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Synthesis and study of two hydrogelators derived from *L*-Valine

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BACHELOR'S DEGREE RESEARCH PROJECT

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Escuela superior de Tecnología y Ciencias Experimentales
Departamento de Química Inorgánica y Orgánica
Grupo de Nanomateriales Moleculares Orgánicos con Aplicaciones Biomédicas

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CHEMISTRY DEGREE RESEARCH PROJECT

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CERTIFICAN

Que el trabajo fin de grado con el título **Synthesis and study of two hydrogelators derived from L-Valine** ha sido realizado por Milla Pitkäranta bajo su dirección, en el grupo de Nanomateriales Moleculares Orgánicos con Aplicaciones Biomédicas 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 Castelló de la Plana a 02 de julio de 2020.

Fdo. Dr. Juan F. Miravet Celades

Fdo. Dr. César A. Angulo Pachón

Abbreviations

Suc	Succinic acid radical
Val	Valine radical
Non	n-Nonyl radical
CMC	Critical micellar concentration
MGC	Minimum gelation concentration
DCC	*N,N'*-Dicyclohexylcarbodiimide
DLS	Dynamic light scattering
NMR	Nuclear Magnetic Resonance
DMSO_*d6*	Dimethyl sulfoxide deuterated
T_{gel}	Transition temperature of gel to solution
6	1,6-hexane diamine
THF	Tetrahydrofuran
MeOH	Methanol

Index

1. Introduction 1
1.1. Supramolecular gels 1
1.2. Hydrogels 2
1.3. Organogels 4
1.4. Surfactant type gels 5
1.5. Techniques for the characterization of supramolecular gels 6
1.6. N-protection and C-activation in peptide synthesis 7

2. Objectives 9

3. Results and Discussion 11
3.1 Synthesis of SucValNon 11
3.2 Synthesis of SucVal6 12
3.3 Minimum gelation concentration 12

4. Conclusions 15

5. Experimental Section 17
5.1. General methods 17
5.2. Synthesis of SucValNon 17
5.2.1. Synthesis of ZValOSu 18
5.2.2. Synthesis of ZValNon 18
5.2.3. Synthesis of HValNon 19
5.2.4. Synthesis of final compound (SucValNon) 20
5.3. Synthesis of SucVal6 21
5.3.1. Synthesis of ZVal6 21
5.3.2. Synthesis of HVal6 22
5.3.3. Synthesis of final compound (SucVal6) 22
5.4. Experimental method for determination of MGC 23

6. Annex 25
6.1. NMR Spectra 25

6.2. Mass sepctometry 38

7. References 39

Introduction

Introduction

1.1 Supramolecular gels

Gels are solid-like viscoelastic materials formed by solvent and gelator. Supramolecular gels are formed by weak interactions between small molecules unlike macromolecular gels which are formed by macromolecules or polymers.[1] Supramolecular gels can be utilized, for example, in tissue engineering, wound healing and drug delivery.[2]

The dynamic and reversible nature of non-covalent interactions that hold supramolecular gels network structures together results in the inherent ability of them to respond to external stimuli such as temperature, pH, solvent, light, and redox reactions.[3] The response to stimuli of supramolecular gels makes them very important in Materials Science. For example, some supramolecular gels are sensitive to light or chemical entities by incorporating a spectroscopically active group or receptor unit as part of a gelator. This makes them easily applicable in many areas.[4]

Supramolecular gels, like other gels, can be classified according to several factors. They can be divided into natural and artificial, i.e. synthetic gels, based on a polymer acting as a gelling agent. Besides, they can be classified into supramolecular and macromolecular based on their structure. Macromolecular gels are those that consist of macromolecules and supramolecular those that are built by the self-assembly of low molecular weight (LMW) molecules.[5] The division into physical and chemical gels is based on their bridging mechanism. Physical gels are made up of non-covalent interactions and chemical gels are made up of covalent bonds.[6,7] Also, gels can be classified according to the solvent used into organo-, hydro-, aero-, and xerogels.

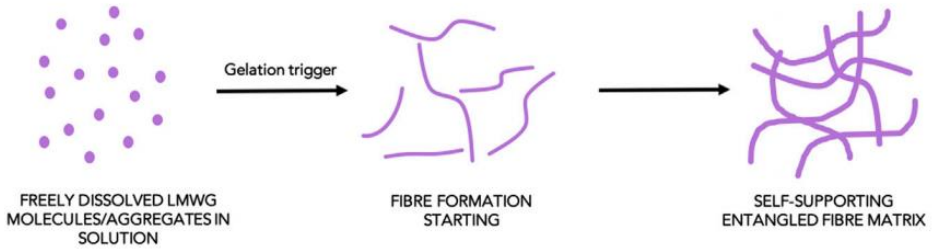


Figure 1.1 Formation of supramolecular gels.[8]

1.2 Hydrogels

Hydrogels are soft materials formed from hydrophilic polymeric networks that can swell in the presence of water or physiological fluids (Figure 1.2). [9,10] Hydrogels can absorb or bind considerable amounts of water relative to their dry weight.[9,8,12]As mentioned above, chemical hydrogels are formed by covalent networks and do not dissolve in water without breaking covalent bonds. However, physical hydrogels are formed through dynamic interactions of synthetic or natural building blocks based on non-covalent interactions, such as hydrophobic and electrostatic interactions or hydrogen bonding.[11]

The nature of the water in the hydrogel can determine the ability of solutes to penetrate the gel and cell products. As the dry hydrogels begin to absorb water, the first water molecules entering the matrix hydrate the most polar, hydrophilic groups, resulting in "primary bound water." As the polar groups hydrate, the network swells and exposes the hydrophilic groups to interactions with water molecules, resulting in hydrophobically bound water or "secondary bound water." Once the polar and hydrophobic sites have interacted and attached to the water molecules, excess water is absorbed into the network due to the osmotic pressure of the network chains. Covalent or physical bridge bonds resist this additional swelling, resulting in an elastic retraction force of the network. Thus, the hydrogel reaches equilibrium in its swelling level.[9]

Hydrogels have been increasingly studied in the field of tissue engineering. Hydrogels designed for use in tissue engineering building platforms may contain pores large enough to accommodate living cells. They can be designed to dissolve or disintegrate, release growth factors, and create pores into which living cells can

penetrate and multiply. However, a significant disadvantage of hydrogels is their low mechanical strength, which causes significant difficulties in their handling. Also, sterilization of hydrogels is very challenging.[12]

Supramolecular hydrogels are probably the most promising soft material substrates in modern biomedical applications. With their inherent reversibility and dynamism, they respond well to environmental stimuli and biochemical cues and can decompose mechanical energy efficiently. These essential features are well suited for cell culture, tissue engineering, controlled release of therapeutic agents as needed (Figure 1.3), tissue adhesion, and molecular recognition. They can also be used as artificial gel substitutes in organs that are not readily achieved by permanently crosslinked covalent hydrogels. Supramolecular interactions that allow gel formation also translate into the properties of their bulk materials and lead to biomedical applications. Because of the diversity and ease of "bottom-up" design, at the molecular level, supramolecular hydrogels offer a wide range of biomedical solutions to modern societal problems, including wound healing, artificial tissue development, cell therapies, and cancer treatment.[13, 14]

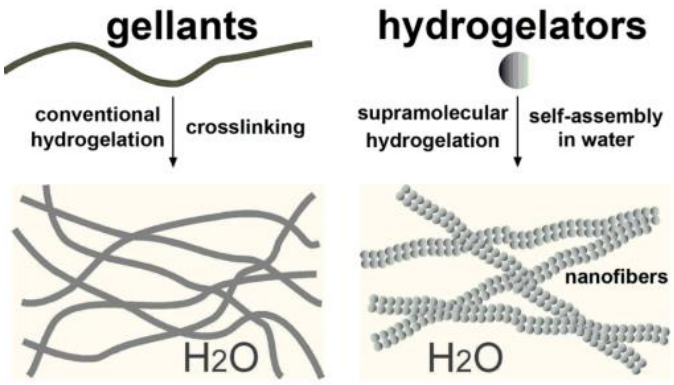


Figure 1.2 Creation of polymeric hydrogels by crosslinking (left) or formation of supramolecular hydrogels by chemical or physical disturbance initiated by self-assembly (right).[14]

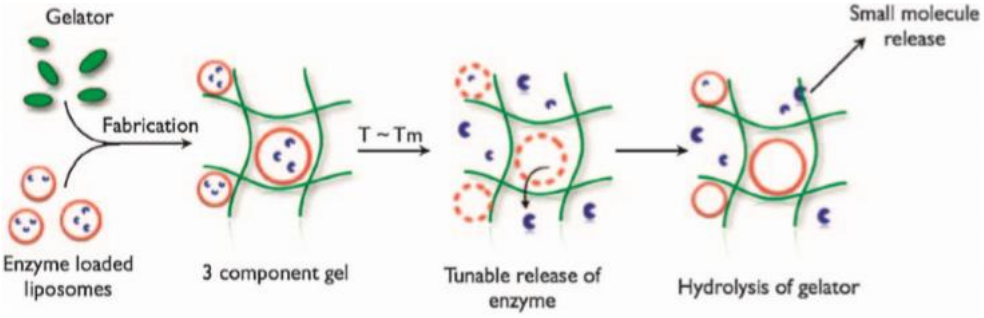


Figure 1.3 Representation of a temperature-triggered drug release system.[1]

1.3 Organogels

Supramolecular organogels are semi-solid materials consisting of a gelator and an organic solvent. Supramolecular organogels have numerous applications. They can serve, for example, as molds for the production of nanoporous materials or nanoparticles, as media for the growth of large, high-quality organic, inorganic and macromolecular crystals, and as electro-optical display materials.[15]

Organogels can be classified into polymeric and supramolecular organogels based on the nature of the gelling molecule. In supramolecular organogels, gel networks are networks formed of either solid or liquid fibers. Liquid fibers gel organic solvents in the same way as solid fibers: the size of the aggregate increases, and the adhesion of the formed structures immobilizes the solvent as a result of surface tension. Solid and liquid fiber networks behave differently from each other. The morphology of solid network structures is permanent throughout the life cycle of the gel, but fluid networks are temporary structures that re-form dynamically throughout time. For example, lecithin and sorbitan monostearate are both of great interest in pharmaceutical applications because they are composed of liquid fiber networks.[16]

Supramolecular organogels are used for topically or transdermally administered drugs. This approach to drug distribution represents a noninvasive method with good tolerance. Pharmaceutical supramolecular organogels are generally formed in biocompatible oils or alcohols, such as n-butanol, n-octanol, ethyl oleate, glycerin, or eucalyptus oil. These substances are usually permeation enhancers that facilitate the passage of drugs through the epidermis.[1]

1.4 Surfactant type gels

Surfactants are molecules consisting of a water-repellent or hydrophobic and hydrophilic moiety. Indeed, in the production of surfactants, it is essential to combine these two different types of groups.[15–18] The hydrophobic moiety is a hydrocarbon chain of 5–18 carbon atoms with low polarizability. The hydrophilic moiety, in turn, may be nonionic, ionic or zwitterionic. The balance between these two different parts gives the surfactants their specific properties, such as accumulation at various interfaces and association in solution.[17]

Surfactants are found in almost every chemical product, such as detergents, paints, dyes, paper coatings, inks, plastics and fibers, personal care products and cosmetics, agrochemicals, pharmaceuticals, and food. Besides, they play an essential role in the oil industry.[16–18] The most common surfactants are soaps, i.e., fatty acid salts containing a chain of at least eight carbon atoms, and detergents, which can be mixtures of several different surfactants. Detergents alter the properties of the interfaces to promote the removal of phase, such as dirt, from solid surfaces.[22]

Amphiphilic mixtures can, in some cases, be gel phases at a specific concentration and temperature range. For example, <70% potassium stearate in water forms clear gels below 50 °C. The area per polar main group is equal to twice the cross-sectional area per paraffin chain. The relationship between these two regions suggests that the soap molecules pack in an interdigitated arrangement within the layers. The formation of hydrogel phases from other alkali metal alkanoates depends on the nature of both the cation and the alkanoate anion species. Different chain lengths for alkanoate anions can lead to different gelator efficiencies. For hydrogels of alkanoate anions having a chain length of 14 to 22 carbon atoms and having the same cation, the disassembly temperature values increase with increasing chain length. Specific concentrations of potassium, rubidium, and cesium salts of alkanoates of the same chain length also produce gel phases in water, whereas sodium and lithium salts do not.[20]

1.5 Techniques for the characterization of supramolecular gels

A variety of skills and techniques must be used to understand gelation (Figure 1.4). For example, nuclear magnetic resonance (NMR) spectroscopy, infrared spectroscopy, circular dichroism, fluorescence, and X-ray diffraction are used to understand molecular packing. While all of these techniques can be informative, they are always accompanied by remarks. For example, circular dichroism is very sensitive to concentrations, and good quality data can often only be collected at concentration values lower than the minimum gelation concentration (mgc). Therefore, it is questionable whether the packing of aggregates below mcg is the same as that of mgc and above. Likewise, fluorescence cannot be easily collected in turbid samples, and higher concentrations make it difficult to obtain due to quenching. Additionally, X-ray diffraction assumes that the diffraction is from the gel phase and not from crystalline impurities. Nevertheless, such techniques can be very informative. For example, NMR spectroscopy can be used to test velocity and infer information about molecular interactions leading to assembly. Similarly, infrared spectroscopy can be used to show that a particular hydrogen bond occurs in the assembly.[8]

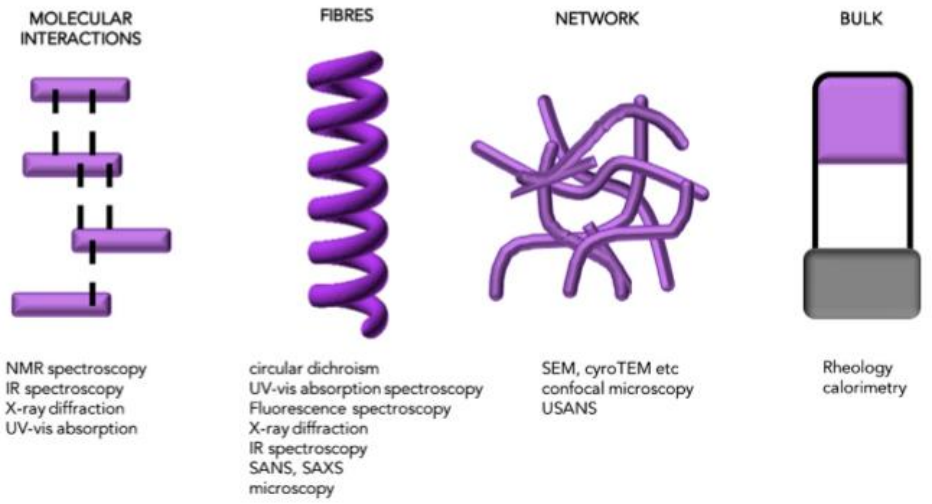


Figure 1.4 As a result of assembly, low molecular weight gels form structures on several length scales that can be analyzed by different techniques in each length scale.[8]

1.6 N-protection and C-activation in peptide synthesis

Peptide synthesis has become one of the most reliable and predictable fields of practical organic chemistry, mainly because of the effectiveness of the protecting groups it uses. Biology makes peptides and proteins by selectively linking members of a pool of about 20 amino acids. To do the same in the laboratory, we must overcome several challenges. For example, by reacting two amino acids together, one could make a dipeptide such as leucine and glycine (Figure 1.5). For the NH_2 group of glycine to react with the CO_2H group of leucine, the carboxylic acid must first be activated towards nucleophilic substitution. For example, by making an acyl chloride or a particularly reactive ester, designated RCOX. The biggest problem is that there is another free CO_2H that can react with the COX group to form an anhydride, and two different free amines, each of which can react. For this reason, both the NH_2 group of leucine and the CO_2H group of glycine should be protected. The protecting groups must be removable under mild conditions, but two groups (one for each of NH_2 and CO_2H) are also required, which can be removed under different conditions. It is then possible to modify both ends of the dipeptide if desired [22]

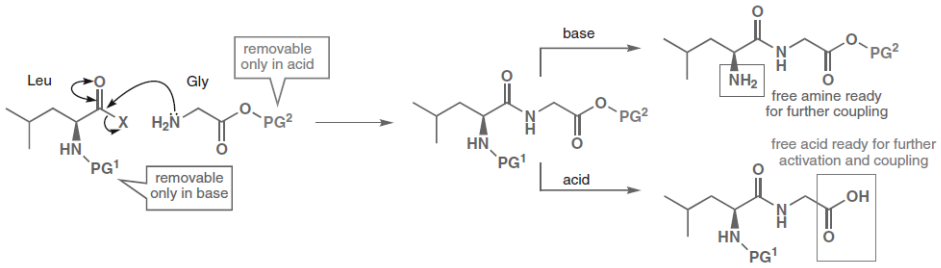


Figure 1.5 Schematic representation of the synthesis of a dipeptide.[22]

Molecules that are prepared in this work require methodology commonly used in peptide synthesis. Cbz (benzyloxycarbonyl), Boc (t-butyloxycarbonyl) and Fmoc (9-fluorenylmethoxycarbonyl) groups are widely used to protect the amino group. On the other hand, activation of the carboxyl group for peptide bond formation is generally performed azide, mixed anhydride and activated ester systems.[21]

Objectives

Objectives

Molecule SucValNon (Scheme 2.1) had been studied previously as a low molecular weight hydrogelator in the research group where this work has been carried out. Studies by the group have shown spherical particles of the gel corresponding to the early stages of aggregation into fibers.[23] SucValNon, and related molecules, form gels in water in its neutral form. However, accidentally it was found that its ionic form, carboxylate, present at basic pH values, tended to form also hydrogels. This fact is, a priori, unexpected due to the improved solubility in water of the ionic form compared to the neutral one. With this consideration mind, the following objectives were established.

1) Reproduce the synthesis and characterization of SucValNon, using conventional organic synthesis procedures.
2) Synthesize an analogue SucVal6, dicarboxylic acid, related to SucValNon
3) Evaluate the capability of gel formation by the carboxylate form of SucValNon.
4) Evaluate the capability of gel formation by the carboxylate form of SucVal6. This point was not developed because of the stop of academic activity associated with the Covid19 pandemics.

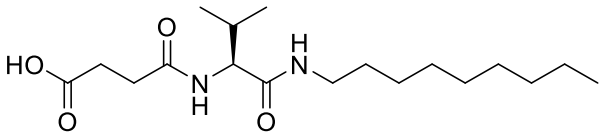


Figure 2.1 Molecular structure of SucValNon.

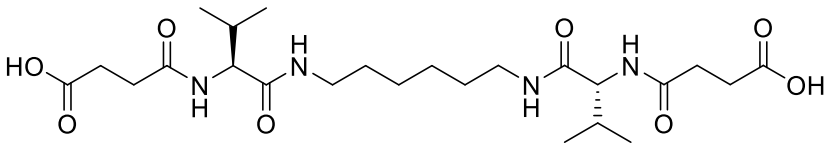
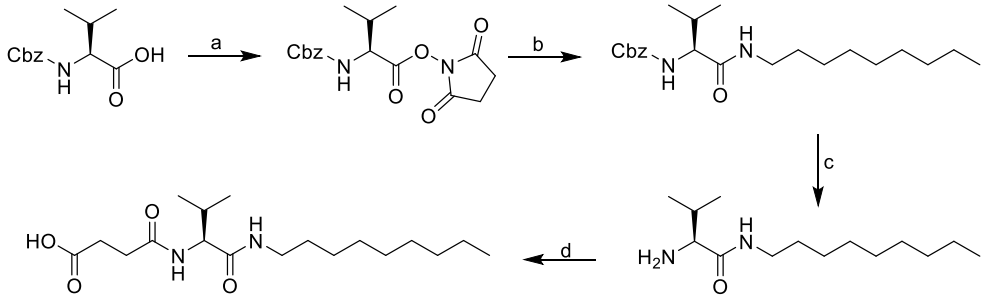


Figure 2.2 Molecular structure of SucVal6.

Results and Discussion

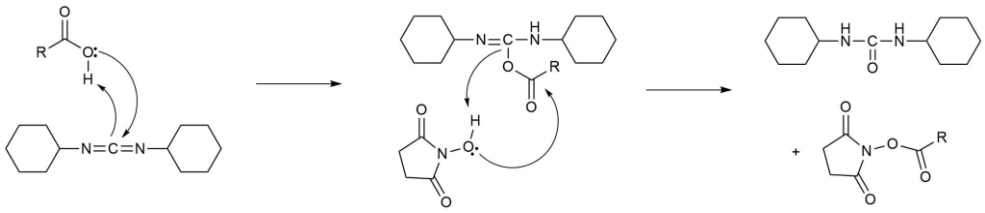
Results and Discussion

3.1 Synthesis of SucValNon

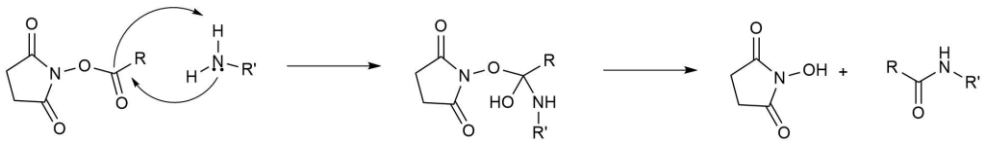


Scheme 3.1 Reagents and conditions: a) *DCC*, *N*-hydroxisuccinimide, THF, 0 °C, 1 h, 84 %; b) *n*-nonylamine, THF, overnight, 50 °C, 77 %; c)) Pd/C, H_2, MeOH, 2 h, 85 %; d) Succinic anhydride, K_2CO_3, THF, overnight, 70 %.

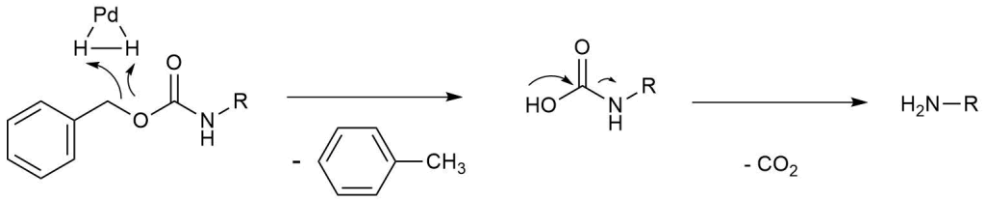
Scheme 3.1 shows the general process of synthesis of SucValNon. The preparation is divided into four main steps. The first step was the activation of Carbobenzyloxy-*L*-valine as an activated ester using *N,N'*-Dicyclohexylcarbodiimide and *N*-hydroxisuccinimide (Scheme 3.2). The second step was an aminolysis coupling of the activated ester and *n*-nonylamine, forming an amide bond (Scheme 3.3). The third step was the removal of the Cbz protecting group by hydrogenolysis using Pd/C as a catalyst (Scheme 3.4). Finally, SucValNon was obtained by reaction between the amine and succinic anhydride.



Scheme 3.2 Amino acid activation with DCC and NHS.

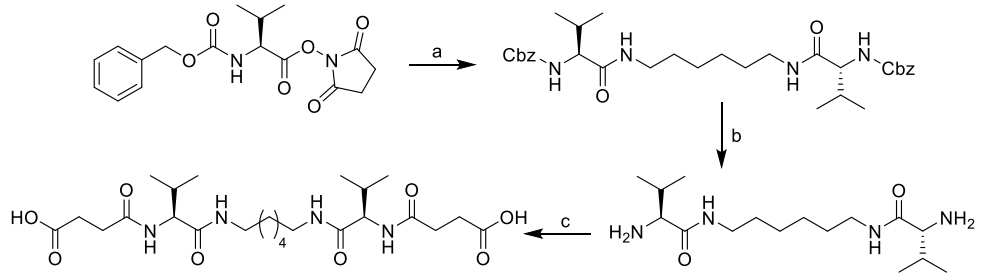


Scheme 3.3 Peptide bond formation.



Scheme 3.4 Hydrogenolysis reaction for removal of the protecting group Cbz.

3.2 Synthesis of SucVal6



Scheme 3.5 Reagents and conditions: a) 1,6-Hexanediamine, THF, N_2, 50 °C, overnight, 99%; b) Pd/C, H_2, MeOH, 3 h, 71%; c) Succinic anhydride, K_2CO_3, THF, overnight, 37%.

Scheme 3.5 shows the synthesis of SucVal6. The stages in the synthesis of SucVal6 are the same as in the synthesis of SucValNon. The only difference is that *n*-nonylamine was used instead of 1,6-Hexanediamine.

3.3 Minimum gelation concentration

Minimum gelation concentration (MGC) describes the lowest possible concentration at which the gelator forms a gel at a given temperature. The MGC

value for the ionic form of SucValNon was studied in the presence of Na^+ and K^+ cations. SucValNon was dissolved in basic aqueous medium using alkaline (Na or K) hydroxides with a final pH of ca. 12, which assures the formation of the anionic carboxylate species. The amount of alkaline cation was regulated by the addition of the corresponding chlorides, affording systems with a final alkaline cation concentration of 0.1 M, 0.5 M and 1.0 M. The maximum concentration of SucValNon tested was 20 mg/mL. It can be observed that with a 0.1 M concentration of the alkaline cation, no gel was formed. Upon increasing to 0.5M, a gel was formed only in the presence of sodium. Finally, for a concentration of 1 M, both systems, containing sodium and potassium cations, developed a gel. The results show that gel formation is sensitive to alkaline cation's nature and concentration. The bigger the ionic strength, the better is the gelation capability. Additionally, it seems that sodium favors gel formation to a higher degree than potassium cation. Figures 3.1 and 3.2 show pictures of the samples used in the experiments carried out to assess the gelation capabilities.

Table 3.1. Minimum gelation concentration of SucValNon in water, pH > 12, in the presence of sodium and potassium cations.

M^+	$[M^+]$ / M	MGC / mg mL^{-1}
Na^+	0.1	No gel
Na^+	0.5	20 ± 1
Na^+	1	20 ± 1
K^+	0.1	No gel
K^+	0.5	No gel
K^+	1	20 ± 1

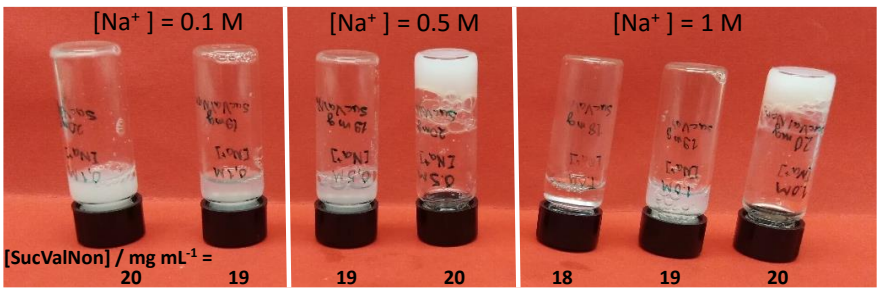


Figure 3.1 Pictures of vial inversion tests for SucValNon in Na^+ solutions.

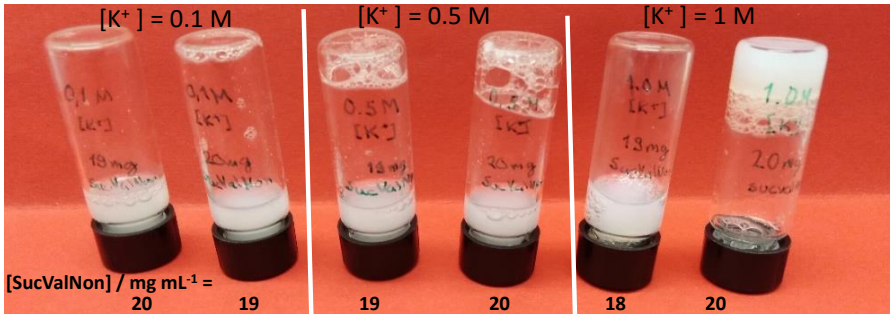


Figure 3.2 Pictures of vial inversion tests for SucValNon in K^+ solutions.

Conclusions

Conclusions

- SucValNon and SucVal6 were successfully prepared in gram scale in pure form.
- The MGC for SucValNon in 0,5 M [Na^+] solution and in 1,0 M [K^+] solution is 20 mg mL^{-1}
- Hydrogel formation is dependant on the concentration and nature of the alkaline cation present in the medium.

Experimental Section

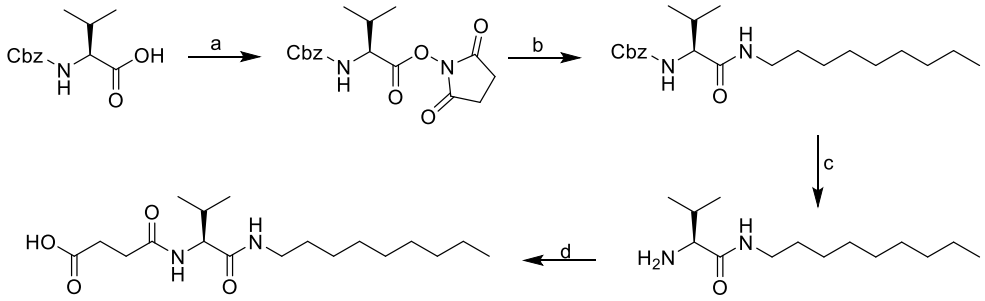
Experimental Section

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 ($DMSOd_6$ 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.

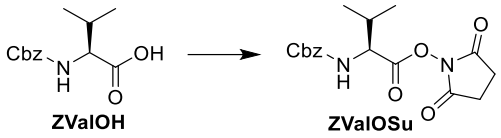
Mass spectra were run by the electro-spray mode (ESMS). Masses spectra were recorded at Mass Spectrometry triple Quadrupole Q-TOF Premier (Waters) with simultaneous Electrospray and APCI Probe.

5.2 Synthesis of SucValNon



Scheme 5.1 Reagents and conditions: a) *DCC*, *N*-hydroxisuccinimide, THF, 0 °C, 1 h, 84 %; b) *n*-nonylamine, THF, overnight, 50 °C, 77 %; c)) Pd/C, H_2, MeOH, 2 h, 85 %; d) Succinic anhydride, K_2CO_3, THF, overnight, 70 %.

5.2.1 Synthesis of ZValOSu



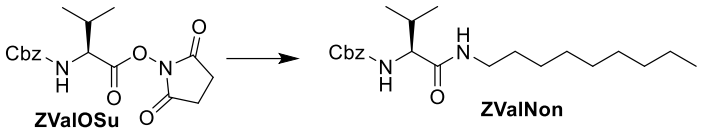
Scheme 5.2 Synthesis of ZValOSu

A solution of commercial available carbobenzyloxy-*L*-Valine and *N*-hydroxysuccinimide (1.0 eq.) in dry THF (150 mL) was added dropwise under N_2 at 0 ºC with a dropping funnel to a solution of *N,N*'-dicyclohexylcarbodiimide (1.01 eq.) in dry THF (75 mL). The mixture was further stirred for 1 h at 0 ºC. The solution was then allowed to stand into refrigerator for 2 h, which caused precipitation of *N,N'*-dicyclohexylurea. After this time, the mixture was filtered under vacuum, and the filtrate was removed under reduced pressure and the crude residue was purified by crystallization in isopropanol to yield the respective activated ester.

2,5-dioxopyrrolidin-1-yl ((benzyloxy)carbonyl)-L-valinate (**ZValOSul**): A white solid was obtained (11,69 g, yield 84%); the NMR spectra were consistent with those described in the literature.[1]

^{1}H NMR (DMSO-*d*$_6$): δ 8.04 (s, 2H), 7.36 (t, 4H), 7.30 (s, 1H), 5.09 (t, 4H), 4.35 (m, 1H), 2.72 (d, 6H), 2.27–2.11 (), 1.09 – 0.93 (m, 6H).

5.2.2 Synthesis of ZValNon



Scheme 5.3 Synthesis of ZValNon

A solution of carbobenzyloxy-*L*-amino ester activated (ZValOSu, 1.0 eq.mmol) in THF (25 mL) was added dropwise under N_2 at room temperature with a dropping

[1] J., Becerril; M., Bolte; M., I., Burguete; F., Galindo; E., García-España; S., V., Luis; J., F., Miravet. *J. Am. Chem. Soc*. **2003**, *125*, 6677 – 6686.

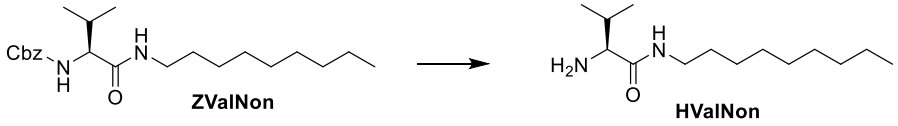
funnel to a solution of commercial available *n*-nonylamine (1.1 eq.) in THF (15 mL). The mixture wasfurther stirred for 5 h at 55 ºC. After this time, the mix was cooled to room temperature and solvent was removed under reduced pressure and the residue was poured into dissolution aq. HCl 0.1 M, then the mix was sonicated during 5 minutes. It was filtered under vacuum, and the residue was washed with water until pH = 7. The residue was dried under reduced pressure at 50ºC overnight.

Benzyl (S)-(3-methyl-1-(nonylamino)-1-oxobutan-2-yl)carbamate (**ZValNon**): A white solid was obtained (1,53 g, yield 77%).

^{1}H NMR (DMSO–*d*$_6$): δ 7.86 (t, *J* = 3 Hz, 1H), 7.41–7.27 (br s, 5H), 7.19 (d, *J* = 9 Hz, 1H), 5.03 (s, 2H), 3.79 (t, *J* = 9 Hz, 1H), 3.16–2.93 (m, 2H), 1.93 (m, 1H), 1.37 (s, 2H), 1.29 (m, 2H), 1.24 (s, 12H), 0.86 (d, *J* = 6 Hz, 9H).

^{13}C NMR (DMSO-*d*$_6$): δ 170.8, 156.0 (C=O), 137.1, 128.2, 127.7, 127.5 (CH), 65.3 (CH_2), 60.3 (CH), 38.3 (CH_2), 31.2 (CH), 30.2, 28.9, 28.9, 28.7, 28.6, 26.3, 25.2, 22.0 (CH_2), 19.1, 18.2, 13.9 (CH_3).

5.2.3 Synthesis of HValNon



Scheme 5.4 Synthesis of HValNon

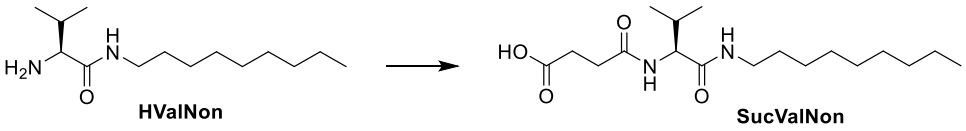
Palladium catalyst (20% *w/w*) was suspended in MeOH (30 mL) and stirred under H_2 at room temperature for 10 min. Subsequently, a solution of ZValNon (500 mg) in MeOH (10 mL) was added via syringe, followed by stirring under H_2 at room temperature for 2 h. The reaction mixture was then filtered through Celite®, and the solvent was removed under reduced pressure to yield respective amine.

*(S)-2-amino-3-methyl-N-nonylbutanamide (**HValNon**):* White solid was obtained (250 mg, yield 85%). The compound was used in crude form for the next reaction.

^{1}H NMR (DMSO – *d*$_6$): δ 7.75 (t*, J* = 3.5 Hz, 1H), 3.14–2.93 (m, 2H), 2.88 (d, *J* = 5 Hz, 1H), 1.84 (m, 1H), 1.36 (t, *J* = 5 Hz, 2H), 1.30 (, 1H), 1.24 (s, 12H), 0.91–0.71 (d, 3H).

^{13}C NMR (DMSO-*d*$_6$): δ 174.2 (C=O), 60.0 (CH), 39.6 (CH_2), 31.6 (CH), 31.2, 29.2, 29.0, 28.6, 26.3, 22.1 (CH_2), 19.4, 17.1, 13.9 (CH_3).

5.2.4 Synthesis of the final compound SucValNon



Scheme 5.5 Synthesis of SucValNon

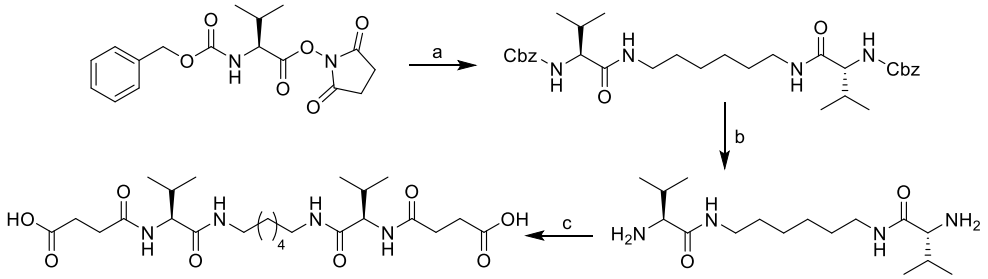
A solution of respective **HValNon** (1 eq.) in THF (50 mL) was treated at 0 ºC under N_2 with solid K_2CO_3 (3.8 eq.). The mixture was stirred for 15 minutes at 0 ºC, after with a dropping funnel to a solution of commercial available succinic anhydride (2.0 eq.) in THF (20 mL). The mixture was further stirred vigorously for 16 h 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 a white precipitate to pH = 4. 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.

*(S)-4-((3-methyl-1-(nonylamino)-1-oxobutan-2-yl)amino)-4-oxobutanoic acid (**SucValNon**):* A white solid was obtained (304 mg, yield 70%) as a white solid.

^{1}H NMR (DMSO–*d6*): δ 12.06 (s, 1H), 7.83 (t, *J* = 10 Hz, 1H), 4.07 (t, *J* = 10 Hz, 1H), 3.18–2.87 (m, 1H), 2.44–2.36 (m, 2H), 1.93 (m, 1H), 1.47–1.35 (m, 1H), 1.23–1.14 (s, 12H), 0.88 (t, *J* = 5 Hz, 3H), 0.81 (d, *J* = 5 Hz, 6H).

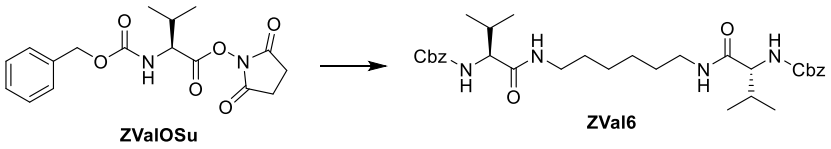
^{13}C NMR (DMSO-*d6*): δ 174.3 (COOH), 171.4, 171.2 (C=O), 58.3 (CH), 41.8, 40.4, 40.1, 40.0, 39.9, 39.6, 39.3, 38.7, 31.7, 30.9, 30.4, 29.8, 29.2, 26.8, 22.5 (CH_2), 19.6, 18.6, 14.3 (CH_3).

5.3 Synthesis of SucVal6



Scheme 5.6 Reagents and conditions: a) 1,6-Hexanediamine, THF, N_2, 50 °C, overnight, 99%; b) Pd/C, H_2, MeOH, 3 h, 71%; c) Succinic anhydride, K_2CO_3, THF, overnight, 37%.

5.3.1 Synthesis of ZVal6



Scheme 5.7 Synthesis of ZVal6

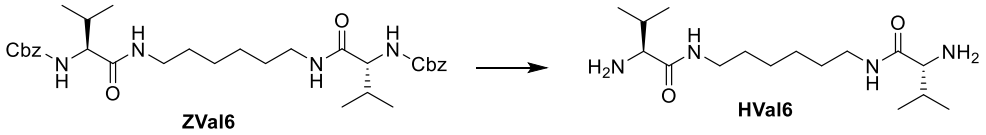
ZVal6, was obtained following the same procedure for the synthesis of ZValNon , except that two equivalents of ZValOSu was used by one equivalent of 1,6-diamine hexane.

Benzyl ((5R,16S)-5-isopropyl-17-methyl-3,6,15-trioxo-1-phenyl-2-oxa-4,7,14-triazaoctadecan-16-yl)carbamate ***(ZVal6)**:* A white solid was obtained (4,10 g, yield 99%) as a white solid.

^{1}H NMR (DMSO–*d*$_6$): δ 7.86 (t, *J* = 5.3 Hz, 1H), 7.42 -7.20 (m, 5H), 7.18 (d, *J* = 8.9 Hz, 1H), 5.02 (s, 2H), 3.86 – 3.69 (m, 1H), 3.03 (ddd, *J* = 19.3, 13.4, 6.7 Hz, 2H), 1.90 (dt, *J* = 13.4, 6.7 Hz, 1H), 1.41 – 1.35 (m, 2H), 1.34 – 1.23 (m, 2H), 0.83 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (DMSO-*d6*): δ 170.9, 156.1 (C=O), 137.1 (C), 128.3, 127.7, 127.6(CH), 65.3 (CH_2), 60.3 (CH), 38.3, 30.2, 28.9, 26.0 (CH_2), 19.2, 18.2 (CH_3).

5.3.2 Synthesis of HVal6



Scheme 5.8 Synthesis of HVal6

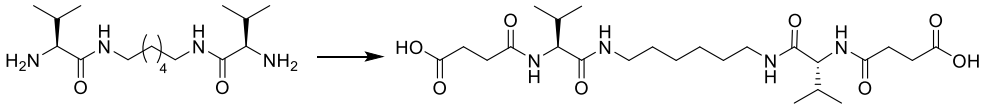
HVal6, was obtained following the same procedure for the synthesis of HValNon.

(S)-2-amino-N-(6-((R)-2-amino-3-methylbutanamido)hexyl)-3-methylbutanamide ***(HVal6)****:* A white solid was obtained (2,35 g, yield 71%) as a white solid. The compound was used in crude form for the next reaction.

^{1}H NMR (DMSO–*d6*): δ 7.76 (t, *J* = 5.4 Hz, 1H), , 3.04 – 2.90 (m, 2H), 2.88 (d, *J* = 5.3 Hz, 1H), 1.96 – 1.80 (m, 1H), 1.55 – 1.21 (m, 2H), 1.21 – 1.12 (m, 2H), 0.85 (d, J = 6.8 Hz, 3H), 0.77 (d, *J* = 6.8 Hz, 6H). The amines signals (-NH_2) were very broads and cannot distinguish in the spectrum.

^{13}C NMR (DMSO-*d6*): δ 174.4 (CO), 48.6 (CH), 38.1, 31.6, 29.2, 26.1 (CH_2), 19.5, 17.1 (CH_3).

5.3.3 Synthesis of the final compound SucVal6



Scheme 5.9 Synthesis of SucVal6

SucVal6, was obtained following the same procedure for the synthesis of SucValNon , except that double of equivalents of succinic anhidre and K_2CO_3 was used by one equivalent of HVal6.

(6R,17S)-6,17-diisopropyl-4,7,16,19-tetraoxo-5,8,15,18-tetraazadocosanedioic acid (***SucVal6***). A white solid was obtained (917 mg, yield 37%) as a white solid.

^{1}H NMR (DMSO_*d*$_6$): δ 12.04 (s, 1H), 7.91 – 7.83 (m, 2H), 4.07 (dd, *J* = 8.6, 7.1 Hz, 1H), 3.01 (dtt, *J* = 25.7, 12.8, 6.6 Hz, 2H), 2.47 – 2.27 (m, 4H), 1.92 (dq, *J* = 13.5, 6.7 Hz, 1H), 1.37 (dd, *J* = 13.8, 6.7 Hz, 1H), 1.19 (dt, *J* = 17.1, 5.8 Hz, 1H), 0.81 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (DMSO-*d6*): δ 174.4, 171.5, 171.2 (C=O), 58.3 (CH), 38.8, 30.9, 30.4, 29.7, 29.1, 24.2 (CH_2), 19.67, 18.59 (CH_3).

5.4 Experimental method for determination of MGC

A stock dissolution of MOH 0.1 M (M= Li, Na or K) was prepared; the concentration of cation (M^+) was adjusted by the addition of solid MCl.

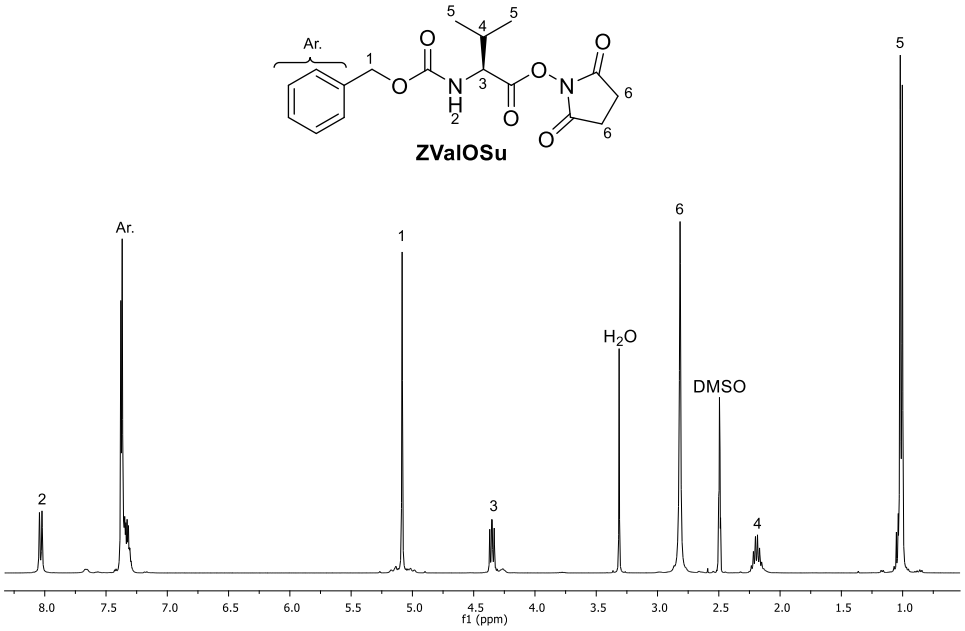
In a typical experiment, 20 mg of SucValDoc and 1 mL of stock dissolution were introduced into a cylindrical screw-capped glass vial (8 mL, diameter =1.5 cm). The system was heated up with heat air to 100ºC with a heat gun. Once the solid was dissolved, the system was cooled by immersion into a water bath at 25ºC for 30 minutes. Gel formation was checked with the inverted vial test.

ANNEX

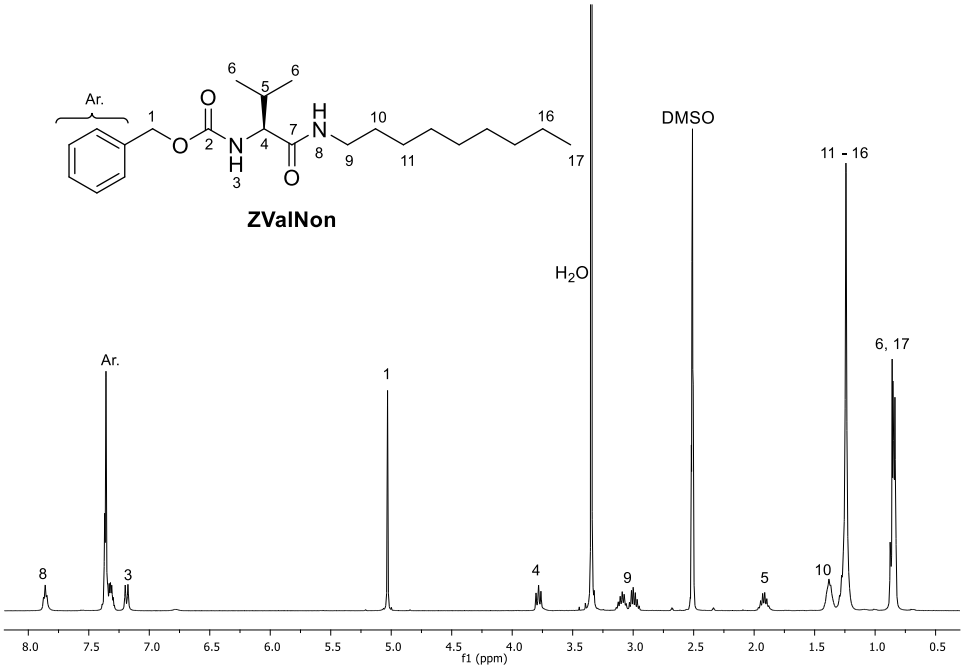
Annex

6.1 NMR spectras

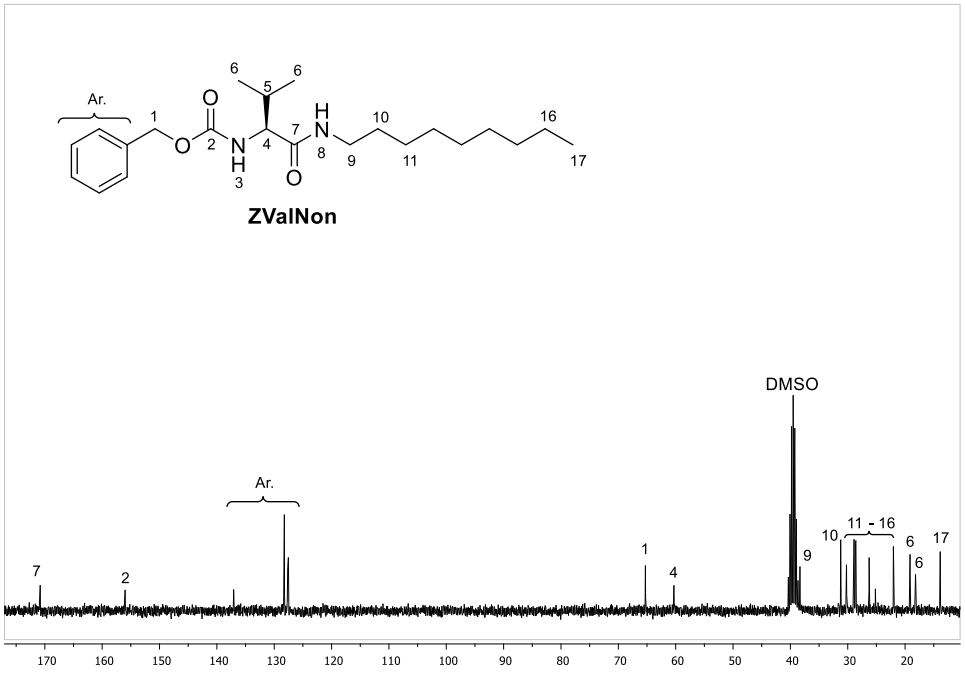
ZValOSu ^{1}H NMR spectrum



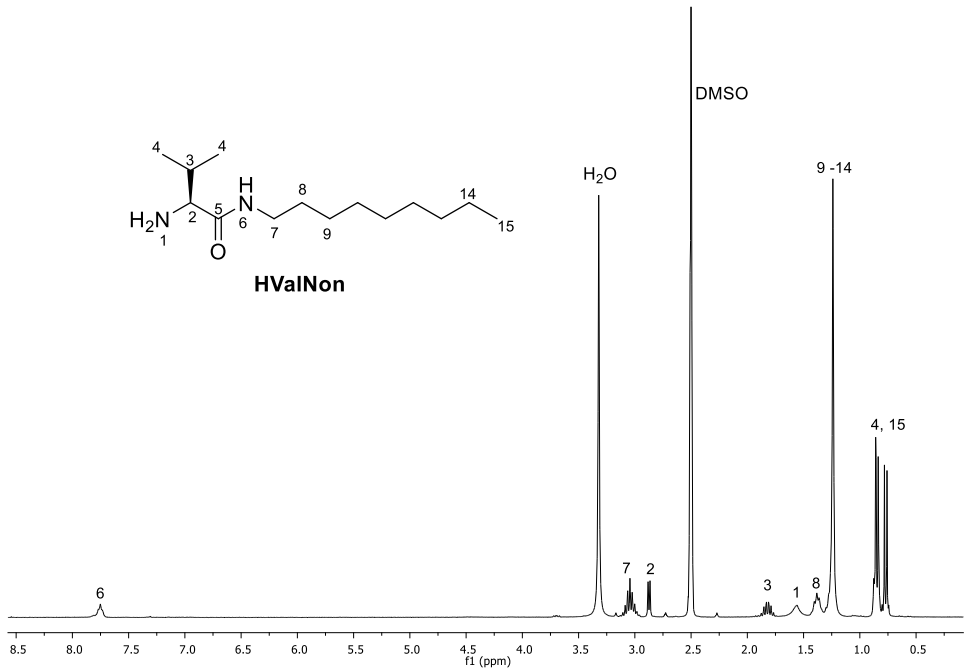
ZValNon ^{1}H NMR spectrum



ZValNon ^{13}C NMR spectrum

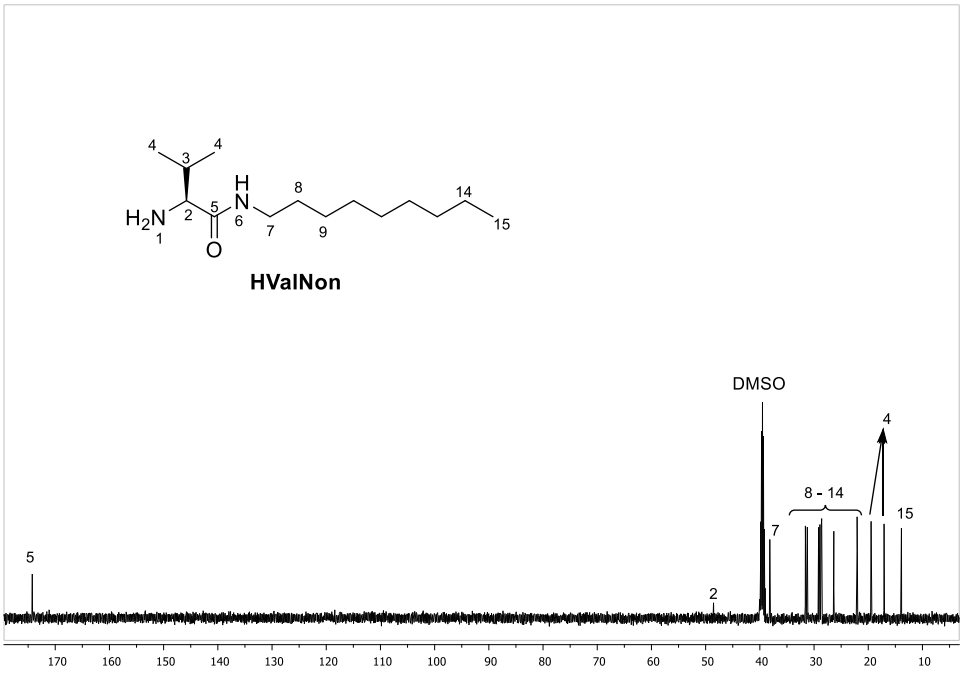


HValNon ^{1}H NMR spectrum

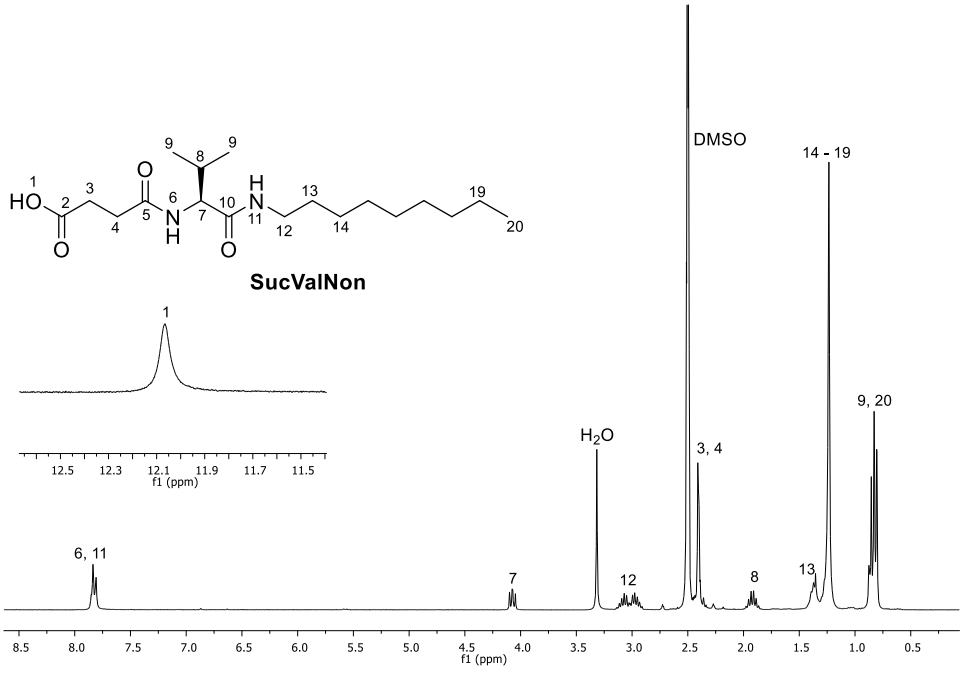


HValNon

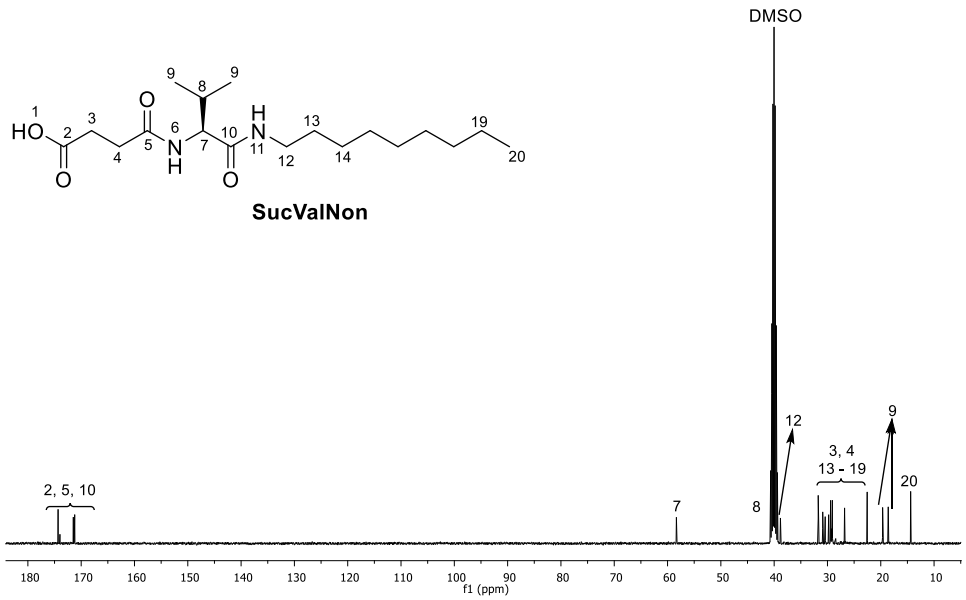
HValNon ^{13}C NMR spectrum



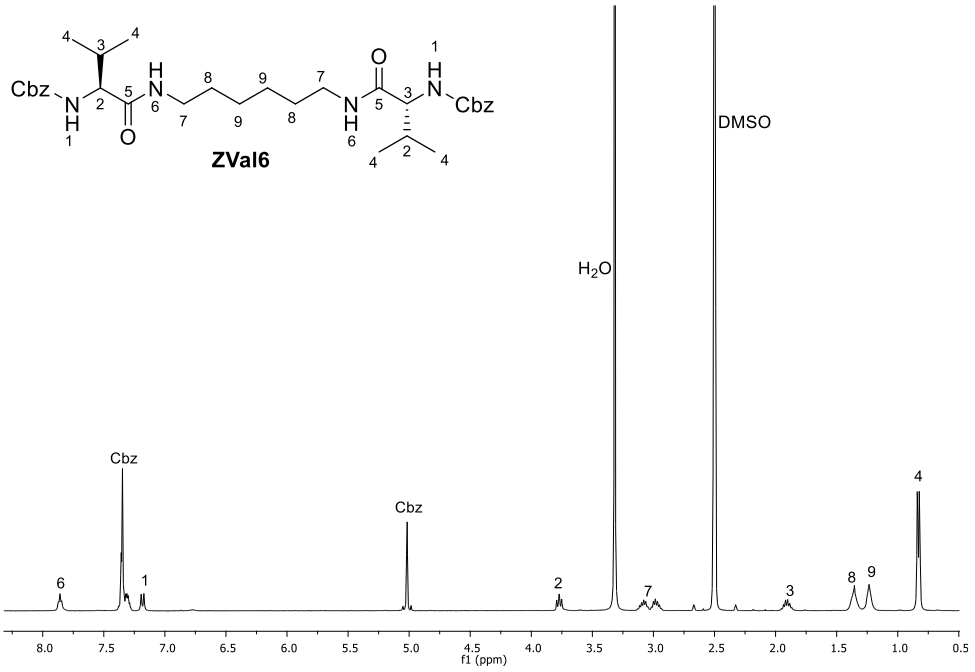
SucValNon ^{1}H NMR spectrum



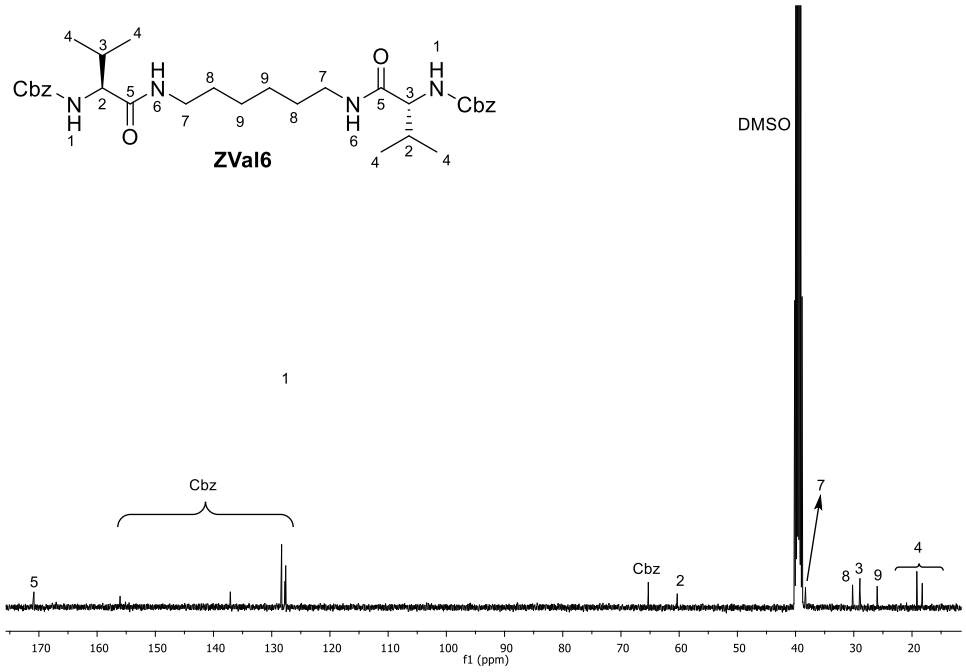
SucValNon ^{13}C NMR spectrum



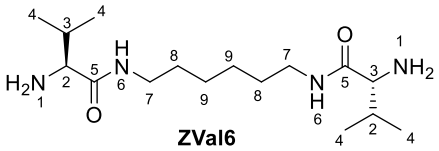
ZVal6 ^{1}H NMR spectrum



ZVal6 ^{13}C NMR spectrum

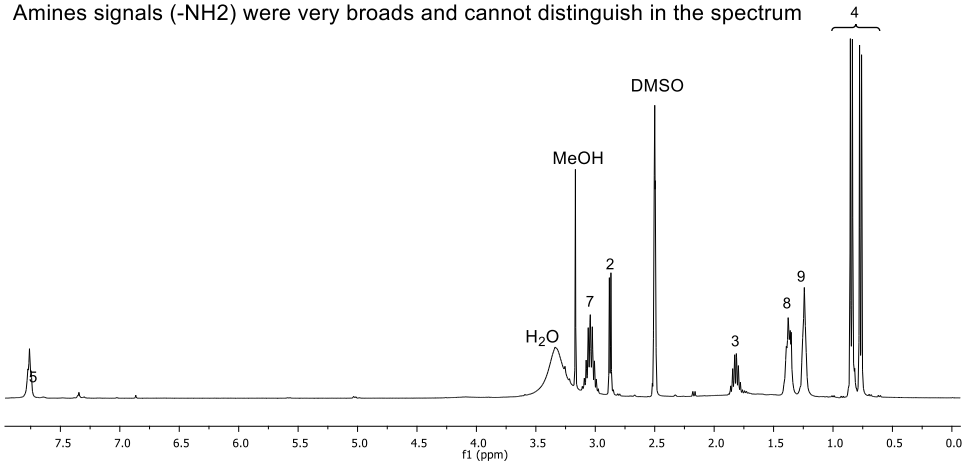


HVal6 ^{1}H NMR spectrum

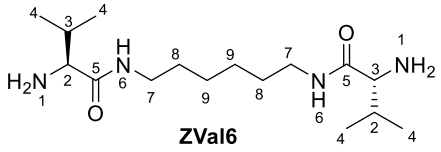


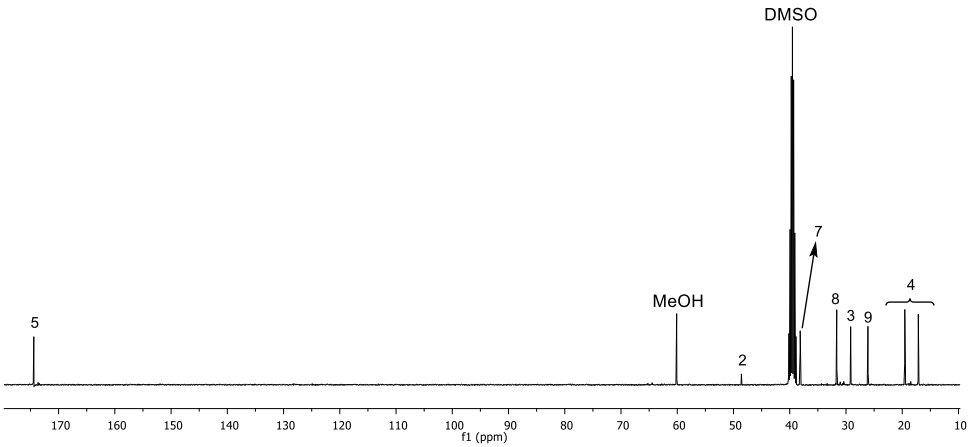
ZVal6

Amines signals (-NH2) were very broads and cannot distinguish in the spectrum

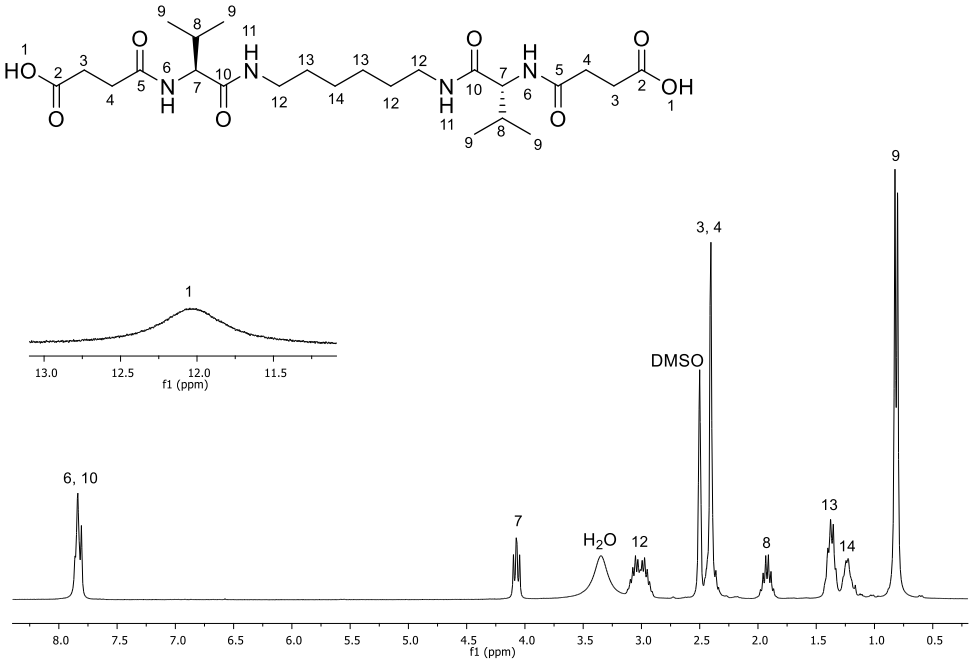


HVal6 ^{13}C NMR spectrum

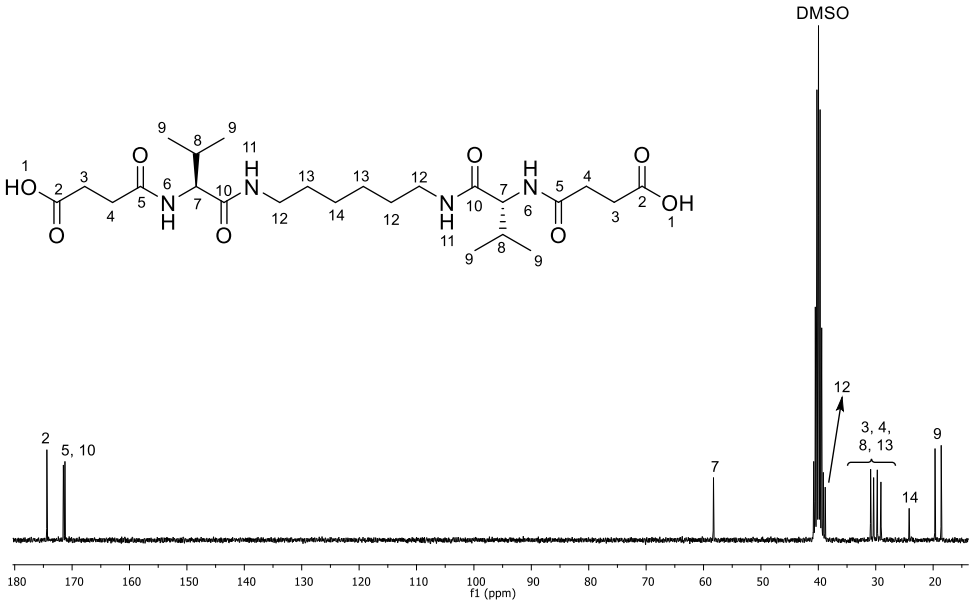




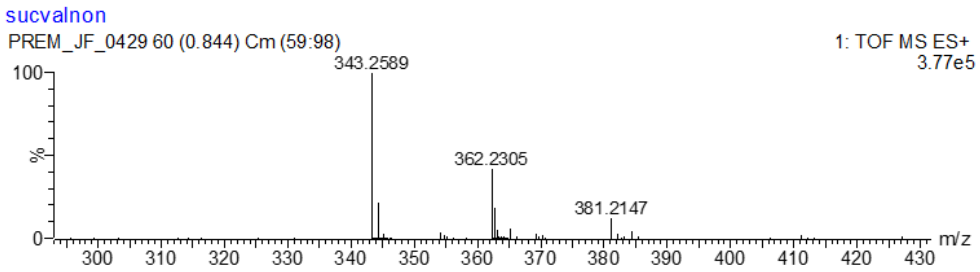
SucVal6 ^{1}H NMR spectrum

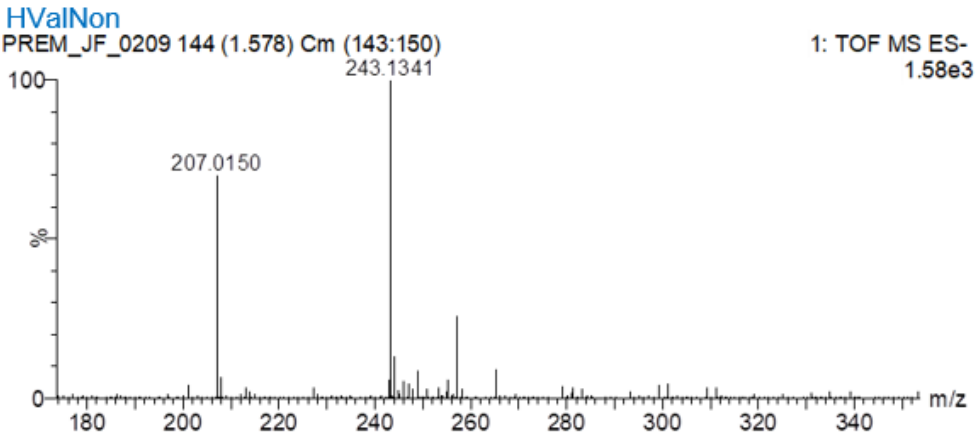


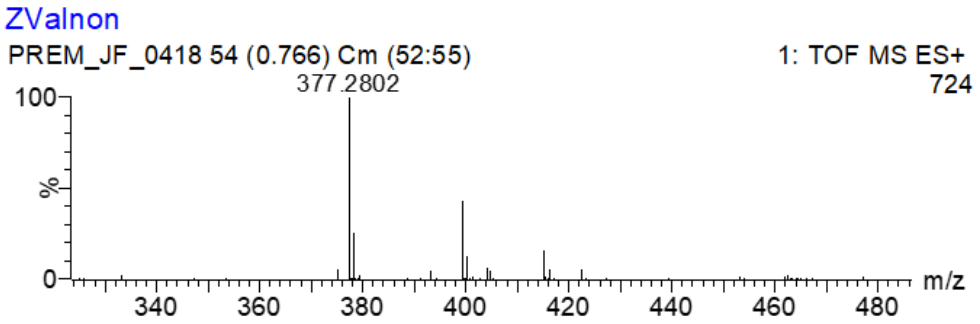
SucVal6 ^{13}C NMR spectrum



6.2 Mass sepctometry







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