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Optimizing Biomimetic Gelators Constructed from Amino Acid Building Blocks

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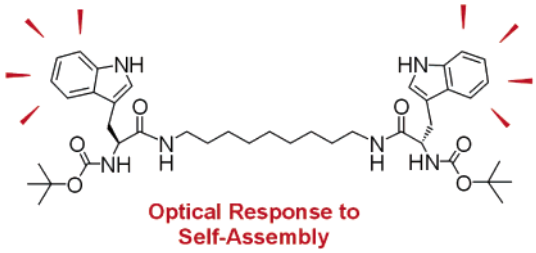

This paper reports the use of a range of amino acids to construct diverse gelators, employing structures in which Boc-protected amino acids are attached to either end of an aliphatic diamine spacer chain. The choice of amino acid determines whether nanoscale self-assembly takes place and controls the properties of the resultant material, while the function of the amino acid (e.g., the optical properties of tryptophan) is translated into the self-assembled nanostructured gel.

The use of noncovalent interactions to reversibly assemble molecular-scale building blocks into nanoscale architectures is a research area of intense current interest.[1] Indeed, the assembly of low-molecular-weight organic molecules to yield soft gel-phase materials has been a particular focus.[2] The assembly of synthetic molecules generating fibrillar gel-phase assemblies is a biomimetic process—the amyloid fibrils associated with Alzheimer's disease are assembled via protein−protein interactions.[3] Furthermore, the assembly of peptides in lipid phases plays a key role in the development of functional cell membranes.[4] A wide range of different organic building blocks assemble into gels, with saccharides,[5] nucleic acids,[6] cholesterol derivatives,[7] and ureas/amides/peptides[8] being widely used.

Previously, we have used lysine as a building block to construct dendritic molecules which act as gelators.[9] Lysine (Lys) is one of the most widely exploited amino acids in gelator synthesis because it has multiple points of functionalization and is a dense source of hydrogen bonding functional groups.[10] Innovative studies have also explored other amino acids, although in general, amino acids with relatively nonfunctional side chains (e.g., phenylalanine, alanine, and valine) have been the most widely investigated.[11,12] In a recent interesting paper, Chow and co-workers employed dendritic structures containing different amino acid building blocks.[13] There is still a need for systematic studies of the effect of amino acid modification on gelation. Furthermore, because proteins achieve their function by using an array of amino acids with different properties, we reasoned that, in analogy, generating gelators using different amino acids would ultimately provide us with access to soft materials which could exhibit biomimetic function.

Our previously reported Lys-based gelator (**1-Lys**)[14] provided the starting point. Similar "bolaform amides" of this type have previously been reported by other researchers, but generally using amino acids with relatively simple side chains.[11a,h] This class of molecule assembles primarily as a consequence of hydrogen bond interactions between the amide groups which connect the head groups to the bolaform structure (and to a lesser extent hydrogen bonds between carbamates).[11a,h,14] For our initial study, we synthesized compounds **1**−**6** based on Boc-protected lysine (Lys), alanine (Ala), phenylalanine (Phe), glutamine (Gln), protected cysteine (Cys), and tryptophan (Trp), respectively (Figure 1). These compounds were synthesized by coupling the appropriate protected amino acid with the diaminononane spacer, using DCC or EDC methodology. All products were characterized using standard spectroscopic methods, and full data are included in the Supporting Information.

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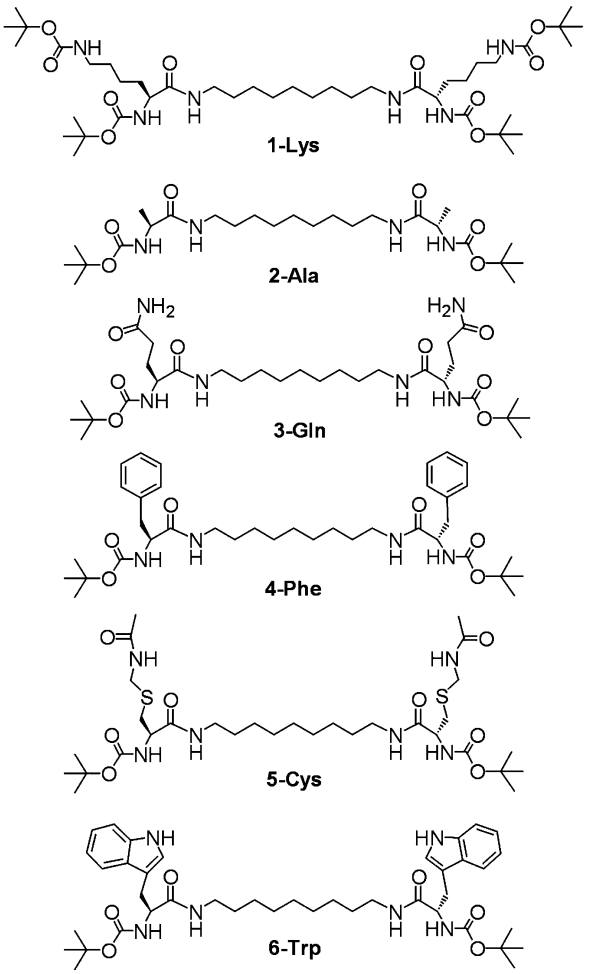

FIGURE 1. Compounds investigated for self-assembly and gelation properties.

We monitored the ability of these compounds to form gels in toluene. Compound **2-Ala** did not form gels, yielding instead a precipitate. Unlike **1-Lys**, **2-Ala** does not possess additional hydrogen bonding functionality in the amino acid side chain to assist gelation. However, related molecules based on valine (a bulkier analogous amino acid) have been previously demonstrated to form gels.[12] We suggest that the lack of hydrogen bonding groups *and* steric bulk means that **2-Ala** prefers to precipitate, rather than assemble in a dimensionally constrained manner (e.g., forming fibrils or platelets)—a prerequisite for gelation.[15] Compound **3-Gln**, perhaps surprisingly given it has additional hydrogen bonding functionality, also did not form gels in toluene, yielding a precipitate. This compound did form a gel in chloroform and acetonitrile; however, a full solvent study is beyond the scope of this Note, where we are primarily interested in comparing related gelators within a single solvent system.

All of the other compounds yielded gels in toluene which were investigated using tube inversion methodology to generate a phase diagram (Figure 2).[16] Thermal stabilities and minimum gelation concentration (MGC) values were strongly dependent

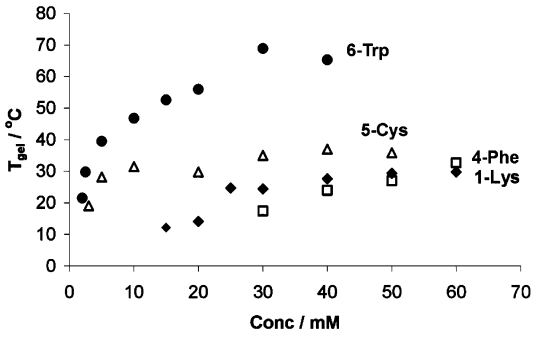

FIGURE 2. Phase diagram for compounds **6-Trp** (closed circles), **5-Cys** (open triangles), **4-Phe** (open squares), and **1-Lys** (closed diamonds) in toluene, indicating effect of gelator concentration on T_{gel} value.

on the amino acid. Gels based on **4-Phe** were, at low concentration, slightly less thermally stable than those based on **1-Lys**, but comparable at higher concentrations. Unlike **1-Lys**, **4-Phe** does not have hydrogen bonding functionality in the amino acid side chain; however, it does have an aromatic ring, which may be capable of $\pi-\pi$ interactions within a fibrillar assembly. Gels based on **5-Cys** were more thermally stable than equivalent systems based on **1-Lys**, and the MGC value was as low as 5 mM. Gelator **5-Cys** contains an amide within the thiol side chain protecting group, and this may make a significant contribution to the formation of a sample spanning intermolecular hydrogen bonded network (see below for NMR evidence to support this). Gelator **6-Trp** gave the most thermally stable gels, with an MGC at room temperature as low as 1−2 mM (<0.2 wt %/vol). Trp contains an indole ring in its side chain. The N−H group in the indole may become involved in intermolecular hydrogen bond networks, enhancing the stability of the gel (see below for NMR evidence). Furthermore, the extended aromatic system of Trp may be capable of more effective packing or $\pi-\pi$ interactions, although we did not see any direct evidence.

Field emission gun scanning electron microscopy (FEGSEM) was used to provide comparative visualization of the gels (Figure 3). On a qualitative level, gelator **6-Trp** gave the most effective nanoscale interpenetrating network, with fiber diameters of approximately 40 nm. The fibers formed by the other gelators were all significantly wider. Narrower fibers have more effective interpenetration and a greater surface area, factors which might be expected to increase the thermal stability of the gel. As such, these SEM observations are in agreement with the ability of **6-Trp** to form the most thermally stable gel.

Given that **6-Trp** gave the most thermally stable gels, we decided to investigate its behavior in more detail. Trp is a particularly interesting amino acid because the indole ring on the side chain has unique optical properties; indeed Trp is often used as a fluorescent "reporter group" in order to provide information about the three-dimensional folded structures of biomolecules.[17]

In the circular dichroism (CD) spectrum of **6-Trp**, a CD band corresponding to the indole heterocycle was observed at ca. 297 nm (Figure 4). Variable temperature (VT) circular dichroism studies demonstrated that on *increasing* the temperature the CD band *decreased* in intensity. This indicates that this CD band is associated with a temperature-sensitive process and can be

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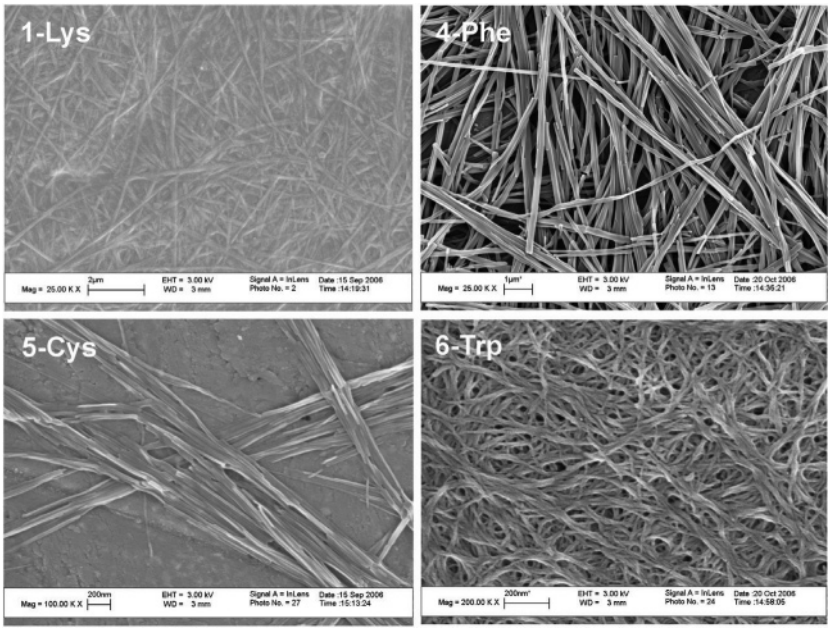

FIGURE 3. Field emission gun scanning electron microscopy (FEGSEM) images of gelators **1-Lys**, **4-Phe**, **5-Cys**, and **6-Trp**, indicating the presence of fibrillar self-assembled nanostructures. Samples were prepared by drying gels (3 mM) under ambient conditions (i.e., not vacuum drying).

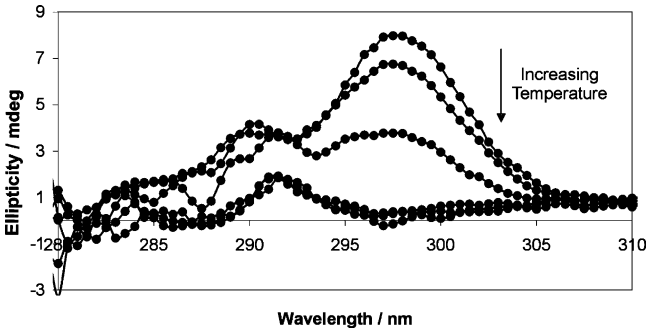

FIGURE 4. Variable temperature CD spectroscopy performed on gelator **6-Trp** as a viscous liquid (1 mM) in toluene. Temperature increased from 20 to 70 °C in increments of 10 °C.

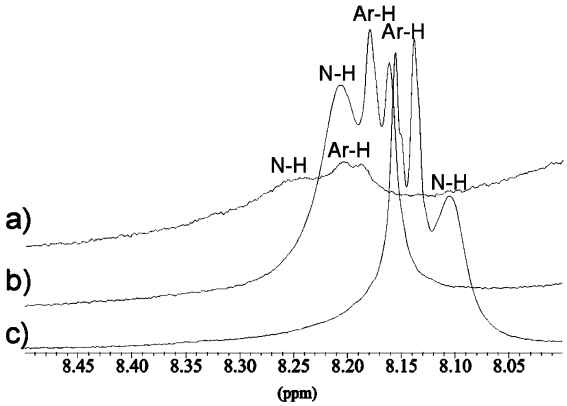

FIGURE 5. Variable temperature ^{1}H NMR spectroscopy performed on **6-Trp** (5 mM) in d_8-toluene at temperatures of (a) 30 °C, (b) 50 °C, and (c) 70 °C.

assigned to the self-assembly of **6-Trp**. This CD band demonstrates that the indole ring is located in an assembled nanoscale chiral environment and experiences an induced CD effect. Usefully, the CD band of **6-Trp** was well separated from signal associated with the solvent (toluene) and could therefore easily be monitored, whereas in previous investigations of **1-Lys**,[14] we had to use the CD band associated with the organization of the CONH groups at ca. 220 nm—preventing the use of toluene as solvent due to overlapping spectral bands.

VT ^{1}H NMR studies on **6-Trp** (Figure 5) were used to monitor changes in the Ar−H and N−H resonances of the indole ring on increasing the temperature from 30 to 70 °C. The N−H proton shifted upfield from ca. 8.25 to 8.11 ppm and sharpened (n.b. the N−H proton shifts through an aromatic doublet across this temperature range, Figure 5). The sharpening of the peaks is associated with increased molecular mobility as temperature breaks down the assembled structures. The upfield shifts demonstrate that the indole ring of tryptophan is involved in the molecular recognition pathways responsible for gelation, consistent with the N−H group of the indole forming hydrogen bond interactions within the gel which are broken by heat.[16]

The aromatic protons also sharpened and shifted upfield, albeit to a lesser extent (from ca. 8.20 to 8.15 ppm). The N−H (amide and carbamate) protons of **6-Trp** also shifted upfield on increasing temperature, indicating their primary importance in self-assembly. This latter observation is consistent with NMR changes observed for **4-Phe** and **5-Cys**. For **5-Cys**, it was also clear that the N−H proton in the amino acid side chain was perturbed by increasing temperature, indicating the involvement of this side chain functionality in the formation of an intermolecular hydrogen bonded network within the gel.

Tryptophan displays fluorescent emission at ca. 330 nm (when excited at 290 nm), which is known to respond to the local environment of the indole ring.[18] Gels with responsive fluorescent behavior have been widely studied and are of considerable recent interest.[19] In many of these literature examples, it has been argued that excimer formation becomes favored within

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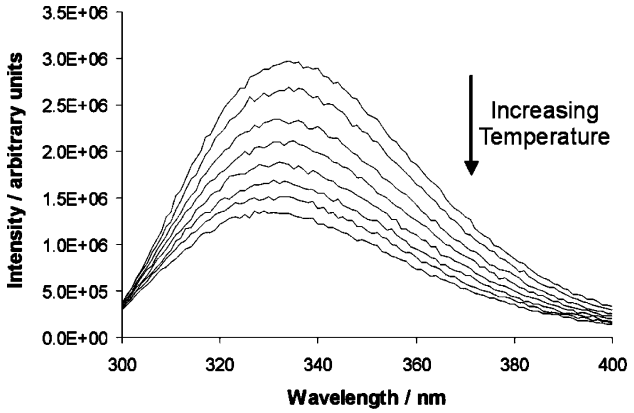

FIGURE 6. Variable temperature fluorescence spectroscopy performed on 6-Trp (0.4 mM) in toluene at temperatures of 25, 35, 45, 55, 65, 75, 85, and 90 °C.

the gel. We probed the gels formed from **6-Trp** for evidence of excimer formation; it is known that tryptophan excimers emit at 450 nm and can be excited at 385 nm.[20] However, in our materials, only a small excimer peak was observed (ca. 1% of the intensity of the emission peak observed at 330 nm). Furthermore, the excimer peak was not dependent on temperature; hence, we argue that excimer formation does not play a role in the optical behavior of these materials.[21]

However, when exciting at a wavelength of 290 nm, VT fluorescence spectroscopy in toluene did indicate a decrease in emission intensity of >50% on increasing the temperature from 25 to 90 °C (Figure 6). However, carrying out VT fluorescence in methanol (a solvent which does not support gelation) led to no change in intensity on increasing the temperature. Clearly in toluene, fluorescence responds to the self-assembly process. The absolute intensity of the fluorescence spectrum of **6-Trp** in methanol was much higher than that in toluene. This indicates that fluorescence is disfavored in apolar toluene (e.g., via nonradiative processes). This therefore provides a potential mechanism for the decrease in fluorescence intensity observed on disassembly of the gel. As **6-Trp** is released from the assembled fibers, which are relatively polar, into the solvent (toluene), nonradiative processes associated with solvent−gelator interactions can lower the intensity of emission.

In addition to the temperature-induced change in intensity in toluene, there is also a small shift in λ_{max} on heating from 25 °C (334 nm) to 90 °C (329 nm). For tryptophan, a hypsochromic shift of this type is usually ascribed to the indole ring being in a *less polar*, *less hydrogen bonded environment* (as might be expected in this case as the gel disassembles and the gelator experiences the "toluene" solvent environment).[18] This is the first example of an optically responsive gel in which the fluorophore is biologically derived—a feature which may have uses in biological applications.

In combination, these results indicate that the spectroscopic properties (CD, NMR, fluorescence) of the indole in **6-Trp** are responsive to temperature and hence self-assembly. The tryptophan unit endows the gel with distinctive optical properties. Indeed, the indole ring can be considered as an optical marker which reports on self-assembly, analogous to its use as a fluorescent marker in biomolecule analysis.[17] It is possible that these systems will also exhibit useful sensory properties.

Gelator **6-Trp** provides a clear example of how extending the range of amino acids can lead to gelators with enhanced thermal stability and, in addition, provides a simple method of endowing gels with function (in this case, optical properties). By learning from the functional roles of amino acids in biology, we anticipate that gelators based on different amino acids, or combinations thereof, will provide access to a fascinating range of functional nanostructured materials. Work in this direction is currently in progress in our laboratories.

Experimental Section

General Synthetic Method for Peptide Coupling. 1,9-Diaminononane (0.8 g, 5.05 mmol) was suspended in dichloromethane (100 mL). Triethylamine (2.00 mL, 1.45 g, 14.34 mmol) was added, followed by the appropriate protected amino acid (13.14 mmol). The mixture was stirred under nitrogen for 30 min and cooled to 0 °C. 1-Hydroxybenzotriazole (HOBt, 3.99 g, 29.29 mmol) and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC, 3.91 g, 18.9 mmol) were added simultaneously as a mixture of solids. The reaction mixture was allowed to return to room temperature and was stirred for 24 h. The precipitate was removed by filtration, washed with dichloromethane, and then discarded. The filtrate was then washed with an aqueous saturated solution of $NaHCO_3$, aqueous $NaHSO_4$ (16 g in 100 mL of water), before being washed again with aqueous $NaHCO_3$ and finally with water. The solution was dried over magnesium sulfate then rotary evaporated to produce a white solid.

Compound 6-Trp. Synthesized using the general method. The crude product was purified by column chromatography (silica, CH_2Cl_2/MeOH 98:2 and 0.1% triethylamine) to give a white solid with a yield of 1.29 g (1.77 mmol, 56%): mp 102−106 °C; R_f 0.51 (CH_2Cl_2/MeOH, 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (2H, br), 7.65 (2H, d, J = 7.6 Hz), 7.36 (2H, d, J = 8.2 Hz), 7.20 (2H, t, J = 7 Hz), 7.11 (2H, t, J = 7.4 Hz), 7.03 (2H, s), 5.72 (2H, br), 5.24 (2H, br), 4.41 (2H, br), 3.30 (2H, dd, J = 14.0, 8.0 Hz), 3.15−3.05 (6H, m), 1.70−1.00 (32H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 155.6, 136.4, 127.5, 123.4, 122.2, 119.6, 118.9, 111.4, 110.5, 80.1, 55.4, 39.5, 29.1, 29.0, 28.9, 28.8, 28.4, 26.5; IR ν_{max} 3337*m* (N−H), 2974*m* (C−H), 2928*m* (C−H), 2854*m* (C−H), 1682*s* (C═O), 1654*s* (C═O), 1519*s*, 1365*m*, 1319*s*, 1246*s*, 1165*s*, 1050*m*; $[\alpha]_D^{589}$ −1.8 (c = 1.0, acetone); ESI-MS $C_{41}H_{58}N_6O_6$ (M_r = 730.9) m/z 753 ([M + Na]$^+$, 100%), 754 (45%); HRMS-FAB, calcd for $C_{41}H_{58}N_6O_6Na$ 753.4316, found 753.4318.

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Supporting Information Available: Materials and Methods, experimental data for compounds **2-Ala**, **3-Gln**, **4-Phe**, and **5-Cys**, and ^{1}H and ^{13}C NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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