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Cite this: DOI: 10.1039/c2cc32921b

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Structure–activity effects in peptide self-assembly and gelation – Dendritic *versus* linear architectures†

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Received 24th April 2012, Accepted 12th June 2012
DOI: 10.1039/c2cc32921b

We demonstrate that linear-dendritic shape-isomerism can have an impact on the gelation potential of peptides based on lysine, but the architectural effects can be inverted depending on the choice of functional groups, with the nature of these protecting groups dominating the gelation ability.

There has recently been intense interest in understanding the self-assembly of molecular-scale building blocks into fibrillar materials, a process which can take place in a range of different solvent environments to give rise to gel-phase behaviour.[1] Peptides are particularly versatile building blocks for gelation[2] and have been used to create organogels and hydrogels with high-tech applications ranging from nanoelectronics to tissue engineering.[3] We have been interested in peptide organogels based on a dumb-bell shaped architecture (Fig. 1) which assemble as a consequence of intermolecular peptide-peptide hydrogen bond interactions.[4] In particular, we have focussed on examples where the peptide head groups are dendritically branched.[5]

There has been considerable interest in exploring structure–activity relationships in self-assembling dendritic molecules – with a number of reports outlining the importance of the dendritic structure in controlling gelation.[6] We have previously explored structure–activity effects using our dendritic gelators, and reported the dendritic effects of branched peptide head groups,[7] modifications in the spacer chain,[8] variation of amino acids,[9] role of protecting groups[10] and solvent effects.[11] We also recently reported that linear peptide oligomers form effective organogels, even when present as ill-defined mixtures.[12] However, there have been no explorations of the way in which dendritic architectures differ from their linear analogues in terms of underpinning gels, which would allow the 'true dendritic effect' to be elucidated. This paper therefore explores a comparison between dendritic and linear peptide gelators with identical molecular formulae.

We synthesised compounds **1–4** (Fig. 2) using stepwise solution-phase peptide chemistry with appropriate protecting group methodologies. Gelators derived from lysine have been a particularly rich source of organo–and hydro-gelators over the last 10 years,[13] as it is both a rich source of hydrogen bond functionality and also relatively easily functionalised to give a wide range of different structures. Compounds **1** and **2** are architectural/structural isomers with Boc protecting groups and 'linear' and 'dendritic' structures respectively. Compounds **3** and **4** are equivalent to **1** and **2** but have Z protecting groups instead of Boc. We reasoned that these compounds would allow us to deduce the effect of peptide architecture, and the consequent organisation of hydrogen bonding amide groups, on gelation. The synthesis and characterisation of novel gelators **1–4** is described in full detail in the supporting information.†

Characterisation of the compounds by NMR showed the difference between linear and dendritic systems in terms of organising the hydrogen bonding CONH groups. This is reflected in the resonances of the chiral C–H protons of the lysine groups (see experimental data in supporting information†). For linear compounds **1** and **3**, two *C–H are adjacent to two amide groups ('chain-like' and appear more downfield at *ca.* 4.2 ppm), and one is between an amide and a carbamate ('surface-like' and appears more upfield at 3.8 ppm). For dendritic compounds **2** and **4**, the situation is reversed with one 'chain-like' *C–H at *ca.* 4.2 ppm and two 'surface-like' groups at *ca.* 3.8 ppm. These kind of architectural differences, reflected in the NMR spectra, may underpin different molecular recognition pathways within the self-assembly of these architectural isomers.

We initially explored the gelation ability of these compounds in 1,2-dichlorobenzene (DCB), and where appropriate determined gel–sol transition temperatures (T_{gel} values) using the reproducible tube inversion methodology.[14] Dendritic compound **2** was a more effective gelator than the linear analogue, compound **1**, which might suggest that the dendritic architecture is preferable for self-assembly and gelation (Table 1). However, linear gelator **3** was a much more effective

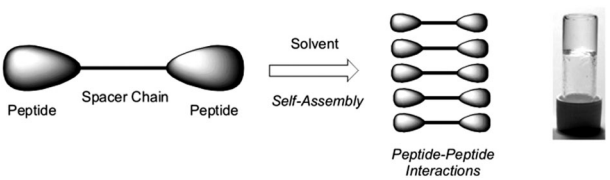

Fig. 1 Schematic diagram of the self-assembly of a dumbbell-shaped peptide into a fibrillar gel-phase material.

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† Electronic supplementary information (ESI) available: Synthesis and characterisation, IR spectra, Job plots and titration curves. See DOI: 10.1039/c2cc32921b

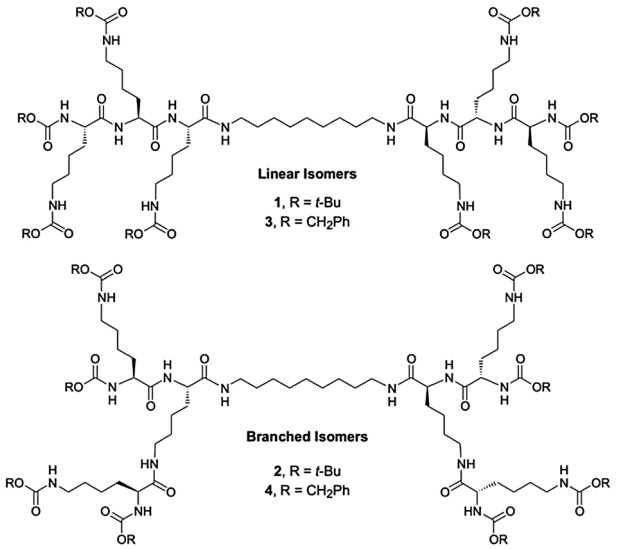

Fig. 2 Structures of linear and branched gelators derived from lysine investigated in this paper.

Table 1 Visual observations of gelation for compounds **1–4** (and T_{gel} values at 1% wt/vol), in a range of different solvents. G = gel, PG = partial gel, S = solution, O = opaque

	1	**2**	**3**	**4**
	Boc-protected		Z-protected	
Solvent	Linear	Branch	Linear	Branch
Chlorobenzene	OS	OG (29)	G (72)	OG (27)
1,2-Dichlorobenzene	PG	OG (30)	G (120)	G (31)
1,2-Dibromoethane	OS	OG (26)	G (110)	G (41)
Phenetole	OS	PG	G (85)	OG (24)

gelator than dendritic compound **4**, which would suggest that a linear architecture was more effective for underpinning gel-phase materials (Table 1). Fig. 3 illustrates the dependence of T_{gel} on gelator concentration for compounds **3** and **4**. Linear compound **3** forms a room temperature gel above *ca.* 2 mg ml^{-1} (0.2% wt/vol), while dendritic compound **4** forms an effective gel above *ca.* 4 mg ml^{-1} (4% wt/vol) indicating they are both efficient gelators. Compound **3** has a maximum T_{gel} value of 125 °C, while for compound **4** it is only 40 °C. Furthermore, compounds **3** and **4** are both more effective gelators than compounds **1** and **2** – clearly the nature of the protecting groups (*i.e.*, the molecular formula of the gelator) plays a more important role than whether the architecture is linear or dendritic. Variable temperature NMR

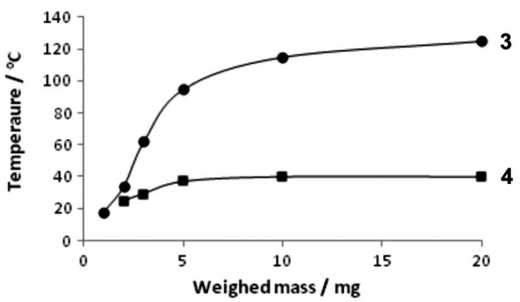

Fig. 3 Effect of weighed mass of gelator (in 0.5 mL of DCB), on the T_{gel} value as determined by the inverted vial method.

studies were in agreement with the T_{gel} values reported above, with NMR peaks emerging from the baseline at around the T_{gel} value, as the gelator molecules become mobile.

To probe this further, we investigated gelation in a wider range of solvents. As we have observed before, gelation was best supported in solvents with effectively no hydrogen bonding ability, such as chlorobenzene, dibromoethane and toluene. Limited gelation was observed in phenetole, which has some ability to accept hydrogen bonds with its oxygen atom. In each case, the trends observed in dichlorobenzene were replicated *i.e.*, Z-protected compounds are more effective than Boc-protected analogues, with Boc protection the branched architecture is preferred, whereas with Z protection the linear architecture is better. In more polar, hydrogen bond acceptor solvents, such as acetonitrile and dimethoxyethane, gelation was not observed. Gels were also not observed in hydrogen bond donor solvents such as methanol. In decane, there was no gelation – as a very low polarity solvent, it is unable to sufficiently solubilise the compounds and self-assembly is therefore unable to take place. These solvent effects demonstrate how gelation occurs at a borderline of solubility – compounds must be soluble enough to allow self-assembly, but also must not interact too strongly with the solvent, or self-assembly is disrupted.

The solvent effects indicate that, as expected, peptide–peptide hydrogen bond interactions are primarily responsible for gelation in this case. However, the data also indicate that the choice of protecting groups has a profound impact on the self-assembly of these peptides, with Z groups encouraging hierarchical self-assembly and gelation in comparison with the the Boc groups. We suggest that this is a consequence of the ability of these groups to participate in π–π interactions. The better ability of Z-protected compounds to aggregate is supported by their lower observed solubility – which will also help drive the gelation process.[10] In addition, changing the protecting groups inverts the dendritic/linear architectural preferences of the overall assembly process. We suggest that the linear compound is better able to organise the aromatic rings of the Z groups for mutual interaction than the branched isomer can. Indeed, for the branched isomer, there is little difference between Z and Boc derivatives, suggesting ineffective organisation of the π-stacking groups in this morphology. For the Boc protected system, we suggest that steric hindrance between the bulky Boc groups plays a role in limiting the assembly of the linear isomer, but has less impact on the dendritic system where all of these groups are on the periphery of the molecule. We attempted to probe this hypothesis further using circular dichroism spectroscopy, but unfortunately, the aromatic/halogenated solvents which support gelation obscured the bands associated with peptide self-assembly.

We employed scanning electron microscopy (SEM) to gain further insight into the self-assembly process. Initially, the xerogels formed by **3** and **4** were imaged after drying from DCB and phenetole under ambient conditions. In DCB relatively similar looking fibres were observed (Fig. 4A and B), whereas in phenetole it appeared that the linear compound (Fig. 4C) formed a somewhat better defined gel fibre network than the dendritic analogue (Fig. 4D) – consistent with its higher T_{gel} value. We were unable to image the Boc-protected gelators by SEM, as they did not form such effective extended gel-fibre networks as their Z-protected analogues.

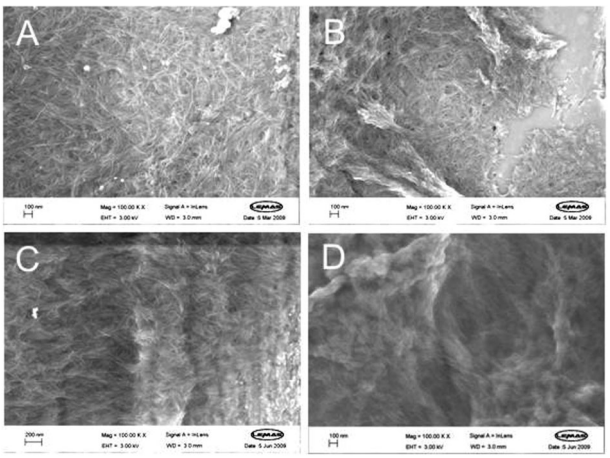

Fig. 4 SEM images of xerogels formed by Z-protected gelators. A: Linear gelator **3** dried from DCB, B: gelator **4** dried from DCB, C: gelator **3** dried from phenetole, D: gelator **4** dried from phenetole. Scale bars: 100 nm (A,B,D) and 200 nm (C).

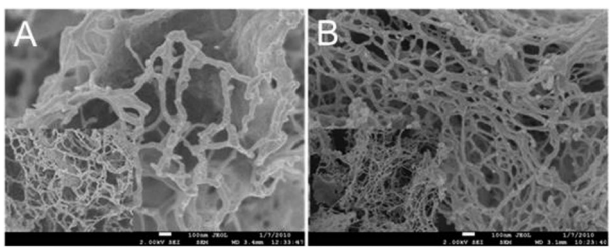

Fig. 5 SEM images of A: compound **3**, and B: compound **4**, after drying from DCB under low temperature conditions to create an aerogel. Scale bar: 100 nm. Insets show larger area images.

We also applied a new approach to sample preparation in order to image the gels formed by compounds **3** and **4** under more representative conditions. We used critical point drying to remove solvent under low temperature supercritical conditions from samples of the gel under high vacuum, on the SEM stub. We were able to do this in our laboratory and then transfer the samples into the SEM machine. Remarkably, the expanded aerogels formed during this drying process were highly stable. Because the samples are dried at liquid nitrogen temperatures, they are less susceptible to thermal collapse during drying, and should be more representative of the three dimensional solvated structure of the gel.[15] This was indeed the case, as can be seen in Fig. 5. There were some differences in the gel networks, with linear compound **3** appearing to form larger fibres (*ca.* 40 nm) than those of dendritic compound **4** (*ca.* 20 nm). We suggest that these larger, more aggregated fibres may reflect greater fibril aggregation, perhaps driven by π-stacking of the Z groups.

In summary, we have demonstrated that by careful synthesis it is possible to make linear and dendritic gelators with identical molecular formulae, but which exhibit significantly different organogelation characteristics, and thus elucidate the 'true dendritic effect' on gelation. This demonstrates that molecular architecture plays a role in enabling the effective organisation of hydrogen bonding groups to underpin gelation. Furthermore, this relationship is not a simple one – the nature of functional groups present (Boc or Z) inverts the architectural effect – indeed these protecting groups play a dominating role in controlling gelation. This indicates the subtlety of molecular recognition pathways within self-assembled peptide nanomaterials, and demonstrates the importance of carefully optimising molecular structures to obtain the desired materials behaviour.

We acknowledge EPSRC and Givaudan for funding (Industrial CASE award).

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