Cyclohexane-Based Low Molecular Weight Hydrogelators: A Chirality Investigation

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Abstract: Seven new 1,3,5-cyclohexyltricarboxamide-phenylalanine derivatives were synthesized in order to investigate the effect of the amino acid chirality on the gelating properties of these small molecules in water. Gelation tests have shown that enantiomerically pure homochiral 1,3,5-cyclohexyltricarboxamide-L-phenylalanine is a non-hydrogelator as it crystallizes from water, whereas the heterochiral derivatives with either two L-phenylalanine moieties and one D-phenylalanine (LLD), or vice versa (DDL), are very good hydrogelators. Concentration-dependent gel-to-sol transition-temperature (T_{gs}) curves for LLD or DDL gels show a sigmoidal behaviour, which is in contrast to the logarithmic curves generally observed for gels derived from low molecular weight gelators (LMWGs). Such sigmoidal behaviour can be related to interactions between fibre bundles, which give rise to intertwined bundles of fibres. Transmission electron microscopy (TEM) images of LLD and DDL gels show a network of thin, unbranched, fibre bundles with diameters of 20 nm. Right-handed twisted fibre bundles are present in the LLD gel, whereas left-handed structures can be found in the DDL gel. Each bundle of fibres consists of a finite number of primary fibres. Gels consisting of mixtures of gelators, LLD and DDL, and nongelators (LLL or DDD) were investigated by means of T_{gs} measurements, CD spectroscopy and TEM. Results show that the incorporation of nongelator molecules into gel fibres occurs; this leads to higher T_{gs} values and to changes in the helicity of the fibre bundles. Furthermore, it was found that peripheral functionalization of the homochiral derivatives LLL or DDD by means of a second amino acid or a hydrophilic moiety can overcome the effect of chirality; this process in turn leads to good hydrogelators.

Keywords: amino acids · chirality · cyclohexane · gels · self-assembly

Introduction

Low molecular weight gelators (LMWGs) can self-assemble in water leading to the formation of hydrogels; these gels are jelly-like materials usually consisting of intertwined fibrous structures, in which water remains entrapped, thus resulting in their characteristic consistency.[1] Such materials are of considerable interest since they are intermediates between liquids and solids and as such combine properties of both of these states, as well as presenting new properties of their own.[1,2] Recently, LMWG hydrogel matrices have shown great potential in the field of drug delivery as controlled release systems,[3,4] as scaffolds providing cell storage as well as directionality for cellular differentiation[5] and for the construction of protein microarrays compatible with enzyme assays.[6] A class of particularly interesting LMWGs is based on the 1,3,5-benzene- and 1,3,5-cyclohexyltricarboxamide cores. A variety of 1,3,5-benzenetricarboxamide derivatives synthesized by Meijer and co-workers have shown interesting behaviour in organic polar and apolar solvents. In both media chiral columnar aggregates can form,[7] and in some cases the so-called "sergeants-and-soldiers" effect could be observed for gelators with relatively small peripheral substituents (C_8-alkyl chains).[8] Furthermore, derivatives with polymerizable peripheral moieties can undergo photoinitiated polymerization, forming very stable colum-

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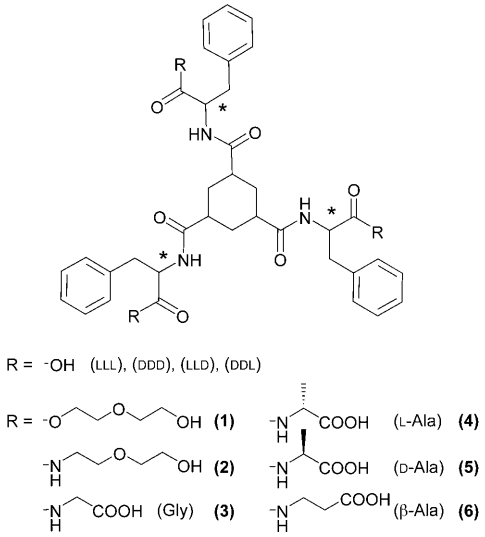
Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the authors.

 DOI: 10.1002/chem.200500007

nar, mesoscopic objects.[9,10] The gelation capabilities of alkyl-substituted 1,3,5-cyclohexyltricarboxamide derivatives in organic solvents were initially investigated by Hanabusa et al.[11] More recently we showed that amino acid substituted derivatives function as gelators for water.[12] Moreover, peripheral functionalization of such molecules with pH-sensitive moieties leads to responsive gels that can be switched reversibly from gel to sol by changing the pH, thus making these materials particularly interesting for controlled drug-delivery systems.[12]

Both 1,3,5-benzenetricarboxamide- and 1,3,5-cyclohexyltricarboxamide derivatives make excellent scaffolds for the study of chiral supramolecular architectures in protic solvents. To obtain well-defined architectures of synthetic molecules in such media the right balance between forces of limited directionality, such as hydrophobic forces, and more directional but less stable interactions, such as hydrogen bonds, has to be found. For natural proteins, the possibility of creating stable hydrogen bonds, even in aqueous media, derives from the presence of hydrophobic regions within the protein where such interactions can take place. Similarly, for 1,3,5-cyclohexyltricarboxamide amino acid based gelators, the hydrophobic moieties of the amino acid substituents are needed for the protection of the hydrogen-bonding network from the surrounding solvent, allowing self-assembly and subsequently gelation in water to take place.[12] In some cases, as has often been observed with many other gelator systems, very slight changes to the gelator structure can influence the gelation behaviour dramatically.[13] Chirality, for example, often determines whether a compound will function as a gelator or not. Whereas racemic mixtures are sometimes less efficient gelators than the corresponding enantiomerically pure products,[14,15] the opposite effect has been observed in other cases.[13,16,17] Very recently, Hirst et al. have shown that changing the chirality of one amino acid of a dendritic organogelator can lead to changes in the gelation behavior in organic solvents.[18]

Herein we report that changes in chirality effect the gelation behaviour of amino acid-substituted 1,3,5-cyclohexyltricarboxamide derivatives in water (Scheme 1), which lead to novel hierarchical self-assembled systems. In particular, we show that the enantiomerically pure homochiral 1,3,5-cyclohexyltricarboxamide-phenylalanine derivative (LLL or DDD) is a non-hydrogelator that can be transformed into a good gelator for water simply by changing the chirality of one of the three substituents (LLD or DDL). The consequences of the chirality changes made to the structure of this cyclohexyltricarboxamide derivative are not only reflected in the gelation behaviour of the system, but are also apparent at the microscopic level through changes in the gel fibre morphology. CD spectroscopy, transmission electron microscopy (TEM) and gel-to-sol transition-temperature measurements demonstrate that interactions between homochiral and heterochiral derivatives can take place, thus leading to changes in supramolecular chirality and thermostability of the gels. Furthermore, we show that although the chirality of the amino acid plays a very important role in determining the



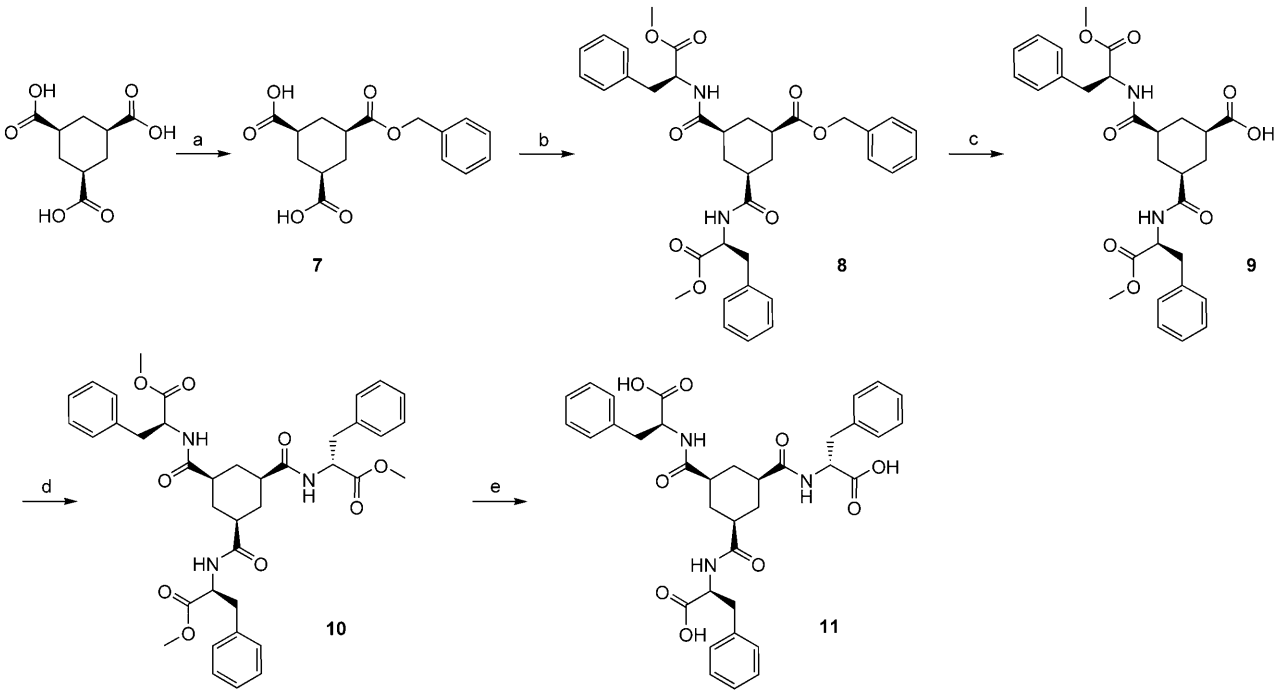
Scheme 1. Molecular structure of amino acid substituted 1,3,5-cyclohexyltricarboxamide derivatives.

gelating capability of 1,3,5-cyclohexyltricarboxamide-phenylalanine, this effect can be counteracted. For nongelating homochiral derivatives, substitution at the acid terminus of the amino acid with a second amino acid or with hydrophilic R groups generally leads to the formation of very good hydrogelators.

Results

Synthesis: The synthesis of enantiomerically pure homochiral 1,3,5-cyclohexyltricarboxamide-phenylalanine (either LLL or DDD) was carried out according to literature procedures.[12] The products were obtained in good yield. Protecting-group chemistry (Scheme 2) was used for the synthesis of the heterochiral compounds (LLD and DDL). Reaction of L-phenylalanine methyl ester with **7** (obtained via monofunctionalization of *cis*,*cis*-1,3,5-cyclohexyltricarboxylic acid), deprotection to the cyclohexanecarboxylic acid **9**, subsequent reaction with D-phenylalanine methyl ester, and finally hydrolysis of the three methyl esters gave LLD-product **11**. Switching the L- and D-amino acids in this synthetic route results in the isolation of the heterochiral DDL-product **12**.

Gelation experiments: Gelation tests were carried out by dissolving the compound of interest in water by heating, and subsequently allowing the sample to cool. Owing to the pH-sensitive nature of the synthesized compounds, gelation tests could also be performed by first dissolving the compound of interest with 0.1 N NaOH, and subsequently adding 0.2 N HCl to cause gelation. The enantiomerically pure homochiral compounds, LLL and DDD, did not show gelation behaviour in water, nor in a variety of organic solvents, up to a gelator concentration of 15 mM (1 wt%). Soft gels could only be obtained in ethanol and 2-propanol; in all other solvents



Scheme 2. Synthesis of LLD. a) Benzyl alcohol, CDI, DMSO/ethyl acetate; b) H-L-Phe-OMe·HCl, Et_3N, DMT-MM, MeOH; c) Pd/C, H_2, MeOH/*i*PrOH/CH_2Cl_2; d) H-D-Phe-OMe·HCl, Et_3N, DMT-MM, MeOH; e) NaOH (aq)/MeOH. CDI: 1,1′-carbonyldiimidazole, DMT-MM: 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride.

tested, including water, formation of a crystalline needle-like precipitate could be observed with the naked eye. The heterochiral compounds, LLD and DDL, on the other hand, are very good gelators for water, yielding clear, homogeneous gels which become slightly opaque at high gelator concentrations. These compounds possess a critical gelation concentration (cgc) of 0.5 mM (0.04 wt%) (Figure 1).[19] All gels displayed very good stability over time, as no changes were observed in these systems for more than one year. Noticeably, the shape of the curve depicting T_{gs} (that is the temperature at which the gel turns into a solution) as a function of the gelator concentration is sigmoidal, whereas for LMWGs such curves are usually logarithmic. At low gelator concentrations (0.5–2.0 mM) the phase-transition temperatures are between 20 and 40 °C, whereas at high gelator concentrations (3.0–6.0 mM) T_{gs} values are around 120 °C. This seems to indicate that distinct gelator aggregates form at these two different concentration levels. Since a difference in the clarity of the samples was also observed, turbidity measurements as a function of the gelator concentration were performed to see if the parallel between phase transition temperature changes and aggregation could further be substantiated. A small but significant jump in turbidity was observed at around 2 mM (Figure 2), which corresponds quite well to the concentration at which the large change in

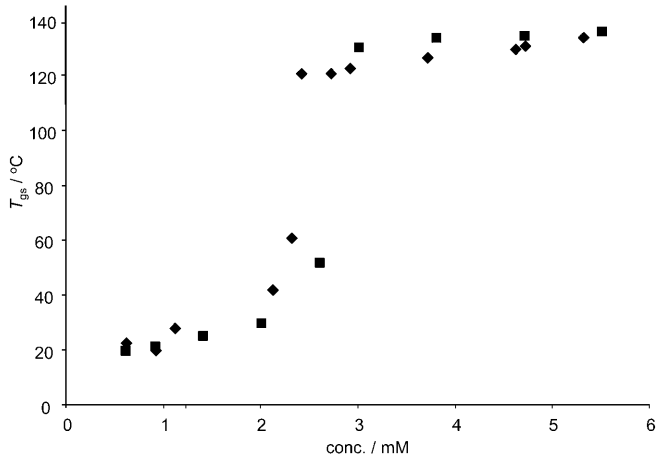


Figure 1. Gel-to-sol transition temperatures (T_{gs}) as a function of gelator (◆: LLD, ■: DDL) concentration in water.

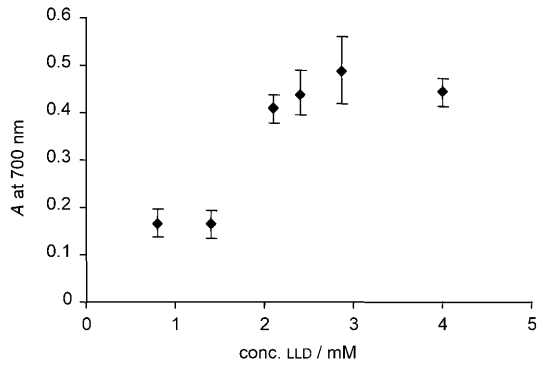


Figure 2. Concentration dependent absorption (at 700 nm) of LLD gels in water.

T_{gs} occurred. The presence of different gelator aggregates was furthermore confirmed by cryo-TEM images of low (1.0 mM) and high (4.0 mM) gelator concentration gels (see below).

Mixtures of LLD with DDL, LLL or DDD, and of DDL with LLL and DDD, of different ratios, respectively, were also tested for gelation in water. The appearance of the gels obtained was investigated (Table 1). Gels consisting of either LLD or DDL compounds were homogeneous and did not contain any crystalline material at all ratios of the two components. On the contrary, mixtures of LLD with LLL or DDD (and of DDL with LLL or DDD) led to the formation of clear gels at high percentages of the heterochiral component (i.e., the gelator), and to gels containing more and more needle-like crystals as the percentage of the homochiral component (i.e., the nongelator) increased. For LLD samples containing LLL or DDD, the lowest percentage of LLL, which led to the formation of a gel containing crystalline material, was approximately 48%; the lowest percentage of DDD was about 36%. These results provide a strong indication that nongelators LLL and DDD are the cause of crystallization in the mixed samples, as presented in Table 1.[20]

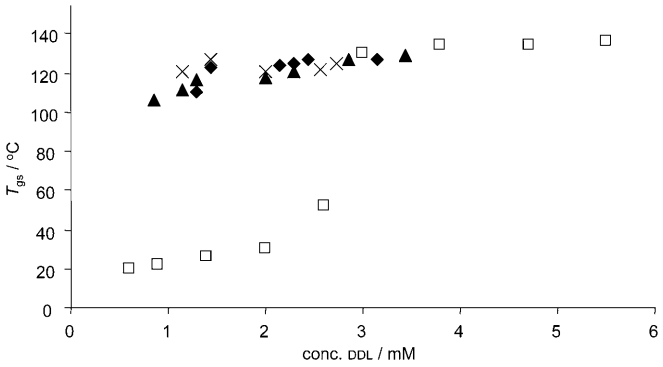


Figure 3. Gel-to-sol transition temperatures (T_{gs}) as a function of gelator concentration for DDL (□) and 1:1 mixtures of DDL and DDD (◆), DDL and LLD (×), or DDL and LLL (▲) in water.

Table 1. Aggregation state and appearance (app.) of mixtures of DDL with LLL or DDD, and of LLD with LLL, DDD, or DDL, at room temperature (RT) in water (total concentration of each sample: 4.5 mM).[a]

	LLL		DDD			LLL		DDD		DDL	
DDL (%)	RT	App.	RT	App.	LLD (%)	RT	App.	RT	App.	RT	App.
77	g	t	g	c	79	g	c	g	t	g	o
71	g	t	g	c	64	g	c	cg	w	g	o
53	cg	w	cg	w	52	cg	w	cg	w	g	o
48	cg	w	cg	w	34	cg	w	cg	w	g	o
32	cg	w	cg	w	32	cg	w	cg	w	g	o
30	cg	w	cg	w	30	cg	w	cg	w	g	o
21	cg	w	cg/p	w	22	cg/p	w	cg	w	g	o

[a] g: gel, cg: gel containing crystalline material, p: precipitate, c: clear, t: turbid, w: white, o: opaque.

To determine whether gels containing two components consist of self-assembled aggregates (generally fibres) in which both components are present, or of a mixture of aggregates of the individual components, the temperatures at which the gels turn into solutions (T_{gs}) were measured. Such measurements can provide an indication of molecular interactions in LMWG gels.[4,21] T_{gs} values of mixtures of DDL and DDD, LLD, or LLL, as depicted in Figure 3, show that at low concentrations of DDL the mixed gels exhibit much higher T_{gs} values than the pure DDL gel. Analogous results were obtained with mixtures of LLD and DDD or LLL (data not shown). Higher T_{gs} values are generally obtained for LMWGs upon increasing the gelator concentration, whereby a greater number, longer or thicker gel fibres are formed, making the gel network thermally more stable. However, it is remarkable that at comparable gelator concentration, for example, 1.5 mM, the samples that also contain non-hydrogelator show much higher T_{gs} values than the sample containing only DDL (Figure 3). Therefore, a larger number of gel fibres, longer or thicker fibres, must be present in the mixed sample, which can only be achieved if the non-hydrogelator is also incorporated into the gel fibres.

Although the amino acid chirality in 1,3,5-cyclohexyltricarboxamide phenylalanine determines the gelating properties of the product, this effect can be overcome by simple changes to the molecular structure. Scheme 1 shows a variety of gelators based on 1,3,5-cyclohexyltricarboxamide-L-phenylalanine with the R group being either a hydrophilic moiety or a second amino acid. All of these molecules (**1**–**6**) are good hydrogelators (Figure 4), even though the three phenylalanines are of L chirality.[12] In particular, the gelation tests carried out on compounds **3**–**6** show that, although some differences in T_{gs} values occur between the different gelators, the chirality, or non-chirality, of the second amino acid does not strongly influence the gelation capability of these molecules.

Circular dichroism studies: CD measurements were performed to determine if the chirality of the different 1,3,5-cy-

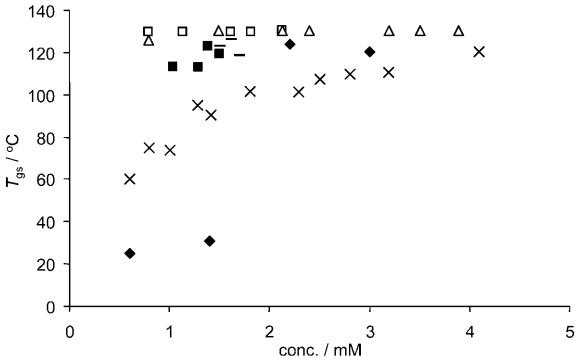


Figure 4. Gel-to-sol transition temperatures (T_{gs}) as a function of gelator (**1**: ×, **2**: ■, **3**: –, **4**: □, **5**: ◆, **6**: △) concentration in water.

clohexyltricarboxamide phenylalanine compounds is reflected in the self-assembled gelator aggregates. LLD and DDL gels in water present opposite CD absorptions (as was to be expected) at 227–228 nm,[22] and a gel consisting of a 1:1 mixture of the two didn't give any CD absorption in this region (Figure 5). Variable-temperature CD performed on a

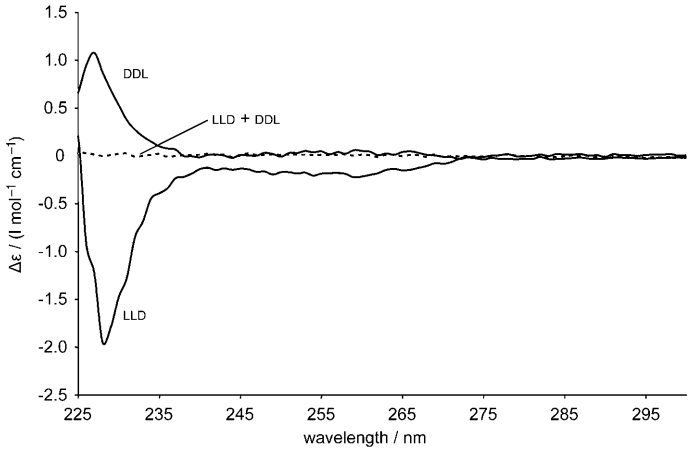


Figure 5. CD spectra of 2.4 mM gels of DDL, LLD, and DDL+LLD 1:1 in water.

2.40 mM gel of LLD showed that the intensity of the CD signal decreases upon heating of the sample, that is, upon going from a gel to a solution (Figure 6). The CD signal ob-

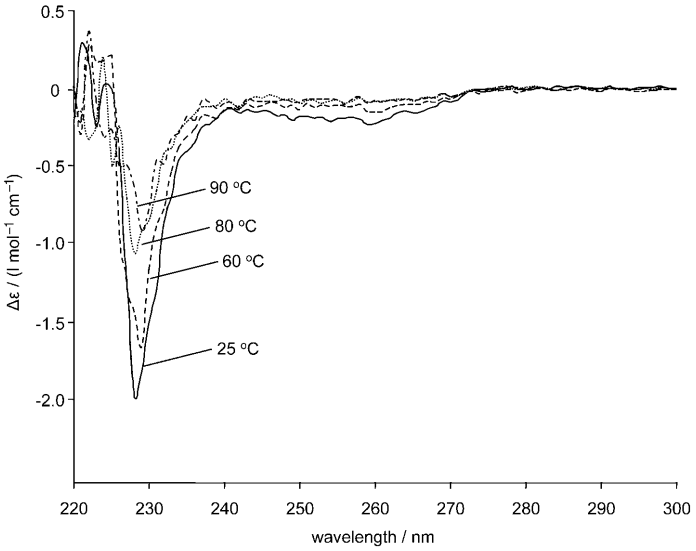


Figure 6. Temperature dependent CD spectra of a 2.4 mM LLD hydrogel.

served for the gel therefore derives from the chirality of the self-assembled aggregates making up the gel, and not from the chirality of the individual gelator molecules.[7,23] Complete loss of the CD signal did not occur even at 90 °C, because the sample though liquid still contained some small gelator aggregates.[24]

CD spectroscopy of gel samples of LLD or DDL mixed with LLL and DDD was carried out to determine if interactions between the gelator and nongelator molecules can be detected by using this technique. Interestingly, 1:1 gels of gelator LLD and of nongelator LLL showed a modest positive CD signal, whereas both LLD and LLD + DDD gels gave rise to negative signals (Figure 7).[25] In a similar fashion, gels of gelator DDL

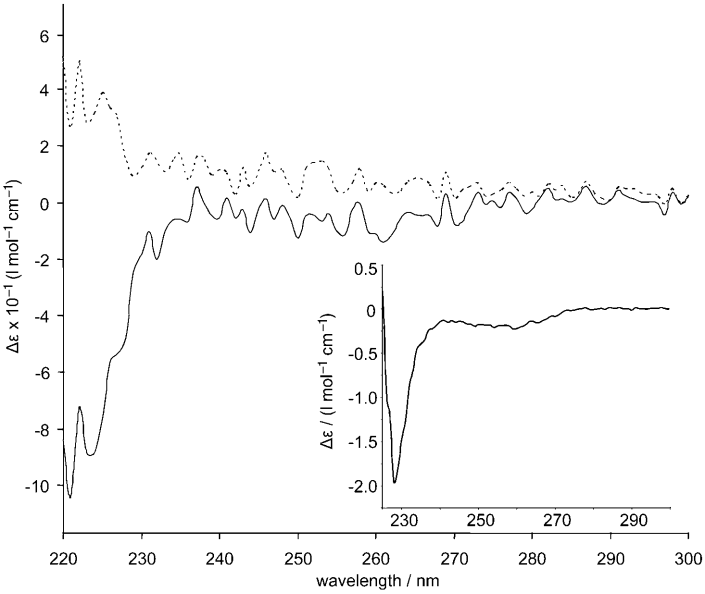


Figure 7. CD spectra of 2.4 mM LLD + LLL (-----) and LLD + DDD (——) hydrogels in 1:1 ratios (inset: spectrum of 2.4 mM LLD gel).

with nongelator DDD gave a negative CD signal, whereas gels of DDL only, or of DDL + LLL, gave positive peaks (see Supporting Information for figure).[25] CD sign inversions have previously been observed by Shinkai and co-workers for cholesterol-based gelators in organic solvents, and they were related to the variable cooling speeds of samples during gel preparation.[23] However, in this case, all samples were prepared and handled identically and the CD sign inversions observed for duplicate samples were found to be reproducible. Therefore, these results prove that interactions are taking place at the molecular level in the gels between gelators LLD and DDL, and non-hydrogelators LLL and DDD, respectively. Such interactions most probably derive from the incorporation the nongelator molecules into the gel fibres, as was also suggested from the T_{gs} measurement results, thus leading to a change in the morphology of the gels. With respect to eventual molecular interactions taking place in a gel containing LLD and DDD (or DDL and LLL), no changes in CD sign were observed in the spectra. Such results are indicative of either a lack of molecular interactions between these particular gelator and nongelator combinations, which seems highly improbable due to the large changes observed in the T_{gs} measurements, or of the fact

that the CD sign of the mixed gelator/nongelator samples is determined by the nongelator; that is, mixed samples containing the nongelator LLL lead to a positive CD sign, while samples containing the nongelator DDD lead to a negative CD sign. The gels were investigated by TEM to clarify this matter further.

Transmission electron microscopy studies: TEM was employed to obtain visual insight into the gel structure morphology, and to shed light on the interactions between the different derivatives of 1,3,5-cyclohexyltricarboxamide phenylalanine in the gels. Gels of LLD or DDL (2.0 mM) consist mainly of very long, thin fibre bundles with very uniform diameters of approximately 20 nm (Figure 8). A few thicker

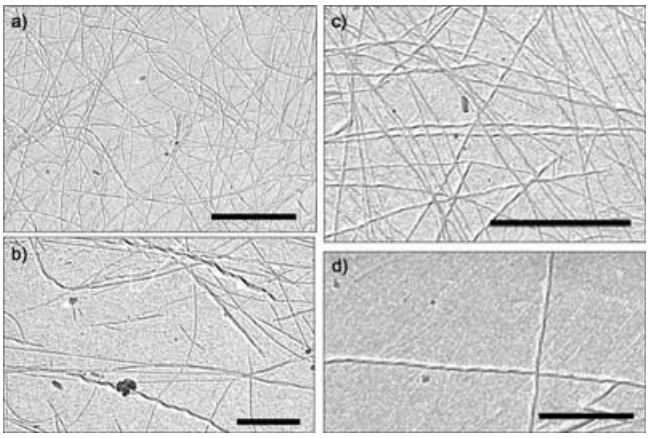


Figure 8. TEM images 2.0 mM LLD (a, b) and DDL (c, d) hydrogels (scale bars a and c: 1 μm, b and d: 500 nm).

bundles, 50–80 nm, which seem to arise from the intertwining of thinner bundles are also present (Figure 8c); fibre branching was not observed. Considering that each gelator molecule is approximately 14–15 Å wide, the cross-section of a gel fibre bundle 20 nm in diameter should therefore consist of 13–15 monomolecular-thick fibres (primary fibres). Furthermore, several right-handed fibres can be distinguished in the Pt-shadowed electron micrographs of the LLD gel (Figure 8a and b), whereas left-handed helices are present in the DDL gel (Figure 8c and d). To determine whether gelator concentration affects gel morphology, 1.0 and 4.0 mM gels of LLD were investigated by cryo-TEM. Figure 9a shows that in the 1.0 mM gel fibre bundles of approximately 15 nm in diameter are mainly present, whereas in the 4.0 mM gel 100–200 nm bundles, deriving from intertwined thinner bundles, can predominantly be found (Figure 9b and c). The presence of such larger intertwined bundles at high gelator concentrations is in excellent agreement with the higher turbidity and T_{gs} values observed for these gels.

Mixing of gelators LLD and DDL with nongelators LLL and DDD led to detectable changes in gel fibre morphology that reflect the results obtained with CD spectroscopy. TEM images of a gel containing LLD + DDD show straight fibres as well as some right-handed helical fibres, while for a LLD + LLL gel left-handed helical fibres are visible (Figure 10). Such a change in handedness, brought about by mixing the LLL component with the LLD gelator, confirms the change in CD sign observed for the same system (see above). In the case of DDL, mixing with LLL did not cause changes in the fibre helicity, whereas mixing with DDD led to the formation of right-handed helical fibres. Therefore, it seems that the presence of the nongelator LLL leads to left-handed helical fibres, and vice versa for the nongelator DDD. Interestingly, this behaviour has been observed in other peptide-based aggregates. The L-chirality of such peptides, although the structure is quite different from that of 1,3,5-cyclohexyltricarboxamide-phenylalanine gelators, leads to β-sheet ribbons with an intrinsically left-handed twist.[26] Finally, a gel containing the two gelators LLD and DDL is observed to consist mainly of straight, non-helical fibres (see Supporting Information for figure), which agrees with the fact that no signal was observed in the corresponding CD spectrum. Such a lack of specific fibre handedness could be due to incorporation of the two types of gelator molecules, LLD and DDL, within the same fibre.

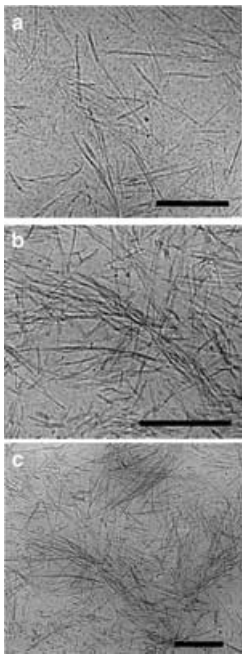


Figure 9. Cryo-TEM images of 1.0 mM (a) and 4.0 mM (b, c) LLD hydrogels (scale bars: 500 nm).

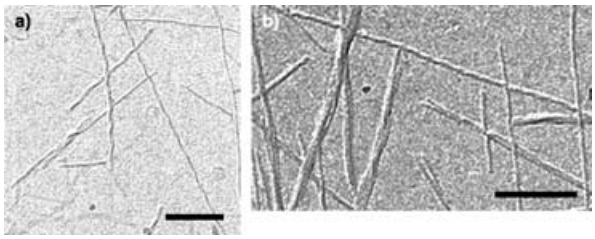


Figure 10. TEM images of 2.4 mM LLD + DDD (a) and LLD + LLL (b) hydrogels in 1:1 ratios (scale bars: 250 nm).

Discussion

These results highlight the importance of different types of interactions and their relative contributions with respect to the gelating capability of a compound in water. In the case of the homochiral 1,3,5-cyclohexyltricarboxamide-phenylalanine (LLL or DDD) compounds, molecular interactions occur

not only in a one-dimensional fashion, but also in two and three dimensions, thereby causing precipitation of the compound in water. Although a crystal structure of this compound could not be obtained, that of 1,3,5-cyclohexyltricarboxamide-L-tyrosine was solved (Figure 11).[12] It can therefore be assumed that 1,3,5-cyclohexyltricarboxamide-L-phenylalanine molecules would be arranged in a similar fashion. The molecules stack through the formation of a triple chain of intermolecular hydrogen bonds and the individual stacks are packed hexagonally, giving rise to hydrophobic areas in which the phenyl rings of neighbouring tyrosines (or phenylalanines) come together.[12] Changing the chirality of one phenylalanine from L to D causes the molecules to lose their symmetry, thus assuming a different overall shape. However, their ability to aggregate in a stack-like fashion remains unimpaired. The loss of molecular symmetry, or the fact that the resulting fibres can be constituted of stacked molecules with either matching (D on D) or different (D on L) chiral centres above and below each other, gives rise to a more disordered system which makes crystallization unfavourable, and thus causes gelation to occur. Similarly, the peripheral substituents (R groups) of compounds **1**–**6** also inhibit strong 2D and 3D interactions from taking place, making these 1,3,5-cyclohexyltricarboxamide-L-phenylalanine derivatives good hydrogelators in spite of the fact that all three amino acid have the same chirality.

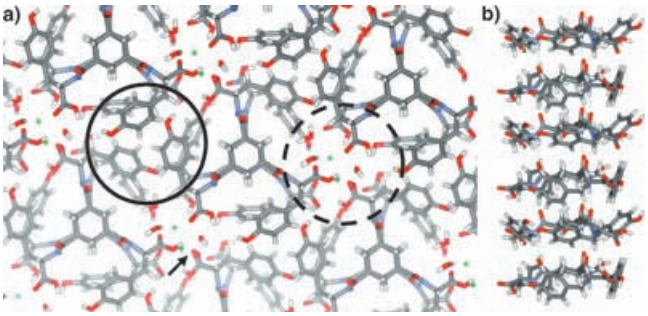


Figure 11. X-ray crystal structure of 1,3,5-cyclohexyltricarboxamide-L-tyrosine: a) view along the *a* axis showing the packing of the individual stacks (arrow: disordered carbonyl, solid circle: hydrophobic area, dashed circle: hydrophilic area); b) side view of a single stack showing the intermolecular triple hydrogen bonding motif.

In the case of homochiral derivatives LLL and DDD crystallization occurs independently of concentration; this indicates that the interactions between the stacks of molecules (in the second and third dimensions) are energetically favourable. However, for the heterochiral derivatives LLD and DDL, primary gel fibres form at low gelator concentrations, reflecting the fact that one-dimensional aggregation is in this case energetically more favourable than 2D and 3D interactions. These primary fibres subsequently aggregate into bundles with very monodisperse diameters of approximately 20 nm. The formation of such monodisperse aggregates by self-assembling peptides was recently ascribed by Boden et al.[26] to the intrinsic chirality of their system. They showed that aggregation occurs via increasingly complex structures and leads to the formation of fibre bundles (referred to as fibrils) of finite width due to the competition stemming from the free energy gain from the attraction between primary fibres, and the energy loss from the elastic distortion that occurs when the twisted fibres aggregate into a bundle of fibres. In this paper, we have furthermore shown that LLD gels consist of larger fibre bundles deriving from intertwined thinner bundles (cryo-TEM) at high gelator concentrations. The transition from the thinner aggregates to the larger ones follows a sigmoidal relationship as a function of the gelator concentration (see Figures 1 and 2: dropping ball and turbidity measurements). Such sigmoidal behaviour is similar to that generally encountered in concentration dependent studies of surfactant solutions, where the cooperative formation of micelles occur.[27] Evidently, once fibres bundles are formed in LLD gels, interactions between bundles arise that lead to the formation of larger intertwined aggregates. Apparently, a certain bundle concentration is needed to make the formation of large intertwined bundles more favourable, in analogy to micellar systems.

Conclusion

In summary, we have shown how the gelation behaviour of 1,3,5-cyclohexyltricarboxamide phenylalanine derivatives in water can be controlled by either varying the chirality of one of the amino acids, or by peripheral functionalization of the compound with a second amino acid or a hydrophilic moiety. Whereas homochiral 1,3,5-cyclohexyltricarboxamide phenylalanine does not gelate water, its heterochiral variants are good hydrogelators. The chiral nature of the investigated gelators is reflected microscopically in their CD activity, and at a macroscopic level in the helical fibre morphologies observed by TEM. These two techniques, as well as gel-to-sol transition temperature measurements, have made it possible to conclude that heterochiral gelator and homochiral nongelator molecules interact with each other within individual gel fibres. Such interactions can lead to changes in the gel fibre helicity, and in the gel-to-sol transition temperatures of the materials. Furthermore, once the fibre bundles are formed, interactions between them can also take place. These interactions lead to large intertwined bundles; their concentration-dependent formation is strikingly analogous to the formation of micelles in surfactant solutions. The straightforward design of amino acid substituted 1,3,5-cyclohexyltricarboxamide-based molecules has enabled us to investigate the apparently serendipitous effect of chirality on gelation in a methodical fashion, thus obtaining a better understanding of these hierarchical self-assembled architectures.

Experimental Section

General: All chemicals were purchased from Aldrich or Fluka and used without further purification. NMR experiments were performed by using a Varian Gemini NMR spectrometer operating at 200 MHz, or a Varian VXR NMR spectrometer operating at 300 MHz. All spectra were recorded in [D_6]DMSO unless stated otherwise. MS spectra were measured on a JOEL JMS-600H or a Science API 3000 mass spectrometer.

Synthesis: The syntheses of compounds **1**–**3** were carried out according to literature procedures.[12] Yields were not optimized.

CHex(Am-L-Phe-L-Ala)$_3$ (4): Compound **4** was synthesized according to literature procedures,[12] by using H-L-Phe-L-Ala-OMe·*x*TFA (4.8 g, 13 mmol). After the reaction was completed, the precipitate was filtered off, washed with MeOH, and dried in vacuo to give **4** (0.54 g, 24%). ^{1}H NMR: δ=1.03–1.32 (m, 2H), 1.27 (d, ^3J=7.3 Hz, 3H; CH_3), 2.12 (brm, 1H), 2.68 (t, 1H; CH_2), 2.98 (d, ^3J=13.9 Hz, 1H; CH_2), 4.19 (m, 1H; CH), 4.48 (m, 1H; CH), 7.20 (m, 5H; Ar), 7.97 (d, ^3J=9.2 Hz, 1H; NH), 8.37 (d, ^3J=7.0 Hz, 1H; NH); ^{13}C NMR: δ = 17.19, 37.54, 42.06, 47.63, 53.34, 126.17, 127.93, 129.17, 138.01, 171.31, 174.01; elemental analysis calcd (%) for $C_{45}H_{54}N_6O_{12}\cdot 5H_2O$: C 56.24, H 6.71, N 8.74; found: C 56.38, H 6.51, N 8.60; EI-MS: *m*/*z*: calcd for $C_{45}H_{54}N_6O_{12}$: 870.38; found 434.1 $[M-2)/2]^{2-}$, 870.5 $[M]^-$.

CHex(Am-L-Phe-D-Ala)$_3$ (5): Compound **5** was synthesized similarly to **4** by using H-L-Phe-D-Ala-OMe·*x*TFA (4.8 g, 13 mmol); yield: 0.54 g, 24%; ^{1}H NMR: δ=1.08–1.39 (m, 2H), 1.18 (d, ^3J=7.3 Hz, 3H; CH_3), 2.15 (brm, 1H), 2.72 (t, 1H; CH_2), 2.90 (q, 1H; CH_2), 4.17 (m, 1H; CH), 4.53 (m, 1H; CH), 7.18 (m, 5H; Ar), 7.92 (d, ^3J=9.2 Hz, 1H; NH), 8.28 (d, ^3J=7.7 Hz, 1H; NH); ^{13}C NMR: δ = 17.47, 31.15, 42.11, 47.38, 53.33, 126.26, 127.93, 129.23, 137.72, 170.95, 173.91; elemental analysis calcd (%) for $C_{45}H_{54}N_6O_{12}\cdot 4.25H_2O$: C 57.04, H 6.65, N 8.87; found: C 56.70, H 6.10, N 8.68; EI-MS: *m*/*z*: calcd for $C_{45}H_{54}N_6O_{12}$: 870.38; found: 893.5 $[M+Na]^+$.

CHex(Am-L-Phe-β-Ala)$_3$ (6): Compound **6** was synthesized similarly to **4** by using H-L-Phe-β-Ala-OMe ·*x*TFA (4.6 g, 13 mmol); yield: 1.18 g, 80%; ^{1}H NMR: δ = 1.03–1.40 (m, 2H), 2.14 (m, 1H), 2.32 (m, 2H), 2.71 (t, 1H; CH_2), 2.89 (d, 1H; CH_2), 3.23 (m, 2H), 4.40 (m, 1H; CH), 7.19 (m, 5H; Ar), 7.94 (d, ^3J=8.4 Hz, 1H; NH), 8.07 (s, 1H; NH), 12.24 (brm, 1H); ^{13}C NMR: δ = 31.11, 33.79, 34.80, 37.89, 42.14, 53.60, 126.20, 127.95, 129.14, 137.90, 171.30, 172.87, 173.94; elemental analysis calcd (%) for $C_{45}H_{54}N_6O_{12}\cdot 2H_2O$: C 59.01, H 6.49, N 9.17; found: C 58.92, H 6.38, N 8.91; EI-MS: *m*/*z*: calcd for $C_{45}H_{54}N_6O_{12}$: 870.38; found: 869.3 $[M-H]^-$.

CHex(COOH)$_2$(COOBz) (7): CDI (37.5 g, 0.24 mol) was added to a solution of *cis*,*cis*-1,3,5-cyclohexanetricarboxlic acid (50 g, 0.24 mol) in a mixture of DMSO (250 mL) and ethyl acetate (1 L). The solution was stirred for an additional 2 h. Benzyl alcohol (24.8 g, 0.24 mol) was subsequently added and the mixture was stirred overnight. The main part of the solvent was removed in vacuo. To the remaining viscous solution water (500 mL) was added and the aqueous fraction was extracted with ethyl acetate (3×300 mL). The aqueous layer was brought to pH 1 by using 2N HCl (aq). The acidified aqueous layer was extracted with ethyl acetate (3×300 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, and evaporated to dryness to give pure **7** as a white solid (38.16 g, 52%). ^{1}H NMR: δ=1.26 (m, 3H; CHex), 2.09 (m, 3H; CHex), 2.25–2.65 (m, 3H; CHex), 5.09 (s, 2H; CH_2Ar), 7.35 (m, 5H; Ar), 12.27 (brs, 2H; COOH); ^{13}C NMR: δ = 29.3, 29.5, 39.5, 64.5, 126.8, 127.0, 127.4, 135.2, 172.8, 174.5; elemental analysis calcd (%) for $C_{16}H_{18}O_6\cdot{}^1/_2H_2O$: C 60.93, H 6.08; found C 60.94, H 6.01; EI-MS: *m*/*z*: calcd for $C_{16}H_{18}O_6$: 306.1; found 306.0 $[M]^+$.

CHex(Am-L-Phe-OMe)$_2$(COOBz) (8): A solution of **7** (3.06 g, 10.0 mmol), H-L-Phe-OMe·HCl (4.73 g, 22.0 mmol), Et_3N (3.03 g, 30.0 mmol), and DMT-MM[28] (6.08 g, 22.0 mmol) in MeOH (50 mL) was stirred overnight at room temperature. The resulting precipitate was filtered off, rinsed with MeOH (50 mL), and dissolved in CH_2Cl_2 (200 mL). The organic layer was washed with 0.5N HCl (3×100 mL), brine (100 mL), dried with $MgSO_4$, and evaporated to dryness. The crude product was dissolved in CH_2Cl_2 and MeOH (75 + 110 mL). Slow evaporation of the CH_2Cl_2 resulted in the precipitation of pure **8** as a white solid (2.80 g, 45%). ^{1}H NMR: δ=1.05–1.35 (brm, 3H; CHex), 1.55, 1.70, 1.85 (3×brm, 3×1H; CHex), 2.1–2.4 (brm, 3H; CHex), 2.8–3.1 (m, 4H; CH_2Ar), 3.57, 3.58 (2×s, 2×3H; OMe), 4.43 (m, 2H; CH), 5.08 (s, 2H; OCH_2Ar), 7.17 (m, 10H; Ar), 7.35 (m, 5H; Ar), 8.19, 8.23 (2×d, J= 3.7 Hz, 2×1H; NH); ^{13}C NMR: δ=29.7, 35.5, 39.9, 40.6, 40.8, 50.8, 52.1, 52.3, 64.5, 125.4, 126.8, 127.0, 127.1, 127.4, 128.0, 135.1, 136.2, 136.3, 171.1, 172.7, 172.8, 172.9; elemental analysis calcd (%) for $C_{36}H_{40}N_2O_8$: C 68.77, H 6.41, N 4.46; found: C 68.55, H 6.43, N 4.72; EI-MS: *m*/*z*: calcd for $C_{36}H_{40}N_2O_8$: 628.3; found: 627.4 $[M-H]^-$.

Similarly, with H-D-Phe-OMe·HCl, CHex(Am-D-Phe-OMe)$_2$(COOBz) was obtained as a white solid (4.00 g, 64%). elemental analysis calcd (%) for $C_{36}H_{40}N_2O_8$: C 68.77, H 6.41, N 4.46; found: C 68.50, H 6.49, N 4.66.

CHex(Am-L-Phe-OMe)$_2$(COOH) (9): 10% Pd/C (ca. 100 mg) was added to a solution of **8** (2.4 g, 3.82 mmol) in MeOH/*i*PrOH/CH_2Cl_2 (100 + 100 + 200 mL); after the addition the mixture was degassed and subsequently stirred vigorously under a H_2 atmosphere for 3 d. After removal of the catalyst by filtration (double paper filter) the resulting solution was evaporated to dryness to give pure **9** as a white solid (2.00 g, 97%). ^{1}H NMR: δ=1.1–1.3 (brm, 3H; CHex), 1.55, 1.70, 1.85 (3×brm, 3×1H; CHex), 2.1–2.3 (brm, 3H; CHex), 2.75–3.1 (m, 4H; CH_2Ar), 3.6 (s, 6H; OMe), 4.42 (m, 2H; CH), 7.20 (m, 10H; Ar), 8.23 (m, 2H; NH), 12.2 (brs, 1H; COOH); ^{13}C NMR: δ=24.4, 29.8, 35.5, 40.9, 50.8, 52.3, 125.4, 127.1, 128.0, 136.2, 171.1, 173.0, 174.7; elemental analysis calcd (%) $C_{29}H_{34}N_2O_8$: C 64.66, H 6.37, N 5.20; found: C 64.56, H 6.41, N 5.40; EI-MS: *m*/*z*: calcd for $C_{29}H_{34}N_2O_8$: 538.2; found: 539.3 $[M+H]^+$.

Similarly, CHex(Am-D-Phe-OMe)$_2$(COOH) was obtained as a white solid (3.13 g, 95%). elemental analysis calcd (%) for $C_{29}H_{34}N_2O_8\cdot{}^1/_4H_2O$: C 64.14, H 6.40, N 5.16; found: C 64.13, H 6.52, N 5.19.

CHex(Am-L-Phe-OMe)$_2$(Am-D-Phe-OMe) (10): DMT-MM (0.95 g, 3.60 mmol) was added to a solution of **9** (1.85 g, 3.44 mmol), H-D-Phe-OMe·HCl (0.78 g, 3.60 mmol), Et_3N (0.50 g, 5.00 mmol) in MeOH (50 mL). The mixture was stirred overnight after which the formed precipitated was filtered off, washed with MeOH (3×30 mL), and dried to give pure **10** as a white solid (1.09 g, 45%). ^{1}H NMR: δ=1.05–1.80 (brm, 6H; CHex), 2.15 (brm, 3H; CHex), 2.75–3.10 (m, 6H; CH_2Ar), 3.58 (s, 9H; OMe), 4.43 (brm, 3H; CH), 7.19 (s, 15H; Ar), 8.20 (brs, 3H; NH); ^{13}C NMR: δ=30.0, 35.5, 41.1, 50.8, 52.2, 125.4, 127.1, 128.0, 136.2, 171.1, 173.1; elemental analysis calcd (%) for $C_{39}H_{45}N_3O_9\cdot{}^1/_2H_2O$: C 66.09, H 6.54, N 5.93; found: C 66.16, H 6.48, N 5.99; EI-MS: *m*/*z*: calcd for $C_{39}H_{45}N_3O_9$: 699.3; found: 700.2 $[M+H]^+$.

Similarly, CHex(Am-D-Phe-OMe)$_2$(Am-L-Phe-OMe) was obtained as a white solid (1.64 g, 54%). Elemental analysis calcd (%) for $C_{39}H_{45}N_3O_9\cdot{}^1/_2H_2O$: C 66.09, H 6.54, N 5.93; found C 66.00, H 6.41, N 5.91.

CHex(Am-L-Phe-OH)$_2$(Am-D-Phe-OH) (11, LLD): A suspension of **10** (0.90 g, 1.29 mmol) in a mixture of MeOH (50 mL) and 2N NaOH (aq) (50 mL) was stirred for 2 d, after which the now-clear solution was filtered, added to H_2O (100 mL), and subsequently acidified to pH 1 by using concentrated HCl (aq). The formed gel precipitate was filtered off, washed with H_2O (3×50 mL), and dissolved in hot MeOH/H_2O (5:1, approximately 400 mL). This solution was evaporated to dryness, the remaining solvent was stripped off using a mixture of toluene and MeOH (1:1, 3×50 mL), and the solid was dried in vacuo to give pure **11** as a white solid (0.63 g, (74%). ^{1}H NMR: δ=1.05–1.70 (m, 6H; CHex), 2.13 (m, 3H; CHex), 2.75–3.10 (m, 6H; CH_2Ar), 4.36 (brm, 3H; CH), 7.18 (s, 15H; Ar), 8.04 (m, 3H; NH), 12.6 (brs, 3H; COOH); ^{13}C NMR: δ=30.1, 35.5, 41.1, 52.1, 125.3, 127.0, 128.0, 136.7, 172.1, 173.0; elemental analysis (%) calcd for: $C_{36}H_{39}N_3O_9\cdot 2H_2O$: C 62.33, H 6.25, N 6.06; found: C 62.22, H 5.94, N 6.03; EI-MS: *m*/*z*: calcd for $C_{36}H_{39}N_3O_9$: 657.3; found: 656.5 $[M-H]^-$.

Similarly, CHex(Am-D-Phe-OH)$_2$(Am-L-Phe-OH) (**12**, DDL) was obtained as a white solid (0.83 g, 59%). Elemental analysis for $C_{36}H_{39}N_3O_9\cdot H_2O$: C 63.99, H 6.12, N 6.22; found: C 63.85, H 6.11, N 6.13.

Gelation tests: Gelation was considered to have occurred when a homogeneous substance was obtained which exhibited no gravitational flow upon inversion of the container (generally a glass vial) in which it was

found. Gel-to-sol transition temperatures (T_{gs}) were determined by using the "dropping ball" method, which consisted of carefully placing a steel ball (65 mg, 2.5 mm in diameter) on top of prepared gels in 2 mL glass vials and subsequently placing these vials in a heating block.[4] The temperature of the heating block was increased by 5 °C h^{-1} and the T_{gs} was defined as the temperature at which the steel ball reached the bottom of the vial, as observed by a CCD camera. All T_{gs} measurements were carried out in duplicate or triplicate. The error on T_{gs} values was ±5 °C. All gels were allowed to stand at room temperature for one week before any measurements/characterization was carried out.

CD spectroscopy: CD spectra were measured using a JASCO J-715 spectropolarimeter. Gel samples were prepared in closed vials and after standing at room temperature for one week the gels were transferred to an optical cell (0.5 mm optical path length) for measurement. Variable-temperature CD on a 2.28 mM LLD gel was carried out by increasing the temperature in steps of 5 °C, allowing ample equilibration time, to a maximum of 96 °C to prevent drying out of the samples due to water evaporation. All measurements were carried out in duplicate. No contribution from linear dichroism was observed, as no change in absorption was measured when the sample cell was rotated by 90°.

TEM measurements: Samples were prepared by taking a piece of the gel, placing it on a carbon-coated copper grid (400 mesh), and removing most of it after 1 min. Samples were shadowed with platinum at a 30° angle. All samples were examined using a JEOL 1200 EX transmission electron microscope operating at 100 kV. For cryo-TEM measurements, a few microliters of gel were deposited on a bare 700 mesh copper grid. After blotting away the excess of liquid the grids were rapidly plunged into liquid ethane. Frozen hydrated specimens were mounted in a cryo-holder (Gatan, model 626) and observed in a Philips CM 120 electron microscope, operating at 120 KV. Micrographs were recorded under low-dose conditions on a slow-scan CCD camera (Gatan, model 794).

[1] P. Terech, R. G. Weiss, *Chem. Rev.* **1997**, *97*, 3133–3159; D. J. Abdallah, R. G. Weiss, *Adv. Mater.* **2000**, *12*, 1237–1247.
[2] L. A. Estroff, A. D. Hamilton, *Chem. Rev.* **2004**, *104*, 1201–1217.
[3] A.-C. Couffin-Hoarau, A. Motulsky, P. Delmas, J.-C. Leroux, *Pharm. Res.* **2004**, *21*, 454–457.
[4] A. Friggeri, B. L. Feringa, J. van Esch, *J. Controlled Release* **2004**, *97*, 241–248.
[5] G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler, S. I. Stupp, *Science* **2004**, *303*, 1352–1355.
[6] S. Kiyonaka, K. Sada, I. Yoshimura, S. Shinkai, N. Kato, I. Hamachi, *Nat. Mater.* **2004**, *3*, 58–64.
[7] L. Brunsveld, H. Zhang, M. Glasbeek, J. A. J. M. Vekemans, E. W. Meijer, *J. Am. Chem. Soc.* **2000**, *122*, 6175–6182.
[8] J. J. van Gorp, J. A. J. M. Vekemans, E. W. Meijer, *J. Am. Chem. Soc.* **2002**, *124*, 14759–14769.
[9] M. Masuda, P. Jonkheijm, R. P. Sijbesma, E. W. Meijer, *J. Am. Chem. Soc.* **2003**, *125*, 15935–15940.
[10] M. de Loos, J. van Esch, I. Stokroos, R. M. Kellogg, B. L. Feringa, *J. Am. Chem. Soc.* **1997**, *119*, 12675–12676.
[11] K. Hanabusa, A. Kawakami, M. Kimura, H. Shirai, *Chem. Lett.* **1997**, 191–192.
[12] K. J. C. van Bommel, C. van der Pol, I. Muizebelt, A. Friggeri, A. Heeres, A. Meetsma, B. L. Feringa, J. van Esch, *Angew. Chem.* **2004**, *116*, 1695–1699; *Angew. Chem. Int. Ed.* **2004**, *43*, 1663–1667.
[13] J. Makarevic, M. Jokic, Z. Raza, Z. Štefanic, B. Kojic-Prodic, M. Žinic, *Chem. Eur. J.* **2003**, *9*, 5567–5580, and references therein; V. Caplar, M. Žinic, J.-L. Pozzo, F. Fages, G. Mieden-Gundert, F. Vögtle, *Eur. J. Org. Chem.* **2004**, 4048–4059.
[14] J.-H. Fuhrhop, P. Schnieder, J. Rosenberg, E. Boekema, *J. Am. Chem. Soc.* **1987**, *109*, 3387–3390.
[15] K. Hanabusa, K. Okui, K. Karaki, M. Kimura, H. Shirai, *J. Colloid Interface Sci.* **1997**, *195*, 86–93.
[16] Y. Watanabe, T. Miyasou, M. Hayashi, *Org. Lett.* **2004**, *6*, 1547–1550.
[17] M. de Loos, J. van Esch, R. M. Kellogg, B. L. Feringa, *Angew. Chem.* **2001**, *113*, 633–636; *Angew. Chem. Int. Ed.* **2001**, *40*, 613–616.
[18] A. R. Hirst, D. K. Smith, M. C. Feiters, H. P. M. Geurts, *Chem. Eur. J.* **2004**, *10*, 5901–5910.
[19] The cgc is the lowest concentration of gelator molecules capable of gelling a liquid at room temperature.[1]
[20] The racemic mixture that was obtained by carrying out the synthesis with racemic phenylalanine, was also capable of gelating water with a cgc similar to that of the pure heterochiral gelators LLD and DDL. However, the appearance of the gels was turbid to white, depending on the concentration, due to the presence of needle-like crystalline material from the homochiral components, LLL and DDD. Such crystalline material was observable with the naked eye and was birefringent when observed by optical microscopy.
[21] H. Kobayashi, K. Koumoto, J. Hwa Jung, S. Shinkai, *J. Chem. Soc. Perkin Trans. 2* **2002**, 1930–1936; A. Friggeri, O. Gronwald, K. J. C. van Bommel, S. Shinkai, D. N. Reinhoudt, *J. Am. Chem. Soc.* **2002**, *124*, 10754–10758; H. Kobayashi, M. Amaike, J. Hwa Jung, A. Friggeri, S. Shinkai, D. N. Reinhoudt, *Chem. Commun.* **2001**, 1038–1039.
[22] The spectra of LLD and DDL showed slight differences around the 200–225 nm due to noise that can occur in this spectral region.
[23] K. Murata, M. Aoki, T. Suzuki, T. Harada, H. Kawabata, T. Komori, F. Ohseto, K. Ueda, S. Shinkai, *J. Am. Chem. Soc.* **1994**, *116*, 6664–6676.
[24] The T_{gs} of a 2.40 mM LLD sample as measured by the dropping ball method should be between 60 and 120 °C. Temperature-dependent CD spectroscopy was attempted with 3 mM samples, however, no effect was observed due to the very high temperature necessary to melt the gel. Experiments at gelator concentrations below 2 mM were not carried out due to the very small temperature range available for the measurement. LLD solutions did not give rise to CD signals.
[25] All CD spectroscopy experiments, excluding temperature-dependent experiments, were carried out at three different concentrations: 1.5, 2.4, and 5.3 mM; however, no significant differences were observed in the obtained spectra. For mixed gel samples containing gelators (LLD or DDL) and nongelators (LLL or DDD) the CD signal intensities were considerably lower than for samples containing only gelators (LLD or DDL) due to increased turbidity of the mixed samples with respect to the pure gelator samples.
[26] A. Aggeli, I. A. Nyrkova, M. Bell, R. Harding, L. Carrick, T. C. B. McLeish, A. N. Semenov, N. Boden, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11857–11862.
[27] R. J. Hunter, *Foundations of Colloid Science*, 2nd ed., Oxford University Press, Oxford, **2001**.
[28] M. Kunishima, C. Kawachi, J. Morita, K. Terao, F. Iwasaki, S. Tani, *Tetrahedron* **1999**, *55*, 13159–13170.

Received: January 4, 2005
Published online: July 8, 2005

