white product crystals.

-Compound 3.a: 2,5-dioxopyrrolidin-1-yl ((benzyloxy)carbonyl)-L-phenylalaninate, *(ZPheOsu)*



The general procedure described above was used to synthesize **3.a**. Yield (5,57 g, 84%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, *J* = 8.5 Hz, 1H), 7.41 – 7.19 (m, 10H, H_{Arom}), 4.99 (s, 2H), 4.69 (ddd, *J* = 10.8, 8.5, 4.4 Hz, 1H), 3.23 (dd, *J* = 13.9, 4.4 Hz, 1H), 3.01 (dd, *J* = 13.9, 10.8 Hz, 1H), 2.84 (br s, 4H). Compound 3**.a** was previously described in literature and ^{13}C NMR spectra were in good agreement with the literature spectra.[17]

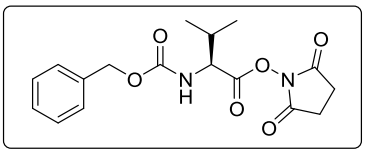
-Compound 3.b: 2,5-dioxopyrrolidin-1-yl ((benzyloxy)carbonyl)-L-alloisoleucinate, *(ZIleOsu)*



The general procedure described above was used to synthesize **3.b.** Yield (5.690 g, 83.5%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (d, *J* = 8.2 Hz, 1H), 7.45 – 7.26 (m, 5H, H_{Arom}), 5.08 (s, 2H), 4.37 (dd, *J* = 8.2, 6.6 Hz, 1H), 2.81 (s, 4H), 1.99 – 1.81 (m, 1H), 1.59 – 1.46 (m, 1H), 1.38 – 1.24 (m, 1H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.86 (t, *J* = 7.4 Hz,

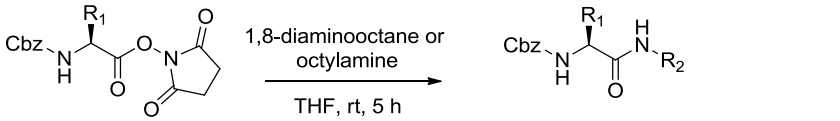
3H); Compound 3**.b** was previously described in literature and ^{13}C NMR spectra were in good agreement with the literature spectra.[17]

-Compound 3.c: 2,5-dioxopyrrolidin-1-yl ((benzyloxy)carbonyl)-L-valinate, *(ZValOsu)*



The general procedure described above was used to synthesize **3.c**. However, in this case the synthesis was carried out with 20 g of starting material. Yield (20.19 g, 73%); ^1H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, *J* = 8.3 Hz, 1H,), 7.47 – 7.25 (m, 5H, H_{Arom}), 5.08 (s, 2H), 4.34 (dd, *J* = 8.3, 6.4 Hz, 1H), 2.82 (br s, 4H), 2.27 – 2.09 (m, 1H), 1.01 (d, *J* = 6.7 Hz, 6H). Compound **3.c** was previously described in literature and ^{13}C NMR sprectra were in good agreement with the literature spectra.[17]

5.3.2 Amine coupling to the activated acids 3.a-d to form the compounds 4a-d.

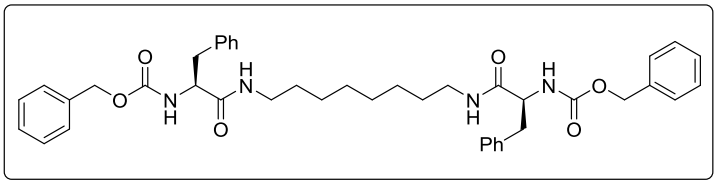


3a; R_1: Phe
3b; R_1: Ile
3c; R_1: Val
3d; R_1: Ala

4a; R_1: Phe; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$
4b; R_1: Ile; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$
4c; R_1: Val; R_2: $(CH_2)_8$
4d; R_1: Ala; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$

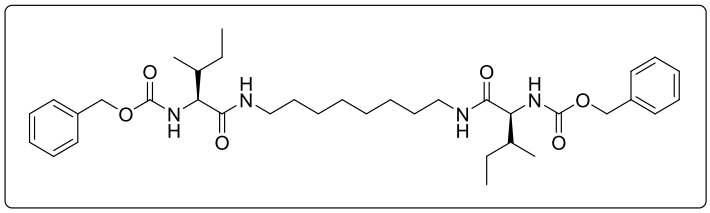
The *N*-hydroxysuccinimide ester(1.0 eq.) was dissolved in anhydrous THF (3.55 mL/mmol). 1,8-diaminooctane (0.55 eq.) or octylamine (1.01 eq.) dissolved in THF (2.18 mL/mmol) was added drop wise, under N_2 atmosphere at 25°C, with dropping funnel. The resulting solution was further stirred for 5 h at 50 °C. The solvent was removed under reduced pressure and the white solid obtained was washed first with HCl 0.1 M (30 mL) twice, then was washed several times with H_20 (20 mL each time) and KOH 0.1 M (30 mL) twice. Finally, the product was washed with H_20 until neutral pH and cold diethylehter.

-Compound 4.a: dibenzyl ((2S,2'S)-(octane-1,8-diylbis(azanediyl))bis(1-oxo-3-phenylpropane-1,2-diyl))dicarbamate, *(ZPhe8)*



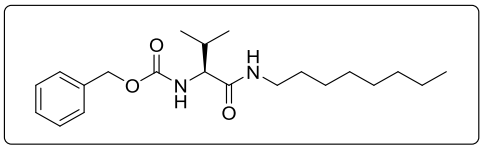
The general procedure described above was used to synthesize **4.a.** Although, 20 mL of THF was further added because the mixture was too thick. Yield (4.79 g, 96%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89 (t, *J* = 5.2 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.37 – 7.12 (m, 20H, H_{Arom}), 4.94 (s, 4H), 4.19 (ddd, *J* = 10.2, 8.4, 4.9 Hz, 2H), 3.13 – 2.96 (m, 4H), 2.93 (dd, *J* = 13.5, 4.9 Hz, 1H), 2.75 (dd, *J* = 13.5, 10.2 Hz, 2H), 1.43 – 1.26 (br s, 4H), 1.27 – 1.13 (br s, 8H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.1, 155.8 (C=O), 138.1, 137.1 (CH), 129.2 (C), 128.3, 128.0, 127.7, 127.4, 126.2 (CH), 65.2 (CH_2), 56.3 (CH), 38.5, 37.8, 29.0, 28.7, 26.3 (CH_2); HRMS (ESI): calcd. or: $C_{42}H_{50}N_4O_6$ $[M+H]^+$ = 707.3809; found = $[M+H]^+$ = 707.3798 (Δ = 1.6 ppm).

-Compound 4.b: dibenzyl ((2S,2'S,3R,3'R)-(octane-1,8-diylbis(azanediyl))bis(3-methyl-1-oxopentane-1,2-diyl))dicarbamate, *(ZIle8)*



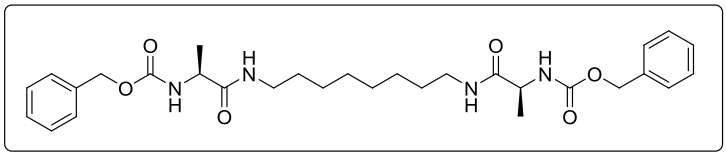
The general procedure described above was used to synthesize **4.b.** Yield (4.458 g, 90%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 – 7.81 (m, 2H,), 7.44 – 7.27 (m, 10H, H_{Arom}), 7.23 (d, *J* = 8.6 Hz, 2H), 5.01 (s, 4H), 3.81 (t, *J* = 8.6 Hz, 2H), 3.20 – 2.87 (m, 4H), 1.78 – 1.56 (m, 2H), 1.47 – 1.03 (m, 16H), 0.92 – 0.68 (m, 12H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.0, 156.0 (C=O), 137.1 (C), 128.3, 127.6, 127.6 (CH), 65.3 (CH_2), 59.2 (CH), 38.4, 36.3, 28.9, 28.7, 26.3 (CH_2), 24.4, 15.4, 10.9 (CH_3); HRMS (ESI): calcd. or: $C_{36}H_{54}N_4O_6$ $[M+H]^+$ = 639.4122; found = $[M+Na]^+$ = 639.4118 (Δ = 0.6 ppm).

-Compound 4.c: benzyl (S)-(3-methyl-1-(octylamino)-1-oxobutan-2-yl)carbamate, *(ZValOct)*



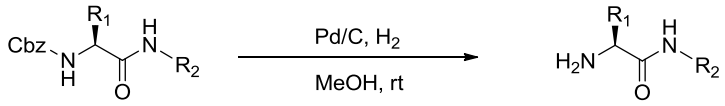
The general procedure described above was used to synthesize **4.c**, but in this case THF wasn't further added because the resulting solution wasn't thick. Moreover, the ether wash wasn't done because it dissolves the product. Yield (4.96 g, 95%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (t, *J* = 5.4 Hz, 1H), 7.42 – 7.28 (m, 5H, H_{Arom}), 7.20 (d, *J* = 8.8 Hz, 1H), 5.02 (s, 2H), 3.77 (dd, *J* = 8.8, 7.5 Hz, 1H), 3.15 – 3.03 (m, 1H), 3.02 – 2.91 (m, 1H), 2.00 – 1.82 (m, 1H), 1.46 – 1.32 (m, 2H), 1.32 – 1.15 (m, 10H), 0.89 – 0.79 (m, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 156.0 (C=O), 137.1 (C), 128.3, 127.7, 127.6 (CH), 65.3 (CH_2), 60.3(CH), 38.3, 31.2, 30.2, 29.0, 28.6, 26.3, 22.1(CH_2), 19.2, 18.2, 13.9 (CH_3); HRMS (ESI): calcd. or: $C_{21}H_{34}N_2O_3$ $[M+Na]^+$= 385.2467; found = $[M+Na]^+$ = 385.2463 (Δ = 1 ppm).

-Compound 4.d: dibenzyl ((2S,2'S)-(octane-1,8-diylbis(azanediyl))bis(1-oxopropane-1,2-diyl))dicarbamate, *(ZAla8)*



The general procedure described above was used to synthesize **4.d**. Yield (4.10 g, 95%); 1H NMR (500 MHz, DMSO-d6) δ 7.75 (t, J = 5.1 Hz, 2H), 7.39 – 7.30 (m, 10H), 7.31 (d, J = 7.4 Hz, 2H), 5.03 (d, J = 12.7 Hz, 2H), 4.99 (d, J = 12.7 Hz, 2H), 4.02 – 3.94 (m, 2H), 3.08 – 2.96 (m, 4H), 1.42 – 1.31 (m, 4H), 1.27 – 1.20 (m, 8H), 1.18 (d, J = 7.1 Hz, 6H); 13C NMR (101 MHz, DMSO-d6) δ 172.7, 156.1 (C=O), 137.5 (C), 128.8, 128.2, 128.2 (CH), 65.8 (CH_2), 50.6 (CH), 38.9, 29.5, 29.1, 26.7 (CH_2), 18.9 (CH_3); HRMS (ESI): calcd. or: $C_{30}H_{42}N_4O_6$ $[M+H]^+$ = 555.3183; found = $[M+H]^+$ = 555.3179 (Δ = 1.6 ppm).

5.3.3 The cleavage of the Cbz group by hydrogenolysis.

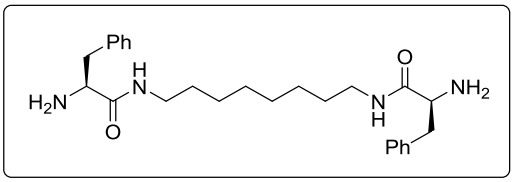


4a; R_1: Phe; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$
4b; R_1: Ile; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$
4c; R_1: Val; R_2: $(CH_2)_8$
4d; R_1: Ala; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$

5a; R_1: Phe; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$
5b; R_1: Ile; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$
5c; R_1: Val; R_2: $(CH_2)_8$
5d; R_1: Ala; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$

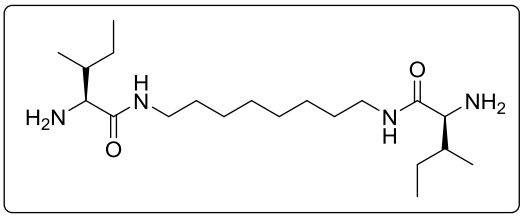
The corresponding *N*-benzyloxycarbonyl protected derivate and catalytic amount of Pd over activated carbon (20% w/w) were placed in a two necked round bottom flask and suspended in MeOH (4 mL/mmol) with stirring at room temperature under N_2 atmosphere. MeOH (20 mL) was further added so that nothing remained on the walls of the flask. The system was purged to remove the air with N_2 and connected to H_2atmosphere. The grey suspension was stirred until it turned completely black (also checked with NMR spectra). The black mixture was filtered over Celite and the solvent was evaporated under reduced pressure. The resulting oil was dried in vacuum pump to yield the product as a white solid.

-Compound 5.a: (2S,2'S)-*N,N'*-(octane-1,8-diyl)bis(2-amino-3-phenylpropanamide), *(HPhe8)*



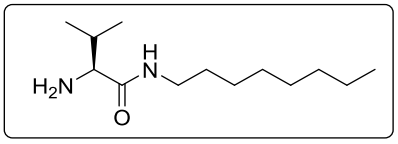
The general procedure described above was used to synthesize **5.a.** In this case, the grey suspension was stirred for 7.5 h. Yield (2.85 g, 96%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (t, *J* = 5.7 Hz, 2H), 7.36 – 7.10 (m, 10H), 3.33 (m, 2H, overlapped with water signal) 3.14 – 2.94 (m, 4H), 2.88 (dd, *J* = 13.3, 5.5 Hz, 2H), 2.61 (dd, *J* = 13.3, 7.9 Hz, 2H), 1.63 (br s, 4H), 1.41 – 1.27 (m, 4H), 1.26 – 1.09 (br s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.1 (C=O), 138.8(CH), 129.3 (C), 128.1, 126.1, 56.3(CH), 41.4, 38.3, 29.1, 28.7, 26.3(CH_2).

-Compound 5.b: (2S,2'S,3R,3'R)-*N,N'*-(octane-1,8-diyl)bis(2-amino-3-methylpentanamide), *(HIle8)*



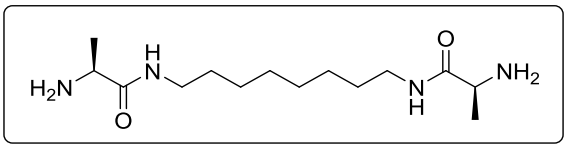
A similar procedure to that described above was used, but in this case the mixture was only further stirred for 2h to turn completely black and the product was obtained as a yellow waxy solid. Yield (2.51 g, >99%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 (t, *J* = 5.6 Hz, 2H), 3.10 – 2.97 (m, 4H), 2.92 (d, *J* = 5.6 Hz, 2H), 1.63 – 1.49 (m, 2H), 1.47 – 1.32 (m, 8H), 1.30 – 1.17 (m, 8H), 1.12 – 0.96 (m, 2H), 0.85 – 0.79 (m, 12H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.4 (C=O), 59.3(CH), 38.5, 38.2, 29.1, 28.7, 26.3, 23.8 (CH_2), 15.8, 11.5(CH_3); HRMS (ESI): calcd. or: $C_{20}H_{42}N_4O_2$ $[M+H]^+$= 371.3386; found = $[M+H]^+$ = 371.3376 (Δ = 2.7 ppm).

-Compound 5.c: (S)-2-amino-3-methyl-N-octylbutanamide, *(HValOct)*



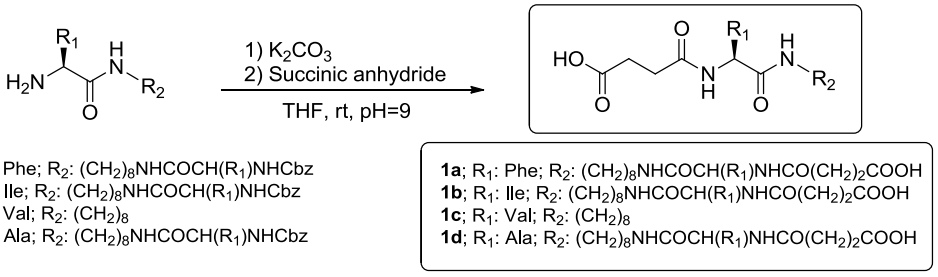
A similar procedure to that described above was used, but in this case the mixture was only further stirred for 1.5h to turn completely black and the product was obtained as a yellow waxy solid. Yield (2.96 g, 95%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 (t, *J* = 5.4 Hz, 1H), 3.14 – 2.96 (m, 2H), 2.88 (d, *J* = 5.3 Hz, 1H), 1.91 – 1.74 (m, 1H), 1.45 – 1.33 (m, 2H), 1.31 – 1.16 (m, 10H), 0.89 – 0.81 (m, *J* = 6.9, 3.3 Hz, 6H), 0.77 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.3(C=O), 60.1(CH), 38.2, 31.6, 31.2, 29.2, 28.7, 28.6, 26.4, 22.1 (CH_2), 19.5, 17.1, 13.9 (CH_3); HRMS (ESI): calcd. or: $C_{13}H_{28}N_2O$ $[M+H]^+$ = 229.2280; found = $[M+H]^+$ = 229.2274 (Δ = 2.6 ppm).

-Compound 5.d: (2S,2'S)-N,N'-(octane-1,8-diyl)bis(2-aminopropanamide), *(HAla8)*



A similar procedure to that described for above was used, but in this case the mixture was only further stirred for 2h to turn completely black and the product was obtained as a yellow waxy solid. Yield (2.32 g, >99%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72 (t, 2H), 3.23 – 3.16 (m, 2H), 3.07 – 2.99 (m, 4H), 1.89 (s, 4H), 1.43 – 1.34 (m, 4H), 1.28 – 1.21 (m, *J* = 2.2 Hz, 8H), 1.09 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.5 (C=O), 50.3 (CH), 38.2, 29.2, 28.7, 26.3, 21.8 (CH_2); HRMS (ESI): calcd. or: $C_{14}H_{30}N_4O_2$ $[M+H]^+$ = 287.2447; found = $[M+H]^+$ = 287.2437 (Δ = 3.5 ppm).

5.3.4 Synthesis of final products.



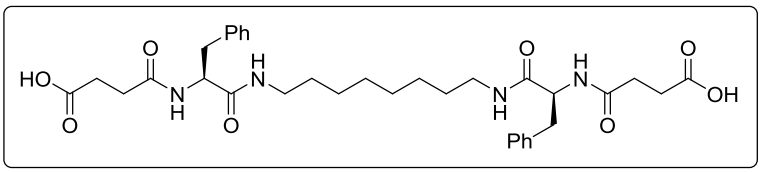
5a; R_1: Phe; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$
5b; R_1: Ile; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$
5c; R_1: Val; R_2: $(CH_2)_8$
5d; R_1: Ala; R_2: $(CH_2)_8NHCOCH(R_1)NHCbz$

1a; R_1: Phe; R_2: $(CH_2)_8NHCOCH(R_1)NHCO(CH_2)_2COOH$
1b; R_1: Ile; R_2: $(CH_2)_8NHCOCH(R_1)NHCO(CH_2)_2COOH$
1c; R_1: Val; R_2: $(CH_2)_8$
1d; R_1: Ala; R_2: $(CH_2)_8NHCOCH(R_1)NHCO(CH_2)_2COOH$

A solution of corresponding amine (1.0 eq.) in THF (30mL/mmol) was treated with K_2CO_3 (1.9 eq.) and was introduced in a ultrasonic bath for 10 min. The mixture was stirred for 15 min at 0°C under N_2 atmosphere, and then a solution of succinic anhydride or glutaric anhydride (4 eq.) in THF (6mL/mmol) was added dropwise. After the addition, K_2CO_3 (1.9 eq.) was added to the mixture at room temperature in order to achieve pH 9-10, and several portions of K_2CO_3 was further added to make it. The mixture was stirred vigorously for 16h at room temperature. After this time, the solution was concentrated under reduced pressure and the crude residue was dissolved in water (200 mL), then HCl concentrate was added at 0°C until the formation of a white precipitate to pH 3-4. The frothy white solution

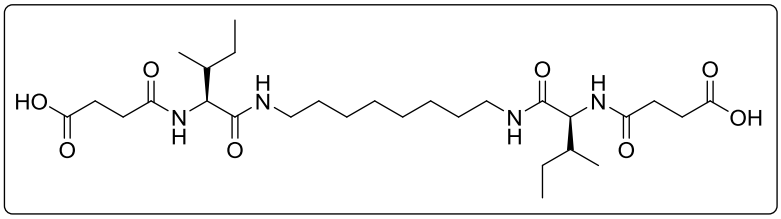
obtained was filtered off under vacuum, and the residue was washed with water until neutral pH to yield a white solid.

-Compound 1.a: (6S,19S)-6,19-dibenzyl-4,7,18,21-tetraoxo-5,8,17,20-tetraazatetracosane-1,24-dioic acid, *(SucPhe8)*



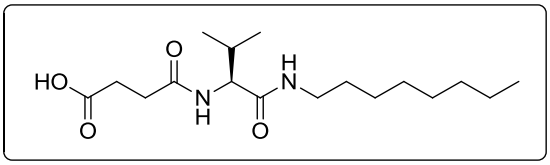
The general procedure described above was used to synthesize **1.a.** Yield (3.82 g, 92%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (s, 2H, H_1), 8.07 (d, *J* = 8.5 Hz, 2H, H_5), 7.80 (t, *J* = 5.5 Hz, 2H, H_{13}), 7.36 – 7.09 (m, 10H, H_{Arom}), 4.42 (ddd, *J* = 9.0, 8.5, 5.8 Hz, 2H, H_6), 3.08 – 3.00 (m, 2H, H_{14}), 3.00 – 2.90 (m, 4H, $H_{14'}$, H_7), 2.74 (dd, *J* = 13.6, 9.0 Hz, 2H, $H_{7'}$), 2.40 – 2.20 (m, 8H, H_2, H_3), 1.41 – 1.24 (m, 4H, H_{15}), 1.26 – 1.10 (m, 8H, H_{16}, H_{17}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.9 (C_1), 170.8, 170.7($C_{Arom,}C_{Arom}$), 138.0 (C_8), 129.1, 128.0($C_{9,}C_{10}$), 126.2 (C_{11}), 54.1 (C_6), 38.5 (C_{14}), 37.9 (C_7), 30.0 (C_2), 29.2, 28.9, 28.7 (C_{15}, $C_{17,}$ C_3), 26.2 (C_{16}); HRMS (ESI): calcd. or: $C_{34}H_{46}N_4O_8$ $[M+Na]^+$= 661.3213; found = $[M+Na]^+$ = 661.3215 (Δ = 0.3 ppm).

-Compound 1.b: (6S,19S)-6,19-di((R)-sec-butyl)-4,7,18,21-tetraoxo-5,8,17,20-tetraazatetracosanedioic acid, (SucIle8)



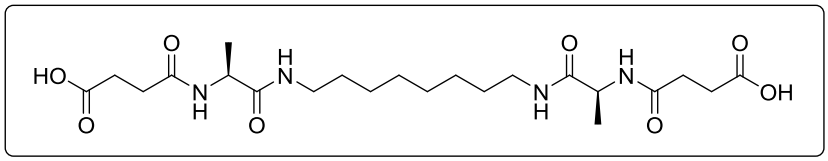
The general procedure described above was used to synthesize **1.b**. Yield (3.17 g, 83%); ^{1}H NMR (300 MHz, DMSO-d_6) δ12.00 (s, 1H, H_1), 7.87 (t, *J* = 5.6 Hz, 2H, H_{11}), 7.85 (d, *J* = 7.8 Hz, 2H, H_5), 4.10 (dd, *J* = 8.5, 7.8 Hz, 2H), 3.08 (m, 2H), 2.95 (m, 2H), 2.45 – 2.32 (m, 8H), 1.68 (m, 2H), 1.53 – 1.30 (m, 8H), 1.29 – 1.14 (m, 8H), 1.12 – 0.97 (m, 2H), 0.85 – 0.73 (m, 12H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.9 (C_1), 170.9, 170.8 (C_4 , C_{11}), 56.9 (C_6), 38.3 (C_{13}), 36.6 (C_7), 29.9, 29.3, 28.9, 28.7 (C_2, C_3, C_{14}, C_{16}), 26.3 (C_{15}), 24.3 (C_9), 15.4 (C_8), 11.1 (C_{10}). HRMS (ESI): calcd. or: $C_{28}H_{50}N_4O_8$ $[M-H]^-$= 569.3550; found = $[M-H]^-$ = 569.3559 (Δ = 1.6 ppm).

-Compound 1.c: (S)-4-((3-methyl-1-(octylamino)-1-oxobutan-2-yl)amino)-4-oxobutanoic acid, (SucValOct)



The general procedure described above was used, but in this case the product was purified by washing with H_20 (30 mL), after with diethyl ether. Yield (3.88 g, 91%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (s, 1H, H_1), 7.84 – 7.78 (m, 2H, H_5, H_{10}), 4.07 (dd, *J* = 8.9, 6.9 Hz, 1H, H_6), 3.12 – 3.04 (m, 1H, H_{11}), 3.01 – 2.93 (m, 1H, $H_{11'}$), 2.45 – 2.38 (m, 4H, H_2, H_3), 1.98 – 1.87 (m, 1H, H_7), 1.43 – 1.33 (m, 2H, H_{12}), 1.30 – 1.17 (m, 10H, H_{13}, H_{14}, H_{15}, H_{16}, H_{17}), 0.85 (t, *J* = 7.0 Hz, 3H, H_{18}), 0.83 (d, *J* = 2.4 Hz, 3H, H_8), 0.81 (d, *J* = 2.4 Hz, 3H, $H_{8'}$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.9 (C_1), 171.0, 170.73 (C_4, C_9), 57.8(C_6), 38.3(C_{11}), 31.2, 30.4, 29.9, 29.3, 29.0, 28.6(C_2, C_3, C_7, C_{12}, C_{14}, C_{15}, C_{16}), 26.3 (C_{13}), 22.1 (C_{17}), 19.2 (C_8), 18.1($C_{8'}$), 13.9 (C_{18}); HRMS (ESI): calcd. or: $C_{17}H_{32}N_2O_4$ $[M+Na]^+$= 351.2260; found = $[M+Na]^+$ = 351.2258 (Δ = 0.6 ppm).

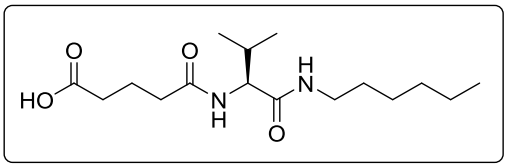
-Compound 1.d: (6S,19S)-6,19-dimethyl-4,7,18,21-tetraoxo-5,8,17,20-tetraazatetracosanedioic acid, (SucAla8)



A similar procedure to that described for **4.a** was used, but in this case the product remained dissolved in the water and it was impossible to obtain it by filtration. The liquid-liquid extraction was not a good method. So, the solution was putted in the fridge and the product precipitated and was filtrated off under vacuum. Yield (1.93 g, 53 %); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 2H, H_1), 8.04 (d, *J* = 7.6 Hz, 2H, H_5), 7.72 (t, *J* = 5.6 Hz, 2H, H_9), 4.18 (m, 2H, H_6), 3.06 – 2.95 (m, 4H, H_{10}), 2.44 – 2.30 (m, 8H, H_2, H_3), 1.42 – 1.31 (m, 4H, H_{11}), 1.26 – 1.19 (m, 8H, H_{12}, H_{13}), 1.16 (d, *J* = 7.1 Hz, 6H, H_7); ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.07 (C_1), 172.1 (C_4), 170.8 (C_8), 48.2 (C_6), 38.5 (C_{10}), 30.0, 29.2, 29.0, 28.7 (C_2, C_3, C_{11},

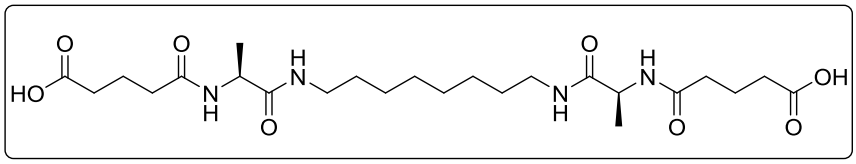
C_{13}), 26.2(C_{12}), 18.3(C_7); HRMS (ESI): calcd. or: $C_{22}H_{38}N_4O_8$ $[M+H]^+$= 487.2768; found = $[M+H]^+$ = 487.2756 (Δ = 2.5 ppm).

-Compound mono glutaric acid derived: (S)-5-((3-methyl-1-(octylamino)-1-oxobutan-2-yl)amino)-5-oxopentanoic acid, (GluValHx)



The general procedure described above was used, but in this case the product was purified by washing with H_20 (30 mL), after with diethyl ether. Yield (2.88 g, 95%); ^{1}H NMR (400 MHz, DMSO) δ 7.94 (t, *J* = 5.6 Hz, 1H, H_{11}), 7.82 (d, *J* = 14.1 Hz, 1H, H_6), 4.06 (dt, *J* = 15.7, 7.9 Hz, 1H, H_7), 3.14 – 2.88 (m, 2H, H_{12}), 2.23 – 2.08 (m, 4H, H_2, H_4), 1.90 – 1.94 (m, 1H, H_8), 1.75 – 1.64 (m, 2H, H_3), 1.43 - 1.33 (m, 2H, H_{13}), 1.32 – 1.16 (m, 6H, $H_{14–16}$), 0.91 – 0.76 (m, 9H, $H_{9–9'}$, H_{17}) (carboxyl OH not detected).

-Compound double glutaric acid derived: (7*S*,20*S*)-7,20-dimethyl-5,8,19,22-tetraoxo-6,9,18,21-tetraazahexacosanedioic acid, (GluAla8)



A similar procedure to that described for **GluAla8** was used, but in this case the product remained dissolved in the water and it was impossible to obtain it by filtration. The liquid-liquid extraction with $CHCl_3$ and removal of all volatiles under reduced pressure. Yield (0.83 g, 69 %); ^{1}H NMR (400 MHz, DMSO) δ 7.98 (d, *J* = 7.7 Hz, 2H, H_6), 7.82 (t, *J* = 5.6 Hz, 2H, H_{10}), 4.21 (p, *J* = 7.1 Hz, 2H, H_7), 3.07 – 2.95 (m, 4H), 2.28 – 2.09 (m, 8H, H_2, H_4), 1.75 – 1.64 (m, 4H. H_3), 1.43 – 1.29 (m, 4H, H_{11}), 1.29 – 1.18 (m, 6H, $H_{12–14}$), 1.16 (d, *J* = 7.1 Hz, 6H, H_8)) (carboxyl OH not detected).

NMR Spectra

6 Chapter: NMR Spectra

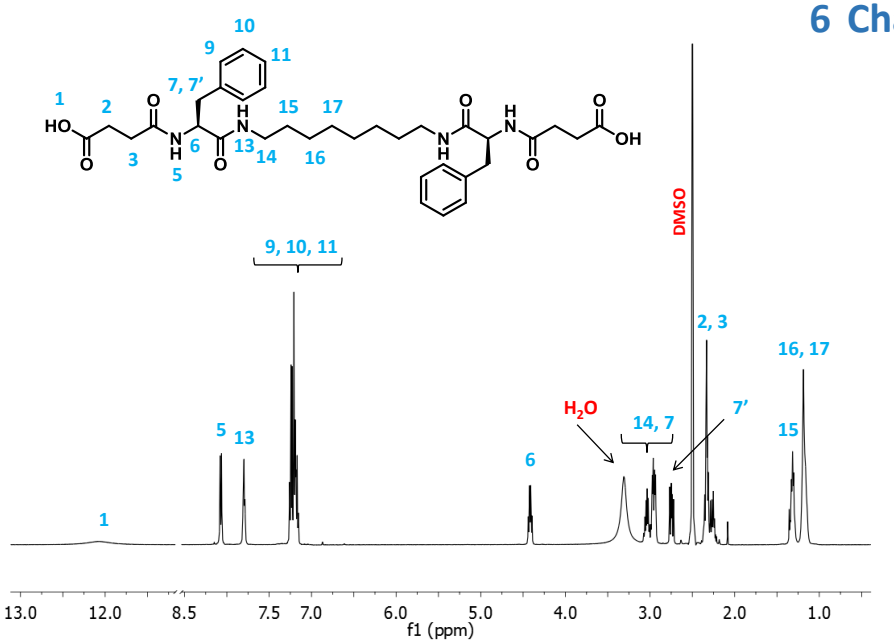


Figure 6-1. ^{1}H NMR spectrum of compound **1.a**

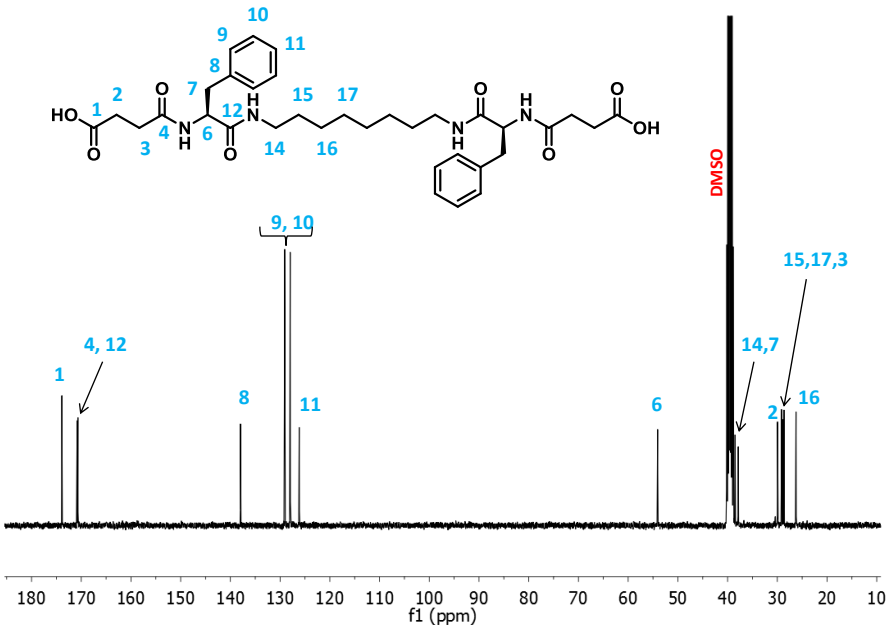


Figure 6-2. ^{13}C NMR spectrum of compound **1.a**

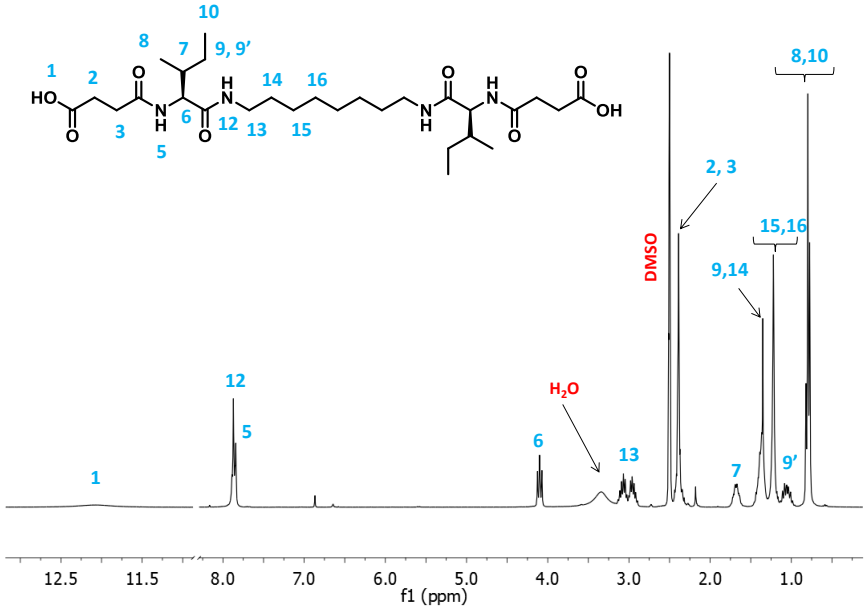


Figure 6-3. ^{1}H NMR spectrum of compound **1.b**

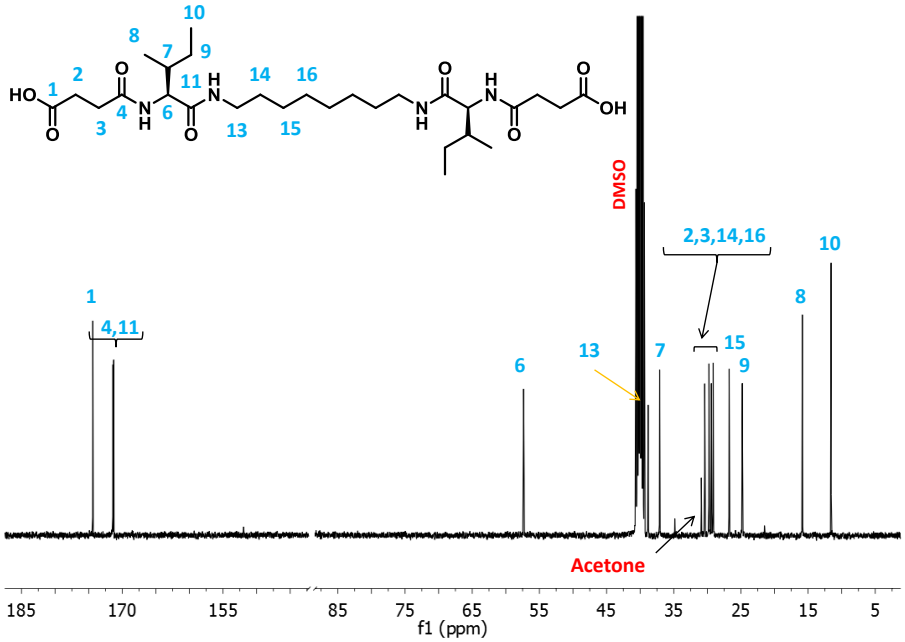


Figure 6-4. ^{13}C NMR spectrum of compound **1.b**

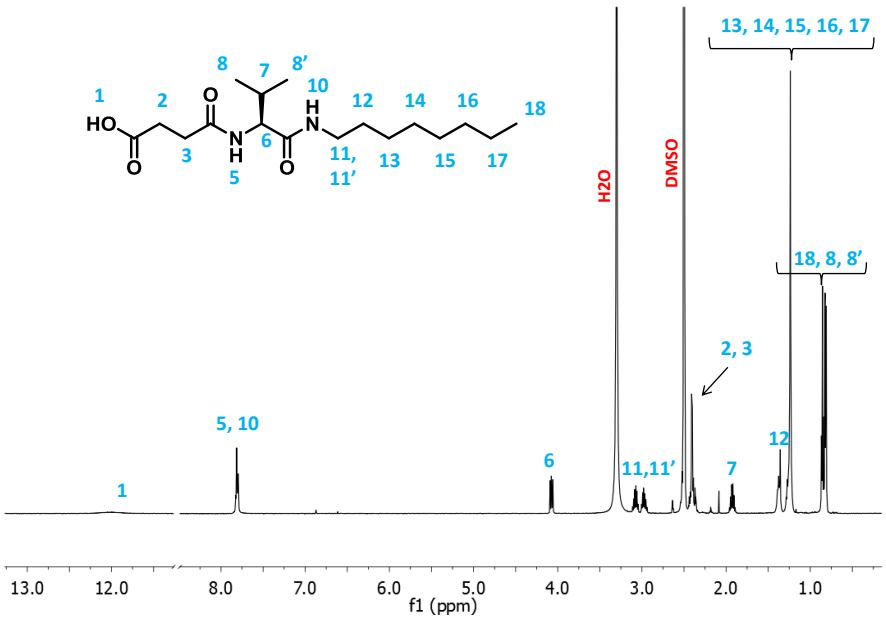


Figure 6-5. ^{1}H NMR spectrum of compound **1.c**

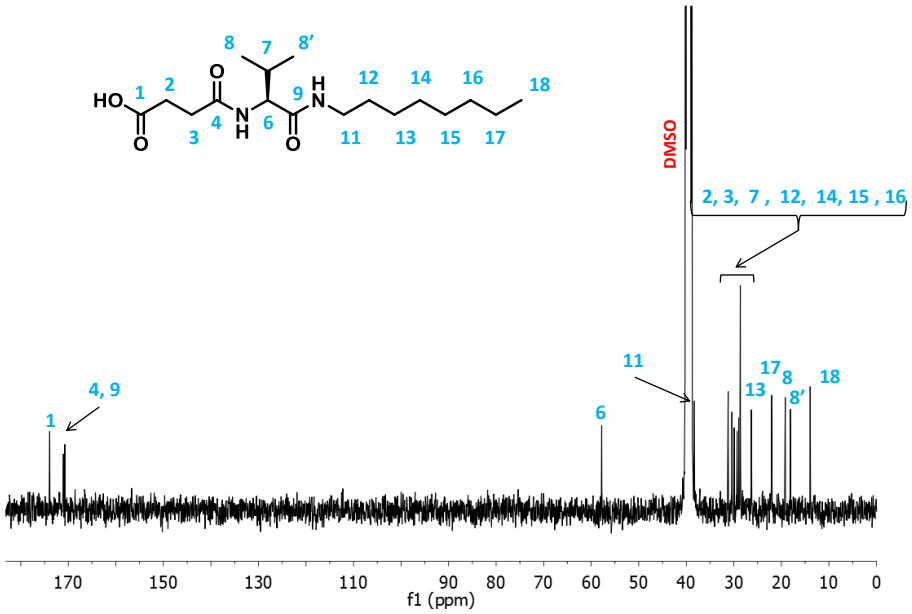


Figure 6-6. ^{13}C NMR spectrum of compound **1.c**

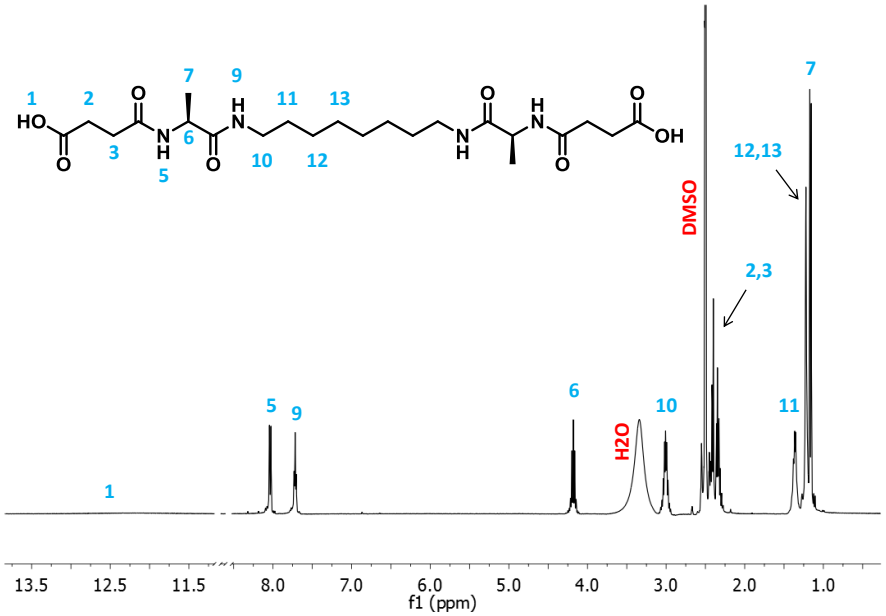


Figure 6-7. ^{1}H NMR spectrum of compound **1.d**

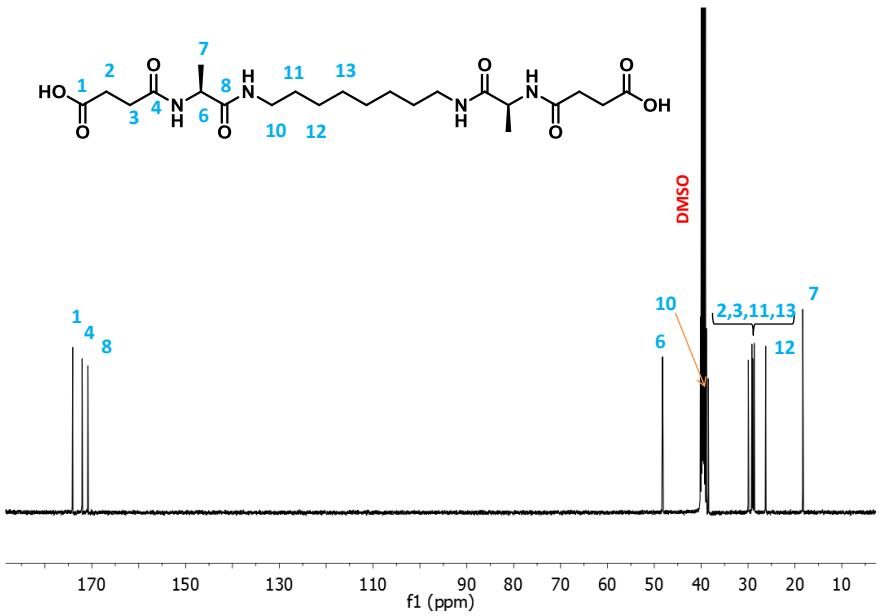


Figure 6-8. ^{13}C NMR spectrum of compound **1.d**

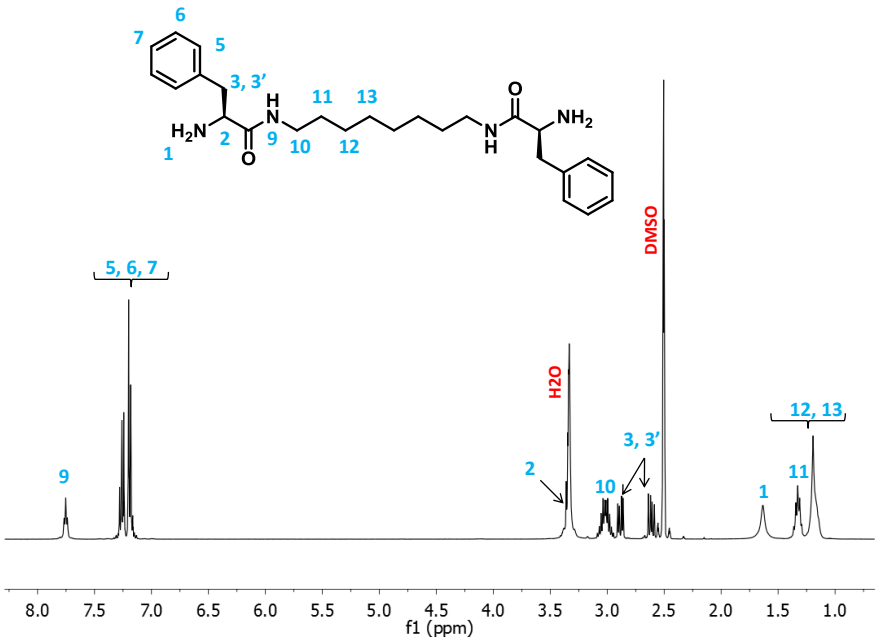


Figure 6-9. ^{1}H NMR spectrum of compound **5.a**

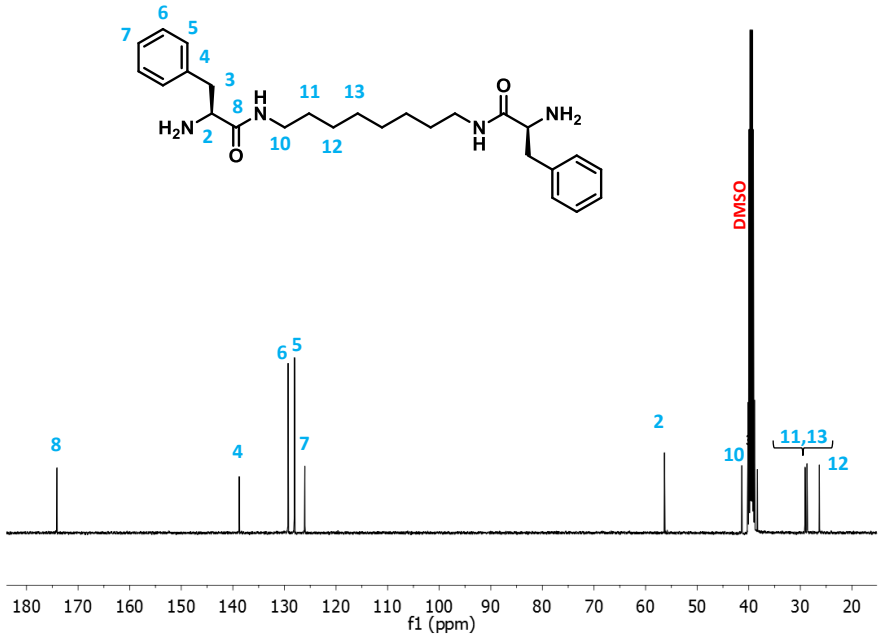


Figure 6-10. ^{13}C NMR spectrum of compound **5.a**

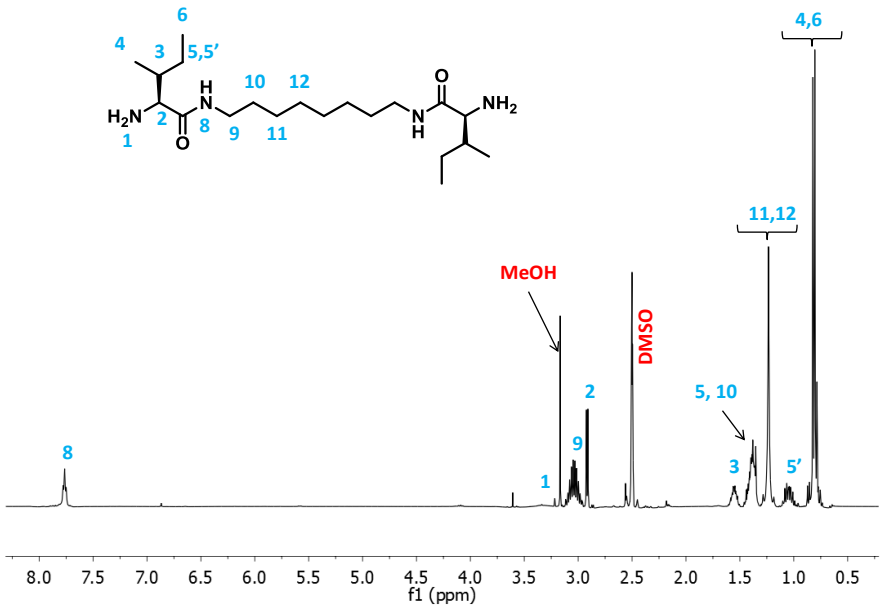


Figure 6-11. ^{1}H NMR spectrum of compound **5.b**

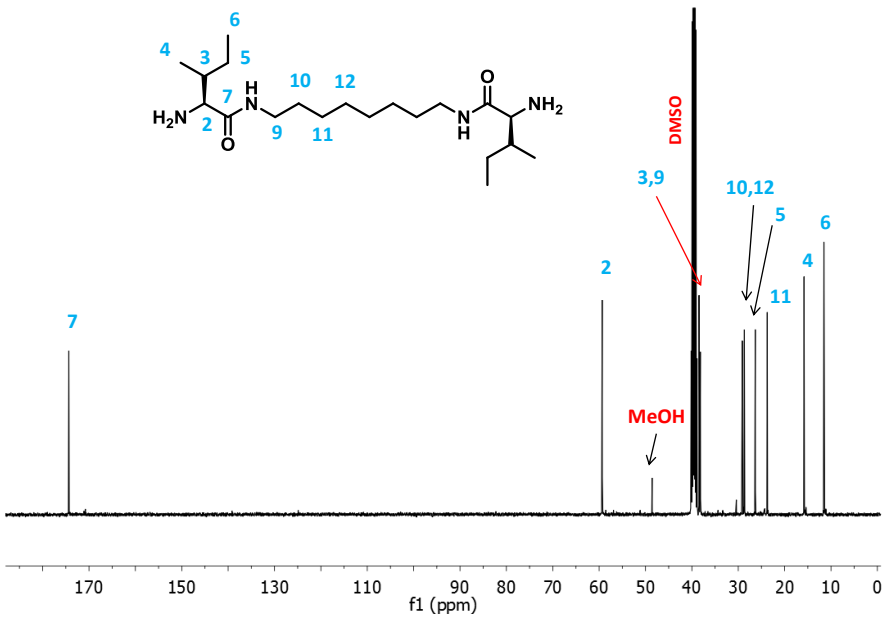


Figure 6-12. ^{13}C NMR spectrum of compound **5.b**

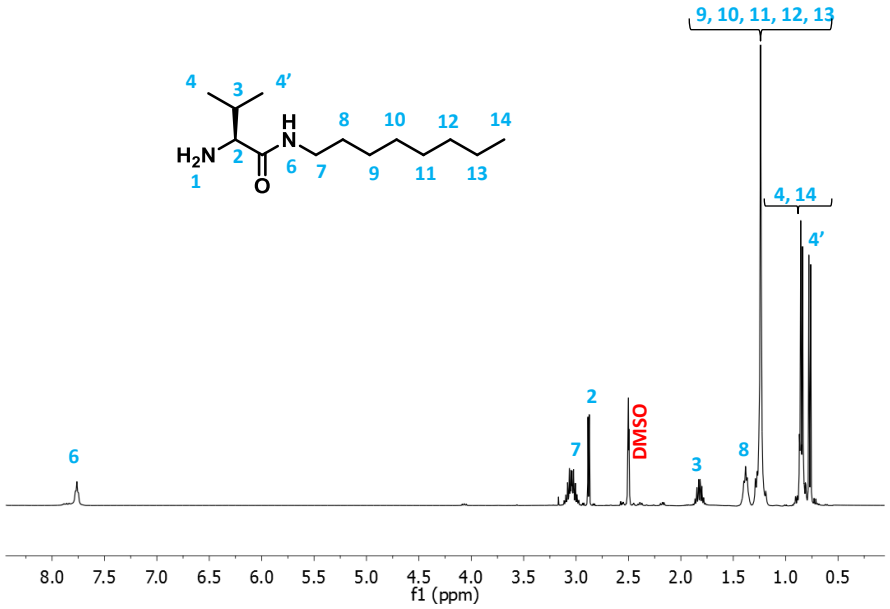


Figure 6-13. ^{1}H NMR spectrum of compound **5.c**

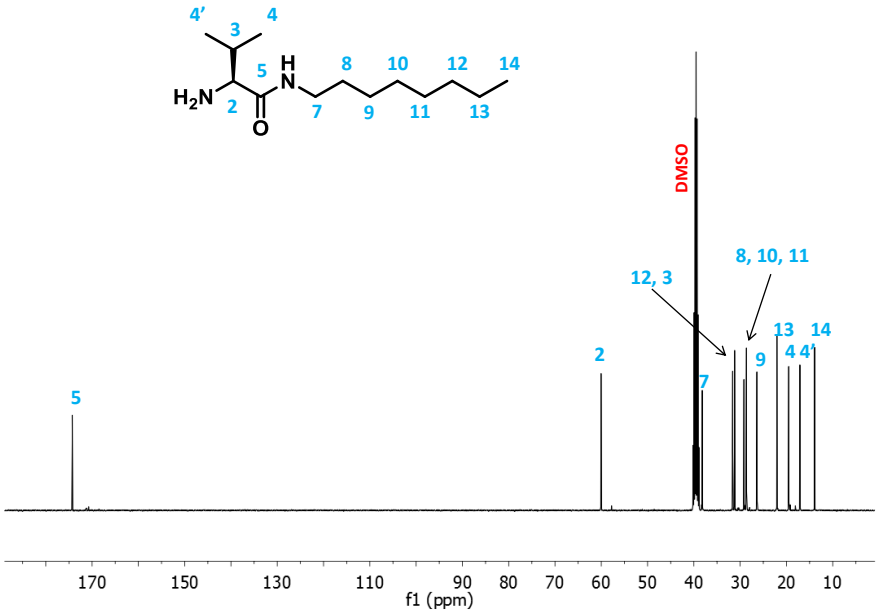


Figure 6-14. ^{13}C NMR spectrum of compound **5.c**

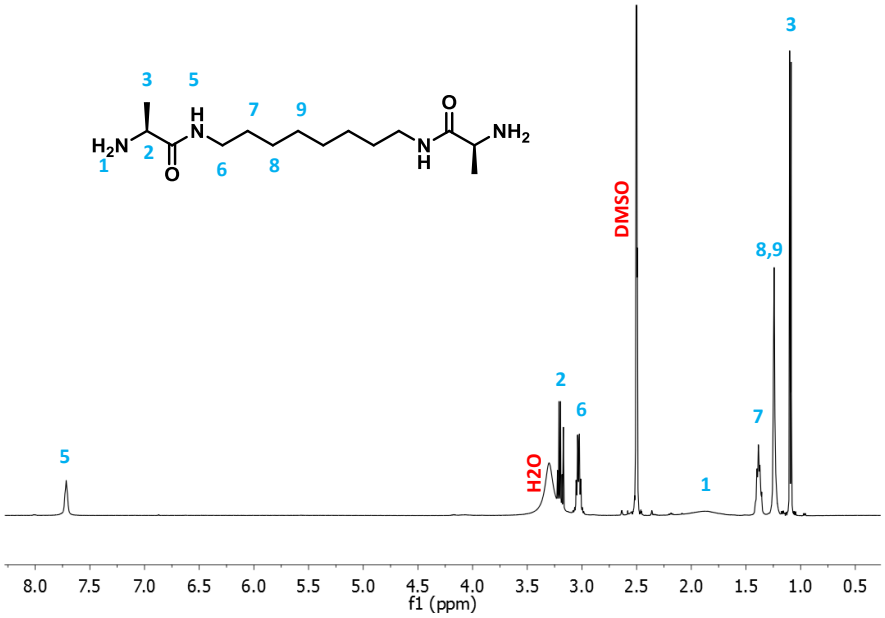


Figure 6-15. ^{1}H NMR spectrum of compound **5.d**

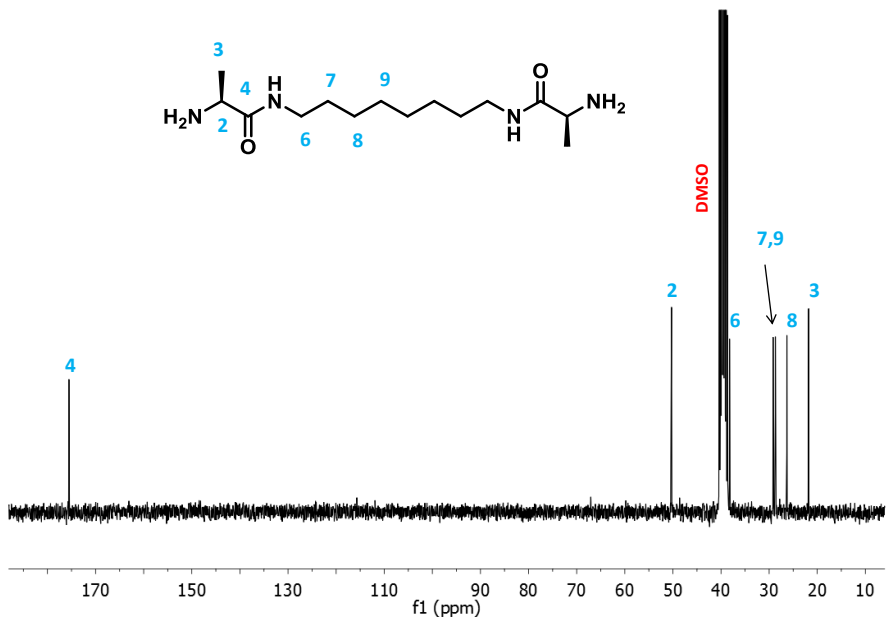


Figure 6-16. ^{13}C NMR spectrum of compound **5.d**

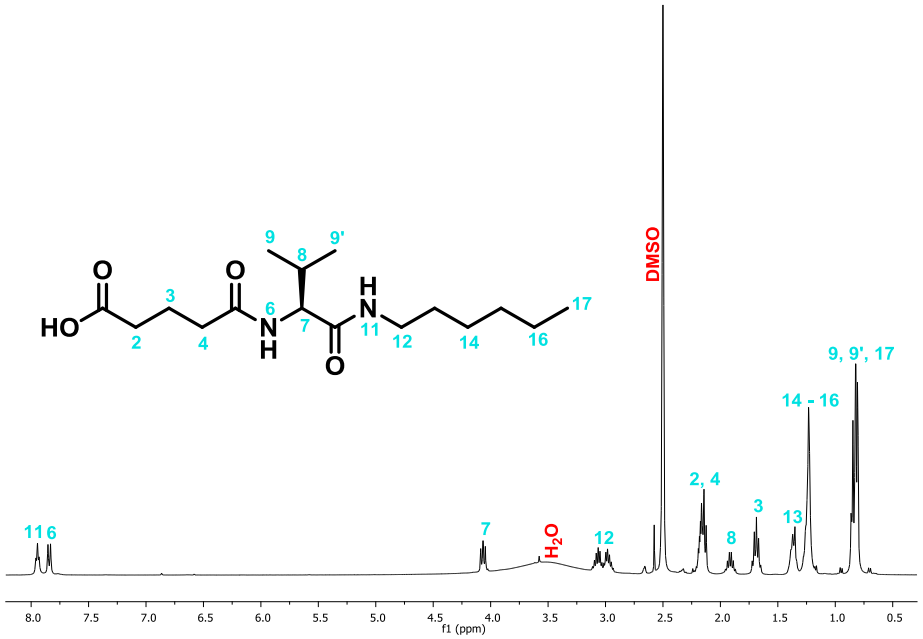


Figure 6-17. ^{1}H NMR spectrum of compound **GluValHx**

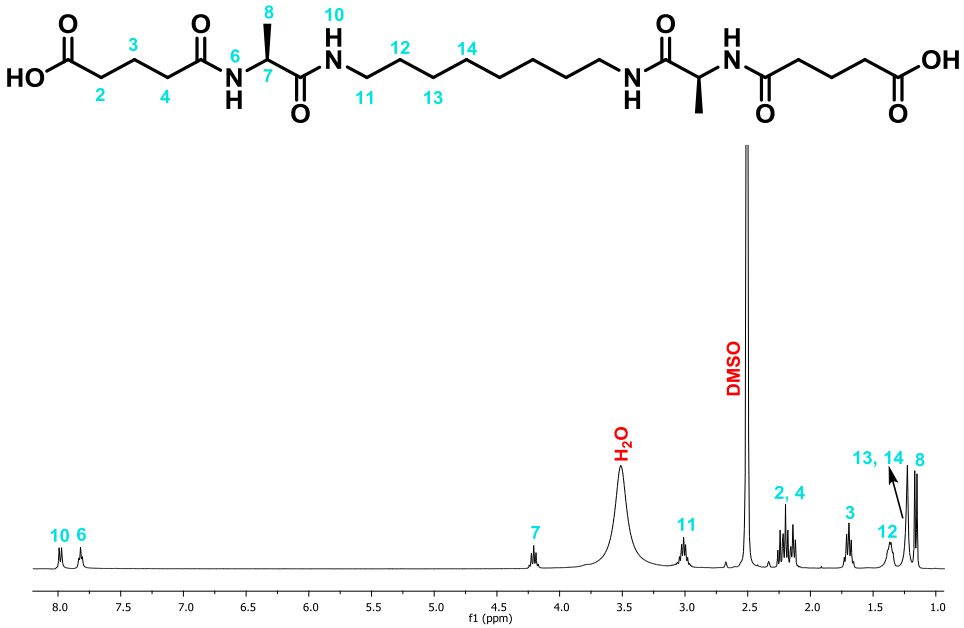


Figure 6-17. ^{1}H NMR spectrum of compound **GluAla8**