Escuela Superior de Tecnología y Ciencias Experimentales

Departamento de Química Inorgánica y Orgánica

Group of Organic Molecular Nanomaterials with Biomedical Applications

SYNTHESIS, ACID-BASE PROPERTIES AND HYDROGEL FORMATION STUDIES OF A BIS-QUINOLINE BASED LOW MOLECULAR WEIGHT GELATOR

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CHEMISTRY DEGREE RESEARCH PROJECT

JULY 2018

El Dr. Juan Felipe Miravet Celades, Catedrático del Departamento de Química Inorgánica y Orgánica de la Universitat Jaume I de Castellón de la Plana,

CERTIFICA

Que el trabajo fin de grado, con el título "***SYNTHESIS, ACID-BASE PROPERTIES AND HYDROGEL FORMATION STUDIES OF A BIS-QUINOLINE BASED LOW MOLECULAR WEIGHT GELATOR",*** ha sido realizado por Lorena Nicoleta Grigorescu bajo su dirección, en el grupo Organic Molecular Nanomaterials with Biomedical Applications del Departamento de Química Inorgánica y Orgánica de la Universitat Jaume I de Castellón de la Plana.

Lo que certifico a los efectos oportunos en Castellón de la Plana a 2 de Julio de 2018.

Fdo. Dr. Juan F. Miravet Celades

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Finalmente a ti, Diego, por inspirarme a crecer y a mejorar, por ayudarme a alcanzar todos los retos que la vida me pone y por ser el mejor compañero, gracias.

Abbreviations

Cbz	Benzyloxycarbonyl
COCl	Oxalylchloride
COSY	2D correlation spectroscopy
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCU	*N,N'*-Dicyclohexylurea
DMF	*N,N'*-Dimethylformamide
DMSO	Dimethyl sulfoxide
Et_3N	Triethylamine
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum coherence
HRMS	High resolution mass spectrometry
HVal3	*N,N'*-(propane-1,3-diyl)bis(2-amino-3-methylbutanamide)
LMWGs	Low molecular weight gelators
mgc	minimum gelator concentration
NHS	N-hydroxysuccinimide
NMR	Nuclear magnetic resonance
QuiCl	Quinoline-4-carbonyl chloride
QuiOH	Quinoline-4-carboxylic acid
rt	Room temperature
THF	Tetrahydrofuran
TMS	Trimethylsilane
Val	L-Valine
ZValOH	Carbobenzyloxy-L-valine acid

ZValOSu	(S)-2,5-dioxopyrrolidin-1-yl((benzyloxy)carbonyl)-L-valinate
ZVal3	Dibenzyl ((propane-1,3-diylbis(azanediyl))bis(3-methyl-1-oxobutane1,2-diyl))dicarbamate
wt	Weight

Index

Chapter 1

Introduction

1 Chapter: Introduction

1.1 What are gels?

Gels are soft solids that are common in everyday life and their applications can be found in a great variety of forms. Their properties, like flexibility, malleability and storage capacity, make them suitable for different fields such as medicine, cosmetics, materials science, pharmacology as well as food or electronics.

Although gels have been recognised and studied since the 1860's, it is still difficult to find a proper definition because of their variety and complexity. In 1926, Dorothy Jordon Lloyd stated that "the gel, is one which is easier to recognize than to define" and made the following statement:[1]

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

Thenceforth, many different definitions have been made. One of the most comprehensive was stated by Flory in 1974. He defined a gel as a colloid system of at least two components with two different features:

i. An essential characteristic of a gel is its solid-like behaviour. When deformed its response is that of an elastic body.

ii. Gels must possess a continuous structure, being of macroscopic dimensions, which must be permanent at least for a period of time while the experiment lasts.[2]

[1] Lloyd, D. J. *Colloid Chem.* **1926**, *1*, 767.
[2] Flory, P. J. *Faraday Discuss. Chem. Soc.* **1974**, *57*, 7.

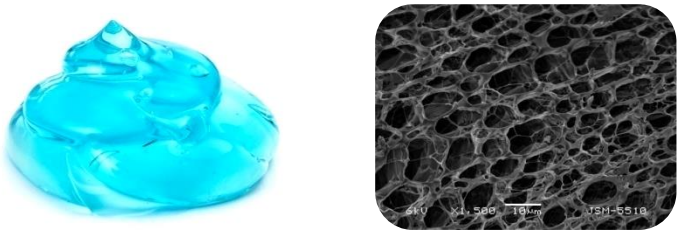


Figure 1-1. Solid-like appearance (left) and field emission scanning electron microscopy image showing the continuous structure of a gel (right).

Therefore, a gel consists of two or more components, one of which is a liquid and the other one is a small amount of solid (0.1-1 wt%) named gelator. The gelator is able to immobilize a large volume of solvent, i.e., the major one. And the solid-like appearance of a gel is a result of the entrapment and adhesion of the solvent in the network formed by the gelator (Figure 1-1).

1.2 Types of gels

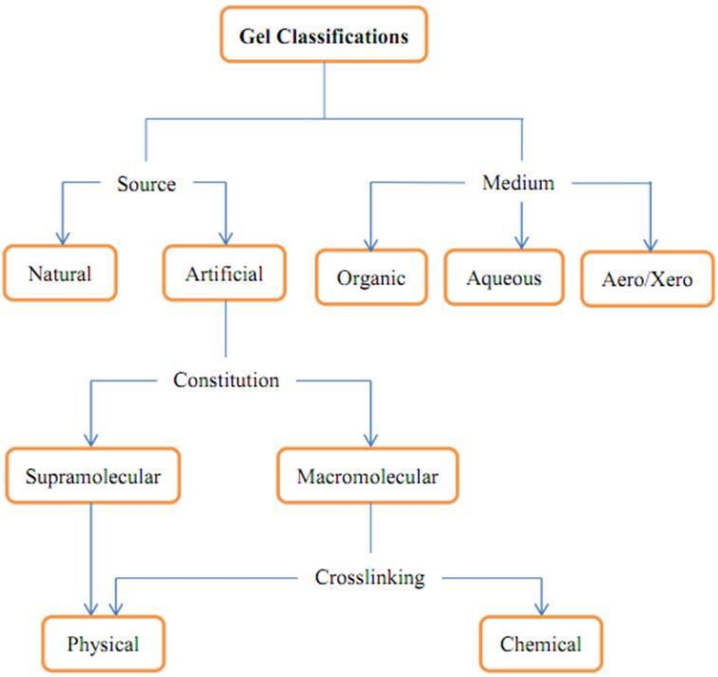
Gels can be classified in different ways depending on their source, the medium they hold, constitution or type of interaction that creates their 3D network (Scheme 1-1).[3]

If we classify them according to their constitution, they can be polymeric gels which are formed by the cross-linking of macromolecules or molecular gels which are formed by small organic compounds called low molecular weight gelators (LMWGs).

Respect to the medium, if it is an organic solvent the gels are called organogels, however if the solvent is water they are called hydrogel. In an aerogel and xerogel the solvent have been removed. In the case of the aerogel, the liquid phase of a gel is replaced by a gas, however in the case of xerogels the liquid has been evaporated. Finally, gelators which are capable of hardening both organic solvents as well as water are termed as amphoteric.

[3] Sangeetha, N. M.; Maitra, U. *Chem. Soc. Rev.* **2005**, *34*, 821.

On the other hand, according to the type of crosslinking, the gels can be chemical or physical. Chemical gels are crosslinked covalently, resulting in a permanent network structure unless the covalent crosslinks are broken, therefore, the gelation is irreversible. Physical gels present a non-covalent network links consisting in a combination of interactions such as hydrogen bonding, π-π stacking or van der Waals, and hence, the formation of physical gels is usually thermoreversible.[3]



Scheme 1-1. Classification of gels.

1.3 Low molecular weight gels

A low molecular weight gel can be defined as a physical gel which is formed by small organic compounds (LMWGs) with a molecular weight of less than 2000 Da, that is capable of gelling organic solvents or water even at extremely low concentration.[4]

[4] Zweep, N.; van Esch, J. H. *Funct. Mol. Gels* **2014**, No. 1, 1.

Molecular gels or supramolecular gels, have the following three main properties:

i. The assembly is completely reversible and can be regulated by stimuli giving rise to responsive soft materials,

ii. The process of self-assembly implies an accurate organization determined by the structure and functionalities of the individual molecules,

iii. In general, molecular gels are more biodegradable than polymeric or macromolecular gels due to being formed by molecules of low molecular weight.

With respect to the first, as physical gel, the network is formed by weak noncovalent interactions. Usually the self-assembly of the gelator molecules is not the result of only one type of noncovalent interaction but rather a combination of different interactions (Table 1-1). These interactions differ from a covalent bond in a way that the energy released in their formation is usually <40 kJ/mol, which is very less in comparison to covalent bond energies.

Table 1-1. Supramolecular interactions.

Supramolecular Interactions (kJ/mol)	
Hydrogen bond	12-30
Hydrophobic	<40
Electrostatic	~20
Van der Waals	0.4-4
π-π stacking	<5

Due to physical interactions, the LMWG network can be reversed by the input of energy (for example, by heating) making them thermoreversible, i.e., they can be cycled repeatedly with their pregelation (sol) phases. Moreover, a gel can be reversed by means of chemical stimuli such a pH, redox or physical stimuli as electric or magnetic fields (Figure 1-2).[5]

[5] Segarra-Maset, M. D.; Nebot, V. J.; Miravet, J. F.; Escuder, B. *Chem. Soc. Rev.* **2013**, *42*, 7086.

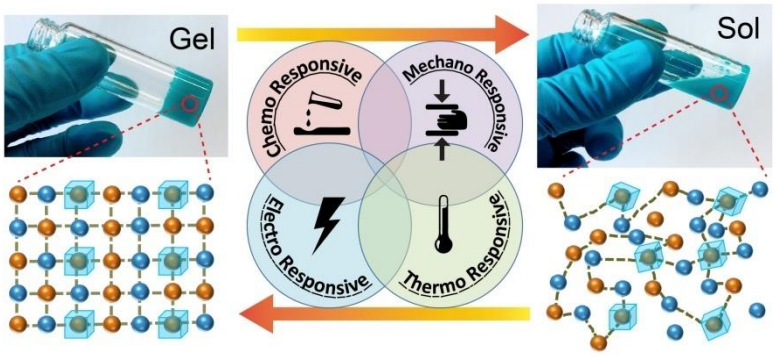


Figure 1-2. Reversible conversion of a gel subject to physical and chemical stimuli.[6]

With respect to the second property, the gelation process requires that the individual molecules acquire an adequate conformation that allows the generation of the gel. Because of this, molecules are well organized in a preferred direction, leading to highly organized supramolecular materials. Moreover, the properties of the gels are extremely process dependent, which means that it is possible to access materials with very different properties from a single gelator.[7]

With respect to the latter, property the use of natural compounds such as amino acids sugars and fatty acids as starting materials make molecular gels biocompatible. Furthermore, the presence of weak physical interactions makes molecular gels easily biodegradable, and hence non-cytotoxic, unlike their polymeric counterparts where C-C covalent bonds are strong.[8]

1.3.1 Process of assembly

Molecular gels are formed from the self-assembly of small molecules in long anisotropic structures, such as, tapes, tubes and helical structures but most commonly fibers. At a sufficiently high concentration, these fibers become

[6] Chaudhari, A. K.; Han, I.; Tan, J. C. *Adv. Mater.* **2015**, *27*, 4438.
[7] Singh, N.; Kumar, M.; Miravet, J. F.; Ulijn, R. V.; Escuder, B. *Chem.- A Eur. J.* **2017**, *23*, 981.
[8] Skilling, K. J.; Citossi, F.; Bradshaw, T. D.; Ashford, M.; Kellam, B.; Marlow, M. *Soft Matter* **2014**, *10*, 237.

entangled or cross-linked, which creates the network that is able to immobilize the solvent through surface tension and capillary forces. However, developing LMWGs is still a difficult task. It is complicated to predict which solvent will be hardened by a particular LMWG, i.e., whether a molecule will form a gel or not; indeed, gelation has been described as an empirical science.[9]

In general terms, a molecular gel is formed by solubilizing or dispersing a molecule, and then triggering gel formation (Figure 1-3).

It is necessary to initially have the molecule in a solvated solution state. This can be achieved, for example, by heating to improve the solubility, dissolving initially in a good solvent or choosing a pH in which the molecule is soluble. Then by cooling, adding an anti-solvent or adjusting the final pH to one the which the molecules start to self-assemble with each other generating the network that is able to form the gel.

Other ways could be to use a progelator with a cleavable solubilizing group, or use a chemical or enzymatic reaction to generate the gelator in situ from suitable precursors.

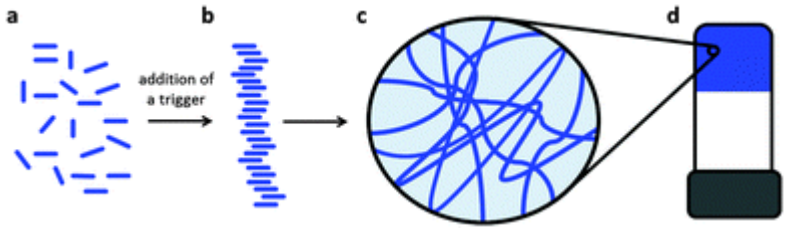


Figure 1-3. The assembly of a LMWG into fibers, resulting in a network and gel formation. (a) LMWG dissolved in solution (b) self-assembly of LMWG starts to occur after application of a trigger (c) formation of the gel network by entanglement (d) a self-supporting bulk gel.[10]

[9] Weiss, R. G. *J. Am. Chem. Soc.* **2014**, *136*, 7519.
[10] Draper, E. R.; Adams, D. J. *Chem.* **2017**, *3*, 390.

1.3.2 Applications

LMWGs started to have great interest in the last two decades, because of their applications and their morphological and physical properties. For a long time, molecular gels have been employed in relatively low-cost bulk applications, such as lubricants, grease and personal care products. However, with a more clear understanding of nanoscale self-assembly processes, gelators can be designed to use in more high-tech applications such as catalysis, controlled drug release, cell growth, tissue engineering, nanoelectronics and environmental detection.

Herein, some of the most outstanding applications like drug delivery and tissue engineering are explained.

1.3.2.1 Drug delivery

In recent years, LMWGs have emerged as new carriers for therapeutic agents. Developing new drug delivery methods is paramount to improving the therapeutic efficacy of drugs that are limited by poor solubility, low bioavailability and short plasma half-life.

There are three strategies for drugs delivery using LMWGs:

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

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

iii. A functionalized linker can be conjugated covalently with a therapeutic, after the enzymatic cleavage of part of the linker the amphiphilic prodrug can self-assemble.[8]

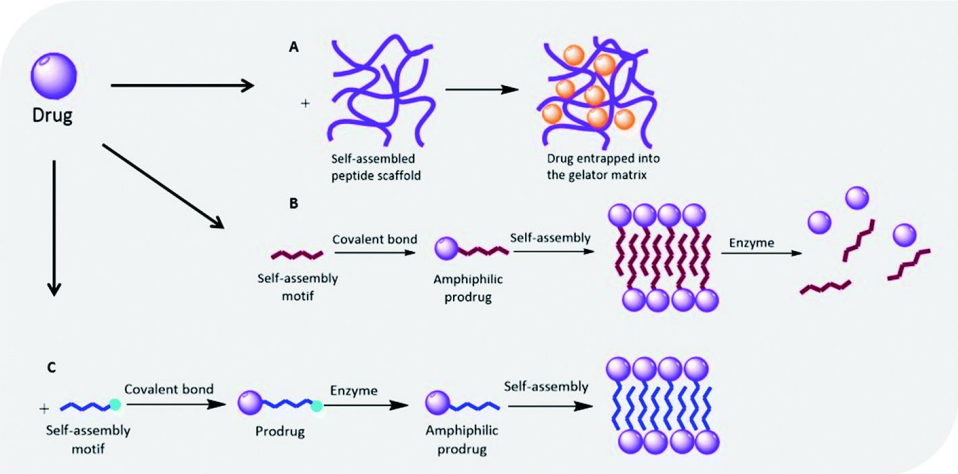


Figure 1-4. Strategies for drugs delivery using LMWGs.

1.3.2.2 Tissue engineering

This area of biomedical technology uses gels prepared from small molecules to create a platform to mediate the growth of tissues and organs for regenerative medicine. Indeed, hydrogels have been extensively studied in the field of tissue engineering and have been found to have many potential applications.[11]

Molecular gelators presenting increased mechanical strength, good biocompatibility and ease of manipulation are suitable platforms on which to culture cells. In addition to the ability of a gel structure to act as a relatively inert matrix, it is also possible to modify self-assembling molecules containing biologically active motifs. In this way, small molecule hydrogels can be designed to mimic natural tissues extracellular matrix (ECM).

The Ulijn group reported the design of a biomimetic nanofibrous hydrogel as a 3D-scaffold for anchorage-dependent cells. To form it, Fmoc-FF (Fluorenylmethoxycarbonyl-diphenylalanine) was mixed with Fmoc-RGD (arginine-glycine-aspartate), which are the simplest self-assembling moieties. Hence, this hydrogel provides a highly hydrated, stiff and nanofibrous network that uniquely

[11] Zhou, M.; Smith, A. M.; Das, A. K.; Hodson, N. W.; Collins, R. F.; Ulijn, R. V.; Gough, J. E. *Biomaterials* **2009**, *30*, 2523.

presents bioactive ligands at the fibre surface; therefore it mimics certain essential features of the extracellular matrix.[11]

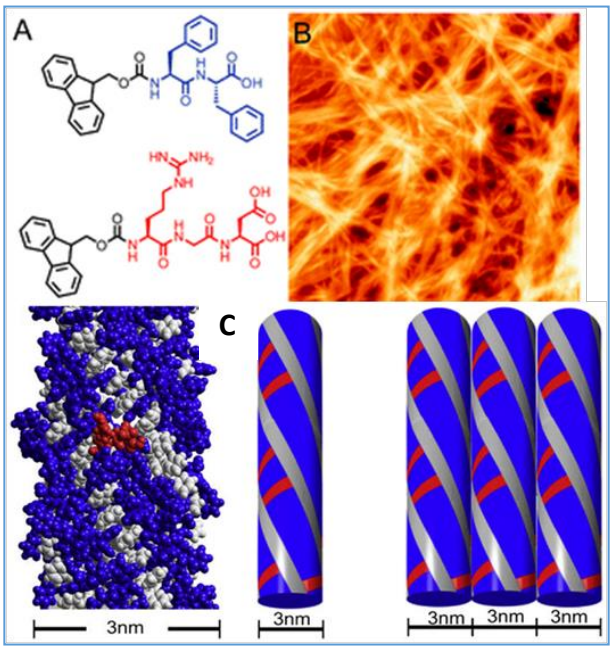


Figure 1-5. (A) The chemical structures of: Fmoc-FF and Fmoc-RGD. (B) AFM height image of self-assembled Fmoc-peptides. (C) The proposed supramolecular model demonstrates. The formation of the 3 nm fibrils of Fmoc-FF and Fmoc-RGD and their further lateral assembly into larger ribbons.

Chapter 2

Objectives

2 Chapter: Objectives

The research group, where this final degree project was carried out, was interested in the use of molecule **QVal3** (see structure below) as a hydrogelator which could be used for entrapment and release of bioactive substances and for the preparation of nano/microparticles. This compound is related to previouslyl reported structures prepared in the group. As a first step towards that goal, this project aims to optimize the synthetic preparation of **QVal3** and study its hydrogel formation properties.[12, 13]

In detail the objectives are the following:

i. The synthesis and characterization of **QVal3**. In particular, optimization of the amide formation step implied in its preparation.

ii. Determination of the minimum gelator concentration in aqueous media and evaluation of the influence of the concentration of DMSO present, in a small proportion due to gel preparation procedure, on the gelation efficiency.

iii. Evaluation of the acid-base constants of **QVal3** to assess the range of pH where the molecule is neutral and prone to aggregation. Likewise, the influence of different amounts of DMSO in the aqueous system on pKa values will be tested.

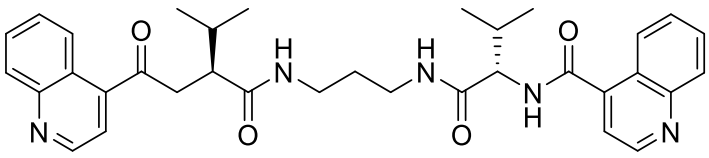


Figure 2-1. Structure of **QVal3**.

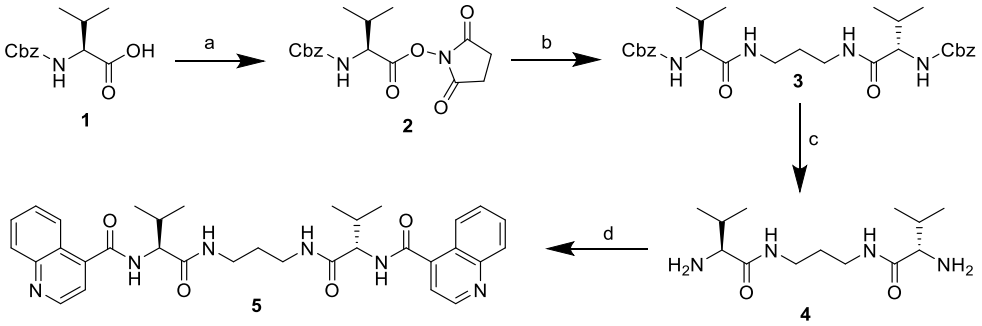
[12] Segarra-Maset, M. D.; Escuder, B.; Miravet, J. F. *Chem. - A Eur. J.* **2015**, *21*, 13925.
[13] Nebot, V. J.; Ojeda-Flores, J. J.; Smets, J.; Fernández-Prieto, S.; Escuder, B.; Miravet, J. F. *Chem. - A Eur. J.* **2014**, *20*, 14465.

Chapter 3

Results and Discussion

3 Chapter: Results and Discussion

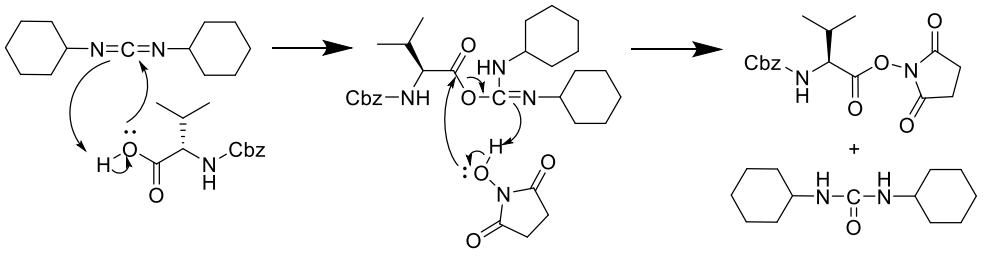
3.1 Synthesis of QVal3



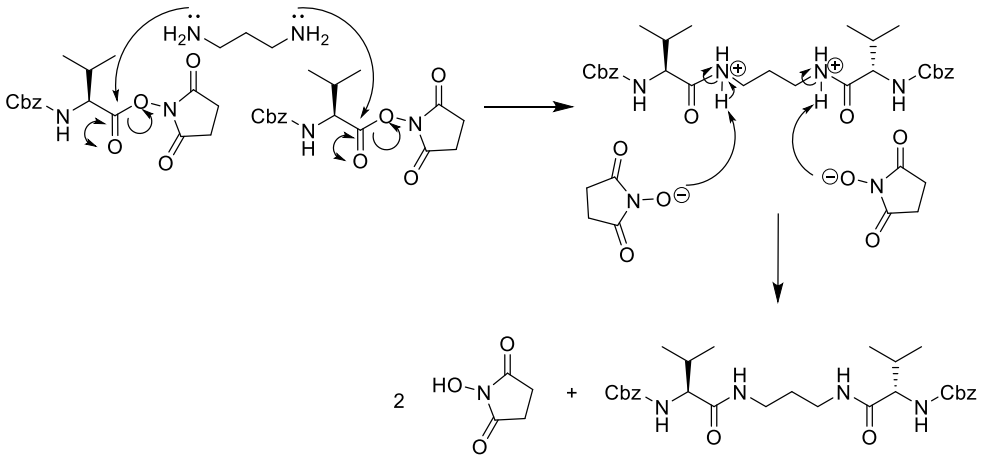
Scheme 3-1. Synthesis of **QVal3.**

Scheme 3-1 shows the total synthesis of **QVal3** and the following ones (Scheme 3-2 to 3-4 and Scheme 3-9 to 3-14) illustrate the mechanisms of each step in particular. The first step (step a) was the activation of Carbobenzyloxy-L-Valine with *N,N'*-dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (NHS) to produce the activated ester **2** (Scheme 3-2) as reported previously.[14] The second step (step b) consists of an aminolysis coupling of the activated ester with 1,3-diaminopropane to form two amide groups (Scheme 3-3), yielding the compound **3**. After that, the protecting Cbz group was eliminated by hydrogenolysis using Pd/C as a catalyst, obtaining the diamine **4** (Scheme 3-4). Finally, the diamine **4** and quinoline-4-carboxylic acid reacted to produce **5**, **QVal3**.

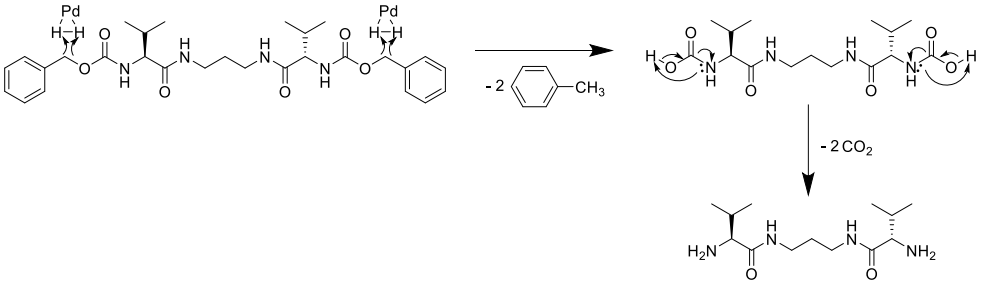
[14] Becerril, J.; Bolte, M.; Burguete, M. I.; Galindo, F.; Luis, S. V; Miravet, J. F. **2003**, No. 5, 6677.



Scheme 3-2. Mechanism of amino acid activation with DCC and NHS.



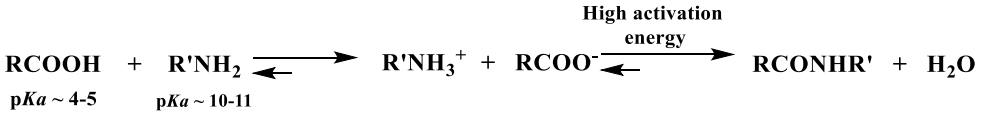
Scheme 3-3. Mechanism of formation of the peptide bond.



Scheme 3-4. Mechanism of hydrogenolysis (removal of the protection group).

3.1.1 Optimization of step d

The formation between a carboxylic acid and an amine to form the amide bond are as a rule, condensations. However, when mixing an amine with a carboxylic acid, an acid-base reaction occurs first to form a stable salt. Hence, the amide bond formation has to fight against a high kinetic barrier (Scheme 3-5).



Scheme 3-5. Acid-base equilibrium versus amide bond formation.

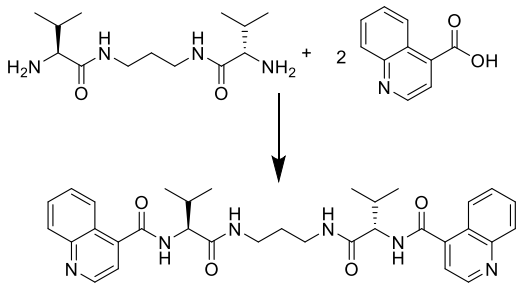
For this reason, the direct condensation of the salt can be achieved only at high temperature (160-180ºC), which is usually quite incompatible with the presence of other functionalities. Therefore, the activation of the acid, by binding of a leaving group to the acyl carbon, is necessary to allow the attack of the amino group (Scheme 3-6).

Scheme 3-6. Acid activation and aminolysis steps.

A plethora of methods and strategies have been developed to activate the acid in order to form the amide bond, for instance, as acyl halides, anhydrides, esters, etc. However, a variety of such conditions must be examined to find the method best suited to the situation that is required. For example, the optimum reaction should avoid racemization, optimize yield, reduce the amount of by-products, improve selectivity, facilitate final purification, exploit cheaper reagents and so forth.[15]

[15] Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827.

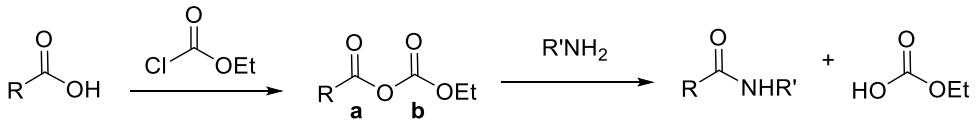
Herein, we aimed to perform the coupling reaction between valine derivative, HVal3, and quinoline-4-carboxylic acid (Scheme 3-7).



Scheme 3-7. Coupling reaction between HVal3 and quinoline-4-carboxylic acid.

This reaction was not previously studied, so that, different procedures were performed in order to optimize this final step.

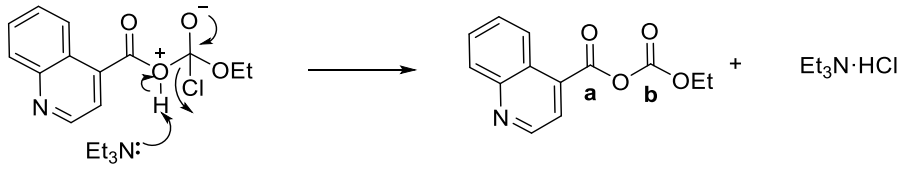
i) The first procedure (Procedure A, in Experimental Section) consisted in the formation of a carbonic anhydride. This strategy is based on the different chemical nature of both reactive centers. Generally, the electrophilic carboxylic center **a** is more reactive than the carbonate site **b** because it is less stabilized by resonance (Scheme 3-8).



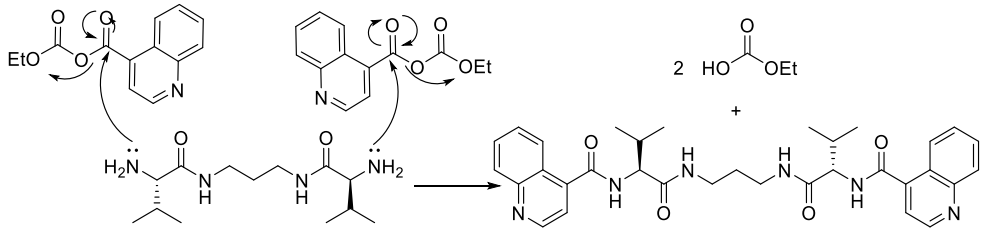
Scheme 3-8. Two-step coupling via ethyl carbonic anhydride.

In the schemes below, the mechanism of the reaction that has been studied is proposed. The first step was the nucleophilic attack of the acid moiety to ethyl chloroformate, yielding the carbonic anhydride, followed by the amine attack which resulted in the formation of **QVal3**.

Scheme 3-9. Nucleophilic attack of the acid moiety to ethyl chloroformate.



Scheme 3-10. Carbonic anhydride formation.



Scheme 3-11. Nucleophilic attack of the amine moiety to the carbonic anhydride.

This was the first procedure that was attempted to coupling HVal3 with QuiOH, but the compound was not obtained pure. As organogelator, this compound tends to form gels in organic solvents. Therefore, when it is purified by column chromatography, generally using organic solvents, it tends to aggregate inside the column, hence, it doesn't run with the eluent, making purification unworkable. Additionally purification was also attempted was by precipitation, with no good results.

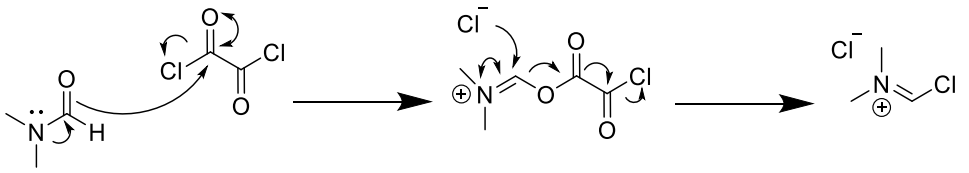
ii) The second procedure (Procedure B, in Experimental Section).

Acyl chlorides (also called acid chlorides) are one of the easiest methods to activate an acid and numerous acyl chlorides are commercially available. This is

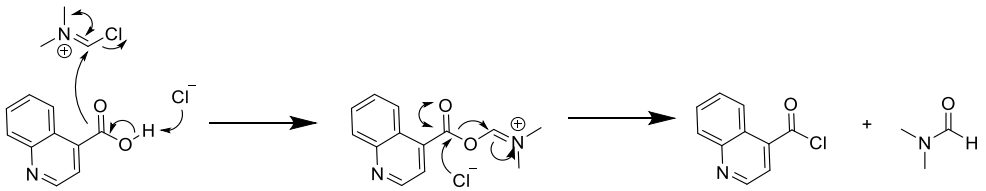
usually a two-step process, involving first the conversion of the acid into the acyl halide followed by the coupling itself.

In our case oxalyl chloride in the presence of DMF was employed to prepare the corresponding acyl chloride (Scheme 3-12 and Scheme 3-13).

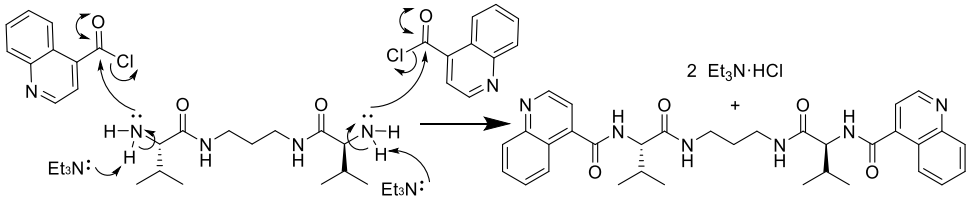
The amide bond is formed by reacting the acyl chloride with the desired amine. An additional base is usually required to trap the formed HCl and to avoid the conversion of the amine into its unreactive HCl salt. Couplings are usually performed in inert dry solvents, in the presence of a non-nucleophilic tertiary amine such a NEt_3 (Scheme 3-14).



Scheme 3-12. Activation of DMF with $C_2O_2Cl_2$.



Scheme 3-13. Mechanism of formation of quinoline-4-carbonyl chloride.



Scheme 3-14. Mechanism of reaction of QVal3 with QuiCl and HVal3.

The use of acid chlorides may cause racemization. Studies of the preservation of chirality in a **QVal3** need to be done in future work.

3.2 Characterization of the compound

To characterize the product, the acid-base constants and the minimum gelator concentration were determined.

3.2.1 Determination of the acid-base constants of QVal3

The determination of acid-base constants of a low molecular weight gelator is important to control the gelation process, because they allow us to known approximately what species are predominant in any pH. In aqueous media, we are interested in that range where the compound is not ionized, since the decrease in solubility allows the molecules begin to interact forming fibers as consequence of its hydrophobic nature.

Potentiometric titrations were done to determine the acidity constant of **QVal3** and also to study if the presence of DMSO has an effect on the acid-base constants.

The titration curves are shown below.

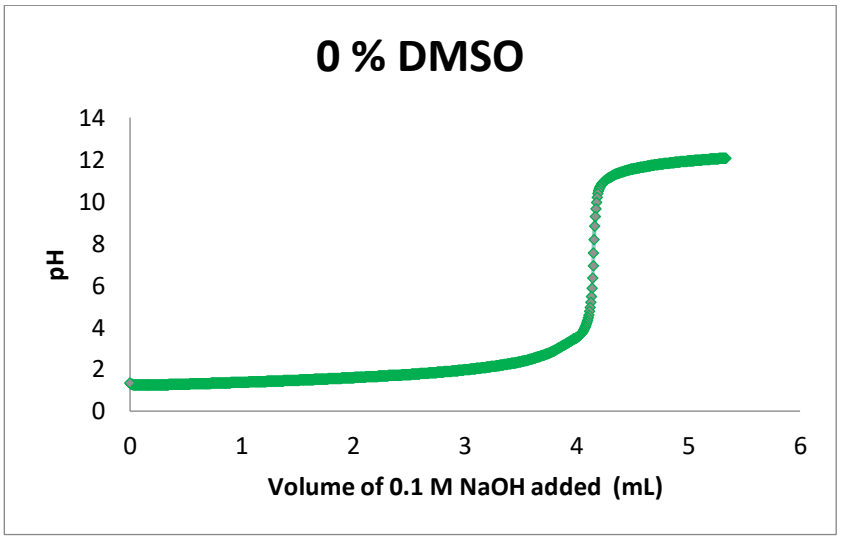


Figure 3-1. Curve titration of 1.5 mM **QVal3** in water.

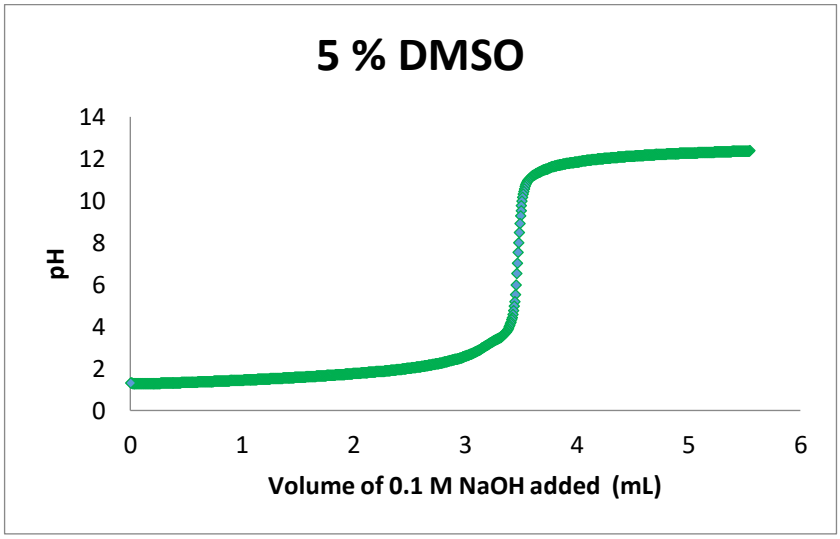


Figure 3-2. Curve titration of 1.5 mM **QVal3** in a mixture of DMSO:H_2O 5:95.

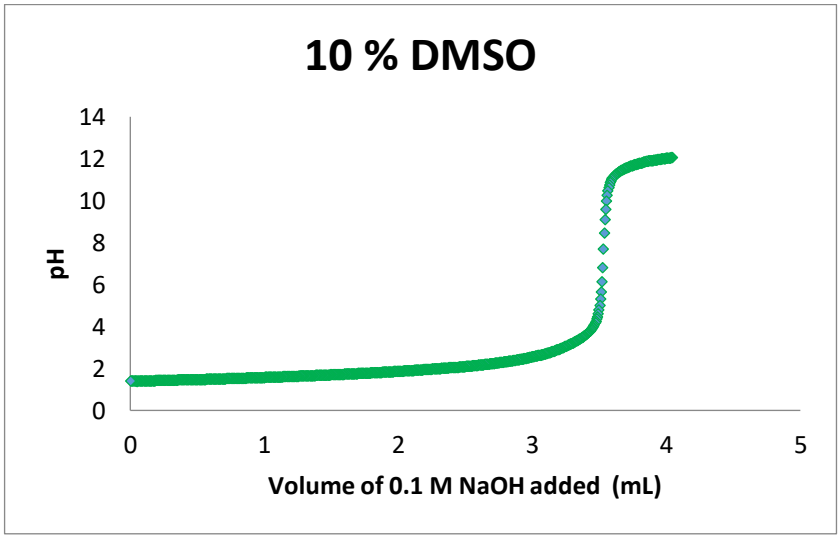


Figure 3-3. Curve titration of 1.5 mM **QVal3** in a mixture of DMSO:H_2O 10:90.

The titration data were analyzed with the program HYPERQUAD which fits the experimental data to thermodynamic protonation constants.

The results show that **QVal3** has two pKa values in the aggregated state (Table 3-1).

Table 3-1. Acid-base constants (log k) depending on the % of DMSO. *

	0% DMSO	5% DMSO	10% DMSO
QVal3 → QVal3-H	3.34	3.61	3.80
QVal3-H → QVal3-H_2	2.58	2.71	2.77

*Estimated errors are ± 0.03.

Where **QVal3** is the neutral compound, **QVal3-H** is the mono-protonated and **QVal3-H_2** is the double-protonated species.

Once the acidity constants of **QVal3** were determined, the speciation diagram was made with the HySS program.

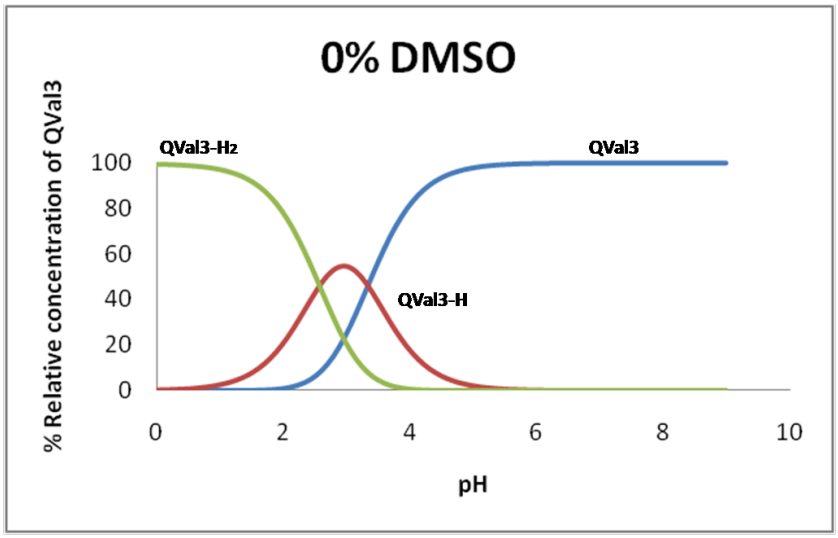


Figure 3-4. Speciation diagram of 1.5 mM **QVal3**, 0% DMSO.

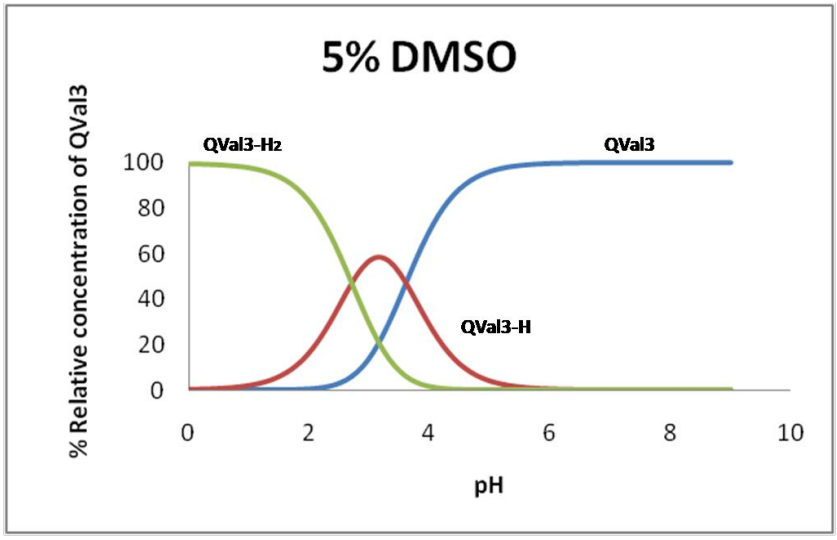


Figure 3-5. Speciation diagram of 1.5 mM **QVal3**, 5% DMSO.

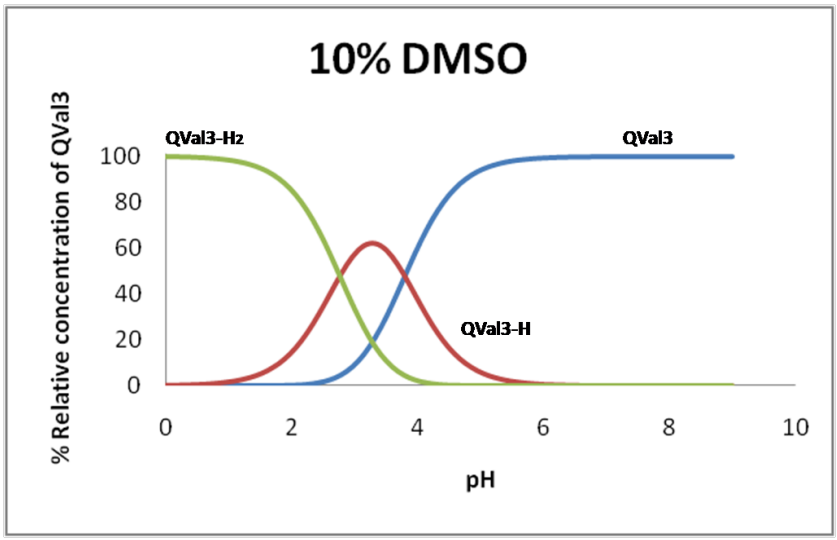
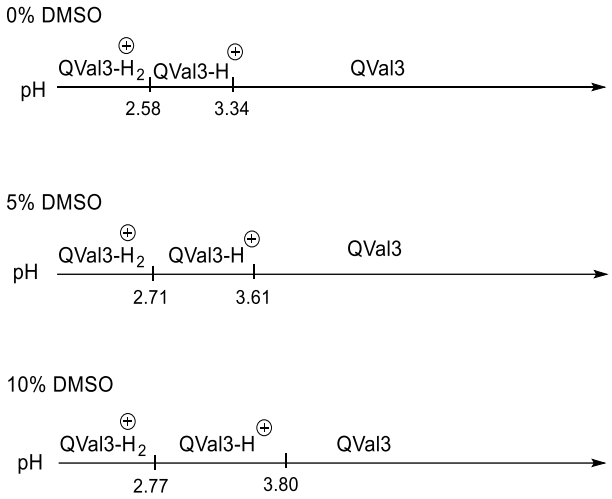


Figure 3-6. Speciation diagram of 1.5 mM **QVal3**, 10% DMSO.

In view of the results, is possible to known what type of species are predominant at different pH values (Scheme 3-15).



Scheme 3-15. Predominant species of **QVal3** at different pH values.

The results indicate that when increasing the % of DMSO, a slight increasing tendency in the acid-base constants is observed, this may be due to solvation effects.

3.2.2 Minimum gelation concentration (mgc)

To know the efficiency of a gelator, it is necessary to determine the minimum amount required to form a gel in the solvent. This parameter is called minimum gelator concentration (mgc).

For LMWG, the minimum gelator concentration tends normally to be lower than 1% wt, therefore, it is possible that when a molecule does not form a gel in a specific solvent, it could simply be that the mgc has not been reached. To determine this value, the most widespread method is the inverted tube, because this method provide a fast test to identify the formation of a gel. However, viscous liquids may also be stable to inversion, therefore, the results must be carefully analyzed.

There are many methods to obtain a gel, such as pH change, heating-cooling or precipitation.

The precipitation method was chosen to obtain the gel. This consists of dissolving the gelator in a small amount of a suitable solvent, in our case, DMSO, and then adding a solvent in which the gellant is poorly soluble.

3.2.2.1 Influence of DMSO/water ratio in the minimum gelator concentration of QVal3

It was decided to determine the minimum gelation values of **QVal3** at different ratios DMSO:H_2O. First, to determine the minimum gelation, water was used as solvent, and the tests were done with two different ratios. DMSO:H_2O 10:90 and 5:95.

Table 3-2. Minimum gelation concentration assays. Ratio DMSO:H_2O 10:90. *

[] (mM)	µL stock solution **	µL DMSO	µL H_2O	Gelation
2	100 (B)	0	900	Yes
1.6	**80 (B)**	**20**	**900**	**Yes**
1.5	75 (B)	25	900	No
1.1	55 (B)	45	900	No
0.6	30 (B)	70	900	No
0.5	25 (B)	75	900	No

* All the experiments have been done in triplicate.
** The letters in parentheses indicate the stock solution used (see Chapter 5, page 36).

Table 3-3. Minimum gelation concentration assays. Ratio DMSO:H_2O 5:95. *

[] (mM)	µL stock solution **	µL extra DMSO	µl H_2O	Gelation
1.5	25 (C)	25	950	Yes
1	16.6 (C)	33.3	950	Yes
0.9	15 (C)	35	950	Yes
0.8	13.3 (C)	36.6	950	Yes
0.7	11.6 (C)	38.3	950	Yes
0.6	**10 (C)**	**40**	**950**	**Yes**
0.5	50 (A)	0	950	No
0.4	40 (A)	10	950	No

* All the experiments have been done in triplicate.
** The letters in parentheses indicate the stock solution used (see Chapter 5, page 36).

We observe that at ratio DMSO:H_2O 10:90 the minimum gelation is 1.6 mM,while at ratio DMSO:H_2O 5:95 is 0.6 mM. Therefore, the amount of DMSO does influence in the minimum gelation. The higher the concentration of DMSO, the greater the mgc. This is because if a higher concentration of DMSO is used, the solubility of the compound in the resulting mixture is increased, therefore, a greater amount of compound is required to reach the saturation situation that results in the aggregation process, obtaining the gel.

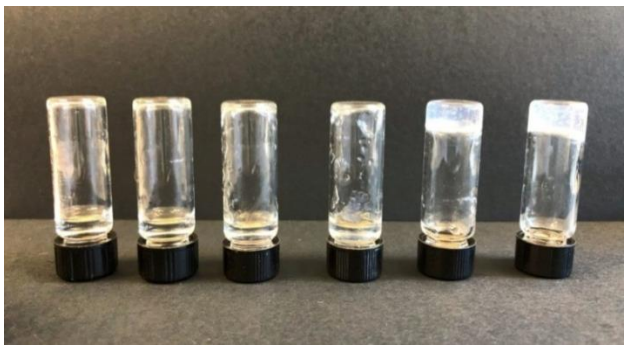


Figure 3-7. Vial inversion test for **Ratio DMSO:H_2O 10:90**. Left to right concentrations (mM): 0.5 - 0.6 - 1.1 - 1.5 - 1.6 - 2. It is shown that the gel is formed above 1.6 mM. Images for the ratio **DMSO:H_2O 5:95** are not included, but the same criterion was followed.

3.2.3 Study of pH effect though buffer phosphate 0,1 M

Then it was decided to assay mgc values at different pH values. For this purpose different phosphate buffers pH=5, 7 and 9, were used.

Table 3-4. Effect of pH on gelation. Ratio DMSO:Buffer 5:95. *

[] (mM)	µL stock solution **	µL DMSO	µL buffer	Gelation		
				pH 5	pH 7	pH 9
1.5	25 (C)	25	950	Yes	Yes	Yes
1	50 (B)	0	950	Yes	Yes	Yes
0.7	35 (B)	15	950	Yes	Yes	Yes
0.6	**30 (B)**	**20**	**950**	**Yes**	**Yes**	**Yes**
0.5	25 (B)	25	950	No	No	No

* All the experiments have been done in triplicate.
** The letters in parentheses indicate the stock solution used (see Chapter 5, page 36).

Table 3-5. Effect of pH on gelation. Ratio DMSO:Buffer 10:90. *

[] (mM)	µL stock solution **	µL DMSO	µL buffer	Gelation		
				pH 5	pH 7	pH 9
1.5	75 (B)	25	900	Yes	Yes	Yes
1	**50 (B)**	**50**	**900**	**Yes**	**Yes**	**Yes**
0.8	40 (B)	60	900	No	No	No
0.7	35 (B)	65	900	No	No	No
0.6	30 (B)	70	900	No	No	No
0.5	25 (B)	75	900	No	No	No

* All the experiments have been done in triplicate.
** The letters in parentheses indicate the stock solution used (see Chapter 5, page 36).

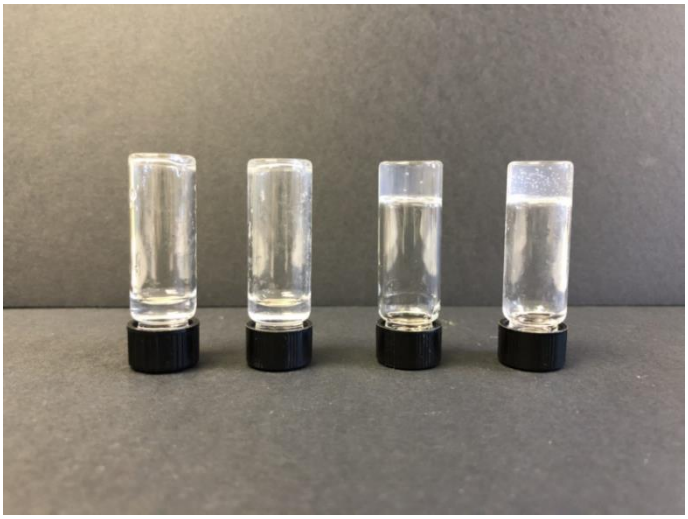


Figure 3-8. Vial inversion test for **ratio DMSO:H_2O 10:90 at pH 5.** Left to right concentrations (mM): 0.7 - 0.8 - 1 - 1.5.

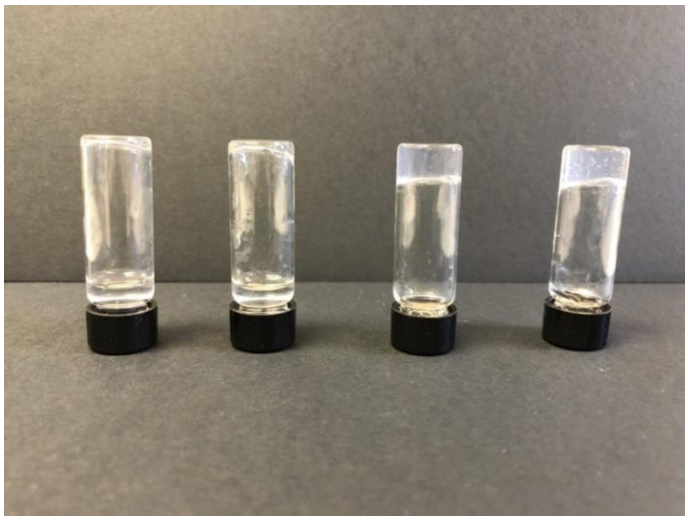


Figure 3-9. Vial inversion test for **ratio DMSO:H_2O 10:90 at pH 7.** Left to right concentrations (mM): 0.7 - 0.8 - 1 - 1.5.

Figure 3-10. Vial inversion test for **ratio DMSO:H_2O 10:90 at pH 9.** Left to right concentrations (mM): 0.7 - 0.8 - 1- 1.5.

It is observed that at pH 5, 7 and 9 gel is obtained above 1 mM, being negligible the influence of medium acidity, in this range of pH values.

Chapter 4

Conclusions

4 Chapter: Conclusions

- **QVal3** was obtained, purified and characterized. The amide formation step was successful using the carbonic anhydride activation procedure.

- Potentiometric titration of **QVal3** revealed that this molecule presents two ionization equilibria, being the pKa values in water respectively 2.6 and 3.3. This values increase only slightly with the addition of DMSO (5% or 10%) as cosolvent.

- Minimum gel concentration (mgc) of **QVal3** was determined by tube inversion. Minimum gelator concentration in 95 % water/DMSO was 0.6 mM, being the molecule a super hydrogelator. The mgc value increases to 1 mM for 90% water/DMSO systems.

- Gels were successfully formed in buffered media at pH 5, 7 and 9 with no significant changes in the mgc value. This range of pH values, 5-9 is therefore suitable for future applications of the gels.

Chapter 5

Experimental Section

5 Chapter: Experimental Section

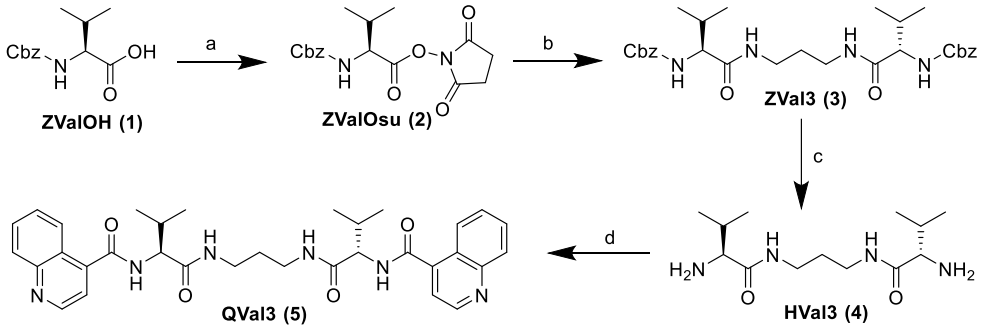
5.1 General methods.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 500, in the indicated solvent at 30 ^{o}C. The data are reported as follows: chemical shifts were given in parts per million (ppm, δ), referenced to the peak of tetramethylsilane, defined at δ =0.00 (^{1}H NMR) or using solvent residual signals 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), were reported in Hertz (Hz), and ^{1}H NMR Spectroscopy splitting patterns were designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). ^{1}H and ^{13}C signals were assigned with the aid of 2D methods (COSY, HSQC and HMBC).

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 performed under N_2 and all reagents and solvents were used as received from commercial suppliers with purity higher than 98 % and were used without further purification unless otherwise stated.

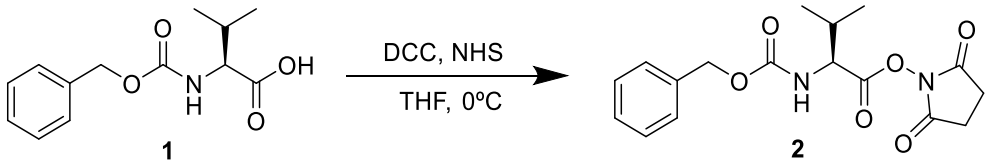
Analytical thin layer chromatography was performed on 0.25 mm extra hard silica gel plates with UV254 fluorescent indicator. Chromatography was performed using 300-400 mesh silica gel (SiO_2).

5.2 Experimental procedure for synthesis of QVal3



Scheme 5-1. Reagents and conditions: a) DCC, N-hydroxysuccinimide, THF, 2 h, 75%; b) 1,3-diaminopropane, THF, 16 h, 76%; c) Pd/C, H_2, MeOH, 4h, >99%; d) Quinoline-4-carboxylic acid, $C_2O_2Cl_2$, DMF, CH_2Cl_2, 5 h, 22 %.

5.2.1 Activation of N-Cbz-L-Valine

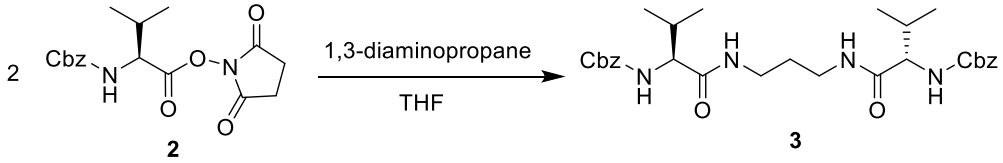


N-Cbz-L-Valine (10 g, 39.8 mmol, 1.0 eq.) and *N*-hydroxysuccinimide (NHS) (4.58 g, 39.8 mmol, 1.0 eq.) were dissolved in THF (140 mL). When a clear solution had been obtained, *N,N'*- dicyclohexylcarbodiimide (DCC) (8.29 g, 40.2 mmol, 1.01 eq.) in THF (40 mL) was added dropwise under N_2 atmosphere at 0 ^oC with a dropping funnel. The reaction mixture was further stirred for 2h. At this time, the mixture was left to settle into freezer overnight. Next day, *N,N'*-dicyclohexylurea (DCU) formed was filtered off under vacuum. A paste was formed and it was concentrated to dryness. The crude product was purified by crystallization in 2-propanol to furnish **2** as white product crystals.

Yield (10.302g, 75%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.04 (d, *J* = 8.1 Hz, 1H), 7.47 – 7.26 (m, 5H), 5.08 (s, 2H), 4.34 (dd, *J* = 10.1, 4.3 Hz, 1H), 2.82 (br s, 4H), 2.18 (m, 1H), 1.01 (d, *J* = 6.5 Hz, 6H).

Compound **2** was previously described and ^{13}C NMR spectra were in good agreement with the literature spectra.[16]

5.2.2 Amine coupling to the activated ester



2 **3**

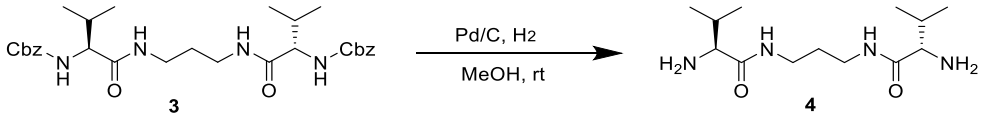
ZValOsu (2) (10.4g, 29.85mmol, 1.0 eq.) was dissolved in THF (106 mL). 1,3-diaminopropane (1.26 mL, 14.925 mmol, 0.5 eq.) was dissolved in THF (33 mL) and was added dropwise, using a dropping funnel under N_2 atmosphere at 25°C. The resulting solution was further stirred for 16 h at 55 °C in a silicone bath. The solvent was concentrated under vacuum. The white solid obtained was washed first with HCl 0.1 M (300 mL) and then with NaOH 0.1 M (300 mL). It was filtered off and was washed several times with H_2O until neutral pH. Finally, the product was left to the vacuum oven overnight.

Yield (6.09 g, 76%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.90 (t, *J* = 4.6 Hz, 2H), 7.48 – 7.27 (m, 10H), 7.22 (d, *J* = 8.7 Hz, 2H), 5.02 (s, 4H), 3.77 (t, *J* = 7.9 Hz, 2H), 3.17 – 2.92 (m, 4H), 1.98 – 1.87 (m, 2H), 1.61 – 1.46 (m, 2H), 0.85 (d, *J* = 6.7 Hz, 12H).

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

[16] Fontanillo, M.; Angulo-Pachón, C. A. *J. Colloid Interv. Scl.***2003**, *412*, 65-71.
[17] Becerril, J.; Bolte, M.; Burguete, M. I.; Galindo, F.; García-España, E.; Luis, S. V.; Miravet, J. F. *J. Am. Chem. Soc.***2003**, *125*, 6677.

5.2.3 Deprotection of the Cbz amine protecting group



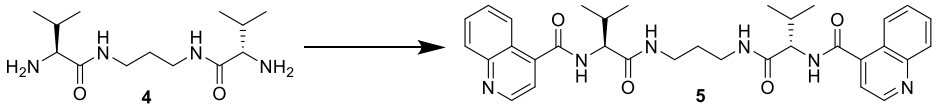
3 4

ZVal3 (6.09 g, 11.26 mmol) and catalytic amount of Pd over activated carbon (10% w/w, 600 mg) were placed in a two necked round bottom flask and suspended in MeOH (225 mL) with stirring at room temperature under N_2 atmosphere. The system was purged to remove the air with N_2 and connected to H_2 atmosphere. The grey suspension was stirred until it turned completely black 4h approximately (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. The crude product **4** was used for the next reaction without more purification.

Yield (>99%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.86 (t, *J* = 5.3 Hz, 2H), 3.08 (m, 4H), 2.91 (d, *J* = 5.2 Hz, 2H), 1.91 – 1.76 (m, 2H), 1.59 – 1.46 (m, 2H), 0.86 (d, *J* = 6.8 Hz, 6H), 0.78 (d, *J* = 6.8 Hz, 6H).

Compound **4** was previously described and ^{13}C NMR spectra were in good agreement with the literature spectra.[18]

5.2.4 Synthesis of final products



4 5

Procedure A:

Quinoline-4-carboxylic acid (2,75 g, 15.88 mmol, 2 eq.) was dissolved in THF (90 mL) and was added, under N_2 atmosphere at 0°C, over a solution of Et_3N (2.20 ml, 15.88 mmol, 2 eq.) in THF (10 mL). Then, ethylchloroformate (1.5 ml, 15.88 mmol, 2 eq.) was added by syringe through the septum. After 30 minutes, **HVal3** (2.16 gr,

7.94 mmol, 1 eq.) was dissolved in THF (10.5 mL) and was added by syringe pump for 20 minutes. Elapsed time, the resulting solution was further stirred for 24 h and allowed to warm to room temperature. At this time, the solvent was removed under reduced pressure and the solid obtained was washed first with NaOH 0.1 M and then with H_2O several times until neutral pH to yield the product as a white solid. The NMR characterization is not included here, because the pure compound was not obtained by means of this procedure (see Chapter 3, page 17).

Procedure B:

Quinoline-4-carboxylic acid (850 mg, 4.9 mmol, 1.0 eq.) were placed in a two necked round bottom flask and suspended in CH_2Cl_2 (90 mL) with stirring at 0 ^oC under N_2 atmosphere. After 10 min, $C_2O_2Cl_2$ 2 M in methylene chloride was added (2.5 ml, 4.95 mmol, 1.01 eq.) by syringe. Then, 4 drops (40 µl) of dried DMF was added and the mixture was stirred constantly for 4h. The emission of bubbles evidenced that the reaction took place when CO_2 is formed as a by-product. Then, the ice bath was removed and the mixture was left to warm to room temperature. **HVal3** (667 mg, 2.45 mmol, 0.5 eq.) was dissolved in 65 mL CH_2Cl_2 and added over the initial reaction slowly by syringe with stirring at 25 ^oC under N_2 atmosphere for 1 h. At this point Et_3N (1.5ml, 10.8 mmol,2.2 eq.) was added was left to react overnight at the same conditions. The reaction mixture was changed to a one necked bottom flask and was rotaevaporated to dryness and then put under vacuum for 1 h. Then the solid was washed with 0.1 M NaOH, sonicated and filtered. The product was dried overnight in the oven at 60 ^oC.
Yield (22.3%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (d, J = 4.2 Hz, 2H), 8.81 (d, J = 8.4 Hz, 2H), 8.22 – 8.01 (m, 6H), 7.78 (t, J = 7.2 Hz, 2H), 7.65 (t, J = 7.5 Hz, 2H), 7.54 (d, J = 4.2 Hz, 2H), 4.37 (t, J = 8.0 Hz, 2H), 3.25 – 3.06 (m, 4H), 2.16 – 2.02 (m, 2H), 1.71 – 1.59 (m, 2H), 0.97 (d, J = 6.5 Hz, 6H), 0.96 (d, J = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.6, 166.8, 150.1, 147.8, 142.1, 129.6, 129.2, 127.1, 125.4, 124.3, 119.2, 59.0, 36.4, 30.0, 29.2, 19.3, 18.5. HRMS (ESI): *m/z* calcd for: $C_{33}H_{38}N_6O_4$ [M+Na] + = 583.3033; found = 583.3038 [M + H^+].

5.3 Experimental procedure to determinate acid-base constants

Potentiometric titrations to determine acid-base constants were carried out at 298 K. In a typical experiment **QVal3** (9.61 mg, 0.0165 mmol, 1.5 mM) was dissolved in HCl 0.06 M. For the 0% DMSO experiment, only 7 mL 0.06 M HCl (0.42 mmol) was added. In 5% DMSO experiment, were added 6.45 mL 0.06 M HCl (0.387 mmol) and 0.55 mL DMSO, and finally, in 10 % DMSO experiment, were added 5.9 mL 0.06 M HCl (0.354 mmol) and 1.1 ml DMSO. Therefore, in our initial solution, we always had 7 mL. These solutions were titrated with 4 mL 0.1 M normalized solution of aqueous NaOH with vigorous stirring. Addition rate was 0.0333 mL/min. The pH was monitored every 10 seconds with a New Era Pump Systems NE300 pHmeter. Thermodynamic constants for the different species were determined analysing the data with HYPERQUAD 2008 software. Titrations were performed at a very slow flow rate in order to avoid kinetic problems associated to aggregation.

5.4 Experimental procedure to prepare the gels

First stock solutions of **QVal3** in DMSO were prepared. For this purpose the corresponding amounts of **QVal3** were weighed and dissolved in 2 mL of DMSO in 2 mL vials.

Table 5-1. Composition of **QVal3** stock solutions.

Stock solutions	[] (mM)	QVal3 (mg)	QVal3 (mmol)	DMSO (mL)
A	10	11.65	0.020	2
B	20	23.31	0.040	2
C	60	69.93	0.12	2

To prepare gels water (950 µL or 900 µL) was added to the DMSO solution of the gelator (50 µL or 100 µL). Micropipettes of the adequate range were used to carry out the discharges.

The formed gel was let stand for 5 minutes, and after it was checked whether gelation has occurred through the inverted vial test.

When we inverted vial test there are three situations possible:

i. The product forms a gel.

ii. The product aggregates, but not form a gel.

iii. The product remains completely in solution.

Chapter 6

NMR Spectra

6 Chapter: NMR Spectra

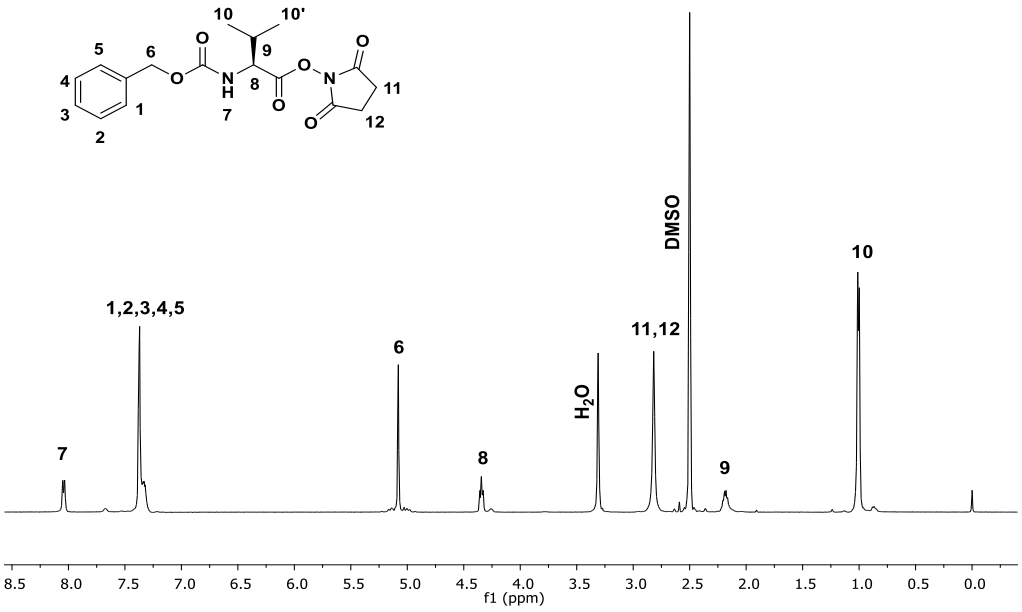


Figure 6-1. ^{1}H NMR spectrum of **ZValOsu.**

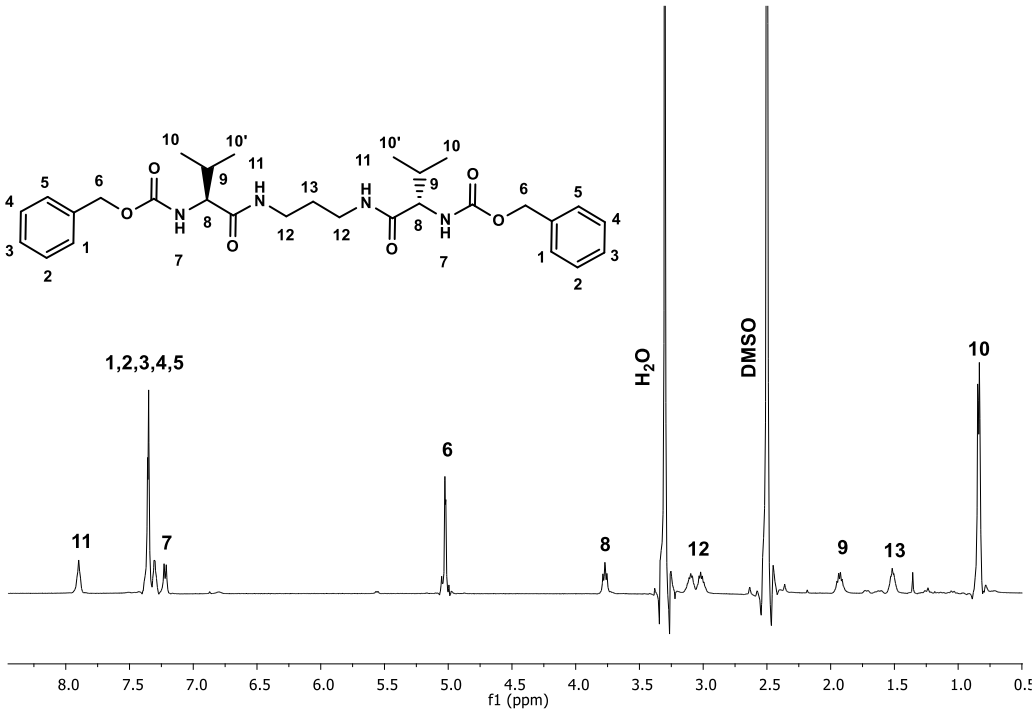


Figure 6-2. ^{1}H NMR spectrum of **ZVal3.**

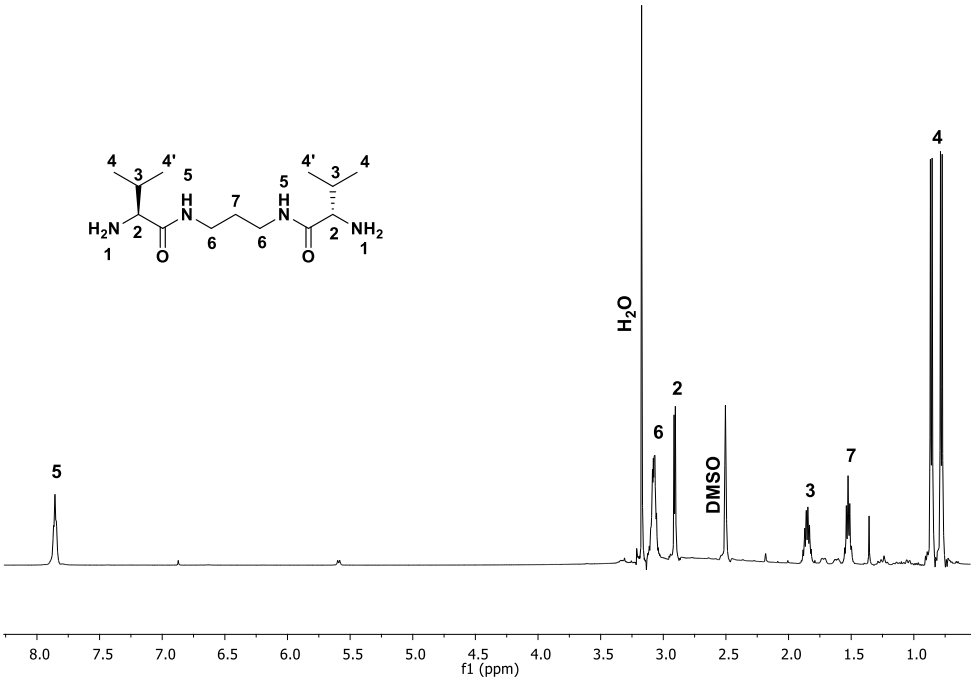


Figure 6-3. ^{1}H NMR spectrum of **HVal3.**

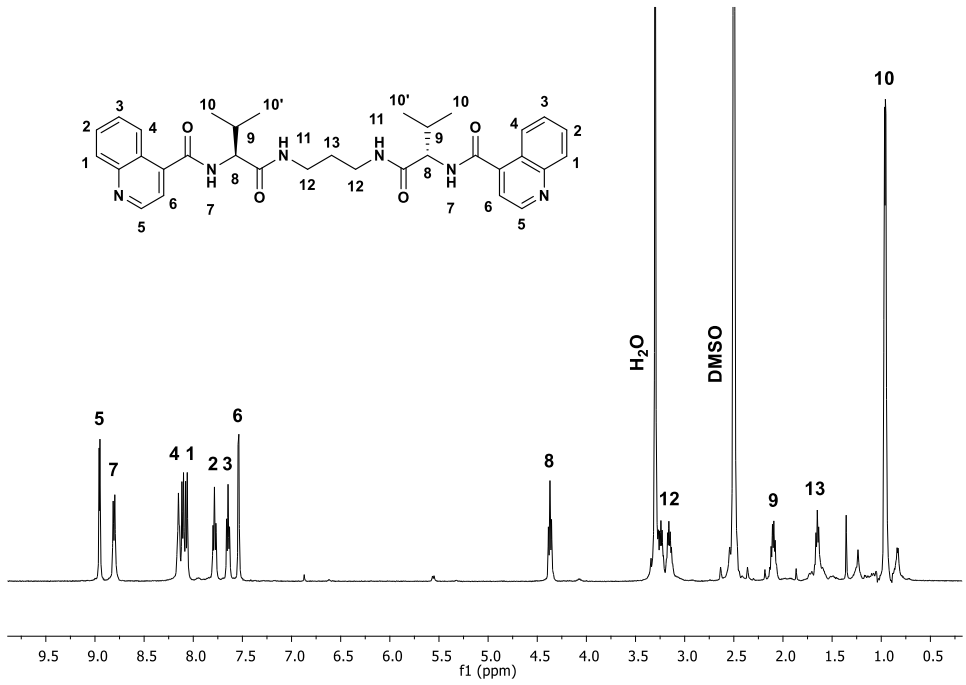


Figure 6-4. ^{1}H NMR spectrum of **QVal3.**

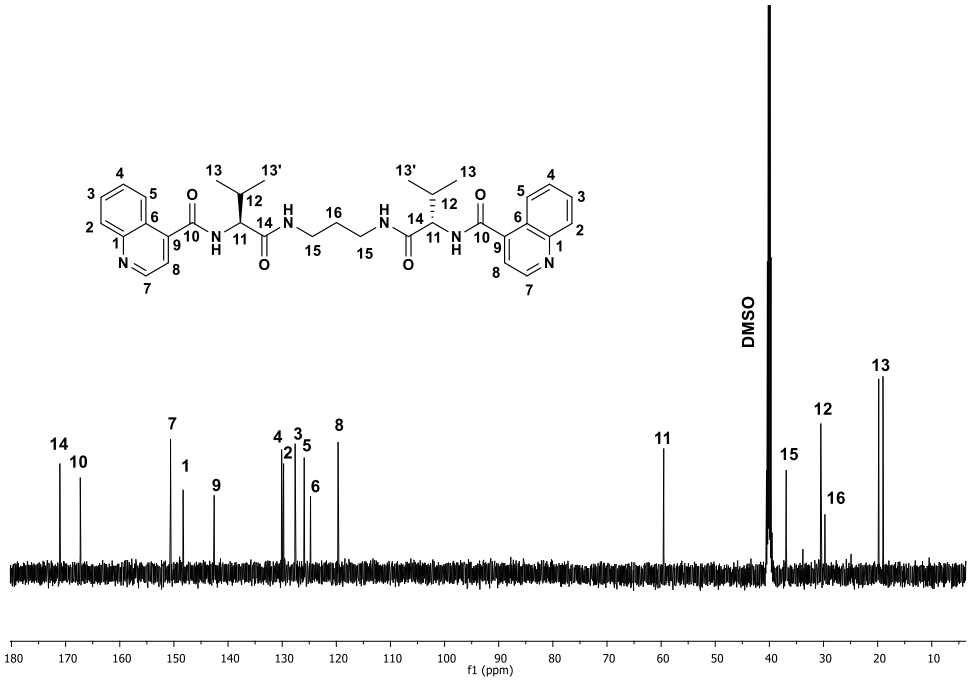


Figure 6-5. ^{13}C NMR spectrum of compound **QVal3.**