Electronic Supplementary Material (ESI) for Chemical Communications


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

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## SUPPLEMENTARY INFORMATION

### Contents

## 1. General Experimental Methods

All solvents and reagents were used as supplied – protected lysine amino acids with orthogonal Z and Boc protecting groups were purchased, while the lysine with two Boc protecting groups was synthesised using published methods.[1] Compounds **G1-Boc** and **G1-Boc/Z** (see retrosynthetic analysis) were synthesised as previously reported.[2] Silica gel column chromatography was carried out on silica gel provided by Fluka (60 Å, 35-70 µL). Thin layer chromatography was carried out on commercially available Merck aluminium backed TLC plates (60, $F_{254}$). $^{13}C$ NMR titration and Job plot experiments were carried out on a Bruker AMX 300 spectrometer in undeuterated ethyl acetate ($^{1}H$ 300 MHz, $^{13}C$ 75 MHz) and the spectra were referenced to TMS. For compound characterisation a Jeol ECX spectrometer ($^{1}H$ 400 MHz, $^{13}C$ 100 MHz) and reference to residual solvent were used. All chemical shifts (δ) are quoted to ppm. Coupling constant values (*J*) are reported in Hz. ATR-FTIR was carried out using a Jasco FT/IR 4100instrument fitted with a Pike MIRacle ATR sampling accessory. Positive ion electrospray mass spectra were recorded on a Finnigan LCQ mass spectrometer. Melting points were measured on a Stuart SMP3 apparatus. Optical rotation was measured on a Jasco DIP-370digital polarimeter. Column chromatography was performed on silica gel (30-70 µm) and thin layer chromatography (TLC) was performed using Merck silica gel $60F_{254}$ pre-coated aluminium backed plates. Spots were visualised by absorption of UV light or use of an appropriate stain; phosphomolybdic acid (PMA) solution [PMA hydride (12 g), conc. sulfuric acid (10 mL), ethanol (250 mL)], ninhydrin solution (0.2% by mass in ethanol) or cerium molybdate (or Hanessian's stain) [$Ce(NH_4)_2(NO_3)_6$ (2 g), $(NH_4)_6Mo_7O_{24}.4H_2O$ (24 g), $H_2SO_4$ (28 mL), $H_2O$ (180 mL)]. The eluant for TLC and column chromatography was 9:1 DCM/MeOH.

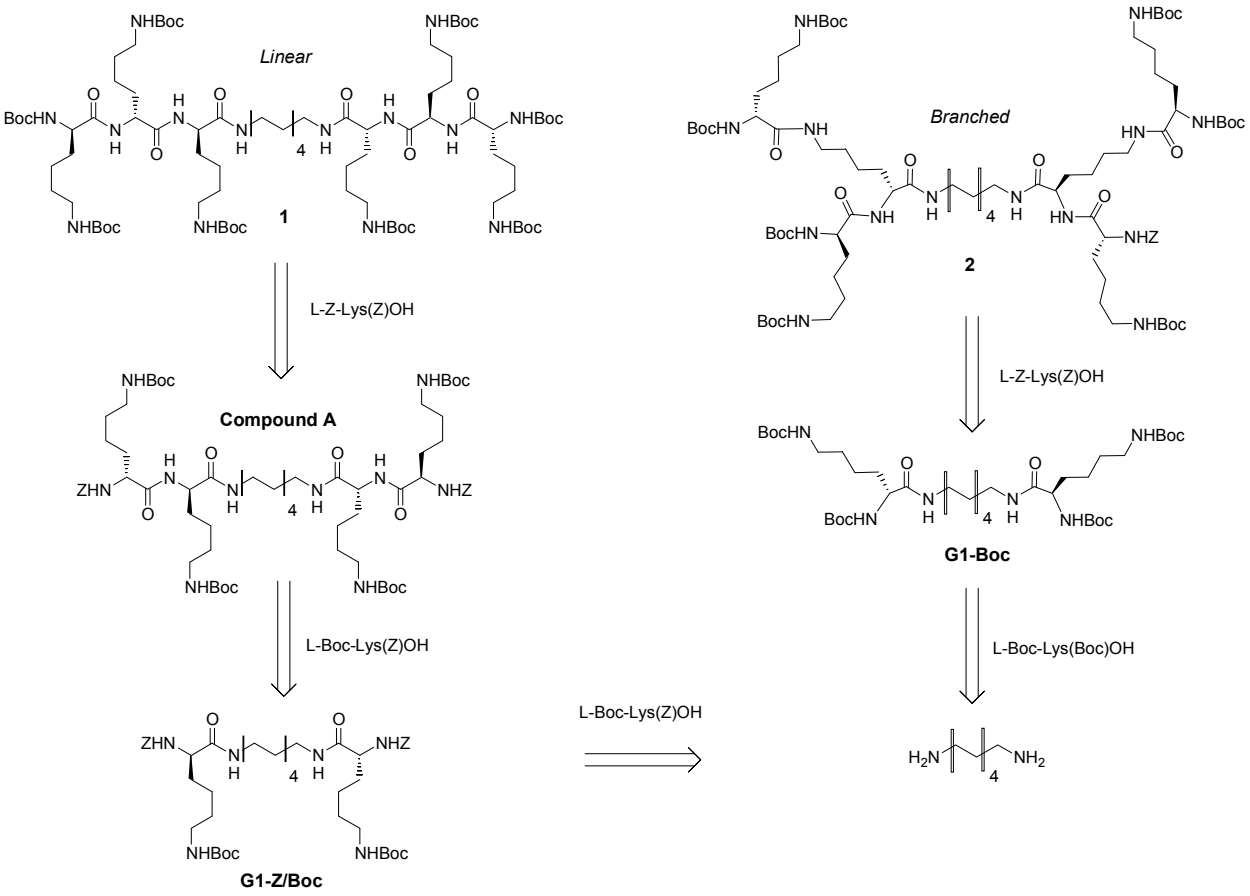
### General procedure for Z deprotection

The appropriate compound was dissolved in MeOH (100 mL) and placed under an argon atmosphere. Then, $Pd(OH)_2$ (215 mg, 40 wt%) was added, and the solution was purged three times with argon before being placed under a $H_2$ atmosphere. The reaction mixture was then stirred at room temperature for 1 h (monitoring by TLC). The reaction mixture was then filtered through Celite, and rotary evaporated to dryness.

### General procedure for Boc deprotection

The appropriate boc protected compound was dissolved (or suspended) in dichloromethane (10-15 mL) and then trifluoroacetic acid (TFA, 1-2 mL) was added slowly to the solution, the reaction mixture was stirred at room temperature for 30 min, then rotary evaporated and dried under high vacuum to remove traces of TFA. Then 2 equivalents of triethylamine were added to the deprotected compound to protonate the amines.

## 2 Retrosynthetic Analysis for Boc-Protected Shape-Isomeric Gelators



## 3. Synthesis and Characterisation of Gelators 1 and 2 (and Precursors)

### Compound A

Compound **G1-Z/Boc** (1.00g, 1.14 mmol) was Z deprotected following the appropriate procedure. The product (1 eq) was solubilised in a mixture of EtOAc (100 mL) and THF (50 mL), triethylamine (2.5 eq) was added followed by the protected amino acid (2.2 eq). The mixture was stirred at room temperature, and 1-propylphosphonic acid cyclic anhydride (T3P, 2.2 eq) was added dropwise to the solution, which was then stirred overnight. After rotary evaporation of the THF, water (50 mL) and EtOAc (50 mL) were added to the reaction mixture, which was stirred for a further 10 min. After separation of the organic and aqueous phases, the organic phase was washed with sodium hydrogen sulfate solution (8 g in 50 mL water), aqueous sodium hydrogen carbonate (1 M) then finally water. The organic layer was and then rotary evaporated to dryness. The product was obtained as a white solid in a yield of 1.02 g (0.765 mmol, 67%). $^{1}$H NMR (DMSO-$d_6$) δ 7.76 (4H, br, CON*H*$(CH_2)_9$, N*H*$_{\alpha G1}$,), 7.38 (2H, br, N*H*$_{\alpha G2}$), 7.37-7.25 (10H, m, $C_6H_5$), 6.72 (4H, br, N*H*Boc), 4.99 (4H, s, $CH_2C_6H_5$), 4.12 (2H, br, C*H*$_{\alpha G1}$), 3.92 (2H, br, C*H*$_{\alpha G2}$), 2.97-2.89 (4H, m, NHC*H*$_2(CH_2)_8$), 2.87-2.80 (8H, m, C*H*$_2$-NHBoc), 1.59-1.00 (38H, m, C*H*$_2$), 1.33 (36H, s, C(C*H*$_3)_3$). $^{13}$C NMR (DMSO-$d_6$) δ 173.5, 171.7 (CH$_\alpha$*C*O x 4), 156.5, 156.0, 154.6 (NH*C*OO x 6), 137.5, 128.8, 128.2 ($C_6H_5$), 77.8 (*C*$(CH_3)_3$ x 4, overlapping), 65.8 ($CH_2C_6H_5$ x 2), 55.3, 53.8 (*C*H$_\alpha$ x 4), 43.2, 40.3, 39.5 (C*H*$_2$NHBoc x 4, NH*C*H$_2(CH_2)_7$*C*H$_2$), 32.3, 32.0, 29.7 (*C*H$_2$, overlapping), 28.7 (C(*C*H$_3)_3$ x 12), 24.7, 23.3, 23.1, 22.4 (*C*H$_2$, overlapping). IR $\nu_{max}$ cm$^{-1}$ 3286*m*, 2931*m*, 2862*m*, 1681*s*, 1643*s*, 1520*s*, 1450*m*, 1365*m*, 1242*s*, 1165*s*, 1049*m*. ESI-MS (*m/z*) calculated value for $C_{69}H_{114}N_{10}NaO_{16}$ $[M+Na]^+$ requires 1356.85145; found: 1356.8531 (100%, $[M+Na]^+$), 1339.8297 (20%, $[M+H]^+$). $R_f$ = 0.45. m.p. 94-97$^{o}$C. Calcd. For $C_{69}H_{114}N_{10}O_{16}$: C, 61.86; H, 8.58. Found: C, 61.62; H, 8.57.

### Target Compound 1

Compund **A** (0.591 g, 0.44 mmol) was Boc-deprotected following the appropriate procedure. The product (1 eq) was solubilised in a mixture of EtOAc (100 mL) and THF (50 mL), triethylamine (2.5 eq) was added followed by compound **G1-Boc** (2.2 eq). The mixture

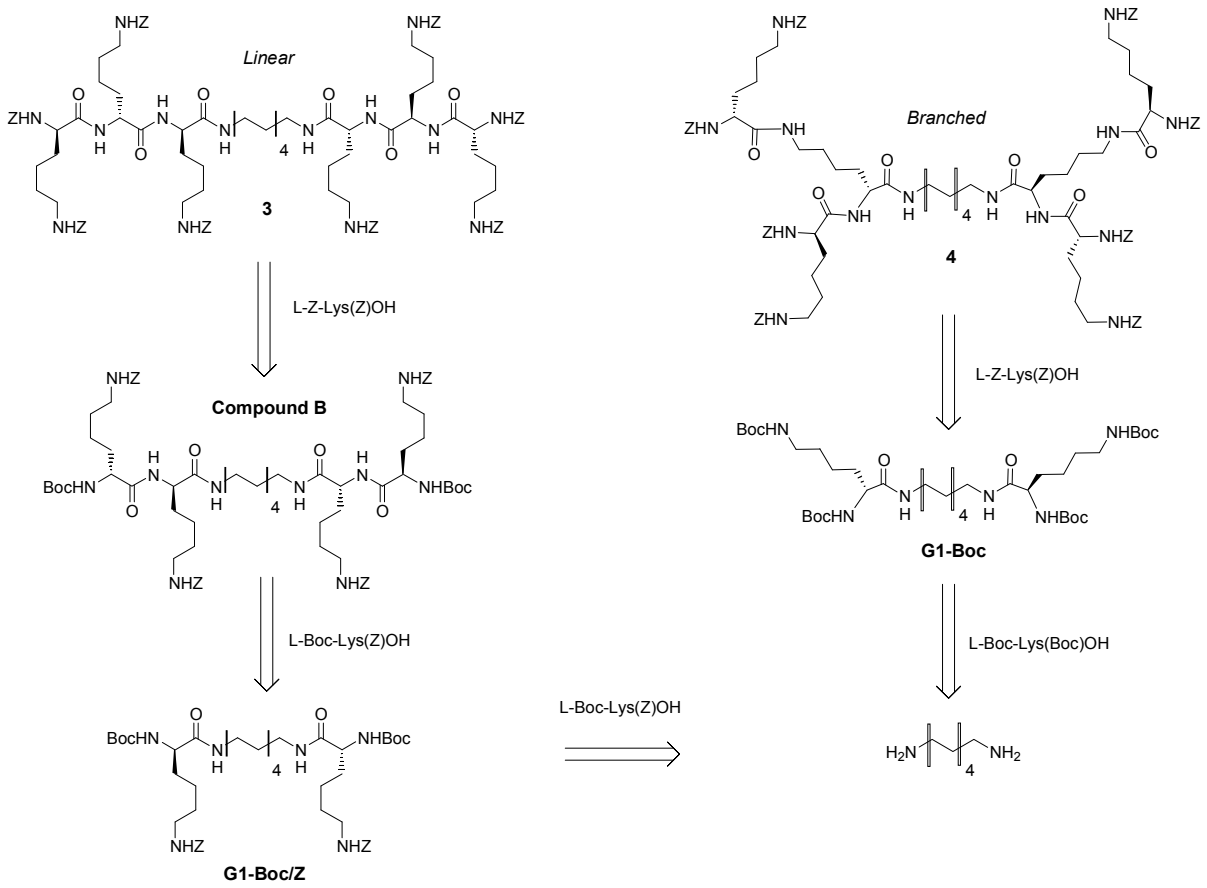
was stirred at room temperature, and 1-propylphosphonic acid cyclic anhydride (T3P, 2.2 eq) was added dropwise to the solution, which was then stirred overnight. After rotary evaporation of the THF, water (50 mL) and EtOAc (50 mL) were added to the reaction mixture, which was stirred for a further 10 min. After separation of the organic and aqueous phases, the organic phase was washed with sodium hydrogen sulphate solution (8 g in 50 mL water), aqueous sodium hydrogen carbonate (1 M) then finally water. The organic layer was and then rotary evaporated to dryness. The product was obtained as a white solid in a yield of 365 mg (0.21 mmol, 47%). $^{1}$H NMR (DMSO-$d_6$) $\delta_H$ 8.49 (2H, br, CON*H*$(CH_2)_9$), 7.99-7.82 (4H, br, N$H_{\alpha G1+2}$), 6.92 (2H, br, N$H_{\alpha G3}$), 6.72 (6H, br, $CH_2$N*H*Boc), 4.16-4.09 (4H, br, C$H_{\alpha G1+2}$), 3.81 (2H, br, C$H_{\alpha G3}$), 2.98 (4H, br, C$H_2$-NH$(CH_2)_9$), 2.83 (12H, br, C$H_2$NHBoc), 1.75-1.22 (50H, m, C$H_2$), 1.32 (72H, s, C(C$H_3$)$_3$). $^{13}$C NMR (DMSO-$d_6$) $\delta_C$ 177.9 ($CH_\alpha$*C*O x 6, overlapping), 156.0 (NH*C*OO x 8, overlapping), 77.8 (*C*$(CH_3)_3$ x 8, overlapping), 55.7, 54.7, 53.2 (*C*H$_\alpha$ x 6), 46.9, 45.5, 42.7, 41.2 (NH*C*H$_2$$(CH_2)_7$*C*H$_2$, *C*H$_2$NHBoc x 6, overlapping), 32.5, 32.2, 31.9, 29.7 (*C*H$_2$, overlapping), 28.8, 28.7 (C(*C*H$_3$)$_3$ x 24, overlapping), 27.1, 23.2, 13.7, 18.4 (*C*H$_2$, overlapping). IR $\nu_{max}$ cm$^{-1}$ 3289*m*, 2931*m,* 1681*s*, 1643*s*, 1512*s*, 1396*m*, 1365*m*, 1249*m*, 1165*s*, 1049*m*, 1018*m*. ESI-MS (*m/z*) calculated value for $C_{85}H_{158}N_{14}NaO_{22}$ $[M+Na]^+$ requires 1750.1567; ($ES^+$) found: 1750.1579 (100%, $[M+Na]^+$). $R_f$ = 0.42 m.p. 109-111$^o$C.

**Target Compound 2**

Compound **G1-Boc** (2.00 g, 2.46 mmol) was Boc-deprotected following the standard method. The deprotected product was dissolved in ethyl acetate (100 mL) and triethylamine (10 mL), L-Boc-Lys(Boc)-OH (4.00 g, 10.5 mmol). The reaction mixture was heated to 40°C and T3P (6.5 mL, 10.9 mmol) was added dropwise over 1 h. After further stirring for 2 h, the reaction mixture was cool down to room temperature, water (100 mL) was added and the mixture stirred for 5 min. The two phases were separated and the organic phase was dried over magnesium sulfate and then the solvent was removed under reduced pressure. The crude solid was solubilised in methanol and crashed out with diethylether to produce a white solid in a yield of 1.95 g (46%). $^{1}$H NMR (DMSO-$d_6$) δ 7.80 (2H, br, $(CH_2)_9$N*H*), 7.68 (4H, br, N$H_\alpha$), 6.89 (2H, br, N$_\alpha$*H*Boc), 6.72 (4H, br, $CH_2$N*H*Boc), 6.70 (2H, br, N$H_\alpha$Boc), 4.13 (2H, br, C$H_{\alpha G1}$), 3.80 (4H, br, C$H_{\alpha G2}$), 2.97 (4H, br, C$H_2$-NH$(CH_2)_9$), 2.84 (12H, br, C$H_2$-NHBoc), 1.75-

1.22 (50H, m, C$H_2$), 1.32 (72H, s, C(C$H_3$)$_3$). $^{13}$C NMR (DMSO-$d_6$) δ 172.9, 172.4, 171.3 (CH$_\alpha$*C*O x 6), 156.0, 155.9 (NH*C*OO x 8), 78.6, 78.4, 77.8 (*C*(CH$_3$)$_3$ x 8, overlapping), 56.9, 55.1, 54.8 (*C*H$_\alpha$ x 6), 40.1, 40.0 (NH*C*H$_2$(CH$_2$)$_7$*C*H$_2$, *C*H$_2$NHBoc x 6, overlapping), 32.4, 31.2, 29.8, 29.7, 29.5, 29.2 (*C*H$_2$, overlapping), 28.8, 28.7 (C(*C*H$_3$)$_3$ x 24, overlapping), 26.8, 23.4 (*C*H$_2$, overlapping). IR: $\nu_{max}$ cm$^{-1}$ 3289*m*, 2931*m,* 1681*s*, 1643*s*, 1519*s*, 1365, 1249*m*, 1165*s*, 1018*m*. ESI-MS (*m/z*) calculated value for $C_{85}H_{158}N_{14}NaO_{22}$ [M+Na]$^+$ requires 1750.1567; (ES$^+$) found: 1750.1505 (100%, [M+Na]$^+$), 1729.1719 (15%, [M+H]$^+$). m.p. 103-106$^o$C.

## 4 Retrosynthetic Analysis for Boc-Protected Shape-Isomeric Gelators



## 5. Synthesis and Characterisation of Gelators 3 and 4 (and Precursors)

### Compound B

Compound **G1-Boc/Z** (600 mg, 0.68 mmol) was deprotected using the standard method for Boc deprotection and used without further purification. The deprotected intermediate was dissolved in triethylamine (2 mL, > 20 eq) and ethyl acetate (100 mL), and Boc-Lys(Z)-OH (4.8 g, 12.6 mmol) was then added. The reaction mixture was heated to 55°C and 1-propylphosphonic acid cyclic anhydride (T3P, 1.2 mL, 2.04 mmol) was added dropwise into the reaction mixture. After the addition, the reaction mixture was stirred at 60°C overnight. Water was then added and the reaction mixture stirred for another 30 min. Aqueous and organic phases were separated and the organic phase washed with a solution of sodium hydrogen sulfate (8 g in 50 ml), (NB: the product was not completely soluble in ethyl acetate), and rotary evaporated to produce the crude product, which was then purified by column chromatography (silica, $CH_2Cl_2$: MeOH 98:2) to give the target compound as a white solid with a yield of 630 mg (66%). $^1$H NMR ($CD_3OD$) δ 7.36-7.30 (20H, m, $C_6H_5$), 5.08 (8H, s, $CH_2C_6H_5$), 4.31 (2H, dd, $CH_{\alpha G1}$, $J$= 5.5 Hz, $J$ = 8.56 Hz), 4.00 (2H, dd, $CH_{\alpha G2}$, $J$ = 5.5 Hz, $J$ = 8.2 Hz), 3.25-3.20 (4H, m, $NHCH_2(CH_2)_8$), 3.13 (8H, dd, $CH_2NHZ$, $J$ = 6.4 Hz, $J$ = 10.4 Hz), 1.80-1.62 (4H, br, $CH_\alpha CH_2$), 1.52-1.10 (38H, m, $CH_2(CH_2)_7CH_2$, $CH_2$), 1.31 (18H, s, $C(CH_3)_3$). $^{13}$C NMR ($CD_3OD$) δ 175.2, 173.9 ($CH_\alpha CO$ x 4), 158.9, 158.8 ($NHCOO$ x 6), 138.4 (Ar*C*), 129.5, 128.8 ($C_6H_5$), 80.7 ($C(CH_3)_3$ x 2), 67.3 ($CH_2C_6H_5$ x 4, overlapping), 56.3, 54.6 ($CH_\alpha$ x 4), 41.5, 41.3, 40.4 ($NHCH_2(CH_2)_7CH_2$, $CH_2NHZ$ x 4), 32.7 ($CH_\alpha CH_2$ x 4), 30.5 ($CH_2$, overlapping), 30.3 ($C(CH_3)_3$ x 2), 27.9, 24.0 ($CH_2$, overlapping). IR $\nu_{max}$ $cm^{-1}$ 3302*m*, 2928*m*, 2855*m*, 1686*s*, 1639*s*, 1535*s*, 1454*m,* 1365*w*, 1254*s*, 1138*m*. ESI-MS (*m/z*) 1429.8 $[M+Na]^+$. $R_f$ = 0.45. m.p. 106-109°C**.**

### Target Compound 3

Compound **B** (0.492 g, 0.350 mmol) was Boc-deprotected following the standard procedure. The product (1 eq) was solubilised in a mixture of EtOAc (100 mL) and THF (50 mL), triethylamine (2.5 eq) was added followed by the protected amino acid (2.2 eq). The mixture was stirred at room temperature, and 1-propylphosphonic acid cyclic anhydride

(T3P, 2.2 eq) was added dropwise to the solution, which was then stirred overnight. After rotary evaporation of the THF, water (50 mL) and EtOAc (50 mL) were added to the reaction mixture, which was stirred for a further 10 min. After separation of the organic and aqueous phases, the organic phase was washed with sodium hydrogen sulfate solution (8 g in 50 mL water), aqueous sodium hydrogen carbonate (1 M) then finally water. The organic layer was and then rotary evaporated to dryness. The crude product was suspended in acidic water and agitated for 1 h and then filtered using a sintered glass funnel the product was obtained as a white solid in a yield of 393 mg (0.196 mmol, 56%). $^{1}$H NMR (DMSO-$d_6$) δ 7.92 (2H, d, N$H_{\alpha G1}$, $J$ = 8.0 Hz), 7.79 (4H, br, N$H_{\alpha G1}$, N$H$(CH$_2$)$_9$), 7.40 (2H, d, N$H_{G3,}$ $J$ = 8.4 Hz), 7.32-7.28 (40H, m, C$_6H_5$), 7.21 (6H, d, N$H$Z, $J$ = 5.6 Hz), 5.00 (4H, s, C$H_2$C$_6$H$_5$), 4.98 (12H, s, C$H_2$C$_6$H$_5$), 4.18 (2H, dd, C$H_{\alpha G1,}$ $J$ = 11.4 Hz, $J$ = 4.9 Hz), 4.12 (2H, dd, C$H_{\alpha G2,}$ $J$ = 14.0 Hz, $J$ = 5.9 Hz), 3.95 (2H, br, C$H_{\alpha G3}$), 3.05-2.91 (16H, m, C$H_2$NHZ, NHC$H_2$(CH$_2$)$_8$), 1.67-1.42 (12H, m, CH$_\alpha$C$H_2$), 1.41-1.78 (38H, m, CH$_\alpha$C$H_2$). $^{13}$C NMR (DMSO-$d_6$) δ 171.8, 171.7 (CH$_\alpha$CO x 6, overlapping), 156.6 (NHCOO x 8, overlapping), 137.8, 135.5, 128.9, 128.2 ($C_6$H$_5$), 65.9, 65.6 ($C$H$_2$-C$_6$H$_5$ x 8, overlapping), 55.2, 53.0 ($C$H$_\alpha$ x 6, overlapping), 40.4, 40.2 (NH$C$H$_2$(CH$_2$)$_7C$H$_2$, $C$H$_2$NHZ x 6, overlapping), 31.7, 31.2, 29.7, 29.5, 29.3, 29.1, 26.9, 23.3, 23.0 ($C$H$_2$, overlapping). IR $\nu_{max}$ cm$^{-1}$ 3299*m*, 2937*m*, 2865*w*, 1684*s*, 1653*s*, 1636*s,* 1521*s*, 1507*s*, 1457*w*, 1231*s*, 1136*m*, 1028*m.* ESI-MS (*m/z*) calculated value for C$_{109}$H$_{143}$N$_{14}$O$_{22}$ [M{$^{13}$C$_1$}+2H]$^{2+}$requires 1001.0305; (ES$^+$) found: 1001.0298 (90%, [M{$^{13}$C$_1$}+2H]$^{2+}$), 1009.5431 (100%, [M{$^{13}$C$_1$}+H+NH$_4$]$^{2+}$). $R_f$ = 0.45. m.p**.** 134-137°C.

**Target Compound 4**

Compound **G1-Boc** (1.49 g, 1.83 mmol) was deprotected using the standard method. The deprotected intermediate was dissolved in triethylamine (10 mL) and ethyl acetate (200 mL), L-Z-Lys(Z)-OH (3.30 g, 8.05 mmol) was then added. The reaction mixture was heated to 55°C and 1-propylphosphonic acid cyclic anhydride (T3P, 4.7 mL, 8.05 mmol) was added dropwise into the reaction mixture. After stirring at 60°C overnight, the reaction mixture was cooled to room temperature and filtered under vacuum to produce a yellow solid. The crude product was dissolved in pyridine, the solution filtered and the filtrate rotary evaporated. The resulting solid was suspended in acidic water to remove the remaining pyridine and filtered using a sintered glass funnel to a slightly yellow solid with a yield of 2.2

g (60%). $^{1}$H NMR (DMSO-$d_6$) δ 7.85 (6H, br, CON*H*$(CH_2)_9$, N$H_{\alpha G1}$, $CH_2$N*H*CO ), 7.39 (4H, br, N$H_{\alpha G2}$), 7.33-7.28 (40H, m, $C_6H_5$), 7.22 (4H, br, N*H*Z), 5.00 (8H, s, C$H_2C_6H_5$), 4.95 (8H, s, C$H_2C_6H_5$), 4.17 (2H, d, C$H_\alpha$, *J* = 4.3 Hz), 3.96 (2H, br, C$H_\alpha$), 3.87 (2H, br, C$H_\alpha$), 2.95 (16H, br, C$H_2$NH), 1.68-1.40 (12H, m, $CH_\alpha CH_2$), 1.39-1.04 (38H, m, C$H_2$). $^{13}$C NMR (DMSO-$d_6$) δ 172.0, 171.3, 171.1 ($CH_\alpha$*C*O x 6), 156.1 (NH*C*OO x 8, overlapping), 137.2, 137.0, 128.7, 128.4, 127.7, 127.3 ($C_6H_5$), 65.4, 65.1 (*C*$H_2C_6H_5$ x 8, overlapping), 54.7, 53.9, 52.5 (*C*$H_\alpha$ x 6), 39.3 (NH*C*$H_2(CH_2)_7$*C*$H_2$, *C*$H_2$NHZ x 6, overlapping), 32.0, 31.5, 29.6, 29.3, 29.1, 29.0, 28.8, 26.9, 26.4, 23.1, 22.8, 22.6, 22.5 (*C*$H_2$, overlapping). IR $\nu_{max}$ cm$^{-1}$ 3299*m*, 2937*m*, 2865*w*, 1685*s*, 1632*sh*, 1539*s*, 1507*sh*, 1457*sh*, 1256*s*, 1136*m*, 1026*m*. ESI-MS (*m/z*) calculated value for $C_{109}H_{143}N_{14}O_{22}$ $[M\{^{13}C_1\}+2H]^{2+}$requires 1001.0305; ($ES^+$) found: 1001.0284 (100%, $[M\{^{13}C_1\}+2H]^{2+}$), 2018.0961 (30%, $[M+NH_4]^+$). m.p. 138-141$^o$C.

## 6. Gelation Studies

### Procedure for Making Gels

An accurately measured mass of gelator was weighed out into a 2 ml glass vial. The solvent (0.5 ml) was then added using a Gilson pipette. The sample was sonicated for 30 minutes and heated with a heat gun until a homogeneous solution had been obtained which was then left to cool down. All gels were left overnight to set.

### Procedure for the measurement of $T_{gel}$ values

Once the gel as formed, in a 2 mL glass sample vial, it was placed into a high precision thermoregulated oil bath and the temperature was increased at the rate of 0.5°Cmin$^{-1}$. The temperature at which solvent started to leach from the sample was recorded as the $T_{gel}$ value, which reflects the onset temperature of the gel-sol transition.

### Procedure for the Preparation of SEM Samples

A small amount of gel sample was removed from its glass vial with a spatula and it was spread thinly onto an aluminium stub and left to dry overnight under ambient conditions in a fume hood. Before imaging, the sample was covered in a thin layer (4 nm) of Pd/Pt using an Agar sputter coater before being placed in the microscope.

### Procedure for the Preparation of the Freeze-Dried Samples

As before a small amount of gel sample was placed on a metal stub, which was placed into a critical point dryer to remove the solvents under supercritical conditions. Again before imaging the sample were coated with a layer of gold/palladium then placed into the machine.

## 7. References

1. O. Keller, W. E. Keller, G. van Look, and G. Wersin, *Org. Synth.* **1985**, *63*, 160-170.

2. A. R. Hirst, I. A. Coates, T. Boucheteau, J. F. Miravet, B. Escuder, V. Castelletto, I. W. Hamley and D. K. Smith, *J. Am. Chem. Soc.* 2008, **130**, 9113-9121.