

# Synthetically accessible, tunable, low-molecular-weight oligopeptide organogelators†‡

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**We report a synthetically simple approach to effective and tunable low molecular weight gelators based on amine-initiated oligomerisation of an amino acid *N*-carboxyanhydride.**

Self-assembling nanostructured gels, in which non-covalent interactions between molecular building blocks assemble a nanoscale ‘solid-like network’ suspended within a ‘liquid-like’ solvent phase, are of intense interest.<sup>1</sup> The components used to assemble such materials can either be small molecules or polymers. However, there has been relatively little overlap between researchers in these distinct areas.<sup>2</sup> Peptides are capable of mutually complementary hydrogen bond interactions, and there has therefore been considerable interest in using peptides to assemble gels.<sup>3</sup> The diversity of amino acid side chains, combined with their biocompatibility, opens a variety of applications.<sup>4</sup> Low molecular weight peptides, often incorporating hydrophobic amino acids, have been widely employed, as organogelators.<sup>5</sup> The peptides aggregate through hydrogen bonding whilst the apolar groups maintain the solubility in the organic solvent and avoid precipitation. Polymeric peptides have also been assembled into physical organogels. For example, poly( $\gamma$ -benzyl-L-glutamate) forms organogels in toluene due to aggregation between polypeptide helices.<sup>6</sup> Polypeptides have been incorporated into block co-polymers which can form gels at low loadings.<sup>7</sup> Amino acids have also been attached to polymers, either as side chains, or on the termini, in order to provide the polymers with greater propensity to self-assemble into gels.<sup>8</sup> For some time, we have investigated gelators which are intermediate between low and high molecular weight, working extensively with dendritic peptides,<sup>9</sup> and gaining a good understanding of their assembly.<sup>10</sup> However, dendrimers are relatively complex and require multi-step syntheses. We therefore became interested in developing alternative approaches to gelator structures of intermediate size. The preliminary results are reported here. We decided to apply ring opening oligomerisation using an amino acid *N*-carboxyanhydride to yield relatively small oligopeptides.<sup>11</sup> Clearly, the products will not be as well-defined as traditional low molecular weight gelators, but the ease of synthesis is highly attractive. We were interested to know whether the polydisperse crude products from this type of reaction could still support gelation.

To demonstrate this approach we used *N*<sup>ε</sup>-(benzyloxycarbonyl)-L-lysine *N*-carboxyanhydride (NCA), as we have previously shown that well-defined peptides constructed from *Z*-protected lysine can form effective gels.<sup>12</sup> Compound NCA was synthesised in 84% yield *via* cyclisation of *N*<sup>ε</sup>-(benzyloxycarbonyl)-L-lysine with triphosgene under argon in anhydrous THF (see ESI†).<sup>13</sup> We then explored the reaction of NCA using a range of amines which can initiate ring opening oligomerisation of a peptide chain (Scheme 1). A 1 : 6 initiator : NCA ratio was employed to target relatively short peptide oligomers. Initiators **1–6** (Scheme 1, for other initiators see ESI†) were investigated using a parallel reaction station, with the crude products being dissolved in a minimum of DMF and the desired oligomers precipitated by the addition of diethyl ether.

Electrospray mass spectrometry (ESMS) showed that, in most cases, the crude solid products formed from initiators **1–6** had succeeded in growing an oligo-lysine chain to give the desired **I-Lys<sub>n</sub>**. Typically, between two and six lysine units were attached to the initiator (Table 1). However, the products obtained in some cases also had ESMS peaks for unattached oligo-lysine (**Lys<sub>n</sub>**, Fig. S1, ESI†)—oligomerisation was initiated with adventitious water, possibly because these initiators were difficult to dry fully. Two initiators (**1** and **4**) did not produce any unattached oligo-lysine, but initiator **6** mainly gave **Lys<sub>n</sub>**, with a smaller amount of **I-Lys<sub>n</sub>**.§

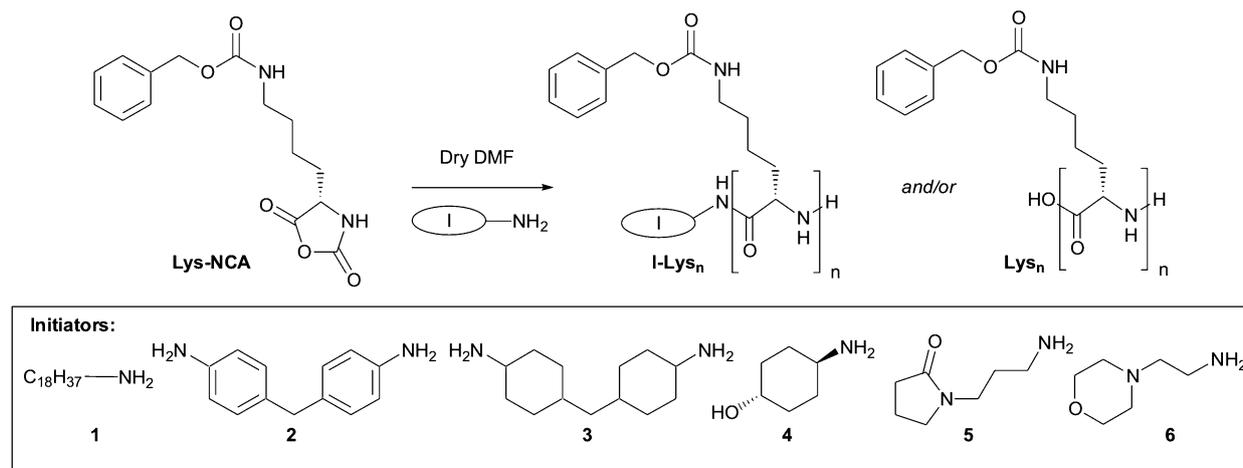
The gelation of the crude oligopeptide products was then studied in a range of solvents. Pleasingly, most compounds formed gels in 1,2-dichlorobenzene (DCB, Table 2). The exception to this was **6-Lys**—interestingly, the product which was primarily unattached **Lys<sub>n</sub>**—suggesting that this peptide does not assemble into effective gels, and may even suppress gelation. Only **1-Lys** formed gels in toluene (TOL), and only **3-Lys** gelled trichloroethylene (TCE), with **1-Lys** forming a viscous liquid in TCE. This demonstrates that the choice of initiator directly impacts on gelation ability.

Considering the solvent Kamlet–Taft parameters (Table 2),<sup>14</sup> gelation only occurred if the  $\alpha$  parameter was zero, *i.e.*, hydrogen bond donor solvents such as acetonitrile (ACN) and nitromethane (NM) disrupt gelation. Of those solvents with an  $\alpha$  parameter equal to zero, only DCB and to some extent TCE and TOL supported gelation, whereas decane (DEC) and mesitylene (MES) did not. It is likely that gelation occurred best in DCB because it has quite high  $\pi^*$  (polarisability) and  $\beta$  (hydrogen bond acceptor) values—which are required in order to solubilise the moderately polar oligopeptide. If the  $\pi^*$  and  $\beta$  values are too low (*e.g.* DEC and MES), then the oligopeptides will not dissolve, hence preventing gelation from taking place. We reason that TCE and TOL are somewhat intermediate

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**Scheme 1** Amine-initiated oligomerisation of Z-protected lysine N-carboxyanhydride. Initiators 1–6 were tested in this reaction.

**Table 1** Mass spectrometric peak intensities associated with different oligomeric products from the parallel synthesis (normalised to the largest peak in each case)

	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
Lys <sub>4</sub>	—	39	18	—	16	88
Lys <sub>5</sub>	—	39	—	—	16	100
Lys <sub>6</sub>	—	18	—	—	11	55
Lys <sub>7</sub>	—	6	—	—	3	24
Lys <sub>8</sub>	—	—	—	—	—	6
I-Lys <sub>2</sub>	100	—	—	100	100	9
I-Lys <sub>3</sub>	94	67	58	60	73	9
I-Lys <sub>4</sub>	53	100	100	20	40	4
I-Lys <sub>5</sub>	56	56	33	5	3	2
I-Lys <sub>6</sub>	22	22	33	—	3	2
I-Lys <sub>7</sub>	12	11	42	—	—	—
I-Lys <sub>8</sub>	44	6	—	—	—	—

**Table 2** Gelation ability (10 mg in 0.5 mL) of the different crude products in a range of solvents and Kamlet–Taft parameters ( $\alpha$ ,  $\beta$  and  $\pi^*$ ) for these solvents. Ins = insoluble/precipitate; V = viscous fluid; G = gel

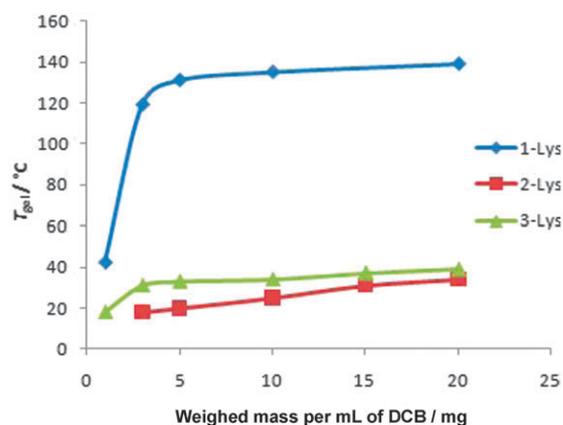
Sol <sup>a</sup>	$\alpha$	$\beta$	$\pi^*$	1-Lys	2-Lys	3-Lys	4-Lys	5-Lys	6-Lys
DEC	0.00	0.00	0.30	Ins	Ins	Ins	Ins	Ins	Ins
MES	0.00	0.13	0.41	Ins	Ins	Ins	Ins	Ins	Ins
TOL	0.00	0.11	0.49	G	Ins	Ins	Ins	Ins	Ins
TCE	0.00	0.50	0.53	V	Ins	G	Ins	Ins	Ins
DCB	0.00	0.30	0.80	G	G	G	G	G	V
ACN	0.19	0.40	0.75	Ins	Ins	Ins	Ins	Ins	Ins
NM	0.22	0.06	0.85	Ins	Ins	Ins	Ins	Ins	Ins

<sup>a</sup> DEC (decane); MES (mesitylene); TCE (trichloroethylene); TOL (toluene); DCB (1,2-dichlorobenzene); CAN (acetonitrile); NM (nitromethane).

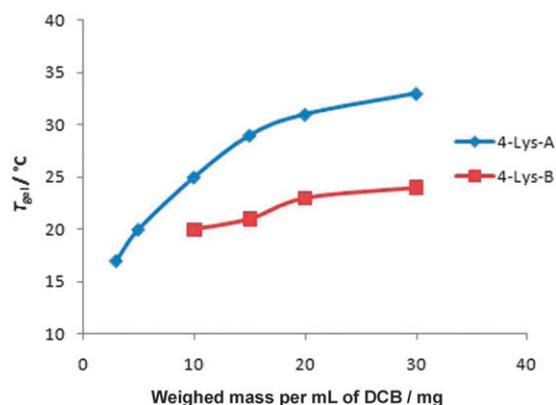
in terms of their  $\pi^*$  and  $\beta$  values and therefore only support gels for some of the molecular frameworks.

We investigated 1-Lys in more detail and monitored the gelation ability of this compound as a function of concentration, using a vial inversion method to record  $T_{\text{gel}}$  values (Fig. 1). The  $T_{\text{gel}}$  value rose to  $>120$  °C at approximately 3 mg mL<sup>-1</sup> (0.3 wt/vol%) in DCB. The minimum gelation concentration (MGC) at room temperature was *ca.* 0.15 wt/vol%. This is

very impressive gelation at such low loading and compares very favourably to other gels. Typically, peptide organogels<sup>3,5,9,10</sup> have MGC values of  $>0.2$  wt/vol% at room temperature—often significantly more. Importantly, this clearly demonstrates that the polydisperse nature of compound 1-Lys does not inhibit its ability to act as an effective organogelator. Compounds 2-Lys and 3-Lys were also investigated (Fig. 1). These gelators also had low MGC values—the MGC of 3-Lys was *ca.* 0.15 wt/vol%, whereas 2-Lys was less effective (0.65 wt/vol%). However, these gelators had lower  $T_{\text{gel}}$  values than 1-Lys. Gelator 3-Lys had a higher  $T_{\text{gel}}$  value (*ca.* 40 °C) than 2-Lys (*ca.* 35 °C). It is possible that 2-Lys is less effective due to structural features of the initiator, although this may be a consequence of 2-Lys containing more unattached Lys<sub>n</sub>, which is not an effective gelator—and may even inhibit the gelation process somewhat. Interestingly, 4-Lys (see below) also gave less thermally stable gels than 1-Lys, even though it contains no Lys<sub>n</sub>. This demonstrates that the chemical structure of the initiator plays an active role in controlling the thermal properties of the gel. It is possible that the apolarity of initiator 1 enhances gel stability, for example through van der Waals interactions or solubility modification.<sup>12</sup>



**Fig. 1** Thermal behaviour of gels formed from 1-Lys, 2-Lys and 3-Lys in 1,2-dichlorobenzene as solvent.



**Fig. 2** Thermal behaviour of gels formed from **4-Lys-A** and **4-Lys-B** in 1,2-dichlorobenzene as solvent.

The influence of oligomerisation was studied in more detail using initiator **4** with different **4** : **NCA** molar ratios—1 : 2, 1 : 4 and 1 : 10. The 1 : 2 ratio led to a product which could not be precipitated—it was assumed that any oligomers obtained were too short. The other reactions produced solid products. ESMS indicated that the compound formed from the 1 : 4 ratio reaction (**4-Lys-A**) had 2 or 3 lysines linked to the initiator, with the major peak being for 2 lysines and with no **Lys<sub>n</sub>** being observed (Fig. S3, ESI†). On the other hand, the crude product from the 1 : 10 reaction (**4-Lys-B**) exhibited ESMS peaks for longer oligomers having 2–5 lysine units attached to the initiator, with the major peaks for 3 and 4 lysine repeat units—however, some peaks were also observed for **Lys<sub>n</sub>** (Fig. S4, ESI†). Clearly, when larger amounts of **NCA** were used, the oligomer chain length increases, but so does the chance of oligomerisation initiated by adventitious water. Compound **4-Lys-A** formed moderately stable gels with a maximum  $T_{gel}$  value around 35 °C (Fig. 2). Compound **4-Lys-B** had a lower thermal stability (<25 °C). Clearly, the presence of longer oligomers does not benefit gel stability—indeed, relatively short oligo-lysines attached to the initiator appear sufficient to enable efficient self-assembly and avoid the formation of **Lys<sub>n</sub>**. Scanning electron microscopy (SEM) performed on the xerogels indicated that although both these gels had a fibrillar nature (Fig. S5 and S6, ESI†), similar to related low molecular weight well-defined peptide gels,<sup>10</sup> **4-Lys-A** had a more nanostructured appearance.

This communication demonstrates that oligomerisation of amino acid **NCA** derivatives is a simple approach to generating oligomers, which even in crude form, can be excellent organogelators. In this case, the use of initiator **1** is optimal. Structurally perfect small molecules are not, therefore, a necessary pre-requisite for gelation. Features such as choice of initiator, and length of oligomer control gel behaviour, making these materials highly tunable. Future work will focus on high-throughput screening of further oligopeptides, minimising water-initiated oligomers, and exploiting potential applications. For example, we anticipate that modifying the amino acid, will give rise to a versatile class of synthetically simple nanomaterials.

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## Notes and references

§ MS peak height does not necessarily correlate directly with product composition. However, for comparison of structurally related compounds it provides useful insight into product composition. For diamines, it is hard to say whether the growth of the oligomer occurs on both amines simultaneously or only on one.

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