

Improvement of molecular gel formation efficiency by adjusting preorganization of amino acid-derived flexible molecules. A NMR and thermodynamic study.

César Angulo-Pachón, Carolina Gascó-Catalán, Juan J. Ojeda-Flores and Juan F. Miravet^{*[a]}

Dedication ((optional))

Efficiency of molecular gel formation of simple derivatives from L-valine and L-isoleucine is much improved in different organic solvents when replacing a hexyl fragment by a bulkier cyclohexyl one. A study using NMR and IR provides information on the preferred conformations of the molecules indicating that the cyclohexyl moiety precludes intramolecular H-bonding and preorganises the system for intermolecular interactions responsible for fiber formation. NMR data of the gels provide thermodynamic data for fibrillization revealing that the origin of this effect is mainly entropic. Electron microscopy (SEM and TEM) images show fibrillar and tape-like objects observed commonly in molecular gels. Rheological measurements reveal significant differences between cyclohexyl and hexyl appended gelators. The conclusions reached could contribute to the rational design of small, flexible, building blocks for self-assembly.

Introduction

Rational design of self-assembling systems is a very challenging task due to the large number of possibilities of intermolecular interactions that arise from the relative disposition of the molecules in the supramolecular complex and their conformational variability.^[1] For example, much effort is devoted in this direction in the field of crystal engineering.^[2] Conformational polymorphism introduces an important factor of complexity in crystal packing and this issue has been reviewed recently.^[3] A particular case of self-assembly is the formation of molecular gels. Small molecules can self-assemble into elongated, fibril-like, objects that percolate the solvent and form gels.^[4] Molecular gels have attracted wide interest due to potential applications in fields such as optoelectronics or biomedicine among others. Their intrinsic reversibility and stimuli responsiveness together with their biodegradable nature provide distinct advantages compared with many polymeric analogues.^[5,6,7] Rationalizing gelation efficiency of small

molecules is puzzling and interesting studies on this respect have been described aiming to factors such as solubility, chirality or the presence of the proper units to achieve intermolecular bonding.^[8,9,10,11,12] Summarizing, molecules capable of self-assembly should present a preferential direction of self-assembly, giving place to of 1-D, fibril-like, aggregates with a sufficient free energy of aggregation in the required solvent. A compromise between solubility and aggregation is generally accepted as a requirement for successful molecular gel formation.

Here we report an remarkable case of improvement of gelation efficiency, namely, a reduction of the concentration required for gelation to take place, by substituting a hexyl moiety by a cyclohexyl one in a family of structurally simple organogelators derived from amino acids and succinic acid (Figure 1). The results are somewhat unexpected because cyclohexyl radical should, a priori, preclude self-assembly when compared to the linear hexyl radical. Indeed steric hindrance disfavoring intermolecular interactions has been pointed out to explain reduced or null gelation efficiency in series of compounds.^[13,14,15] No precedent results describing cyclohexyl appended groups in gelators are reported because the use of linear alkyl chains is taken in many cases as granted.^[16,17,18] Such linear chains are used to modulate the solubility and favor intermolecular interactions by means of solvophobic and van der Waals interactions. As a matter of fact, the cyclohexyl appended molecules described here were initially designed to disfavor gel formation, but serendipitously, the result was just the opposite one. A rationale for the presented results is given which highlights the sum importance of preorganization and conformational flexibility in the self-assembly of small molecules such as those described below.

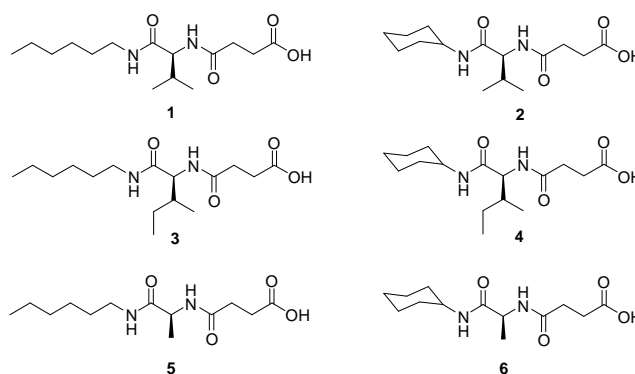


Figure 1. Structure of the studied compounds.

[a] Dr. C. Angulo-Pachón, Ms. C. Gascó-Catalán, Mr. Juan J. Ojeda-Flores and Dr. J. F. Miravet
Departament de Química Inorgànica i Orgànica
Universitat Jaume I
Avda. Sos Baynat s/n, 12071 Castellón, Spain
E-mail: miravet@uji.es

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Results and Discussion

Compounds **1-6** (Figure 1) were easily prepared from L-valine and L-isoleucine. Amino side of the amino acids was reacted with succinic anhydride and the carboxylic moiety converted either to hexyl or cyclohexyl amide (details in SI). The gelation efficiency of the studied molecules was compared considering the minimum concentration required to form a self-sustained gel in vials (mgc). The gelators were dissolved by gentle heating and allow reaching room temperature, yielding the gels (see details in SI).

As revealed in Table 1, gels were formed in a variety of solvents from a non-polar one such as toluene to a fairly polar one such as acetonitrile or even in water. A very noticeable improvement of gelation efficiency was observed upon substituting the hexyl radical by the cyclohexyl one.

Table 1. Minimum concentration (mg/mL) of compounds **1-6** required for gelation in different solvents.

Solvent	1	2	3	4	5	6
AcOEt	S	3	S	20	S	7 ^a
Toluene	5 ^a	1	3	2	0.6	1
CHCl ₃	8	2	15	3	28	16
CH ₂ Cl ₂	10	2	15 ^a	5	10	3
MeCN	20 ^a	3	20 ^a	15 ^a	1	1
H ₂ O	10 ^a	5	5	2	S	S

^a particulate gel

I : insoluble; S: soluble at 20 mg/mL

For example, the hexyl derivative of valine, **1**, did not form self-supported gels in acetonitrile at a concentration of 20 mg/mL but the cyclohexyl derived analogue **2** was able to gel this solvent at a concentration of 3 mg/mL. Interestingly this tendency was also observed in ethyl acetate, chloroform and dichloromethane. A similar effect was observed for isoleucine derivatives **3** and **4**. For example compound **3** formed gels in chloroform with a mgc value of 15 mg/mL but the cyclohexyl derivative **4** showed much improved gelation capability affording gels at a concentration of 3 mg/mL. This effect seems to represent a general tendency which is also observed for gelators derived from alanine **5** and **6**, which present a less bulky side chain. Hexyl derivative **5** formed gels in chloroform at 28 mg/mL but the cyclohexyl analogue **6** afforded self-sustained gels at 16 mg/mL.

Electron microscopy of xerogels from chloroform gels (Figure 2) revealed the presence of elongated fibrillary objects as commonly observed for self-assembled molecular gels. It has to be noted that it is known that the structures observed by electron microscopy and those in the wet gel can differ due to drying effects. Compound **1** revealed thin twisted fibres but compound **2** is found to form thick tape-like objects composed of thinner fibrils. Compound **3** afforded xerogels that contain straight

elongated objects formed by association of fibrils but compound **4** presented elongated and leaf-like branched objects.

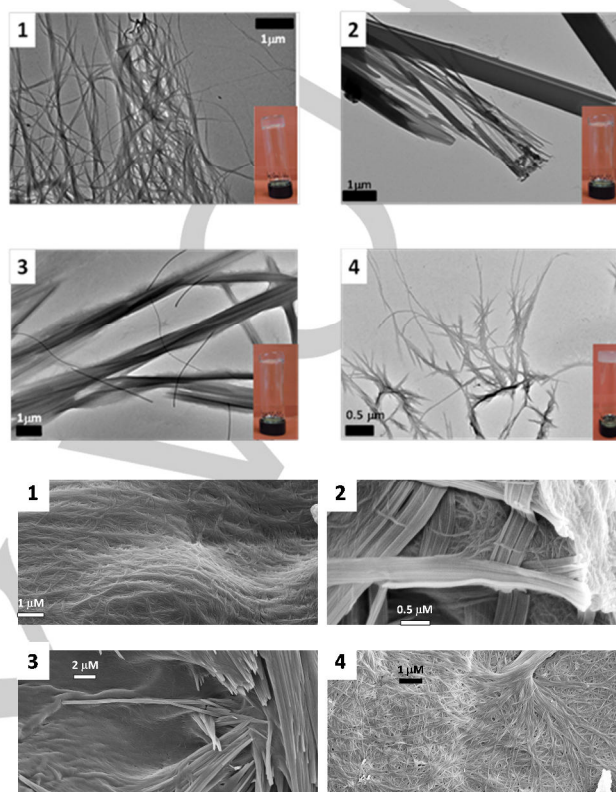


Figure 2. Transmission (top) and scanning (bottom) electron microscopy images of xerogels from chloroform gels obtained for compounds **1-4**. Inset: images of vials containing the gels.

A simple ¹H NMR analysis of the gelators in solution provided key information to rationalize the reported “cyclohexyl effect” (Figure 3). As illustrated in the graph of Figure 3, upon dilution the chemical shift of the amide protons of compounds **3** and **4** experiences an upfield shift associated to the dissociation of intermolecular H-bonded species. For the most dilute solutions (0.1 mM) the signal from compound **3** reaches a plateau indicating no aggregation. At this concentration, both amide protons of the cyclohexyl derivative **4** are shifted upfield when compared to hexyl analogue **3** (Figure 3). The substitution of hexyl by cyclohexyl units can't account for such differences merely by different electron induction effects which would result in minor shifts, specially for the amide labelled as “b” which is four bonds away from the hexyl/cyclohexyl moieties. Therefore the shift of the signals must be ascribed to the formation of intramolecularly hydrogen bonded species in the case of **3**.^[19, 20] Both amide protons seem to act as hydrogen donors becoming deshielded and producing the observed chemical shifts. Similar results were obtained for the case of compounds **3** and **4**. IR studies were carried out for in deuterated chloroform which agreed with the results obtained by NMR (Figure 4). When diluted solutions of compounds **3** and **4** were compared, only

compound **3**, the hexyl derivative, showed associated NH bands ascribable to intramolecular H-bonding. Additionally, IR analysis of gels formed by **3** and **4** reveals a strong signal of associated NH confirming the relevance of H-bonding in the aggregation process. Such differences were not detected in the amide I signals.

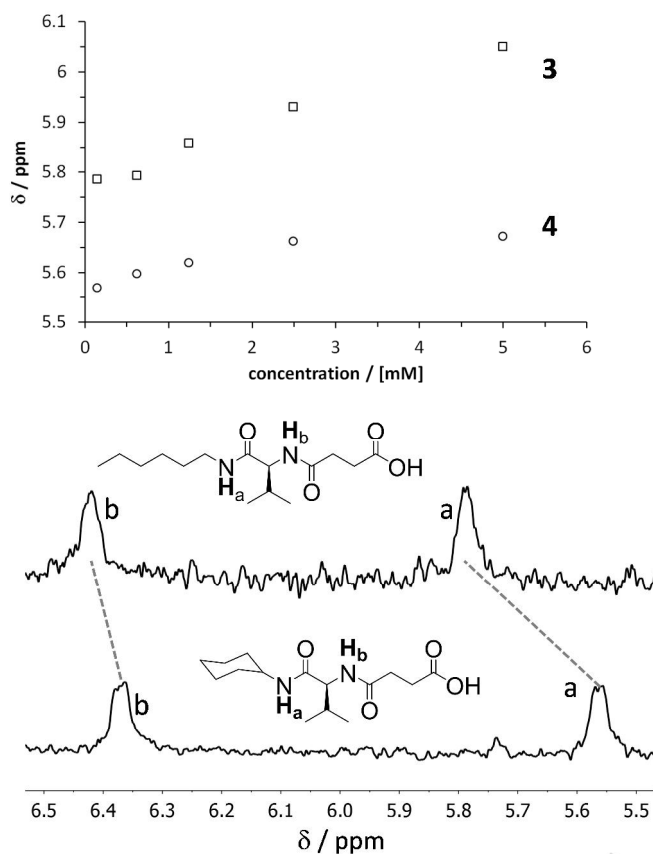


Figure 3. Partial ^1H NMR spectra for solutions of compounds **3** and **4** in CDCl_3 ($c = 0.1$ mM).

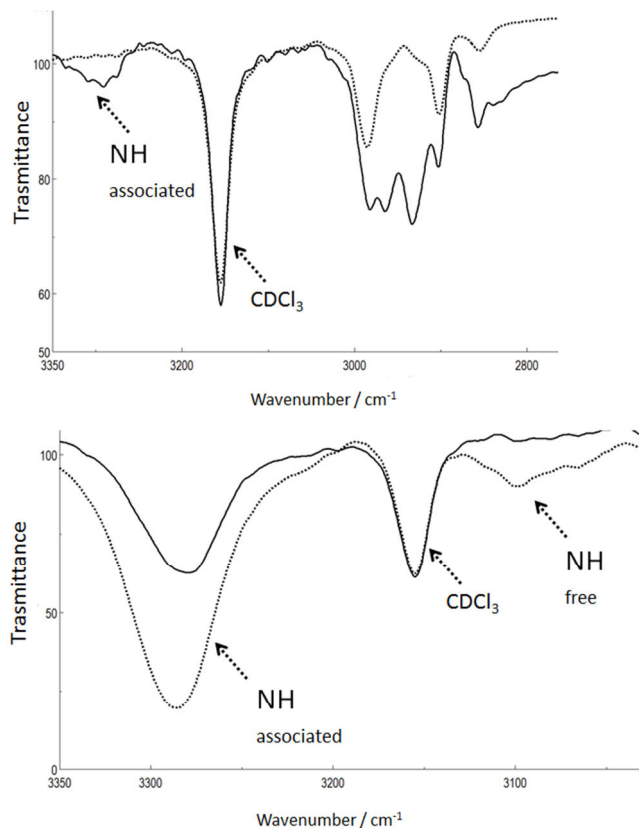


Figure 4. IR study of compounds **3** and **4** in CDCl_3 . Solid line: **3**; dotted line: **4**. Top: 0.1 mM solutions. Bottom: gel samples at minimum gelator concentration.

A sensible energy minimized computational model (semiempirical, AM1) for the postulated folded species is shown in Figure 5A. There both amide protons are H-bonded to the carbonyl from the carboxylic acid moiety. For the case of cyclohexyl derivatives **2** and **4**, NMR results point to the presence of extended, non-hydrogen bonded, conformations.

As can be seen in Figure 5A, and schematically represented in Figure 5B, the cyclohexyl moiety disfavors intramolecularly H-bonded conformations due to steric crowding. It seems reasonable to argue that extended conformations are properly preorganised to form linear aggregates, responsible of fiber formation, like those proposed schematically in Figure 5C. Intramolecular hydrogen bonding is proposed to take place in the aggregates involving both amido and carboxylic acid units. Such packing model is reasonable considering the literature on related, valine-derived gelators.^[21] Therefore, the much improved gelation efficiency can be linked to the favourable preorganization introduced by the cyclohexyl moiety which precludes folded conformations.

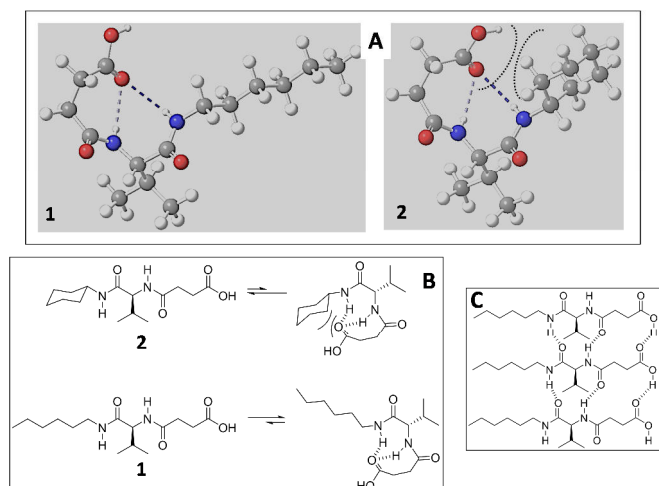


Figure 5. A: Energy minimized models (semiempirical, AM1) of folded conformations of compounds **1** and **2**. B: Schematic representation of unfolded and folded conformations for compounds **1** and **2**. C: Schematic representation of the intermolecular interactions responsible of gelation.

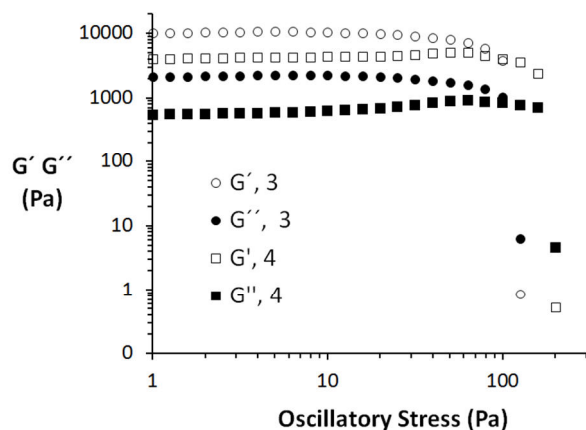


Figure 6. Comparative of stress sweep (1 Hz) between gels in chloroform of compounds **3** and **4** at 25 °C at their minimum gelation concentration. The storage modulus (G'), loss modulus (G'') and oscillatory stress are in logarithmic scale.

Next, a study was carried out to compare the rheological properties of gels formed by **3** and **4** in chloroform. As can be seen in Figure 6, the gel formed by the cyclohexyl derivate, **4**, presents a significant lower storage modulus than that formed by **3**. These results highlights that gelation efficiency and rheological properties are not necessarily directly related. Interfiber contacts are key for the rheology of the gels. It seems that the linear hexyl chains provide better crosslink points than the cyclohexyl moieties, probable due to interdigitation facilitated by the former units.

Further insight could be obtained by means of a thermodynamic analysis of the solubilization of the gel network. It is reported that solubility is a thermodynamic parameter that correlates well with gelation efficiency.^[22] NMR is an ideally suited technique to evaluate the solubility of the gelators because the solid fibrillar

network is NMR-silent but the free gelator molecules in equilibrium with it are NMR visible.^[23,24] Variable temperature analysis of the solubility in chloroform can provide with thermodynamic solubility values. To avoid kinetic effects in the solubilisation, the samples are heated progressively and rested to the desired temperature until equilibrium is reached.^[22] The results revealed noticeable differences between cyclohexyl and hexyl derivatives.

As shown in Figure 7, hexyl derivatives are quite more soluble and present a much more significant dependency on the temperature. A van't Hoff analysis (see SI) of the variable temperature data allows extracting enthalpy and entropy values associated to fibrillization (Table 2 and 3). Noticeably, the major impact of substitution of a hexyl radical by a cyclohexyl one is on the entropy values. The presence of the cyclohexyl moiety significantly reduces the unfavorable entropic cost associated to aggregation. In the case of valine derivatives **1** and **2**, a reduction of the entropy penalty for aggregation of 4 kJ mol⁻¹ is measured for the cyclohexyl derivative. This value overcomes the less favorable enthalpy of aggregation associated to the presence of the cyclohexyl unit (Table 3).

This effect is much enlarged in the case of isoleucine derivatives **3** and **4**. The presence of cyclohexyl unit in **4** affects dramatically to the enthalpy of aggregation which is much less favorable than in the case of hexyl derivative **3** with a difference of 14 kJ mol⁻¹. Again the reduction of the entropic penalty of fibrillization (19 kJ mol⁻¹) overcomes the unfavorable enthalpy change, resulting in a "cyclohexyl effect" favorable for aggregation.

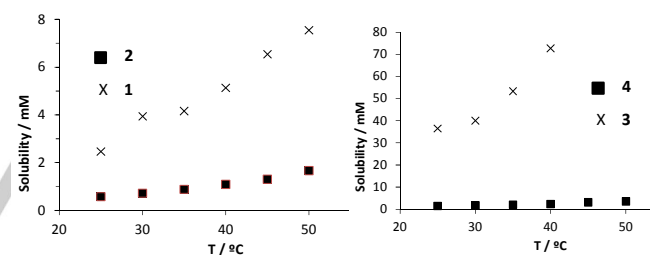


Figure 7. Solubility vs temperature graphs for compounds **1-4** in chloroform.

Table 2. Thermodynamic data for the fibrillization of compounds **1-4** in chloroform at 25 °C. Estimated error is ± 0.4 .

Compound	$\Delta H_{\text{dis-gel}} / \text{kJ mol}^{-1}$	$-T\Delta S_{\text{dis-gel}} / \text{kJ mol}^{-1}$	$\Delta G_{\text{dis-gel}} / \text{kJ mol}^{-1}$
1	-36	26	-10
2	-34	22	-12
3	-43	35	-8
4	-29	16	-13

Table 3. Energetic contributions to the fibrillization process in chloroform associated to the substitution of hexyl by cyclohexyl moieties in compounds **1-4**. Estimated error is ± 0.4 .

Compared compounds	$\Delta\Delta H_{\text{hex-cyc}}/\text{kJ mol}^{-1}$	$-T\Delta\Delta S_{\text{hex-cyc}}/\text{kJ mol}^{-1}$	$\Delta\Delta G_{\text{hex-cyc}}/\text{kJ mol}^{-1}$
1, 2	-2	4	2
3, 4	-14	19	5

Conclusions

Summarizing, the results presented above reveal that the mentioned “cyclohexyl effect” which boosts the gelation efficiency is linked to the reduction in conformational mobility introduced in the gelator molecules when compared to hexyl derivatives. Cyclohexyl compounds don't show intramolecularly hydrogen bonded conformations and seem to be preorganised for intermolecular hydrogen bonding responsible of fibrillisation. Indeed thermodynamic data reveal that the origin of the much improved gelation efficiency is on the reduction of entropic penalty of fibrillization. Preorganization is a common topic in the realm of supramolecular chemistry, the case exposed here reveals how such simple compounds as **1-6** can be outstandingly affected in their self-assembly by changes in conformational population. The results show that introducing bulkier groups in a potential gelator can result in much improved formation of molecular gels. These findings could contribute to the rational design of new molecular gelators and in general to improved self-assembling of small molecules.

Experimental Section

Experimental details are provided as supporting information.

Acknowledgements

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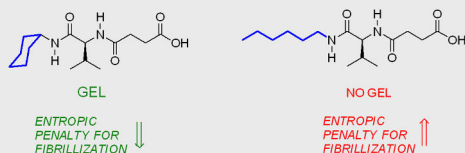
Keywords: molecular gel, self-assembly, preorganisation, NMR, thermodynamics of aggregation.

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Layout 2:

ARTICLE



César Angulo-Pachón, Carolina Gascó-Catalán and Juan F. Miravet*

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Improvement of molecular gel formation efficiency by adjusting preorganization of amino acid-derived flexible molecules. A NMR and thermodynamic study

Self-assembly of simple amino acid derivatives into gel fibrillar networks is boosted when replacing a hexyl moiety by a cyclohexyl one. The improvement is ascribed to the key role of preorganization (entropy) which overcomes the energetic penalty associated to the bulkier cyclohexyl moiety