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**STEROIDAL DERIVATIVES OF NITROGEN CONTAINING
COMPOUNDS AS POTENTIAL GELATORS**

BY

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Academic Dissertation for the Degree of
Doctor of Philosophy

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ABSTRACT

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This work describes the design and synthesis of new low molecular weight gelators (LMWGs) based on steroids combined with nitrogen containing moieties and the analytics of the resulting gels. The research is focused particularly on the preparation and study of acid-responsive and metal-organic gels. The highlights of these studies are LMWGs with switches embedded into their structures to be addressed by hydrogen protons. Furthermore, LMWGs are described with metal binding sites forming gels upon exposure to metal ions with properties depending on the metal used. In another approach, acid-sensitive gels are described, which form *in situ* from two components upon heating. Finally and most interestingly, combining this approach with metal ions in the so called subcomponent self-assembly, the generation of gels with properties depending on the metal ion used is described. These gels form spontaneously at room temperature at very low gelator concentrations.

For the purpose to explain and combine all these different studies into one thesis, the following structure was chosen: first, the basic principles of gels and gel formation are explained. Then the reader is led through the chapters “supramolecular gels” and “steroid based supramolecular gels” to show the versatility of the topic and modern approaches like metallo gels and stimuli-responsive gels. Finally, these chapters describe the state-of-the-art in this topic by explaining the key articles and recent publications to show the basis for the work done in this thesis. The literature part closes with a chapter concerning the methods applicable to analyze gels and gel formation and the challenges gels pose for analysis.

The experimental part defines the aims for the work, followed by detailing the logic and the design of the different LMWGs. Then the different topics are sorted into the sub-fields defined and explained in the literature part. The short successive chapter describes the synthesis of the compounds and is followed by the most challenging part of these studies: the elucidation of the gelation process and most important the confirmation of the implementation of the planned properties and the activity of the triggers designed into the LMWGs to make the gels smart materials.

This work closes with a summary and the conclusions derived from this work to be picked up and used for future studies.

Keywords: supramolecular gel, metallo gel, self-assembly, stimuli-responsive, *in situ*, subcomponent, cholesterol, stigmasterol, amino acid, pyridine, imine.

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PREFACE

This work was carried out at the Department of Chemistry, University of Jyväskylä, Finland, between 2009 and 2013.

Looking back at the years that have passed, it has been a busy and challenging time, but also an exciting and pleasant experience. I wish to thank Professor Emeritus Erkki Kolehmainen for providing me the opportunity to work in his research group, introducing me to the exciting field of supramolecular gels, and for his encouragement and support he has given me throughout these years. I also would like to thank Academy Professor Kari Rissanen for supervising the second half of this work and him and his group for their support and inspiration.

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I dedicate my everlasting gratitude to my family for their limitless support throughout my life. Finally, last but not least, I wish to thank my husband Jens for his love and unlimited support over these years.

The National Doctoral Program of Organic Chemistry and Chemical Biology (OCCB) from the Finnish Ministry of Education are gratefully acknowledged for financial support.

Jyväskylä, April 9th 2013

Hana Bunzen

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which in the text are referred to by their Roman numerals.

- I H. Svobodová, V. Nojonen, E. Kolehmainen, and E. Sievänen, Recent advances in steroidal supramolecular gels, *RSC Adv.*, **2012**, 2, 4985 - 5007.
- II H. Svobodová, Nonappa, Z. Wimmer, and E. Kolehmainen, Design, synthesis and stimuli responsive gelation of novel stigmasterol-amino acid conjugates, *J. Colloid Interface Sci.*, **2011**, 361, 587 - 593.
- III H. Bunzen and E. Kolehmainen, *In situ* formation of steroidal supramolecular gels designed for drug release, *Molecules*, **2013**, 18, 3745 - 3759.
- IV H. Svobodová, Nonappa, M. Lahtinen, Z. Wimmer, and E. Kolehmainen, A steroid-based gelator of A(LS)₂ type: tuning gel properties by metal coordination, *Soft Matter*, **2012**, 8, 7840 - 7847.
- V H. Bunzen, Nonappa, E. Kalenius, S. Hietala and E. Kolehmainen, Subcomponent self-assembly: a quick way to novel metallogels, *manuscript*.

Author's contribution

In paper I, the author has written the chapters about stimuli-responsive gels, metallogels, and two-component gels, and contributed to the chapter about organogels. The author is the primary author of papers II-V. She carried out all of the syntheses and the self-assembly studies, and performed or participated in most of the experimental work, apart from X-ray crystallographic measurements in the paper IV, and mass spectrometry and rheological measurements in the paper V. The author is responsible for writing the papers II-V.

ABBREVIATIONS

AFM	atomic force microscope
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
CA	cholic acid
CD	circular dichroism
CDCA	chenodeoxycholic acid
CNC	critical network concentration
CPMAS	cross-polarization magic angle spinning
DCA	deoxycholic acid
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
DSC	differential scanning calorimetry
EDTA	ethylenediaminetetraacetic acid
EM	electron microscopy
Fmoc	fluorenylmethyloxycarbonyl group
FTIR	Fourier transform infrared spectroscopy
IR	infrared
LCA	lithocholic acid
LMWG	low-molecular-weight gelator
MGC	minimum gelation concentration
MOF	metal-organic framework
NMR	nuclear magnetic resonance
PXRD	powder X-ray diffraction
SAFIN	self-assembled fibrous networks
SANS	small-angle neutron scattering
SAS	small-angle scattering
SAXS	small-angle X-ray scattering
SEM	scanning electron microscope
STM	scanning tunneling microscope
TEA	triethylamine
TEM	transmission electron microscope
THF	tetrahydrofuran
T _g	gel-sol phase transition temperature
TTF	tetrathiafulvalene
UV-Vis	ultraviolet-visible spectroscopy
w/v	mass concentration (weight/volume percentage, g/L)
XRD	X-ray diffraction

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1 INTRODUCTION

The synthesis and design of low-molecular-weight gelators (LMWGs), which form supramolecular gels,¹⁻⁶ has been an expanding research area in the last two decades. The motivation behind this surge was not only to understand the fundamentals of the molecular aggregation, but mainly to explore their properties and potential applications.⁷⁻¹¹ In the last few years, there has been an increased trend in preparing “smart” soft materials, *i.e.* materials responsive to various stimuli,¹²⁻¹⁴ and metallogels, *i.e.* gels containing metal ions.¹⁵⁻¹⁷ With regard to their potential applications, they both are considered as one of the most attractive and promising materials in the field.

This thesis represents my efforts to contribute to the area of steroidal stimuli-responsive gels and steroidal metal-organic gels. In the following chapter I will give a literature review to show the background for my work and my inspiration. First, I will give an overview on the gel topic in general and then focus on supramolecular gels, particularly on steroid-based supramolecular gels.

2 LITERATURE REVIEW

2.1 What is a Gel?

Although, gels were extensively studied for several decades and are nowadays commonly used in everyday life, answering the question “What is a gel?” is not easy and a short, straightforward definition of a gel is still missing. As Dorothy Jordon Lloyd stated already in 1926: “... gel is one which is easier to recognize than to define”.¹⁸ And despite the great scientific progress in this area, after all this time of 87 years she is still absolutely right.

The first scientific observation of a gel was reported in 1841 by Lipowitz, who observed a gelation of aqueous solutions by lithium urate.¹⁹ Few years later - in 1861, Thomas Graham described gels by the following statement: “While the rigidity of the crystalline structure shuts out external expressions, the softness of the gelatinous colloid partakes of fluidity, and enables the colloid to become a medium for liquid diffusion, like water itself”.²⁰ Since then, the research was more focused on preparing novel gels than explaining their fundamentals.

According to Flory (1974), a material is a gel “if it has a continuous structure with macroscopic dimensions that is permanent on the time scale of an analytical experiment and is solid-like in its rheological properties”.²¹ In 1997 Terech and Weiss tried to simplify the whole issue by claiming that “if it looks like “Jello”, it must be a gel!”.⁶ Nowadays gel is usually defined with the help of rheology as a viscoelastic material with rheological properties of solids despite being mostly liquid; typically 99 % by weight of the gel is a liquid while the remaining 1 % is the gelator. However, in laboratory practices the first designation of a sample as a gel is based on a visual observation of its fluidity characteristics, which are typically examined by a crude rheological measurement known as an inverted vial (or inverted tube) method. In this method, the weighed amount of gelator is taken into a vial with weighed amount of organic solvent. The vial is then properly closed and carefully heated to dissolve its content completely. After cooling down, the vial is inverted upside down. If the content remains at the

top of the inverted vial and does not flow under the influence of gravity, it is assigned as a gel.

One of the reasons why it is so hard to create a simple definition of a gel is that the term “gel” covers many very different systems. The overview of these systems and the differences between them are given in the following chapter.

2.2 Classification of gels

“Gel” is a term used for a large group of different materials with various properties. Therefore naturally, there have been attempts to categorize them. Basically, there are two main ways to classify them – either based on the nature of their liquid phase or based on the nature of their solid phase (Fig. 1). If the liquid phase is water or an aqueous solution, then the system is called hydrogel; if the liquid phase is formed by other solvents, then the system is called organogel. If a gelator can gel both groups of solvents then it is called amphiphilic. To be complete, there are also aerogels, *i.e.* systems with air as a medium, and xerogels, *i.e.* systems which are formed from gels by drying.

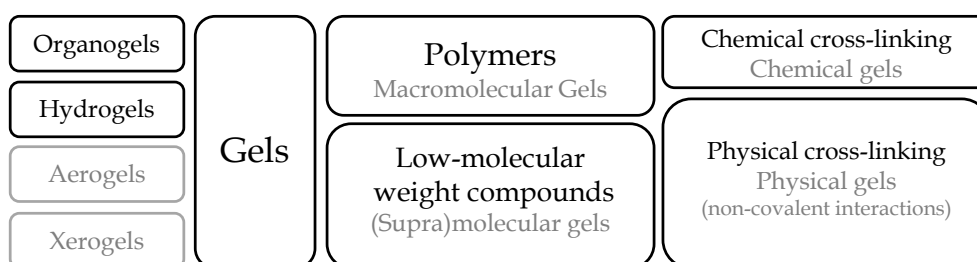


Figure 1. Classification of gels based on the nature of their liquid and solid phases, and on interactions involved in the gel formation process.

The other way to categorize gels is based on the composition of their solid phase. If the gelator is a macromolecule, the systems are called polymer gels (or polymeric gels),²²⁻²⁵ which can be further divided based on the nature of cross-linking in their network into two groups - chemical cross-linked and physical cross-linked gels. In the first group, the three-dimensional network is maintained by cross-linked covalent bonds, which make these gels robust and tolerant to physical deformation. In physical cross-linked gels, non-covalent interactions are responsible for the network stabilization. Polyacrylamide gel, which is commonly used for example as a medium in gel electrophoresis, is an example of a chemical cross-linked polymer gel, whereas gelatin and agarose are physical cross-linked polymer gels (Fig. 2).

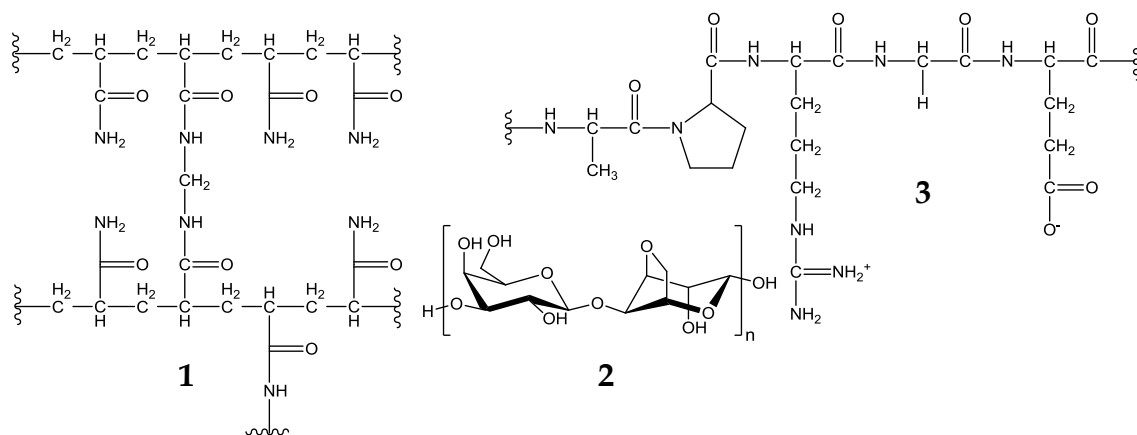


Figure 2. Substructures of common polymer gels such as polyacrylamide (1, chemical cross-linked gel), agarose (2) and gelatin (3).

Another group is called supramolecular gels (or molecular gels).¹⁻⁶ In contrast to the polymer gels, their network is built from small molecules (< 2000 Da), often called low-molecular-weight gelators (LMWGs). These subunits interact *via* non-covalent interactions to form a three-dimensional self-assembled fibrous network (SAFIN) with solvent molecules immobilized in the structure.⁴ Many examples of supramolecular gels utilizing different types of non-covalent interactions, such as hydrogen-bonding, π - π stacking, van der Waals and solvophobic interactions, have been reported. In most cases a combination of different types of non-covalent bonding is utilized and is responsible for an effective gel formation. The nature of these secondary interactions depends on the structure of the gelator and on the properties of the liquid being gelled. The liquid part can either promote or discourage interactions among SAFINs. In organogels, a combination of hydrogen-bonding, π - π stacking and van der Waals interactions is common, whereas in hydrogels, hydrophobic interactions are often the driving forces of a gel formation.²⁶

Many chemically different molecules, ranging from polymers to LMWGs, belong to the group of physical gels. Despite the differing nature of their constituents, most of them exhibit a characteristic thermally reversible gel-sol transition due to the thermally labile nature of non-covalent interactions which are responsible for holding the subunits together.

This work is about supramolecular gels based on steroids. Therefore, the next chapter focuses on supramolecular gels in general and is followed by a chapter about steroid-based supramolecular gels.

2.3 Supramolecular gels

Supramolecular gels¹⁻⁶ are formed by self-assembly of small molecules to fibrous three-dimensional networks. Their advantages to conventional polymer gels include:

- synthetic simplicity (“bottom-up” self-construction),
- synthetic efficiency,
- structural diversity, and
- tailor-made functionalization.

2.3.1 Types of supramolecular gels

Similar to gels in general, supramolecular gels can be classified based on the solvent either as hydrogels or as organogels. However, the classification based on the chemical nature of gelator molecules is more interesting with regard to potential properties of the system. Many reports on supramolecular gels based on chemically totally different molecules (Fig. 3) can be found in literature.^{6, 27} Examples include ureas,²⁸⁻³⁰ anthryl derivatives,⁶ nucleobases,^{31, 32} amino acids and oligopeptides,³³⁻³⁶ steroids,³⁷⁻⁴⁰ sugars,^{26, 41} fatty acids,⁶ and dendrimers,⁴² among many others. A comprehensive review of organogels based on LMWGs was reported for the first time by Terech and Weiss in 1997,⁶ and in 2004 Hamilton and Estroff summarized the field of LMWG hydrogels.⁵ Since then a number of reviews^{2, 3, 9} and books^{1, 4, 27} appeared, including review articles focused on steroidal supramolecular gels.^{37, 38}

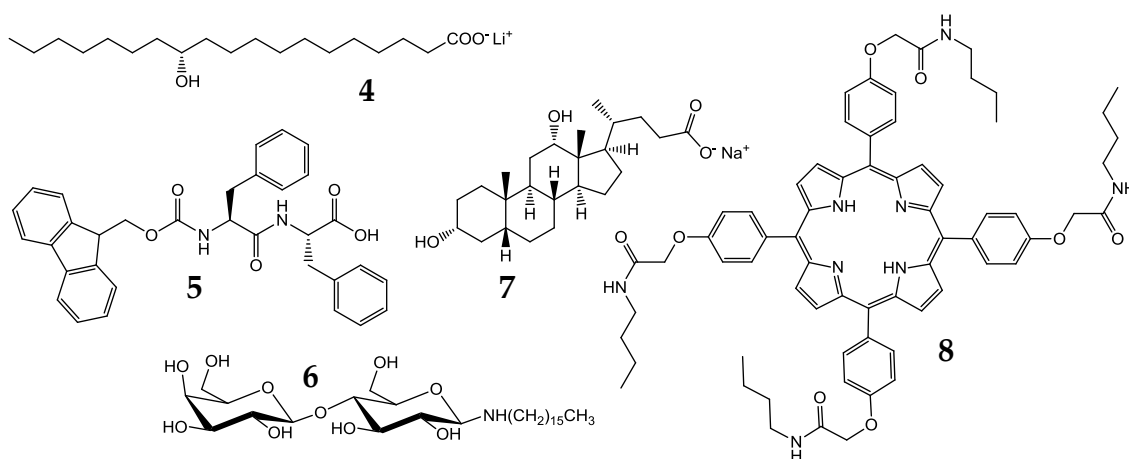


Figure 3. Chemical structures of various LMWGs based on chemically different molecules including fatty acid (4),⁴³ dipeptide (5),⁴⁴ sugar (6),⁴⁵ steroid (7),⁴⁶ and porphyrine (8).⁴⁷

In earlier years the discovery of new gelators was almost exclusively a matter of serendipity – they were usually found during recrystallization processes; however, a recent application of supramolecular principles to design new gelators has boosted the field significantly. Moreover, there still is a deep interest towards the synthesis of new gelators in general, but in the last few

years more and more attention has been given to the design of multi-component gel systems,^{48, 49} stimuli-responsive gels,¹²⁻¹⁴ and metallogels.¹⁵⁻¹⁷

Multi-component gels

The past decade has seen the production of an interesting range of supramolecular gels containing two or more chemically different molecules. These systems are called multi-component gels and can be seen as gel analogues to co-crystals. Most of the reports deal with two-component gels.^{48, 49} However, recently several examples of three-component systems were published.^{50, 51} According to Buerkle and Rowan,⁴⁸ there are three general classes of two-component gels that have been investigated (Fig. 4):

1. Systems where neither of the compounds can form gels alone but in combination they self-assemble to yield a gel (Fig. 4a).
2. Systems where both compounds are themselves gelators. In such systems there are two extremes, where (a) both gelators interact with each other to form co-fibers or (b) the two gelators self-sort resulting in two different nanofibers within the gel (Fig. 4b)
3. Systems where one compound is a gelator and the other molecule is non-gelating and is designed to impact the gel's thermomechanical or functional properties by, for example, impacting the assembly of the gelator (Fig. 4c).

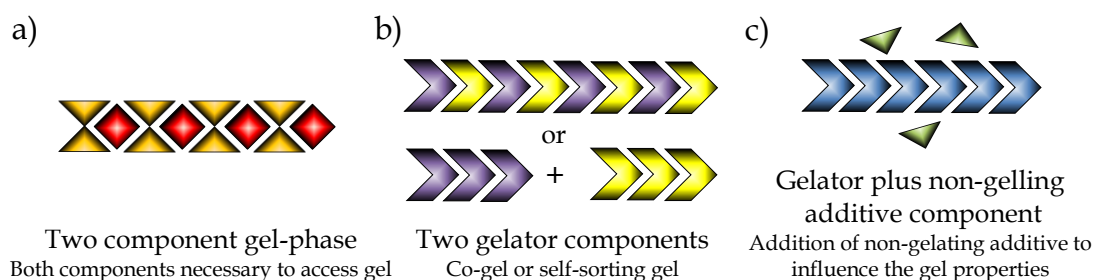


Figure 4. Different types of two-component gel systems.⁴⁸

In comparison to classic one-component gels, two-component systems offer an extra level of control and tunability in the self-assembly process. Moreover, a variation of one of the components provides a simple way to introduce functionality and diversity. Because of these reasons, it can be expected that the number of reports on two- and more component gels will increase quickly in the future.

Stimuli-responsive gels

Supramolecular gels are often called “smart” materials due to their ability to be responsive to various stimuli.¹²⁻¹⁴ Based on the character of the stimulus, they can be divided into two groups (Fig. 5):

1. Materials responsive to physical stimuli such as temperature, mechanical stress and ultrasound, and light.
2. Materials responsive to chemical stimuli - such as ions and neutral molecules, redox changes, *etc.*

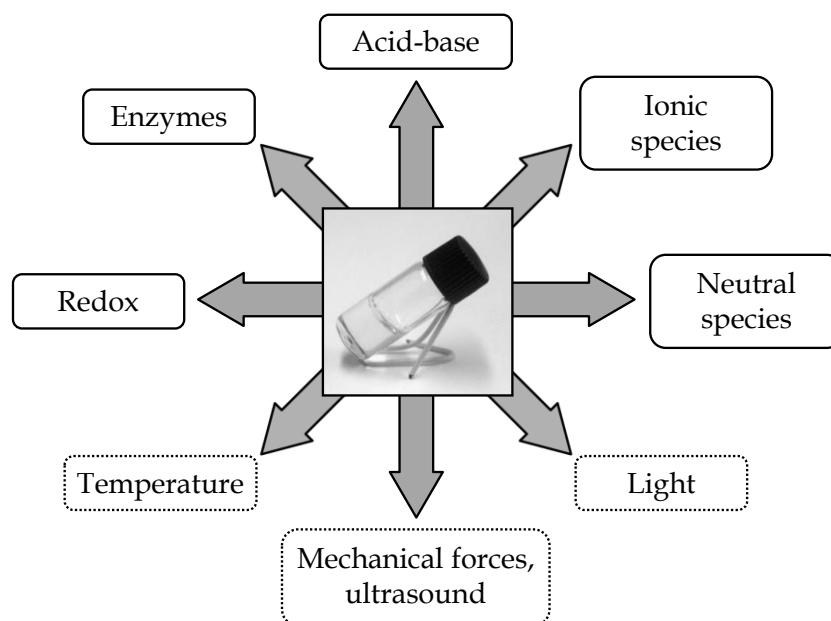


Figure 5. Classification of different stimuli: physical (dashed), chemical (solid).

Furthermore, systems responsive to more than one stimulus (without including thermal stimulus), *i.e.* multi-responsive gels, are known as well.⁵²⁻⁵⁶ The type of response depends on the applied stimulus and the mode of interaction between the stimulus and the gel network. The most common response of the systems is a transition from gel to sol (or *vice versa*). Other responses include changes in chemical or physical properties, such as conductivity, color, and light emission. Another important aspect that has to be taken into account is a reversibility of the process, which is desirable in many cases with regard to potential applications and places a high demand on the gelator design.

Physical stimuli

A temperature change is the most common physical stimulus and it is characteristic for physical gels in general. Since the association of the fibrous network of physical gels is enthalpy-driven and an increase in temperature shifts the equilibrium to the non-aggregated state, most gel systems react to temperature changes by a gel-sol phase transition. This state is usually temporary and after removing the stimulus - in this case a heating source, the gel is reformed. However, several unusual responses have been observed, including a reversible

shrinking and swelling of an LMWG gel induced by a temperature change.⁵⁷ Another common stimulus is an application of mechanical stress, which can deform or destroy the gel, depending on its viscoelastic properties and the magnitude of the applied stress.⁵⁸ Many gel systems are sensitive to the stress and if the stimulus is applied, the gel network breaks down to become a viscous fluid. For many systems, the gel state can be restored only by a heating-cooling cycle through the gel-sol-gel phase transitions. However, some of the gels are thixotropic, which means that the gel state is recovered if the stress is removed.

Light-responsive gels represent a special case of physically responsive LMWG gels because here a physical stimulus (photons) invokes a photochemical reaction which transforms the LMWG compound to a different species with its own characteristic self-assembly and gelation properties, or induces a color change of the system. The photon absorption is a very attractive way to change the gel properties, because it allows a photo-responsive group incorporated into the gel to be addressed selectively. A problem related to potential applications might be the possible lack of reversibility of the process. Azobenzene derivatives are especially useful, because they change their configuration from *cis*-to-*trans* (and *vice versa*) under specific wavelength exposure and that can trigger environmental changes in the self-assembly of LMWGs often resulting in a reversible gel-to-sol or sol-to-gel transition. Other often utilized photochromic groups include stilbene and dithienylethene moieties (Fig. 6).

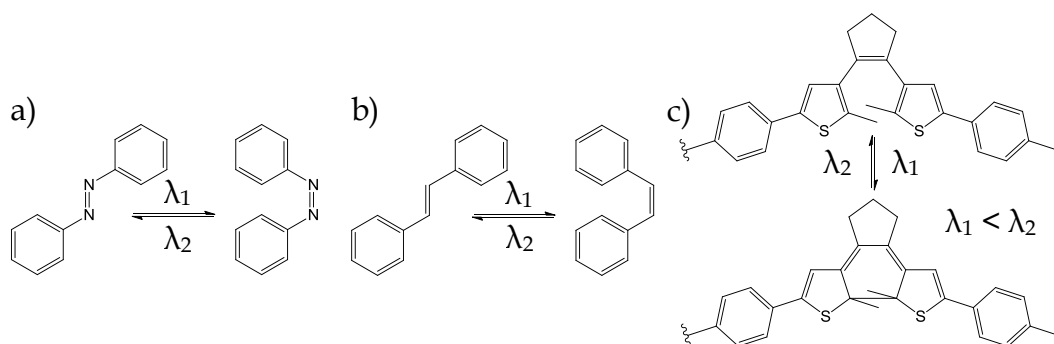


Figure 6. Photoisomerization of common photochromic moieties often used in gelator design: (a) azobenzene, (b) stilbene, and (c) dithienylethene.

Chemical stimuli

LMWG systems can be responsive to a wide variety of chemical triggers, such as pH changes, host-guest interaction, metal-ion coordination, or redox changes. Because of a better understanding of such triggers over the last decade and more powerful tools, like computational chemistry and modeling, nowadays LMWG systems can often be designed for a certain purpose. A promising approach towards development of new “smart” gels relies on an integration of an appropriate switching or responsive functionality, which is addressable by the stimulus, into the gelator structure. Similarly, how it was shown in the light-responsive gels (Fig. 6).

In gels with gelators containing acidic or basic groups, the gel-sol transition can be often reversibly switched by appropriate changes in the pH value,

which causes protonation/deprotonation of the functionalized groups. These chemical changes in the gelator structure affect the way of self-assembly and often result in a gel-sol transition. Especially pH-responsive hydrogels, often based on oligopeptides and peptides,⁵⁹⁻⁶³ are very attractive materials with regard to potential applications in regenerative medicine and drug delivery. However, the pH-sensitive hydrogelation is not restricted only to peptides. For instance, resorcinarene⁶⁴ and calixpyrrole⁶⁵ based compounds, or bile acid derivatives⁶⁶ can gel water at certain pH values as well.

Cation and anion responsive systems are another interesting group of smart materials. Recently, metal sensitive gels¹⁵⁻¹⁷ have received a lot of attention as attractive responsive materials because of the prominent role of metal ions in many enzymes, catalysts and electronic devices. Another quickly developing area is an incorporation of anions into gels and their use in tuning and switching gel behavior.^{16, 28, 67} The presence of an ion in a particular compound may have a large impact on its solubility, conductivity, or spectroscopic properties, which often make the systems easier to study.

For gelators with receptor units, host-guest chemistry is often utilized. The molecular recognition leads to changes in the supramolecular organization and alters physical properties of the gel.

Incorporation of a redox-active unit into a gelator structure leads to redox-sensitive gels.¹³ The response of the moiety may proceed in two different ways – either as a change of oxidation state of the sensitive center, or in a formation of new covalent bonds or in a destruction of sensitive fragments. In all cases the chemical changes alter properties of the LMWG and often results in a gel-sol transition or color changes. The first type is typical for metallo gels, whereas the second type corresponds to non-metallic gelators, for instance gelators with a tetrathiafulvalene (TTF) unit, which can be reversibly transformed into the respective radical cations and dication by either chemical or electrochemical redox reactions (Fig. 7).

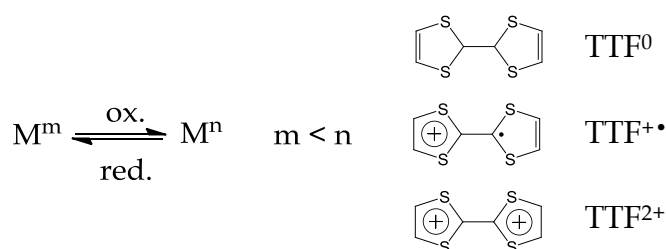


Figure 7. Common redox-sensitive moieties often used in gelator design – a metal ion (left) and the tetrathiafulvalene unit (right).

Enzyme sensitive systems are another attractive group of smart materials with potential applications in medicine and drug delivery.⁶⁸⁻⁷⁰ Two main strategies can be applied in this field – the specific activation of a pro-gelator molecule that forms a fibrillar network in a response to the presence of an enzyme and the use of an enzyme as a trigger for the disassembly of the gel phase (Fig. 8).

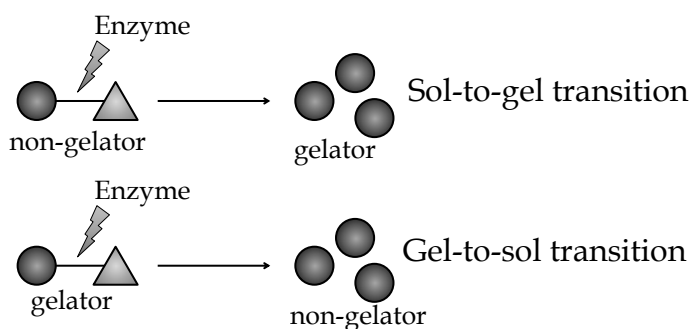


Figure 8. Schematic representation of enzyme triggered sol-to-gel and gel-to-sol transition.

Metal-organic gels

Metal-organic gels (or metallo gels) are another quickly growing area of supramolecular gels. In a simplified way they could be described as gels containing metal ions, where the metal can form a part of the covalent structure of a gelator, or can be present as a metal ion forming weaker coordination interactions that nevertheless affect gelation properties of the gelator. In many cases the systems can be chemically tunable (according to the identity of the metal ion), or stimuli responsive (*e.g.* redox-responsive, or metal ion responsive).¹⁵⁻¹⁷

There are two main reasons for introducing metal ions to gel systems. Firstly, by introducing metal ions to the gel, the properties of metal ions are introduced. This gives a large opportunity to prepare materials with very attractive properties such as luminescence, magnetism, catalytic and redox activity, conductivity, etc. Second, coordination bonds are relatively strong interactions compared to conventional secondary interactions, which are usually employed in the gel formation and therefore they can be used to strengthen the gel microstructure. In some cases a gelator is designed so that after adding metal ions, polymer-like structures are formed,^{16, 71} which can be seen as a gel analogue of metal-organic frameworks (MOFs).⁷²

2.3.2 Gelation process

Despite the structural diversity among LMWGs, they have the common property of self-assembly *via* non-covalent interactions into fibres very efficiently in solution. Subsequently, these fibres form entangled networks (SAFINs) with immobilized solvent molecules inside the microstructure, essentially preventing the macroscopic fluidity.^{4, 73}

Supramolecular gels are usually prepared by dissolving a gelator in an appropriate liquid, usually assisted by warming and an ultrasound treatment. At this point, the gelator is unaggregated or in small aggregates and this system is referred to as a solution (sol). By cooling to ambient temperature, an isotropic supersaturated solution is formed, molecules start to condense and the following three modes¹¹ are possible (Fig. 9):

- A highly ordered aggregation giving rise to crystals, *i.e.* crystallization;
- A random aggregation resulting in an amorphous precipitate;

- An aggregation process intermediate between these two, yielding a gel!

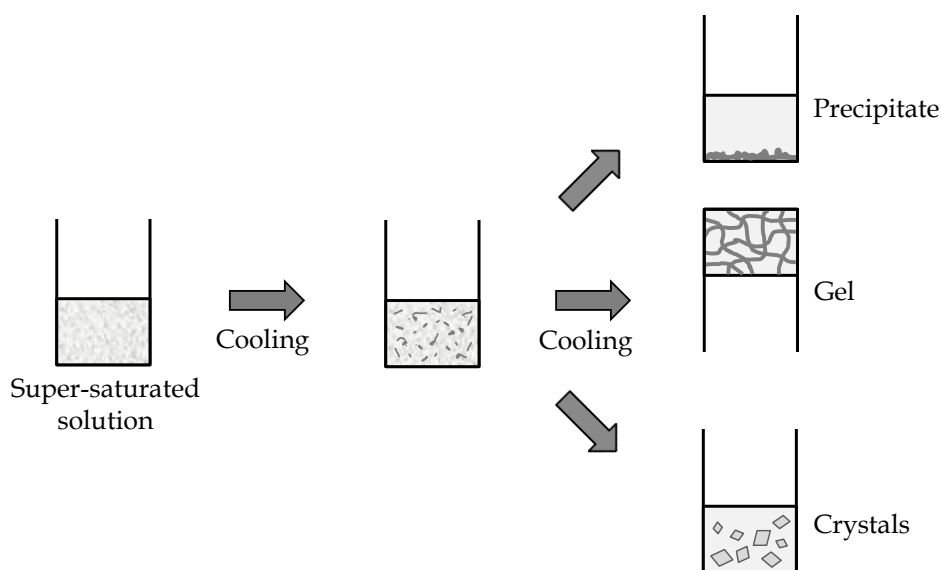


Figure 9. Schematic representation of the possible aggregation modes.

In a simplified way, gelation can be seen as an uncompleted crystallization, because a microscopic phase separation occurs, rather than the macroscopic phase separation common for the crystallization process. From the self-assembly mechanism point of view, gelation can be seen as “supramolecular polymerization” of LMWGs. The gelator molecules self-assemble in stochastic nucleation events involving highly specific interactions that allow preferentially one-dimensional growth, usually to form fibers that are frequently crystal-like.⁴ The “junction zones” between fibers (formed by strands, tapes, ribbons, tubules, *etc.*) provide rigidity to the microstructure. The resulting network of micro- or nanoscopic objects with a high aspect ratio forms a three-dimensional porous lattice that permeates the volume of the sample, encapsulating the liquid component and inhibiting its fluidity.

2.3.3 Potential applications of supramolecular gels

Many different forms of gels are commonly used in everyday life. Their applications range from personal care products (toothpaste, shampoo, deodorants, soft contact lenses *etc.*) to foodstuffs (puddings, syrups, jelly *etc.*), electronic devices, drug delivery and tissue engineering. Most of these systems are based on polymer gels, although, a number of applications based on supramolecular gels is rising.⁷⁻¹¹

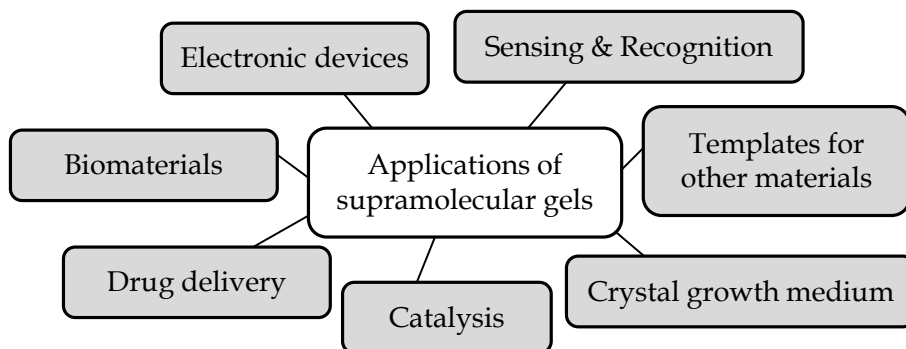


Figure 10. Examples of potential applications of supramolecular gels.

It was shown that supramolecular gels can be used as attractive materials, for instance:

- for molecular recognition and sensing;^{7, 9}
- as templates for nanoparticles and silica materials;^{7, 9}
- as crystal growth material;⁷⁴
- as water cleaning agents;⁷⁵⁻⁷⁷
- for optical and electronic applications;^{9, 10}
- as light-harvesting systems;^{7, 9}
- in catalysis;⁸
- as reaction media;^{8, 9}
- in drug delivery;^{9, 78-81}
- as biomaterials in regenerative medicine and tissue engineering.^{9, 10, 79}

Especially stimuli-responsive supramolecular gels¹²⁻¹⁴ have a great potential in the construction of novel smart functionalized soft materials for applications as sensors, or molecular devices, and systems for controlled drug release.^{7, 9} For instance, incorporating a drug into a gelator which forms a stable gel under physiological conditions but which breaks in the presence of a particular physiological stimulus (change in pH, presence of a particular ion or enzyme) would allow for the targeted delivery of a drug to a diseased site (Fig. 11). This would reduce the dosage of drug required and potentially reduce any adverse side effects.

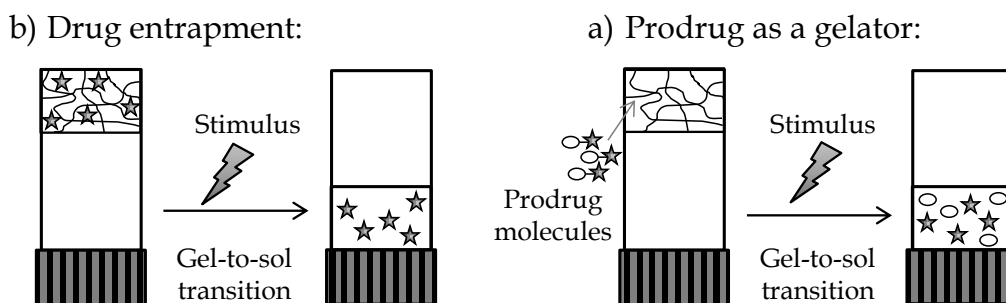


Figure 11. Stimuli triggered substance release from a gel.

2.4 Supramolecular steroid-based gels

Steroids are widespread natural compounds having many various and important functions in living organisms. This variety in their functions is reflected in a diversity of their structures. However, one feature is common for all steroids – they all have a steroidal backbone formed from the tetracyclic cyclopentanoperhydrophenanthrene. This large and conformationally restricted skeleton, together with a presence of different functional groups which can be quite easily derivatized, has made them attractive building blocks for organic and supramolecular chemists for numerous applications.^{82, 83}

Steroids have become favorite building blocks in the preparation of supramolecular gels^{38, 39} due to their strong tendency to aggregate. Additionally, their natural character and biological activity can be very attractive, especially with regard to potential medical and pharmacological applications.^{84, 85} Despite all these excellent properties, steroidal molecules are responsible mostly only for the aggregation process in most cases. When a functional system (*e.g.* stimuli responsive soft material) is required, a derivatization with suitable moieties is often necessary. Additionally, by introduction of suitable functional groups, the overall polarity profile of these gelators can be tailored and the self-assembly characteristics adjusted.

George and Weiss showed that molecular systems comprised of an aromatic moiety (A) connected to a steroidal unit (S), which is usually cholesterol, *via* a functionalized linker (L) can display an effective, and in some cases predictable, gelation ability.⁸⁶ Later on, new generations of steroidal gelators having dimeric structures, named as an $A(LS)_2$ or LS_2 type, have appeared (Fig. 12). Since then, many steroidal derivatives with similarly looking structures have been prepared and proven as effective gelators.³⁷

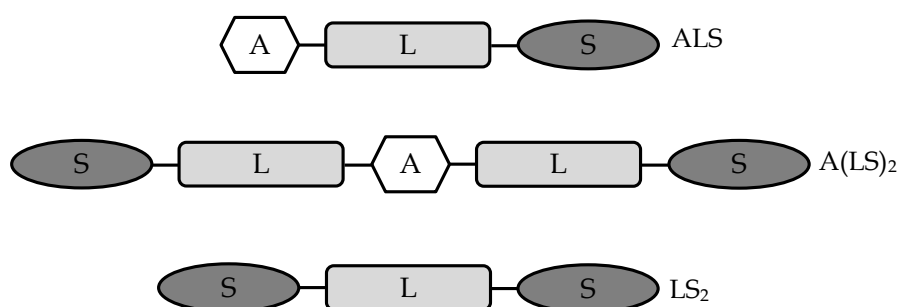


Figure 12. Schematic presentation of ALS, $A(LS)_2$ and LS_2 architectures of steroid-based gelators.

2.4.1 Steroidal building blocks

There are two main groups of steroids which have been extensively used as building blocks for the construction of supramolecular gels. They are bile acids, and sterols with cholesterol as the main representative. However, recently a few

examples of steroidal supramolecular gels based on other steroids, such as pregnane and estradiol, have been reported (Fig. 13). Di Chenna and co-workers synthesized a pregnane derivative (**9**) with a silyl ether group at a C-3 position combined with a 6 β ,19-oxo bridge, and showed the possible usage of its tetraethyl orthosilicate gel as a template for the preparation of SiO₂ nanotubes.⁸⁷ Another example is a family of estradiol-based low-molecular-weight gelators (Fig. 14), readily accessible using click-chemistry, which gels different organic solvents in the presence of water even at concentrations as low as 0.04 wt%.⁸⁸

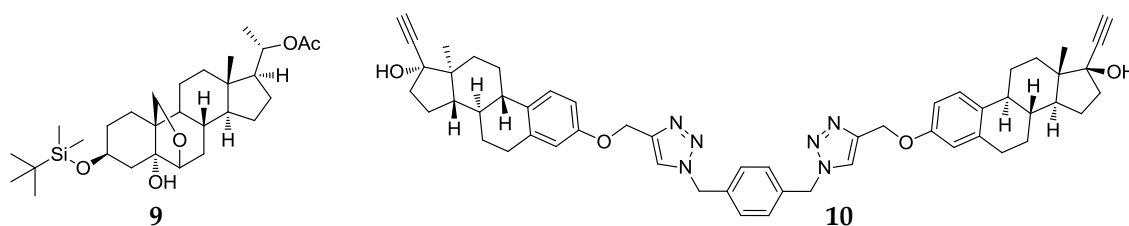


Figure 13. Examples of gelators based on pregnane (**9**) and estradiol (**10**).

Cholesterol (**11**) is the best known representative of the group of sterols. It is a constituent of the cell membranes and is found in all animal tissues, where it maintains membrane permeability and fluidity. Further modifications of the stereochemistry and oxidation states of the fused rings, the side chain, as well as the functional groups of cholesterol lead to a wide variety of biologically important molecules. These include steroidal saponins, cardioactive glycosides, bile acids, and steroidal hormones. Phytosterols are plant sterols and are characterized by the presence of additional one- or two-carbon substituents attached at C-24 of the side chain and may vary in a number of double bonds in the rings and/or the side chain. The widespread plant sterols, campesterol (**12**) and β -sitosterol (**13**), respectively, are 24-methyl and 24-ethyl derivatives of cholesterol, whereas stigmasterol (**14**) contains additional unsaturation of the side chain (Fig. 14).^{89, 90}

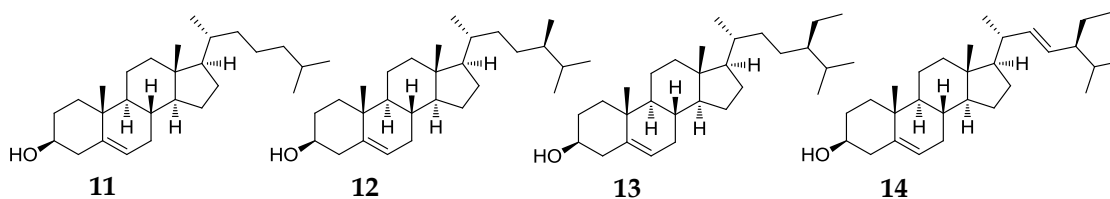


Figure 14. Chemical structures of cholesterol (**11**) and common phytosterols: campesterol (**12**), β -sitosterol (**13**), and stigmasterol (**14**).

Bile acids (Fig. 15) are amphiphilic steroidal acids derived from enzymatic catabolism of cholesterol and can be found predominantly in the bile of mammals. Their main function is to facilitate the formation of micelles, which promotes absorption of dietary fat.⁸⁴ Due to their easily derivatized functional

groups and availability, they have become favorite building blocks for various applications in supramolecular chemistry.^{82, 85, 91}

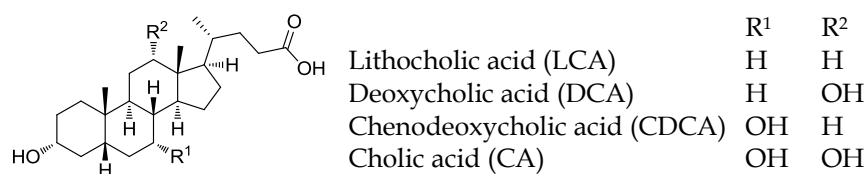


FIGURE 15. Chemical structures of common bile acids.

The unique chemical and physical properties of bile acids and sterols combined with their availability and low costs have made them very popular building blocks for designing new soft materials.^{37, 38} In the following review chapter, examples of supramolecular gels based on steroidal derivatives of nitrogen compounds, including attractive stimuli-responsive gels and metallogels, are summarized. Among these, also other steroidal gels based on chemically different molecules can be found in literature, and recent advances in this field are summarized in article I. In the following chapter, the state of the art in steroidal gelators derived from nitrogen compounds is described by presenting the key articles and other interesting and recent studies to provide a basis for the work done in this thesis.

2.4.2 Steroidal supramolecular gels based on nitrogen compounds

One of the earliest reports on steroidal gelators containing nitrogen compounds was given on the organogelation properties of *N*-isopropyl cholamide.⁹² Later, several other conjugates of steroids and simple amines were found to be effective gelators. For instance, Willeman and co-workers reported on gelling abilities of simple amide and urea derivatives of cholic acid.⁹³ Since then many different conjugates of steroids and nitrogenous compounds have been synthesized and their properties as gelators for various solvents have been studied. It was revealed that the use of steroidal conjugates of amino acids, amino alcohols, or diamines, has been very successful in preparing new gelators. In most of these cases, the nitrogen moieties are introduced due to their potential to participate in hydrogen bonding and to adjust polarity of the conjugates.

The steroidal part is usually responsible only for the aggregation process, whereas, functionality can be introduced to the systems by incorporating suitable nitrogen moieties. For instance, basic amino groups, which can be reversibly protonated, can be used in the preparation of acid/base responsive systems; nitrogen aromatic moieties can often efficiently bind metal ions and therefore, can be used in preparation of metal sensitive gels and metallogels; azobenzene unit can be used in order to prepare light-responsive systems, *etc.*

Herein, interesting examples of steroidal gels containing nitrogen compounds are summarized (based on the nature of the nitrogen molecule).

Steroidal derivatives of amino acids

Various amino acid derivatives of bile acids, cholesterol or other sterols (e.g. stigmasterol⁹⁴) have been reported. In most of the cases amino acids are attached to a bile acid unit *via* an amide bond, whereas to a sterol moiety *via* an ester bond. For instance, *N*-cholyl amino acid alkyl esters (Fig. 16) were shown to act as organogelators for aromatic solvents and cyclohexene affording stable, transparent, and thermoreversible gels.⁹⁵ Modification of the molecular structure and IR measurements showed that gelation took place by means of a hydrogen-bond network and involves at least the amide bond and several hydroxy groups of the cholic acid component. Although a wide variety of derivatives displayed gelation behavior, a small change in molecular structure or chirality of the amino acid unit affected the gelling capability significantly. In order to study the effect of the bile acid moiety, a series of *L*-leucine methyl ester conjugates of other bile acids (**16a-c**) was prepared and their gelation properties were tested.⁹⁵ Unfortunately, these reference compounds did not gel any of the tested solvents indicating that the nature of the bile acid unit, *i.e.* a number of hydroxyl groups, is crucial for a successful gelation.

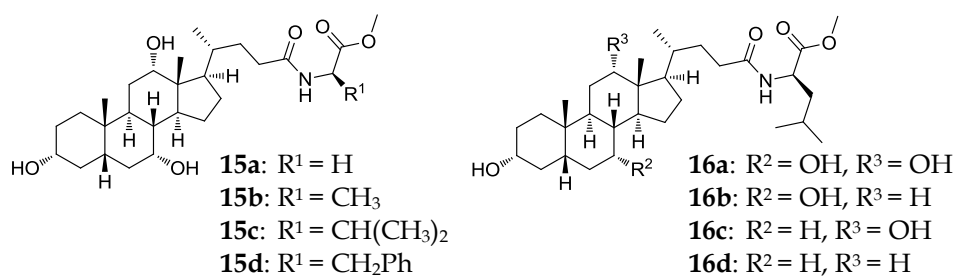


Figure 16. Selected *N*-cholyl amino acid alkyl ester organogelators (**15a-d** and **16d**), and their bile acid-based equivalents (**16a-c**).

Bile acid derivatives of methionine, cysteine and cysteamine represent another extensively studied group of steroidal conjugates (Fig. 17). A series of three bile acids (LCA, DCA, and CA) conjugated with *L*-methionine methyl ester was prepared.⁹⁶ Two of the conjugates, namely lithocholyl and cholyl derivatives (**17a** and **17b**), were found to undergo self-assembly leading to organogelation in certain aromatic solvents. In another study, bile acid derivatives of *L*-cysteine ethyl ester were examined.⁹⁷ Only the cholyl conjugate **18** was found to gelate some of the tested aromatic solvents, tetrachloromethane and diethylether. Similar results were observed, when derivatives of bile acids with cysteamine (2-aminoethanethiol) were studied.⁹⁸ Only the derivatives of dehydrocholic (**19**) and cholic acid (**20**) were found to form organogels.

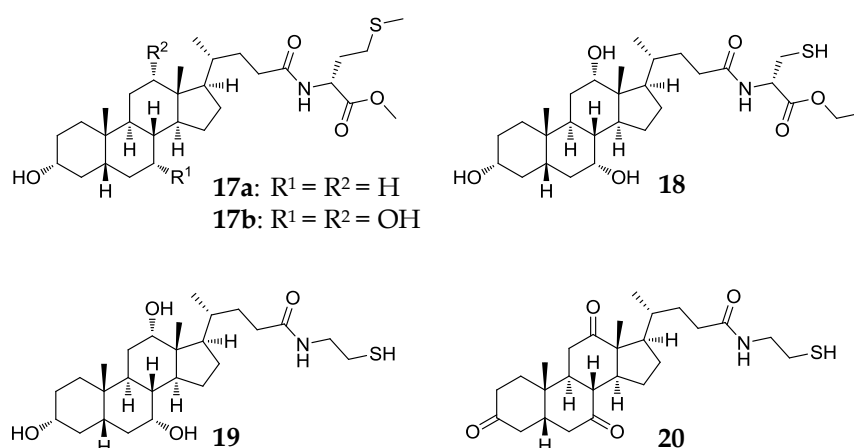


Figure 17. Selected gelators based on bile acid conjugates of methionine (**17a-b**), cysteine (**18**) and cysteamine (**19,20**).

In the following, examples of utilizing amino acid fragments as convenient proton accepting/donating functional groups in order to prepare acid/base sensitive systems are shown. For instance, cholesteryl derivatives of selected amino acids, namely glycine, L- and D-alanine, and L- and D-phenylalanine, were found to be gelators only when transformed to hydrochloride salts, whereas as neutral molecules, they did not gel any of the tested solvents.⁹⁹ Additionally, it was found that the gelling abilities not only depended on the character of the amino acid unit itself, but also on its chirality.

Similar results were obtained when compounds **21a-c** were studied.¹⁰⁰ The gelators, having a cationic polar head (**22a-c**), showed better water gelation efficiency (minimum gelation concentration of 0.9-3.1 % w/v) than the analogous free amines (**21a-c**). Importantly, these cholesterol-based amphiphiles showed improved biocompatibility compared to similar structures having a long alkyl chain instead of a steroidal unit. Thus, introducing a steroidal moiety can be an effective strategy for decreasing cytotoxicity of the system, which is very important with regard to potential applications of steroidal hydrogels as smart soft biomaterials.

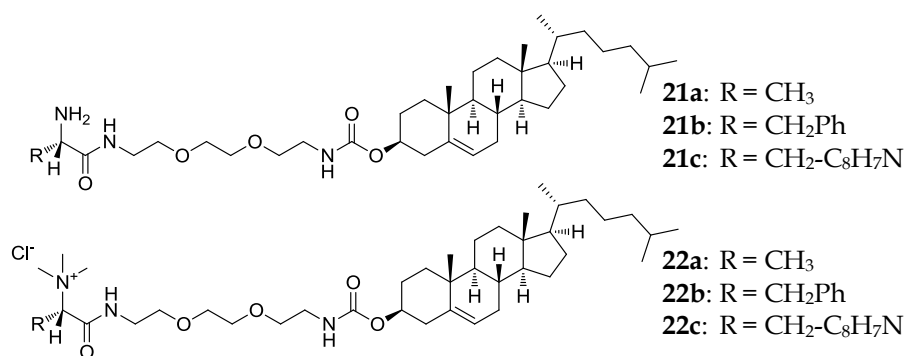
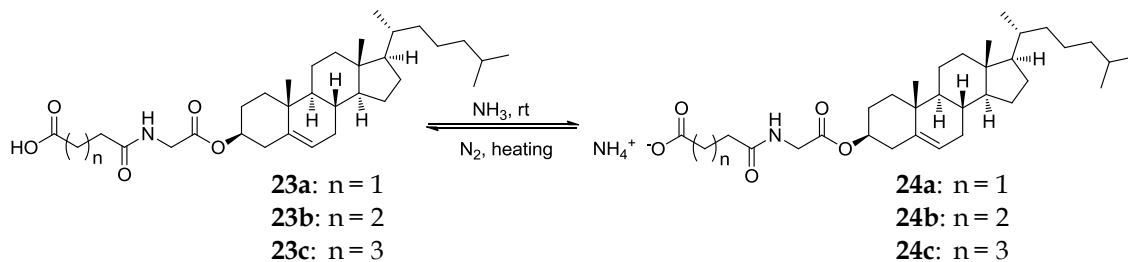


Figure 18. Cholesterol-based amphiphiles.

In another work, base-responsive steroidal gelators were prepared (Scheme 1).¹⁰¹ It was found that the acids (**23a-c**) formed only weak gels, but upon neutralization with ammonia, their gelation ability was significantly enhanced. The amino salt **24a** behaved as an amphiphilic gelator (gelling both apolar solvents and water) suggesting two different ways of self-assembly, hydrophobic surface-mediated in aprotic and hydrophilic surface-mediated in polar solvents. Interestingly, some of the alkyl alcohols and water could be gelled at room temperature simply by bubbling ammonia through the system.



Scheme 1. Chemical conversion of diacid monoamides of cholesteryl glycinates (**23a-c**) to their corresponding ammonium salts (**24a-c**).

Amino acids (and diamines) are also often used as linkers in the ALS, A(LS)₂, and LS₂ gelator types (Fig. 12). Among others,¹⁰²⁻¹⁰⁴ the group of Fang has put a lot of effort into designing and preparing new cholesterol-based gelators along with extensive studies of the formed gels. They prepared several series of cholesterol-based gelators of the A(LS)₂ type with various amino acids as linkers, for instance dimeric cholesteryl gelators (**25-27**) with glycine residues as linkers and benzenedicarboxylic acid moieties as an aromatic central molecule (Fig. 19).¹⁰⁵ Compounds **26** and **27**, bearing *m*- and *p*-substituents, were shown to be more efficient gelators than the compound **25** possessing an *o*-substitution. Interestingly, the compound with the *m*-substitution (**26**) formed a gel in xylene spontaneously at room temperature and the gel displayed a thixotropic behavior. Later on, analogous dimeric cholesteryl gels with either hydrazine¹⁰⁶ or diaminobenzene moieties¹⁰⁷ as linkers were prepared. This linker molecule offered more hydrogen bonding sites, which resulted in the improvement of the gelation behavior of the compounds. Many of these systems formed gels spontaneously at ambient temperature and possessed smart thixotropic properties as revealed by rheological studies.

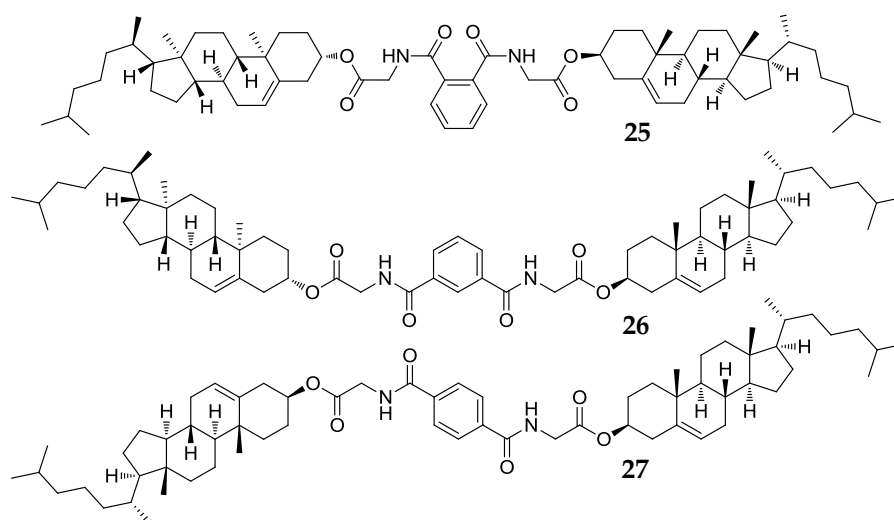


Figure 19. Cholesterol-based gelators of A(LS)₂-type with glycine residues as linkers.

Dicholesteryl derivatives **28a-d**, with spacers containing two L- or D-alanine residues and three to six carbon atoms between them, were found to be thixotropic too. These gelators were prepared in order to investigate the effect of the length of the spacer and the chirality of the amino acid residue on the gelation properties of the compounds.¹⁰⁸ The compounds containing D-alanine residues and shorter spacers were shown to be able to gel more solvents than their analogues with an opposite chirality of the amino acid residues. However, for the compounds having longer spacers, an opposite result was obtained. Additionally, some of the gel systems were shown to form gels spontaneously at ambient temperature. Later on, analogous thixotropic organogelators with either L-phenylalanine (**29a-c**)¹⁰⁹ or glycine¹¹⁰ residues as linkers (instead of the alanine moieties) were prepared.

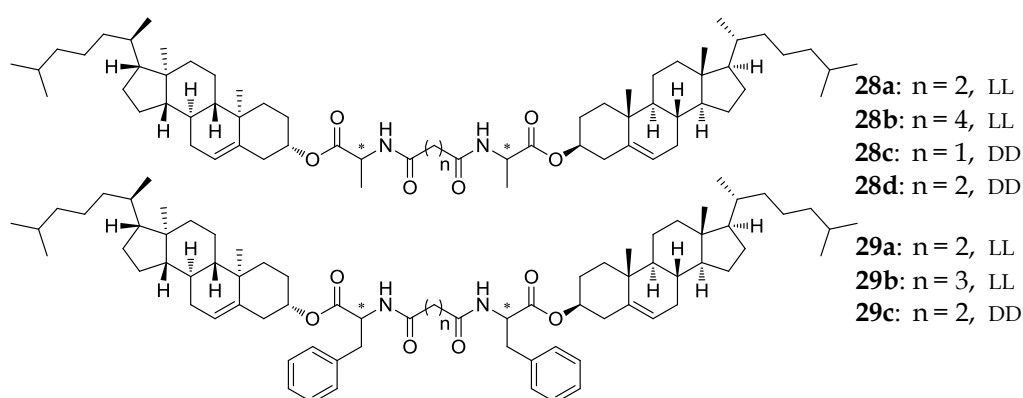


Figure 20. Selected examples of cholesterol-based gelators of LS₂-type with L(D)-alanine and L-phenylalanine residues as linkers.

Recently, ultrasound triggered gelation of fluorescent cholesterol-based gelators of the ALS type (with a naphthalimide unit as the aromatic group and different linkers varied from an alkyl chain with a variable number of acyla-

mino linkages to an alkyl chain with an L-alanine moiety embedded in it) was reported (Fig. 21).¹¹¹⁻¹¹³ It was shown that the ultrasonic irradiation could be used to reversibly control aggregation of the gelators. According to the results, it was suggested that the sonication-switch phenomenon could only happen in a compound with two hydrogen-bonding sites through a competition of intra- and intermolecular hydrogen bonds, as well as hydrophobic interactions. The location of the hydrogen bonds (determined by the lengths of the alkyl chains) had a strong effect on the solubility and gelling properties of the compounds. Interestingly, the morphology of their xerogels was strongly dependent not only on the solvents, but also on the external stimuli triggering the gelation. However, some trends could be observed; in most cases a thermal process afforded hollow spherical motifs, while ultrasound irradiation resulted in the spontaneous formation of the intermolecular hydrogen bonds and aggregation-induced helical motifs.

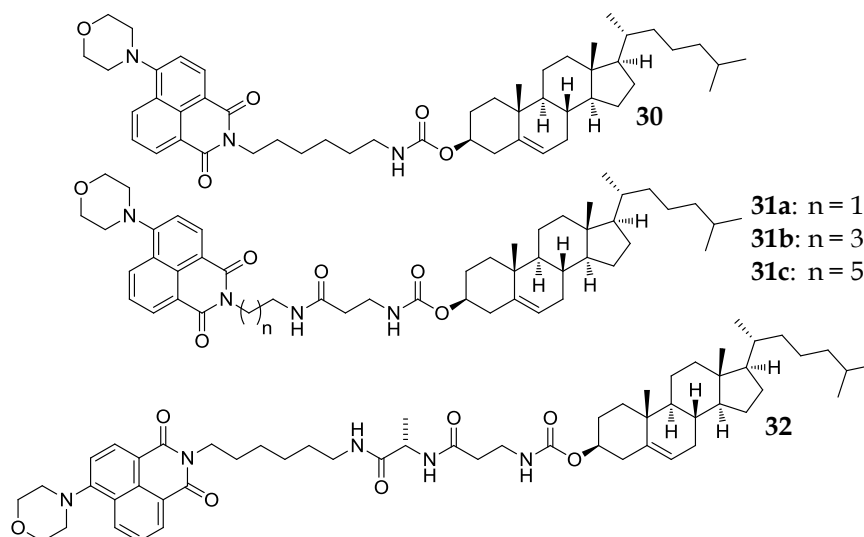


Figure 21. Ultrasound-responsive cholesterol-based gelators.

Steroidal derivatives of aminoalcohols, diamines, and amines

Steroidal derivatives of aminoalcohols, diamines, and amines represent another interesting group of potential gelators. For instance, simple bile acid amides of lithocholic and deoxycholic acids with 2-aminoethanol and 3-aminopropanol were found to be effective gelators for various chlorinated organic solvents or aromatic solvents, depending on the length of the side chain. Moreover, the compounds were capable of thickening neutral and acidic water solutions.¹¹⁴ Later, a similar study was carried out for bile acid conjugates of simple diamines, namely ethane-1,2-diamine, propane-1,3-diamine, and butane-1,4-diamine. It was observed that apart from one exception, only lithocholic acid derivatives formed gels in some of the tested solvents.¹¹⁵

Recently, gelation properties of four amphiphilic conjugates (**33a-d**) based on cholesterol and linear glucose, with various diamines as linkers, were reported.¹¹⁶ All of the studied compounds could gel both protic and aprotic sol-

vents, and it was shown that the number of gelled solvents and the possibility of forming transparent gels increased with increasing the length of the linker chain. Moreover, three of the reported compounds (**33b-d**) could gel xylene at very low concentrations (below 0.09 % w/v).

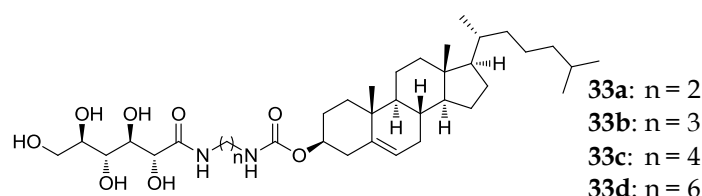


Figure 22. Sugar containing cholesterol-based amphiphilic gelators.

Amide conjugates of deoxycholic acid and 2-amino-2-hydroxymethyl-1,3-propanediol (**34**) and 2-amino-2-methyl-1,3-propanediol (**35**) respectively, represent examples of rare bile acid-based hydrogelators with neutral side chains. Although, they are insoluble in water, in the presence of varying amounts of organic solvents, such as methanol, ethanol, DMSO, and DMF, they form thermoreversible and transparent gels.¹¹⁷ These systems, together with related cationic bile-acid based hydrogelators (Fig. 26),¹¹⁸ were further studied by small-angle scattering, rheology, and microscopy techniques. This provided a precise structural description of the network architecture and its variation as a function of concentration, aging time, solvent composition, and the type of the gelator.¹¹⁹

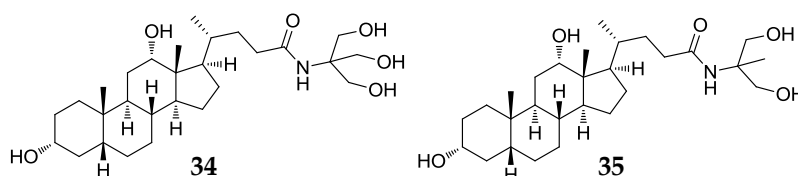


Figure 23. Bile acid-based hydrogelators with neutral side chains.

Tripodal cholamide **36** has turned out to be a supergelator of aqueous fluids.¹²⁰ The minimum gelation concentration was as low as 0.15 mM (*i.e.* 0.02 % w/v). The “best” gels, meaning transparent and thermally stable ones, have been obtained in acetic acid–water mixtures ranging from 0.01 to 30 % AcOH in water, depending on the gelator concentration. Furthermore, the formation of a hydrophobic “pocket” during the gelation was inferred using 8-anilino-1-naphthalenesulfonate as a polarity-sensitive probe. It was shown that the system doped with the probe molecule was highly fluorescent, but only in a gel state. Based on this result, a thermochromic gel using bromophenol blue as a dopant was developed.

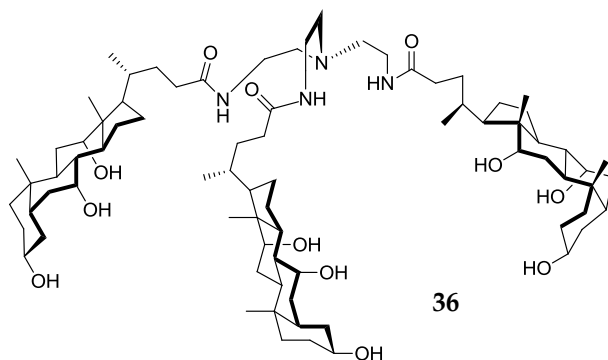


Figure 24. Tripodal cholamide.

As it was shown for steroidal derivatives of amino acids, ⁹⁹⁻¹⁰¹ conjugates containing basic amino groups can be protonated that can lead to a preparation of acid/base responsive systems. For instance, organogelator **37** was found to be a non-gelator in its neutral form, whereas, as its iodide salt, it formed a strong gel in 1,2-dichlorobenzene and chlorobenzene at very low concentrations (0.05 % w/v). To illustrate the acid–base switching of the gel, a simple experiment was performed. Upon exposure to ammonia vapor, the gel transformed to a sol and could be re-formed upon exposure of the sol to HI vapor. For the hydrogel derived from compound **38**, the situation was reversed. The neutral amine formed a gel in a DMSO/water 1:1 mixture (0.5 % w/v), whereas exposure to HI vapor disrupted the gel framework.⁶⁶

Cationic bile-acid based hydrogelators (Fig. 25) were found to form thermoreversible gels in water or in water in the presence of NaCl. ¹¹⁸ The use of NaCl was shown to be essential for better gelation of aqueous solvents, since NaCl provides an ionic environment in addition to the salting out effect. However, salt concentrations above 4 M were found to prevent the gelation process. Gels have been obtained also in a presence of some other inorganic salts. For the quinuclidine-grafted cationic bile salt **39a**, the influence of electrolytes and counter-ions (*e.g.* chloride and iodide ions) on the rheological properties of the gels was investigated.⁶⁶ The concentration of the electrolyte was shown to produce a dramatic effect on the gel stability. Increase in the salt content led to weaker gels. Moreover, the fibrillar network of the conjugate with iodide as a counter-ion appeared to be more sensitive to the added salt compared to the conjugates with other counter-ions.

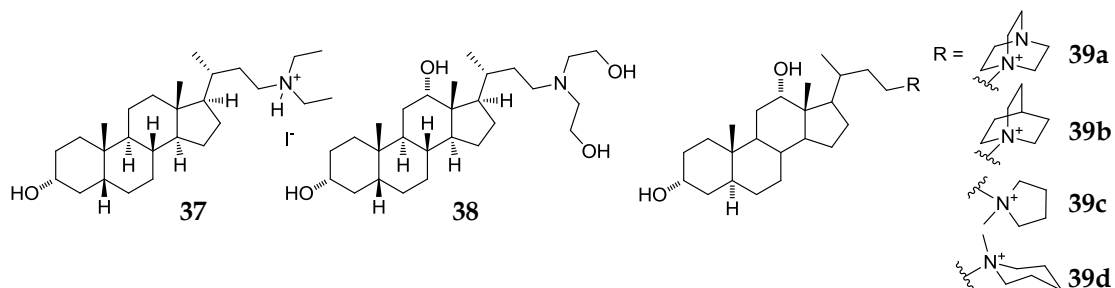


Figure 25. Chemical stimuli responsive bile acid-based hydrogelators.

Steroidal derivatives of aromatic nitrogen moieties

Many steroidal derivatives of aromatic nitrogen compounds were found to be effective gelators. The reasons for introducing aromatic moieties include their possibilities to participate on aggregation *via* π - π stacking, abilities to bind metal ions in order to prepare metal responsive gels or metallo gels with interesting properties (*e.g.* redox-responsive), *etc.*

Compound **40** is one of the first reported steroidal derivatives that forms metallo gels.^{121, 122} The porphyrin-based gelator (bearing a cholesterol moiety with Zn^{2+} ions irreversibly incorporated into the porphyrin unit) was found to form gels in aromatic solvents at 5 °C, which turned to a sol at higher temperatures. It was observed that by adding [60]fullerene, the thermal stability of the gels was improved. π - π stacking, hydrophobic interactions, and van der Waals forces were proven to be the leading forces for the self-assembly, whereas the contribution of metal-ligand interactions to the process was not significant.

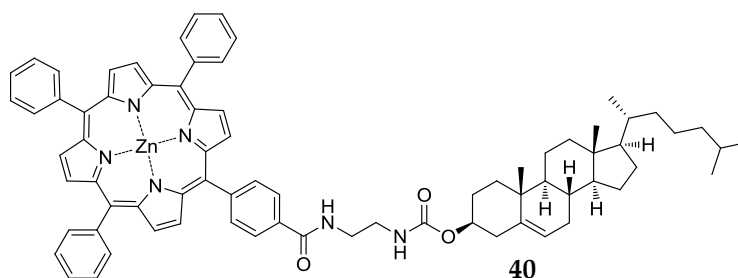


Figure 26. Steroidal porphyrine-based gelator.

Compounds **41a-b** and **42** represent examples of gelators based on steroidal Pt-complexes (Fig. 27). It was determined that π - π stacking of the heteroarene moiety and metal-metal interactions were the key contributors to the gelation process of **41a-b** and that the replacement of the chloro-ligand in the Pt-complex to a bulky ligand blocked the assembly process between the heteroaromatic rings inducing a collapse of the gel network.¹²³ The theory was further confirmed by utilizing chiral BINAP as the bulky ligand. The addition of one equivalent of (*R*)-or (*S*)-BINAP followed by a heating/cooling cycle resulted in a collapse of the metallo gels. Surprisingly, when the amount of the chiral phosphine was reduced to 0.1 eq., a striking difference in the behavior of the respective gels was observed. The gel sample containing (*S*)-BINAP survived the heating and cooling sequence as a robust gel, whereas, the gel sample containing the (*R*)-BINAP enantiomer collapsed. Similar behavior was observed for other chiral phosphine ligands, demonstrating the potential use of the metallo gels in a simple protocol for visual chiral recognition.

Gelator **42** based on a steroidal photochromic spironaphthoxazine-containing platinum(II) bipyridine complex was found to form stable thermoreversible gels in aliphatic solvents like heptane, octane, decane, as well as dodecane at room temperature. Interestingly, upon prolonged irradiation at $\lambda=405$ nm at 15 °C, the yellow gels turned blue with the growth of a new ab-

sorption band at about 600 nm, which was attributed to the photochromic reaction of the spironaphthoxazine moiety.¹²⁴

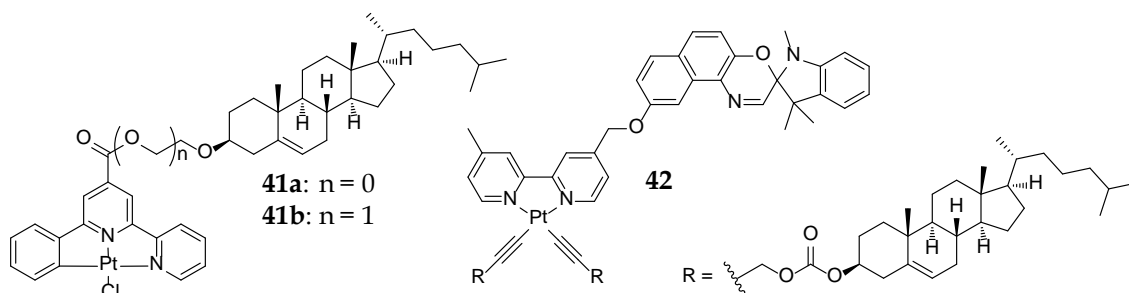


Figure 27. Selected steroidal gelators based on Pt-complexes.

In 2004, the first redox-responsive metallogel was reported.¹²⁵ It was formed by a Cu(I) complex of a ligand based on a 2,2'-bipyridine derivative bearing two cholesteryl groups (**43**). It was observed that the temperature stability of its gel was highly dependent on the metal-ligand molecular ratio. As an optimal ratio, a 1:2 stoichiometry was determined, which is in a good agreement with the expectable stoichiometry of the complex. Moreover, a thermo-chromic sol-gel transition in 1-butyronitrile was accompanied with a color change from reddish brown to greenish blue, which is untypical for Cu(I) complexes (Fig. 28). As the phase-transition behavior could be reversibly repeated, the possibility of the air oxidation of Cu(I) to Cu(II) was ruled out. It was concluded that the chromatic change in the complex was induced by the sol-gel phase transition, which is associated with the distortion of the coordination complex in the specific cholesteryl gel fibril. When an oxidative agent was added to the gel of the Cu(I) complex and the mixture was heated, the deep green gel turned into a sol. This gel-sol transition could be reversibly induced by the addition of the oxidizing and reducing reagents, so it is evident that the redox state of the Cu ions plays a critical role in the stability of the gel system.

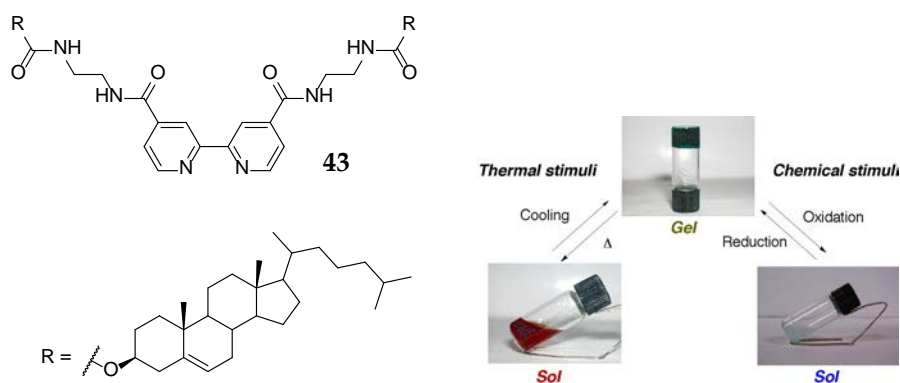


Figure 28. Structure of cholesteryl 2,2'-bipyridine-based gelator and images of its reversible gel-sol transition triggered by either temperature or redox stimulus.¹²⁵ Adapted with permission. Copyright © 2004 American Chemical Society.

Cholesteryl conjugates of pyridines (**44a-c**) were found to form gels in various solvents.¹²⁶ Compound **44c** was reported to be an efficient gelator capable of forming gels in 16 solvents out of the 19 studied. Additionally, it was shown that by using metal–ligand interactions, for example between Ag(I) and the ligands, the morphology and stability of the gels could be tuned. Selected gels were further decorated by fluorescent molecules, like tetraphenyl porphyrin units, in order to prepare attractive materials for applications in photochemical and electrochemical devices.

A 1,10-phenanthroline-appended cholesterol-based gelator (**45**), which forms gels in alcohols, polar aprotic solvents, organic acids, and TEA, was found to be acid-sensitive.¹⁰⁴ It was observed that a protonation of 1,10-phenanthroline nitrogen in the gel state had a big influence on the fluorescence properties of the systems. In fluorescence measurements, most gels afford an emission maximum at 394 nm (purple emission), whereas only the acetic acid gel afforded an emission maximum at 522 nm (yellow emission). However, upon addition of acetic acid to a gel formed in 1-propanol, yellow emission was observed too, clearly indicating a response of the system to acid. The fluorescence intensity of the protonated gelator became particularly strong in the gel, presumably due to the energy transfer from the neutral compound to the protonated form, and the restriction of the molecular motion of the protonated form in the gel phase.

Phenanthroline-containing ligand (**46**) represents one of few examples where the presence of metal ions leads to weaker gels.¹²⁷ The gelator is able to gel methanol-water mixtures in low concentrations starting from 0.1 % w/v. However, upon coordination of the gelator with Zn(II) in a 2:1 ratio, the gel formation required higher concentrations of the ligand and the gel tended to break down over time to a low-viscosity liquid. It was shown that the gel could be reversibly reformed after a rapid heating to 70 °C and subsequent cooling. Clearly, the metal coordination influences the way of the gelator self-assembly significantly.

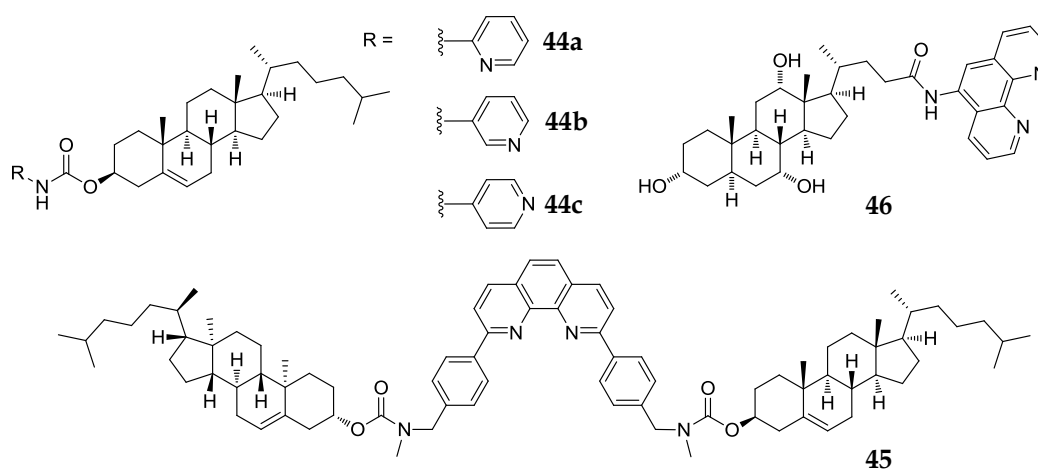


Figure 29. Steroidal gelators containing pyridine or phenanthroline moieties.

Uracil-appended cholesterol gelator (Fig. 30) represents an example of utilizing host-guest chemistry in order to improve the gelation process. Compound **47** was found to form stable gels in polar organic solvents. Interestingly, upon an addition of the complementary polyadenylic acid **48**, the gels were not only stabilized, but they created helical structures. Based on the results, it was concluded that the single-stranded polynucleotide templates can force the gelator molecules to adopt a highly ordered structure through the complementary hydrogen-bonding interactions.¹²⁸

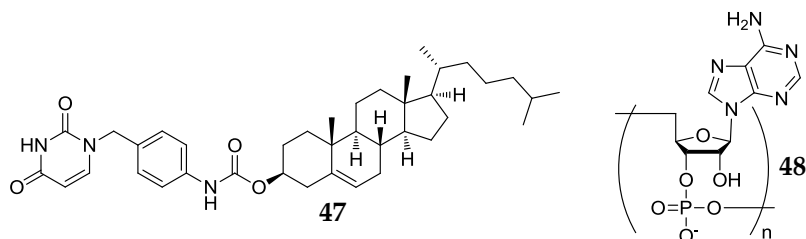


Figure 30. Uracil-appended cholesterol-based gelator and its complementary polyadenylic acid.

Steroidal gelators **49** and **50** were designed as barbiturate receptors (Fig. 31). It was observed that the gelation ability of the compounds could be markedly changed (improved or worsened) by interaction with appropriate guests.¹²⁹

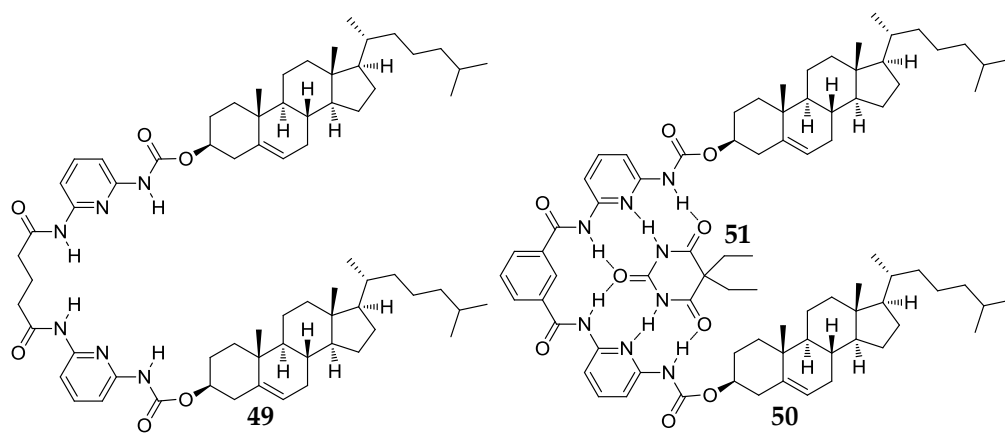
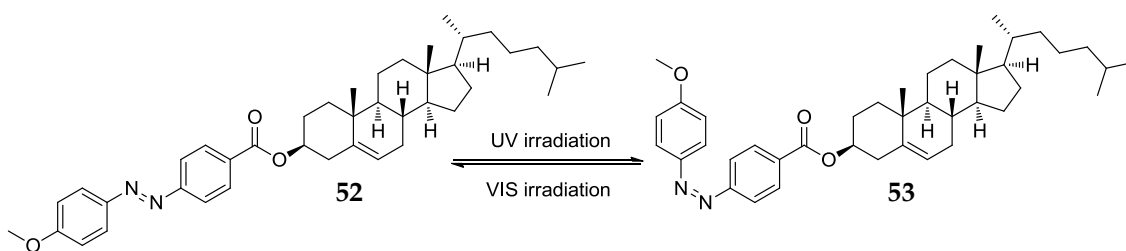


Figure 31. Cholesterol-based barbiturate receptors (**49**, **50**) and an example of tested barbiturate (**51**) bound at the binding site of receptor **50**.

Steroidal derivatives containing an azobenzene moiety

An azobenzene unit is a popular light switchable moiety, often utilized in order to prepare light-responsive gels. One of the first examples of this kind was a series of cholesterol-based gelators reported several years ago.¹³⁰ As expected, the UV irradiation of the gel system led to *cis-trans* isomerization of the azobenzene unit (Scheme 2) causing severe changes in the way of the gelator self-assembly, which resulted in a gel-to-sol transition. It was shown that this process was reversible and the gels could be re-formed repeatedly by a visible light

irradiation of the sol. Later on, several related light-responsive gelators containing cholesterol and the azobenzene moiety were reported.^{54, 131}



Scheme 2. *Cis-trans* photoisomerization of the azobenzene moiety of cholesterol-based gelator.

2.5 Methods for gel study and characterization

A general issue in contemporary supramolecular chemistry is that current experimental techniques remain inadequate to provide structural details at the molecular level for anything other than crystals or proteins. As van Esch stated, nowadays the challenge in the field of supramolecular gels is not to design molecules but to understand them.¹³²

The determination of structures formed in various aggregates is an invaluable help to propose a mechanism of the aggregation. The gel structure and the gelation can be studied by several techniques (Fig. 32). Usually, a combination of more than one technique, often with the help of molecular modeling, is required in order to gain detailed information on the assemblies.

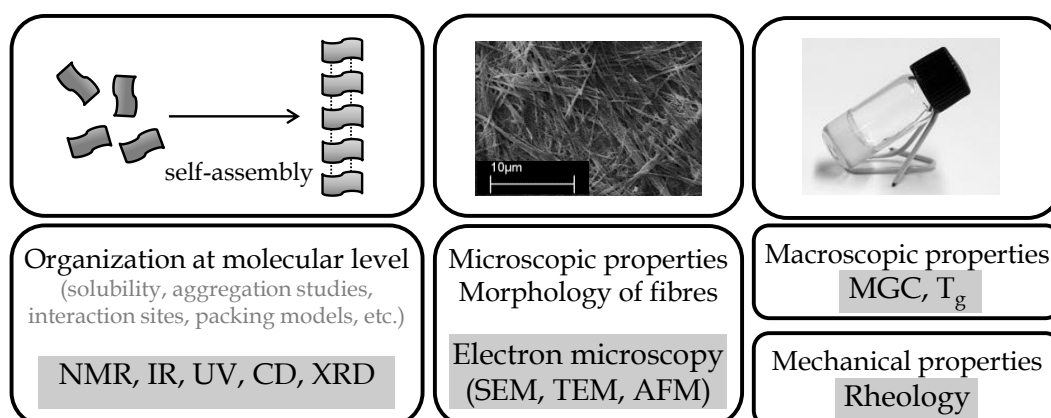


Figure 32. Common methods used to study and characterize supramolecular gels.

The analysis of gel samples is difficult mainly due to the inherent disorder of gel systems. Among others, spectroscopic techniques such as IR, NMR, and UV-Vis together with X-ray diffraction or small-angle scattering methods are commonly employed. Structural information on SAFIN can be gained at different resolution levels using complementary techniques. At the microscopic level,

the structure and morphology of supramolecular gels can be investigated by conventional microscopy techniques such as SEM, TEM, and AFM, while thermal and mechanical studies are used to understand the interactions between these structures. At the nanoscale scale level, X-ray diffraction and small angle scattering (SANS and SAXS) are used. Whereas X-ray powder diffraction is used for elucidating molecular structure packing, small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) can provide information about size, shape and orientation of structures in the gel.

The specific properties of the gel may depend upon its history and the method of its formation, temperature at which it is kept, its age (because many molecular gels are not thermodynamically stable and SAFIN may change with time), LMWG concentration, and the type of liquid used. Therefore, these parameters should be always considered and results should be handled with caution.

2.5.1 Determination of T_g and MGC

A temperature at which the gel turns to a sol is called the gel-sol phase transition temperature, T_g (or T_{gel}), and it is a common criterion to determine the gel stability. To clearly show the thermal stability of gels, T_g is often plotted against a gelator concentration (Fig. 33). Typically, the higher the gelator concentration, the higher is the gelation temperature. The increase of gelator concentration with temperature is usually exponential.

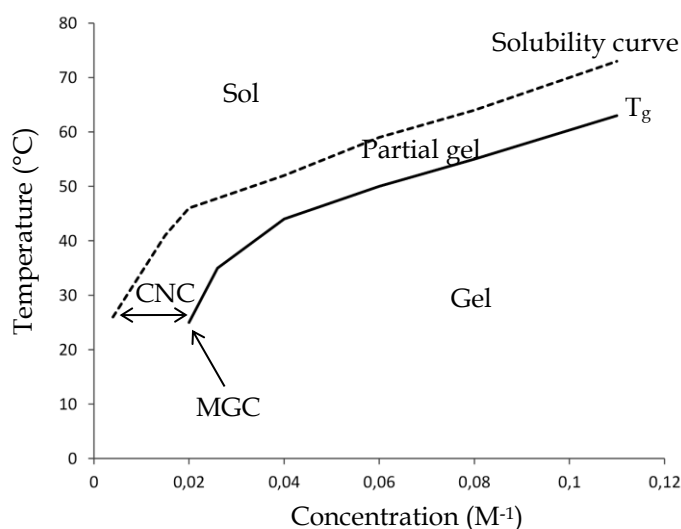


Figure 33. Schematic phase diagram of gelator; the dashed curve represents a solubility curve.

The thermodynamic properties of gels can be strongly dependent on the measurement technique, meaning that results from different techniques (*i.e.* methods with different heat transportation and different characteristics being measured) should be interpreted with caution. Basic methods for the determination of the gel-sol transition include simple “tabletop” rheological methods

such as test tube inversion, falling sphere¹³³ or bubble motion methods,¹³⁴ as well as DSC. Also other methods such as variable temperature NMR experiments and temperature sweep rheological measurements can be used as well.

In the inversion tube measurement, the sample is sealed in a glass vial and slowly gradually heated (usually 1-2 °C/min). The temperature at which the gel starts to flow because of gravity is defined as the gel-sol phase transition temperature. In a dropping ball measurement, a small steel ball is placed on top of the gel and the gel is slowly heated while the position of the ball is monitored. The temperature at which the gel is no longer able to withstand the weight of the ball and the ball drops to the bottom of the vial is determined as T_g . In the bubble movement experiment, bubbles can be either injected into the gel sample or may remain in the sample after the gel preparation. The sample is heated slowly while the position of the bubble is monitored. When the bubble moves through the sample with the same speed compared to the liquid, the T_g has been reached.

By DSC, the influence of an increase/decrease of temperature on heat capacity can be studied. The peak in a heating curve corresponds to the gelation temperature and the area under the peak yields the enthalpy of gelation (melting). Obviously, there is a hysteresis in the gelation behavior, because usually the curve of the gel-to-sol transition does not coincide with that of the sol-to-gel transition. Additionally, DSC can provide information on the strength of the intermolecular interactions in the gel. The network strength is proportional to the magnitude of the enthalpy change - the higher the enthalpy change, the more tightly bound is the network.

Minimum gelation concentration (MGC) is another easily accessible gel characteristic. It is the sum of the critical network concentration (CNC), *i.e.* the concentration of gelator required to form a network able to immobilize the solvent, and the gelator solubility.

$$\text{MGC} = \text{CNC} + \text{Solubility}$$

There is a constant interest in the synthesis of gelators with very low values of MGCs. Typically, the minimum gelation concentration of common LMWGs varies from 1 to 2 % w/v. However, gelators with a MGC below 1 % w/v are known and they are sometimes called “supergelators”. Recently, even systems with extremely low MGC values (less than 0.03 % w/v) were reported.^{116, 120, 135}

2.5.2 Rheology

Rheology is a quantitative study of the deformation and the fluidity of matter under the influence of an applied stress. In a simplified way, it shows a response of material to force. Rheological criteria involving the magnitudes and ratios of the elastic (G') and loss (G'') moduli and viscosity are probably the most useful and physically quantifiable criteria for gels. They allow a differentiation between a “true gel” and “jelly-like” gel. In order to be considered as a gel

(*i.e.* viscoelastic solid-like material), the elastic modulus G' should be invariant with frequency up to a particular yield point and should exceed G'' by at least an order of magnitude.^{16, 136}

The elastic (“storage”) modulus G' represents the ability of the deformed material to “snap back” to its original geometry, whereas the viscous (“loss”) modulus G'' represents the tendency of the material to flow under stress. The common experiments include:

- Frequency sweep measurements
- Strain sweep measurements
- Temperature sweep measurements
- Flow curve measurements

The G' and G'' moduli are usually measured as functions of applied stress or oscillation frequency. The linear response is determined by varying the frequency at a fixed small amplitude of stress (*i.e.* by a frequency sweep measurement), such that the loss and storage modulus are independent of the applied stress. Nonlinear behavior is probed using a fixed frequency and varying the amplitude of the yield stress (*i.e.* by a strain sweep measurement), the storage modulus remains constant. Above the yield stress, G' is observed to decrease rapidly, suggesting that the network from which the gel is formed has collapsed as a consequence of the application of force. In a temperature sweep experiment, the G' and G'' moduli are measured as function of temperatures and the data can be used to determine the gelation temperature as an alternative technique to a conventional “inverted tube” method or DSC. In a flow curve experiment, viscosity is measured as a function of shear rate.

2.5.3 Electron microscopy

Molecular self-aggregation features can be observed by an electron microscope.¹³⁷⁻¹³⁹ The morphology of dried gels is usually studied by scanning electron microscopy (SEM), or transmission electron microscopy (TEM) analysis. Although, other techniques such as atomic force microscopy (AFM) and scanning tunneling microscope (STM) are possible as well. These methods enable the detection of gel structures, which can be formed for instance by fibrous, tapes, ribbons, rods, helices, sheets, micelles, or vesicles, and provide high-resolution images in micro or even nano dimensions.

Conventional SEM and TEM measurements require dried samples. Therefore, careful drying of a gel (*i.e.* without a network collapse) is necessary and is the crucial step of the whole measurement. Fortunately, there are methods which allow examining samples containing high concentrations of liquids without drying them beforehand. They are called cryo-SEM and cryo-TEM and enable to study gels directly without affecting their structure by drying. In these methods, the vapor pressure is reduced by thermal fixation (*i.e.* ultra-fast cooling of the liquid specimens into a vitrified state). This is achieved by rapidly plunging the sample into a suitable cryogen (often liquid ethane) and by studying it at cryogenic temperatures.

2.5.4 Small angle scattering

There are two small angle scattering (SAS) techniques, small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS), whose resolutions are approximately that of TEM. They work similarly to XRD except that small angles ($0.1 - 10.0^\circ$) are used and in SANS, a neutron beam is used instead of an X-ray beam. By analysing the scattering pattern information about the size, shape and orientation of structures in partially ordered materials can be obtained. Both SAXS and SANS can provide useful data on the atomic scale at 50-250 and 10-1000 Å respectively, which are perfectly suited for studying physical parameters of gels. SAS methods have the great advantage of using samples as they are and provide an immediate picture that is a statistical average over the irradiated volume of the sample (usually ca. 0.1 cm^3). They can be used to determine parameters such as average particle sizes, shapes, or distribution. Considerations for choosing between X-rays and neutrons include the required penetration depth and the chemical composition of the material, because in SANS there is no incoherent scattering for nuclei with a zero nuclear spin (*e.g.* ^{12}C and ^{16}O) since they produce no interaction with the neutron spin. Therefore, a change in the isotopic composition of the solvent (deuteriated versus protiated) or the isotopic structure of the gelator itself is often needed.

2.5.5 X-ray diffraction

Structure determination by X-ray diffraction is an important method to understand molecular shapes, the modes of molecular packing, and related physical and chemical properties of gels. Nowadays, solving structures from single crystal data has become relatively routine using well-proven modern methods and software packages. However, using this method for supramolecular gels is very limited due to the fact that growing single crystals of intrinsically disordered molecules is very challenging and most of the time unsuccessful. Additionally, single crystal structures obtained from different solvent systems than the gelling solvents should be always critically evaluated and additional studies showing relationships between these systems should be carried out.

Crystal structure determination from powders, a much more recent technique, is less straight-forward compared to the single crystal method. The main drawback of the powder X-ray diffraction (PXRD) is the projection of the three-dimensional reciprocal lattice onto a single axis which very often produces a severe overlap of the Bragg reflections, but this can be improved by using synchrotron radiation. Nevertheless, with regard to the difficulties with growing gelator single crystals, powder diffraction techniques seem to be very promising for studying supramolecular gels.

Recently, there have been interesting developments in diffraction techniques which has resulted in a method for comparing the structure of the pure gelator (obtained by a single crystal or powder diffraction method) with gel strand structure in gels by subtraction of the dominant amorphous liquid diffraction component from the powder diffraction pattern of the gel (Fig. 34).^{30, 140}

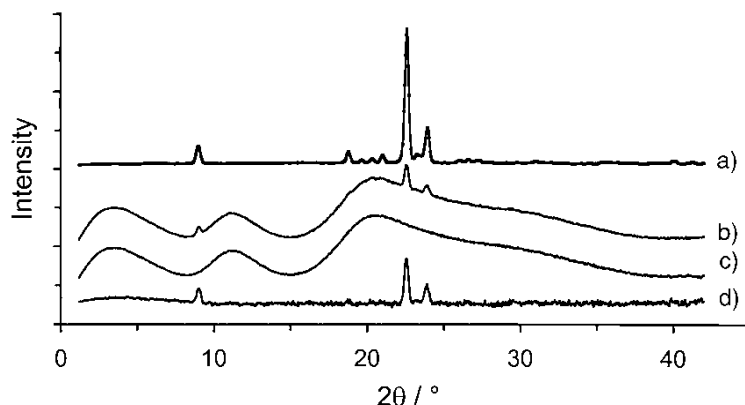


Figure 34. X-ray diffraction patterns at room temperature of *N,N'*-di-*n*-propylurea: a) powder, b) gel, 2 wt% in silicone oil, c) silicone oil, and d) subtraction of diffractogram b) subtracted from diffractogram c).³⁰ Adapted with permission. Copyright © 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

2.5.6 NMR spectroscopy

Solution and recently solid state NMR methods have been used to obtain information on the molecular organization of the LMWGs systems. Traditionally used liquid-state NMR spectroscopy can be used to detect for instance the formation of hydrogen bonds or π - π stacking during the gelation. In order to investigate interactions involved in the gel formation, variable-temperature and concentration-dependent ^1H -NMR experiments are often carried out. Spectra at different temperatures (or different concentrations) are recorded and compared with regard to the chemical shift changes of the signal and the sharpness of the signals.^{141, 142} The limitation of these methods is availability of deuterated solvents at reasonable prices. One also has to be careful when interpreting the observed phenomena of the measured gel samples.

As Miravet and co-workers proposed,¹⁴³ it seems that no structural information on the gel assembly can be obtained by NMR, if the observed signals correspond to free gelator molecules. Therefore, a control measurement at a non-gelling concentration should be always carried out. However, very valuable information such as intermolecular binding points or the conformation of the molecules in the assemblies could be obtained, for instance by analysis of chemical shift variations and techniques such as NOE, respectively.

Solid-state NMR spectroscopy is in many ways complementary to X-ray diffraction as it probes short-range ordering and local structure around the nucleus of interest. It is a relatively new technique in terms of studying supramolecular gels and related materials.^{144, 145} It allows examining LMWG materials in various forms such as solid, xerogel, or even gel itself (if deuterated solvent is available). However, to interpret the spectra in order to obtain very detailed information about the molecular interactions is still quite challenging and supporting data from other methods (*e.g.* diffraction techniques) is often needed.

2.5.7 UV-Vis, CD and IR spectroscopy

UV-Vis spectroscopy can provide useful information on the gel structure, *e.g.* interactions involved in the gel formation, but can also be used to determine gel parameters such as a critical gelation concentration and gelation temperature. If there is a fluorescent group present in the gelator, the system can be additionally studied by fluorescent spectroscopy. Although UV-Vis and fluorescence spectra are not sufficient to characterize molecular gel phases that are self-assembled, they can reveal unique features of intermolecular interactions that are not detectable by other analytical tools, because many gels show characteristic absorption and emission properties arising from their specific aggregation modes. Generally these methods detect changes in surroundings of particular groups, which are either covalent parts of the gelator molecules, or can be added as probes to identify charge-transfer interactions, π - π stacking, or metal coordination. For instance, the usage of pyrene as a probe in fluorescence measurements of europium cholate hydrogelation was reported.¹⁴⁶

Chiral gelators, including those which are not suitable for the UV-Vis measurements due to weak UV-Vis signals, can be studied by the circular dichroism (CD) method, in which an absorption of left and right circularly polarized light by a chiral sample as a function of wavelength is recorded. This method is especially useful for studying helical aggregates, which are the common basis of gel formation by chiral gelators, and for studying the self-assembly of chiral monomers in general.

Infrared spectroscopy (IR), together with the complementary Raman spectroscopy, is a commonly used method for the determination of chemical structures and for the identification of compounds. Common FTIR spectrometers allow very fast recording of spectra of small size and low concentration samples in gaseous, liquid, or solid state, in the 4000–200 cm^{-1} region. In the gel structure investigation, IR spectroscopy is most useful in the study of molecular aggregates, particularly to detect and characterize hydrogen bonding, because the stretching vibrations νOH , νNH and $\nu\text{C}=\text{O}$ are very sensitive to the environment of these functional groups.

3 EXPERIMENTAL

This work represents my efforts to contribute to the field of steroidal supramolecular gels, especially to the area of pH-responsive and metal-organic gels.

3.1 Aims and background of the work

Although, utilizing steroids, especially cholesterol, for synthesizing new gelators is common, stimuli-responsive systems and metallogels are still relatively new areas of research and not many examples based on steroids have been reported (see the paper I and references therein).

Based on the literature review, the following topics were identified as relevant and interesting areas for my studies:

- Design and synthesis of new pH- or acid-responsive gel systems for various applications (*e.g.* in drug release systems);
- Design and synthesis of steroid-based ligands for metal-assisted gelation;
- The impact of different metal ions on the properties of gels;
- Possibilities for *in situ* gelation;
- Gelation at ambient temperature without the need of a heating/cooling cycle.

Herein are summarized and highlighted the most important results of this work. Detailed information can be found in articles II-V, which are referred in the text by roman numbers in brackets.

3.2 Design strategy

Although, reports on new gels found by serendipity (usually during crystallization attempts) are still published, the number of systems which are results of a thorough design is constantly growing. This is mostly due to recent advances in applying principles of supramolecular chemistry and molecular modeling to the field of LMWGs, together with an increase of knowledge about supramolecular gels in general.

In contrast to polymer gels, supramolecular gels involve discrete molecular compounds with well-defined chemical structures. As a consequence, slight changes in the chemical composition of the gelator backbone allow the morphology, chirality, size of aggregates, and ultimately the macroscopic properties of gels, to be exquisitely controlled and tuned. For instance, by introducing a suitable switching or responsive functionality at a molecular level, stimuli-responsive systems can be prepared.

The objectives were to design:

- steroidal LMWGs containing suitable pH- or acid-sensitive moieties;
- and steroidal LMWGs containing suitable binding sites for metal coordination.

3.2.1 Building blocks

In this work sterol units were used as moieties responsible mostly for aggregation processes, whereas nitrogen compounds were used in order to introduce function into the systems.

The utilized building blocks include:

- As steroidal units
 - Cholesterol
 - Stigmasterol
- As nitrogen compounds
 - Amino acid residues
 - Pyridine moieties
 - Diamines

Cholesterol (**11**) has become one of the most often used building blocks when designing new LMWGs. This happened mainly because of its unique properties which include a rigid skeleton, several stereogenic centers, and a strong tendency to assemble *via* van der Waals and hydrophobic interactions. In this work, it was chosen as the main steroidal unit due to its availability, low price, easy derivatization, and aggregation properties.

Stigmasterol (**14**) belongs to a group of plant sterols.^{89, 90} Although, it has a very similar chemical structure to that of cholesterol (it varies only in the constitution of the side-chain), its usage in the preparation of new supramolecular

gels is rare and only very few examples can be found in literature.^{94, 147} Stigmasterol was chosen for the purpose as a second steroidal representative for two reasons: i) to investigate similarities and differences of the prepared systems in a comparison to analogous cholesterol-based systems, and ii) to prepare systems with potentially interesting properties for biomedical applications. Recently stigmasterol was found to reduce cholesterol levels by reducing the absorption in the intestinal tract¹⁴⁸ and to have potential anti-inflammatory and anti-osteoarthritic activity.¹⁴⁹

Amino acids are biologically important organic compounds. They act as building blocks of proteins and intermediates in metabolism. There are 23 proteinogenic amino acids, which can be classified into four groups based on the properties of their side-chain. The side-chain can make an amino acid a weak acid or a weak base, or hydrophilic if the side-chain is polar or hydrophobic if it is nonpolar. In this work residues of glycine (**E1a**), L-leucine (**E1b**), and L-phenylalanine (**E1c**) were used. Leucine and phenylalanine have nonpolar side-chains, which are frequently engaged in van der Waals interactions. Additionally, the aromatic side chain of phenylalanine can sometimes participate in weakly polar interactions. Furthermore, the amino acid main-chain acts as a hydrogen bond donor and acceptor and therefore, amino acids represent suitable moieties for linker molecules in gelators of ALS, A(LS)₂, or LS₂ types.

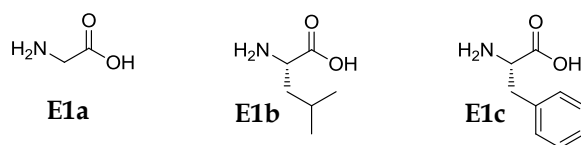


Figure 35. Selected amino acids.

Pyridines are heterocyclic six-membered aromatic compounds consisting of five carbon atoms and one nitrogen atom. They are a class of important heterocycles appearing in many naturally occurring bioactive compounds and pharmaceutical molecules. The pyridine unit can be found in countless molecules with applications as varied as catalysis, drug design, agricultural products, molecular recognition, and natural product synthesis. It is also known as a suitable ligand for d-transition metal ions. For this reason, the pyridine moiety was chosen to be used in the design and synthesis of new steroidal metallogels.

Diamines, namely *p*-phenylenediamine and ethane-1,2-diol, were used as linkers and as potential hydrogen bond donors and acceptors in the preparation of gelators of an ALS and A(LS)₂-type.

3.2.2 pH- and acid-responsive gels (papers II, III)

Sterols are not acid-sensitive on their own. Therefore, the molecular design rationale for these stimuli-responsive gels was to incorporate pH- or acid-sensitive segments into the LMWGs. For this purpose, two different approaches

were implemented. First, we introduced an amino acid moiety containing a proton accepting amino group (II). Second, we incorporated an acid labile imine group (III).

As it was shown several times in the past,^{66, 99-101} a combination of steroids and moieties having proton accepting or donating groups is often a good choice for preparing acid/base-responsive systems. The chemical change is usually reversible. Therefore, a construction of switches sensitive to chemical stimuli is possible. In this work (paper II) amino acid units, namely residues of glycine, L-leucine, and L-phenylalanine (Fig. 35), were used in order to prepare acid-responsive stigmasterol-amino acid conjugates (**E2a-c**).

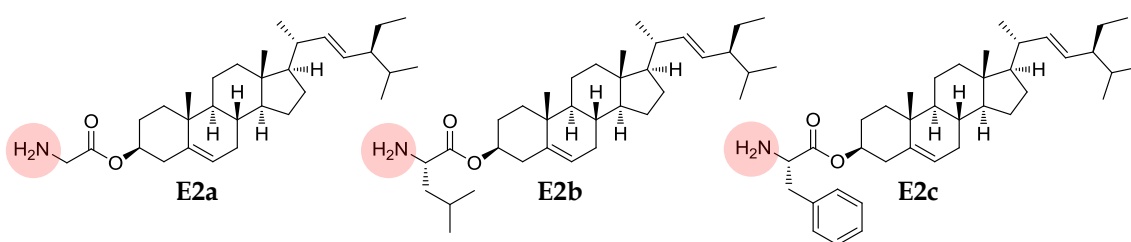


Figure 36. Studied stigmasterol-amino acid conjugates with highlighted amino groups which can be protonated.

Incorporating an acid labile moiety, for example an imine bond, represents another design route to synthesize acid-responsive systems. The imine bond is typically prepared by a reversible condensation of a primary amine and an aldehyde. The equilibrium in this reaction usually favors the carbonyl compound and the amine, so that azeotropic distillation or the use of a dehydrating agent is often required to push the reaction in favor of the imine formation. This equilibrium can be generally used in order to prepare two different types of acid-sensitive systems. If the imine compound is a gelator, then the acid-induced hydrolysis causes a gel-to-sol transition, whereas, if the mixture of the amine and carbonyl compound forms a gel, then the acid-induced hydrolysis leads to a sol-to-gel transition.

In this work (paper III), the cholesteryl derivative containing an imine group was prepared (Fig. 37). In the design *p*-phenylenediamine was chosen as a linker in order to synthesize a stable (conjugated, *i.e.* stabilized by resonance) imine, and to introduce an amide moiety (*i.e.* an acceptor and donor for intermolecular hydrogen bonds) to the structure. Obviously, the reversibility of the hydrolysis process might be challenging. However, there are potential applications, *e.g.* in drug release systems, where the irreversibility of the transition is not a disadvantage.

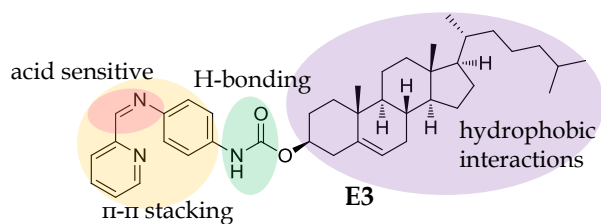


Figure 37. Cholesterol-based gelator containing an imine functional group with highlighted non-covalent interaction sites.

3.2.3 Metallogels (papers IV, V)

One of the objectives was to prepare LMWGs with the ability to bind metal ions. Since steroids do not contain a strong metal binding site in their structure, suitable derivatives have to be prepared. For these purposes, pyridine moieties were used. In most reports on metallogels, systems based on only one kind of metal ion were studied.^{15, 16} In this work, the investigation was focused on utilizing different metal ions and exploring how these can be used in terms of affecting the self-assembly of LMWGs and tuning the properties of the gel systems. Generally, there are several options how systems can be affected by metal ions. The presence of a metal ion can cause a gel-to-sol transition, or contrary to promote a gel formation (paper V), or did not influence the phase change at all, but still affect gels properties (paper IV).

Based on the knowledge that steroidal molecules of A(LS)₂ type are often found to be good gelators, the cholesteryl conjugate **E4** with an aromatic central unit was designed (paper IV, Fig. 38). A pyridine-2,6-dicarboxylic acid moiety was chosen as the central building block, because it is known to be a good chelating agent for various metals, including zinc,¹⁵⁰ iron,¹⁵¹⁻¹⁵⁴ copper and other transition metals.¹⁵⁵

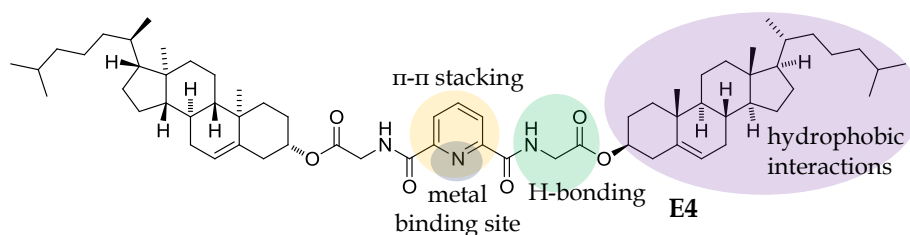


Figure 38. Cholesterol-based gelator of A(LS)₂ type with highlighted non-covalent interaction sites.

Gelator **E5** (= **26**), recently reported by Fang and co-workers,¹⁰⁵ was an inspiration for this design (**E4**). It was proposed, that besides van der Waals interactions between cholesteryl moieties of the gelator, intermolecular hydrogen bonding formation between the neighboring amide groups and the π-π stacking between the neighboring benzene rings plays an important role in the gel formation (Fig. 39).

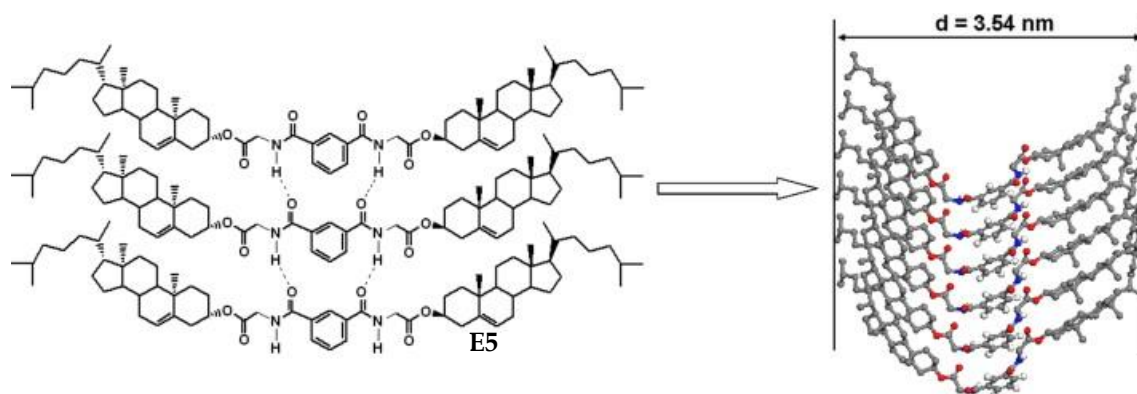


Figure 39. Proposed model of self-assembly of gelator **E5**.¹⁰⁵ Reproduced by permission of Elsevier.

It can be expected that a metal coordination to the nitrogen atom of the pyridine unit of gelator **E4**, affects the self-assembly packing mode of the gelator molecules significantly and results in the build-up of a metal-responsive system. For this purpose, metal ions with different coordination geometries were chosen, namely Ag^+ , Zn^{2+} , and Pd^{2+} ions, in order to examine how their coordination will affect the way of self-assembly of the gelator molecules. Moreover, to explore the relevance and importance of each type of non-covalent binding site in the design, three reference compounds (each of them lacking one moiety in its structure) were prepared (Fig. 40).

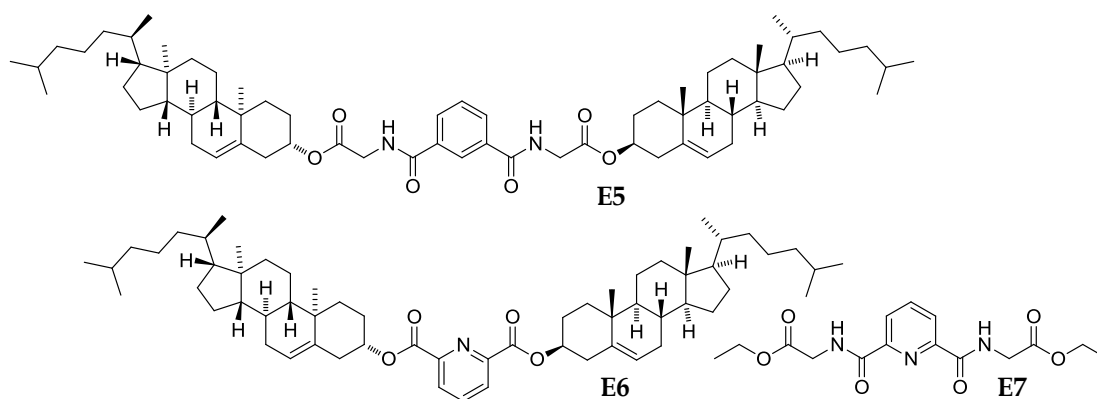


Figure 40. Chemical structures of reference compounds of gelator **E4**.

In the second design approach (paper V), subcomponent self-assembly was utilized as a route towards new metallogels. Subcomponent self-assembly allows the construction of diverse architectures from simple building blocks *via* formation of covalent bonds around metal templates.¹⁵⁶ This concept has been effectively used in supramolecular chemistry to build complicated complex structures,¹⁵⁶⁻¹⁵⁸ but so far it has not been explored in the field of supramolecular gels.

The aim was to design a suitable steroidal amine, which would form subcomponent self-assembled gels after the addition of a suitable aldehyde and a metal ion. Based on the knowledge that ALS systems are often good gelators,

metal complexes with steroidal imine ligands were prepared (Fig. 41). In the design ethylene-1,2-diamine was used as a flexible linker and 2-pyridinecarboxaldehyde as an aromatic moiety. As metal ions, Cu^{2+} , Ni^{2+} , and Zn^{2+} cations were chosen. These metals, having similar properties (*e.g.* octahedral geometry is their common coordination geometry), were selected in order to investigate an influence and significance of the metal choice on gel properties.

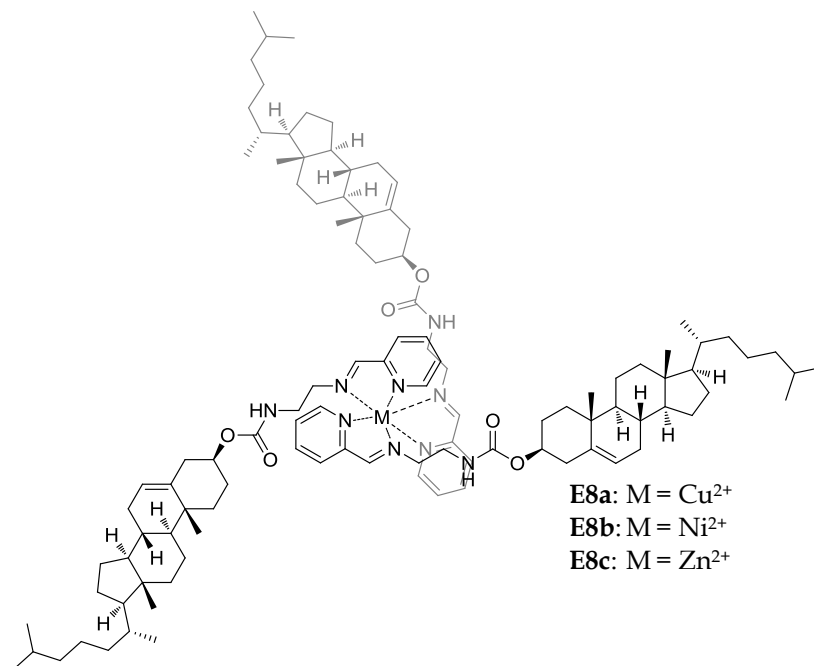


Figure 41. Chemical structure of steroidal subcomponent gelators.

Subcomponent self-assembly offers an effective and easy way to construct new supramolecular gels. Moreover, the systems are very versatile and can be easily tuned by simply exchanging one of the components, *i.e.* by utilizing a different type of amine, aldehyde, or metal ion.

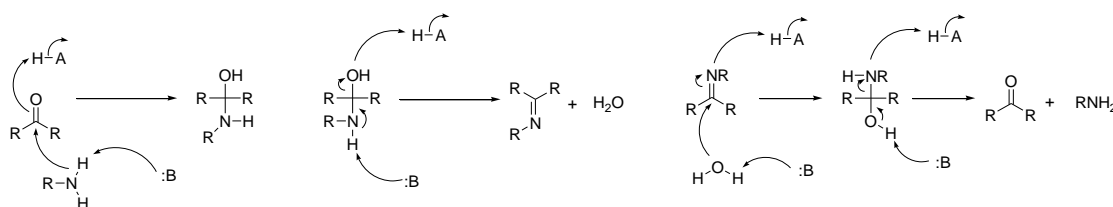
3.2.4 *In situ* gelation (papers III, V)

An *in situ* gelation represents a modern approach to a supramolecular gel formation and so far only few reports can be found in literature.¹⁵⁹⁻¹⁶¹ Whereas in a conventional gel preparation, LMWGs are synthesized beforehand and then dissolved in suitable solvents, in an *in situ* gelation, a gelator molecule is prepared directly in a gelling solvent from suitable precursors. It was shown¹⁶⁰ that in comparison to the classic method, the *in situ* gelation often:

- occurs at room temperature,
- requires shorter gelation time,
- takes place also in solvents which are not gelled by the conventional gelation.

Moreover, the components are usually needed in a certain ratio to achieve an effective gelator formation, therefore, a lack or excess of one of the components can be used as a tool for controlling the gelation process.

On the one hand a separate step of a beforehand gelator synthesis is not needed, which shortens the overall gelator preparation time, but on the other hand it puts high demands on the design of its precursors. The chemical reaction between the precursors, leading to the gelator formation, should be rapid, and in order to carry out a gelation at ambient temperature, it should occur at mild conditions and with a high yield. Imine bond formation represents such a type of reaction and therefore, it was chosen for the purpose of this work (gelator **E3** and **E8a-c**). Imines are typically prepared by condensation of primary amines and aldehydes. In terms of mechanism, such reactions proceed *via* a nucleophilic addition giving a hemiaminal $-C(OH)(NHR)-$ intermediate, followed by an elimination of water to yield the imine (Scheme 3). In the case of gelator **E3**, the imine moiety was utilized not only because of the *in situ* formation concept, but also because of its sensitivity towards acids.

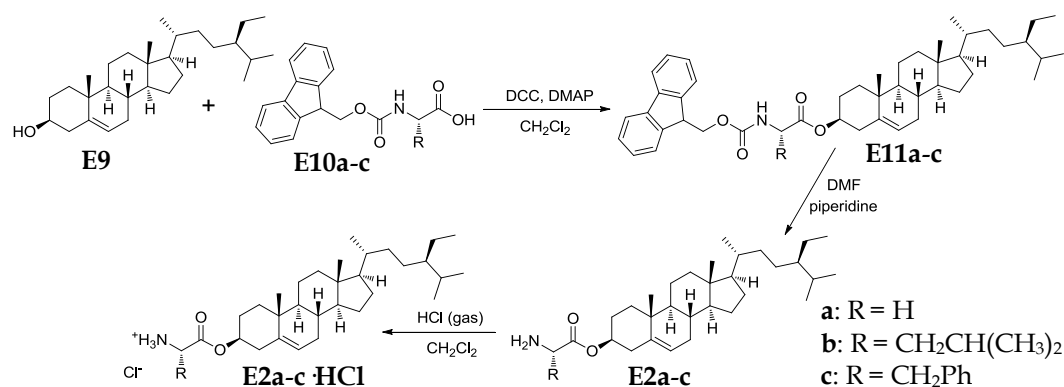


Scheme 3. Mechanism of imine formation and its hydrolysis.

As it was reported,¹⁶⁰ *in situ* gelation often occurs at ambient temperature. This represents an attractive way of a gel preparation compared to the traditional method (*i.e.* method in which a gelator is heated in a particular solvent or a mixture of solvents until all solids are completely dissolved to form clear solutions), because it does not require a heating/cooling cycle.

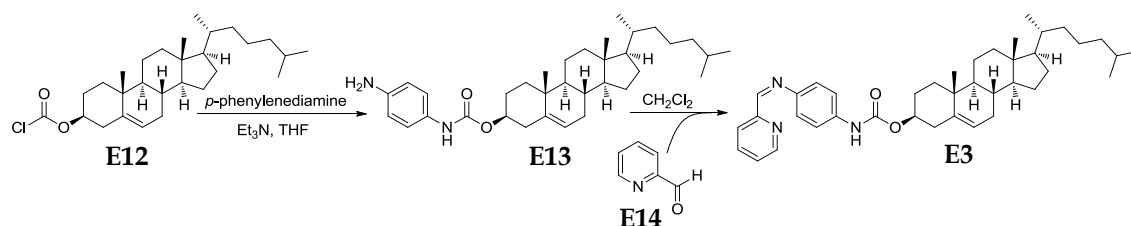
3.3 Synthesis

(Paper II): The stigmaterol-amino acid conjugates (**E2a-c**, Scheme 4) were synthesized by employing a peptide coupling procedure¹⁶² using DCC and DMAP as coupling reagents (81–88 % yield after purification). The Fmoc-protecting group was then removed using a 20 % solution of piperidine in DMF (from 96 % to quantitative yield). The corresponding hydrochlorides (**E2a-c HCl**) were subsequently prepared by bubbling hydrochloric gas through the dichloromethane solution of the prepared conjugates.



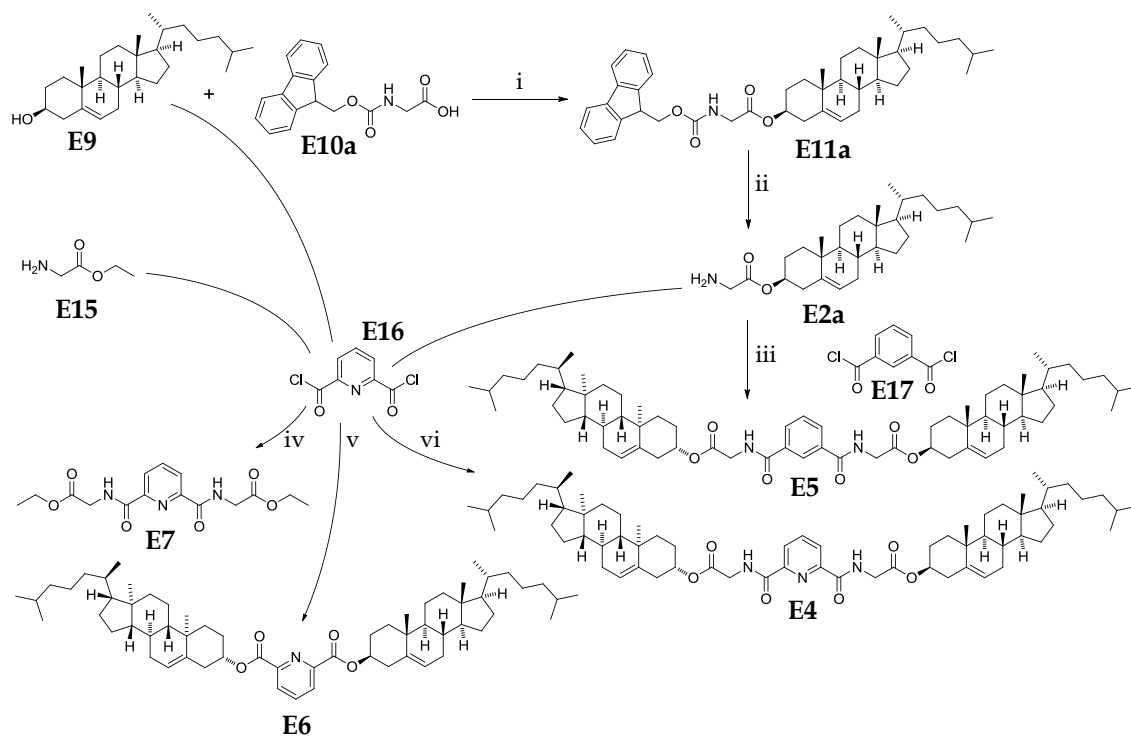
Scheme 4. Synthesis of stigmasterol-amino acid conjugates **E2a-c**.

(Paper III): The steroidal monosubstituted diamine **E13** was prepared by a reaction of cholesteryl chloroformate (**E12**) with *p*-phenylenediamine in dry THF in the presence of TEA (in a 34 % yield). The acid-sensitive steroidal imine **E3** could be prepared either synthetically by a condensation of steroidal amine **E13** and 2-pyridinecarboxaldehyde (**E14**) in dry DCM (Scheme 5), or directly *in situ* in a suitable gelling solvent. Because of the usage of aromatic imine and aromatic aldehyde, the reaction occurred at room temperature giving conjugated (resonance stabilized) imine without the need of any catalysis.



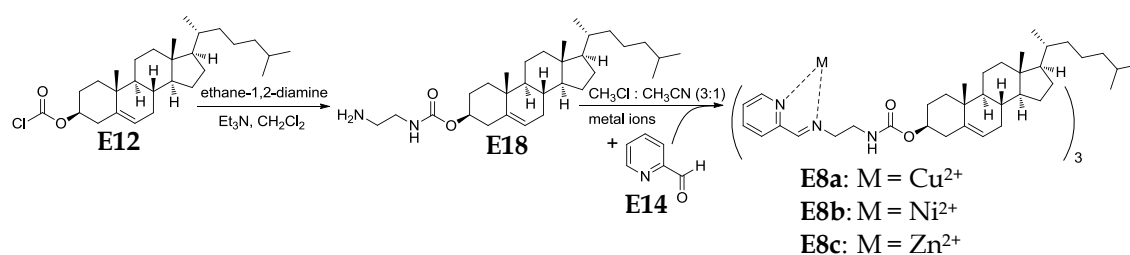
Scheme 5. Synthesis of acid-responsive gelator **E3** based on steroidal imine.

(Paper IV): Cholesteryl glycinate (**E2a**) – which was prepared by employing a peptide coupling procedure using DCC and DMAP and subsequently a 20 % solution of piperidine in DMF for removing the Fmoc-group (Scheme 4) – was used as the initial building block for the synthesis of gelator **E4** and **E5**. The coupling with the aromatic central unit was done by reaction with 2,6-pyridinedicarbonyl dichloride or isophthaloyl dichloride in dry dichloromethane in a presence of TEA. Compounds **E6** and **E7** were prepared from different starting materials (cholesterol and ethyl glycinate respectively) utilizing the same procedure. All these reactions were achieved in good yields (> 85 % after purification).



Scheme 6. Synthesis of compounds **E4-E7**; (i) DCC, DMAP, CH_2Cl_2 ; (ii) piperidine, DMF; (iii)-(vi) Et_3N , CH_2Cl_2 .

(Paper V): The steroidal amine **E18** was prepared by a one-step reaction of cholesteryl chloroformate (**E12**) with 1,2-ethylenediamine in dry DCM in the presence of TEA (96 % yield). After the addition of 2-pyridinecarboxaldehyde (**E14**, 3 equiv.) and a metal salt (1 equiv., $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ or $\text{Ni}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ or $\text{Zn}(\text{BF}_4)_2 \cdot 6\text{H}_2\text{O}$) to the solution of the steroidal amine (**E18**, 3 equiv.) in a chloroform-acetonitrile mixture (3:1), the reaction mixture changed its color indicating that a metal complex formed. The solution turned from colorless to the color from the complex formed by the used metal salt - the Cu^{2+} -complex was green, the Ni^{2+} -complex was yellow, and the Zn^{2+} -complex exhibited a light yellow color. Similarly to the acid-responsive gelator **E3**, also gelators **E8a-c** could be prepared either synthetically beforehand, or *in situ* by simple mixing of all three components - *i.e.* amine **E18**, aldehyde **E14** and a metal salt - in a suitable solvent.



Scheme 7. Preparation of gelators **E8a-c** via subcomponent self-assembly.

3.4 Self-assembly and solid state studies

3.4.1 Gelation

The gelation abilities of the prepared compounds (**E2-4**, and **E8**) were investigated in numerous solvents and the results are summarized in Table 1. It was determined that van der Waals and hydrophobic interactions between steroidal units accompanied by non-covalent interactions between the attached nitrogen containing moieties were the driving forces responsible for the gel formation. In most cases, the nature of the attached molecules had a determinative influence on the type of a gelled solvent. Moreover, it was found that even a minor structural change had a big impact on the gelation properties of the agent. For instance, whereas steroidal derivatives of aliphatic compounds, *e.g.* gelators **E2a HCl** and **E2b HCl**, gelled only higher alcohols, some of the conjugates containing aromatic moieties, *e.g.* gelators **E4** and **E8b**, could form gels also in aromatic solvents in addition to higher alcohols. Furthermore, some of the gelators could also gel CCl_4 (**E2c HCl**, **E4** and **E8b-c**), DMF (**E3** and **E4**), or DMSO (**E3**). Based on visual properties, gels were denoted as opaque, translucent, or clear transparent (Fig. 42). Interestingly, gelator **E4** forms opaque gels in higher alcohols, whereas, the corresponding gels of its metal complexes are clear transparent (Fig. 45).

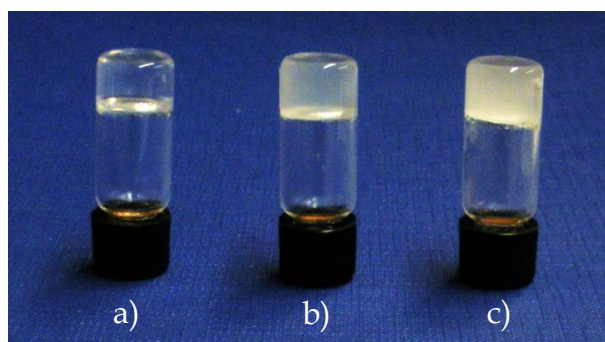


Figure 42. Images of gels (1.0 % w/v) of **E2a HCl**: a) clear transparent gel in CCl_4 , b) a translucent gel in chlorobenzene, and c) an opaque gel in benzene.

Some of the compounds can be called “supergelators” since they can gel selected solvents at very low concentrations - lower than 1 % w/v. For instance, compound **E2c HCl** is a supergelator for aromatic solvents and compound **E4** for DMF with a minimum gelation concentration lower than 0.3 % w/v. Also all three metal complexes (**E8a-c**) exhibit great gelation abilities in higher alcohols. For instance, Ni-complex **E8b** was found to gel octan-1-ol even at a concentration of 0.07 % w/v, which makes it one of the best metal containing gelators ever reported.

Table 1. Summary of gelation tests (2.0 % w/v).

Solvents	E2a·HCl	E2b·HCl	E2c·HCl	E3	E4	E4+Ag ⁺	E4+Zn ²⁺	E8a	E8b	E8c
benzene	I	I	OG ₍₅₎	-	pCG	S	S	S	pCG ₍₄₀₎	S
toluene	I	I	OG ₍₅₎	P	S	S	S	S	pCG ₍₄₀₎	S
CH ₂ Cl ₂	S	S	S	P	OG	S	pCG	S	S	S
CHCl ₃	OG	S	S	S	S	S	S	S	S	S
CCl ₄	I	I	CG ₍₁₀₎	P	pCG	P	pCG	S	pCG ₍₂₅₎	pCG ₍₃₀₎
methanol	P	P	S	I	I	I	I	P	P	P
ethanol	P	P	S	I	I	I	I	P	P	P
propan-1-ol	P	P	S	OG ₍₁₅₎	OG	P	pCG	P	P	P
butan-1-ol	OG	P	S	OG ₍₂₀₎	OG	CG	CG	P	P	P
pentan-1-ol	OG	pOG	S	OG ₍₂₀₎	OG	CG	CG	OG ₍₁₅₎	CG ₍₅₎	OG ₍₁₅₎
hexan-1-ol	pOG	pOG	S	pOG	OG	CG	CG	OG ₍₁₀₎	CG _(2.5)	OG ₍₁₀₎
heptan-1-ol	pOG	pOG	S	pOG	OG	pCG	CG	CG _(7.5)	CG _(2.5)	CG ₍₁₀₎
octan-1-ol	pOG	pOG	S	pOG	OG	S	CG	CG ₍₅₎	CG _(0.7)	CG _(7.5)
ethane-1,2-diol	OG	OG	S	-	I	I	I	-	-	-
water	E	E	E	I	I	I	I	I	I	I
DMF-H ₂ O (3:7)	-	-	-	-	OG	OG	OG	-	-	-
DMF	-	-	-	pOG	CG ₍₃₎	CG _(2.5)	CG _(2.5)	S	S	S
DMSO	-	-	-	OG ₍₂₀₎	-	-	-	S	S	S
chlorobenzene	I	I	TG ₍₁₀₎	-	-	-	-	-	-	-
<i>m</i> -xylene	I	I	OG ₍₅₎	-	-	-	-	-	-	-
mesitylene	I	I	OG ₍₅₎	-	-	-	-	-	-	-
anisole	I	I	pCG	-	-	-	-	-	-	-
formic acid	pOG	P	S	-	-	-	-	-	-	-
acetic acid	pOG	pOG	pTG	-	-	-	-	-	-	-
propionic acid	pOG	pOG	S	-	-	-	-	-	-	-

Note. S = solution, P = precipitated upon cooling, I = insoluble at the solvent boiling point, G = gel (O = opaque, T = translucent, C = clear transparent), pG = partial gel, E = emulsion.

Values in parentheses denote minimum gelation concentration (mg/mL).

It was observed for the gels of **E8**, that their visual, mechanical and thermal stability properties are dependent on the type of metal ion used. The Cu²⁺-complex (**E8a**) forms green gels with similar properties, meaning T_g and MGC, like the light yellow gel of the Zn²⁺-complex (**E8b**). Contrarily, the yellow gels formed by the Ni²⁺-complex (**E8b**) in higher alcohols are much stronger and more thermally stable compared to corresponding gels of **E8a** and **E8b**. This is probably due to the different ligand field stabilization energies and the different type of metal ions (Ni²⁺ *d*⁸, Cu²⁺ *d*⁹, Zn²⁺ *d*¹⁰) used in the gel.

3.4.2 Stimuli triggered and *in situ* gelation, and gel tunability

(Paper II): Unexpectedly, the stigmastrol-amino acid conjugates (**E2a-c**) did not form gels in any of the tested solvents, with one exception of **E2c** forming a partial gel in anisole. In order to improve their solubility in polar solvents, the corresponding hydrochlorides (**E2a-c HCl**) were prepared. Interestingly, the hydrochloride salts were found to be gelators for various solvents (Table 1) indi-

cating that electrostatic interactions play an important role in SAFIN formation. This striking finding brought the idea to test these systems as pH- and acid-responsive. The acid-based triggered sol-to-gel transition could be carried out *in situ* either by bubbling of hydrochloric gas through a solution of the stigmasterol-amino acid conjugate, or by adding a drop of concentrated hydrochloric acid to the solution. The reverse gel-to-sol transition was achieved by addition of TEA to the gel sample, and the acid-base cycle could be repeated several times (Fig. 43).

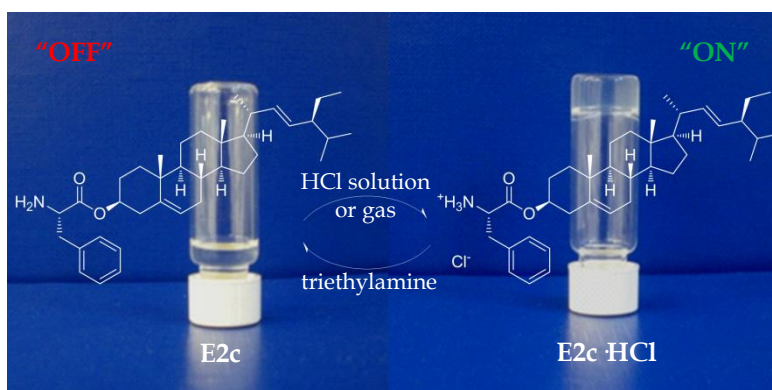


Figure 43. Schematic image of an acid-base triggered reversible sol-to-gel transition of **E2c·HCl** in CCl_4 (2.0 % w/v).

(Paper III): Gelator **E3** containing an imine bond was designed in order to prepare an acid-sensitive compound. The acid-response of gels, prepared in propan-1-ol, butan-1-ol, pentan-1-ol, and DMSO, was tested. It was shown that when a catalytic amount of *p*-toluenesulfonic acid or hydrochloric acid was added to a gel, a gel-to-sol transition occurred (Fig. 44). The hydrolysis of the imine linkage affected the assembly of the gelator molecules, which resulted in the dissociation of the fibrillar network and gave rise to an acid-mediated gel-sol transition. In addition, it was shown that the gelator could be prepared either synthetically beforehand or generated from its precursors *in situ* directly in the solvent to be gelled. The possibilities to use acid-responsive gels as carriers for aromatic drugs were examined with emphasis on their potential applications in drug delivery, particularly in a pH-controlled drug release.

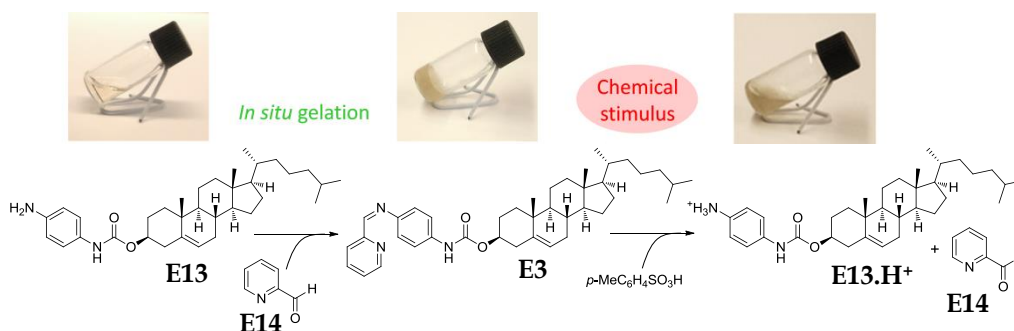


Figure 44. Schematic image of *in situ* formation and an acid-induced irreversible gel-to-sol transition of steroidal gelator **E3** in propan-1-ol (2.0 % w/v).

(Paper IV): The pyridine moiety was introduced into gelator **E4** because of its ability to bind metal ions. It was expected that a coordination of a metal ion will affect self-assembly of the gelator molecules, which could result in a gel-to-sol transition. For this purpose, various metal ions with different coordination geometries were used. Surprisingly, after addition of a metal ion, the transition did not occur. However, changes in visual properties were observed. The opaque gels (formed in higher alcohols) turned to clear transparent gels indicating changes in morphology of the gels (Fig. 45), which was confirmed by electron microscopy (Fig. 49) and PXRD measurements (Fig.56).

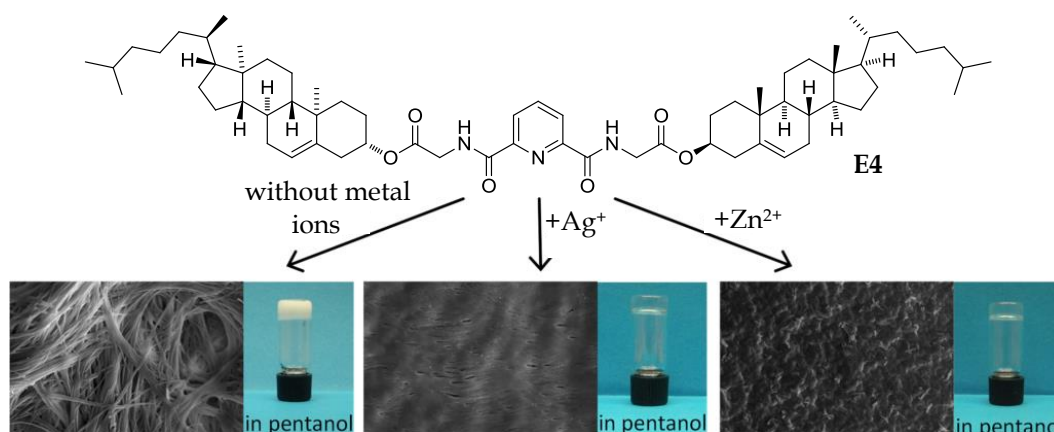


Figure 45. Schematic image of tuning visual and morphological properties of gels of **E4** in pentan-1-ol (2.0 % w/v) by metal coordination.

(Paper V): Similarly to gelator **E3**, the gelation of the metal complexes **E8a-c** could be carried out either by dissolving a beforehand prepared complex in a suitable solvent or *in situ* by simple mixing of the components (amine **E18**, aldehyde **E14**, and a metal salt) in a gelling solvent. It was observed that all three components were needed for an effective gelation, but the order in which they were added, was not important (Fig. 46). For instance, when amine **E18** and 2-pyridinecarboxaldehyde (**E14**) were dissolved in higher alcohols, no gel was observed. However, when a metal ion was added, a sol-to-gel transition was observed which makes the systems responsive to a chemical stimulus. Moreover, it was shown that the *in situ* gelation occurred at ambient temperature, *i.e.* without the need of a heating/cooling cycle (if amine **E18** was dissolved beforehand).

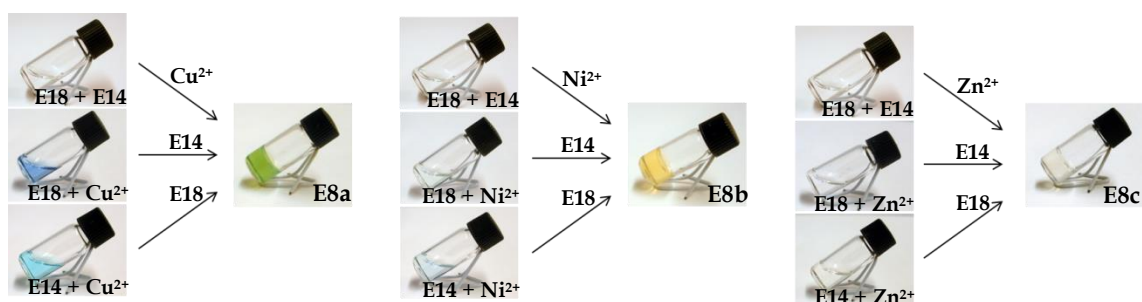


Figure 46. Schematic image of *in situ* gelation of **E8a-c** in octan-1-ol (1.0 % w/v) at room temperature.

Gels based on LMWGs are often called smart materials because of their ability to be responsive to various physical and mechanical stimuli, from which the most common is temperature. However, in this case, temperature was not the only trigger to induce a gel-to-sol transition (Fig. 47). It was observed that the amount of metal ions was crucial for an effective gel formation. If an additional portion of metal was added to the 3:1 complexes, then non-gelator metal complexes were formed and no gelation was observed. The importance of the metal ions for the formation of the gelator complexes **E8a-c** was shown when metalogels were treated with a strong chelating agent such as EDTA. After an addition of the chelate, a gel-to-sol transition occurred. However, the gels could be restored after phase separation and an addition of a new portion of **E14** and metal ions - either of the same kind like in the original gel or interestingly of a different kind, showing a unique possibility for switching gel properties by a metal ion exchange.

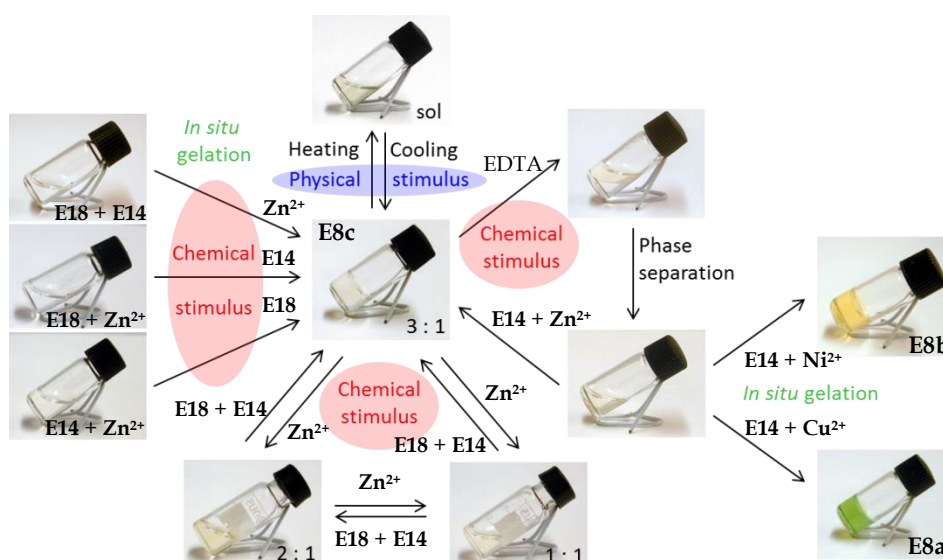


Figure 47. Schematic images of the response of the system of **E8c** in octan-1-ol (1.0 % w/v) to various chemical and physical stimuli. Similar results were observed when the response to stimuli of **E8a** and **E8b** was studied.

3.4.3 Morphology of the gels

SEM and TEM measurements were used to study the morphology of the gels. Typically, fibrous structures of xerogel samples were detected. It was observed that the structure of the fibrillar network did not depend only on the chemical nature of the gelator, but also on the type of gelling solvent. For instance, the micrographs (Fig. 48) of the xerogel of **E4** (IV) obtained from pentan-1-ol (2.0 % w/v) showed a high density network made from tapes, whereas, the image of its xerogel from DMF (1.0 % w/v) revealed an entangled three-dimensional network constructed from bundles of fibers and large pores (residues of evaporated solvent molecules).

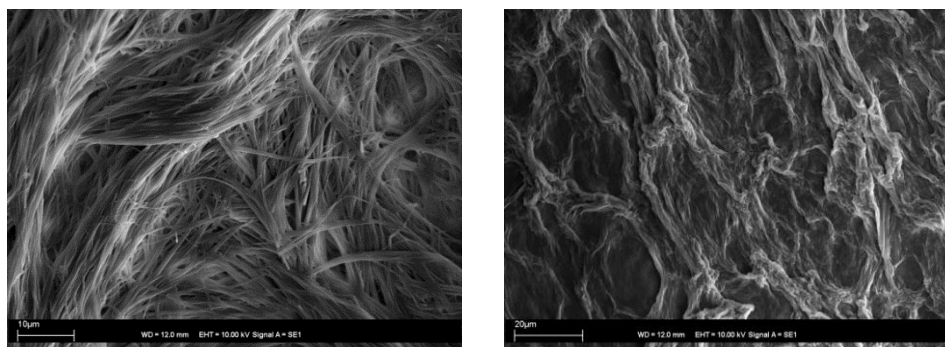


Figure 48. SEM micrographs of xerogels of **E4** obtained from pentan-1-ol (left) and DMF (right).

SEM measurements were also used to study differences in the morphology between gels of gelator **E4** in the presence and absence of metal ions (IV). It was found that the microstructures of the xerogel of **E4** in absence of any metal ions are very different from those of **E4** in presence of metal ions, which was in a good agreement with visual observations (Fig. 45 and 49). Whereas the images of the xerogel of **E4** from pentan-1-ol showed a high density network made from tapes, in the presence of Ag^+ ions, only hard to distinguish thin fibers were observed, and in the presence of Zn^{2+} ions, the structure of the xerogel turned from fibrillar nature to small partly rounded particles. Analogous observations were also found when TEM micrographs were recorded (Fig. 49). The image of the xerogel of **E4** from pentan-1-ol displayed a fibrillar network, whereas in the presence of Zn^{2+} ions, cluster-like microstructures were observed, which was in a good agreement with the results obtained from the SEM measurements. Interestingly, the TEM images of xerogel **E4**+ Ag^+ revealed an *in situ* formation of silver nanoparticles in the gel phase, similarly to a recent report.¹⁶³ These results clearly indicate that a metal coordination has an impact on the way of self-assembly of the gelator molecules and that properties of the gel system can be tuned by using metal ions of different geometry.

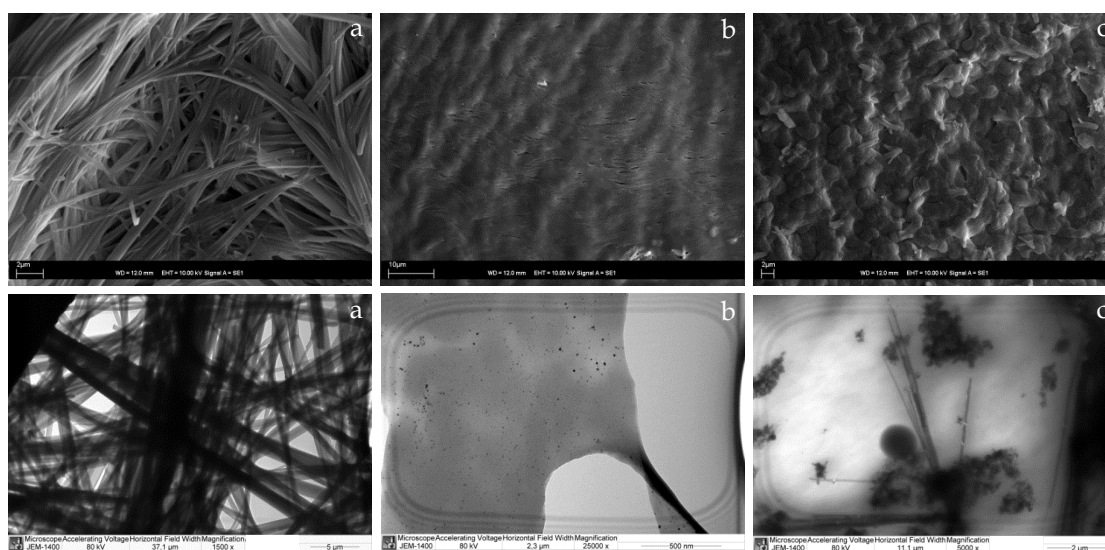


Figure 49. SEM (on top) and TEM micrographs of xerogels of (a) **E4**, (b) **E4**+ Ag^+ , and (c) **E4**+ Zn^{2+} , obtained from pentan-1-ol (2.0 % w/v).

Generally, it was observed that when a gel was opaque, the three-dimensional network of its xerogel was more robust compared to the xerogel structure of a transparent gel, which was more sensitive to solvent removal and upon a solvent evaporation at ambient temperature, the network often collapsed. For transparent gels, better results were often obtained by TEM than by SEM when thinner gel layers were used.

3.4.4 NMR studies of gels

In this work, NMR spectroscopy was utilized for different purposes:

- NMR characterization of newly synthesized compounds (II-V);
- Concentration-dependent ^1H NMR experiments of **E3** in CDCl_3 in order to study the self-assembly of the gelator molecules (III);
- ^1H NMR measurements of the formation of imine **E3** (III) and the imine ligand formed by **E14** and **E18** (V), and of the hydrolysis of imine **E3** (III);
- ^1H NMR for monitoring the drug release from the gel of **E3** in pentan-1-ol (III);
- ^1H NMR titration and Job plot measurements for investigating the ligand-metal ratio of Ag^+ and Zn^{2+} complexes of **E4** (IV);
- Variable-temperature ^1H NMR measurements of gel samples of **E2c HCl** in benzene- d_6 (II), **E4** and **E4+Zn $^{2+}$** in DMF- d_7 (IV), and **E3** in DMSO- d_6 (III) for investigating interactions involved in the gel formation;
- ^{13}C and ^{15}N CPMAS solid state NMR measurements of solids, gels or xerogels of **E2a,c HCl** (II), **E3** (III) and **E4** (IV).

Liquid state NMR

In order to investigate the non-covalent interactions involved in the gelation process, variable-temperature ^1H NMR measurements of **E2c HCl** in benzene- d_6 (II), **E4** and **E4+Zn $^{2+}$** in DMF- d_7 (IV), and **E3** in DMSO- d_6 (III) were carried out. The ^1H NMR spectra of the gel samples were recorded at different temperatures and compared with regard to the chemical shift values and sharpness of the signals. Signals of the gel samples were not sharp at ambient temperatures suggesting a restriction in the molecular movement. With an increase of temperature, the signals became sharper and the temperature, when well-resolved signals were observed, could be referred to as a gel-sol transition temperature (T_g).

However, one has to be careful when interpreting NMR spectra of gels. As Miravet and co-workers pointed out,¹⁴³ ^1H signals observed in a gel state are often from those of non-aggregated molecules, whereas the aggregated structures are "NMR silent". In such situations not much information about SAFIN can be obtained by this method. In order to check if the observed signals belong to molecules in an aggregated or non-aggregated state; control experiments of gelators at non-gelling concentrations were carried out. Interestingly, the ^1H NMR chemical shift values of **E2c HCl** in benzene- d_6 below the gelling concentration did not correspond to that observed in the gel state (Fig. 50); rather, they showed some similarity to the spectrum at high temperature (sol state), indicating that the observed ^1H NMR signals in the gel sample do not correspond to

the non-aggregated state and that the data can be used for the clarification of the gel formation. With an increase of temperature the chemical shifts of the signals did not change remarkably except those of protons on the α - and β -carbons of the phenylalanine part and of NH-protons indicating hydrogen bonding between C=O...H-N functional groups of the gelator molecules **E2c HCl**.

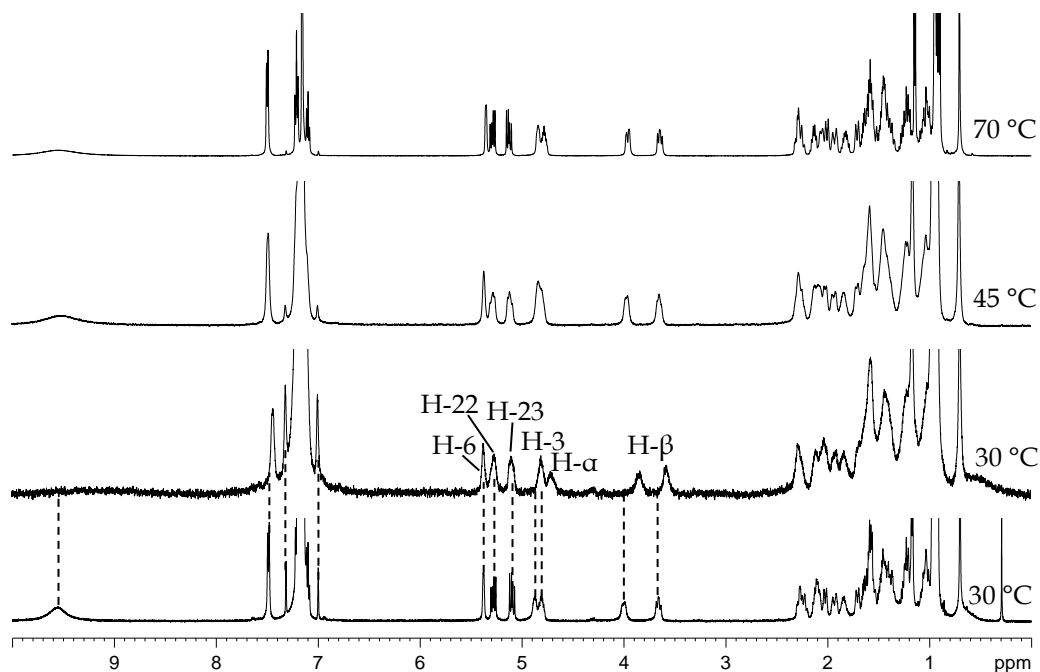


Figure 50. ^1H NMR spectra of **E2c HCl** in benzene- d_6 at non-gelling concentration (0.3 % w/v, bottom spectrum), and as a benzene- d_6 gel (2 % w/v) at different temperatures (30-70 °C).

In the case of gelator **E3** (Fig. 51) and gelator **E4** (Fig. 52), the ^1H NMR chemical shift values of the signals in the gel state correspond to those measured in non-gelling concentrations. Indicating that the observed signals refer to molecules in a non-aggregated state. Nevertheless, the variable-temperature measurements of the gel samples reveal changes in the chemical shift values of protons of amide groups suggesting a participation of these molecules in hydrogen bonding.

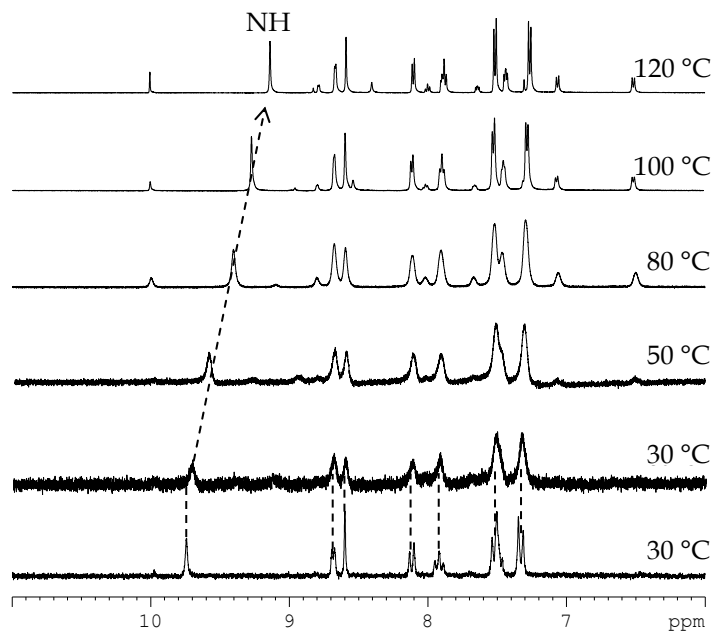


Figure 51. ^1H NMR subspectra of E3 in $\text{DMSO-}d_6$ at non-gelling concentration (0.2 % w/v, bottom spectrum), and as a $\text{DMSO-}d_6$ gel (2.5 % w/v) at different temperatures (30-120 °C).

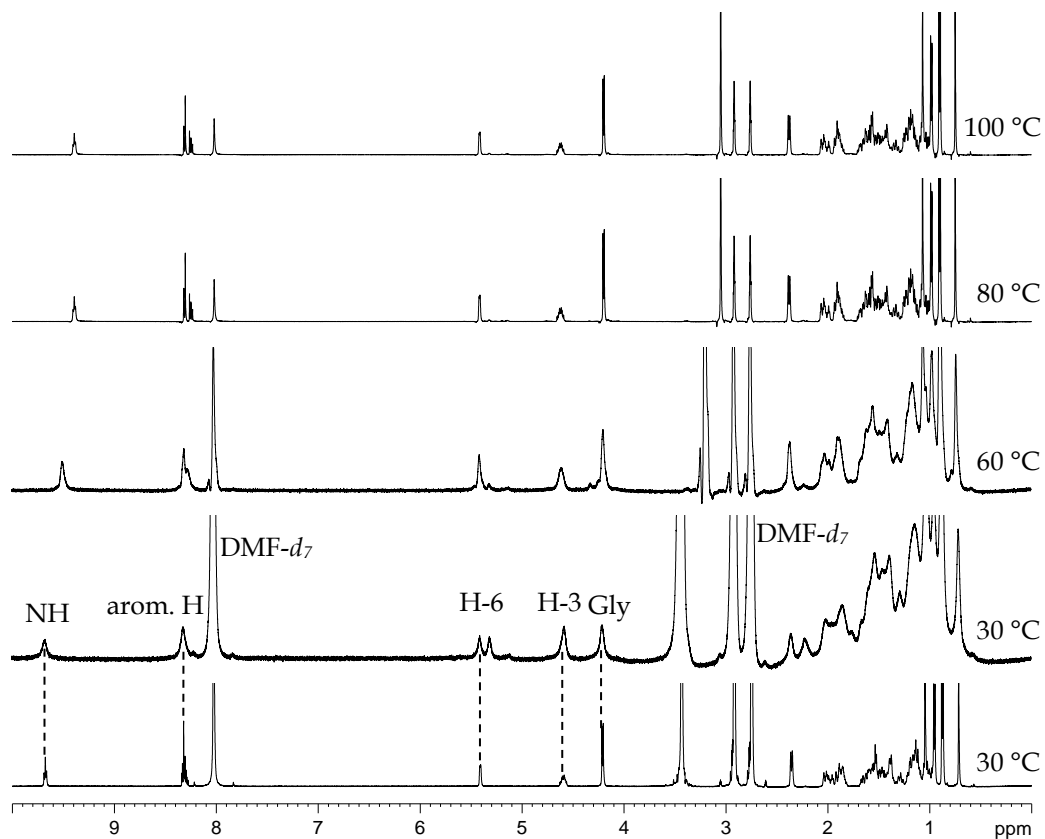


Figure 52. ^1H NMR spectra of E4 in $\text{DMF-}d_7$ at non-gelling concentration (0.1 % w/v, bottom spectrum), and as a $\text{DMF-}d_7$ gel (2% w/v) at different temperatures (30-100 °C).

Solid state NMR

Solid state NMR spectroscopy has emerged as a powerful and complementary tool to XRD to study crystalline solids, semisolids, and liquid crystalline materials.^{144, 145} In this work, solid state NMR techniques were utilized in order to investigate structural properties of selected gelators in different forms - as solids, xerogels, and gels. The structural studies were carried out for gelators **E2a,c ·HCl** (II), **E3** (III), and **E4** (IV). Following, the most interesting results are reported.

¹³C and ¹⁵N cross polarization magic angle spinning (CPMAS) NMR measurements¹⁶⁴ were carried out for gelator **E2c ·HCl** in order to investigate its molecular packing (Fig. 53).

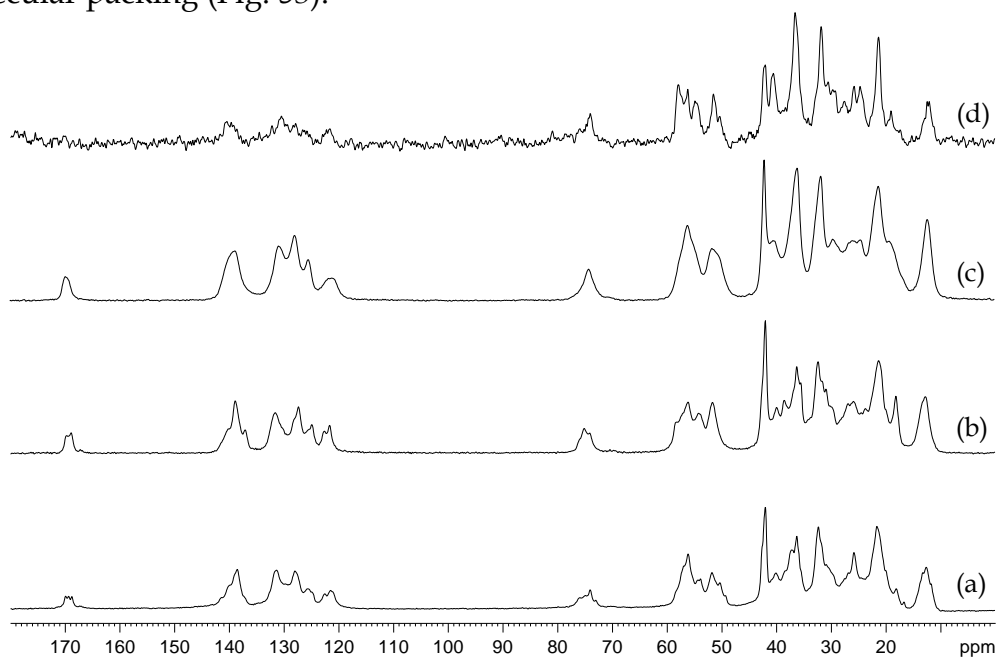


Figure 53. ¹³C CPMAS NMR spectra of **E2c ·HCl** as a) synthetically prepared solid, b) xerogel obtained from benzene, c) xerogel obtained from CCl₄, and d) CCl₄-gel (4 % w/v).

The ¹³C CPMAS NMR spectra of the synthetic solid of **E2c ·HCl** showed multiple signals of some of the carbon atoms indicating the presence of more than one crystalline form of **E2c ·HCl** in the sample. Interestingly, the ¹³C CPMAS NMR of a xerogel from benzene showed a doublet resonance pattern of some of the carbon signals. The doublet resonance pattern is known to occur in crystalline organic solids for two reasons; either (i) two different crystalline forms/polymorphic forms or (ii) due to the presence of two crystallographically non-equivalent molecules in an asymmetric unit. A similar observation was reported for bile acid-based gelators and in that case it was shown to be because of the presence of two non-equivalent molecules in an asymmetric unit of the crystal lattice.⁹⁶ The xerogel obtained from CCl₄ displayed a singlet resonance pattern and differed from the xerogel from benzene supporting observations from SEM measurements that the molecular packing is dependent on the type of the used solvent. In addition, a spectrum of native CCl₄-gel of **E2c ·HCl** was acquired and it exhibited some similarities to the spectrum of the xerogel ob-

tained from CCl_4 , indicating that there is a similarity in the packing mode of the gelator molecules in the native gel and its xerogel.

In order to study the influence of metal ions on the molecular packing of gelator **E4**, ^{13}C CPMAS NMR spectra of its solid and its xerogels from pentan-1-ol were recorded (Fig. 54). The spectra of the synthetic solid and the xerogel from pentan-1-ol show some similarities suggesting similar molecular packing. The broad signals in the spectrum of the xerogel of **E4**+ Ag^+ from pentan-1-ol indicates an amorphous nature of the sample which is in agreement with data obtained from the SEM and TEM measurements (Fig. 49). The spectrum of the xerogel of **E4**+ Zn^{2+} from pentan-1-ol shows a quite crystalline nature and deviates from the others indicating a very different packing mode.

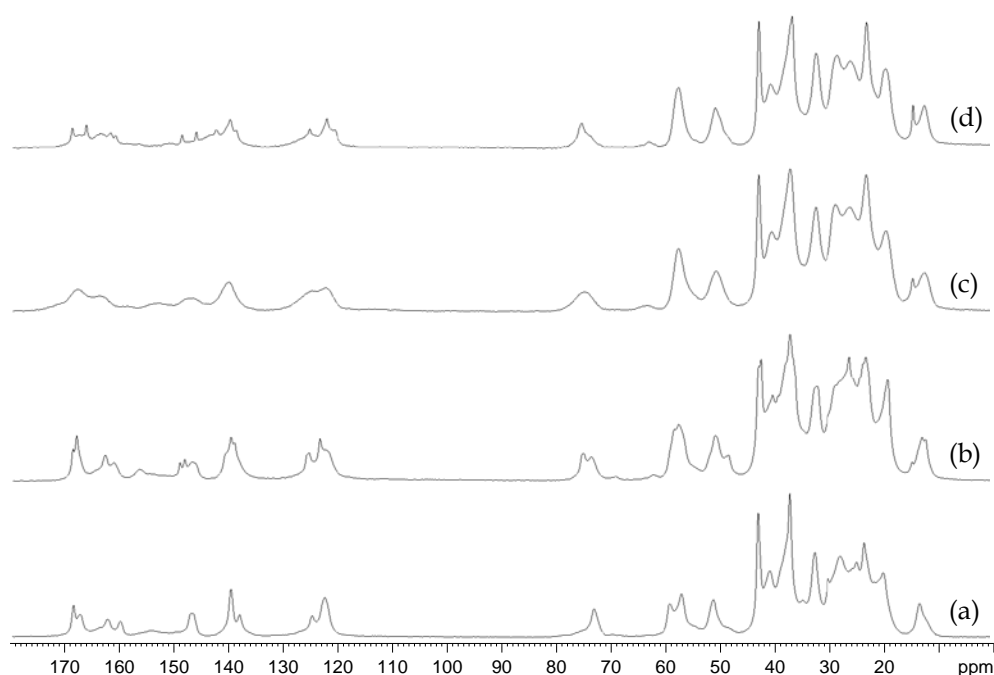


Figure 54. ^{13}C CPMAS NMR spectra of a) solid of **E4** from CHCl_3 , b) xerogel of **E4** from pentan-1-ol, c) xerogel of **E4**+ Ag^+ from pentan-1-ol, and d) xerogel of **E4**+ Zn^{2+} from pentan-1-ol.

3.4.5 X-ray studies

Most gelators do not undergo crystallization to yield good quality single crystals. Moreover, the crystallization conditions are often different (either a non-gelling solvent is used or a non-gelling concentration) from those used in the gel formation. However, in this work (paper IV) a single crystal of gelator **E4** was obtained from DMF, so from a gelling solvent. The crystal was obtained by a very slow evaporation of the solvent from the DMF-gel of **E4**+ Ag^+ . The structure was determined as a DMF solvate (Fig. 55) and surprisingly did not contain silver ions. This finding was unexpected, but it was in a good agreement with observed visual and thermal stability properties of the DMF-gels of **E4** and it was further confirmed by X-ray powder diffraction measurements (Fig. 56).

The PXRD data of xerogels obtained from DMF-gels of **E4**, **E4+Ag⁺** and **E4+Zn²⁺** displayed a very similar pattern indicating a similar packing mode and suggesting that the presence of the metal ions does not play an important role in the gelation process of DMF, because most likely strong H-bonds between the solvent and the gelator molecules rule out a metal coordination. Contrarily, the molecular packing of gels in pentan-1-ol is highly dependent on the presence/absence of the metal ions as it was observed visually and supported by electron microscopy (Fig. 49) and PXRD measurements (Fig. 56).

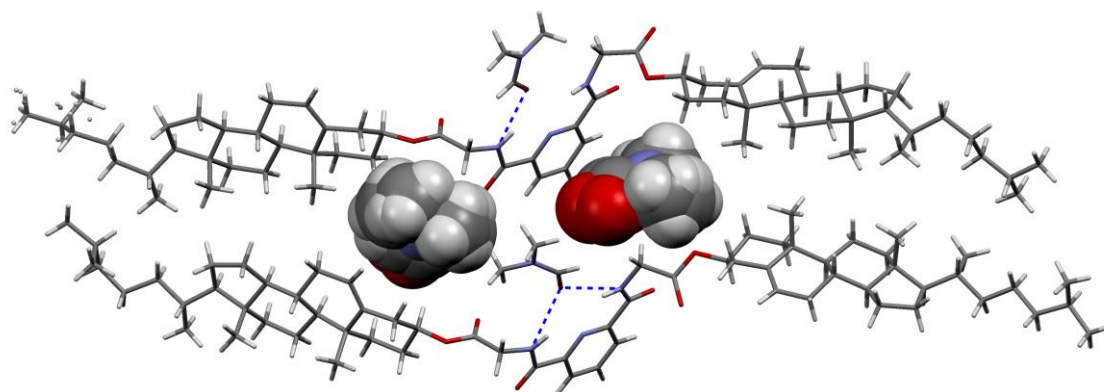


Figure 55. The asymmetric unit of a DMF solvate of **E4** with marked H-bonds between the amide groups of **E4** and the solvent molecules. The DMF molecules are filling two distinct infinite voids running in the directions of the a-axis.

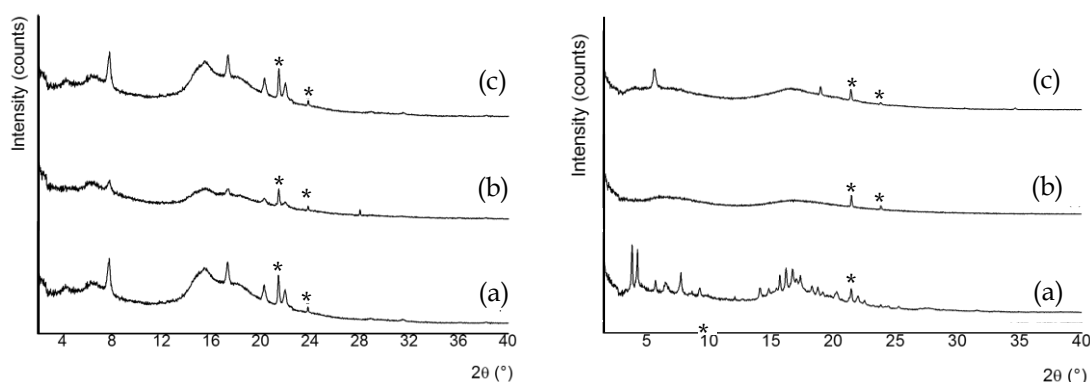


Figure 56. XRD patterns of xerogels from DMF (left) and of xerogels from pentan-1-ol (right): a) **E4**, b) of **E4+Ag⁺**, and c) of **E4+Zn²⁺**; * indicates diffraction peaks originating from an adhesive.

3.4.6 Rheological studies

Metallogels **E8a-c** (paper V), prepared by subcomponent self-assembly, were studied by rheological measurements. The gel formation was monitored by time sweep measurements. The samples were prepared *in situ* by mixing a metal salt with an imine solution (**E14** + **E18**). Upon an aggregation leading to a gelation, a rapid increase of G' and G'' and instantaneous decrease of the phase angle to $\sim 20^\circ$ was observed (Fig. 57 and 58). An effective gelation was achieved

from 20 min to 3 h depending on the type of the used metal ion, and increased in the order $Zn^{2+} < Cu^{2+} < Ni^{2+}$.

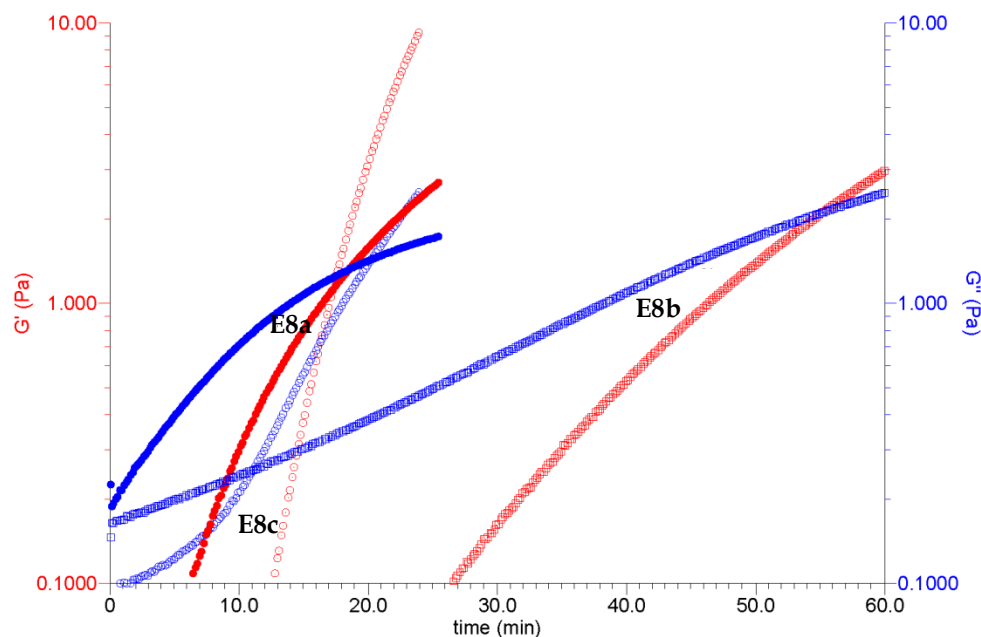


Figure 57. Time sweep experiments of gels of E8a-c formed in octan-1-ol (2 % w/v).

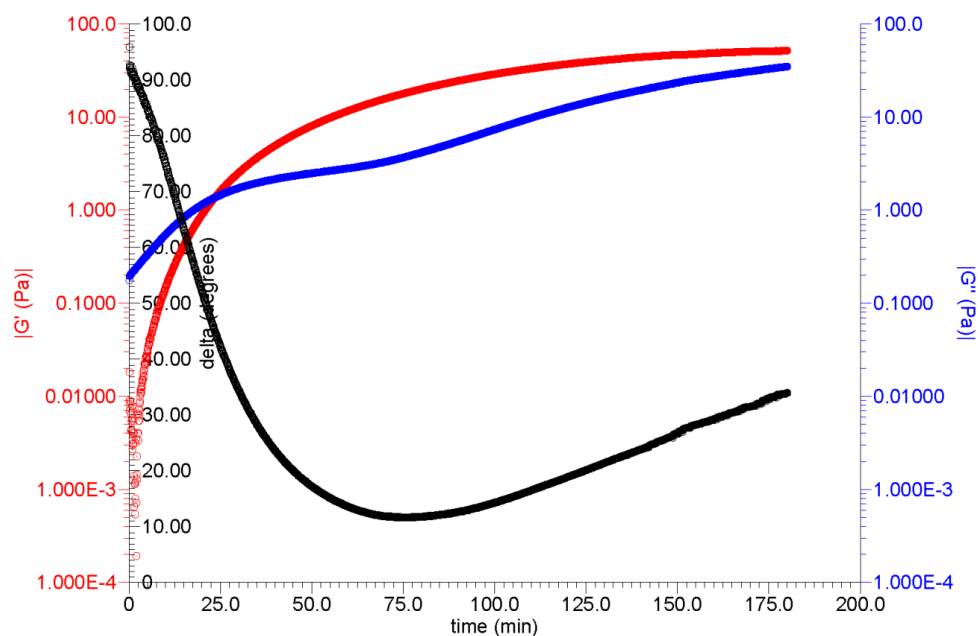


Figure 58. Time sweep experiment of a gel of E8a formed in octan-1-ol (2 % w/v).

The mechanical properties of the gels were studied by frequency sweep and stress sweep experiments (Fig. 59 and 60). These experiments revealed the predominant nature of the elastic modulus G' , which was found to be higher

than the loss modulus G'' , showing that the systems **E8a-c** in octan-1-ol are viscoelastic solids.

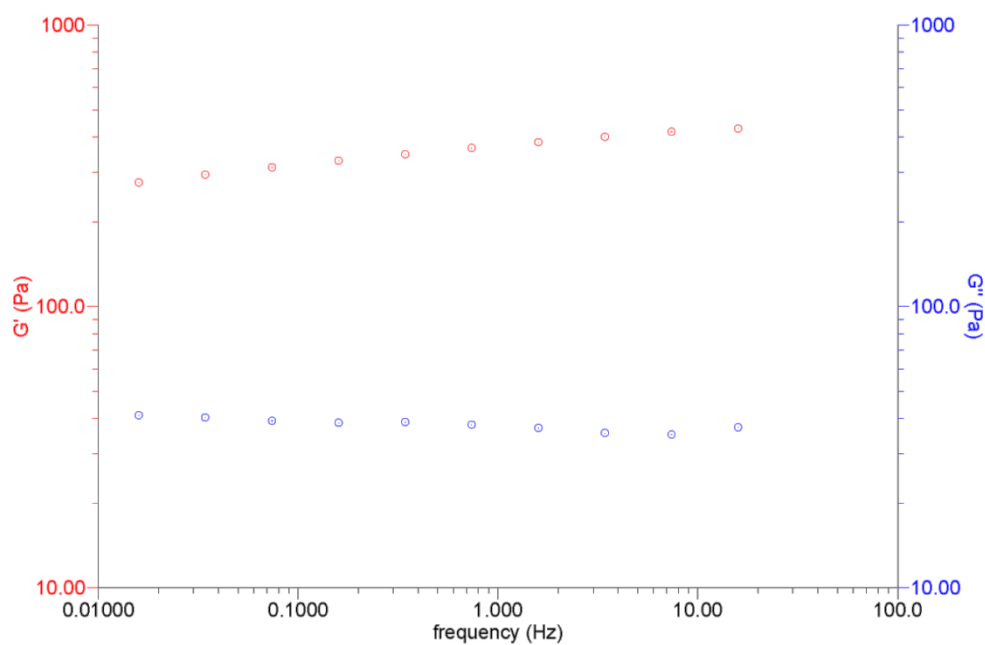


Figure 59. Frequency sweep experiment of a gel of **E8a** formed in octan-1-ol (2 % w/v).

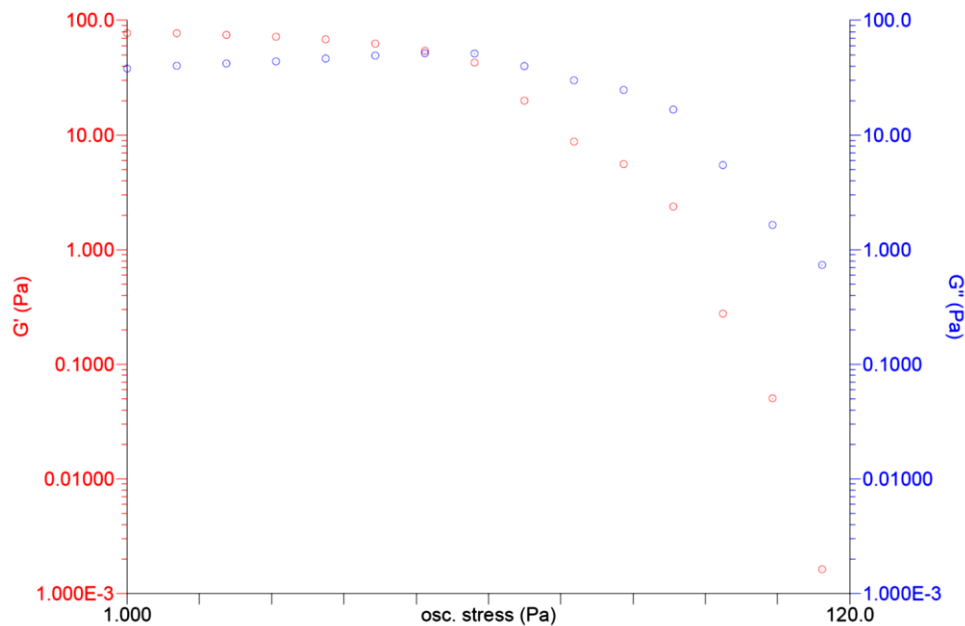


Figure 60. Stress sweep experiment of a gel of **E8a** formed in octan-1-ol (2 % w/v).

4 SUMMARY AND CONCLUSIONS

The aims of this thesis were to synthesize materials based on steroidal derivatives of nitrogen compounds capable to act as efficient gelators and to investigate the properties of their gels and factors affecting gelation, *e.g.* stimuli-response and *in situ* gelation.

In summary, new steroidal acid-responsive gelators containing either a proton accepting amino functional group or an acid-sensitive imine bond were synthesized. Three novel organogelators based on amino acids and stigmaterol - a steroidal molecule rarely used in the synthesis of LMWGs - were prepared. The most striking finding was that the hydrochloride salts (**E2a-c HCl**) of the prepared conjugates acted as gelators, whereas the neutral conjugates were either non-gelators or formed only a weak gel in anisole. The hydrochloride salts of stigmasteryl glycinate and L-leucinate form gels in higher alcohols and ethane-1,2-diol, while stigmasteryl L-phenylalaninate forms gels in aromatic solvents and in tetrachloromethane, indicating that the nature of the amino acid residue had the determinative influence on the gelation abilities. These systems were further explored in order to prepare stimuli responsive materials. It was shown that the compounds could act as pH-responsive gelators with an "acid-base" triggered sol-gel transition, and most importantly the gelation was reversible and could be switched "on and off" by an acid/base-cycle repeatedly.

Compound **E3** represents the first gelator, which forms *in situ* acid-responsive supramolecular gels. It was shown that the gelator could be prepared either synthetically beforehand or *in situ* from its precursors. In both cases the resulting gels exhibited the same macroscopic and microscopic properties. Another specialty of the gelator is the imine moiety designed into the structure not only to facilitate the *in situ* formation, but also to afford an acid-induced hydrolysis. It was shown that after an addition of a catalytic amount of *p*-toluenesulfonic acid or hydrochloric acid, the imine bond was hydrolysed, which resulted in dissolving the gelator self-assembled fibrillar network and as a consequence, a gel-to-sol transition occurred. The gels were also used as carriers for aromatic drugs, namely 5-chloro-8-hydroxyquinoline, pyrazinecarboxamide, and antipyrine, and their potential applications in drug release were

examined. In contact with water, the prepared two-component gels were slowly releasing the drug. Contrarily, by adding an acid, the drug could be instantly released from the gel network. Based on these results, it can be concluded that gels of gelators containing acid-responsive imine bonds are promising soft materials with potential applications in drug delivery and control release systems.

The preparation of metallogels was achieved by incorporating highly efficient coordination sites to the gelator design. Compound **E4** was designed as a gelator of $A(LS)_2$ with a central aromatic unit (pyridine moiety) capable to bind metal ions. It was shown that changes in the chemical composition of the parts A, L, and S, affected the gelation ability significantly. The properties of the systems were studied in the presence and in the absence of various metal ions with different coordination geometries. It was found that not only the choice of the solvent, but also the choice of the metal ion had a big influence on the visual and morphological properties of the gels indicating that a metal coordination can be used as a powerful tool for tuning gel properties.

Based on the knowledge gained by studying *in situ* formation of **E3**, and by investigating possibilities to tune gel properties of **E4** by metal coordination, gelators **E8a-c** were designed. It was observed that similarly to the gelator **E3**, gelators **E8a-c** could be prepared either synthetically beforehand or *in situ*, but in this case without the need of a heating/cooling cycle. It was shown that all three components (steroidal amine **E18**, 2-pyridinecarboxaldehyde, and a metal salt) were needed to achieve an effective gelation, but the order of the addition was not crucial. In all cases the last added component acted as a chemical stimulus causing a sol-to-gel transition. It was demonstrated that visual, rheological, and temperature stability properties of the gels were determined by the type of the metal ion used. However, this could be switched by adding (and removing) a strong chelating agent (which caused a gel-to-sol transition), followed by adding a new portion of metal ions (either of the same or of a different kind compare to the original gel), which resulted in a gel restoration. Furthermore, the ligand-metal ratio of 3:1 was observed to be crucial for an effective gel formation. If an excess of metal salt was added, a gel-to-sol transition occurred. In general, the subcomponent self-assembly gelator preparation represents a novel design approach, which has a potential to become a general design route towards new supramolecular metallogels. It allows one to prepare various materials just by simply exchanging one (or more) of the three components (*i.e.* amine, aldehyde, and metal salt) and by doing so, to design and fine-tune the gel properties.

In this work two systems (gelators **E3**, and gelators **E8a-c**) that enable an *in situ* gelation were prepared. The *in situ* approach represents a modern and still very rare method to the gelation and a tremendous increase in popularity is expected for the future considering its advantages (primarily shortening of the gelator preparation time). Additionally, one of the systems displays gelation abilities at ambient temperature (gelators **E8a-c** in higher alcohols), which is a very unique and attractive way to gel formation with regard to potential applications.

Although, the area of supramolecular gels was extensively studied over the last two decades, there are still problems which remain to be addressed, such as more powerful methods for studying gels, tools to tune gel properties, general principles for designing gelators, *etc.* I hope that this work and the results therein will contribute to the development of the field of supramolecular gels and will help to solve some of the problems of the future.

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ORIGINAL PAPERS

I

Recent advances in steroidal supramolecular gels

by

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REVIEW

Recent advances in steroidal supramolecular gels

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During the last decade or two the interest towards small molecules capable of self-assembly leading to gelation has increased intensively. The investigation of these supramolecular gels aims not only at understanding the fundamental processes underlying gel formation but also at development of new materials with a myriad of applications. Steroids are widely-spread natural products with a large and rigid steroidal nucleus combined with derivatizable functional groups leading to an adjustable polarity profile, which makes them attractive building blocks when designing novel low molecular weight gelators. Due to their unique properties, steroid-based supramolecular gels may find use in applications ranging from materials science and nanoelectronics to their application as reaction media or as sensing and responsive materials. Moreover, biomaterials based on steroidal gels may find use in biomedicine, drug delivery, regenerative medicine, and tissue engineering. This article summarizes the most recent advances in the field of steroidal supramolecular gels in terms of steroid-derived hydro- and organogels, metallo-gels, two-component gels, and stimuli-responsive gels. Furthermore, the potential applications of the systems are discussed.

Introduction

The definition of a gel is not straightforward, albeit gels are generally considered to be viscoelastic solid-like materials comprised of an elastic cross-linked network and a solvent. A three-dimensional network of entangled fibers is formed by aggregation of gelator molecules, which with a large surface area entraps and attaches the solvent molecules, resulting in an increase in viscosity.¹

Polymeric gels have been known for centuries and their applications in different fields, such as food, medicine, materials science, cosmetics, pharmacology, sanitation, and environmental clean-up, are renowned. Since they are formed by covalent cross-linking, they cannot be re-dissolved and are thermally irreversible. In contrast to traditional polymeric gels, supramolecular gels organize by self-assembly processes based on non-covalent interactions, including hydrogen bonding, π - π stacking, electrostatic, van der Waals, and dipole-dipole interactions as well as metal coordination. They can be transformed to a fluid (sol) by heating and are thermally reversible.¹ Applications in numerous fields ranging from materials science and nanoelectronics to drug delivery and biomedicine are envisaged for supramolecular

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Hana Svobodová

Hana Svobodová was born in 1984 in Trebic, Czech Republic. She received her MSc degree in chemistry in 2008 from the Institute of Chemical Technology, Prague, Czech Republic, under the supervision of Professor Zdeněk Wimmer. Currently, she works as a PhD student in organic chemistry at the University of Jyväskylä, Finland, under the supervision of Professor Erkki Kolehmainen. She is interested in the design and synthesis of steroid-based molecules toward functional assemblies and materials.



Virpi Noponen

Virpi Noponen was born in 1980 in Mikkeli, Finland. She received her MSc degree in organic chemistry in 2005 from the University of Jyväskylä, Finland. Currently, she is finalizing her PhD project on amides of bile acids and biologically important small molecules under the supervision of Academy Research Fellow, Adjunct Professor Elina Sievänen. The emphasis of her PhD research is placed on the properties and applications of the prepared molecules, particularly in the fields of supramolecular gels and metal nanoparticles.

gels^{2–6} as a result of the intense investigation within the field during the last decade or two.

The rational design of new molecules capable of self-assembly leading to gelation has proven complicated, due to the challenging specification of conditions and structural requirements for a gelator. Structural features known to promote one-dimensional aggregation include amidic, carbamate, urea, or oxalamide groups combined with aliphatic or aromatic molecules with a large surface area, and many efficient gelators of organic solvents (organogelators) as well as water (hydrogelators) have actually been prepared by their structural combination.⁷

Steroids are natural products comprised of a tetracyclic ring system, to which functional groups are attached. Cholesterol typifies the fundamental structure of steroids. It is a constituent of the cell membranes and thus found in all animal tissues, where it maintains membrane fluidity, microdomain structure, and permeability. Further modifications of the stereochemistry and oxidation states of the fused rings, the side chain, as well as the functional groups of cholesterol lead to a wide variety of biologically important molecules. These include steroidal saponins, cardioactive glycosides, bile acids, and steroid hormones. Phytosterols are characterized by the presence of additional one- or two-carbon substituents attached at C-24 of the side chain, and may vary with respect of the oxidation states of the rings and/or the side chain. The widespread plant sterols, campesterol and sitosterol, respectively, are 24-methyl and 24-ethyl derivatives of cholesterol, whereas stigmasterol contains additional unsaturation of the side chain.⁸

The physical and chemical nature of steroids is well-known. Particularly cholesterol and the bile acids (Fig. 1) have risen as attractive starting materials for organic syntheses, due to their easily derivatized functional groups, availability, and low cost.^{9–11} Furthermore, the overall polarity profile of these compounds can be tailored and the self-assembling characteristics adjusted, which makes them potential components of supramolecular gels.

In this article we summarize the most recent advances in the field of steroidal supramolecular gels. First, the latest achievements in the areas of steroid-based hydro- and organogels are discussed, after which the attention is focused on steroidal metallogels, two-component gels, and stimuli-responsive gels. Moreover, the potential applications of these gels are highlighted.

Hydrogels

The earliest reports on the pH-dependent gel formation of bile salts in water date back to the early 20th century.^{12,13} In the late 1950s it was discovered by Blow and Rich¹⁴ that under appropriate conditions sodium deoxycholate aggregated in solution forming a gelatinous helical complex of macromolecular dimensions. Forty years later, the use of sodium deoxycholate hydrogel for drug delivery applications was investigated.¹⁵ However, the number of hydrogel-forming steroidal derivatives other than bile acids/salts and their conjugates is very limited. Moreover, the majority of the recently published articles presenting bile acid-based hydrogelators seem to originate from the laboratories of Maitra and his co-workers.

A tripodal cholamide **1** (Fig. 2) has turned out to be a supergelator (the minimum gel concentration is as low as 0.15 mM) of aqueous fluids. The “best” gels, meaning transparent and thermally stable, have been obtained in acetic acid–water systems ranging from 0.01 to 30% AcOH in water, depending on the gelator concentration. A variety of physical techniques, including cryo-TEM, CD, steady-state fluorescence, time-resolved fluorescence, and dynamic light-scattering, have been employed in order to understand the structure and dynamics of the gel. The studies have revealed that the molecules of **1** aggregate in an asymmetric manner to yield an entangled network structure of nanofibers having a relatively immobilized aqueous compartment. The kinetic data has indicated that this relates to the progressive ordering of the solvent molecules around the network of nanoscale fibers.¹⁶



Erkki Kolehmainen

Erkki Kolehmainen was born in 1947 in Hankasalmi, Finland. He received his MSc degree in physical chemistry in 1973 from the University of Jyväskylä and his PhD degree in organic chemistry in 1990 from the University of Kuopio. At present Erkki Kolehmainen is working as a Professor Emeritus in the Department of Chemistry, University of Jyväskylä. His research interests are focused on the structures and dynamics of biologically interesting organic molecules (bile acids and other steroids as well as nitrogen heterocycles and their tautomerism) studied by liquid and solid state NMR spectroscopy, X-ray crystallography, and thermal analysis.



Elina Sievänen

Elina Sievänen (née Virtanen) was born in 1977 in Jyväskylä, Finland. She received her PhD degree in organic chemistry in 2003 from the University of Jyväskylä, under the supervision of Professor Erkki Kolehmainen. In 2004 she visited the National Centre of Biomolecular Research in Masaryk University, Brno, Czech Republic, as a post-doctoral student. In 2007 Elina Sievänen was appointed as an Adjunct Professor in organic chemistry at the University of Jyväskylä, where she currently works as an Academy Research Fellow. Her research interests focus on all aspects of steroid-based functional materials, including supramolecular gels, metal nanoparticles, and composite materials.

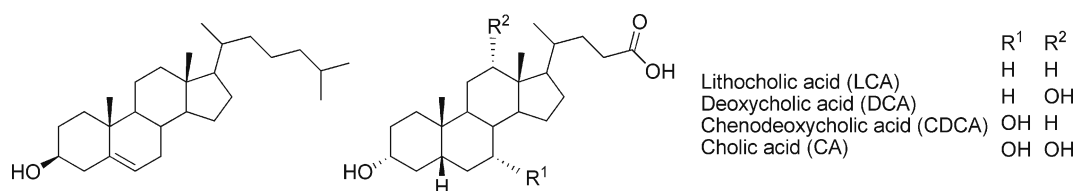


Fig. 1 Structures of cholesterol (left) and four of the most common bile acids (right).

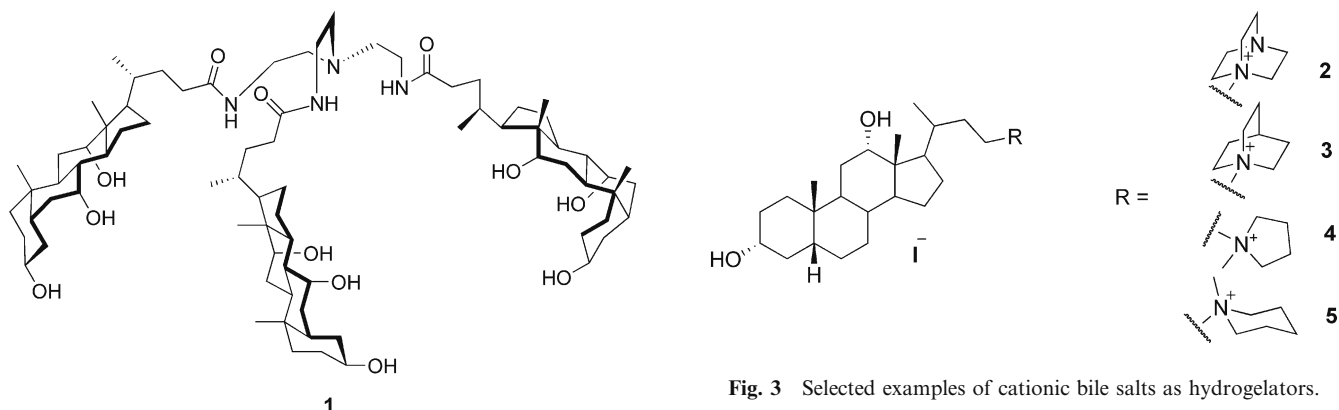


Fig. 3 Selected examples of cationic bile salts as hydrogelators.

Fig. 2 Structure of the tripodal cholamide **1**.

The above-mentioned gel systems have been further studied by the small-angle neutron scattering and rheometry techniques. The self-assembled systems have turned out to exhibit particular scattering and flow properties unusual in the class of molecular gels. Based on the results obtained, the authors were able to propose an aggregation model, in which three gelator molecules per cross-section of fibrillar aggregates are packed in a tail-to-tail fashion. Moreover, it has been observed that the rheological phenomenology of the solutions and gels of **1** is similar to that of DNA solutions.¹⁷ The gel formed by the tripodal cholamide has been exploited in preparing gold nanoparticle–organogel hybrid materials. A bile acid-based thiol derivative has been used as a capping agent in forming the steroidal gold nanoparticles (AuNPs). The hybrid material has been prepared by dissolving the NPs and the gelator compound first in acetic acid, and then diluting the system with water. The formed dispersion has been shown to transform into gel in 4–5 h. The steroid-derived gel has proven to be an excellent medium for stabilizing the structurally analogous AuNPs.¹⁸

A series of cationic analogues of bile salts have been reported as potent hydrogelators. Some of the compounds have been shown to form thermoreversible gels in water in the presence of NaCl. One of the compounds (**2**, Fig. 3) has been shown to form a stable gel in pure water at a concentration above 0.8%. According to the investigations, the use of NaCl with bile salts is essential for better gelation of aqueous solvents, since NaCl provides an ionic environment in addition to the salting out effect. It has been recognized that the concentration of NaCl has a significant influence on the gel strength of the studied bile salts. However, salt concentrations above 4 M were found to prevent the gelation process. Gelation was shown to require at least 0.1 M NaCl solution, and to obtain optically transparent hydrogels, 0.5–1 M NaCl solutions were the optimum. Gels

have been obtained also in 1 M aqueous solutions of NaBr, Na₂SO₄, Na₂CO₃, NaN₃, NaNO₃, KCl, LiCl, and BaCl₂. Some of the compounds (**3–5**, Fig. 3) were shown to form a gel in the presence of organic co-solvents, such as MeOH, EtOH, DMF, and DMSO (up to 20% of organic co-solvent).¹⁹

Maitra and co-workers were the first to design and synthesize a bile acid analogue with a neutral hydrophilic side chain (**6**, Fig. 4) capable of self-assembly promoting gel formation. The amide conjugate of deoxycholic acid and 2-amino-2-hydroxy-methyl-1,3-propanediol was shown to be insoluble in water, but in the presence of varying amounts of organic solvents, such as MeOH, EtOH, DMSO, and DMF, it was shown to form stable, thermoreversible, and transparent gels.²⁰ An analogue of **6** (**7**, Fig. 4), lacking one hydroxyl group in the side chain, was also shown to form gels in aqueous mixtures of MeOH, EtOH, DMSO, and DMF (at 0.2% w/v). However, the gels of **7** in aqueous mixtures of MeOH, EtOH, and acetic acid were unstable and phase-separated to give crystals in 2–24 h. Complementary scattering, diffraction, and microscopy techniques provided a precise structural description of the network architecture and its variation as a function of concentration, aging time, solvent composition, and the type of the gelator. The diameters of the gel fibers of the gels of **6** and **7** were determined. As indicated by the results, the head-to-tail molecular arrangements were shown to be similar in the solid and gel phases.²¹ The structural and rheological features of a series of hydrogels formed by these gelators were scrutinized further rather recently. The results showed that even a small modification to the bile acid backbone leads to striking variations in the self-association properties (structure, stability, and topography–elasticity–strain fields in the related self-assembled fibrillar networks).²²

As an example of the exploitation of bile acid analogue-derived hydrogels as reaction vessels a photodimerization reaction has been investigated. The selectivity of the photodimerization of acenaphthylene was shown to correlate with the

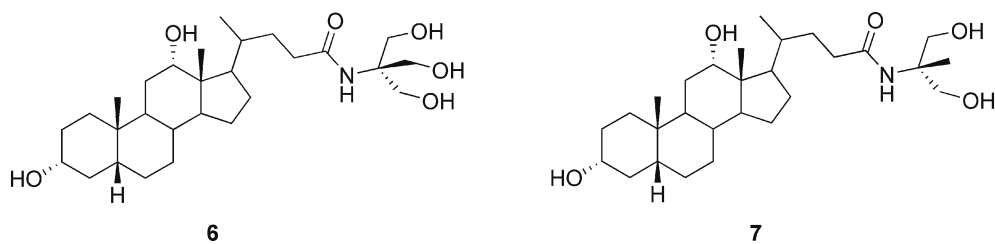


Fig. 4 Structures of bile acid-based hydrogelators with neutral hydrophilic side chains.

rigidity of the gels. However, the selectivity was shown to be lower compared to the reactions reported in other media.²³

A series of 23- and 24-phosphonobiole salts from cholic, deoxycholic, chenodeoxycholic, ursodeoxycholic, and lithocholic acids (Fig. 5) was prepared and the aggregation properties of the compounds investigated. All of the compounds in the series formed hydrogels at different pH values ranging from 1.7 to 7.5. The pH range at which the gelation occurred turned out to be rather narrow. As suggested by the authors, after further development this property might be useful for drug delivery and other pH-responsive systems. The critical micellar concentrations of 23- and 24-phosphonobiole salts were measured using fluorescence and ³¹P NMR methods, the results of which were in good agreement with each other. 23-Phosphonodeoxycholate **8b** was shown to form a reversible thermochromic system, which changes color upon gelation.²⁴

Maitra and co-workers have also introduced novel luminescent materials containing a cholic acid-based europium hydrogel, in which the lanthanide ions were an integral part of the gel matrix, and were essential for the self-assembly process.²⁵ The sensitization of the system was increased by incorporating a hydrophobic chromophore, pyrene, into the hydrophobic gel fibers (Fig. 6). This approach provides a very simple way of making new lanthanide-derived responsive soft materials, whose luminescent properties can be modulated by incorporating suitable hydrophobic chromophores. The applicability of this idea was shown in another work, in which hydrogels based on cholic acid and different lanthanide ions were prepared.²⁶

Very recently, a novel method for the modification of known sodium deoxycholate hydrogels, their application as templates for nanomaterial synthesis, as well as their potential applications in biotechnology and drug delivery have been demonstrated. The gel crystallinity and rigidity have been shown to be enhanced in the presence of increasing concentrations of tris(hydroxymethyl) aminomethane (TRIS). The tunable hydrogel microstructures obtained under various conditions have been successfully utilized as templates to synthesize cyanine-based fluorescent

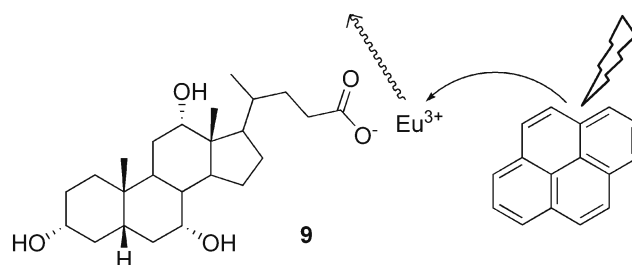


Fig. 6 Schematic representation of energy transfer in the sensitization process in a gel system created from europium cholate and pyrene.

nanoGUMBOS (nanoparticles from a group of uniform materials based on organic salts). The gel microstructures have been shown to direct the size as well as the molecular self-assembly of the nanomaterials, thereby tuning their spectral properties. Moreover, the release profiles of the hydrogel systems have been studied in order to evaluate their utilization for drug delivery purposes.²⁷

The few reported hydrogelators based on cholesterol are represented by the ammonium salts of the organic diacid monoamides of cholesteryl glycinates (Fig. 7). The gels are formed only after excessive bubbling of ammonia gas through the mixture of water and the neutral cholesterol-derivative. Introducing ammonia into the system of, for example, **10a**/water by slow bubbling resulted in a turbid solution. The system was shown to form a turbid, stable gel within 5 min by heating and cooling, or by ultrasonic treatment. The formed salts do not only gelate water, but also apolar solvents, making them typical ambidextrous gelators.²⁸

A very recent and interesting report on steroidal hydrogelators described the use of dexamethasone as a part of a larger molecular hydrogelator system composed of two complementary anti-cancer drugs, namely Taxol and 10-hydroxycamptothecin (HCPT).²⁹ This co-delivery hydrogel system might be introduced in the cavity after surgical tumor removal for the long-term release of anti-cancer drugs.

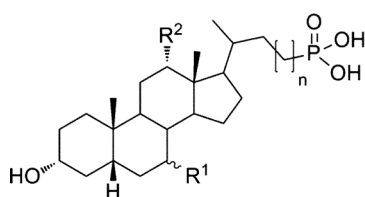


Fig. 5 Structures of 23- and 24-phosphonobiole acids.

8a	R ¹ = α-OH	R ² = OH	n = 1
8b	R ¹ = H	R ² = OH	n = 1
8c	R ¹ = α-OH	R ² = H	n = 1
8d	R ¹ = β-OH	R ² = H	n = 1
8e	R ¹ = H	R ² = H	n = 1
8f	R ¹ = α-OH	R ² = OH	n = 2
8g	R ¹ = H	R ² = OH	n = 2
8h	R ¹ = α-OH	R ² = H	n = 2
8i	R ¹ = β-OH	R ² = H	n = 2
8j	R ¹ = H	R ² = H	n = 2

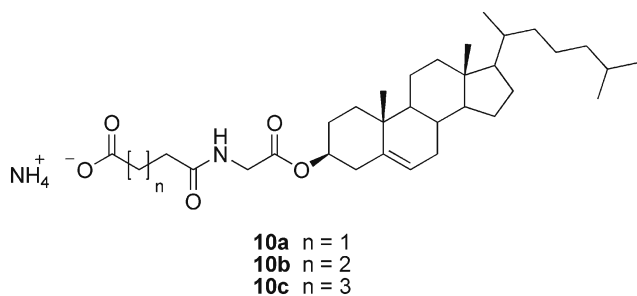


Fig. 7 Structures of cholesterol-based salts capable of hydrogelation.

Organogels

Cholesterol-based organogels

The first rational synthesis of cholesterol-based low molecular weight organogelators was reported by Weiss and co-workers in the 90s.^{30,31} Since then, this class of compounds has been under intensive investigation. Cholesterol-based gelators were comprehensively reviewed by Žinić, Vögtle, and Fages in 2005,³² which is why in this review we concentrate on the most recent achievements in the field.

Weiss and co-workers showed that molecular systems comprised of an aromatic moiety (A) connected to a steroidal group (S) *via* a functionalized linker (L) could display an effective, and in some cases predictable, gelation ability. Later on, new generations of steroidal gelators having dimeric A(LS)₂- or LS₂-type structures have appeared (Fig. 8).

Recently, Weiss and co-workers reported pioneering research aimed at investigating the kinetics of organogel formation.^{33,34} A combination of four techniques – circular dichroism (CD), small-angle neutron scattering (SANS), rheology, and fluorescence spectroscopy – that probe the different aspects of the aggregation process was used to follow the kinetics of the gelation of two *n*-alkanes by 5 α -cholestan-3 β -yl *N*-(2-naphthyl)carbamate (CNC). The gelation was shown to occur *via* instantaneous nucleation and one-dimensional growth, which was further supported by optical microscopy studies. The data collected indicated, that the initial nucleation – being largely dependent on the nature of the gelator, the degree of supersaturation, and heterogeneities within the system – determines the morphology of the final gel.³³ Moreover, Weiss and co-workers extracted the fractal dimensions of the self-assembled fibrous networks

(SAFINs) of the molecular organogel systems from the kinetic data for the first time. In addition, they showed that by varying the incubation temperature and the concentrations of the solutions containing CNC in *n*-alkane, the compound can be directed to form one out of two types of SAFINs corresponding to two crystal forms.³⁴

Professor Shinkai directs another group actively involved in the research of steroidal gelators, particularly those capable of acting as templates for the creation of novel inorganic materials. In 2001 Shinkai and Jung reviewed their studies on crown-appended cholesterol gelators. The superstructures of the prepared gels could be transcribed to a silica gel affording novel inorganic materials with controlled morphologies.³⁵ Furthermore, the gel structure of the dimeric azobenzene-appended cholesterol organogel (compound **11**, Fig. 9) was shown to successfully transcribe into the silica nanotubes yielding monodisperse inner helical hollows of the silica.³⁶ The self-assembled compound **12** (Fig. 9) was shown to exhibit a tubular structure, whereas compounds **14** and **15** formed spherical structures in acetonitrile. Thus the authors concluded that the balance between the solvophilic and solvophobic groups is important for the formation of the tubular structure by self-assembly in organic solvents. The self-assembled compound **12** was further used as a template as well as a catalyst for sol–gel polymerization of inorganic precursors to produce novel double-walled tubular structures of transition-metal oxides (TiO₂, Ta₂O₅, and ZrO₂). Moreover, this sol–gel transcription was able to give binary transition-metal oxide nanotubes of TiO₂/ZrO₂.³⁷

The Shinkai group also designed and prepared pyridine-containing cholesterol-based gelators (**16a–c**, Fig. 9). Compound **16a** proved to be an efficient gelator capable of forming gels in 16 solvents out of the 19 studied. Three of the solvents were gelled at a gelator concentration of 0.5 wt%, making compound **16a** a supergelator in those solvents. Even a solid solvent, naphthalene, was stabilized by compounds **16a** and **16c**. The authors showed that the morphologies of the systems strongly depended on the process of solvent removal from the gel state. By using metal–ligand interactions, for example between Ag(I) and the ligands, the tuning of the morphologies and stabilities of the gels was enabled. Moreover, the gel fibers were decorated by fluorescent molecules, like tetraphenylporphyrin Zn(II), which had photopolymerizable units at the end of the tether groups. The modified fibers were then characterized by UV-VIS absorption spectroscopy, confocal laser scanning microscopy, and TEM.

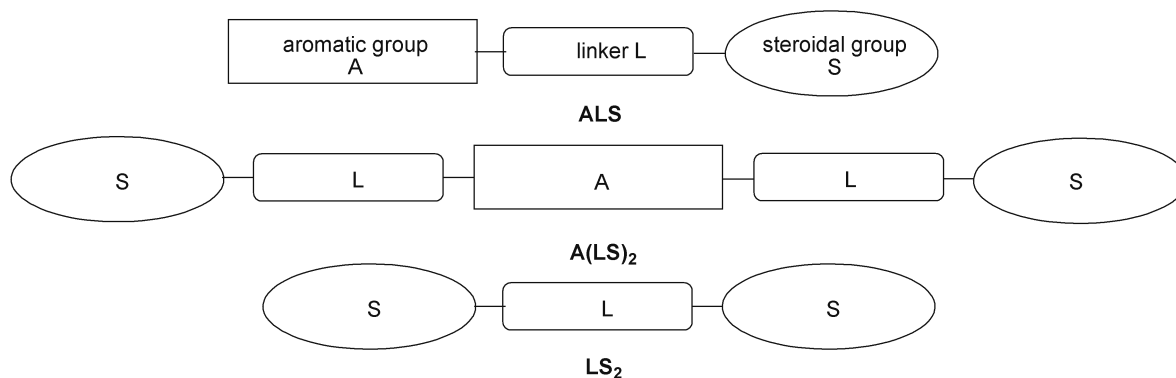


Fig. 8 ALS, A(LS)₂, and LS₂ architectures of cholesterol-based gelators.

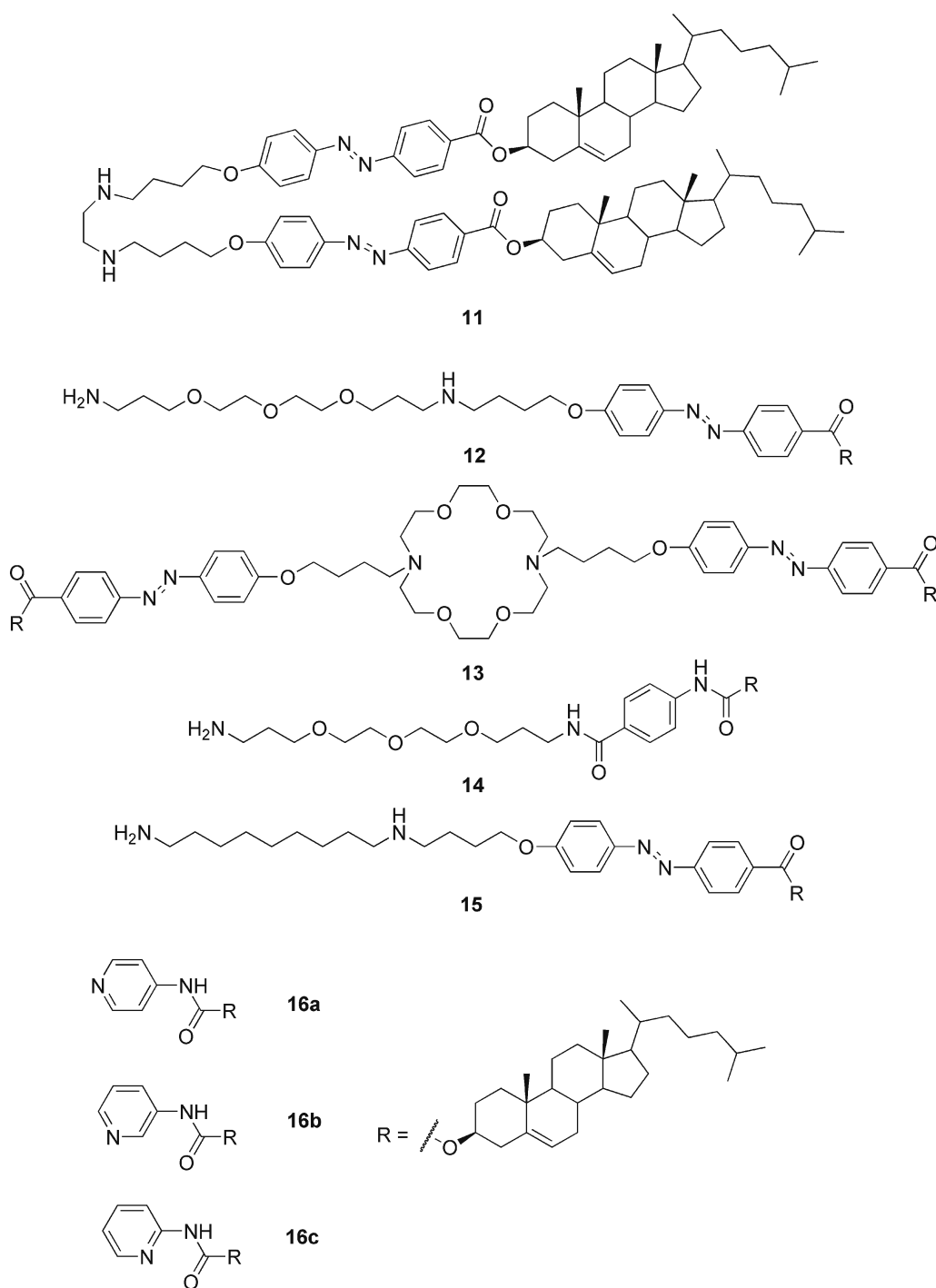


Fig. 9 Representative examples of cholesterol-based gelators reported by Shinkai and co-workers.

Attachment of the fluorescent molecules to the gel fibers and/or tubes might find application in photochemical and electrochemical devices.³⁸

An additional example of exploiting a steroidal organogel as a template for creating inorganic materials has been reported by Lu and co-workers. CuS nanofibers with tunable helical pitches were observed to form using an organogel of a dicholesterol derivative as a template. It was shown that the morphologies of the inorganic nanofibers could be controlled by the binding sites between the inorganic precursor and the organogel.³⁹ Another

interesting example of the applications of organogels composed of cholesterol derivatives has been reported very recently. An all-organic steroid-D- π -A modular design was shown to lead to ferroelectricity in nano-architectures constituted of organogels.⁴⁰

Also Fang *et al.* have put a lot of effort into designing and preparing new cholesterol-based gelators along with extensively studying the formed gels. Quite recently, four novel conjugates of cholesterol and linear glucose were designed and prepared. The compounds differed from each other by the length of the linking diaminoalkyl moiety. The gelation behavior of the compounds

was examined in 26 solvents at a concentration of 2.5% (w/v). All of the studied compounds showed gelation properties in both protic and aprotic solvents, and it was shown that the number of gelled solvents and the possibility of forming transparent gels increased with increasing the length of the linker chain. Three of the compounds started gelating xylene already at concentrations below 0.03% (w/v), making them possible supergelators. More interestingly, the above-mentioned compounds can be used for preparing supramolecular gel films by injecting a hot xylene solution of the gelator into a film mold and then cooling the system to room temperature. These films are stable in the wet state, and can even be slightly stretched as shown in Fig. 10.⁴¹

Also, dimeric cholesterol-based compounds of the A(LS)₂-type were prepared and their gelation properties extensively studied by Fang *et al.* They prepared a series of three gelators differing by the positions of the linkers in the benzene ring. Compounds bearing the *m*- and *p*-substituents, were shown to be more efficient gelators than the compound possessing *o*-substitution. Interestingly, the xylene gel of the compound with *m*-substitution was shown to form spontaneously at room temperature. Shaking of the gel was shown to result in a phase transition from the gel state to solution. Moreover, the gel was shown to recover when the shaking was stopped. This thixotropic behavior of the gel was further proved by rheological studies.⁴² Later, an analogous series of A(LS)₂-type gelators was prepared and the gelation properties of the compounds investigated as well as compared with the above-mentioned studies. The difference between the two series of compounds lies in the linker moieties of the structures having in the latter case more sites for hydrogen bond formation. Numerous gel systems were shown to form spontaneously at room temperature, and also these gels possessed smart thixotropic properties as revealed by the rheological studies.⁴³

The same group has discovered even more examples of thixotropic gels. Dicholesteryl derivatives with spacers containing two L(D)-alanine residues and 3–6 carbon atoms between them were prepared in order to investigate the effect of the length of the spacer and the chirality of the amino acid residue to the gelation properties of the compounds. Indeed, the above-mentioned features were shown to have profound effects on the gelation properties. The compounds containing D-alanine residues and shorter spacers were shown to be able to gelate more solvents than their analogues with the opposite chirality. For the compounds having longer spacers, however, an opposite result was obtained. At least 11 of the gel systems studied were shown to form gels spontaneously at room temperature. Again, the rheological studies proved the thixotropy of the gels.⁴⁴ Very

recently, yet another series of diacid amides of dicholesteryl L-glycinates as organogelators was reported. The length of the linker connecting the two cholesteryl residues was shown to play a crucial role in the gelation behavior of the compounds and in the nature of the microstructures of the gels.⁴⁵

As the first example of cholesterol derivatives, compound **17** (Fig. 11) was shown to form a water-in-oil type gel emulsion by agitating the system at room temperature.⁴⁶ Similar types of compounds, differing only in the L-alanine amino acid being changed to L-phenylalanine, have shown to be versatile organogelators (compounds **18a–b**, Fig. 11). Again, many of the gel systems studied were shown to form spontaneously at room temperature. Furthermore, compounds **18a** and **18b** were capable of selectively gelating xylene or kerosene from their water mixtures. Gels of **18a**/xylene were even shown to be mechanically strong enough for separation, thus making compound **18a** a strong candidate for the practical separation of xylene from its water mixtures (Fig. 12).⁴⁷

Yet another group of LS₂-type dimeric cholesteryl derivatives, now having a linker with three benzene rings in addition to two amide and two carbamate groups, has been reported by Fang and co-workers. This group of compounds was shown to gelate a wide variety of organic solvents by three different ways. In addition to the traditional heating–cooling cycle, some of the gels were shown to form by mixing at room temperature or by ultrasound treatment. The studies revealed that one of the compounds was a supergelator for DMSO (0.04% w/v), and that the DMSO gel could be prepared by any of the three methods mentioned above. Moreover, the gel possesses excellent mechanical strength and smart thixotropic properties.⁴⁸

The microstructures of diphenylbutadiene derivatives linked *via* flexible alkyl chains to one or two cholesterol units have recently been investigated by Das and co-workers. Scanning electron microscopy of the xerogels of the mono-cholesterol derivatives indicated that the molecules self-assemble to form 3D networks consisting of helically twisted fibers. However, the xerogel morphology of the bis-cholesterol derivatives indicated agglomerated spheres. Further investigation of spectroscopic properties has suggested that the morphology of the superstructures formed in these systems may be correlated to the nature of the molecular aggregates formed.⁴⁹

Yi *et al.* have recently introduced and extensively studied the organogelation behavior of novel compounds of the ALS-type with a naphthalic unit as the aromatic group. The linker connecting the steroidal and aromatic parts varies from an alkyl chain with a variable number of acyl amino linkages to an alkyl chain with an L-alanine moiety embedded in it. The introduction of a fluorophore (naphthalic group) into the system makes it convenient to study the molecular aggregation, for example, by absorption spectroscopy and confocal scanning microscopy. The aggregation of these compounds is reversibly controlled by ultrasonic radiation, as presented later in this review.^{50–52}

Cholesterol-based perylene derivatives have been synthesized in order to design novel visible-light-harvesting gel systems.⁵³ The studied conjugate **19** (Fig. 13) created gels in mixtures of alcohols and aromatic solvents. It was shown that the organogels could absorb a wide range of light energy through the perylene-stacked assemblies in the gel phase. Interestingly, the gel phase (but not the sol phase) showed CD activity.

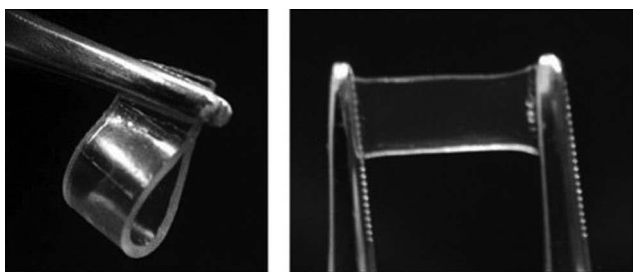


Fig. 10 Photographs of the gel films of a conjugate of cholesterol and linear glucose in xylene.⁴¹ Reproduced by permission of Elsevier.

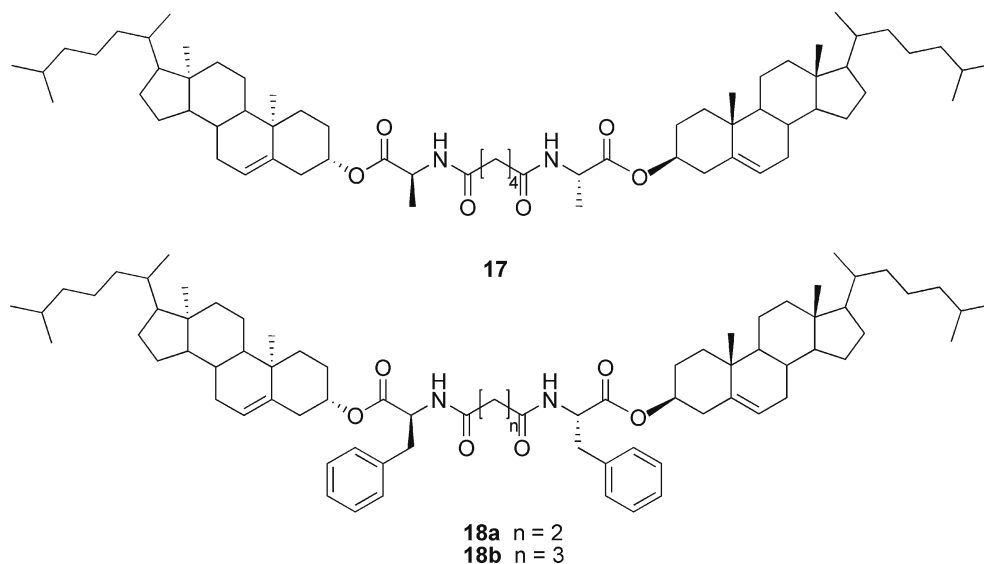


Fig. 11 Structures of compounds **17** and **18a–b**.

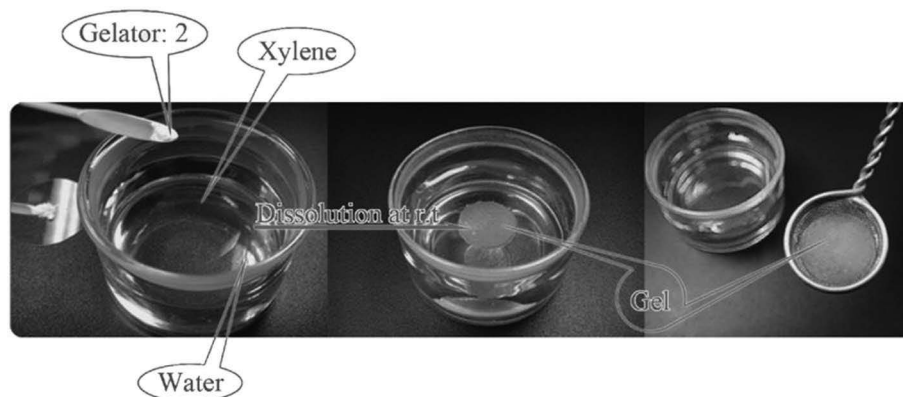


Fig. 12 Selective gelation of xylene from its mixture with water (gelator 2 refers to compound **18a**).⁴⁷ Reproduced by permission of the Royal Society of Chemistry (RSC) for the Centre National de la Recherche Scientifique and the RSC.

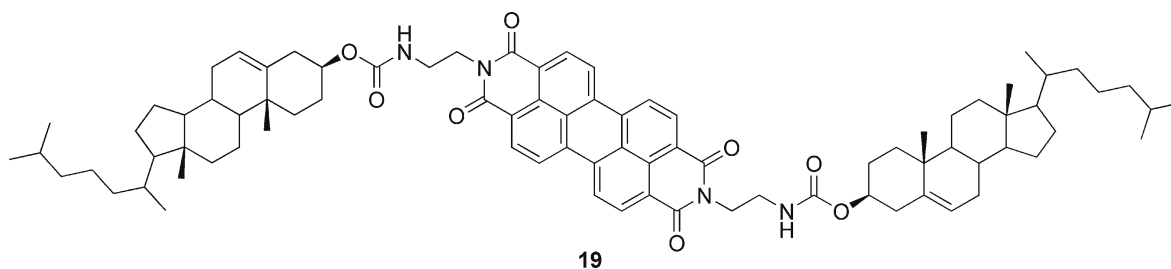


Fig. 13 Perylene-appended cholesterol-based gelator.

Although organogelators with tunable emission properties are well-known, so far only a few examples of white-light-emitting gels have been reported. One of the rare illustrations of such systems are organogelators **20** and **21** (Fig. 14).^{54,55} Compound **20** was found to be a weak gelator for decane, whereas compound **21** created a strong gel in the same solvent. This difference in the gelation behavior was explained as a different packing mode in the gel phase and resulted in the systems having different optical, chiroptical, and morphological properties.⁵⁶ In

order to investigate the light emitting properties of the gels, energy transfer in the presence and absence of a red-light-emitting acceptor **22** was studied.^{54,55} In the case of moderate self-assembly, a partial energy transfer of a blue-light-emitting donor to a red-light-emitting acceptor was expected and a mixture of blue, green, and red emission being generated, leading to a white-light-emitting gel. On the other hand, a strong self-assembly and complete energy transfer should lead to exclusive red emission of the acceptor. These hypotheses were proven by

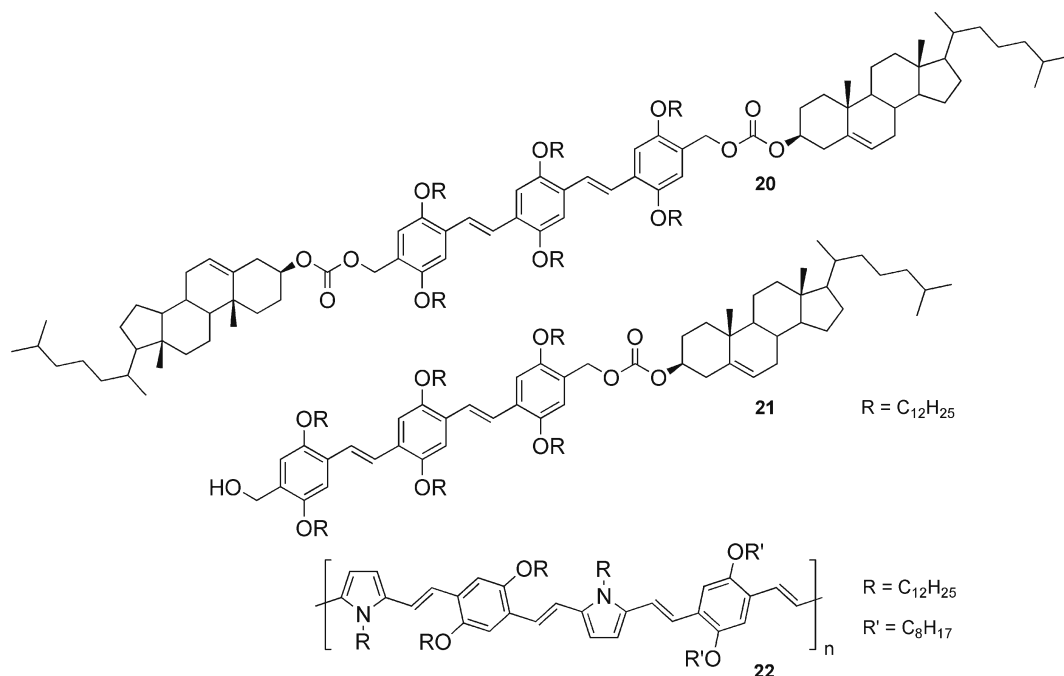


Fig. 14 Structures of steroidal organogelators **20** and **21**, and the red-light-emitting acceptor **22**.

experiments and it was shown that the self-organization of the donor was crucial for energy transfer, suggesting this to be a way to efficiently control energy migration.

Bile acid-based organogels

One of the earliest reports on bile acid-based organogelators introduced the organogelation properties of *N*-isopropyl cholamide in 1998.⁵⁷ Since then, several reports on the organogelation properties of bile acid derivatives have been published. Moreover, a few review articles have touched upon the issue.^{1,58,59}

Very recently a new class of efficient gelators of organic and aqueous–organic media, namely perfluoroalkyl bile esters, as first examples of fluorinated gelators derived from the bile acids, were introduced. The target compounds were prepared by attaching the perfluoroalkyl chains of different lengths to the bile acids through two different ester linkages (Fig. 15). Three different bile acid moieties (LCA, DCA, and CA) were exploited in the syntheses. By varying the above-mentioned variables, the gels were obtained in aromatic hydrocarbons, DMSO, and DMSO/DMF–H₂O mixtures of different proportions. The most efficient gelators among these compounds formed gels well below 1.0% (w/v) and hence they can be termed supergelators. The effect of the bile acid moiety, the nature of the ester linkage, and the length of the fluoroalkyl side chain on the gelation properties of the compounds, as well as on the properties of the formed gels (thermal stability, mechanical properties, and morphology), were carefully and systematically investigated. Rheological studies demonstrated that the gels behaved as viscoelastic materials, and that the mechanical properties of the studied gels could be modulated by changing either the bile acid moiety or by varying the length of the fluoroalkyl segment. The presence of CO₂-philic perfluoroalkyl group is expected to enhance the solubility of the studied compounds in supercritical

CO₂, thus making these compounds promising candidates for forming aerogels.⁶⁰

The simplest esters of bile acids showing the ability to promote self-assembly leading to organogelation were serendipitously discovered by Maitra *et al.* in 2007. The compounds included allyl and propargyl cholates, and simple alkyl (*n*-propyl and ethyl) esters of cholic acid. All of the compounds were able to gelate mesitylene, xylene, benzene, and toluene at concentrations below 1.0% (w/v). However, methyl and butyl esters of cholic acid were not able to form gels in any of the solvents tested.⁶¹ Recently, further studies utilizing the solid state NMR and X-ray powder diffraction methods revealed a close resemblance in the packing pattern between the gelators and the bulk solid, xerogel, and the gel in its native state. A doublet resonance pattern of ¹³C signals in the ¹³C CPMAS NMR spectra were observed for the gelator molecules, whereas the non-gelators were shown to exhibit simple singlet resonances or to result in the formation of inclusion complexes/solvates.⁶²

The gelation abilities of some alkyl esters of cholic acid were studied in 2002. According to these studies, alkyl cholates with

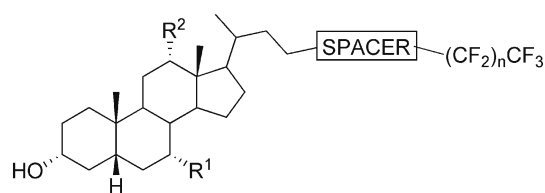


Fig. 15 Fluorinated bile acid-based gelators by Maitra *et al.* In LCA-derivatives R¹ = R² = H; DCA-derivatives R¹ = H, R² = OH; CA-derivatives R¹ = R² = OH. The spacer is either –(CO)–OCH₂– (type P) or –O–(CO)– (type Q); *n* = 1, 6, 8 (with spacer type P) or *n* = 6, 8, 9, 10, 12 (with spacer type Q).

chain lengths of 8, 10, and 12 carbon atoms were not able to form gels in any of the solvents tested. However, in apolar solvents they were shown to increase the solubility of the more polar components, like sugars and water. These findings are further discussed in the context of two-component gels later in this article. Besides the alkyl esters, a group of amide and urea derivatives of cholic acid were prepared and the gelation properties of the compounds investigated. These compounds formed transparent gels in aromatic solvents and cycloalkenes. By using a reversed amide bond, it was shown that the direction of the amide bond had a minor influence on the gelation ability of these compounds, while the introduction of an aryl group decreased the gelling capability. Dimers containing two cholic acid units were shown to form organogels in more polar solvents.⁶³

The same research group reported that *N*-choly amino acid alkyl esters acted as organogelators for aromatic solvents and cyclohexene affording stable, transparent, and thermoreversible gels. Compounds with varying amino acid side chains and ester groups were prepared and their gelation properties systematically investigated. In order to study the effect of the bile acid moiety, *L*-leucine methyl ester conjugates of lithocholic, chenodeoxycholic, and deoxycholic acids were synthesized and their gelation properties studied. No gels formed by these reference compounds were observed.⁶⁴ Detailed network structures of some of the gels formed by the cholic acid derivatives presented above have been further studied by small-angle neutron scattering (SANS).⁶⁵

Our group has been interested in preparing conjugates of bile acids and biologically important small molecules linked *via* an amide bond. Recently, we published the synthesis and detailed gelation studies for a series of three bile acids (LCA, DCA, and CA) conjugated with *L*-methionine methyl ester. Two of the conjugates, namely lithocholyl and cholyl derivatives **23a** and **23b** (Fig. 16), respectively, were shown to undergo self-assembly leading to organogelation in certain aromatic solvents. The

properties of the formed gels were investigated by the conventional methods typical for molecular gel studies along with ¹³C CPMAS NMR spectroscopic studies of the native gel. Estimation of the packing similarities/differences in the solid and gel states exploiting solid state NMR spectroscopy together with X-ray diffractometry was performed. Our results suggest that in some cases the mode of packing in the gel or xerogel may differ from that of the single crystal X-ray structure.⁶⁶

In another paper by our group six derivatives of bile acids with a drug molecule cysteamine (2-aminoethanethiol) were prepared. Two of the conjugates (dehydrocholyl and cholyl derivatives **24a** and **24b**, respectively, Fig. 16) were shown to form organogels. All but one of the solvent systems gelled were aromatic solvents, 1-octanol being the exception in the case of compound **24a**.⁶⁷ Furthermore, two groups of compounds structurally related to the previous cysteamine conjugates of bile acids have been synthesized and their gelation properties extensively studied by us.^{68,69} First, simple bile acid amides of lithocholic and deoxycholic acids with 2-aminoethanol (**25a** and **25b**, Fig. 16) and 3-aminopropanol were shown to be effective gelators for mainly chlorinated organic solvents or aromatic solvents, depending on the length of the side chain. The compounds were shown to be capable of thickening neutral and acidic water solutions, but accurate gel formation in those conditions has not been detected.⁶⁸ Second, the ability of a series of amino- and dihydroxyalkyl amides of bile acids to promote gel formation was clarified.⁶⁹ Out of 396 combinations formed by 11 compounds and 36 different solvents, 22 gel-containing systems (1% w/v) were obtained. Apart from one exception, the compounds capable of gel formation were shown to be lithocholic acid derivatives.

Comparison of the above-obtained results for bile acid amidoalcohols, -amines, and -thiols has enabled the investigation of the effect of the replacement of a single functional group in the side chain on the gelation behavior of these structurally related compounds. Based on the data, the amidoalcohols **25a** and **25b**

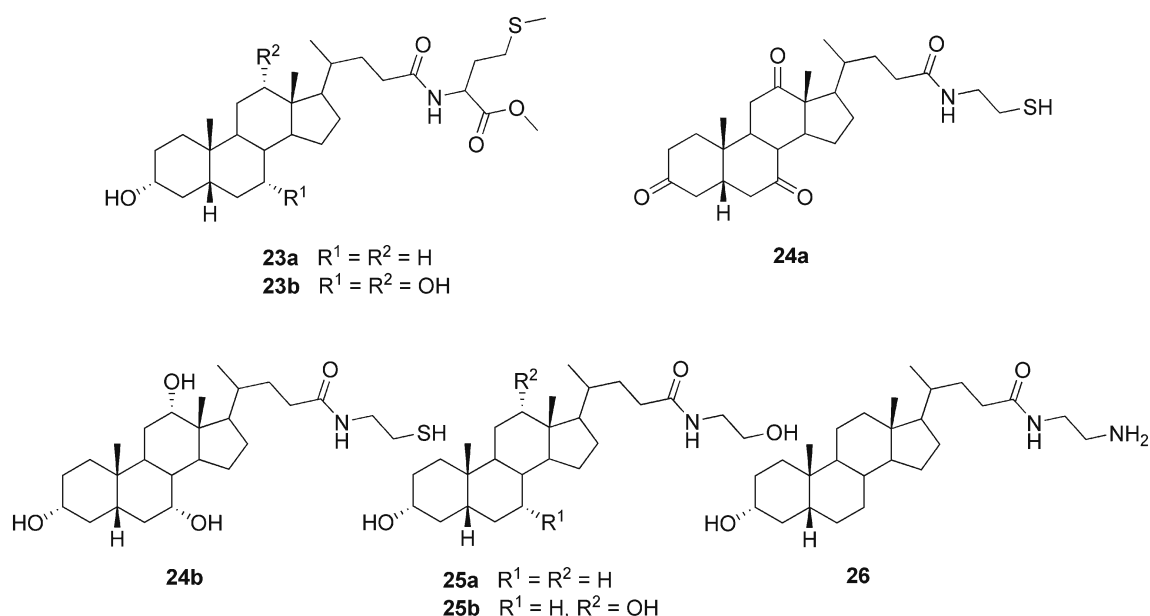


Fig. 16 Selected examples of bile acid-based organogelators prepared by us.

were evidently shown to be the most effective gelators being able to gelate the greatest number of solvents. Of the amidoamines studied, only the lithocholyl derivative **26** (Fig. 16) has shown to be able to undergo self-assembly leading to gelation. However, LCA-, DCA-, and CDCA-derived amidothiols were not shown to form any gels, except a partial gel of the lithocholyl derivative in benzene. It is assumed that this difference in the behavior of structurally rather similar compounds relates to the stronger hydrogen bonding capacity of the OH-group compared to the NH₂- and SH-groups, leading to stronger intergelator interactions. In the case of the studies on the amino- and dihydroxyalkyl amides of bile acids,⁶⁹ a correlation between the values of the Kamlet–Taft parameters and solvent preferences for gelators was observed. Moreover, the morphologies of the solid and gel structures studied by SEM were shown to vary from fibers to spherical microscale aggregates, the latter of which are unique among bile acid-based organogels. The gels were also shown to exhibit rather complex behavior, judging from the microscale diversity present in gelating and non-gelating systems.

Yet another recent example from our laboratory represents two alkylamide–phenylurea derivatives of deoxycholic acid with organogelation properties. The monomeric derivative was shown to form gels in CHCl₃ and chlorobenzene, whereas the dimeric derivative gelated THF and higher alkanols (1-heptanol, 1-octanol, 1-nonanol, and 1-decanol).⁷⁰

Organogels composed of other steroids

There are only a few examples of steroidal organogel systems based on steroids other than cholesterol or bile acids. Di Chenna *et al.* have reported the synthesis of a new pregnane derivative having a silyl ether group at C-3 combined with a 6 β ,19-oxo bridge (**27**, Fig. 17). Compound **27** was capable in gelating hydrocarbons and tetraethyl orthosilicate (TEOS) at very low concentrations (<1 wt%). The self-assembled fibrillar network was studied by FTIR, X-ray powder diffraction, CD spectroscopy, and microscopic techniques (SEM, TEM, and AFM). In addition, the ability of compound **27** to gelate TEOS was exploited in the preparation of SiO₂ nanotubes. The slow, catalyst-free polymerization of the TEOS gel of **27** resulted in fibers of SiO₂. After calcination, the SEM pictures showed hollow, straight fibers with a uniform inner diameter of about 7 nm and an external diameter comparable to that of the non-calcined material. The observations indicated that the organogel structure was successfully transcribed into the silica nanotubes by an *exo*-templating process.⁷¹

An interesting new family of estradiol-based low molecular weight gelators readily accessible using click-chemistry has recently been reported. Extensive structure–property studies for the family of gelators showed that the symmetry of the gelator molecules plays an important role in the gelation ability of the compounds. C₂-symmetric dimeric and tetrameric compounds (**28**, Fig. 17) gelated different organic solvents in the presence of water even at concentrations as low as 0.04 wt%, while their monomeric and C₃-symmetric analogues did not behave as gelators.⁷²

Stigmasterol belongs to the group of plant steroids. Its structure resembles that of cholesterol, but differs with respect

to the side chain. In our laboratory, three amino acid conjugates of stigmasterol, exploited in preparing stimuli-responsive, acid–base triggered reversible sol–gel transitions, as discussed later, have been prepared.⁷³ Another example of stigmasterol-based gels has been reported by Wimmer and co-workers. The compound consisting of stigmasterol and L-phenylalanine interconnected *via* short-chained dicarboxylic acyls by ester and amide bonds, respectively, was shown to gelate 1-octanol when cooled in the refrigerator.⁷⁴

Recently, cholesteryl and stigmasteryl derivatives containing tetraphenylethenes were synthesized.⁷⁵ Both molecules were almost non-luminescent in a solution, but became highly emissive in an aggregated state. Their photoluminescence spectra were measured in THF/water mixtures and it was found that higher water content induced a stronger light emission. This is probably due to a low water solubility of the compounds, which leads to a higher aggregation of the molecules. The stigmasteryl derivative **29** (Fig. 17) formed a gel in methanol in the presence of a few drops of THF (added due to the low gelator solubility in methanol). Under normal room light illumination, the organogel appeared white, but upon UV irradiation, it emitted an intense blue light, whose intensity was 18 times higher compared to that from a hot methanol solution (before the gel was formed).

Metallogels

One of the most rapidly developing areas of functional materials nowadays is metallogels.^{76,77} The incorporation of metal ions to gels brings new properties to the system. Examples are catalytic and redox activity, conductivity, luminescence, and magnetism, which significantly increase the attractiveness of these materials for potential applications as catalysts, electronic devices, sensors, *etc.* Since the ability of a steroidal unit to bind metal ions is limited, the incorporation of a suitable metal binding site is necessary. Metal ions can form part of the covalent structure of the gelator, or most often are bound by weaker coordination interactions. So far an unsolved problem is the reversibility of the gelation process.

One of the first examples of steroidal metallogels was reported by the Shinkai group several years ago.⁷⁸ They prepared a series of porphyrin-based gelators bearing a cholesterol moiety, where Zn(II) ions were irreversibly incorporated into the porphyrin unit, and studied their gelation properties in the presence and absence of [60]fullerene. π – π Stacking, hydrophobic interactions, and van der Waals forces were proven to be the leading forces for the self-assembly, whereas the contribution of metal–ligand interactions to the process was not significant.

Nowadays the research focuses more on situations, where the metal is incorporated into the system either to decrease or, as it is in most cases, to enhance the gel strength by increasing the density of the gelator network. As an example, Shinkai and co-workers have reported the first redox-responsive metallogel; a Cu(I) complex in which the ligand is a 2,2'-bipyridine derivative bearing two cholesteryl groups (compound **30**, Fig. 18).⁷⁹ They have shown that the temperature stability of the gel is highly dependent on the metal–ligand molecular ratio. As an optimal ratio, a 1 : 2 stoichiometry was determined. This is in good agreement with the expectable stoichiometry of the complex. Moreover they found that a thermochromic sol–gel transition in

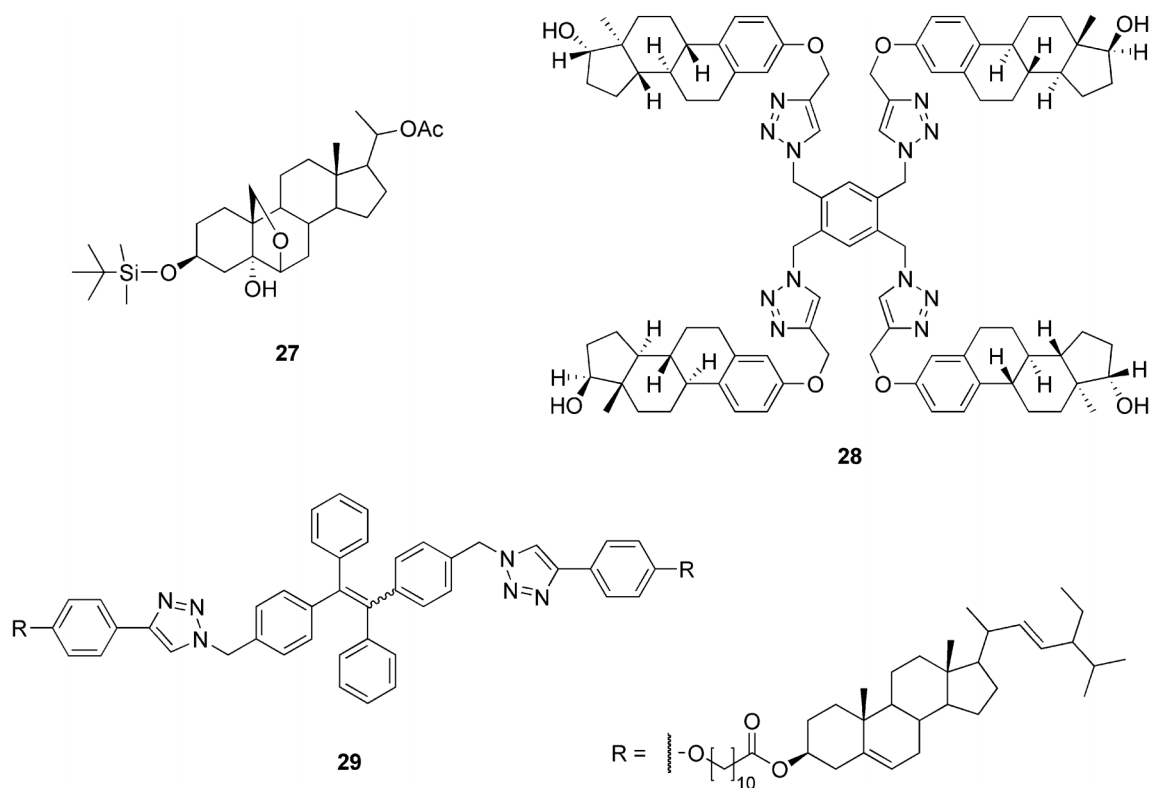


Fig. 17 Selected examples of organogelators based on steroids other than cholesterol or bile acids.

1-butyronitrile is accompanied with a color change, from reddish brown to greenish blue, which is untypical for Cu(I) complexes. As the phase-transition behavior could be reversibly repeated the possibility of the air oxidation of Cu(I) to Cu(II) was ruled out. It was concluded that the chromatic change in the complex was induced by the sol–gel phase transition, which is associated with the distortion of the coordination complex in the specific cholesteric gel fibril. They also reported the gel–sol transition of the complex based on the redox stimuli. When the oxidative agent was added to the Cu(I) complex and the mixture was heated, the deep green gel turned into the sol. This gel–sol transition could be reversibly induced by the addition of the oxidizing and reducing reagents, so it is evident that the redox state of the Cu ions plays a critical role in the stability of the gel system.

In another study, an enantioselective metallogel for a visual chiral recognition of BINAP, 2'-bis(diphenylphosphino)-1,1'-binaphthyl, was reported.⁸⁰ Compounds **31a** and **31b** (Fig. 19) were utilized as steroid-based gelators of the ALS-type. It was shown that π – π stacking of the heteroarene moiety

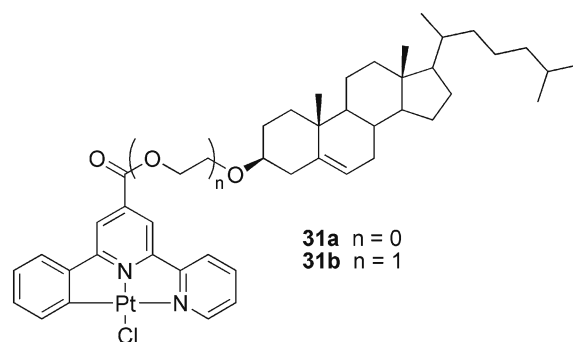


Fig. 19 Steroidal gelators for visual chiral recognition of BINAP.

and metal–metal bonding were key contributors to the gelation process, and that the replacing of the chloro ligand in the Pt complex to a bulky ligand could block the assembly process between the heteroarene rings inducing a collapse of the gel network. The theory was proven utilizing chiral BINAP as the bulky ligand. The addition of one equivalent of (*R*)- or

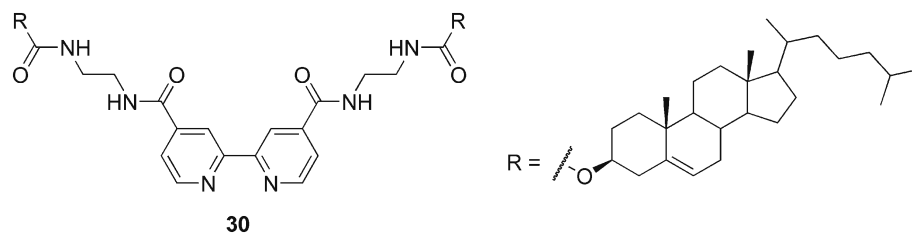


Fig. 18 Steroidal 2,2'-bipyridine ligand for preparation of redox-responsive metallogels.

(*S*)-BINAP to the stable gel with subsequent heating to reflux and cooling to room temperature resulted in a collapse of the metallogels. Surprisingly, when the amount of the chiral phosphine was reduced to 0.1 eq., a striking difference in the behavior of the respective gels was observed. Whereas the gel sample containing (*S*)-BINAP survived the heating and cooling sequence as a robust gel, the gel sample containing the (*R*)-BINAP enantiomer collapsed. Similar behavior was observed for other chiral phosphine ligands, demonstrating the potential use of the metallogels in a simple protocol for visual chiral recognition.

Recent development in the use of metallocene groups in the gelator synthesis was reported by Fang and co-workers. They studied the gelation abilities of cholesterol-appended ferrocene derivatives with glycine⁸¹ and diamines as linkers (compounds **32a–d**, Fig. 20)⁸² in various solvents. It was found that the critical gelation concentration of compound **32a** in cyclohexane was only 0.09% meaning that it is one of the best up-to-now reported metal-containing gelators. The gel was strong and could be moulded into a film, which was moreover bendable. Furthermore, some unusual redox and mechanical sol–gel phase transition phenomena were observed. These include, for example, a very rare heating-free sol–gel phase transition attained by a combination with a chemical oxidation and reduction reaction of the ferrocenyl residue accompanied by a typical color change from orange yellow to dark green, or a stress-triggered reversible sol–gel phase transition induced either by shaking or sonication.

Equivalent to the steroidal ferrocene derivatives are cholesterol-functionalized titanocenes.^{83,84} They form twisted fiber structures and are able to gelate a variety of solvents of different polarity due to the amphiphilic character of the compounds. For example, the steroidal titanocene complex **33** (Fig. 20) creates a gel at low concentrations, even less than 1 wt%, in toluene, benzene, ethyl acetate, and acetone. It was observed that the formation of the bundles and gels proceeded with some degree of stereoselectivity; the majority of fibers assembled in the left-handed *M*-helices, which induced supramolecular chirality in the gel network, as confirmed by CD spectroscopy.

One of the rarer examples, where the presence of the metal leads to a weaker gel was reported by Drašar and co-workers.⁸⁵ The phenantroline-containing ligand **34** (Fig. 21) created a gel on its own in a methanol–water mixture in low concentrations starting from 0.1%. Upon complexation of the gelator with Zn(II) in a 2 : 1 ratio, the gel formation required higher concentrations and the gel tended to break down over time to a low-viscosity liquid. It was shown that the gel could be

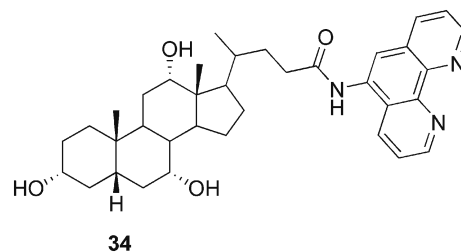


Fig. 21 Cholic acid-based phenantroline gelator.

reversibly reformed after a rapid heating to 70 °C and subsequent cooling.

Two-component gels

Two-component gel systems have recently attained ever increasing interest and have developed rapidly. The first attempts in this field were reported in the late 90s. They were mostly based on host–guest complex interactions, which could markedly change the gelation ability of the components as it was shown, for example, in the work of Shinkai and co-workers, who reported a cholesterol-based barbital receptor⁸⁶ and cholesterol-based crown-ammonium pseudo-rotaxane complexes.⁸⁷ Nowadays the field is more oriented to gel systems, whose gelation abilities can be finely tuned by adding other molecules to the system. Still mostly hydrogen bonds are utilized for the designing purposes due to their strength and directionality. In the “true” two-component gel systems, both components are essential for gel formation. However, several other examples, where one or both components can create a gel on their own but together induce formation of either a stronger gel or a gel with significantly different properties can be found as well.

In general, complicated multi-step syntheses leading to gelator molecules are not needed, because the gel formation can be reached by simple mixing of suitable ready-to-use components, as was reported, for example, by Bhattacharya and co-workers.⁸⁸ They demonstrated that a supramolecular gel was formed in aqueous solution using simple bile acids, such as lithocholic acid, in the presence of various dimeric and oligomeric amines. By carefully choosing the amines, the gelation properties of the mixtures could be modulated. Furthermore, varying the molar ratio of the two components provided an additional level of controlling the properties of the materials. Spectroscopic studies confirmed that the carboxylate and ammonium residues of the two components are involved in the salt (ion pair) formation,

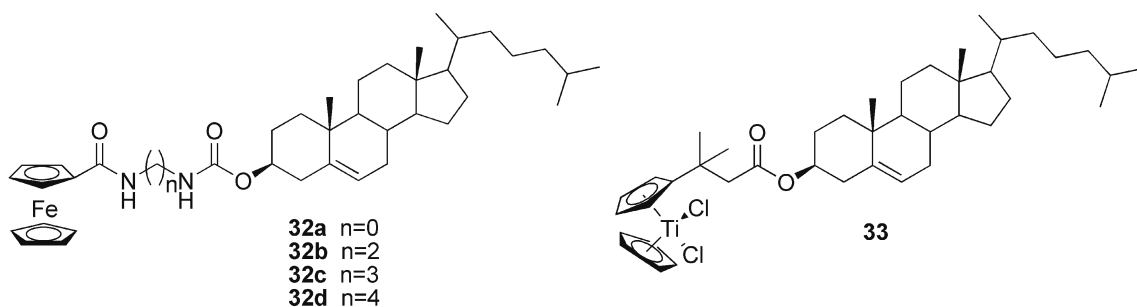


Fig. 20 Cholesterol-appended ferrocene and titanocene derivatives.

which promotes further self-assembly reinforced by a continuous hydrogen-bonded network leading to gel formation.

Another example of a simple two-component gel system was published by Raghavan and co-workers.⁸⁹ They reported sodium deoxycholate-assisted gel formation of a twin-tailed anionic surfactant, sodium bis(2-ethylhexyl) sulfosuccinate, in nonpolar solvents. The surfactant **35** (Fig. 22) is widely known to form spherical reverse micelles in a range of organic solvents. After adding the bile salt **36**, however, the situation was dramatically changed. It was observed that addition of even a trace of sodium deoxycholate to the micellar solution of the surfactant led to the formation of a transparent gel. It was proposed that the bile salt forms hydrogen bonds with the surfactant headgroups, transforming some of the spherical surfactant micelles into semiflexible filaments, which can entrap solvent molecules and create a gel structure.

A uracil-appended cholesteryl gelator **37** (Fig. 23) has been observed to form stable gels in polar organic solvents.^{90,91} Upon addition of the complementary polyadenylic acid **38**, the gel system was not only stabilized, but the gel created a helical structure. Based on the obtained results, it was concluded that the self-assembly mode of nucleobase-appended organogelators could be controlled by the addition of complementary polynucleotides and that the single-stranded polynucleotide templates could force

the gelator molecules to adopt the highly ordered structure through the complementary hydrogen-bonding interactions.

Jiang and co-workers have synthesized an ALS-type cholesterol-based molecule (compound **39**, Fig. 24), which by itself displayed no gelation ability.⁹² Interestingly, when 3-cholesteryloxycarbonylpropanoic acid (compound **40**) was added as a counterpart to the system, a gel was formed. The hydrogen-bonded complex (Fig. 24) displayed strong gelation ability in alcohols and aromatic solvents, and typical mesomorphic behavior of thermotropic liquid crystals.

The construction of two-component gels is possible also without utilizing hydrogen bonds as driving forces for the self-assembly process. It was found that cholesterol-based dinitrobenzoyl esters (Fig. 25) could gelate various organic solvents in the presence of a wide range of polyaromatic hydrocarbons, such as anthracene and its derivatives, due to donor-acceptor interactions and π - π stacking.⁹³ It was shown that both components were essential for gel formation, since the monomeric precursors themselves did not gelate any of the tested solvents.

Maitra and co-workers observed that the gelation ability of bile acid-pyrene derivatives was improved after adding a charge transfer agent, 2,4,7-trinitrofluorenone, to the system.^{94,95} Additionally, it was shown that the gelation properties could

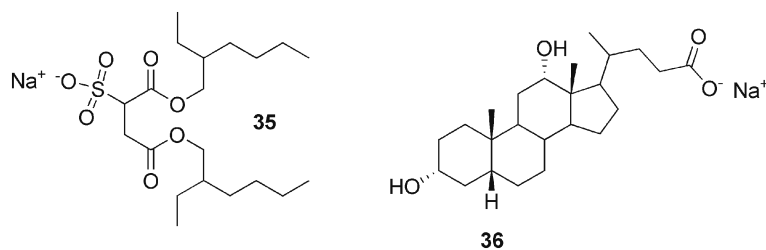


Fig. 22 Moieties of a simple two-component gel system: anionic surfactant and sodium deoxycholate.

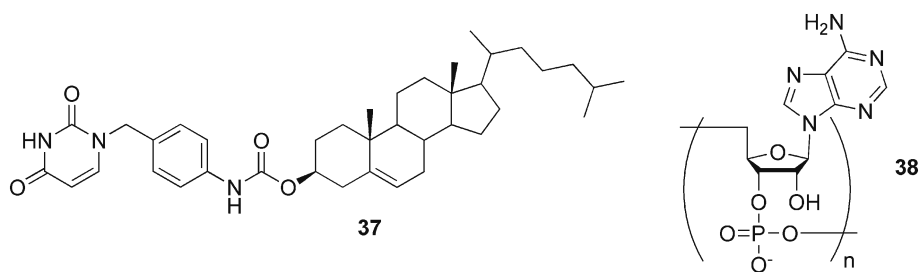


Fig. 23 A uracil-appended cholesteryl gelator and a complementary polyadenylic acid.

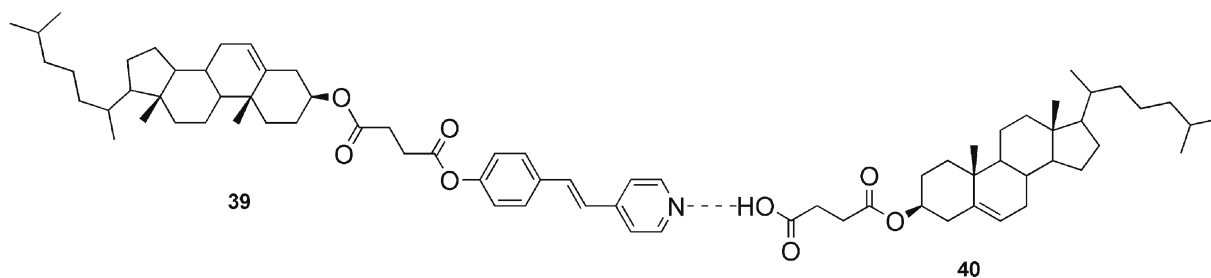


Fig. 24 Hydrogen-bonded complex formed by **39** and **40**.

be finely tuned by inserting different functional groups to the bile acid side chain.

Since the series of alkyl cholates **43a–c** (Fig. 26) prepared by Marcellis *et al.* did not gelate any of the tested solvents, they were then tested for use for improving the solubility of selected carbohydrates (isomannide **44** and isosorbide **45**) in organic solvents, such as hexane or octane.⁶³ Surprisingly, after mixing the components and a heating–cooling cycle, the solution turned into a thermoreversible opaque gel. It was found that the amount of carbohydrate added determined the gel stability. The optimum ratio of both components was close to 1 : 1. The lower concentration of alkyl cholate compared to the carbohydrate led to an incomplete dissolution of the latter.

In order to solubilize carbon nanotubes in water sodium deoxycholate and single-walled carbon nanotubes were mixed.⁹⁶ After ultrasonic treatment, a hydrogel was formed. The hydrogel exhibited excellent viscoelastic properties, *e.g.* it could be extended 50-fold along the direction of additional stretching force. Moreover, it could be used as a “solid” ink in preparing nanowires and nanopatterns. The conductivity of the nanowires was studied in order to examine the potential applications of the material in electronics.

Combining a cholesterol-based gelator **46** (Fig. 27) with Zn(II)–phthalocyanine-containing complementary steroidal structures **47** or **48** led to the formation of thermoreversible organogels.⁹⁷ The chosen cholesterol-based gelator **46** is known to gelate alkanes and alkanols, but not aromatic solvents. Nevertheless, stable photoactive gels in various aromatic solvents were obtained, when the compound and a Zn(II)–phthalocyanine component were mixed in a molar ratio of 20 : 1. Unfortunately, the prepared gels were stable only for a few weeks. In order to improve their temporal and thermal stabilities, the gels were prepared in the presence of tiny amounts (5 mol%) of suitable complementary diacetylene **49**, and diazides **50** and **51**, appropriate for “click”-reactions and able to, at least partially, integrate into the gel structures. All of the prepared

cross-linked gels displayed better thermoreversibility and stability over time.

Stimuli-responsive gels

Although there still is a deep interest towards the synthesis of new gelators in general, in the last few years more and more attention has been given to the design of responsive soft materials.^{3,5,98,99} These materials are characterized by a change in their properties in response to a specific physical (temperature, mechanical stress, light, *etc.*) or chemical (pH, ions, oxidation state, *etc.*) stimulus. The most common response of the systems is a transition from solution to gel (or *vice versa*). Other responses include changes in chemical or physical properties, such as conductivity, color, or light emission. Stimuli-responsive gels have a great potential for designing and constructing new functional materials, such as sensors, actuators, molecular devices, *etc.*^{1,2}

Physical stimuli

Temperature and mechanical stress. Temperature changes and mechanical stress are examples of physical stimuli, which are commonly used to test gel properties. Since the association of the fibrous network of the supramolecular gel system is usually enthalpy-driven and an increase in temperature shifts the equilibrium to the non-aggregated state, most gel systems react to temperature changes by a gel–sol phase transition. The temperature at which the gel turns to solution is called the gelation temperature, T_{gel} , and it is often used to describe the properties of the gel system. Since most of the gel systems are responsive to thermal stimuli, we will not emphasize them further in this article. Mechanical stress, another common physical stimulus, can deform or destroy a gel depending on the viscoelastic properties and the magnitude of the applied stress. Many gel systems are sensitive to the stress and if the

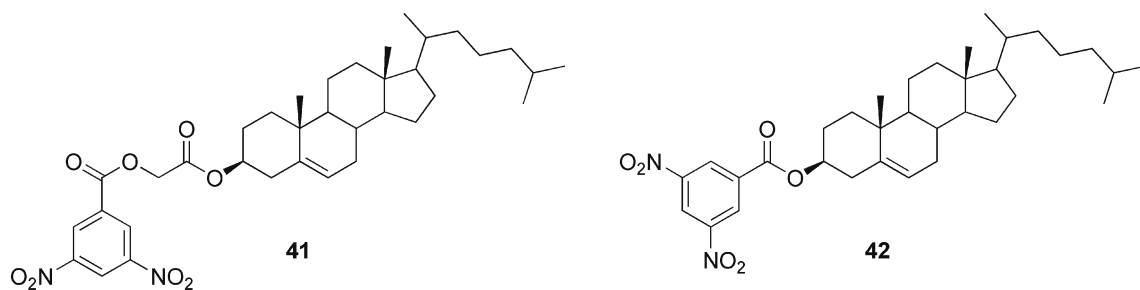


Fig. 25 Cholesterol-based dinitrobenzoyl esters.

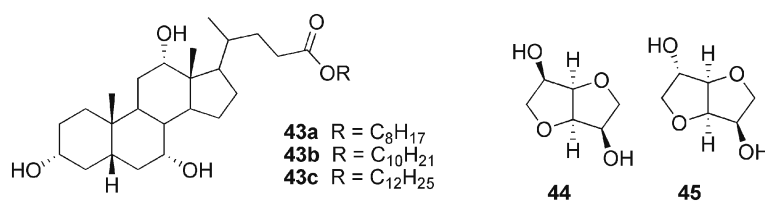


Fig. 26 Selected alkyl cholates **43a–c** and carbohydrates (**44**, **45**) used for forming two-component gels.

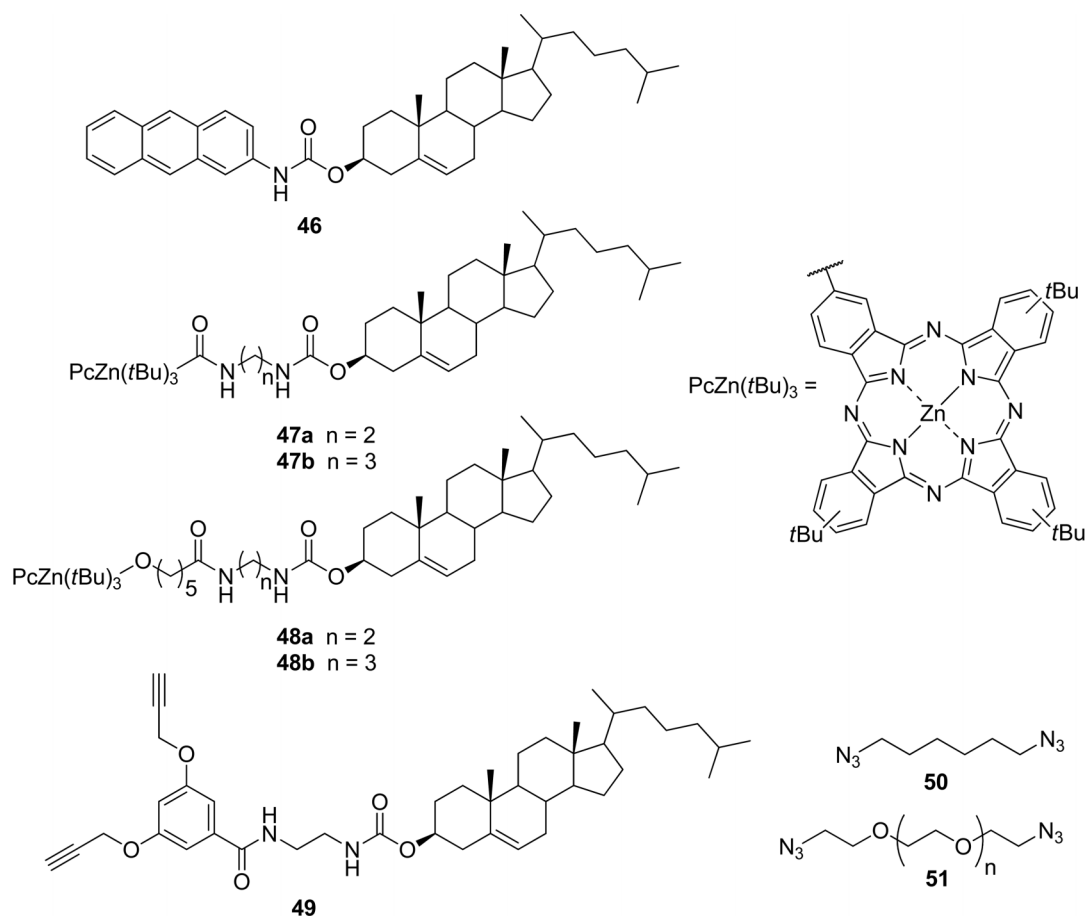


Fig. 27 Moieties of two-component gel systems.

stimulus is applied, the gel network breaks down to become a viscous fluid. For most systems, the gel state can be restored only by a heating-cooling cycle through the gel-sol-gel phase transitions. However, some of the gels are thixotropic, which means that the gel state is spontaneously restored if the stress is removed.⁹⁸

Recently, it was reported that ultrasound could act as a trigger for the instant gelation of organic solvents by certain cholesterol-based compounds.^{50–52} The use of ultrasonic irradiation in order to reversibly control aggregation of asymmetric cholesterol-based fluorescent gelators was studied. According to the results, it was suggested that the sonication-switch phenomenon could only happen in a compound with two hydrogen bonding sites, through a competition of intra- and intermolecular hydrogen bonds, as well as hydrophobic interactions. The location of the hydrogen bonds (determined by the lengths of the alkyl chains) had a strong effect on the solubility and gelling properties of the compounds. The organogelator **52** (Fig. 28) formed gels only upon ultrasonic treatment, while the other external stimuli, such as quick heating or cooling, did not initiate aggregation. Compounds **53a–c** and **54**, for one, could form gels either upon sonication or upon a heating-cooling cycle (though higher concentrations were needed for a successful gel formation). Interestingly, differences in the morphology of the xerogels were observed. Generally, the morphology was strongly dependent on the solvents and external stimuli.

However, some trends could be observed; in most cases a thermal process afforded hollow spherical motifs, while ultrasound irradiation resulted in the spontaneous formation of the intermolecular hydrogen bonds and thus aggregation-induced helical motifs.

Light stimuli. Photo-responsive gels represent a special case of physically responsive gel systems. A physical stimulus (photons) can invoke a photochemical reaction, which transforms the gelator molecule to a different species with its own characteristic gelation properties, or induces a color change of the system. The photon absorption is a very attractive way to change the gel properties, because it allows a photo-responsive group incorporated into the gel to be addressed selectively. The response of the gel depends on several factors, such as a structural level at which the photo-responsive group is incorporated within the gel as well as the chemical changes that are invoked by the photochemical reaction. A problem related to potential applications might be the possible lack of reversibility of the process.⁹⁸ One of the first examples in this field was reported by Shinkai and co-workers several years ago.¹⁰⁰ They synthesized a series of cholesterol-based gelators containing an azobenzene moiety. It was observed that UV irradiation of the gel system led to *cis-trans* isomerization of the azobenzene unit, turning the gel into a solution. It was also shown that the gel could be re-formed by visible light irradiation of the sol (Scheme 1).

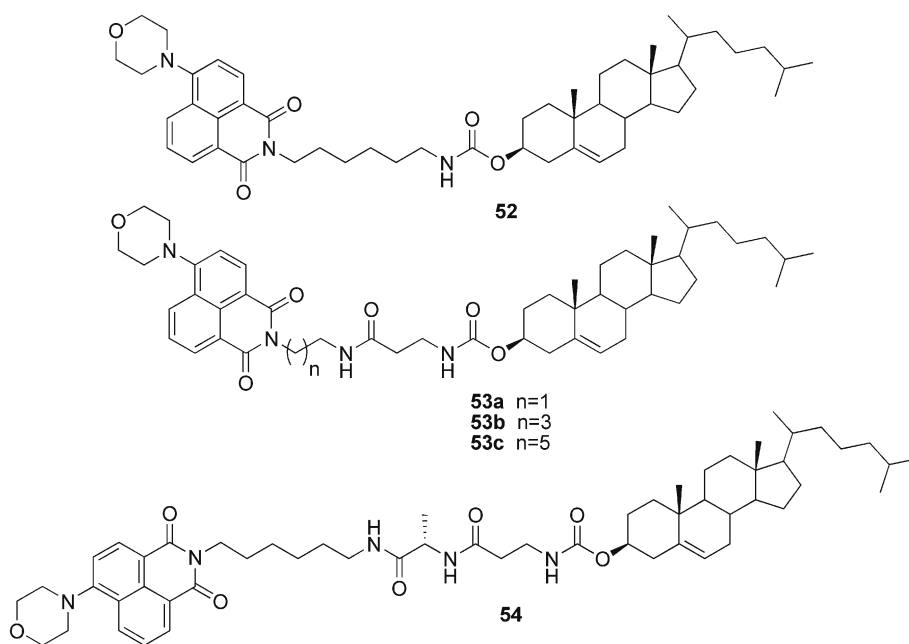


Fig. 28 Ultrasound-responsive cholesterol-based fluorescent gelators.

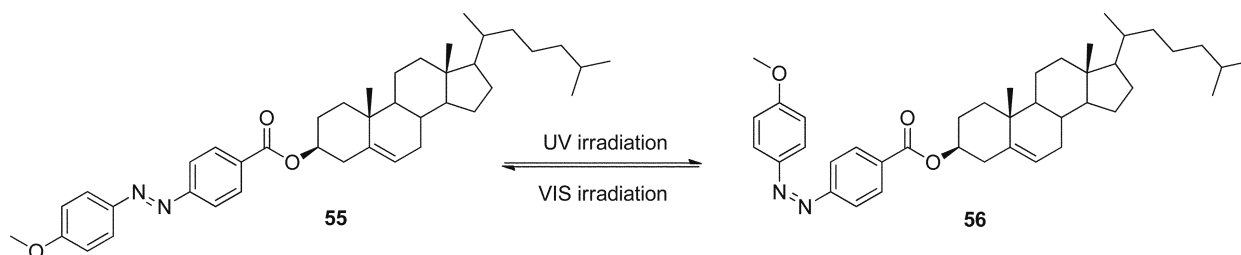
Tian and co-workers synthesized a gelator **57** (Scheme 2) containing bithienylethene-bridged fluorescent naphthalimide units bearing two cholesteryl groups.¹⁰¹ It was shown that the color of the gel system can be turned from yellow to red after absorption of UV irradiation and back after absorption of visible light (Scheme 2). The cycle could be repeated more than 10 times and the gel phase remained stable at room temperature. A difference in luminescence spectra between these two photochromic states was observed. In addition, the system was sensitive to fluoride ions in solution. This makes it a promising fluorescent molecular switch activated by fluoride ions and protons (Fig. 29).

Chemical stimuli

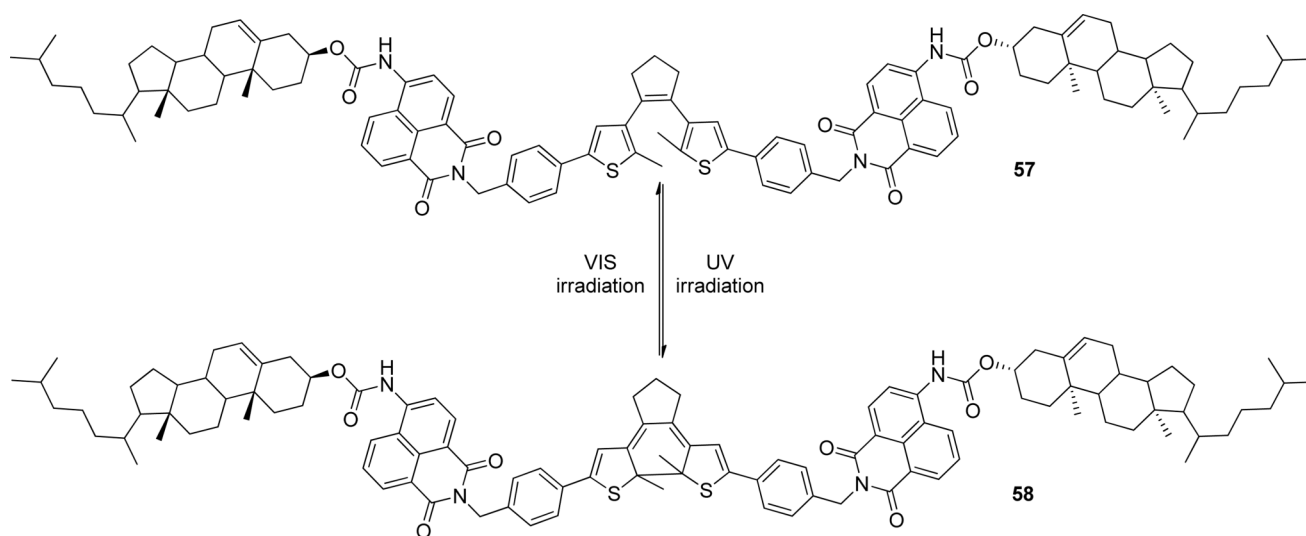
pH and ion stimuli. The design and synthesis of steroid-based gelators responsive to chemical stimuli require an integration of appropriate responsive functionalities into the gelator molecules. Incorporation of suitable amino acid units into a gelator structure, for example, may lead to pH-responsivity of the systems. Recently, we reported the synthesis of stigmasterol–amino acid-based conjugates,⁷³ whose hydrochloride salts acted as gelators. Their neutral conjugates, however, were either non-gelators or formed only weak gels. The reversibility of the “acid–base” triggered

sol–gel process by alternating addition of HCl (as a gas or in solution) and triethylamine, and the repeatability of the acid/base cycle was demonstrated. Moreover, the gelators and gels were characterized utilizing solid state NMR measurements, a non-commonly used technique in gel research. The same phenomenon was observed utilizing cholesterol–amino acid conjugates,¹⁰² which were found to be better gelators as hydrochlorides than as neutral molecules. The influence of chirality of the amino acid unit on the gelation process was investigated.

The Shinkai group showed that a 1,10-phenanthroline-appended cholesterol-based gelator **59** (Fig. 30) created gels in alcohols, polar aprotic solvents, organic acids, and triethylamine.¹⁰³ In fluorescence measurements, most gels afforded an emission maximum at 394 nm (purple emission), whereas only the acetic acid gel afforded an emission maximum at 522 nm (yellow emission). Interestingly, upon addition of acetic acid to the gel of **59**/1-propanol, yellow emission was observed suggesting that the protonation of the 1,10-phenanthroline nitrogen in the gel state has a big influence on the fluorescence properties of the system. The fluorescence intensity of $59\cdot\text{H}^+$ became particularly strong in the gel, presumably due to the energy transfer from neutral 59^* to protonated $59\cdot\text{H}^+$ and the restriction of the $59\cdot\text{H}^+$ molecular motion in the gel phase.



Scheme 1 *Cis*–*trans* photoisomerization of the azobenzene moiety of a cholesterol derivative.



Scheme 2 Structure and photochromic process of bisthienylethene-bridged cholesteryl derivative.

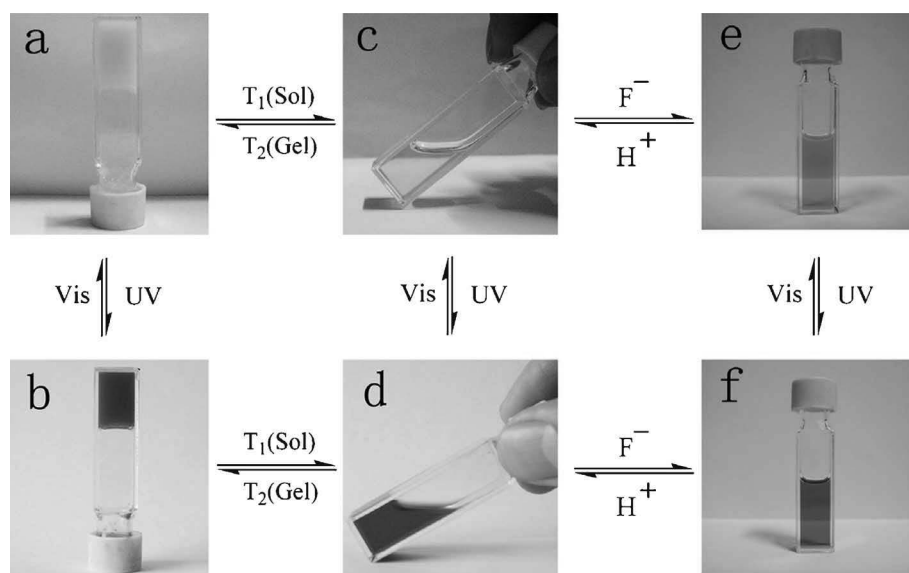


Fig. 29 Multiple switching images of bisthienylethene-bridged cholesteryl derivative under the cooperative effects of light, temperature, fluoride anions, and protons. (a) Gel of **57**; (b) gel of **58**; (c) sol of **57**; (d) sol of **58**; (e) sol of **57** + F⁻; (f) sol of **58** + F⁻.¹⁰¹ Reproduced by permission of the Royal Society of Chemistry.

Fang and co-workers prepared three ammonium salts of diacid monoamides of cholesteryl glycinate (compounds **10a–c**, Fig. 7) and studied their gelation behavior aiming at controlling it.²⁸ It was found that the acids formed only weak gels, but neutralization with ammonia significantly enhanced their gelation ability. The amino salt of the succinic acid derivative behaved as an ambidextrous gelator (gelating both apolar solvents and water) suggesting two different ways of self-assembly, hydrophobic surface-mediated in apolar and hydrophilic surface-mediated in polar solvents. More interestingly, some of the alkyl alcohols and water could be gelatinized at room temperature simply by bubbling ammonia through the system. This could be applied in the design of ammonia sensors.

Maitra and co-workers observed two bile acid-derived molecules containing basic amino groups (Fig. 31) to act as

efficient gelators for organic and aqueous solvents.¹⁰⁴ The organogelator **60** was found to be a non-gelator in its neutral form, whereas, as its iodide salt, it formed a strong gel in 1,2-dichlorobenzene and chlorobenzene at very low concentrations (0.05% w/v). To illustrate the acid–base switching of the gel, a simple experiment was performed. Upon exposure to ammonia vapour, the gel transformed to a solution. Upon exposure to HI vapour, on the other hand, the gel was re-formed. However, for the hydrogel derived from compound **61**, the situation was reversed. The neutral amine formed a gel in 1 : 1 DMSO/water (0.5% w/v), whereas exposure to HI vapour disrupted the gel framework.

As mentioned before, quinuclidine-grafted cationic bile salt **3** (Fig. 3) has been found to form a hydrogel.¹⁰⁵ For this system, the influence of electrolytes and counter-ions on the rheological

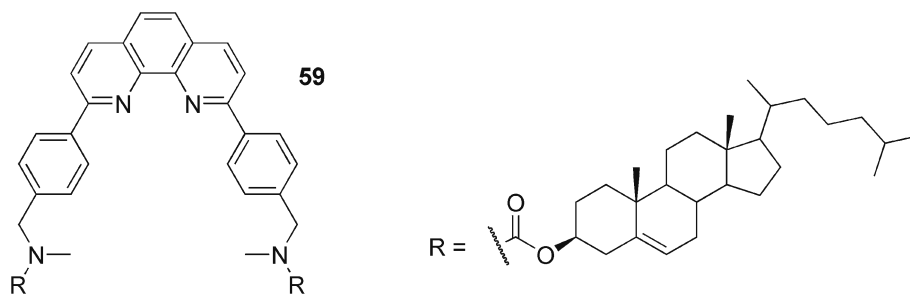


Fig. 30 Proton-sensitive fluorescent 1,10-phenanthroline-appended cholesterol-based gelator.

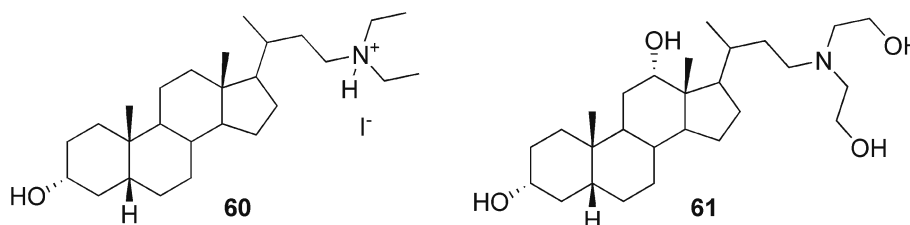


Fig. 31 Bile acid-based pH-responsive gelators.

properties of the gel was investigated. The concentration of the electrolyte was shown to produce a dramatic effect on the gel stability. Increase in the added salt content led to weaker gels. Moreover, the fibrillar network of the conjugate with iodide as a counter-ion appeared to be more sensitive to the added salt compared to the conjugates with other counter-ions.

Similarly to the pH-responsive systems, there is a rapidly growing interest in the incorporation of anions into gels and towards their use in tuning and switching the gel behavior. Examples of the anion-triggered gel–sol transitions as well as of the opposite situations, where the presence of an anion leads to a gel formation, are known.

Lee and co-workers, for example, have reported a fluoride-responsive gel system.¹⁰⁶ They synthesized an organogelator **62** (Fig. 32) containing a cholesterol unit with a fluorophore, 2-(2-hydroxyphenyl)benzoxazole, linked *via* an amide group. The compound created fluorescent supramolecular gels in mixtures of cyclohexanone and cyclohexane or cyclohexane and *p*-xylene. Based on the role of intramolecular hydrogen bonds in the fluorophore, they suggested that addition of fluoride ions, which are known to interact with the N–H bond in the amide group or to deprotonate it, could have an impact on the gelation process. Upon addition of tetrabutyl ammonium fluoride, they observed a rapid transition from an opaque gel to a homogenous solution with an altered fluorescence color. Moreover, the change was

irreversible. They suggested that the gel–sol transition was caused by the change in inter- and intramolecular hydrogen bonding patterns due to newly established interactions by the added fluoride anions.

On the contrary, Pandey and co-workers reported a bile acid-based imidazolium anion-receptor **63** (Fig. 33), which formed a stable gel in a $\text{CHCl}_3/\text{DMSO}$ mixture only in the presence of anions.¹⁰⁷ They observed that among the tested anions (F^- , Cl^- , Br^- , AcO^- , H_2PO_4^- , and HSO_4^-) only the HSO_4^- ion was effective in inducing the gel formation. The system could thus be used for a selective naked-eye detection of hydrogen sulfate ions.

Redox stimuli. Metals incorporated covalently or non-covalently can be used as active centres for redox reactions, as we already described in the metallogel chapter of this article. Redox-responsive gel systems that do not contain metals have also been reported. Shinkai and co-workers, for example, have synthesized a series of quater-, quinque-, and sexithiophene derivatives bearing two cholesteryl moieties at the α -positions (compounds **64a–c**, Fig. 34).¹⁰⁸ It was found that these oligothiophene steroidal derivatives acted as excellent organogelators for various organic solvents and showed unique thermochromic behavior through the sol–gel phase transition. It was also described that the sol–gel phase transition could be implemented by an addition of oxidizing (FeCl_3) and reducing (ascorbic acid) reagents simply

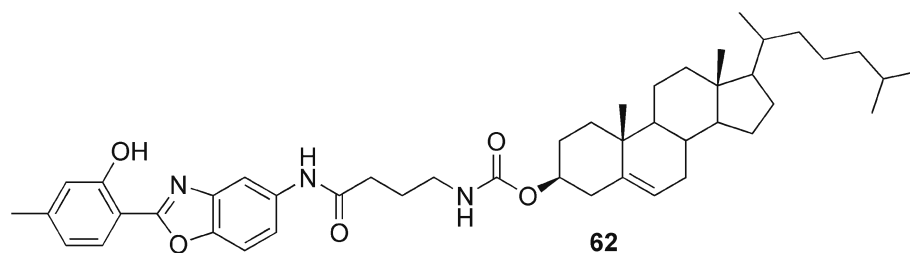


Fig. 32 A fluoride-responsive organogelator.

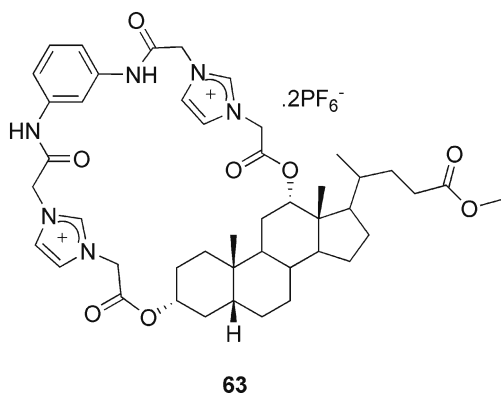


Fig. 33 A bile acid-based imidazolium anion-receptor.

without any heating-cooling process, making these soft materials attractive for applications in electro- and photochemistry.

Recently, the synthesis of a redox active tetrathiafulvalene derivative bearing two cholesteryl units was reported.¹⁰⁹ Compound **65** (Fig. 34) was found to create a gel in *n*-hexane after heating and ultrasonic treatment. It was demonstrated that besides heating, the gel-sol transition occurred upon oxidation of the tetrathiafulvalene unit by I_2 , making the organogel redox-responsive. The reversibility of the process was not studied.

Another example utilizing redox properties of a tetrathiafulvalene moiety was reported by Stoddart and co-workers.¹¹⁰ They synthesized a rotaxane **67** (Fig. 35) with tetrathiafulvalene and 1,5-dioxynaphthalene recognition units situated in the rod and with cholesterol units as stoppers, and investigated its gelation behavior and switching properties. They demonstrated that rotaxane **67** as well as its precursor **66** formed organogels in a $CH_2Cl_2/MeOH$ (3 : 2) mixture and could be liquified by adding an oxidizing agent $[Fe(ClO_4)_3]$. Moreover, they showed that the cholesterol stoppers were essential for the self-aggregation process and suggested that the same design could be used as a general strategy for introducing gelation properties to mechanically interlocked molecules.

Multistimuli. Yet another interesting approach to increase the attractiveness and applicability of gels as functional materials is

to incorporate several moieties responsive to different stimuli to the gelator structure. As an example, a reversible multistimuli-responsive steroidal organogelator with incorporated electroactive and photo-responsive groups was reported (compound **68**, Fig. 36).¹¹¹ It was demonstrated that by manipulating the redox state of the electroactive tetrathiafulvalene group, the gel-sol transition could be reversibly tuned by either chemical or electrochemical oxidation/reduction reactions. Alternatively, the *cis-trans* photoisomerization of the photoresponsive azobenzene group could trigger the gel-sol transition. Therefore, the reported gel system can be considered as a reversible three-stimuli-responsive material, namely thermal, redox, and light.

Recently, the cholesterol-based perylene derivative **19** (discussed in the organogel part of this review, Fig. 13) was used in a mixture with oligothiophene-bridged cholesteryl derivatives **64a** and **64c** (discussed in the redox stimuli part of this review, Fig. 34) to prepare a self-sorting photoactive gel system (Fig. 37).¹¹² The components act as opposite charge carriers of the n-type (electron poor perylene derivative) and p-type (electron rich oligothiophene derivatives), respectively. Because of the structural similarities of the molecules, they could form gels in the same organic solvents, *e.g.* in chlorobenzene. On the other hand, their molecular lengths are different and also the number of hydrogen bonding sites is different (two for the perylene derivative, and four for the oligothiophene derivatives). Upon mixing the components in chlorobenzene and after a heating and cooling cycle, gel formation was observed. The gelation process was monitored by variable temperature UV-VIS spectroscopy. The absorption spectrum of the mixed gel showed no difference to the combined spectra of the components. This indicates an absence of interactions between the oligothiophene and perylene moieties. Interestingly, also the dissociation temperatures of the gelators in the mixed gel matched with those of the individual ones, suggesting that a self-sorting gel system was formed. A fluorescence measurement of the mixed gel of **19** and **64c** showed the same λ_{max} (600 nm) compared to the spectrum of **64c**, but the maximum was partly quenched (~63%), indicating an electron and/or energy transfer between the two components. In another experiment, photo-current measurements were carried out. Upon photo-irradiation of the

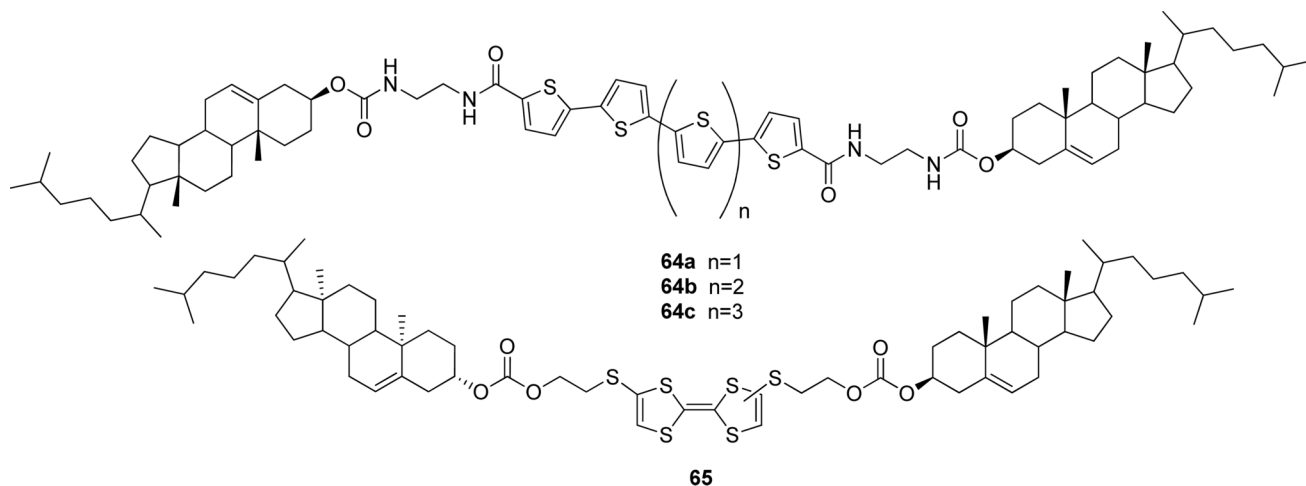


Fig. 34 Oligothiophene- (**64a-c**) and tetrathiafulvalene-appended (**65**) redox-responsive cholesterol-based gelators.

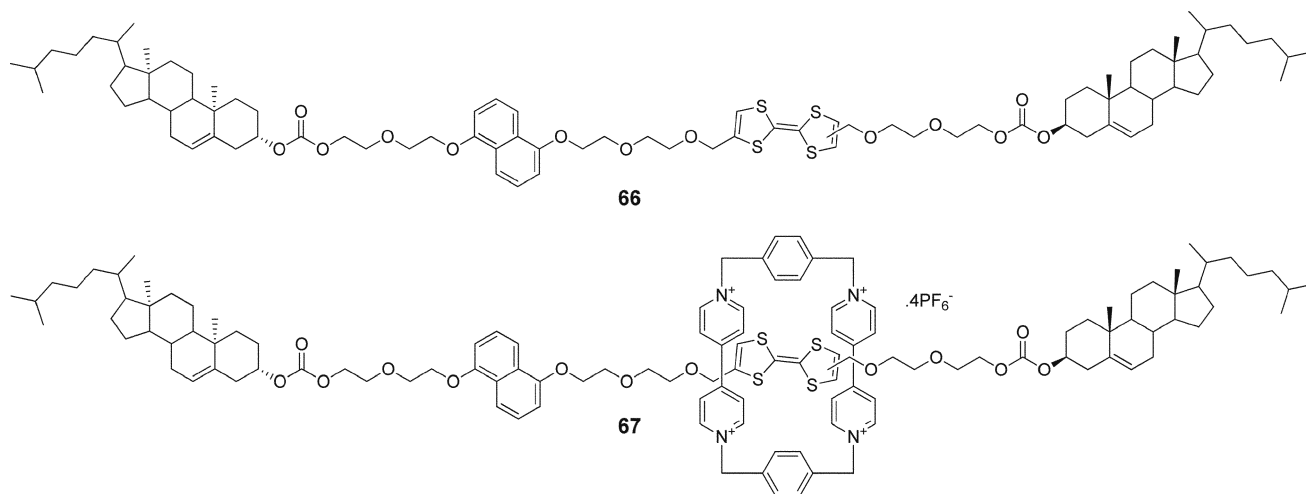


Fig. 35 The steroidal rotaxane **67** with tetrathiafulvalene and 1,5-dioxynaphthalene recognition units in the rod and its precursor **66**.

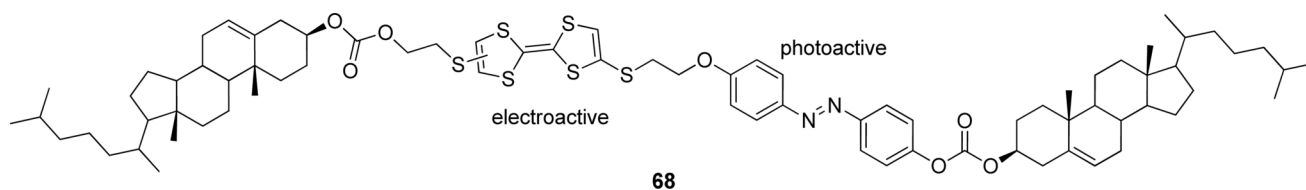


Fig. 36 Electro- and photo-responsive steroidal organogelator.

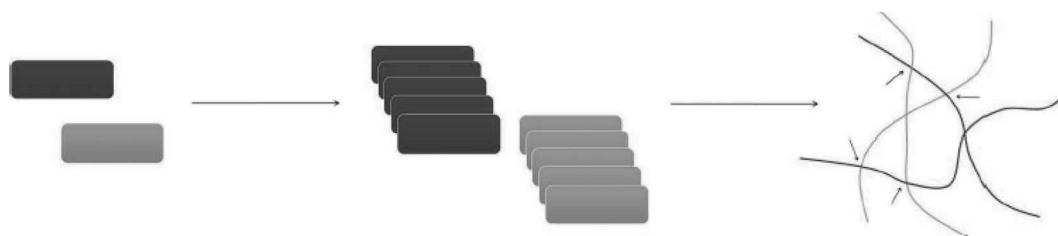


Fig. 37 A schematic representation of a self-sorting assembly of oligothiophene (orange) and perylene (red) derivatives (**19** and **64c**, respectively) with p-n heterojunction nodes (black arrows).

gel film on a working electrode, an anodic photo-current was generated. This photo-responsive phenomenon could be reversibly repeated many times, and the film was sufficiently robust. Fluorescence spectra showed that both the thiophene and the perylene components acted as photoactive species for the photo-current generation.

Conclusions and future prospects

The unique chemical and physical properties of steroids combined with their availability and low cost have raised them among significant building blocks when designing new soft materials. This article summarizes the latest developments within the field of steroid-based supramolecular gels, most of which are derived from cholesterol or bile acids – pregnane, estradiol, and stigmasterol being the few exceptions. The myriad of applications of supramolecular gels derived from steroids include their use as templates for syntheses, hybrid materials, sensing and responsive materials, media for selective reactions, and light-harvesting systems. Due to the endogeneity of cholesterol, bile

acids, and steroid hormones, materials based on them are promising from the biomedical and pharmacological point of views.

Nowadays, physical – or supramolecular – gels belong to one of the most rapidly developing groups of materials. An ever increasing expansion in the field of targeted design of functional and tunable systems is expected in the future. With respect to steroid-based supramolecular gels in particular these might, in our opinion, relate to the exploitation of the chiral gel network as a template in reactions, such as photochemical as well as asymmetric transformations, and as a crystallization medium, not forgetting the wide-ranging applications of stimuli-responsive systems. Biomaterials based on steroidal supramolecular hydrogels may find use in biomedicine, drug delivery, regenerative medicine, and tissue engineering. The ability of the gel forming systems to act in molecular recognition processes will undoubtedly be capitalized upon. Moreover, the amount of hybrid materials containing steroid-based supramolecular gel component(s) for applications in optics, electronics, ionic liquids, mechanics, biology, and catalysis is expected to increase. The use

of steroidal components other than bile acids or cholesterol in supramolecular gel systems opens fascinating opportunities. Finally, the move from potential to active applications is to be expected.

Although the research of low molecular weight gelators and supramolecular gels has experienced extensive development during the last decade or so, several topics remain to be addressed. Undoubtedly, one of the most pivotal problems is the lack of sufficient methods for studying the gel systems in detail at the molecular level. The development of these methods will increase our understanding related to the detailed mechanism of the gel formation, ultimately enabling the rational design of gelator molecules combined with the ability to predict the properties of the forming gel systems. Moreover, the practical challenges, like the stability of the supramolecular gels in a prolonged use, enhancement of the mechanical strength of the systems, and their response to a multi-enzyme environment encountered in the human cells if used in biomedical applications are still to be solved.

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II

Design, synthesis and stimuli responsive gelation of novel stigmasterol-amino acid conjugates

by

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Design, synthesis and stimuli responsive gelation of novel stigmasterol–amino acid conjugates

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ABSTRACT

An efficient synthesis of three novel stigmasterol–amino acid (glycine, L-leucine and L-phenylalanine) conjugates as stimuli responsive gelators is reported. The gelation properties of the prepared compounds were investigated in a variety of organic as well as aqueous solvents. The most striking finding of our investigation was that the hydrochloride salts of the prepared conjugates acted as gelators, whereas the neutral conjugates were either non-gelators or formed only a weak gel in anisole. The hydrochloride salts of stigmasteryl glycinate and L-leucinate form gels in *n*-alcohols (*n* = 4–10) and in ethane-1,2-diol, and that of stigmasteryl L-phenylalaninate forms gels in aromatic solvents and in tetrachloromethane. These unique properties of the gelators were explored to prepare stimuli responsive, “acid–base” triggered reversible sol–gel transitions. The gelators and their gels were characterized by liquid and solid-state NMR as well as FT-IR. The morphology of their corresponding xerogels was investigated by SEM.

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1. Introduction

The last few years showed an enormous increase of interest in design and synthesis of new low-molecular-mass organic gelators (LMOGs) [1–7]. The structure of the gelators varies from simple alkanes [8] to large dendritic conjugates [9]. In suitable solvents, molecules of LMOGs create entangled supramolecular networks by self-assembly of the monomeric species to higher-ordered structures, such as fibers, tapes, strands, and ribbons. The driving forces of such self-assembling processes are usually non-covalent interactions, such as hydrogen bonding, π – π stacking, van der Waals and dipole–dipole interactions, and solvophobicity [10,11]. A great deal of LMOG research has been done not only for probing the relationship of the structure of the gelator and properties of the gel but also for exploiting their potential applications as sensors, shape memory devices, controlled drug delivery, optoelectronics, displays etc. [12–16].

The major classes of mono-component gelators published in the literature include derivatives of long-chain alkanes, fatty acids, carbohydrates, amino acids, steroids, anthryl and organometallic derivatives [6]. In the past there have been reported several steroid–amino acid conjugates which were shown as effective organogelators. However, in most of these cases either bile acids [17,18] or cholesterol [19–23] have been utilized as the steroidal unit. In

this work we used the less common stigmasterol. Stigmasterol (**1**) belongs to a group of naturally occurring steroidal compounds which are called phytosterols (plant sterols) [24]. Its structure resembles cholesterol (**2**), but has a different side-chain configuration (Fig. 1). Stigmasterol (**1**) has a rigid steroidal skeleton, several stereogenic centers, and a strong tendency to form aggregates via van der Waals interaction. Moreover stigmasterol (**1**) is also an interesting molecule from pharmacological point of view. Recently it gained considerable attention in the field of medicine as anti-stiffness factor in the rheumatic disease therapy and as a potential antiosteoarthritic agent [25]. In this work, we prepared three stigmasterol conjugates comprising different amino acids: glycine, as an amino acid without a side chain, leucine, as an example of an amino acid with a hydrophobic side chain, and phenylalanine, as an example of an amino acid with an aromatic side chain. To the best of our knowledge, there has been no work presenting stigmasterol–amino acid conjugates as potential gelators and therefore by this work, we want to contribute to the extending of phytosterols usage for gel preparation.

During the course of our investigation on the structure properties of these compounds we encountered the gelation of some of them. This prompted us to scrutinize the self-assembly of these conjugates in detail. We tested their gelation abilities in 22 different organic solvents and also in water. The most striking findings of our investigation have been the dramatic change in the gelation properties of the conjugates when they are converted into their hydrochloride salts. While the neutral conjugates showed either the formation of a weak gel in anisole or to be non-gelators, the

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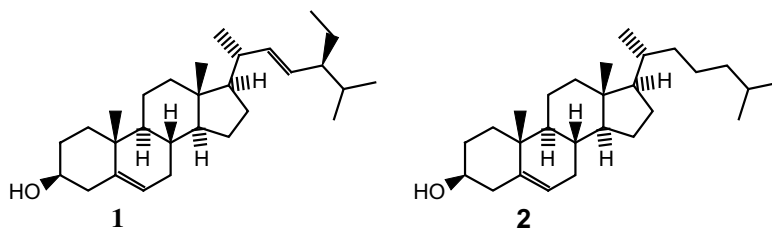


Fig. 1. Chemical structure of stigmasterol (1) and cholesterol (2).

respective hydrochlorides gelled a number of organic solvents studied. Stimuli responsive systems are one of the key areas in supramolecular gels because of a wide spectrum of their potential uses, ranging from medicine to material science [12–16]. Though a number of steroidal gelators reported in the literature have been prepared, this is one of the few examples showing stimuli responsive reversible gelation [26–28]. The non-gelators (neutral analogues) have been tested for its ability to act as a sensor for gaseous hydrogen chloride. The acid–base triggered reversible sol–gel transition has been demonstrated and the versatility of these gelators is presented. The properties of prepared solids, xerogels and gels were studied *via* up to date methods and especially with solid state NMR.

2. Materials and methods

2.1. Synthesis

The syntheses of stigmasterol-amino acid conjugates (Scheme 1) were achieved employing a classic peptide coupling procedure using DCC and DMAP [29] in good yields (81–88% after purification). The Fmoc-protecting group was removed using 20% solution of piperidine in DMF (from 96% to quantitative yield). All prepared compounds were characterized by standard analytic methods (^1H and ^{13}C NMR, MS, IR and EA).

2.2. Methods

2.2.1. Gelation tests

In a typical gelation test a weighed amount of the gelator was mixed with a measured volume of selected solvent in a sealed 5 mL test tube. The sample was sonicated for *ca.* 2–3 min and then

the mixture was heated until the solid was completely dissolved (if soluble). The resulting solution was allowed to slowly cool down to room temperature. Finally the test tubes were inverted to observe if the solution inside could still flow. Upon cooling down, the formation of gel, precipitate (P), solution (S) or emulsion (E) was detected. Three different types of gels were observed regarding light transmission: clear (CG), translucent (TG) and opaque (OG). Minimum gel concentration was determined by scaling a minimum amount of gelator needed for the formation of a stable gel. The gel-melting temperature (T_{gel}) was measured using the “inversion tube” method.

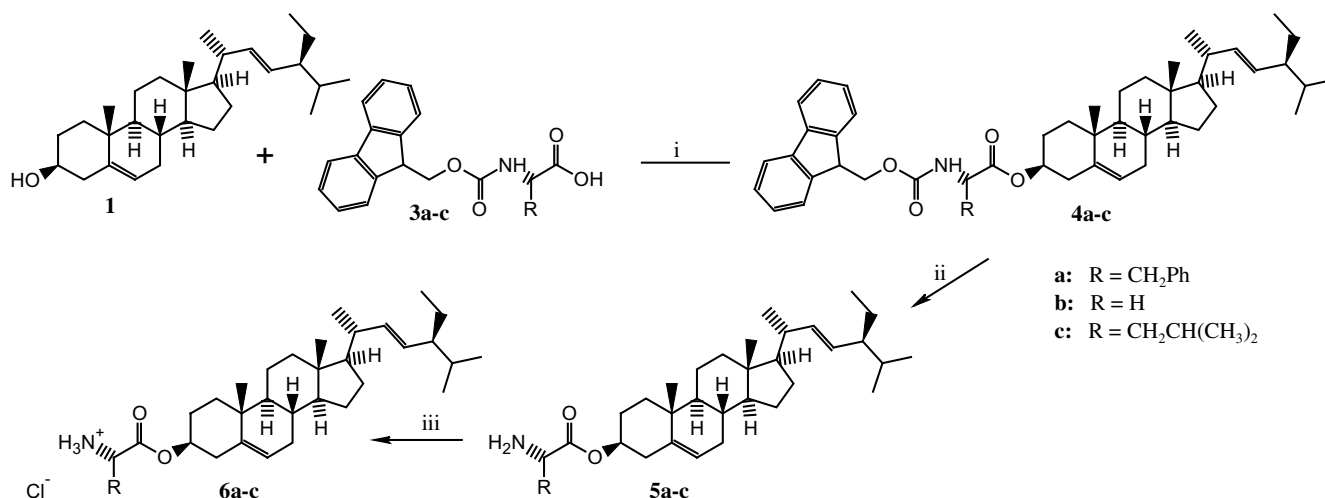
2.2.2. *In situ* gelation and acid–base triggered sol–gel transition

Compound **6a** (10 mg) was taken in a test tube and dissolved in CCl_4 (0.5 mL). Through the clear solution, freshly prepared HCl gas was bubbled for 1 min at room temperature. The solution turned viscous and then into a transparent gel which was resistant to flow upon inversion. A similar experiment was examined using a drop of concentrated hydrochloric acid, hydrobromic acid, formic acid or acetic acid instead of HCl gas.

In the acid–base triggered reversible sol–gel transition experiment compound **6a** (40 mg) was dissolved in CCl_4 (2 mL). Then for each cycle, we added alternately 10 μL of triethylamine and approximately 2–3 μL of conc. HCl. The acid–base cycle was repeated three times.

2.2.3. SEM measurements

Scanning electron micrographs of xerogels were taken on a Bruker Quantax400 EDS microscope equipped with a digital camera. Sample of the xerogels were prepared by placing a hot, clear solution of the gelator on carbon tape over a sample stub. The samples were dried at room temperature and then sputter coated with a thin layer of gold in a JEOL Fine Coat Ion Sputter JFC-1100.



Scheme 1. Synthesis of stigmasterol-amino acid conjugates; (i) DCC, DMAP, CH_2Cl_2 , rt, 48 h; (ii) piperidine, DMF, rt, 1–2 h; (iii) HCl (gas.), CH_2Cl_2 , rt, 30 min.

2.2.4. NMR measurements

VT ^1H spectra were recorded with a Bruker Avance DRX 500 NMR spectrometer equipped with a 5 mm diameter broad band inverse probe head working at 500.13 MHz for ^1H . The gel was prepared by dissolving the gelator in an NMR tube (20 mg/mL) and was stabilized over night. The VT ^1H NMR experiment was conducted varying the temperature by 5 °C steps. The sample was allowed to stabilize for 5 min at each temperature before acquiring the spectrum.

The ^{13}C CP MAS and ^{15}N CP MAS NMR spectra were recorded on a Bruker AV 400 spectrometer equipped with a 4 mm standard bore CP MAS probehead whose X channel was tuned to 100.62 MHz for ^{13}C and 40.55 MHz for ^{15}N , respectively. The other channel was tuned to 400.13 MHz for broad band ^1H decoupling. The dried and finely powdered samples were packed in the ZrO_2 rotor closed with Kel-F cap and spun at 10 kHz. The ^{13}C CPMAS NMR of the gel was measured using 50 μL HR MAS rotor with a spinning frequency of 4–5 kHz. The ^{13}C CP MAS NMR was carried out for all samples under Hartmann–Hahn conditions with TPPM decoupling. The experiments were conducted at contact time of 2–3 ms with 4–5 s recycle delay for each sample. All FIDs were processed by exponential apodization function with line broadening of 10–30 Hz prior to FT. The ^{15}N CP MAS NMR experiments were carried out for all samples at a 10 kHz spinning rate under Hartmann–Hahn condition. The optimized contact time of 2 ms was used for efficient polarization transfer with a 5 s recycle delay to acquire the CP MAS spectra. The ^{13}C CPMAS NMR chemical shifts are referenced to the C=O signal of glycine at 176.3 ppm and ^{15}N CPMAS chemical shifts were referenced to the glycine signal at –345.25 ppm.

2.2.5. FTIR measurements

FTIR measurements were carried out on a Bruker Tensor27 FT-IR spectrometer. Spectra of solids and xerogels were recorded using a Pike GladiATR attenuated total reflectance (ATR) cell equipped with a diamond crystal plate. For measuring FTIR spectra of gels and solutions KBr pellets were coated with a thin film of either the gel or the solution.

3. Results and discussion

3.1. Gelation tests

Due to the amphiphilic nature (hydrophobic steroidal skeleton and hydrophilic amino acid part) of stigmaterol-amino acid conjugates (**5a–c**), we speculated them to create self-aggregated supramolecular structures and possibly gels as well. In order to test our hypothesis, the gelation abilities of **5a–c** were investigated in 22 common organic solvents including alcohols, aromatic solvents, organic acids, and water. However, as shown in Table 1, compounds **5b** and **5c** did not form gels in any of the tested solvents. Interestingly, compound **5a** formed only a weak gel in anisole. Further to improve solubility in water and polar solvents, and test their hydrogelation properties, we converted the compounds **5a–c** into their corresponding hydrochloride salts **6a–c**. A systematic gelation test, revealed a dramatic change in the gelation abilities of the conjugates which was significantly improved. While, compound **6a** was found to be an effective gelator for aromatic solvents and tetrachloromethane, compounds **6b** and **6c** formed gels mostly in alcoholic solvents (Table 1). These results indicate that the driving force of the gel formation is the presence of electrostatic interactions between a quaternary amino group and a chloride anion, although intermolecular hydrogen bonds between carbonyl and amino groups are probably also important. Our hypothesis was further studied in more detail by other techniques including NMR and IR measurements. For the selected gels, the minimum gelation concentration (see data

Table 1

Gelation properties of **6a–c** (2.5% w/v).

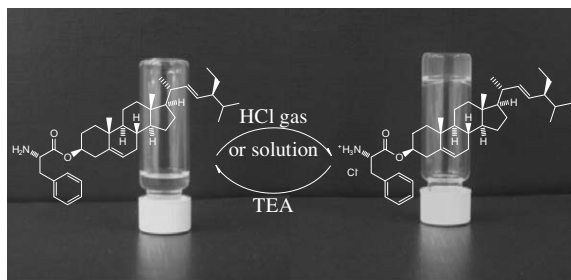
Solvent	5a	6a	5b	6b	5c	6c
Benzene	S	OG _(0.5)	S	I	S	I
Toluene	S	OG _(0.5)	S	I	S	I
Chlorobenzene	S	TG _(1.0)	S	I	S	I
<i>m</i> -xylene	S	OG _(0.5)	S	I	S	I
mesitylene	S	OG _(0.5)	S	I	S	I
Anisole	pCG	pCG	S	I	S	I
CH_2Cl_2	s	S	s	S	s	S
CHCl_3	s	S	s	OG	s	S
CCl_4	s	CG _(1.0)	s	I	s	I
Methanol	P	S	P	P	P	P
Ethanol	I	S	S	P	S	P
1-Propanol	I	S	S	P	S	P
1-Butanol	S	S	S	OG	S	P
1-Pentanol	S	S	S	OG	S	pOG
1-Hexanol	S	S	S	pOG	S	pOG
1-Heptanol	S	S	S	pOG	S	pOG
1-Octanol	S	S	S	pOG	S	pOG
1-Decanol	S	S	S	OG	S	OG
Ethane-1,2-diol	E	S	E	OG	S	OG
Water	I	E	I	E	I	E
Formic acid	S	S	S	pOG	S	P
Acetic acid	S	pTG	S	pOG	S	pOG
Propionic acid	S	S	S	pOG	S	pOG

Note. S = soluble at boiling point, s = soluble at room temperature, I = insoluble at boiling point, G = gel (O = opaque, T = translucent, C = clear transparent), pG = partial gel, P = precipitated upon cooling, E = emulsion. For the cases of stronger gels, the minimum gel concentration (% w/v) is shown in parentheses (tested concentrations: 1.0%, 0.5% and 0.25%).

in Table 1) and the gel-melting temperatures (see Supplementary data, Table S1) were determined.

3.2. In situ gelation and acid–base triggered sol–gel transition

The ability of supramolecular gels to act as sensors for various potential applications has been demonstrated by various research groups [30–32]. There is a steady increase in the number of stimuli responsive gels or gelation triggered by chemical, mechanical or enzymatic reactions [7,15,16,26–28,33]. In such cases the compound itself does not act as a gelator. But upon applying an external stimulus (acid/base, irradiation with UV–VIS light, redox reactions or enzymatic cleavage) it results in the formation of the gelators, thereby leading to the immobilization of the solvent. The striking finding in our investigation is that the formation of the hydrochlorides is crucial for the gelation of the prepared compounds, this warranted for further studies on these compounds in detail. As discussed earlier, the neutral or parent compounds (**5a–c**) were found to be non-gelators, or only formed a weak gel in anisole, whereas their hydrochloride salts (**6a–c**) are organogelators. We speculated that this property can be utilized to generate hydrochlorides in situ thereby leading to spontaneous gelation. On the other hand, the process of gel formation can be used as a sensor for gaseous hydrogen chloride. In order to test our hypothesis, we took a solution of non-gelator **5a** in tetrachloromethane (2% w/v, a clear solution at room temperature) and passed through it freshly generated gaseous hydrogen chloride for 45–60 s. The solution turned immediately into a stable transparent gel (Scheme 2). The same phenomenon was also observed after adding a drop of concentrated hydrochloric acid instead of HCl gas. Unfortunately we did not find any gel after adding a drop of hydrobromic acid, formic acid or acetic acid, suggesting that the anion effect is important and the presence of strong electrostatic interactions between an ammonium group and a chloride anion is crucial for the gel formation. We further explored the possibility of an “acid–base” triggered sol–gel (on–off) transition. In order to achieve this, we prepared a stable CCl_4 gel of **6a** (2% w/v). After the introduction



Scheme 2. Protonation and deprotonation of stigmasteryl phenylalaninate in tetrachloromethane.

of triethylamine to the gel, the gel structure got destroyed. Upon heating, the sample turned into a clear solution which remained a solution also after cooling. Further addition of concentrated hydrochloric acid to the system followed by heating and cooling, resulted in a transparent gel (Scheme 2). We repeated the acid/base cycle three times always with the similar result – the sol-gel transformation was observed.

3.3. Morphology of xerogels

In order to gain more insight into the morphology of the gels, scanning electron microscopy (SEM) was utilized. The micrographs of the xerogel from the 1-butanol gel of **6b** revealed plate like aggregates (Fig. 2a and b). Similar structures were also observed on the SEM images of the xerogels from 1-pentanol and 1-octanol gels (see Supplementary data, Fig. S1a and b). Interestingly, the micrographs obtained from the xerogel of the ethane-1,2-diol gel showed highly entangled fibrous structures (Fig. 2c). The SEM images of the xerogels from the gels of **6a** in aromatic solvents disclosed bundles of fibres (Fig. 2d–f) approximately 250 nm in diameter (see Supplementary data, Fig. S1c). Unfortunately, we were not able to get any SEM images of the xerogel from the gel in CCl_4 which would be interesting for a comparison in regard to the properties of the gels. The careful analysis of the various SEM images clearly shows that the structure and the nature of the fibrillar

networks observed depends on both the gelator as well as the solvent used for the gelation.

3.4. NMR measurements

3.4.1. VT ^1H NMR measurements

Solution NMR experiments have been extensively used to understand the possible interactions involved in the process of gelation [17,20,34–38]. Attempts have also been made recently to study details to extract the self-assembled structural information at various stages of gelation [34,35]. There exist a number of contradictory observations and these are strongly dependent on the gel forming system under investigation. According to some reports, it is believed that the well resolved ^1H signals observed in the gel state are presumably from those of non-aggregated molecules, whereas the aggregated structures are NMR silent [36,38]. According to other reports, the well resolved spectral lines may be due to the presence of mobile portions of the gel fibers [37]. However, all these arguments have been supported by various experimental evidences. It is also believed that the supramolecular gels co-exist in equilibrium between associated and non-associated forms. In order to investigate the interactions involved in the gel formation of compound **6a**, we recorded variable-temperature ^1H NMR spectra of its gel in benzene- d_6 (2% w/v, Fig. 3, and Supplementary data, Fig. S2 and S3). We further carried out some control experiments: ^1H NMR spectra of compound **6a** in benzene- d_6 below non-gelling concentration (0.3% w/v) and in a non-gelling solvent (CDCl_3). Interestingly, the ^1H NMR chemical shift values of **6a** in benzene- d_6 below gelling concentration does not correspond to that observed in the gel state, rather they showed some similarity to the spectrum at high temperature (sol state) (Fig. 3) clearly suggesting that the observed ^1H NMR signals in the gel sample do not correspond to non-aggregated states unlike published earlier [36,38] and the spectral data can be used for the clarification of the gel formation.

VT ^1H NMR spectra were recorded in 5 °C steps for temperatures between 30–70 °C. The broad signals apparent to the gel state gradually turned sharper. The well-resolved spectral patterns were finally observed at 55 °C, suggesting that the gel transfers to the

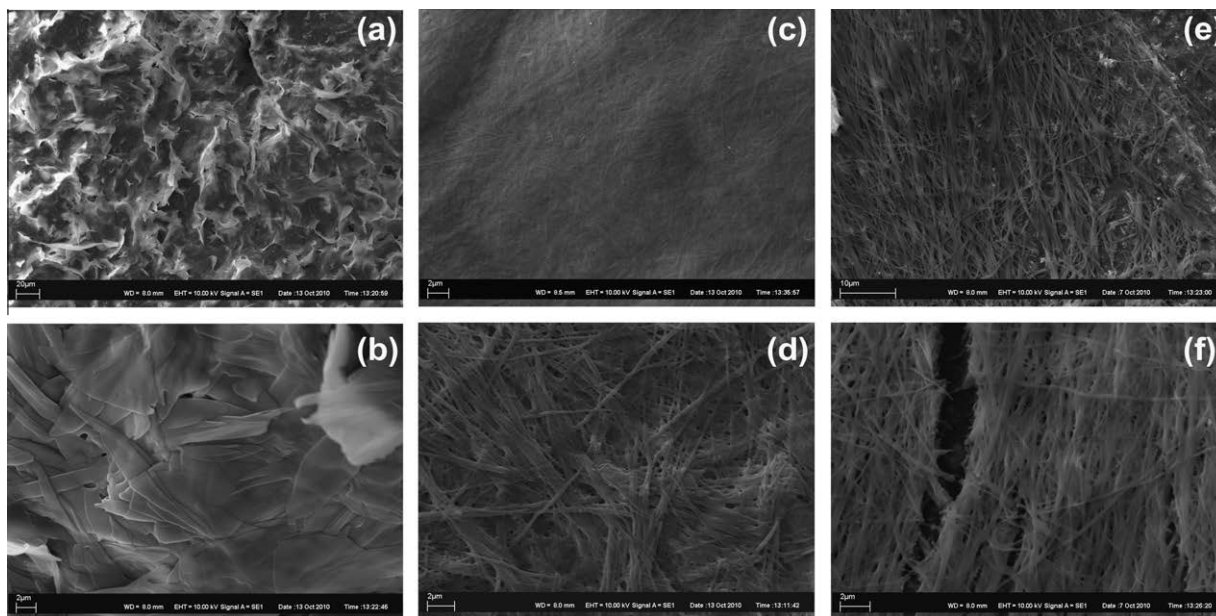


Fig. 2. SEM images of xerogels obtained from the gel of **6b** in (a) and (b) 1-butanol (2% w/v), (c) ethane-1,2-diol (2% w/v), and from the gel of **6a** in (d) benzene (2% w/v) and in (e) and (f) chlorobenzene (2% w/v).

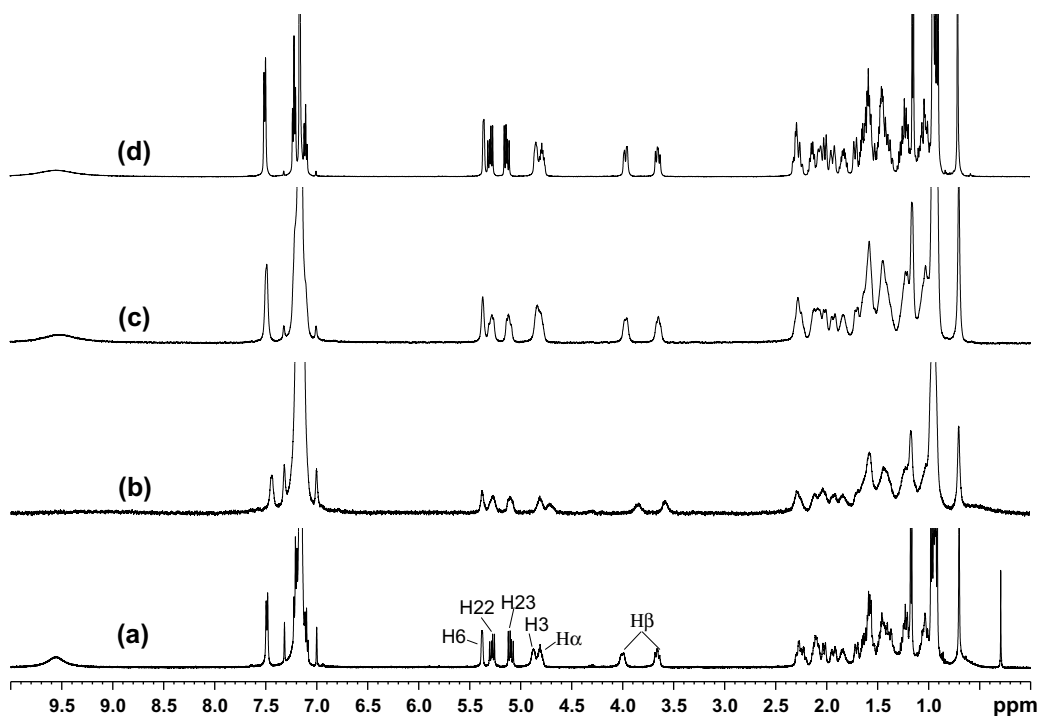


Fig. 3. ^1H NMR spectra of (a) compound **6a** in benzene- d_6 at non-gelling concentration (0.3% w/v) at 30 °C, (b) benzene- d_6 gel of **6a** (2% w/v) at 30 °C, (c) at 45 °C and (d) at 70 °C.

solution state between 50 and 55 °C which is in an agreement with the results obtained using the “inversion test tube” method ($T_{\text{gel}} = 49\text{--}50$ °C, see Supplementary data, Table S1). With the temperature increase the chemical shifts did not change remarkably except of those of protons on the α - and β -carbons of the phenylalanine part. It was found that with an increase of the temperature the chemical shift values of the α -hydrogen were gradually shifting from 4.71 at 30 °C to 4.89 ppm (deshielded) at 55 °C and then showed shielding back to 4.84 ppm at 70 °C. For the β -hydrogens, similar trends were observed. The chemical shifts of the β -hydrogens showed a deshielding upon heating from 3.58 and 3.84 ppm at 30 °C to 3.67 and 4.01 ppm respectively at 55 °C and then a very small upfield shift to 3.65 and 3.96 ppm at 70 °C. A careful analysis of the VT ^1H NMR also revealed the change and variation of the chemical shift values of NH hydrogens. These hydrogen signals are silent in the gel state but at 35 °C a broad singlet appeared at 9.28 ppm showed a downfield shift upon heating. A difference of 0.29 ppm was observed between 35 and 55 °C (see Supplementary data, Fig. S2). Looking at the structure of the gelator molecules, a strong hydrogen bonding similar to small peptides can be expected. The major interactions certainly are between $\text{C}=\text{O}\cdots\text{H}-\text{N}$ in the gel state along with $\pi-\pi$ stacking. Upon heating the hydrogen bonding is disrupted (as can be seen from the $\text{N}-\text{H}$ chemical shift) and the gel breaks. This is indirectly observed by a small change in the chemical shift values of the α -hydrogens. In the gel state the protons are shielded while upon heating, they become more deshielded. However, at this point a slight shielding effect above the gel melting point cannot be explained.

3.5. CPMAS NMR studies

Solid state NMR spectroscopy has emerged a powerful and complementary tool to X-ray crystallography to study crystalline solids, semisolids and liquid crystalline materials (see for example [39–42]) and continued to expand rapidly. The cross polarization magic angle spinning (CPMAS) NMR provides a unique opportunity

to study the gel like materials as solid, xerogel and especially the native gel itself. The study of gels in their native forms utilizing CPMAS solid state NMR experiments is a quite new approach in gel structural investigation and it has been an active area of research in our laboratory [17,43,44]. The ^{13}C solid state NMR spectra were recorded for the solids of the prepared compounds, as well as for their gels and xerogels. The structural study was carried out for **6a** (Fig. 4) and **6b** (Fig. 5). The ^{13}C CPMAS NMR of the synthetic solid showed to be crystalline in nature but the signals were relatively broad. However, a careful analysis of the CPMAS NMR spectral data of **6a** clearly indicates the presence of more than one form (Fig. 4). This is evident from the signals arising from the carbonyl carbon, which shows three signals at 169.88, 169.34 and 168.76 ppm. A ^{15}N CPMAS NMR experiment of the solid also indicated the presence of more than one form as multiple ^{15}N signals were observed (see Supplementary data). Interestingly, the ^{13}C CPMAS NMR of the xerogel prepared from the benzene gel of **6a** showed a doublet resonance pattern with carbonyl signals at 169.85 and 168.92 ppm. The sample also showed better crystallinity compared to that of the synthetic solid (Fig. 4). The doublet resonance pattern is known to occur in crystalline organic solids for two reasons; either (i) two different crystalline forms/polymorphic forms or (ii) due to the presence of two crystallographically non-equivalent molecules in an asymmetric unit. The polymorphs can be separated by choosing either different solvents or adopting a suitable crystallization condition. Similar observations have been reported earlier for bile acid based gelators and it was shown to be due to the presence of two non-equivalent molecules in an asymmetric unit of the crystal lattice [17]. However, the concluding evidence can be drawn only using single crystal X-ray structure and unfortunately none of the gelators formed single crystals.

The xerogel obtained from the CCl_4 -gel, showed a singlet resonance pattern suggesting that the synthetic solid is a mixture of polymorphs which are clearly separated upon changing the solvents. Solid state NMR results also support the observation made on the morphology of the xerogels (SEM measurements) that they

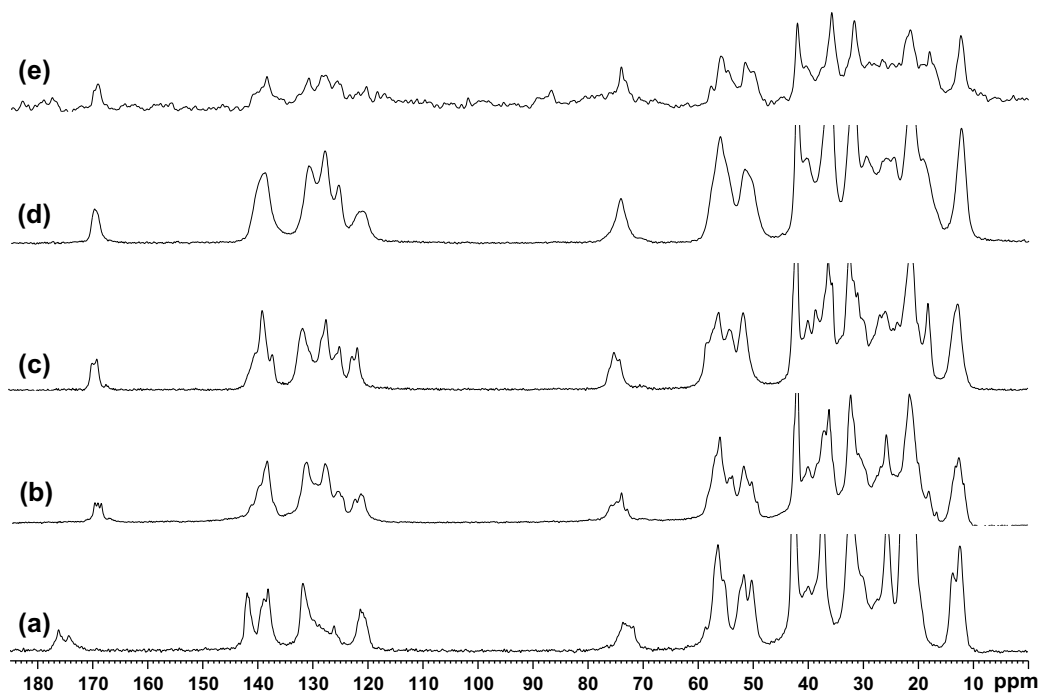


Fig. 4. ^{13}C CPMAS NMR of (a) **5a** solid, (b) **6a** solid, and xerogels of **6a** obtained from (c) benzene gel of **6a**, (d) from CCl_4 gel of **6a**, and (e) CCl_4 gel of **6a** (4% w/v).

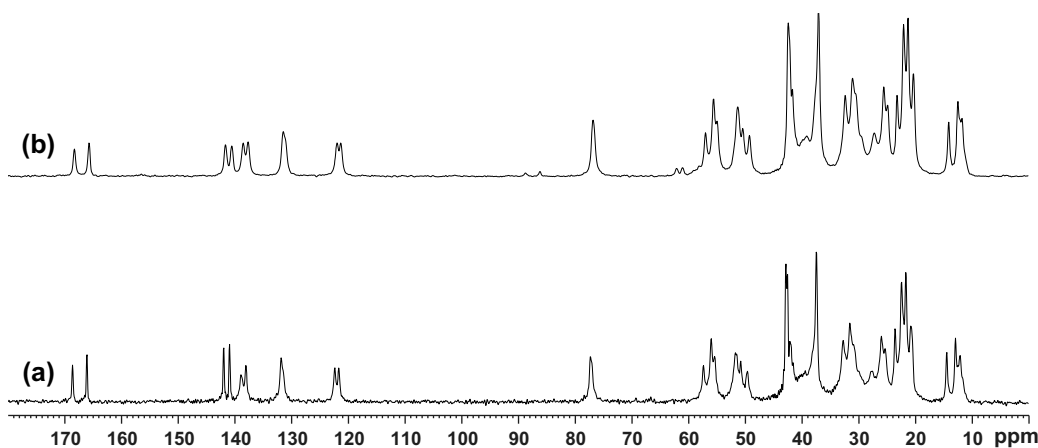


Fig. 5. ^{13}C solid state NMR of (a) **6b** solid and (b) xerogel from 1-butanol gel of **6b**.

are highly dependent on the solvent. Over the years literature has witnessed several reports correlating the packing patterns of the gelators leading to gel formation based on the single crystal structure of gelator molecules. However, most of the gelators do not undergo crystallization to yield quality single crystals. Moreover, the crystallization conditions are different (either a non-gelling solvent or concentration) from the gel formation. As a result, the mode of packing pattern in the xerogel and the gel may be different from that of the single crystal X-rays structure. In this context, solid state NMR has the ability to provide useful information about the packing pattern in the solid, xerogel as well as the native gel as reported from our laboratory. Attempts were made to acquire the ^{13}C CPMAS NMR spectrum of the native CCl_4 -gel of **6a**, although the quality of the spectrum was not as good as that of the xerogel, still it shows the similarity to the xerogel obtained from the CCl_4 -gel (Fig. 4). The above results strongly suggest that there exist a similarity in the packing of the gelator molecules in the xerogel and the native gel. The ^{13}C CPMAS NMR spectrum of **6b** displayed a highly

crystalline nature also showing a doublet resonance pattern. In this case both the xerogel obtained from the 1-butanol gel of **6b** as well as the bulk solid showed similar spectral patterns. Two signals are clearly visible also in the ^{15}N CPMAS NMR spectrum (see Supplementary data).

We also observed a significant difference in the chemical shift values of $\text{C}=\text{O}$ between **5a** and **6a** (Fig. 4), which we attribute partly to the chemical modification of the neutral molecule into its salt and partly to the change in a hydrogen bonding pattern. The shielding of the carbonyl carbon chemical shift value suggests stronger hydrogen bonding interactions between $\text{N}-\text{H}\cdots\text{O}=\text{C}$ in **6a** than in **5a**. Furthermore, similar results were obtained for **5b** and its hydrochloride **6b** as well.

3.6. FTIR measurement

IR spectroscopy is a technique capable of investigating inter- and intramolecular interactions. Therefore, it is frequently used

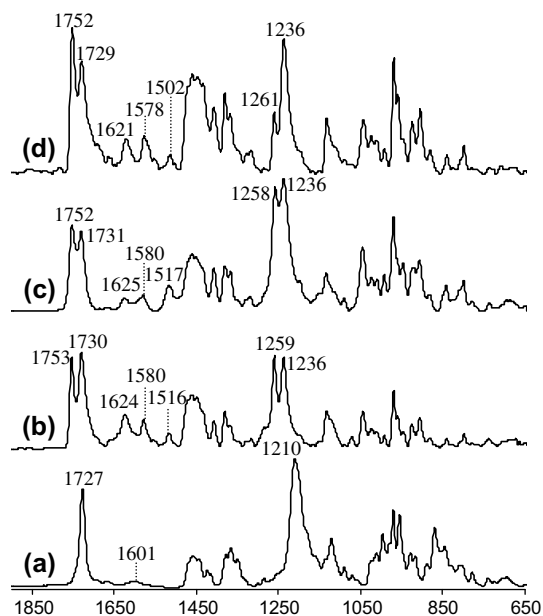


Fig. 6. FTIR spectra of **5b** (a) and **6b** as (b) a thin film in CH_2Cl_2 (non-gelling solvent), (c) xerogel from 1-butanol, and (d) gel in 1-butanol (2% v/w).

in the study of gels. In order to explore interactions, which might be the driving forces of the gelation process, FTIR spectra of **5a**, **6a** (Supplementary data, Fig. S4), **5b** and **6b** (Fig. 6) in different forms were collected and the positions of peaks were studied. The FTIR spectra of the gels and the corresponding xerogel were similar, suggesting that the structure in the gel state is close to that in the crystalline form of the xerogel, which is in agreement with the results obtained from the solid state NMR experiments (Fig. 4). Unfortunately, we did not observe any relevant shifts of the peaks in the FTIR spectra of the compounds in non-gelling solvents, and their gels and xerogels, which could help us to clarify the gelation process. However, we found the significant differences between the FTIR spectra of neutral molecules (Fig. 6a, Fig. S4a) and their hydrochlorides (Fig. 6b, Fig. S4b). Expectantly the peaks of NH bending vibrations were shifted, but also the peaks of the (C–O) and (C=O) vibrations were remarkable moved suggesting a change in hydrogen bonding pattern between hydrochlorides and neutral molecules, which corresponds to the results obtained from solid state NMR measurements (Fig. 4).

4. Conclusions

We prepared three novel organogelators based on amino acids and stigmaterol. A steroid molecule rarely used in the synthesis of LMOGs [45]. The conversion of neutral molecules to the corresponding hydrochlorides was crucial in our system for the gel formation and gave us a tool to switch the gelation ability of the compounds “on and off”. The big advantage of the system is the reversibility of the gelation process, therefore these pH-stimuli responsive organogels may find potential applications in the fields of designing of receptors and sensors [12,16] as well as systems for controlled drug delivery [14]. We analyzed the prepared solids, xerogels and gels via up to date methods (liquid NMR, IR, SEM etc.) and especially with solid state NMR, which is a new and powerful technique so far not often used in the structural study of gels [17,43,44].

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcis.2011.05.084.

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III

***In situ* formation of steroidal supramolecular gels designed for drug release**

by

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Abstract: In this work, a steroidal gelator containing an imine bond was synthesized, and its gelation behavior as well as a sensitivity of its gels towards acids was investigated. It was shown that the gels were acid-responsive, and that the gelator molecules could be prepared either by a conventional synthesis or directly *in situ* during the gel forming process. The gels prepared by both methods were studied and it was found that they had very similar macro- and microscopic properties. Furthermore, the possibility to use the gels as carriers for aromatic drugs such as 5-chloro-8-hydroxyquinoline, pyrazinecarboxamide, and antipyrine was investigated and the prepared two-component gels were studied with regard to their potential applications in drug delivery, particularly in a pH-controlled drug release.

Keywords: organogel; acid-responsive; cholesterol; *in situ* gelation; drug release

1. Introduction

Steroids, as compounds with a wide array of different tasks in Nature, come in many varieties. But all of them combine a large and rigid framework with functional groups making them especially valuable as starting materials for organic synthesis. In this article we want to show the application of steroids in supramolecular gels. Supramolecular gels [1–6] are smart functional nanoscale materials with high potential for a wide range of advanced applications, among others [7–11], in drug delivery [12–14], tissue engineering [12], light-harvesting systems [7,9,15], and optoelectronics [10,11]. Their formation stems from spontaneous but controlled self-assembly of small molecules, often called low-molecular-weight

gelators (LMWGs), into a three-dimensional network with solvent molecules entrapped in its cavities [5,16]. Despite their mostly liquid composition, these systems demonstrate the appearance and rheological behaviour of solids. Besides steroids [17–20] other examples of supramolecular gels, based on natural building blocks like nucleobases [21–23], amino acids, or oligopeptides [24–28], have been reported.

In recent years supramolecular gels have received increased attention as alternative materials to conventional polymer gels, mostly due to the possibility to install desired properties into a gelator structure, *i.e.*, on a molecular level [7]. By a proper design of LMWGs, the self-assembly of the molecules can be arranged and controlled. Appropriately designed gelators, *i.e.*, decorated with suitable functional groups, can be programmed for example to be responsive to external stimuli such as light, mechanical stress, pH, ionic strength, metal ions, or anions [29–34]. In most cases, a change on a molecular level results directly in a change of macroscopic properties of the whole system. Recently, several stimuli-responsive steroidal supramolecular gels exhibiting the before mentioned properties were reported, among others [17], pH and acid-responsive sterol-amino acid conjugates [35,36] and bile acid derivatives [37].

In a conventional gel preparation, gelators are synthesized beforehand and then dissolved in suitable solvents. However, in some cases gelators can be synthesized by mixing two or more components directly in a gelling solvent, where new covalent bonds between the building blocks are formed. This modern approach is called *in situ* gelation and recently some gelators of this type were reported [38–40]. It was shown that in comparison to the classic method, the *in situ* gelation often: (i) omits heating of the sample; (ii) occurs at ambient temperature; (iii) requires shorter gelation time; or (iv) takes place also in solvents which are not gelled by the conventional gelation [39]. Moreover, the components are needed in a certain ratio to achieve an effective gelator formation, therefore, a lack or excess of one of the components can be used as a tool to control the gelation process.

In this work we present a synthesis of cholesteryl gelator containing an imine bond. Cholesterol and its derivatives are commonly used in the preparation of LMWGs due to the hydrophobic character of the cholesteryl unit and its strong tendency to self-aggregate. Many cholesterol-based gelators have been reported [17–19] and they have been often classified as ALS, A(LS)₂, LS, and LS₂ types according to the numbers of aromatic (A) and steroidal (S) units, and linkers (L) [18].

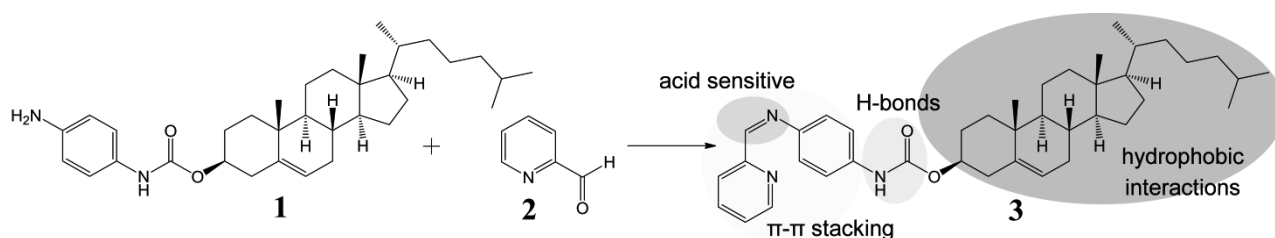
2. Results and Discussion

2.1. Preparation and Gelation Behaviour of Imine 3

The cholesteryl moiety **1** and 2-pyridinecarboxaldehyde (**2**) were used to prepare gelator **3** of an ALS type (Scheme 1). In our design *p*-phenylenediamine was chosen as a linker in order to synthesize stable (conjugated) imine **3**, and to introduce an amide moiety (*i.e.*, an acceptor and donor for intermolecular H-bonds) to the structure. The imine bond was embedded into the gelator structure due to: (i) its known sensitivity towards acids which we wanted to exploit as an acid-sensitive system; and (ii) its relatively rapid and easy formation at mild conditions, which allows an *in situ* gelator formation. To test these hypotheses, a formation and an acid catalysed hydrolysis of imine **3** were carried out and monitored by NMR. The reaction of amine **1** with the aldehyde **2** occurred at room temperature and it was completed within few hours providing time-stable imine **3** (ESI, Figure S1). However, when the imine **3** was treated with a catalytic amount of *p*-toluenesulphonic acid, it decomposed into its two precursors (protonated

compound **1**, and compound **2**) within several minutes (the majority of **3** decomposed within 45 min, see ESI, Figure S2 for details), confirming that the cleavage of the imine linkage under acidic conditions is facile and effective.

Scheme 1. Design and synthesis of imine **3**—gelator of an ALS type [18].



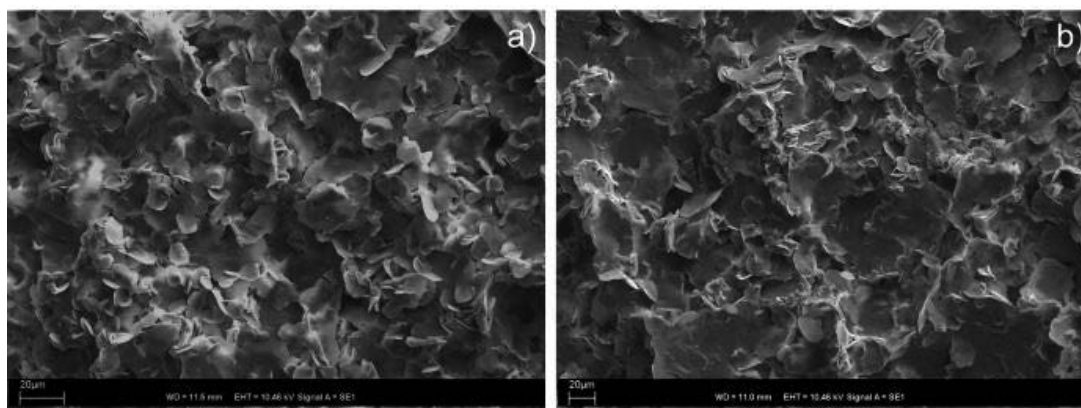
The gelation behaviour of individual compounds was studied in 16 different organic solvents and in water. The tests were carried out for both moieties separately (*i.e.*, for **1**, and for **2**), and for the synthetically and the *in situ* prepared imine **3** (Table 1). Expectably, 2-pyridinecarboxaldehyde (**2**) did not gelate any of the tested solvents at the concentration of 2% w/v, and amine **1** formed gels only in cyclohexane and DMSO. However, after mixing these two components together and the heating/cooling cycle, gel formation was observed in DMF and DMSO, and in higher alcohols starting from propan-1-ol. The same results were obtained when the gelation behaviour of a synthetically prepared imine **3** was tested. The corresponding imine gels (formed by synthetically and *in situ* prepared gelators) exhibited the same visual properties and the morphology of their xerogels, studied by SEM, was also very similar. The SEM micrograph did not reveal any regular fibrous structures. It only showed three-dimensional structures constructed from thin microflakes of different sizes and mostly rounded shapes (Figure 1, and ESI, Figure S11) suggesting that the gel microstructure was very fragile and collapsed upon solvent evaporation. We also tested the effect of the component ratio on the *in situ* gelation (ESI, Tables S2 and S3). As expected, we found that the effective gelation was reached when a 1:1 molar ratio of the components was used. An excess of 2-pyridinecarboxaldehyde did not significantly affect the gelation process, whereas the excess of amine **1** resulted in a sample precipitation (ESI, Table S2). We also investigated a possibility to prepare gel *in situ* omitting the heating/cooling cycle, which was recently reported [39]. Unfortunately, we did not observe an effective gelation at room temperature, not even after 24 h or ultrasonic treatment, suggesting that the reaction does not quantitatively occur in alcohols at room temperature and energy needs to be added to the reaction via heating.

Table 1. Gelation tests (2% w/v).

Solvents	1	2	3 (“ <i>in situ</i> ”)	3	Solvents	1	2	3 (“ <i>in situ</i> ”)	3
toluene	S	S	P	P	hexan-1-ol	S	S	pG	pG
CH ₂ Cl ₂	S	S	P	P	heptan-1-ol	S	S	pG	pG
CHCl ₃	S	S	S	S	octan-1-ol	S	S	pG	pG
CCl ₄	S	S	P	P	water	I	S	-	I
methanol	P	S	I	I	DMF	S	S	pG	pG
ethanol	P	S	I	I	DMSO	G	S	G	G
propan-1-ol	S	S	G	G	cyclohexane	G	I	-	I
butan-1-ol	S	S	G	G	hexane	I	I	-	I
pentan-1-ol	S	S	G	G					

Note: S = soluble, P = precipitate upon cooling, G = gel, pG = partial gel, I = insoluble at the solvent boiling point.

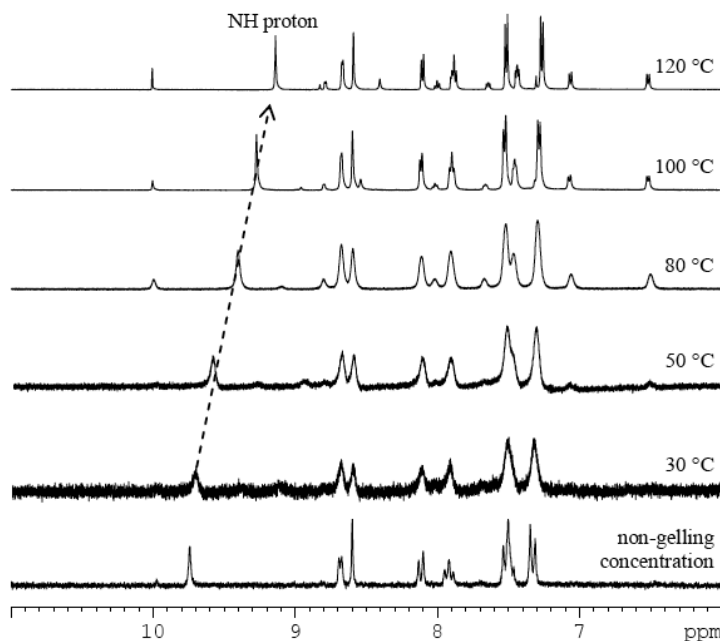
Figure 1. SEM micrographs of xerogels from propan-1-ol of (a) of the *in situ* prepared imine **3**; and (b) the synthetically prepared imine **3**.



In order to discover the non-covalent interactions involved in the gelation process, we carried out variable-temperature $^1\text{H-NMR}$ measurements of the imine **3** gel in $\text{DMSO-}d_6$ (Figure 2, and ESI, Figures S4 and S5). By heating the sample in $10\text{ }^\circ\text{C}$ steps ($30\text{--}120\text{ }^\circ\text{C}$), the broad signals gradually turned sharper. The well-resolved spectral patterns were finally observed at $100\text{ }^\circ\text{C}$, suggesting that the gel fully transformed to the solution state. As a control experiment, a $^1\text{H-NMR}$ spectrum of imine **3** in a non-gelling concentration (0.2% w/v) was recorded (Figure 2). Chemical shifts of proton signals of imine **3** at the non-gelling concentration match those in the gel state at $30\text{ }^\circ\text{C}$ indicating that the observed peaks correspond to the free molecules which are not tightly integrated in the gel network. With an increase of temperature, the chemical shifts of the signals did not change remarkably with the sole exception of the amide proton. The chemical shift values of N-H changed from 9.71 ppm at $30\text{ }^\circ\text{C}$ to 9.13 ppm at $120\text{ }^\circ\text{C}$. This indicates an increase in the strength of the N-H bond, direct evidence of the disentanglement of NH from a bonding state. This disruption of intermolecular hydrogen-bonding can be also indirectly observed by a small deshielding of signals of the protons of the phenyl ring (the strongest change is from 7.33 at $30\text{ }^\circ\text{C}$ to 7.27 ppm at $120\text{ }^\circ\text{C}$). The experiment did not prove the presence of $\pi\text{-}\pi$ stacking between pyridine units in the DMSO-gel, because only small shielding ($0.01\text{--}0.05\text{ ppm}$) of pyridine protons was observed. The spectra also showed that the increase of temperature in the presence of water (from the deuterated solvent) caused partial hydrolysis of imine **3** to the starting components **1** and **2**.

The presence of intermolecular hydrogen bonds was further confirmed by a dilution NMR experiment. The $^1\text{H-NMR}$ spectra were recorded for the imine **3** at different concentration and showed deshielding of the NH signal at higher concentrations indicating the intermolecular hydrogen bond formation (ESI, Figure S3). Overall, we propose that the gel formation is caused by an aggregation of cholesteryl units *via* hydrophobic and van der Waals interactions in polar solvents, and that the structure is further stabilized by intermolecular hydrogen bonds between amide groups and possibly by $\pi\text{-}\pi$ interactions between the aromatic parts of the molecules.

Figure 2. $^1\text{H-NMR}$ subspectra of imine **3** in $\text{DMSO-}d_6$ at non-gelling concentration (0.2% w/v), and as a $\text{DMSO-}d_6$ gel (2.5% w/v) at different temperatures (30–120 °C) with a highlighted shift of the amide proton signal (dotted arrow).



2.2. Acid-Responsive Gels

Considering the difference in the gelation behaviour of amine **1** and imine **3**, and after successful acid catalysed hydrolysis of imine **3** monitored by NMR (ESI, Figure S2), we expected that the addition of a small amount of acid to the gel of imine **3** should result in a gel-sol transition. When we placed a catalytic amount of *p*-toluenesulphonic acid on the top of a gel of imine **3** in propan-1-ol, butan-1-ol, pentan-1-ol, and DMSO , a gel-sol transition occurred (Scheme 2, and ESI, Figure S10). We suppose that the hydrolysis of the imine linkage weakened the intermolecular interactions, resulting in the dissociation of the self-assembled network of the gelators and giving rise to an acid-mediated gel-sol transition. This process occurred within less than 2 h, and could be speeded up to several minutes under an ultrasonic treatment. In another experiment, a drop of hydrochloric acid (1 M aqueous solution) was added resulting in a gel-sol transition within several minutes (approx. 15 min). And again, the process could be speeded up to few minutes by an ultrasonic treatment. These results, together with a recently published report [41], show that the organogels formed by imines are acid-responsive. We believe that such systems could find potential applications in drug release systems and therefore, gels based on imine **3** were further studied.

2.3. Two Component Gel System

In order to study the potential of gelator **3** in drug delivery [12–14], two component gels [42,43] containing different drugs were prepared. As a second component, aromatic drugs of different polarities were chosen: 5-chloro-8-hydroxyquinoline (cloxyquine, **Q**), pyrazinecarboxamide (pyrazinamide, **PC**), and antipyrine (phenazone, **AP**) (Figure 3). The quinoline derivative and pyrazinecarboxamide are drugs used in the treatment of tuberculosis [44,45], and antipyrine is analgesic and antipyretic. These

pharmaceuticals were chosen because of their aromatic character (*i.e.*, possibility to intercalate to the aromatic parts of imine **3**), commercial availability, low price, and gelation potential (recently, 5-chloro-8-hydroxyquinoline was found to be an effective gelator for alcohol-water mixtures [46]).

Scheme 2. *In situ* gelation and acid-induced gel-sol transition of **3** in propan-1-ol.

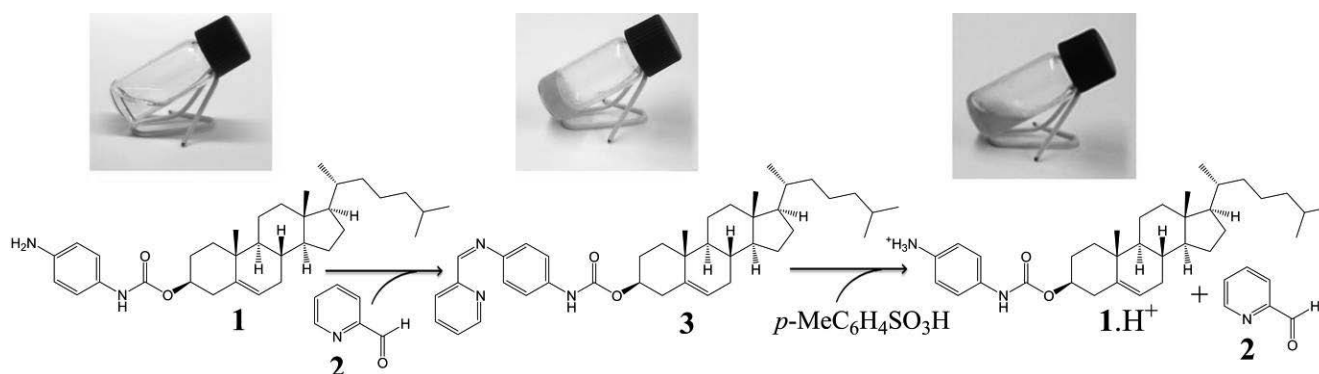
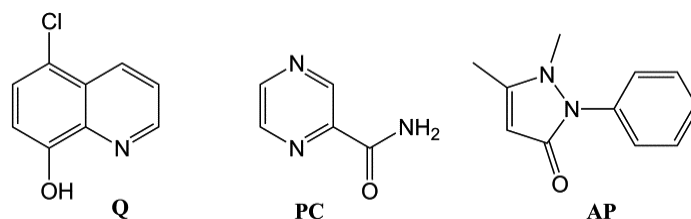


Figure 3. Chemical structures of the chosen drugs: 5-chloro-8-hydroxyquinoline (**Q**), pyrazinecarboxamide (**PC**), and antipyrine (**AP**).

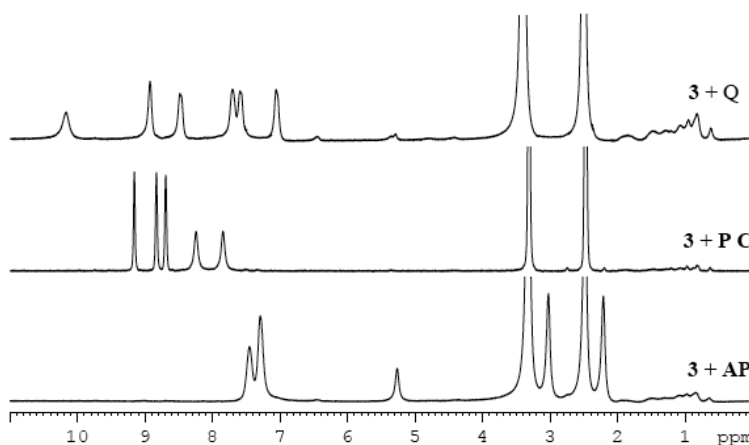


It was found that the mixtures of imine **3** and the drugs in a 1:1 ratio formed gels in most of the selected solvents (Table 2). In order to investigate interactions between the gelator and drug molecules, $^1\text{H-NMR}$ spectra of imine-drug gels (Figure 4) and variable temperature $^1\text{H-NMR}$ spectra of a gel of imine **3** and pyrazinecarboxamide were recorded (ESI, Figures S6 and S7). The drug signals in the spectra are not sharp suggesting that the mobility of the drug molecules is restricted within the gel network. As it was proposed by Miravet *et al.* [47], broadening of the signals, indicating shorter T_2 relaxation times, is often caused by a fast exchange between molecules in liquid-like (“visible” by NMR) and solid-like (“invisible” by NMR) phases, which means in our case between the free dissolved drug molecules and the drug molecules interfering in the gelator network. With an increase of temperature the gel melts and the drug is fully released to the solution and as a consequence, the signals become sharper. The fully sharp signals were observed at 100 °C (see Figures S6 and S7) which corresponds to the gel-sol transition of the gel of imine **3** (Figure 1). Unfortunately, the experiment did not prove the presence of π - π stacking either between drug molecules or between drug and gelator molecules in a DMSO-gel, because additional shifts, besides the small upfield shifts (approximately 0.05 ppm) of the aromatic proton signals expected for an increase of temperature, are missing.

Table 2. Gelation tests (2% w/v).

Solvents	3	Q	3 + Q	PC	3 + PC	AP	3 + AP
ethanol	I	R		P	I	S	I
propan-1-ol	G	G	G	P	G	S	G
butan-1-ol	G	R	G	P	G	S	G
pentan-1-ol	G	R	pG	P	G	S	G
hexan-1-ol	pG	R	pG	P	G	S	G
octan-1-ol	pG	R	pG	P	pG	S	pG
DMF	pG	S	pG	S	pG	S	pG
DMSO	G	S	G	S	G	S	G

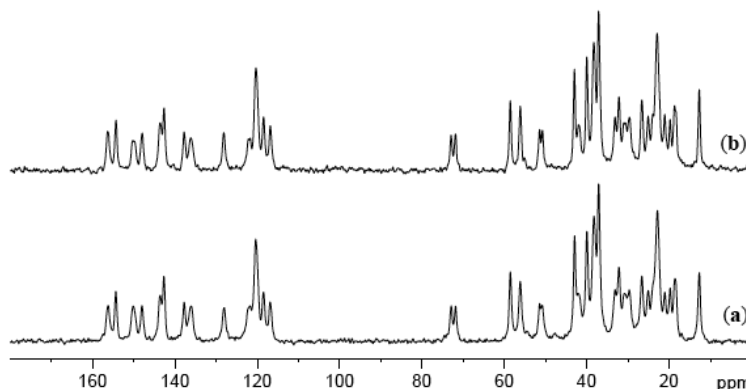
Note: S = soluble, P = precipitate upon cooling, G = gel, pG = partial gel, R = recrystallization upon cooling, I = insoluble at the solvent boiling point.

Figure 4. Comparison of the ^1H -NMR spectra of the different imine-drug gels in DMSO- d_6 (3% w/v).

In order to investigate an influence of a drug on the molecular packing of the gelator, ^{13}C cross polarization magic angle spinning (CPMAS) NMR spectra of xerogels of imine **3**, and of imine **3** and pyrazinecarboxamide in a 1:1 ratio were recorded. As can be seen from Figure 5 (and Figure S8 in ESI), the spectra display very similar patterns suggesting that the presence of a drug does not significantly affect the packing mode of imine **3** in the xerogel state. Moreover, the spectra show that imine **3** is much more crystalline compared to the drug because the drug signals can be hardly seen in the spectrum. Some of the signals reveal a double resonance pattern indicating the samples being either (i) a mixture of different polymorphic forms, or (ii) composed of a form having two non-equivalent molecules present in an asymmetric unit. A similar observation has been reported earlier for bile acid-based gelators and it was shown to be due to the presence of two non-equivalent molecules in the crystal lattice [48].

The morphology of the xerogels of the imine-drug gels from pentan-1-ol and DMSO was studied by SEM (ESI, Figure S11). Micrographs of xerogels of imine **3** from alcohols and from DMSO displayed similar microstructures regardless of the presence or absence of the drugs suggesting that the drug molecules did not significantly affect the way of self-assembly of the gelator. Only images of the xerogel of **3+PC** from pentan-1-ol showed a slightly different arrangement with partly integrated elongated crystals (ESI, Figure S11b). These crystals could represent drug molecules which were not too strongly incorporated to the gel network and could freely crystallize during the sample preparation when the solvent was slowly evaporated.

Figure 5. ^{13}C CPMAS NMR spectra of (a) xerogel of **3** from propan-1-ol; (b) xerogel of **3** and PC (1:1) from propan-1-ol.



2.4. Controlled Drug Release

In order to examine the potential of the two component gels in drug delivery [12–14], the release of a drug from gel to water was studied. For this purpose, we chose a gel of imine **3** and pyrazinecarboxamide in pentan-1-ol. Pyrazinecarboxamide was selected as a model drug due to its suitable solubility in water (15 g/L at 25 °C). Gels in pentan-1-ol were chosen because of their stability and because pentan-1-ol forms a two layered system with water, which made the drug release experiments easy to monitor. Gel samples of imine **3** and pyrazinecarboxamide were treated with water either without or with *p*-toluenesulfonic acid. After 0.5, 1, 2, 4 and 24 h, the water layers were separated off and the amount of the released drug was analysed by NMR measurements. Control experiments, in which pyrazinecarboxamide was dissolved in pentan-1-ol and treated either with water (exp. A) or with acidic water (exp. B), were carried out as well. To check the drug release under non-calm conditions, the samples were treated by ultrasonic for 10 min and after one hour of standing without any additional disturbance, the water layers were checked (in the same way like in the other drug release experiments). The results are summarized in Figures 6 and 7 (for details see ESI, Figure S13 and Table S4).

Due to the fact that water is more dense than the gel from pentan-1-ol, the water put above the gel sample diffused through the gel and thus lifted the gel layer to the top (within the first 2–4 h). Naturally, this water penetration through the gel washed out the major amount of the drug in the first several hours of the experiment as can be seen from Figure 6.

Figure 6. Release of PC from the gel to the water layer under neutral and acidic conditions.

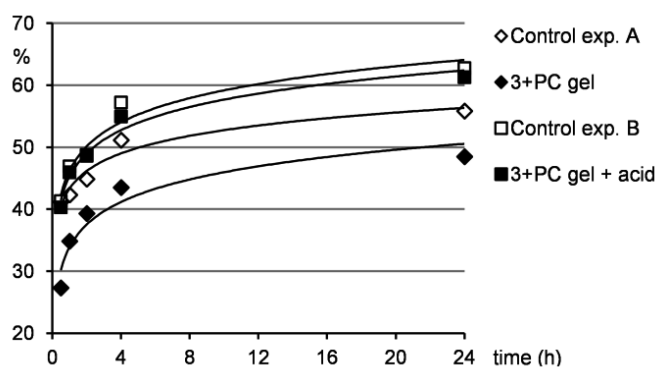
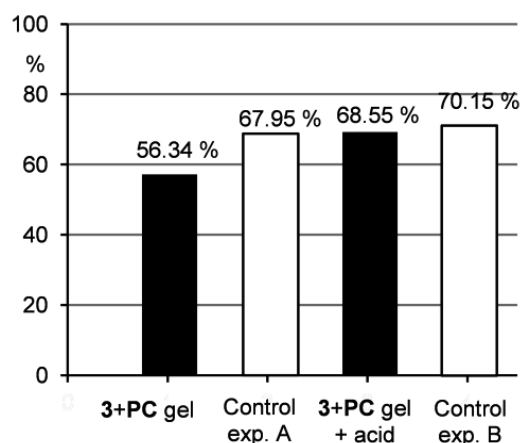


Figure 7. Percentage of PC released to the water layer after ultrasonic treatment.

Furthermore, Figure 6 shows that the drug release from the gel samples was smaller compared to the control experiment A, but still occurred. This indicates that the drug is not strongly integrated to the gel network and can be washed out when water penetrates the gel as described above. Therefore, such system could find potential applications in a slow drug release, where a distribution in small quantities over longer time is needed. Contrarily, when the gel samples were treated with acidic water, the situation changed significantly. Under acidic conditions, a gel-sol transition occurred and as a consequence, the drug release to the water layer was much quicker compared to the situation when the gel was treated only with water. The results of the drug release from the gel under acidic conditions were comparable to those of the control experiment B indicating that the gel hydrolysis and subsequent drug release were efficient. This outcome supports the idea that acid-responsive gel systems with entrapped pharmaceuticals could be effectively used in drug delivery when pH-induced drug release is needed.

3. Experimental

3.1. General

Analytical grade reagents and solvents were used for the synthesis, purification and gelation studies. Cholesteryl chloroformate was purchased from Alfa Aesar (Karlsruhe, Germany), and *p*-phenylenediamine and 2-pyridinecarboxaldehyde from Sigma Aldrich (Steinheim, Germany). 2-pyridinecarboxaldehyde was freshly distilled before use. Pharmaceuticals for the drug release experiments were purchased from Sigma Aldrich (pyrazinecarboxamide and 5-chloro-8-hydroxyquinoline) and from Fluka (Steinheim, Germany) (antipyrine). ^1H and ^{13}C -NMR experiments were run with a Bruker Avance DRX 500 NMR spectrometer equipped with a direct observation BBO probe head working at 500.13 MHz for ^1H and at 125.76 MHz for ^{13}C -NMR spectra were measured in CDCl_3 and the chemical shifts were referenced to the solvent signal ($\delta = 7.26$ ppm for ^1H , and $\delta = 77.0$ ppm for ^{13}C). The numbering of the steroidal part is according to the IUPAC rules. Molecular masses were measured either by using a Micromass LCT ESI-TOF mass spectrometer or by a VG AutoSpace 3500 HR-MS high resolution mass spectrometer. IR spectra were recorded on a Bruker Tensor 27 FTIR spectrometer.

3.2. Preparation of Compound 1

Compound **1** was prepared according a previously reported procedure [49]. The desired product was obtained as a light yellow solid in 34% yield. δ_{H} (500.13 MHz; CDCl_3): 0.68 (3H, s, 18- CH_3), 0.87 (6H, dd, $J = 2.2$; 6.6 Hz, 26- $\text{CH}_3 + 27\text{-CH}_3$), 0.92 (3H, d, $J = 6.5$ Hz, 21- CH_3), 1.03 (3H, s, 19- CH_3), 4.58 (1H, m, 3-CH), 5.39 (1H, m, 6-CH), 6.33 (1H, bs, NH), 6.64 (2H, benzene ring), 7.14 (2H, benzene ring). δ_{C} (125.7 MHz; CDCl_3): 11.86 (C-18), 18.72 (C-21), 19.33 (C-19), 21.06 (C-11), 22.55 (C-27), 22.80 (C-26), 23.84 (C-23), 24.29 (C-15), 28.00 (C-25), 28.14 (C-2), 28.22 (C-16), 31.90 (C-7), 31.91 (C-8), 35.79 (C-20), 36.20 (C-22), 36.59 (C-10), 37.01 (C-1), 38.52 (C-4), 39.52 (C-24), 39.76 (C-12), 42.33 (C-13), 50.04 (C-9), 56.17 (C-17), 56.71 (C-14), 74.66 (C-3), 115.62 (benzene ring), 121.00 (benzene ring), 122.61 (C-6), 129.46 (benzene ring), 139.74 (C-5), 142.59 (benzene ring), 153.58 (C=O); $\nu_{\text{max}}/\text{cm}^{-1}$: 3407, 3332 (NH), 2936, 2866 (CH), 1728 (C=O, -O), 1631 (C=O, -NH), 1525 (NH, bending) and 1208 (C-O); m/z (ES^+) 543.43 ($[\text{M}+\text{Na}]^+$, 100%), 1063.85 ($[\text{2M}+\text{Na}]^+$, 27%); m/z (HR-ESI) 521.4101, $[\text{C}_{34}\text{H}_{52}\text{N}_2\text{O}_2+\text{H}]^+$ requires 521.4102; 543.3933, $[\text{C}_{34}\text{H}_{52}\text{N}_2\text{O}_2+\text{Na}]^+$ requires 543.3921.

3.3. Preparation of Compound 3

Compound **1** (200 mg, 0.384 mmol) was dissolved in dry CH_2Cl_2 (8 mL), then freshly distilled 2-pyridinecarboxyaldehyde (**2**) was added (36.5 μL , 0.384 mmol). The mixture was stirred at rt under N_2 atmosphere for 18 h. After a solvent evaporation, product **3** was obtained as a light orange solid in a quantitative yield (233 mg). δ_{H} (500.13 MHz; CDCl_3): 0.68 (3H, s, 18- CH_3), 0.87 (6H, dd, $J = 2.2$; 6.6 Hz, 26- $\text{CH}_3 + 27\text{-CH}_3$), 0.92 (3H, d, $J = 6.5$ Hz, 21- CH_3), 1.03 (3H, s, 19- CH_3), 4.62 (1H, m, 3-CH), 5.40 (1H, m, 6-CH), 6.73 (1H, bs, NH), 7.30 (2H, benzene ring), 7.34 (1H, pyridine ring), 7.44 (2H, benzene ring), 7.79 (1H, pyridine ring), 8.18 (1H, pyridine ring), 8.62 (1H, s, imine-H), 8.70 (1H, pyridine ring). δ_{C} (125.7 MHz; CDCl_3): 11.85 (C-18), 18.71 (C-21), 19.31 (C-19), 21.05 (C-11), 22.54 (C-27), 22.79 (C-26), 23.84 (C-23), 24.27 (C-15), 27.99 (C-25), 28.09 (C-2), 28.21 (C-16), 31.87 (C-7), 31.90 (C-8), 35.78 (C-20), 36.19 (C-19), 36.58 (C-10), 36.97 (C-1), 38.45 (C-4), 39.51 (C-24), 39.74 (C-12), 42.32 (C-13), 50.02 (C-9), 56.16 (C-17), 56.69 (C-14), 75.08 (C-3), 119.27 (benzene ring), 121.74 (pyridine ring), 122.10 (benzene ring), 122.77 (C-6), 124.91 (pyridine ring), 136.58 (pyridine ring), 137.15 (benzene ring), 139.57 (C-5), 145.97 (benzene ring), 149.66 (pyridine ring), 153.00 (C=O), 154.76 (pyridine ring), 159.21 (C=N); $\nu_{\text{max}}/\text{cm}^{-1}$: 3407, 3332 (NH), 2934, 2866 (CH), 1727 (C=O, -O), 1626 (C=O, -NH), 1524 (NH, bending) and 1225 (C-O); m/z (ES^+) 610.46 ($[\text{M}+\text{H}]^+$, 84%), 632.46 ($[\text{M}+\text{Na}]^+$, 100%), 1241.94 ($[\text{2M}+\text{Na}]^+$, 25%); m/z (HR-ESI) 610.4363, $[\text{C}_{40}\text{H}_{55}\text{N}_3\text{O}_2+\text{H}]^+$ requires 610.4367.

3.4. Gelation Tests

In a typical gelation test a weighed amount of the gelator was mixed with a measured volume of the selected solvent in a sealed 5 mL test tube. The sample was sonicated for *ca.* 2–3 min and then the mixture was heated until the solid was completely dissolved (if soluble). The resulting solution was allowed to slowly cool down to room temperature. Finally the test tubes were inverted to observe if the contents could still flow. Upon cooling down, the formation of gel (G), precipitate (P), or solution (S) was detected.

3.5. NMR Studies

^1H -NMR spectra of imine **3** formation and hydrolysis, ^1H -NMR spectra of imine-drug gels and ^1H -NMR spectra for the drug release experiments were recorded with a Bruker Avance DPX 250 spectrometer equipped with a 5mm 1H/BB inverse detection probe head working at 250.13 MHz for ^1H . Variable-temperature ^1H -NMR spectra of gels were recorded with a Bruker Avance DRX 500 NMR spectrometer equipped with a BBO probe head working at 500.13 MHz for ^1H . The gel samples were prepared directly in an NMR tube; a weighed amount of a gelator, or a mixture of a gelator and drug, was dissolved upon heating in 0.6 mL of DMSO- d_6 , and the gel samples were stabilized overnight. The VT ^1H -NMR experiments were conducted varying the temperature by 10 °C steps. The samples were allowed to stabilize for 5 min at each temperature before acquiring the spectra.

^{13}C CPMAS NMR spectra were recorded with a Bruker AV 400 spectrometer equipped with a 4 mm standard bore CPMAS probe. The dried and finely powdered samples were packed in ZrO₂ rotors. The experiments were carried out at a 10 kHz spinning rate under Hartman-Hahn condition at the contact time being 2 ms with 5 s recycle delay. The number of scans varied between 400–1,000. FIDs were zero filled twice and apodized by 20 Hz exponential window function prior to Fourier transform (FT). The ^{13}C chemical shift was calibrated using a carbonyl signal of a glycine sample at 176.03 ppm as an external standard. Complete lists of acquisition and processing parameters are available by E. K. on request.

3.6. SEM Measurements

Scanning electron micrographs of xerogels were taken on a Bruker Quantax400 EDS microscope equipped with a digital camera. The samples of the xerogels were prepared by placing a hot, clear solution of the gelator on carbon tape over a sample stub. The samples were dried at room temperature and then sputter coated with a thin layer of gold in a JEOL Fine Coat Ion Sputter JFC-1100.

3.7. Drug Release Experiments

The gels of imine **3** and pyrazinecarboxamide (2.8% w/v), prepared in a 1:1 ratio in 0.5 mL of pentan-1-ol ($n_{\text{PC}} = 0.0195$ mmol), were stabilised overnight. Then water (0.5 mL) either without or with *p*-toluenesulfonic acid (0.0053 mmol) was added. The samples stayed without any shaking or other type of disturbance. Water layers (0.4 mL) were separated off at certain times (after 0.5, 1, 2, 4 and 24 h), and after solvent evaporation, they were dissolved in 0.6 mL of D₂O and analysed by NMR with succinic acid (0.0042 mmol) as an internal standard. As control experiments (A and B), pyrazinecarboxamide (0.0195 mmol) was dissolved in pentan-1-ol (0.5 mL), and the samples were treated in the same way as the gel samples (adding of 0.5 mL of water either without or with 0.0053 mmol of *p*-toluenesulfonic acid, and then analysed by NMR with 0.0085 mmol of succinic acid as an internal standard). The percentage of the released drug was calculated from the peak area of drug signals of a sample to the peak area of drug signals of a reference sample which was prepared by dissolving pyrazinecarboxamide (0.0195 mmol) in 0.6 mL of D₂O with succinic acid (0.0085 mmol) as an internal standard.

4. Conclusions

In summary, imine **3** represents the first gelator which forms *in situ* acid-responsive supramolecular gels. The gelator can also be prepared synthetically beforehand. In both cases the resulting gels exhibit the same macroscopic and microscopic properties without any loss by the *in situ* generation of the gel. The modern *in situ* approach to gel formation is still very rare [38–40] but should soon see a tremendous increase in popularity considering the advantages already described for these systems [39]. Besides the shortened gel preparation time integral to all *in situ* formations, the system here described can be controlled by changes in the molar ratio of the components. Another speciality of the described gelator is the imide moiety designed into the structure not just to facilitate the *in situ* formation but also to afford an acid-induced hydrolysis. This acid sensitivity we were able to exploit as a drug release system for different aromatic drugs. The drugs were before embedded into the gel network. In contact with water these two-component gels are slowly releasing the drugs constituting a slow drug release system. Contrarily, by adding acid, the drugs can be instantly released from the gel network. We believe that acid-responsive gels containing imine bonds are promising soft materials with many potential applications in drug delivery and controlled release systems [12–14].

Supplementary Materials

Electronic Supplementary Information (ESI) can be accessed at: <http://www.mdpi.com/1420-3049/18/4/3745/s1>.

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Conflict of Interest

The authors declare no conflict of interest.

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IV

A steroid-based gelator of A(LS)₂ type: tuning gel properties by metal coordination

by

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A steroid-based gelator of A(LS)₂ type: tuning gel properties by metal coordination†

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By utilizing up-to-date knowledge about gelators, we designed and synthesized a novel low-molecular-weight gelator bearing a pyridine-2,6-dicarboxylic acid moiety and two cholesteryl glycinate units. In order to demonstrate the ingenuity of our design, we prepared a series of structurally related compounds and studied their gelation properties. Based on the results, we determined structural features of the gelator molecules which were important for successful gel formation. We showed that the properties of the gel systems (transparency, morphology, *etc.*) can be tuned by coordination with different metal ions, as well as by changing the solvent. Gelators, and their gels and xerogels were studied by combined NMR spectroscopy, X-ray powder and single crystal diffractions, and electron microscopic techniques. Additionally, a systematic investigation of xerogels derived from silver metallogels revealed an *in situ* generation of silver nanoparticles.

Introduction

During the past decade, there has been a substantial increase of interest in the preparation of supramolecular gels.¹ The formation of these gels stems from the spontaneous but controlled self-assembly of low-molecular-weight gelators (LMWGs) into fibrous architectures, where solvent molecules are entrapped by the entangled three dimensional networks.^{1e,2} Supramolecular gels represent smart and functional nanoscale materials with high potential for a wide range of advanced applications, for example in sensors, shape memories, drug delivery devices and displays.³

The use of coordination chemistry as a rational design route toward self-assembled gels is a new, simple and very powerful approach in preparing gels with unusual properties (*e.g.* catalytic and redox activity, conductivity, luminescence, and magnetism).⁴ So far only a few examples of steroid-based metallogels were reported, and they were mostly without

a deeper orientation towards the fine-tuning of gel properties by using different metal ions.

In this work we prepared four molecules (Fig. 1): the gelator **1**, and compounds **2–4**, which were synthesized in order to compare their gelation properties with molecule **1**. The inspiration for the structure of the gelator **1** was a steroid-based molecule **2** of A(LS)₂ type,⁵ recently reported by Fang *et al.*⁶ Based on the knowledge that cholesteryl conjugates with an aromatic central unit can gelate many different organic solvents, we decided to ennoble this idea by incorporating a binding site for metals to the structure. This opened up to us a possibility to tune gel properties and gel formation by coordination with different metal ions. We chose a pyridine-2,6-dicarboxylic acid moiety as a central unit, because it is known to be a good chelating agent for various metals, *e.g.* zinc,⁷ iron,⁸ copper and other transition metals.⁹

Results and discussion

Design of LMWGs

We designed compound **1** to act as a gelator for various organic solvents and to create metallogels with a possibility for tuning gel properties by coordination with different metal ions. The molecule **1** is a gelator of an A(LS)₂ type.⁵ It consists of three parts (Fig. 2): a cholesteryl moiety (S), a pyridine-2,6-dicarboxylic acid group (A), and a glycine residue functioning as a linker (L). The main ideas behind this design are as follows: (1) cholesteryl units can aggregate in solution through van der Waals and hydrophobic interactions;⁵ (2) amino acid moieties have a strong tendency to form hydrogen bonds¹⁰ and increase solubility in polar solvents; (3) pyridine-2,6-dicarboxylic acid components offer a metal-binding site.^{7–9}

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† Electronic supplementary information (ESI) available: Synthetic details; additional photographs of gels; ¹H NMR preliminary coordination studies of **1** and **2**; Job plot of **1** + Ag(I) and **1** + Zn(II); ¹³C NMR spectra of **1** and **2** and their metal complexes; additional SEM and TEM images; analysis of AgNPs; VT ¹H NMR spectra of DMF-d₇ gels; ¹³C CPMAS NMR spectra of **1**; additional data to powder and single crystal diffraction studies of **1**. CCDC 883809. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2sm25259g

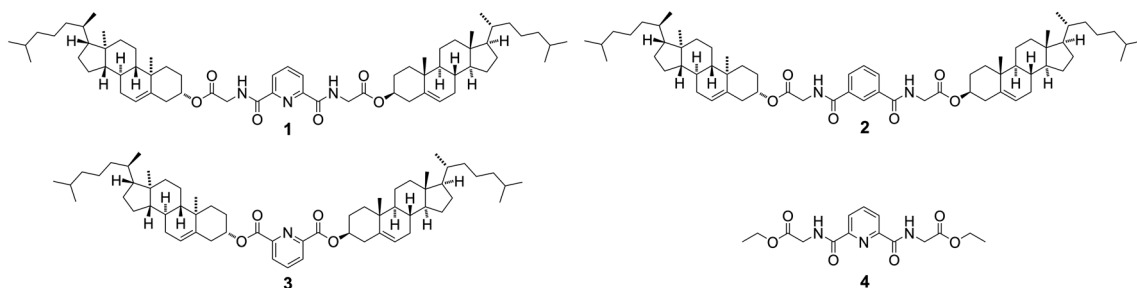


Fig. 1 Structures of compounds 1–4.

In order to investigate the relevance and importance of each part in our design idea (compound **1**), we prepared three reference compounds (**2–4**) – each of them lacking one moiety of **1** in their structures. Compound **2** was prepared with an isophthalic acid moiety as the A unit, substituting the pyridine-2,6-dicarboxylic acid, compound **3** was synthesized without glycine linkers, and compound **4** without steroidal parts. We found that these changes in the chemical composition of the gelator backbones have a big impact on the gelation properties, and are discussed in detail in the gelation test part of this article.

Coordination studies

In the design of gelator **1**, we intentionally introduced a pyridine-2,6-dicarboxylic acid moiety as a central aromatic unit due to its metal-binding potential.^{7–9} As metal cations, we chose Ag(I), Zn(II) and Pd(II)¹¹ ions because of their diamagnetism; thus a complex formation can be easily studied by NMR experiments, and their different coordination spheres which we expected to result in distinct packing modes of the gelator molecules in a gel phase. As the counteranion, we selected tetrafluoroborate, a weak binding anion, in order to minimize the influence of the anion on the complex formation. In order to check coordination properties of prepared compounds and to determine the ligand–metal ratio for gelation tests, we carried out a Job plot and NMR titration experiments (see ESI, Fig. S1 and S2†). Based on the results, the stoichiometry of metal complexes was determined, 1 : 1 for the **1** + Ag(I) complex, and 2 : 1 for the **1** + Zn(II) complex (see ESI, Fig. S3 for Ag(I) and Fig. S4 for Zn(II)†). Unfortunately, we were not able to obtain the ligand–metal ratio for the **1** + Pd(II) complex due to complicated NMR spectra (see ESI, Fig. S7†). For gelator **2**, we carried out similar experiments and ¹³C NMR (see ESI, Fig. S2 and Table S1†, respectively, and Table 1). For the **2** + Zn(II) system, we did not see any changes in

chemical shift values compared to the ¹H and ¹³C NMR spectra of **2**, indicating no coordination. For the silver ions, we observed deshielding of C-5 and C=O, and shielding of C-6. However, we did not see any shifts of signals in the ¹H NMR spectrum, showing that there are only weak interactions between Ag(I) ions and the cholesterol double bond as well as the carbonyl groups.

To determine the exact metal binding site of the gelator **1**, we carried out ¹³C and ¹⁵N NMR experiments in a CDCl₃–CD₃OD (12 : 1) solvent mixture (see ESI, Fig. S8, S9 and Table S1†). Results, listed in Table 1, show that the main coordination interactions are between pyridine nitrogen atoms and the metal ions. However, the evidence of other supporting interactions by surrounding carbonyl or amide groups is not conclusive. Unfortunately, we were not able to obtain any single crystals of the metal complexes for XRD analysis to confirm our observations.

Gelation tests

The gelation abilities of compounds **1–4** were tested for 16 different solvents including alcohols, aromatic solvents, DMF and water in a concentration of 2.0% (w/v), in the absence and presence of various metal ions. The results are summarized in Table 2. While compounds **1** and **2** can gelate most of the tested alcohols and some other solvents, compound **3** is a poor gelator and compound **4** does not form gels at all. Based on these results, the presence of a cholesterol unit is obviously crucial for gel formation. Furthermore, the gelation tests of compound **3** prove that the glycine unit is very important too (as a hydrogen bond acceptor/donor, and perhaps as a metal binding site).

In order to tune the gel properties, we examined the gelation abilities of the metal complexes of compounds **1–4**. As metal ions, we used Ag(I), Zn(II), and Pd(II) in stoichiometry determined by a Job plot for the gelator **1**, *i.e.* 1 : 1 for Ag(I) and 2 : 1 for Zn(II) complexes. For Pd(II) ions, the stoichiometry was assigned as 4 : 5 based on the potential metal binding sites of compound **1**. As we had expected, the presence of metal ions had a significant influence on the gelation properties of gelator **1**, and in addition the gelation ability of compound **3** improved slightly. Most interestingly, the opaque gels of **1** in alcohols turned to clear transparent gels in the presence of Ag(I) or Zn(II) ions (Fig. 3a–c) and the morphology of their xerogels was different (for details see the SEM measurement part of this article). For the **1** + Pd(II) complex, precipitation of palladium black upon heating was found and under these conditions no gel was formed. As expected, no major visual change was observed in the case of compound **2** (Fig. 3d and e), as the gelator **2**

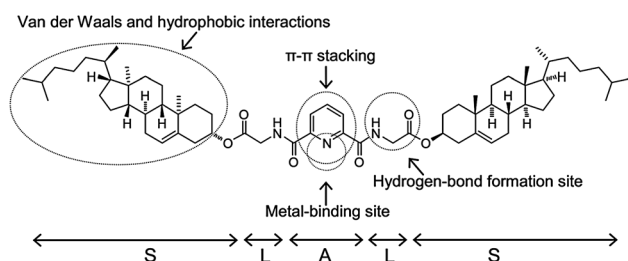
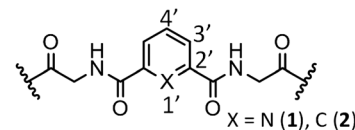


Fig. 2 Structure of compound **1**, a gelator of A(LS)₂ type, with marked non-covalent interaction sites.

Table 1 Chemical shifts of selected signals of ^{13}C and ^{15}N NMR spectra of compounds **1** and **2**, and their metal complexes

	1	1 + Ag(I)	1 + Zn(II)	2	2 + Ag(I)	2 + Zn(II)
^{13}C NMR						
C=O (Gly)	169.51	169.58	169.38	169.55	169.60	169.54
C=O	163.97	164.37	163.94	167.27	167.45	167.24
C-1'	—	—	—	125.44	125.40	125.23
C-2'	148.18	148.17	147.73	133.85	133.86	133.86
C-3'	125.11	125.23	125.18	130.61	130.59	130.61
C-4'	138.92	139.75	139.21	128.93	128.95	128.92
C-5 (chol.)	139.23	139.45	139.21	139.23	139.58	139.23
C-6 (chol.)	122.91	122.28	122.91	122.88	122.45	122.88
C (Gly)	41.45	41.60	41.56	41.88	41.91	41.88
^{15}N NMR						
N-1'	-90.5	-95.1	-91.4			
N (amide)	-282.6	-280.3	-282.6			



is lacking a strong metal-binding site in its structures and based on the results of ^1H and ^{13}C NMR experiments (Table 1 and Fig. S2†), does not create strong complexes with tested metal ions. Surprisingly, the gels of **2**, especially in aromatic solvents were a little bit weaker in the presence of metal ions, which might be explained as the negative influence of a metal salt on the gel packing mode. The gelation abilities of **4** did not improve in the presence of Zn(II) ions.

The gelators **1** and **2** create gels in alcohols in the presence or absence of metal ions, but the process of gelation is very slow. Several hours or even overnight is needed for the effective gelation of the solvents. For the DMF system of **1**, however, the gel can be formed within several minutes and the gels are transparent (Fig. 3f–h), and the time and temperature stable. There was no liquid separated from the gels after being kept for four months in a closed test tube. The minimum gelation concentration (MGC) was less than 0.3% (w/v): 0.3% for **1** in DMF, and 0.25% for **1** + Ag(I) and **1** + Zn(II) in DMF, which means that these systems can be called “supergels” (MGC < 1%

(w/v)). Thus, the DMF systems of **1**, together with the pentan-1-ol systems of **1** and **2**, were selected and have been studied in detail in order to obtain a better understanding of the gel formation process.

Water is the most interesting solvent for gelling with regard to potential applications³ (tissue engineering, drug delivery systems, *etc.*). Unfortunately, none of the tested compounds was soluble in water, and no hydrogel was formed. Compound **1**, however, can gel DMF–water mixtures up to 30% of water. The addition of more than 30% of water results in precipitation.

The gel-to-sol transition temperature (T_{gel}) is an important parameter and is often used to denote the gel stability. T_{gel} was measured for the gel systems of **1** in DMF and pentan-1-ol (Fig. 4). As expected, the values increase with concentration. More interestingly, the gels of metal complexes of **1** are more stable, than the corresponding gel systems of **1**, although the differences in the values are not significant. The better thermal stability of the metalogels could be explained by an increased density of the gelator network due to coordination.

Table 2 Gelation properties of compounds **1–4**^a

2% w/v	1	1 + Ag ⁺	1 + Zn ²⁺	1 + Pd ²⁺	2	2 + Ag ⁺	2 + Zn ²⁺	3	3 + Zn ²⁺	4	4 + Zn ²⁺
Benzene	pCG	S	S	P	OG	pOG	pOG	pOG	pOG	S	P
Toluene	S	S	S	P	OG	pOG	pOG	pOG	pOG	S	P
CH ₂ Cl ₂	OG	S	pCG	pOG	P	P	P	s	pCG	s	P
CHCl ₃	s	s	s	s	s	s	s	s	s	s	P
CCl ₄	pCG	P	P	P	P	P	P	pOG	pOG	S	I
Methanol	I	I	I	Pd(0)	I	I	I	I	I	S	S
Ethanol	I	I	I	Pd(0)	P	P	P	I	I	S	S
Propan-1-ol	OG	P	P	Pd(0)	OG	OG	OG	I	I	S	S
Butan-1-ol	OG	CG	CG	Pd(0)	OG	OG	OG	I	I	S	S
Pentan-1-ol	OG	CG	CG	Pd(0)	OG	OG	OG	I	I	S	S
Hexan-1-ol	OG	CG	CG	Pd(0)	OG	OG	OG	P	P	S	S
Heptan-1-ol	OG	pCG	CG	Pd(0)	OG	OG	OG	P	P	S	S
Octan-1-ol	OG	S	CG	Pd(0)	OG	OG	OG	P	pOG	S	S
Ethane-1,2-diol	I	I	I	I	I	I	I	I	I	S	S
Water	I	I	I	I	I	I	I	I	I	I	P
DMF–H ₂ O (30%)	OG	OG	OG	OG	—	—	—	—	—	—	—
DMF	CG	CG	CG	CG	P	P	P	S	P	I	P

^a Note: S = soluble at boiling point, s = soluble at room temperature, I = insoluble at boiling point, P = precipitated upon cooling, G = gel (O = opaque, C = clear transparent), pG = partial gel, Pd(0) = precipitation of palladium black upon heating.

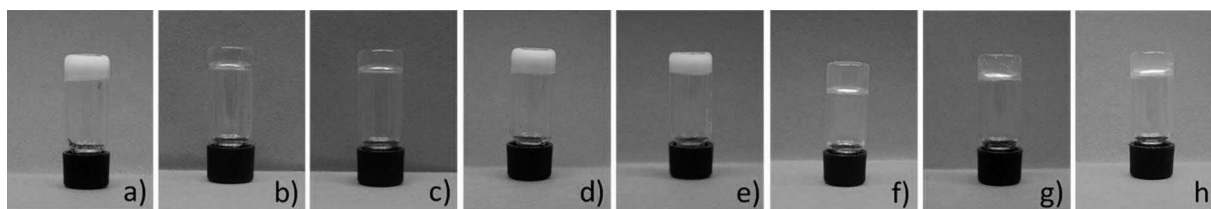


Fig. 3 Photographs of gels of **1** and **2** in an absence and presence of metal ions: (a) **1** in pentan-1-ol, (b) **1** + Ag(I) in pentan-1-ol, (c) **1** + Zn(II) in pentan-1-ol, (d) **2** in pentan-1-ol, (e) **2** + Zn(II) in pentan-1-ol, (f) **1** in DMF, (g) **1** + Ag(I) in DMF, and (h) **1** + Zn(II) in DMF.

SEM and TEM measurements

In order to gain visual insight into the morphology of the gels, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were utilized. The SEM micrographs of the xerogel from pentan-1-ol of compounds **1** and **2**, and from DMF of compound **1** are displayed in Fig. 5 (for more images see ESI†). From the images it can be seen that the microstructures of the xerogel of **1** in the absence of any metal ions (Fig. 5a and b) are very different from those of **1** in the presence of metal ions (Fig. 5d and e). Although the images of the xerogel of **1** show a high density fibrillar network, in the presence of Ag(I) the 3D structure seems to be flatter with hardly distinguished thin fibers, and in the presence of Zn(II) the structure of the xerogel turns from a fibrillar nature to small partly rounded particles. In contrast, the micrographs of xerogels of **2** (Fig. 5c and f) show a similar entangled fibrillar network in the absence and presence of Zn(II) suggesting that the presence of the metal ion has no significant influence on the xerogel morphology which can be expected with regard to the structural nature of molecule **2** (an absence of a strong metal-binding site).

The micrograph of the xerogel of **1** from DMF (Fig. 5g) reveals an entangled three-dimensional network constructed from bundles of fibres and large pores (residues of evaporated solvent molecules). The images of xerogels of **1** + Zn(II) (Fig. 5h) and **1** + Ag(I) (see ESI†) look similar and the porous structure of the xerogels is even more noticeable. The structure of the xerogel

of **1** + Pd(II) is formed from partly rounded particles (Fig. 5i). Without any doubt, not only the absence or presence of metal ions but also the choice of the solvent has an impact on the xerogel morphology which was also confirmed by solid-state NMR and PXRD measurements.

The TEM micrographs were recorded for xerogels from pentan-1-ol of **1**, **1** + Ag(I) and **1** + Zn(II), and are displayed in Fig. 6 (see ESI† for more images). The image of the xerogel of compound **1** shows a high density fibrillar network with an approximate fiber width of 500–700 nm, whereas in the presence of Zn(II) ions, the xerogel is formed by cluster-like microstructures. The above observations are in agreement with the results obtained from SEM measurements. Interestingly, the TEM images of xerogel **1** + Ag(I) revealed an *in situ* formation of silver nanoparticles (AgNPs) in the gel phase. The generation of AgNPs was further supported visually and by UV-spectroscopy, when the metallogel turned to a typical yellow-orange colour after standing on an open bench for several weeks, and exhibited a characteristic plasmon absorption maximum around 430 nm (see ESI, Fig. S10 and S11†). A similar observation, when the silver acts both in a supramolecular gel formation and also in a gel-controlled nanoparticle formation, has been reported recently¹² and the described observations are in agreement with our results. The growth of silver nanoparticles under different conditions (darkness, natural light, UV irradiation, temperature) is in progress in our laboratory.

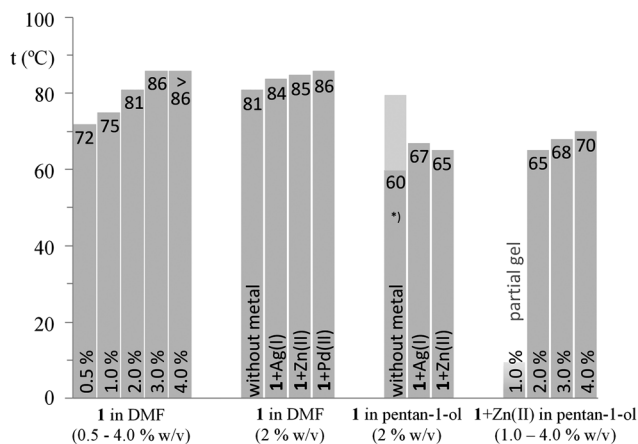


Fig. 4 Gel-to-sol transition temperature (T_{gel}) of gels of **1** in DMF and pentan-1-ol in an absence and presence of metal ions. * The solvent separated from the gel system at 60 °C, but the dried sample stayed at the upper part of the test tube till 80 °C.

Solution NMR measurements

^1H , ^{13}C and ^{15}N NMR experiments were used to study coordination abilities of compounds **1** and **2** with the metal ions (the results are showed and discussed in the “coordination studies” part of this article). Additionally, liquid-state ^1H NMR measurements of DMF- d_7 gels of **1** and **1** + Zn(II) were carried out in order to investigate interactions involved in the gelation process. According to some reports, the well resolved ^1H signals observed in the gel state are presumably from those of non-aggregated molecules, whereas the aggregated structures are NMR silent.¹³ To check this statement, we carried out an experiment, in which ^1H NMR spectra of compound **1** in DMF- d_7 in a non-gelling concentration (0.1% w/v) and a gelling concentration (2% w/v) were recorded (Fig. 7). By comparing the ^1H NMR chemical shift values of signals, we found that the observed ^1H NMR signals in the gel sample corresponds to a non-aggregated state which is in an agreement with the results published earlier.¹³

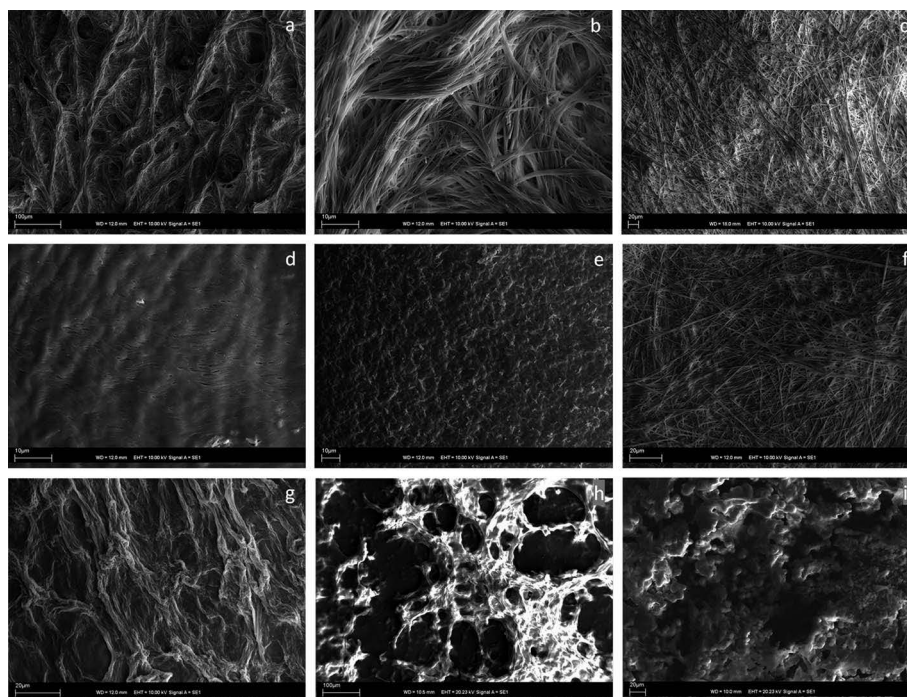


Fig. 5 SEM images of xerogels obtained from the gels of (a) and (b) **1** in pentan-1-ol (2% w/v), (c) **2** in pentan-1-ol (2% w/v), (d) **1** + Ag(I) in pentan-1-ol (2% w/v), (e) **1** + Zn(II) in pentan-1-ol (2% w/v), (f) **2** + Zn(II) in pentan-1-ol (2% w/v), (g) **1** in DMF (1% w/v), (h) **1** + Zn(II) in DMF (1% w/v), and (i) **1** + Pd(II) in DMF (1% w/v).

To study the gelation process of compound **1** in DMF in detail, we recorded variable-temperature ^1H NMR spectra of its DMF- d_7 (2% w/v) gel (Fig. 8, and ESI, Fig. S12–S14 \dagger), and **1** + Zn(II) DMF- d_7 gel (2% w/v, ESI, Fig. S15–S17 \dagger). By heating the sample in steps of 10 °C (30–110 °C), the broad signals gradually turned sharper. The well-resolved spectral patterns were finally observed at 80 °C, suggesting that the gel transfers to the solution state which is in agreement with the results obtained using the “inversion test tube” method for the determination of T_{gel} (Fig. 4, for **1**: $T_{\text{gel}} = 81$ °C and for **1** + Zn(II): $T_{\text{gel}} = 85$ °C). With reference to Fig. 8 (and Fig. S12 in ESI \dagger), the chemical shifts of the signals did not change remarkably with the temperature increase except of those of aromatic protons and of protons of amide groups. The signals of the aromatic and amide protons show downfield shifts upon heating, for signals of proton in a *para*-position from 8.32 ppm at 30 °C to 8.23 ppm at 110 °C. The chemical shift values of N–H shifted from 9.68 ppm at 30 °C to 9.22 ppm at 110 °C. This indicates an increase in the strength of the N–H bond, direct

evidence of the disentanglement of NH from a bonding state. This intermolecular hydrogen-bonding disruption can be also indirectly observed by a small downfield shift of a signal of the α -hydrogens of the glycine unit (see ESI, Fig. S14 \dagger). This shows that the major interactions resulting in the gel formation in DMF certainly are intermolecular hydrogen bonds along with π – π stacking.

Solid-state NMR and XRD measurements

Solid state NMR spectroscopy has emerged a powerful and complementary tool to X-ray crystallography to study crystalline solids, semisolids and liquid crystalline materials.¹⁴ In order to ascertain the molecular packing of the gelator **1** and its metal complexes, we carried out cross-polarization magic angle spinning (CPMAS) NMR and X-ray crystallographic experiments. We recorded ^{13}C CPMAS NMR spectra of compound **1** crystallized from CHCl_3 as well as spectra of its xerogels from pentan-1-ol (Fig. 9, and ESI, Fig. S18–S21 \dagger) and compared

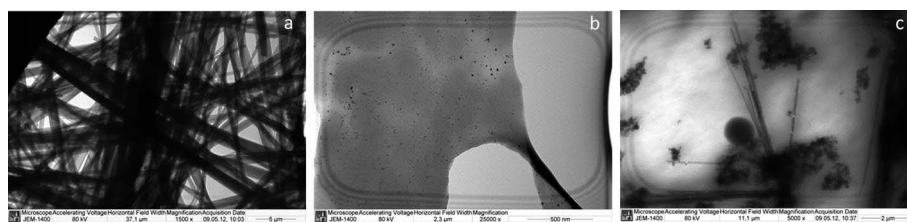


Fig. 6 TEM images of xerogels obtained from the gels of (a) **1** in pentan-1-ol (2% w/v), (b) **1** + Ag(I) in pentan-1-ol (2% w/v), and (c) **1** + Zn(II) in pentan-1-ol (2% w/v).

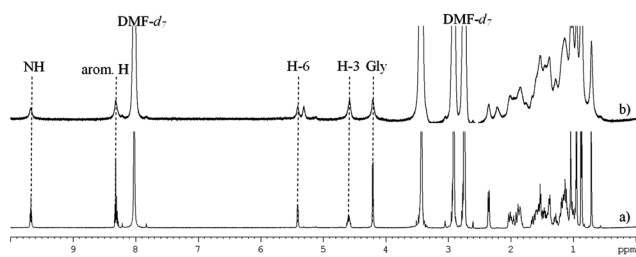


Fig. 7 ^1H NMR spectra of (a) compound **1** in DMF-d_7 at non-gelling concentration, and (b) DMF-d_7 gel of **1** (2% w/v).

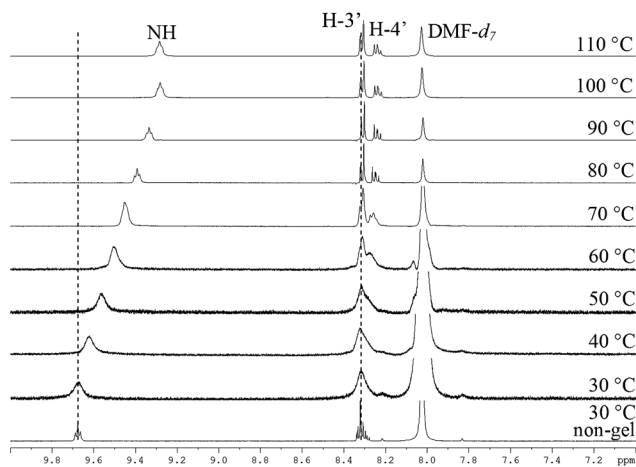


Fig. 8 ^1H NMR spectra of **1** in DMF-d_7 at non-gelling concentration, and as a DMF-d_7 gel (2% w/v) at different temperatures (30–110 °C).

them with the data obtained from the low-angle XRD measurements of the same samples (Fig. 10). A careful analysis of ^{13}C CPMAS NMR spectra of the synthetic solid (Fig. 9a)

and its xerogel from pentan-1-ol (Fig. 9b) showed some similarities suggesting similar molecular packing. The broad signals in the spectrum of the xerogel of **1** + Ag(I) from pentan-1-ol (Fig. 9c) indicates an amorphous nature of the sample which is in agreement with data obtained from the SEM and TEM measurements (Fig. 5d and 6b) and X-ray powder diffraction analysis (Fig. 10c). The ^{13}C CPMAS NMR spectrum of the xerogel of **1** + Zn(II) from pentan-1-ol (Fig. 9d) shows some crystalline nature and deviates from the others indicating very different packing mode.

For DMF gel systems of compound **1**, intermolecular H-bonds and π - π stacking were found to be the main driving forces of gel formation (see results of the VT ^1H NMR measurements of DMF-d_7 gels). This observation was supported by a single crystal X-ray structure which is shown in Fig. 11 (CCDC number 883809, see ESI† for details). The crystal was obtained by very slow evaporation of the solvent from the DMF gel of **1** + Ag(I) . The obtained structure is a DMF solvate and surprisingly does not contain silver. On the other hand this observation is in agreement with X-ray powder diffraction data from xerogels obtained from DMF-gels of **1**, **1** + Ag(I) , and **1** + Zn(II) , all of which showed very similar patterns indicating similar packing mode (see ESI, Fig. S22†). This also explains the similar properties of the gel systems (T_{gel} , morphology, *etc.*) and shows that the presence of the metal ions does not play as important a role in the gelation process of DMF as in the pentan-1-ol gel systems, because the stronger H-bonds between the solvent and gelator molecules rule out metal coordination.

According to the results, it is without doubt that the molecular packing is highly dependent on the solvent and in the case of pentan-1-ol gels also on the presence/absence of metal ions which reflects on different properties of the gel systems (morphology, transparency, *etc.*).

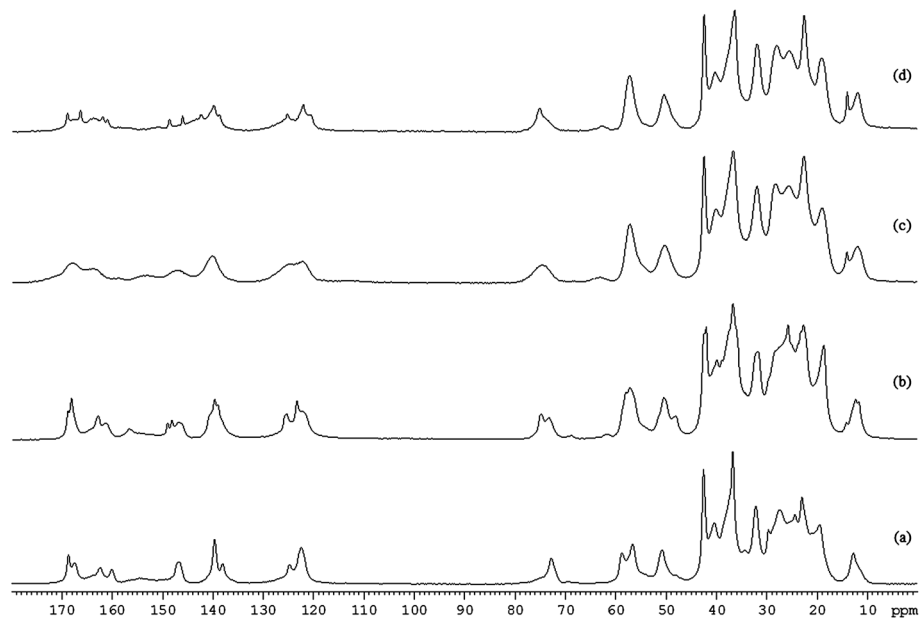


Fig. 9 ^{13}C CPMAS NMR spectra of (a) solid of **1** from CHCl_3 , (b) xerogel of **1** from pentan-1-ol, (c) xerogel of **1** + Ag(I) from pentan-1-ol, and (d) xerogel of **1** + Zn(II) from pentan-1-ol.

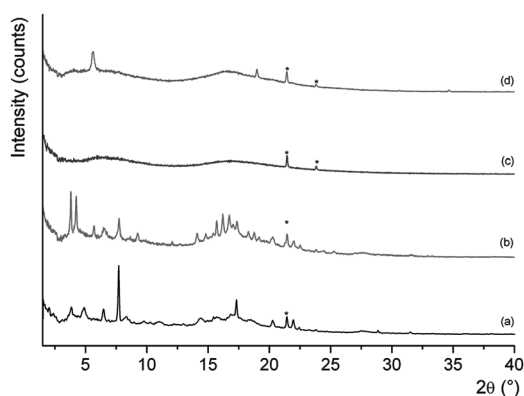


Fig. 10 XRD patterns of (a) solid of **1** from CHCl_3 , (b) xerogel of **1** from pentan-1-ol, (c) xerogel of **1** + Ag(I) from pentan-1-ol, and (d) xerogel of **1** + Zn(II) from pentan-1-ol. Note: * = diffraction peaks originating from an adhesive.

Conclusions

A novel low-molecular-weight gelator of the A(LS)_2 type was designed and prepared. The gelation properties of the gelator and structurally related compounds were studied in various solvents. We found that a slight change in the chemical composition of the gelator backbone can significantly change the gelation abilities. Furthermore, we studied the gelation process in the absence and presence of various metal ions. We reported that not only the choice of solvent, but also the coordination of different metal ions had an influence on the visual properties as well as on the morphology and size of aggregates of the gel systems. In addition, silver metalloids formed *in situ* silver nanoparticles, which possess some potential in pharmacy. This work showed the possibility of using metal coordination as a powerful method for tuning gel properties. Due to this successful work, we plan to prepare more steroidal metalloids in the future and study their properties with regard to potential applications.

Experimental

Synthesis

Cholesteryl glycinate – which was prepared by employing a classic peptide coupling procedure using DCC and DMAP, and a 20% solution of piperidine in DMF for removing the Fmoc-group – was used as an initial building block for the synthesis of **1** and **2**. The coupling with the aromatic central unit was done by

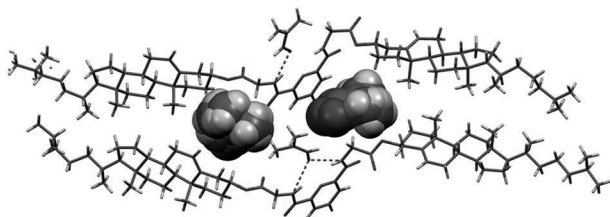


Fig. 11 The asymmetric unit of a DMF solvate of **1** with marked H-bonds between the amide groups of **1** and the solvent molecules. The DMF molecules are filling two distinct infinite voids running in the directions of the *a*-axis.

reaction with 2,6-pyridinedicarbonyl dichloride or isophthaloyl dichloride in dry dichloromethane in the presence of triethylamine. Compounds **3** and **4** were prepared from different starting materials utilizing the same reaction procedure. All these reactions were achieved in good yields (>85% after purification) and all compounds were characterized by standard analytic methods (see ESI† for full characterization and reaction details).

Coordination studies

The Job's method of continuous variations was used for **1** + Ag(I) and **1** + Zn(II) in CDCl_3 – CD_3OD (5 : 1), and for **1** + Pd(II) in CDCl_3 – CD_3CN (3 : 1), for different ligand–metal ratios for the constant total concentration of 0.016 M. ^1H NMR chemical shift titrations in CDCl_3 – CD_3OD (2 : 1) were carried out by varying the concentration of the metal ion for a fixed concentration of the ligand (0.008 M). ^{13}C and ^{15}N NMR spectra were recorded for compound **1**, **1** + Ag(I) (1 : 1) and **1** + Zn(II) (2 : 1) in CDCl_3 – CD_3OD (12 : 1).

Gelation studies

In a typical gelation test a weighed amount of the gelator was mixed with a measured volume of the selected solvent in a sealed test tube. The sample was sonicated for *ca.* 2–3 min and then the mixture was heated until the solid was completely dissolved (if soluble). The resulting solution was allowed to cool down to room temperature. Finally the test tube was inverted to observe if the content could still flow. Upon cooling down, the formation of a gel (G), precipitate (P), or solution (S) was detected. The minimum gelation concentration (MGC) was determined by scaling a minimum amount of gelator needed for formation of a stable gel. The gel-to-sol transition temperature (T_{gel}) was measured using an “inversion tube” method, two times for each sample. Gels, prepared to the sealed test tubes and stabilized overnight at rt, were placed upside-down on a water bath and slowly heated ($2\text{ }^\circ\text{C min}^{-1}$). The temperature at which the gel fell under gravity was recorded as the gel-to-sol transition temperature (T_{gel}).

SEM and TEM measurements

Scanning electron micrographs of xerogels were taken on a Bruker QuantaX400 EDS microscope equipped with a digital camera. The samples of the xerogels were prepared by placing a hot, clear solution of the gelator on carbon tape over a sample stub. The samples were dried at room temperature and then sputter coated with a thin layer of gold in a JEOL Fine Coat Ion Sputter JFC-1100.

Transition electron micrographs of pentan-1-ol xerogels were acquired using a JEOL JEM-1400 Electron Microscope. The samples of the xerogels were prepared by placing a gel or a hot clear solution of the gelator on a grid and were dried at room temperature.

NMR measurements

Variable-temperature ^1H NMR spectra of gels were recorded with a Bruker Avance DRX 500 NMR spectrometer equipped with a 5 mm diameter broad band inverse probe head working at

500.13 MHz for ^1H . Samples were prepared directly in an NMR tube; a weighed amount of the gelator was dissolved upon heating in 0.6 mL of DMF- d_7 , and gel samples were stabilized overnight. The VT ^1H NMR experiment was conducted varying the temperature by 10 °C steps. The sample was allowed to stabilize for 5 min at each temperature before acquiring the spectrum.

The ^{13}C CPMAS NMR spectra were recorded on a Bruker AV 400 spectrometer equipped with a 4 mm standard bore CPMAS probe head whose X channel was tuned to 100.62 MHz for ^{13}C and the other channel was tuned to 400.13 MHz for broad band ^1H decoupling. The dried and finely powdered samples were packed in the ZrO_2 rotor closed with Kel-F cap and spun at 10 kHz. The ^{13}C CPMAS NMR was carried out for all samples under Hartmann–Hahn conditions with TPPM decoupling. Glycine was used as a reference standard for ^{13}C chemical shifts.

Powder and single crystal diffraction studies

The X-ray powder diffraction data of xerogels were measured with a PANalytical X'Pert PRO diffractometer in Bragg–Brentano geometry using Johansson monochromatized Cu $K\alpha_1$ radiation (1.5406 Å; 45 kV, 30 mA). As received fine powder samples were prepared on a silicon-made zero-background holder using petrolatum as an adhesive. The data acquisition was made from a spinning sample by X'Celerator detector in the 2θ range of 1–50° with a step size of 0.017°, counting times of 480 s per step.

The single crystal data for gelator **1** crystallized from DMF were collected at 150.0 ± 0.1 °C (Oxford Cryostream) with Agilent Supernova dual wavelength diffractometer, using a micro-focus X-ray source and multilayer optics monochromatized Cu $K\alpha$ radiation ($\lambda = 1.54184$ Å; 50 kV, 0.8 mA). The data collection, reduction, multi-scan and analytical face-index based absorption corrections were made by program Crystallis^{Pro}.¹⁵ The structure was solved with program Olex2 (ref. 16) (see ESI† for more details).

Acknowledgements

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Salo for recording SEM images, and Spec. Lab. Tech. Paavo Niutanen, Department of Biological and Environmental Science, University of Jyväskylä, for his help with recording TEM images.

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V

Subcomponent self-assembly: a quick way to novel metallogels

by

Hana Bunzen, Nonappa, Elina Kalenius, Sami Hietala & Erkki Kolehmainen

manuscript

Subcomponent self-assembly: a quick way to novel metallogels

Hana Bunzen,^{*[a]} Nonappa,^[b] Elina Kalenius,^[a] Sami Hietala^[c] and Erkki Kolehmainen^[a]

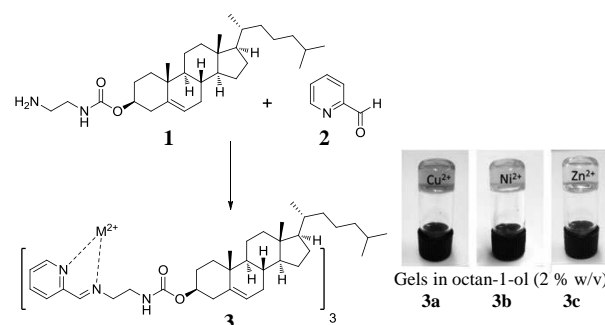
Subcomponent self-assembly, introduced by the Nitschke group,^[1] is a process which allow complex structures to be generated from simple building blocks (generally aldehydes and amines). In this bottom-up approach, the building blocks spontaneously self-assemble around templates (usually metal ions) leading to a simultaneous covalent (C=N) and dative (N-metal) bonds formation. The method has been successfully used to construct well-defined metal-organic macrocycles, helicates, catenanes, rotaxanes, grids,^[2] and cages.^[3] Our field of interest lies not in building-up of defined structures but in designing gelator molecules for a formation of supramolecular gels as functional nanomaterials. Herein, we report on a facile and quick method which leads to an *in situ* preparation of supramolecular metallogels by applying the concept of subcomponent self-assembly. We found that by utilizing this approach, multistimuli-responsive metallogels become easily accessible and tunable.

Metallogels (or metal-organic gels)^[4] belong to the group of supramolecular gels,^[5] *i.e.* gels formed by self-assembly of small molecules, often called low-molecular-weight gelators (LMWGs), into a three-dimensional network with solvent molecules immobilized inside the structure.^[5d, 6] In contrast to conventional supramolecular gels, these gels are formed by gelator molecules containing metal ions. This makes them very attractive materials regarding potential applications, because by incorporating metals into gels, properties such as luminescence, magnetism, catalytic and redox activity can be introduced.^[4a, 4b]

In a conventional gel preparation, gelator molecules are synthesized beforehand and then dissolved in a suitable solvent usually upon heating. This process is time and resource demanding. Therefore, new and simple strategies in a gel preparation are needed. Recently, there were several reports on gels formed by molecules which were synthesized directly in a gelling solvent from suitable components.^[7] This approach of *in situ* gelator synthesis undoubtedly saves time and resources. The subcomponent self-assembly naturally is a good way to apply the *in situ* approach to metallogels. It offers indisputable advantages including (i) shortening synthesis, (ii) gelation at ambient temperature (if components are soluble in the gelling solvent), and most importantly (iii) easy accessibility of a wealth of gel systems by facile exchange of one of the reaction components (or more). In this article we focus on the fine-tunability of the gels by exchanging the metal ions.

The design of a gelator molecule for the subcomponent self-assembly is based on utilizing suitable imine bond precursors. To form strong metal-binding sites, the use of 2-pyridine-carboxaldehyde or its derivative is very convenient because the nitrogen atom of the pyridine unit together with the nitrogen atom from the newly formed imine group constitutes a strong bidentate ligand similar to 2,2'-bipyridine. Therefore, the amine moiety is generally easier to modify and tune for our purpose because the

above mentioned ligand will form with nearly every primary amine. Instead of using rigid di- or triamines like it is usually done in the construction of defined metal-organic compounds,^[2, 3] we used a monoamine attached to a steroidal part responsible for forming non-covalent interactions between the gelator molecules resulting in the formation of a three-dimensional network. In this work commercially available 2-pyridinecarboxaldehyde (**2**), synthetically easily accessible steroidal amine (**1**), and three divalent metal ions, namely Cu(II), Ni(II), and Zn(II), were used as the reactants (Scheme 1). After mixing these components in a 3:3:1 ratio, a gel formation in higher alcohols, selected aromatic solvents and tetrachloromethane was observed (Table 1). Remarkably, the gelation occurred at ambient temperature without the need of a heating/cooling cycle otherwise commonly used in a gel preparation. Interestingly, the order of the components, in which they were added, was not crucial and in all cases the addition of the final component acted as a chemical stimulus triggering the gel formation (Scheme S1 in the Supporting information).



Scheme 1. Preparation of gelators **3a-c** via subcomponent self-assembly.

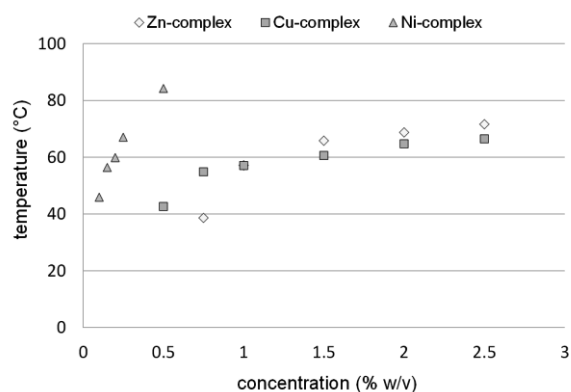
We observed that the rate of the gel formation depends on the type of the used metal and decreases in the order Zn>Cu>>Ni. The sol-to-gel transition was monitored in detail by time sweep rheological measurements (Fig. S12 in the Supporting information). Upon aggregation leading to gelation, there was a rapid increase in G' and G'' and instantaneous decrease in the phase angle to $\sim 20^\circ$. Based on the results, the gelation time was revealed as 15, 18 and 55 min for gels of **3c**, **3a** and **3b**, respectively, which is a relatively quick process. However, upon an ultrasonic treatment, the gel formation was much faster and the gelation time reduced to several minutes (10 min for **3b**) or even seconds (50 s for **3c** and 90 s for **3a**). Additionally, we investigated the rheological properties of the gels by frequency sweep and stress sweep experiments which revealed the predominant nature of the elastic modulus G' , which was found to be higher than loss modulus G'' , confirming the systems under investigation are viscoelastic solids (see the Supporting information for details).

Table 1. Gelation tests of **3a-c** (2 % w/v).^[a, b]

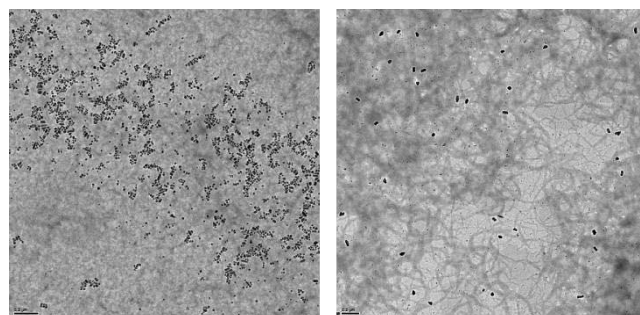
Solvent	1	2	1+2	3a	3b	3c
benzene	pCG ₍₃₀₎	S	S	S	pCG ₍₄₀₎	S
toluene	pCG ₍₃₀₎	S	S	S	pCG ₍₄₀₎	S
CHCl ₃	S	S	S	S	S	S
CCl ₄	pCG ₍₅₀₎	S	S	S	pCG ₍₂₅₎	pCG ₍₃₀₎
methanol	S	S	P	P	P	P
butan-1-ol	S	S	S	P	P	P
pentan-1-ol	S	S	S	OG ₍₁₅₎	CG _(5,0)	OG ₍₁₅₎
hexan-1-ol	S	S	S	OG ₍₁₀₎	CG _(2,5)	OG ₍₁₀₎
heptan-1-ol	S	S	S	CG _(7,5)	CG _(2,5)	CG ₍₁₀₎
octan-1-ol	S	S	S	CG _(5,0)	CG _(0,7)	CG _(7,5)
water	I	S	I	I	I	I
DMF	P	S	S	S	S	S
DMSO	OG ₍₁₅₎	S	P	S	S	S

[a] S = solution, P = precipitate upon cooling, CG = clear transparent gel, OG = opaque gel, pG = partial gel, I = insoluble at the solvent boiling point. [b] Values in parentheses denote a minimum gelation concentration (MGC, g/L).

We observed that visual, mechanical and thermal stability properties of the metallogels were dependent on both the solvent and the metal ions used. The higher the alcohol, the stronger and thermally more stable gels were formed (Fig. S2 in the Supporting information). Whereas the Cu(II) and Zn(II)-gels obtained from pentan-1-ol and hexan-1-ol were opaque, the gels formed in heptan-1-ol and octan-1-ol, and the Ni(II)-gels were transparent (Fig. S1 in the Supporting information). The Cu(II)-complex (**3a**) forms green gels with similar properties (meaning gel-to-sol transition temperature T_g and minimum gelation concentration MGC) to that of the Zn(II)-complex (**3c**). In comparison, the Cu(II)-gels formed in octan-1-ol (with MGC of 0.50 % w/v) are more stable in lower concentrations, whereas the Zn(II)-gels (with MGC of 0.75 % w/v) are more stable at higher concentrations (Fig. 1 and Fig. S3 in the Supporting information), although, the differences are not too significant. Contrarily, the gels formed by the Ni(II)-complex (**3b**) in higher alcohols are more thermally stable compared to the other two systems. For instance, the gel-sol phase transition temperature of its 2 % w/v gels in higher alcohols is higher than 95 °C (Fig. S2 in the Supporting information). Moreover, the Ni(II)-complex was found to be an excellent gelator for octan-1-ol with MGC of 0.07 % w/v (*i.e.* 0.7 mg in 1 mL), which makes the system one of the best metal-organic gels ever reported (regarding MGC).

Figure 1. Phase diagram of gels of **3a-c** in octan-1-ol.

In order to study the morphology of the metallogels, transmission electron microscopy (TEM) measurements were carried out for the octan-1-ol gels. A careful analysis of TEM micrographs (Fig. 2, and Fig. S17 in the Supporting information) revealed a highly entangled three-dimensional fibrillar network of xerogels of **3b** and **3c**. However, electron micrographs obtained from **3a** showed aggregated bundles of spherulite like structures interconnected to each other. Interestingly, an unusual formation of metal nanoparticles (for **3b** and **3c**) was observed when a mixture of components was heated during the gel preparation. These preliminary results, supported by previous reports,^[8] indicate that under heating and aging of metallogels, an *in situ* generation of metal particles can take place.

Figure 2. TEM micrographs of 1 % w/v gel of **3b** (right) and of ten times diluted 1 % w/v gel of **3b** (left) revealing a nanoparticle formation; bar indicates 0.2 μm .

The gelators were designed as 3:1 complexes (ligand-metal ratio) and the formation of these gelator molecules was confirmed by mass spectrometry measurements (Fig. S14-S16 in the Supporting information). Interestingly, an effective gelation occurred only when we used the ligand-metal ratio of 3:1, whereas, higher amounts of metal used did not lead to a gel formation (Fig. S4 in the Supporting information). The systems of different ligand-metal ratios, namely 3:1, 2:1 and 1:1, were studied by mass spectrometry measurements (Fig. S13-S16 in the Supporting information). The mass spectra measured from dissolved gel samples clearly show the abundance of the 3:1 metal complexes decreasing as the ligand to metal ratio (and gelling ability) is reduced, and revealed a formation of non-gelling metal complexes 2:1 and 1:1 instead. Additionally, it was observed that after an addition of excess amount of a metal salt caused a gel-to-sol transition. However, the formation of the non-gelling

Supporting information for

Subcomponent self-assembly: a quick way to novel metallogels

Hana Bunzen, Nonappa, Elina Kalenius, Sami Hietala and Erkki Kolehmainen

Synthesis

General

Analytical grade reagents and solvents were used for the synthesis, purification and gelation studies. Cholesteryl chloroformate was purchased from Alfa Aesar, ethane-1,2-diamine from J.T. Baker, and 2-pyridinecarboxaldehyde from Sigma Aldrich. 2-Pyridinecarboxaldehyde was freshly distilled before use. ^1H and ^{13}C NMR experiments were run with a Bruker Avance DRX 500 NMR spectrometer equipped with a direct observation BBO probehead working at 500.13 MHz for ^1H and at 125.76 MHz for ^{13}C . NMR spectra were measured in CDCl_3 and the chemical shifts were referenced to the solvent signal ($\delta = 7.26$ ppm for ^1H , and $\delta = 77.0$ ppm for ^{13}C from internal TMS). The numbering of the steroidal skeleton is according to the IUPAC rules. Molecular masses were measured either by using a Micromass LCT ESI-TOF mass spectrometer or by a VG AutoSpace 3500 HR-MS high resolution mass spectrometer. IR spectra were recorded on a Bruker Tensor 27 ATR FTIR spectrometer.

Preparation of compound 1

To a solution of ethane-1,2-diamine (2.54 mL, 47.011 mmol, 20 eq.) and dry triethylamine (0.33 mL, 2.366 mmol, 1 eq.) in dry dichloromethane (30 mL) at 0 °C, a solution of cholesteryl chloroformate (1.00 g, 2.352 mmol, 1 eq.) in dry dichloromethane (30 mL) was added dropwise. The mixture was stirred at ambient temperature under nitrogen atmosphere for 18 h. The formed precipitate was filtrated off and the filtrate was washed four times with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness to give the desired product as a white solid in 96 % yield (1.072 g). δ_{H} (500.13 MHz; CDCl_3): 0.68 (3H, s, 18- CH_3), 0.86 (6H, dd, $J = 2.2$; 6.6 Hz, 26- CH_3 + 27- CH_3), 0.91 (3H, d, $J = 6.6$ Hz, 21- CH_3), 1.01 (3H, s, 19- CH_3), 2.81 (2H, t, $J = 5.8$ Hz, ethylenediamine), 3.21 (2H, m, ethylenediamine), 4.50 (1H, m, 3-CH), 4.94 (1H, bs,

NH), 5.37 (1H, m, 6-CH). δ_C (125.7 MHz; CDCl₃): 11.86 (C-18), 18.72 (C-21), 19.32 (C-19), 21.06 (C-11), 22.54 (C-27), 22.79 (C-26), 23.84 (C-23), 24.29 (C-15), 28.00 (C-25), 28.19 (C-2), 28.22 (C-16), 31.90 (C-7), 31.90 (C-8), 35.79 (C-20), 36.20 (C-22), 36.58 (C-10), 37.02 (C-1), 38.59 (C-4), 39.53 (C-24), 39.77 (C-12), 41.83 (ethylenediamine), 42.33 (C-13), 43.74 (ethylenediamine), 50.06 (C-9), 56.18 (C-17), 56.72 (C-14), 74.34 (C-3), 122.46 (C-6), 139.87 (C-5), 156.41 (C=O); $\nu_{\max}/\text{cm}^{-1}$: 3337 (NH), 2937, 2867 (CH), 1715 (C=O, -O), 1696 (C=O, -NH), 1549 (NH, bending) and 1247s (C-O); m/z (ES⁺) 473.33 ([M+H]⁺, 100%); m/z (HR-ESI) 473.4102, [C₃₀H₅₃N₂O₂+H]⁺ requires 473.4107.

Preparation of metal complexes 3a-c

Compound **1**, 2-pyridinecarboxaldehyde (**2**) and a metal salt, namely Cu(ClO₄)₂·6H₂O, or Ni(ClO₄)₂·6H₂O, or Zn(BF₄)₂·6-7H₂O, were mixed in a molar ratio of 3:3:1 in a selected solvent. After mixing the components, a color change was observed – from colorless to a colored solution (Cu²⁺: blue-green, Ni²⁺: yellow, Zn²⁺: light yellow), indicating a complex formation.

Gelation studies

Experimental details

In a typical gelation test the calculated amounts of the components were mixed with the measured volume of the selected solvent in a sealed 5 mL test tube. The sample was shaken (or treated with ultrasound) and if needed also heated (if some of the components were insoluble in the selected solvent at ambient temperature), in order to prepare a clear solution. The resulting solution was allowed to stay without any disturbance and slowly cooled down to room temperature (if the sample was heated). Finally after 30-60 min, the test tubes were inverted to observe if the contents could still flow. Formation of a gel (G), precipitate (P), or solution (S) was detected.

Minimum gelation concentration (MGC) was determined by scaling a minimum amount of gelator needed for a formation of stable gel. The tested concentrations were 5.0, 4.0, 3.0, 2.0, 1.5, 1.0, 0.75, 0.5, 0.25, 0.10, 0.08, 0.07, and 0.06 % w/v.

Photos of gels

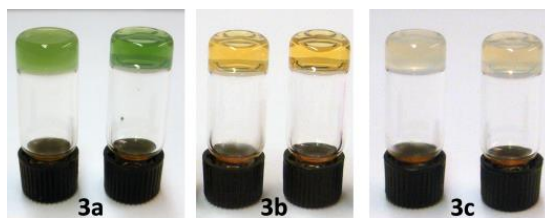
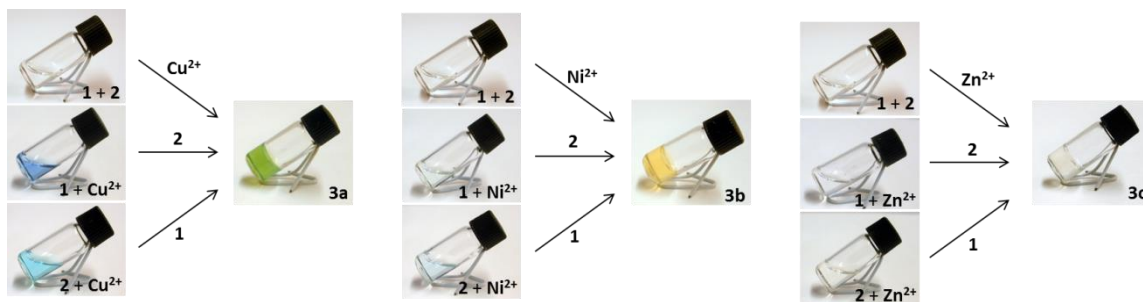


Figure S1. Gels of **3a-c** formed in hexan-1-ol (2 % w/v, left) and octan-1-ol (2 % w/v, right).

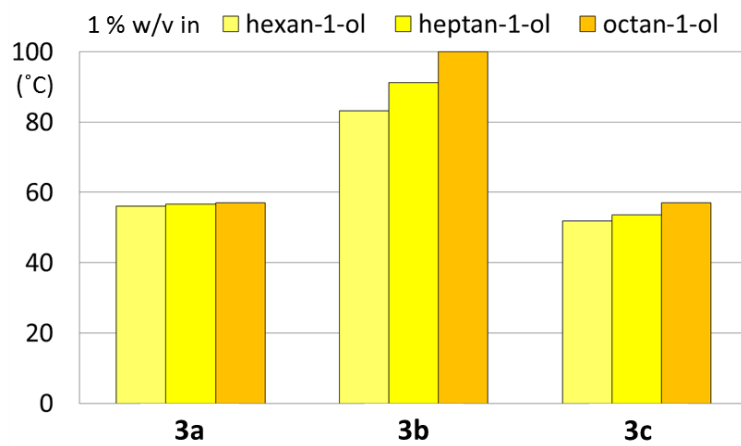
In situ gelation



Scheme S1. *In situ* gelation of **3a-c** in octan-1-ol (1 % w/v) at ambient temperature.

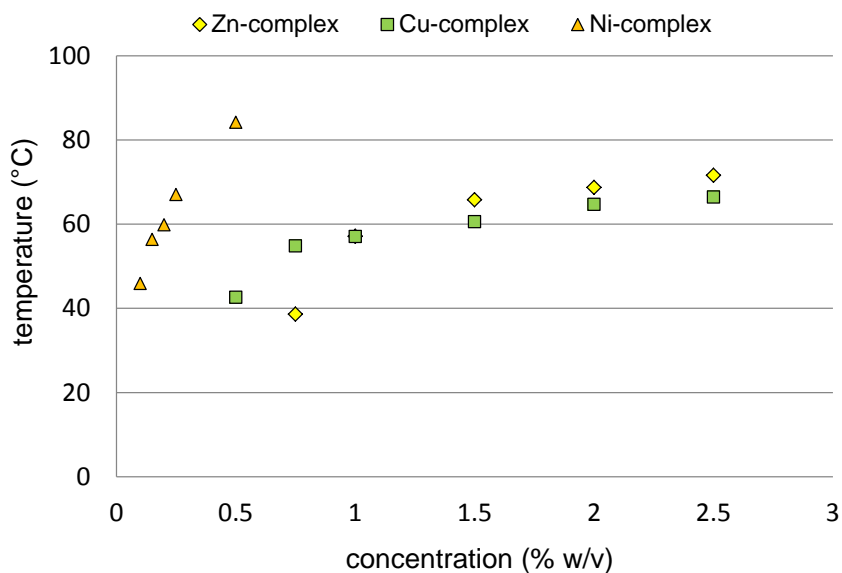
Gel-to-sol phase transition temperature

The gel-to-sol phase transition temperature (T_g) was determined using an “inversion tube” method and the measurements were repeated three times for each sample. Gels of a constant volume were prepared to the sealed containers and stabilized overnight at rt. After that they were placed into a water bath upside-down and gradually heated (1 °C/min). The temperature at which the gel fell under gravity was recorded as the gel-to-sol phase transition temperature (T_g).



% w/v	hexan-1-ol		heptan-1-ol		octan-1-ol	
	2.0	1.0	2.0	1.0	2.0	1.0
3a	63.2	56.1	64.2	56.6	64.7	57.0
3b	>95	83.1	>95	91.2	>95	>95
3c	62.2	51.9	64.9	53.6	68.7	57.1

Figure S2. Gel-to-sol transition temperatures (in °C) of gels of **3a-c** in higher alcohols.



% w/v	2.5	2.0	1.5	1.0	0.75	0.5	0.25	0.20	0.15	0.10	0.05
3a	66.4	64.7	60.6	57.0	54.8	42.6	-	-	-	-	-
3b	>95	>95	>95	>95	>95	84.1	67.0	59.8	56.3	45.9	-
3c	71.6	68.7	65.8	57.1	38.6	-	-	-	-	-	-

Figure S3. Phase diagram of **3a-c** in octan-1-ol.

Different ligand-metal ratios and combinations of metal salts

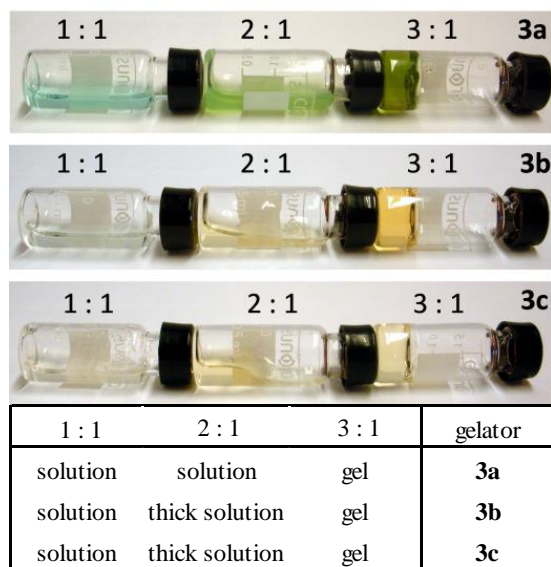
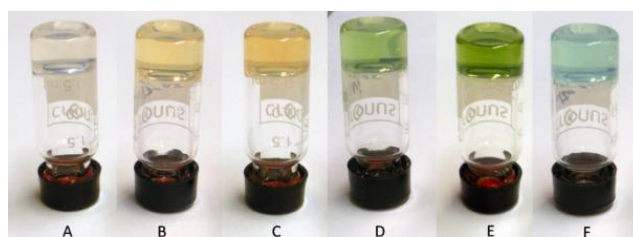


Figure S4. Gelators **3a-c** in octan-1-ol (2 % w/v) at different ligand-metal ratio.



% w/v	Zn ²⁺	Zn ²⁺ /Ni ²⁺	Ni ²⁺	Ni ²⁺ /Cu ²⁺	Cu ²⁺	Cu ²⁺ /Zn ²⁺
2.0	68.7	>95	>95	90.6	64.7	68.3
1.0	57.1	67.9	>95	66.4	57.0	57.5

Figure S5. Gels of metal complexes of a total ligand-metal ratio of 3:1 in octan-1-ol (1 % w/v) and their gel-to-sol transition temperatures (in °C): (A) ligand:Zn²⁺ (3:1, *i.e.* **3c**), (B) ligand:Ni²⁺:Zn²⁺ (3:0.5:0.5), (C) ligand:Ni²⁺ (3:1, *i.e.* **3b**), (D) ligand:Cu²⁺:Ni²⁺ (3:0.5:0.5), (E) ligand:Cu²⁺ (3:1, *i.e.* **3a**), and (F) ligand:Cu²⁺:Zn²⁺ (3:0.5:0.5).

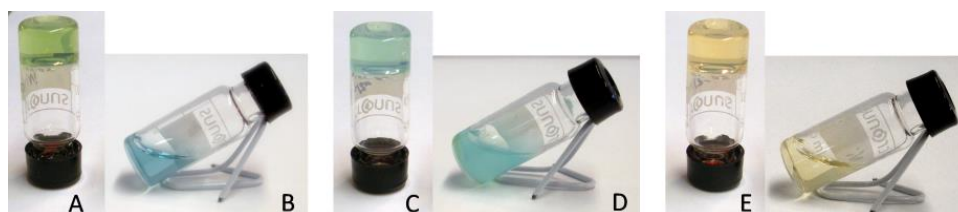
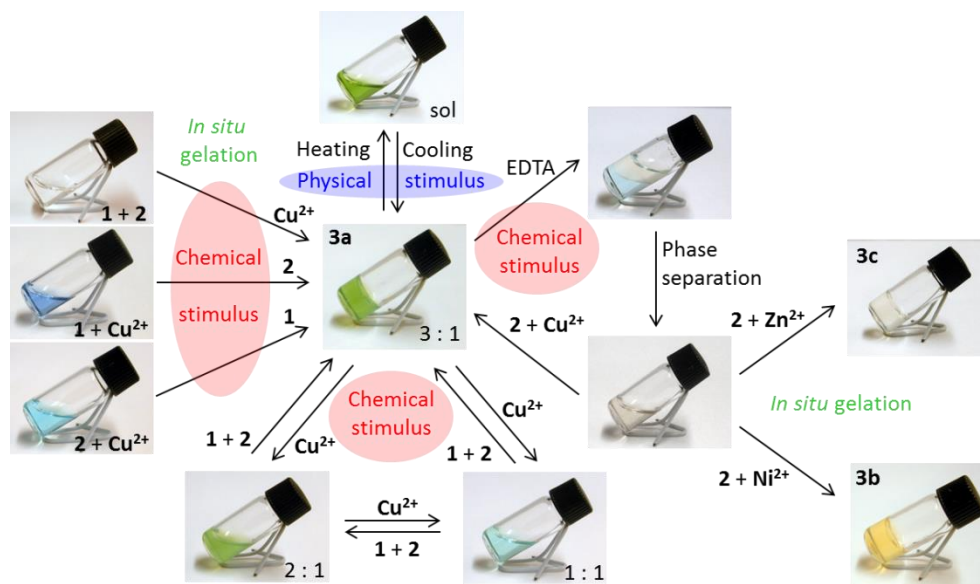


Figure S6. Metal complexes of different ligand-metal ratios in octan-1-ol (1 % w/v): (A) ligand:Cu²⁺:Ni²⁺ (3:0.5:0.5), (B) ligand:Cu²⁺:Ni²⁺ (3:1:1), (C) ligand:Cu²⁺:Zn²⁺ (3:0.5:0.5), (D) ligand:Cu²⁺:Zn²⁺ (3:1:1), (E) ligand:Ni²⁺:Zn²⁺ (3:0.5:0.5), and (F) ligand:Ni²⁺:Zn²⁺ (3:1:1).

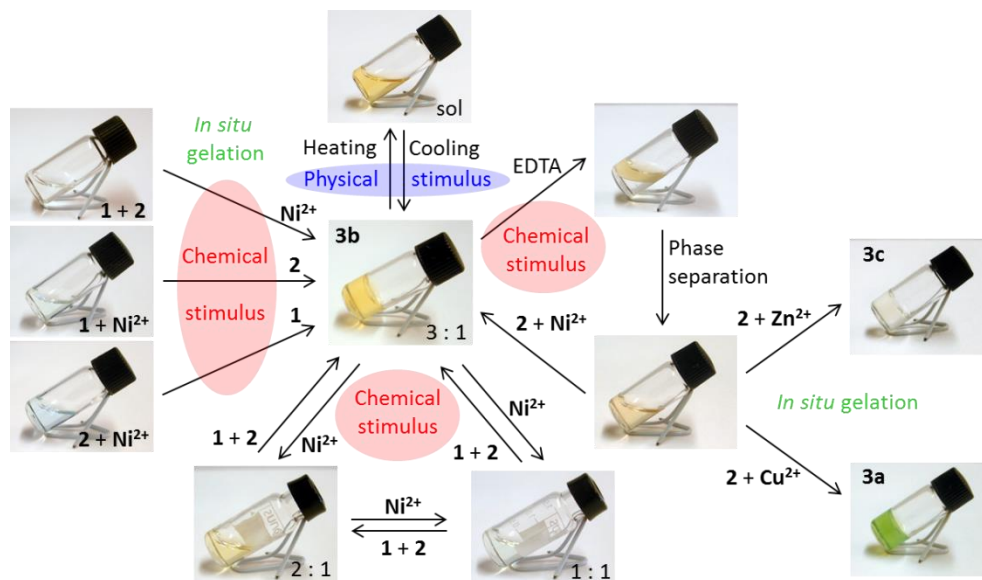
Stimuli-responsive gels

EDTA experiments

In the EDTA experiment, to a 1 % w/v gel in octan-1-ol (1 mL), a saturated aqueous EDTA (sodium salt) solution (1 mL) was added. The sample was treated with ultrasound for 10-15 min, and then was standing for 30 min without any disturbance. The formed phases were separated, and to the organic phase, new portions of aldehyde **2** and a metal salt (Cu^{2+} , Ni^{2+} , or Zn^{2+}) were added. A gel was reformed after intensive shaking of the sample at ambient temperature, or upon a heating/cooling cycle. The drawback of the experiment is the determination of the amount of aldehyde **2**, which has to be added. Since the imine formation is a dynamic reversible reaction, a certain amount of **2** is presented in the sample in a free form and is washed out by the aqueous phase ($\log P_{\text{ow}}$ - partition coefficient for *n*-octanol/water system, of **2** is 0.714). Therefore, an additional amount of **2** has to be added. The exact amount depends on the intensity and duration of the extraction. However, it was found experimentally that an extra addition of 40 - 50 % of **2** was in most cases sufficient and a small excess of **2** did not prevent a gel formation.



Scheme S2. Response of **3a** in octan-1-ol (1 % w/v) to chemical and physical stimuli.



Scheme S3. Response of **3b** in octan-1-ol (1% w/v) to chemical and physical stimuli.

Rheological measurements

The rheological experiments were measured using a TA instruments AR2000 rheometer with 40 mm aluminium 2° cone. The samples for the time sweep experiments were prepared by mixing the solutions of metal salts and the imine before the starting the measurements. Sol to gel transition was followed by an oscillatory time sweep from 20 min to 3 h depending on the gelation time of the components under investigation. The measurements were carried out within the linear viscoelastic regime (10% strain) at 6.28 rad/s frequency. After allowing the gel to attain sufficient stability (monitored by the time sweep experiment) frequency sweep and stress sweep experiments were performed. All the experiments were performed with controlled temperature of 20 °C with a Peltier heated plate.

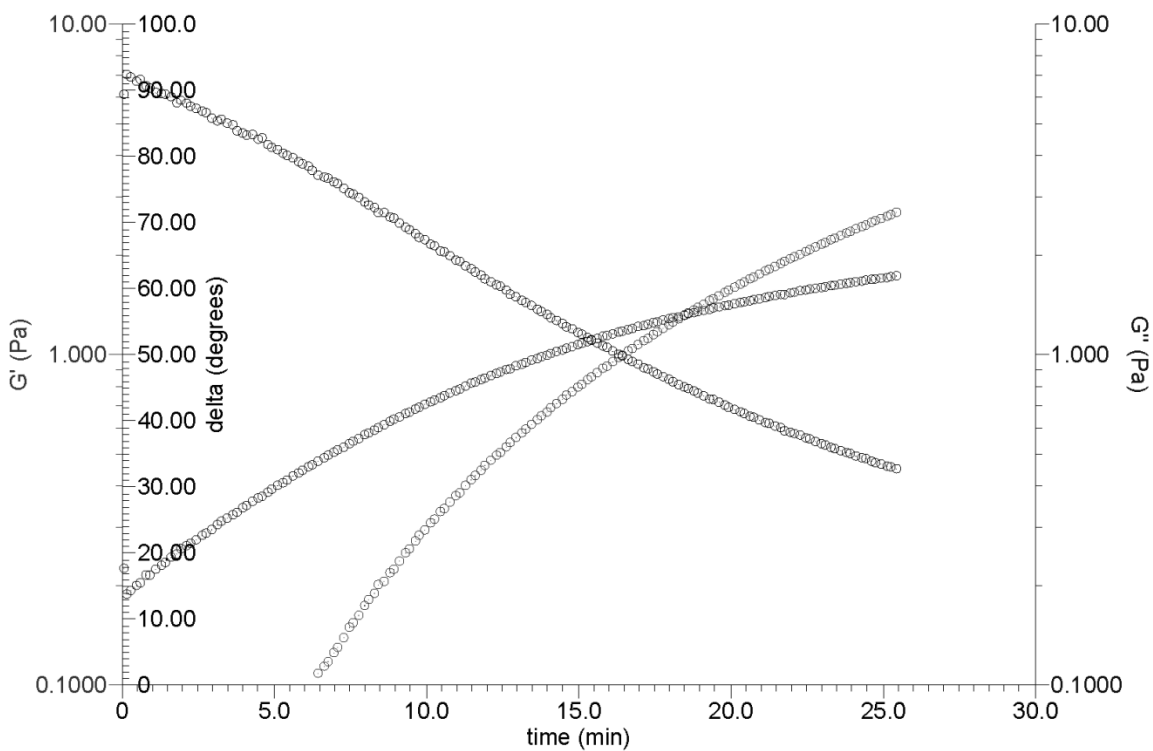


Figure S7. Time sweep experiment of gel of **3a** formed in octan-1-ol (2 % w/v); 30 min.

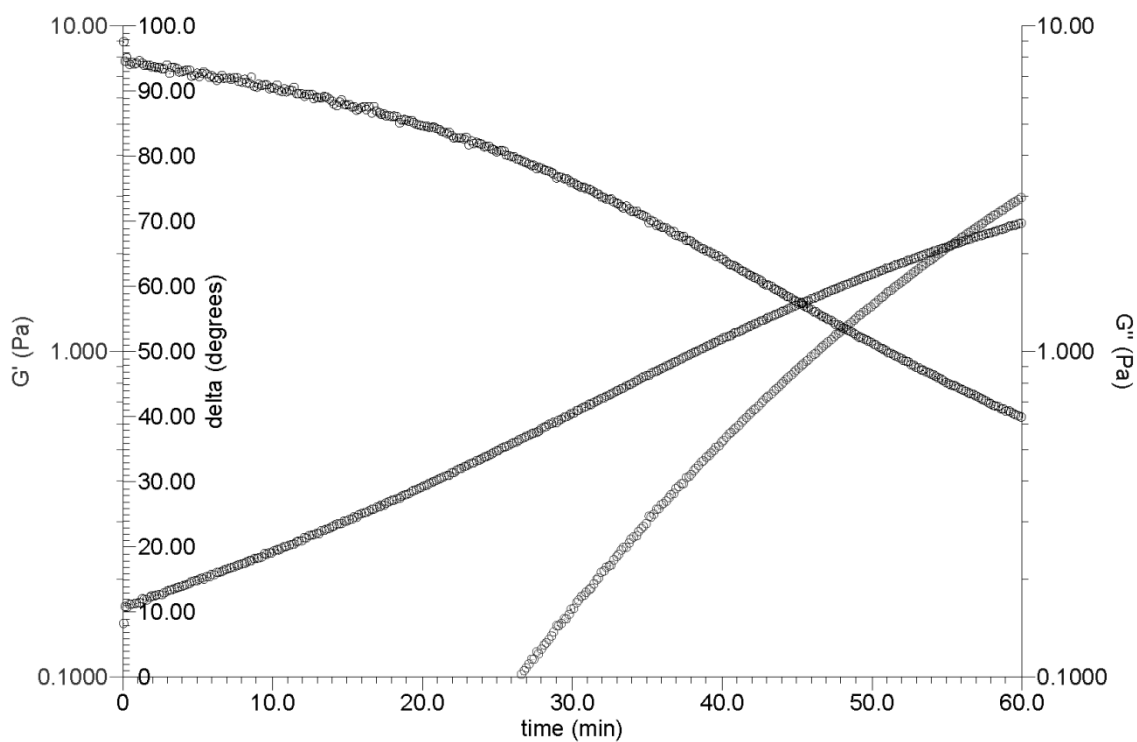


Figure S8. Time sweep experiment of gel of **3b** formed in octan-1-ol (2 % w/v); 60 min.

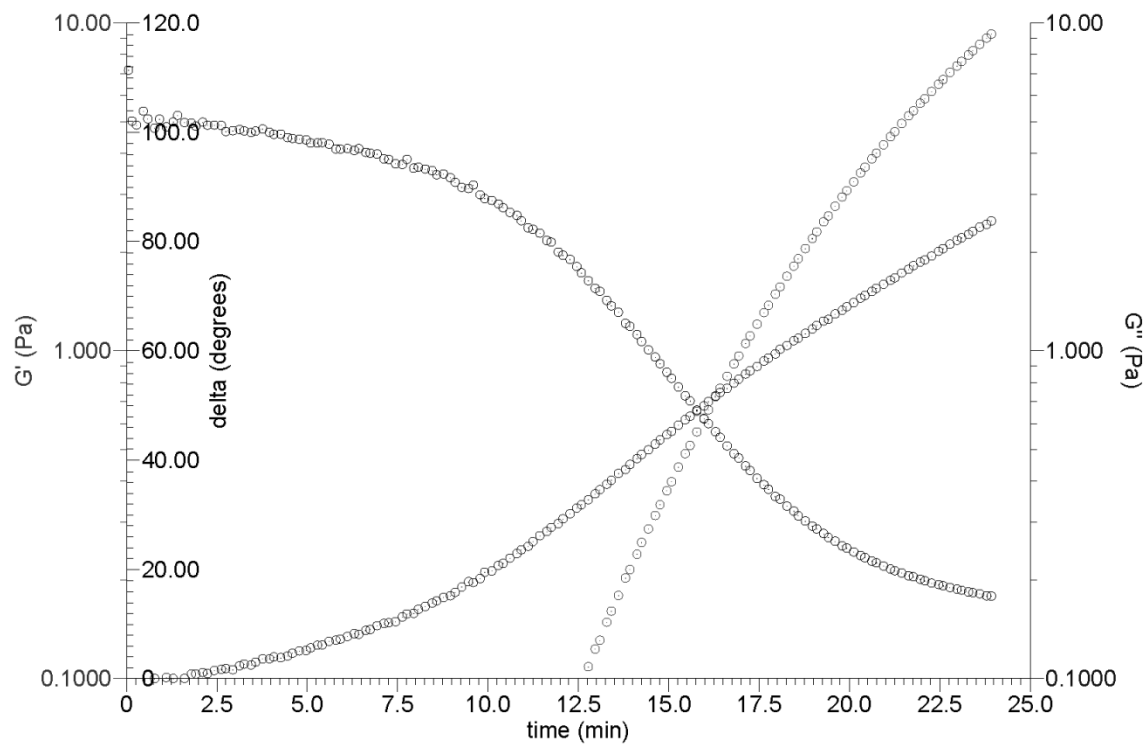


Figure S9. Time sweep experiment of gel of **3c** formed in octan-1-ol (2 % w/v); 25 min.

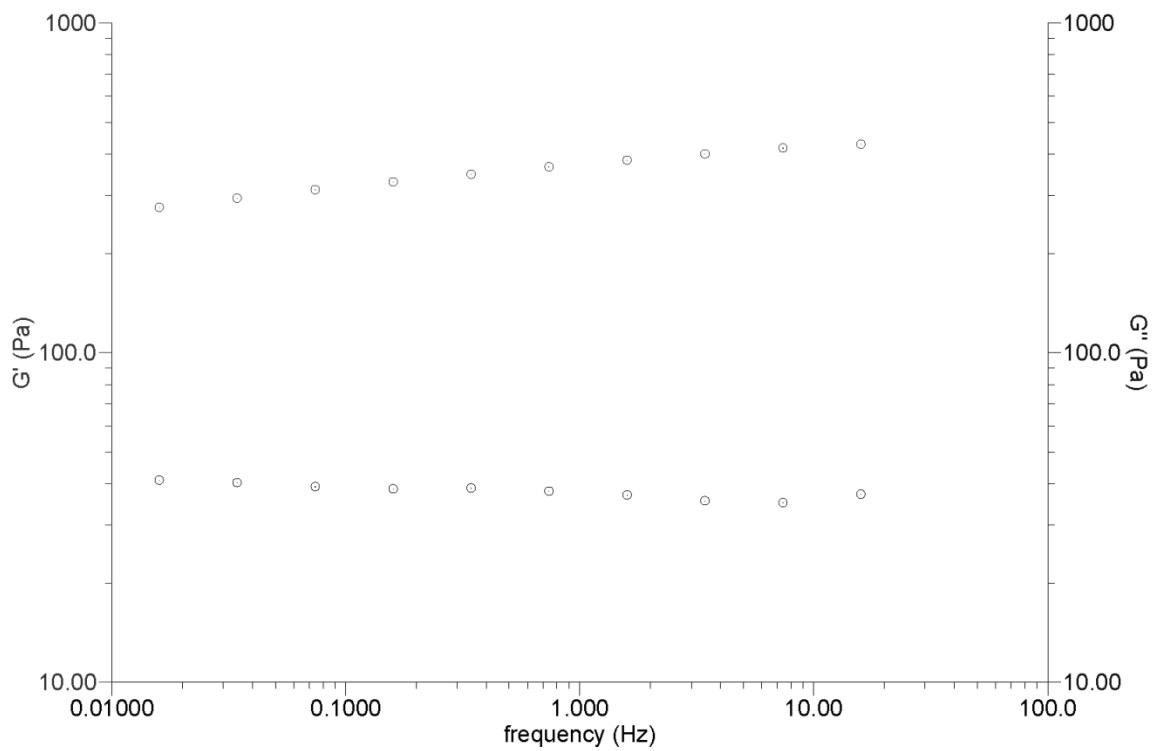


Figure S10. Frequency sweep experiment of gel of **3a** formed in octan-1-ol (2 % w/v); measured after 3 h of the sample stabilization.

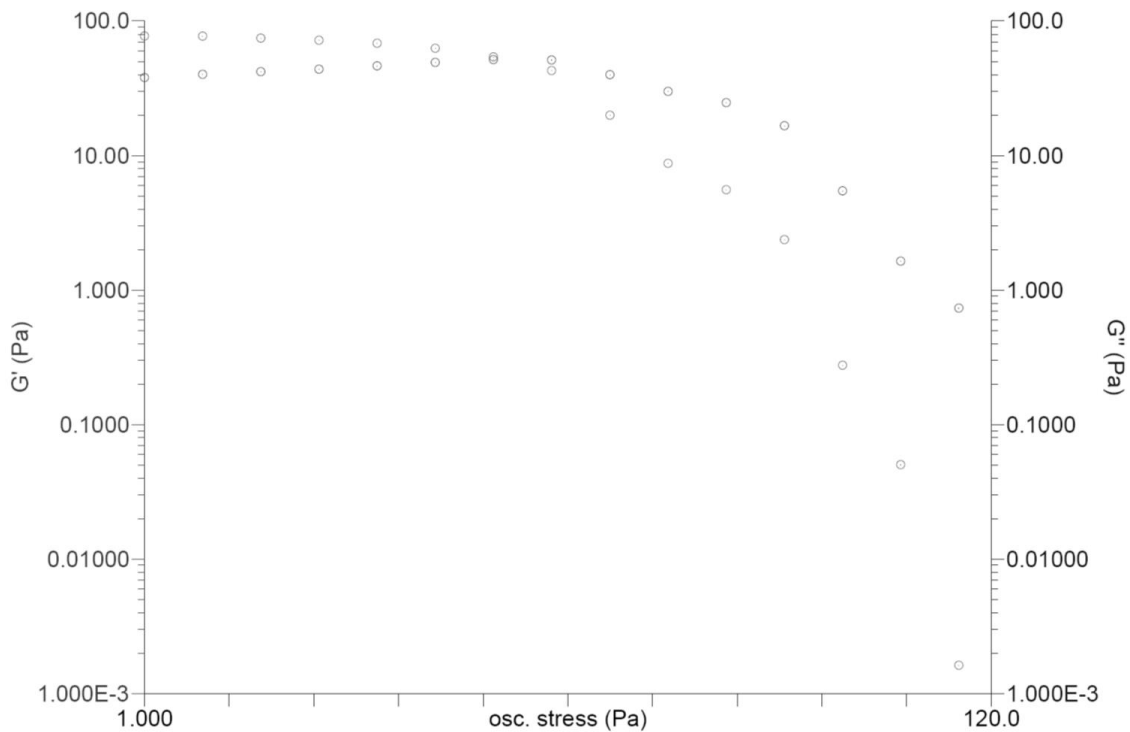


Figure S11. Stress sweep experiment of gel of **3a** formed in octan-1-ol (2 % w/v).

Speed of the gel formation

In order to compare the speed of the gel formation of **3a-c**, *in situ* gelation experiments were carried out for 2 % and 1 % w/v gels of **3a-c** in octan-1-ol.

a) Measured by time sweep experiments:

After mixing the octan-1-ol solution of the amine (**1**) and aldehyde (**2**) with metal salts, oscillatory time sweep experiments were carried out from 20 min to 3 h depending on the gelation time of the system under investigation. The measurements were performed within the linear viscoelastic regime (10% strain) at 6.28 rad/s frequency and the results are summarized in Figure S12.

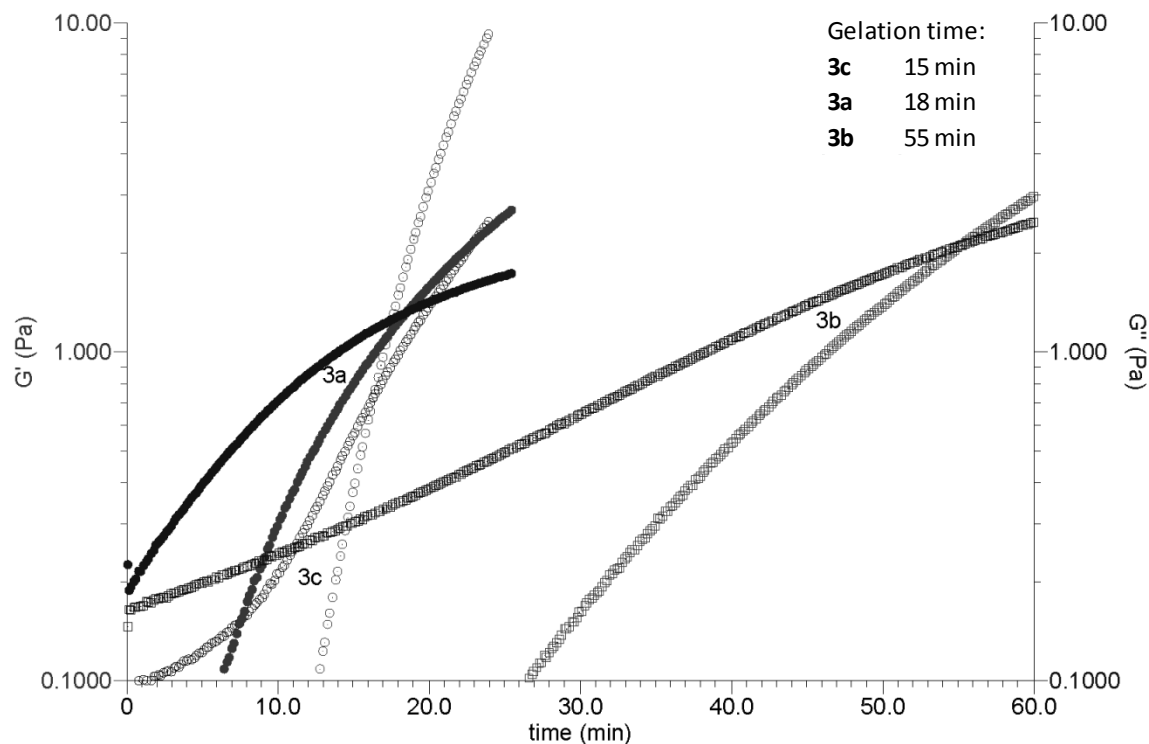


Figure S12. Time sweep experiments of gels of **3a-c** formed in octan-1-ol (2 % w/v).

b) Measured upon ultrasonic treatment:

After mixing the beforehand dissolved components, the samples were treated with ultrasound for 90 s. After that they were carefully checked by an inverted test tube method. The time, when the test tube could be turned up-side-down with the immobilized gel, was determined as the gelation time (Table S1).

Table S1. Time needed for an effective gel formation of **3a-c** in octan-1-ol at different concentrations.

% w/v	Gels in octan-1-ol	
	2.0	1.0
3c	50 s	6.5 min
3a	90 s	9 min
3b	10 min	24 min

Mass spectrometry measurements

The mass spectrometric experiments were performed with a QSTAR Elite ESI-Q-TOF mass spectrometer equipped with an API 200 TurboIonSpray ESI source from AB Sciex (former MDS Sciex) in Concord, Ontario (Canada). The gels of **3a-c** in octan-1-ol (1 % w/v) were first dissolved in CHCl₃ and then diluted in CH₃CN to obtain 20·10⁻⁶ M samples for measurements. The samples were injected into the ESI source with a flow rate of 5 μL/min. Room-temperature nitrogen was used as nebulization (30 psi) and as curtain gas (18 psi). The ion-source voltages were 4.8 kV for capillary, 20 V for the orifice plate (declustering potential), 20 V as potential difference between skimmer and pre-quadrupole, and 250 V for the potential difference between the focusing ring and pre-quadrupole. Accumulation delay of 1s, ion release delay of 6 ms and ion release width of 5 ms were used. Each spectrum was an average of spectra collected within 2 to 5 min, each of these containing 20 individual scans that were averaged before being sent from the instrument to data system. The measurement and data handling was accomplished with Analyst® QS 2.0 Software. Mass spectra were externally calibrated using sodium trifluoroacetate. The composition of the ions was verified by CID measurements as well as by comparison between experimental and theoretical mass values and isotopic distributions.

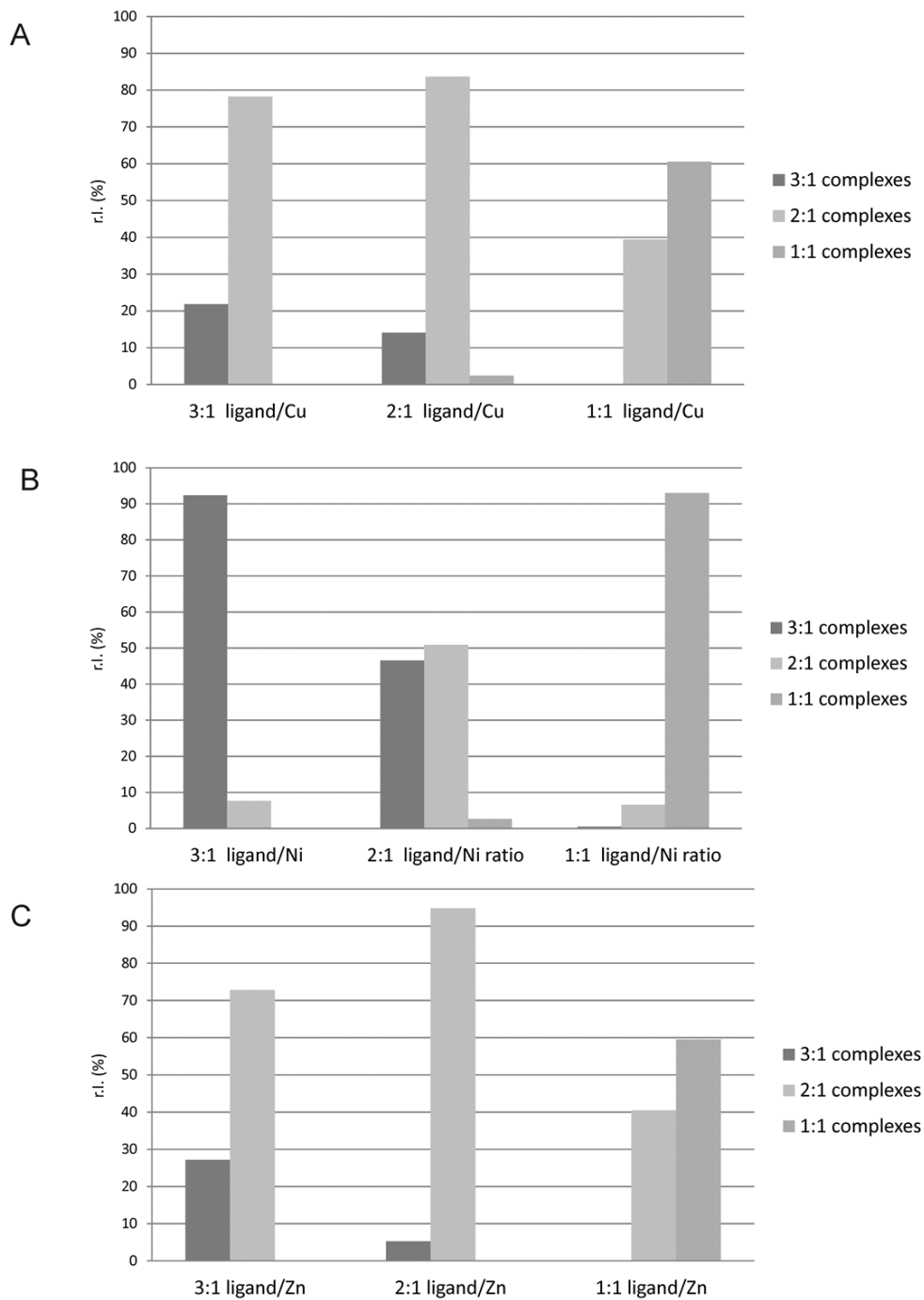


Figure S13. Relative intensities of 3:1, 2:1 and 1:1 complexes observed in ESI-Q-TOF mass spectra measured from Cu^{2+} (A), Ni^{2+} (B) and Zn^{2+} (C) gel samples with the ligand/metal ratios of 3:1, 2:1 and 1:1.

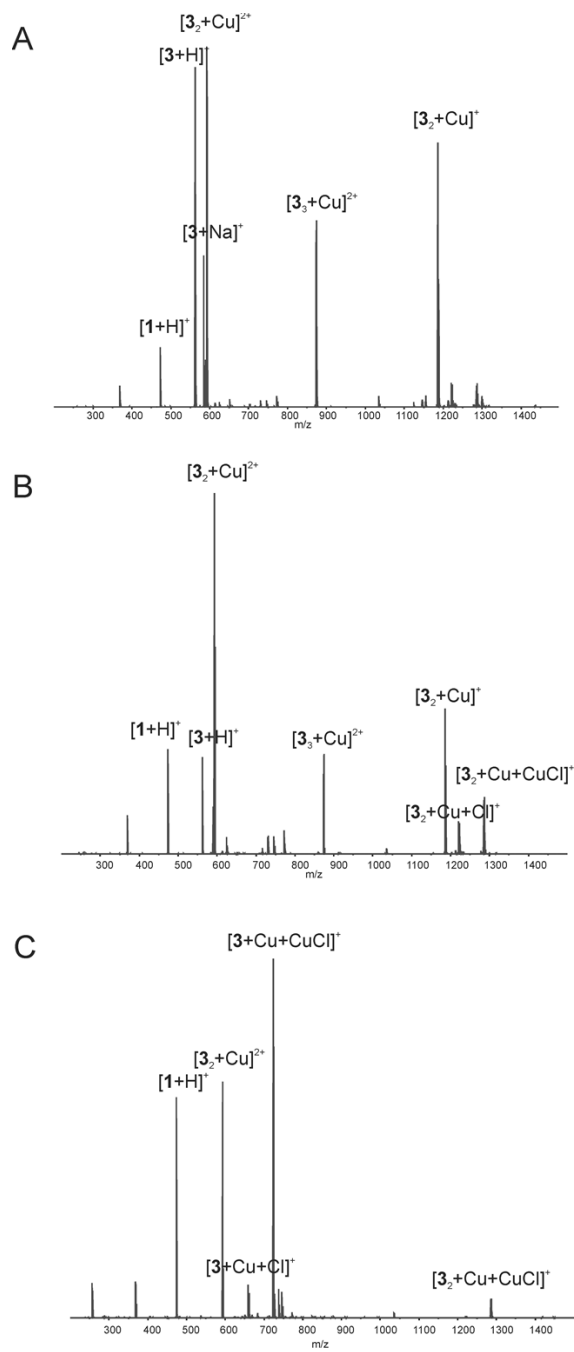


Figure S14. ESI-Q-TOF mass spectra measured from gel samples with the ligand-Cu ratio of 3:1 (A), 2:1 (B) and 1:1 (C).

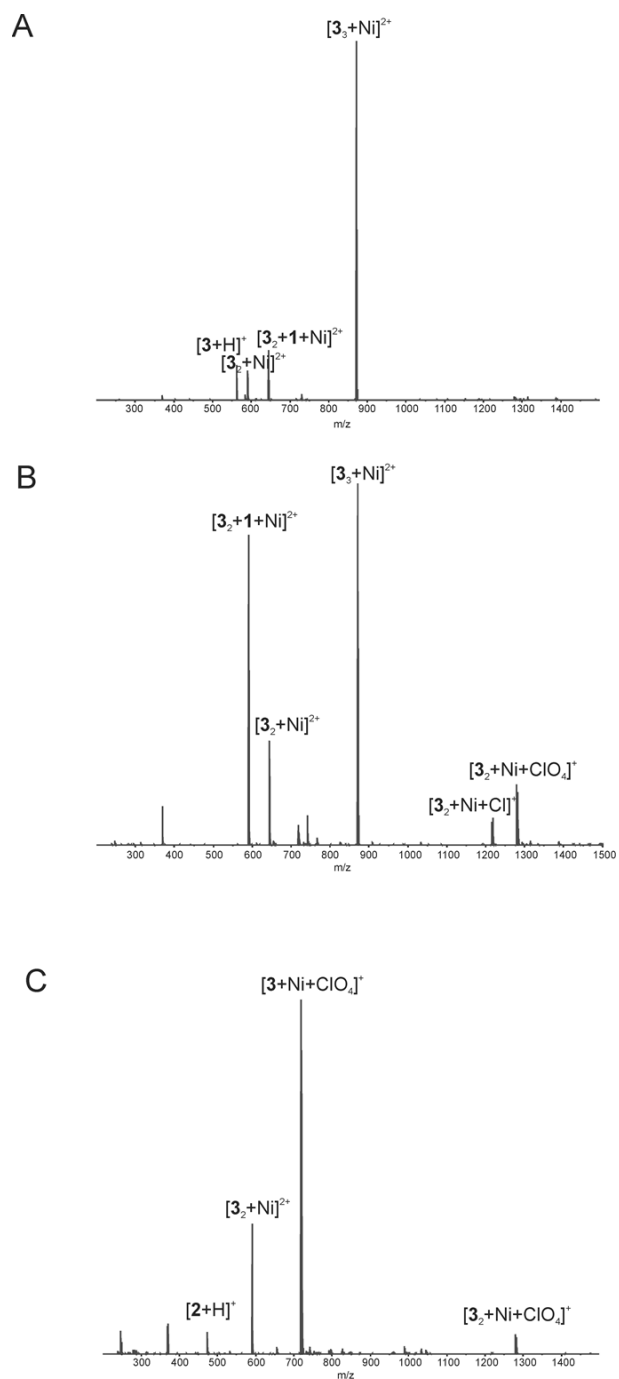


Figure S15. ESI-Q-TOF mass spectra measured from gel samples with the ligand-Ni ratio of 3:1 (A), 2:1 (B) and 1:1 (C).

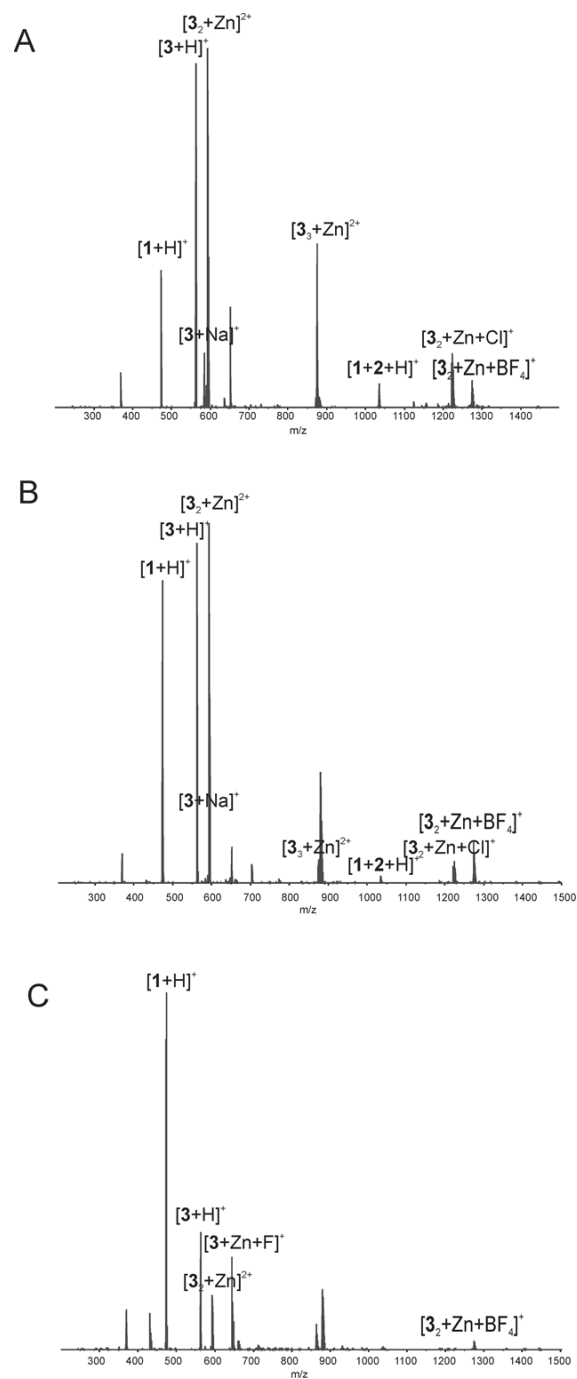


Figure S16. ESI-Q-TOF mass spectra measured from gel samples with the ligand-Zn ratio of 3:1 (A), 2:1 (B) and 1:1 (C).

TEM measurements

Transmission electron micrographs of xerogels of **3a-c** were acquired using a Technai 12 transmission electron microscope (FEI, Hillsboro, OR, USA) operated at 120 kV and equipped with a Gatan Ultrascan 1000 CCD camera (Gatan Inc., Pleasanton, CA, USA). The samples for TEM measurements were prepared by placing 5 μL of a gel solution (1.0 % w/v octan-1-ol gel dissolved by heating) on a TEM grid (carbon film only 200 mesh on gold). For dilute samples (0.1 % w/v), 100 μL of the 1% w/v gel was diluted by octan-1-ol to 1.0 mL. The samples were allowed to dry at ambient conditions for 24 h.

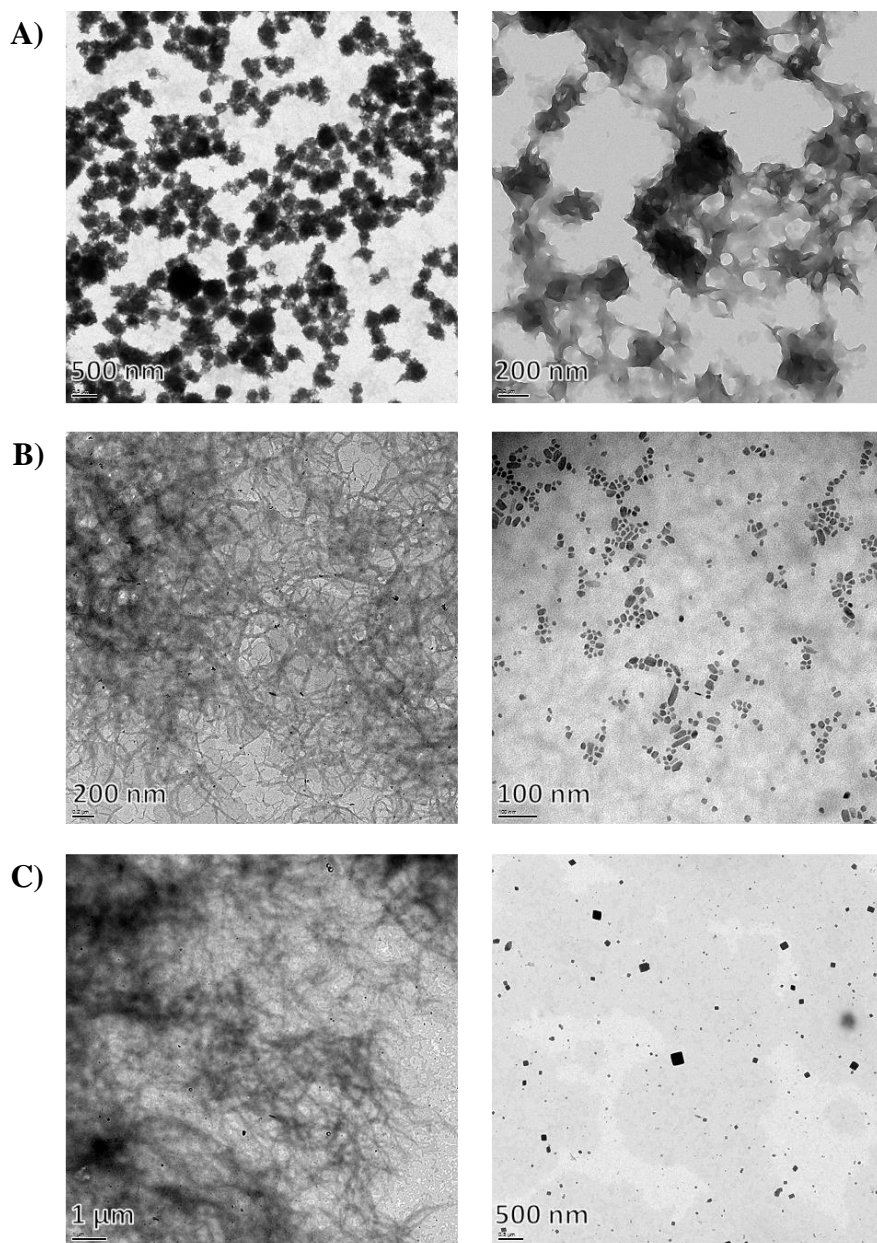


Figure S17. TEM micrographs of xerogels obtained from gels in octan-1-ol (1.0 % w/v) of **3a** (A), **3b** (B), and **3c** (C) revealing a fibrillar network and nanoparticle formation.

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