ELECTRONIC SUPPLEMENTARY INFORMATION

FOR:

Peptide Functionalised Discotic Amphiphiles and Their Self-Assembly into Supramolecular Nanofibres

P. Besenius,*[a,b,#] Y. Goedegebure,[c] M. Driesse,[b] M. Koay,[c] P.H.H. Bomans,[d] A.R.A. Palmans,[a,b] P.Y.W. Dankers,[a,c] E.W. Meijer,*[a,b,c]

[a] *Institute for Complex Molecular Systems, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands.*
[b] *Laboratory of Macromolecular and Organic Chemistry, Department of Chemical Engineering and Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands. Fax: +31 40 245 1036; Tel: +31 40 247 3101.*
[c] *Laboratory of Chemical Biology, Department of Biomedical Engineering, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands.*
[d] *Laboratory of Materials and Interface Chemistry and Soft Matter CryoTEM Research Unit, Department of Chemical Engineering and Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands.*
[#] *Current address: Organic Chemistry Institute and Center for Nanotechnology (CeNTech), Westfälische Wilhelms-Universität Münster, Corrensstrasse 40, 48149 Münster, Germany.*

P.Besenius@uni-muenster.de; E.W.Meijer@tue.nl

Figures S1 – S2

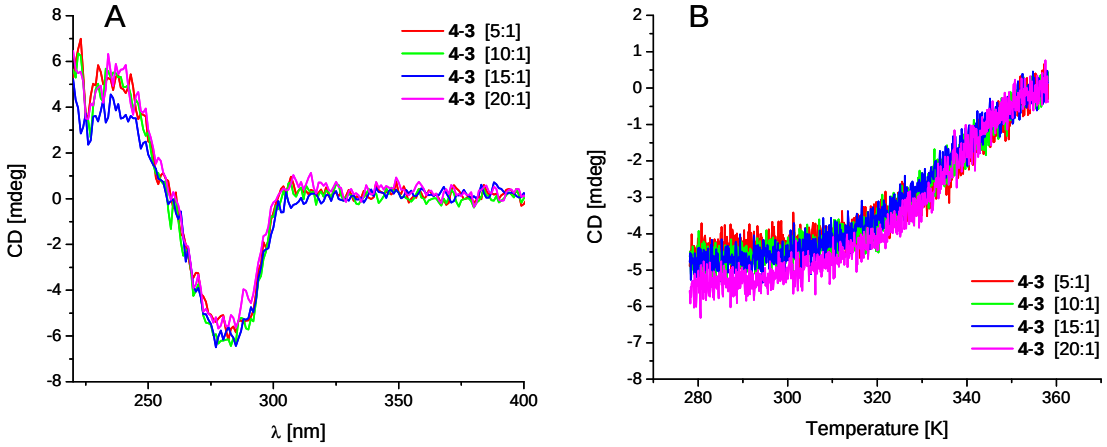


Figure S1 A) CD spectra for discotics **4** and **3** at various ratios (5:1 to 20:1) in PBS buffer [10 mM, pH 8.0] at 293 K; B) the corresponding cooling curves from the molecularly dissolved state, monitored at $\lambda = 280$ nm; in all cases the total discotic concentration was $5 \cdot 10^{-6}$ M.

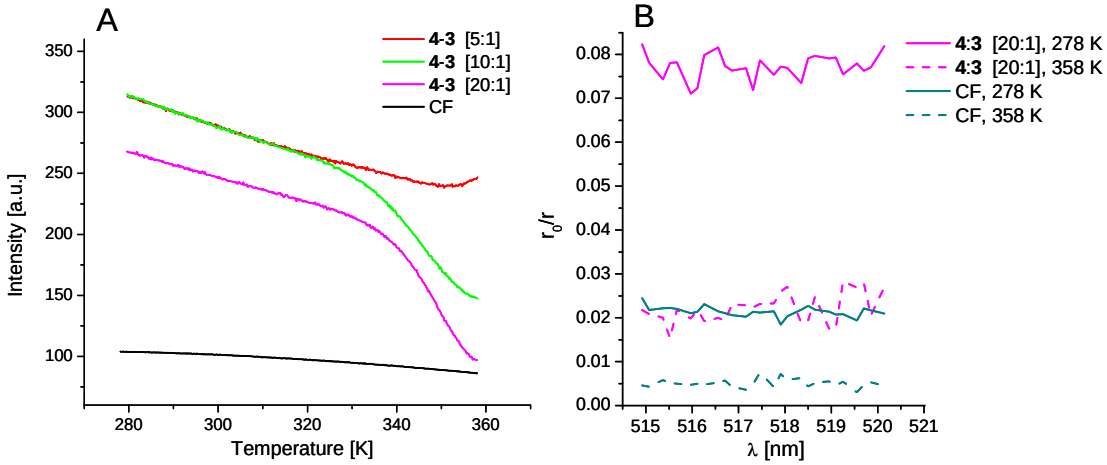


Figure S2 Temperature dependent PL measurements for discotic **4** and **3** at various ratios (5:1 to 20:1) and carboxyfluorescein (CF) in PBS buffer [10 mM, pH 8.0] (λ_{ex} = 485 nm): A) the cooling curves monitored at λ_{em} = 525 nm; B) anisotropy measurements for **4**:**3** at a ratio of 20:1 in the molecularly dissolved and fully aggregated state, as well as for CF at the same temperatures; in all cases the total discotic concentration was $5 \cdot 10^{-6}$ M (detector sensitivity set to 600 V), and the CF concentration was 3.3 10^{-7} M (detector sensitivity set to 450 V).

Experimental Section

Materials

Unless stated otherwise, all reagents and chemicals were obtained from commercial sources at the highest purity available and used without further purification. Water was demineralised prior to use. Freshly made buffer solutions were filtered through a 0.2 μm membrane filter into a sterilised bottle and stored at 4 °C. Phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich:1 tablet dissolved in 200 mL H_2O resulted in a 10 mM phosphate buffer with 2.7 mM KCl and 137 mM NaCl; pH 7.4 or pH 8 were adjusted with 1 M NaOH. Citric acid was obtained from Sigma-Aldrich, dissolved in H_2O, the pH adjusted to 6.0 and the buffer diluted to 100 mM.

Reactions were conducted under an argon atmosphere using standard Schlenk line techniques unless otherwise specified. Thin layer chromatography was performed using 60-F254 (250 nm) silica gel coated plates (Fisher Scientific). Flash chromatography was performed on a Biotage flash chromatography system using 200–425 mesh silica gel (Type 60A Grade 633).

Instrumentation

^{1}H-NMR, ^{1}H-^{1}H COSY, ^{13}C and ^{19}F-NMR spectra were recorded on Varian Mercury 400 and 200 or Gemini 300 spectrometers at 298 K. Chemical shifts are given in ppm (δ) values relative to tetramethylsilane (TMS). Splitting patterns are labeled as s, singlet; br s, broad singlet, d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet.

IR spectra were measured at 298 K on a Perkin-Elmer 1605 FT-IR spectrophotometer.

Matrix assisted laser desorption/ionisation mass spectra were obtained on a PerSeptive Biosystems Voyager DE-PRO spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) and 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) as matrices.

LC-MS analyses were performed using a Shimadzu SCL-10 AD VP series HPLC coupled to a diode array detector (Finnigan Surveyor PDA Plus detector, Thermo Electron Corporation) and an Ion-Trap (LCQ Fleet, Thermo Scientific). Analyses were performed using a reversed phase C-18 column using an injection volume of 1-4 μL, a flow rate of 0.20 mL/min and typically a gradient (5% to 100% in 10 min, held at 100% for a further 3 minutes) of acetonitrile in water (both containing 0.1% formic acid) at 298K. Positive and negative ion mass spectra were acquired in standard enhanced mode using electrospray ionisation (drying temperature: 350 °C; nebuliser pressure: 35 psi; drying gas flow: 8 L/min; HV capillary: 4000 V; ICC target 20,000).

Gd^{III}-contents were determined by means of inductively coupled plasma mass spectrometry (ICP-MS) conducted by the MiPlaza Materials Analysis laboratories (Philips Research Europe) in Eindhoven, The Netherlands.

The cryoTEM experiments were performed on the TU/e CryoTitan (FEI), using a Gatan cryo-holder operating at ~ -170 °C (www.cryotem.nl). The TU/e CryoTitan is equipped with a field emission gun (FEG) operating at 300 kV. Images were recorded using a 2k x 2k Gatan CCD camera equipped with a post column Gatan Energy Filter (GIF). The sample vitrification procedure was carried out using an automated vitrification robot (FEI Vitrobot™ Mark III). CryoTEM grids, R2/2 Quantifoil Jena grids, were purchased from Quantifoil Micro Tools GmbH. Prior to the vitrification procedure the grids were surface plasma treated using a Cressington 208 carbon coater operating at 5 mA for 40 s.

CD measurements were performed on a Jasco J-815 spectropolarimeter, where the sensitivity, time constant and scan rate were chosen appropriately. Corresponding temperature dependent measurements were performed with a Jasco PTC-348WI Peltier-type temperature controller, with a temperature range of 263-383 K and adjustable temperature slope (1 K/min).

UV/VIS spectra were obtained on a Varian Cary 50 Scan UV-Visible Spectrometer.

PL spectra and temperature dependent measurements were obtained on a Varian Cary Eclipse Fluorescence Spectrometer. The temperature slope was the same as for all CD experiments (1 K/min).

Synthetic procedures

Discotics **4**, **12** and all the intermediate compounds **8**-**11**, **20** were synthesised and characterised according to literature procedures.[1-3] For further details and representative ^{1}H-NMR, LC-MS and MALDI-TOF spectra we refer to reference [2].

(7) A magnetically stirred solution of HATU (4.42 g, 11.62 mmol) in dry dimethylformamide (40 mL), under argon, was cooled to 0 °C. After 15 min dry DIPEA (3.8 mL, 21.74 mmol) and Fmoc-L-F_5PheOH (**2**) (4.5 g, 11.62 mmol) were added. This mixture was stirred for 5 min after which *tert*-butyl-4-aminobenzoate (**6**) (1.257 g, 6.5 mmol) was added. Stirring was continued for 25 h at room temperature. The reaction mixture was then diluted with dichloromethane (300 mL). The organic layer was washed with 1 N $KHSO_4$ (3 x 150 mL), sat. $NaHCO_3$ (150 mL) and brine (150 mL) and dried over $MgSO_4$. After evaporation, the crude product was purified by column chromatography (silica, linear gradient of 0% - 80% ethyl acetate in DCM) to yield the pure title compound as a white solid material (3.80 g, 5.82 mmol, 90%).
^{1}H NMR (δ [ppm], $CDCl_3$, 400 MHz): 8.30 (s, 1H, -NH), 7.95 (d, 2H, Ar-H), 7.75 (d, 2H, Ar-H), 7.26-7.50 (m, 8H, Ar-H), 5.42 (q, 1H, -CH), 4.42-4.56 (m, 3H, -NH + -CH_2), 4.17 (m, 1H, -CH), 3.18-3.34 (m, 2H, -CH_2), 1.59 (s, 9H, -CH_3).
ESI-MS (positive mode): *m/z* calcd. for $[M+Na]^+$ 675.2; found: 675.1 $[M+Na]^+$.

(8) Compound **7** (3.73 g, 5.72 mmol) was dissolved in 15 mL DCM and 15 mL of TFA was slowly added. The reaction mixture was stirred for 1.5 h and evaporated to dryness. The resulting brownish solid was dissolved in 15 mL DCM again, reacted with 15 mL of TFA for 1 h and then evaporated to dryness. ^{1}H-NMR showed there was still some *tert*-butylester present, so it was dissolved in 15 mL DCM and 15 mL of TFA again for 3 h. After evaporation, the crude product was purified by column chromatography (silica, linear gradient of 0% - 100% ethyl acetate in DCM) to remove as much TFA as possible. An off white solid was obtained (3.37 g, 5.64 mmol, 98 %).
^{1}H NMR (δ [ppm], DMSO-d_6, 400 MHz): 10.28 (s, 1H, -NH), 7.98 (d, 2H, Ar-H), 7.87 (d, 2H, Ar-H), 7.29-7.69 (m, 8H, Ar-H), 4.47 (q, 1H, , -CH), 4.22 (m, 3H, -CH + -CH_2), 3.20-3.36 (m, 2H, -CH_2).
^{19}F NMR (δ [ppm], DMSO-d_6, 188 MHz): -74.9 (s, 3F, TFA), -141.9 (d, 2F, Ar-F), -157.3 (t, 1F, Ar-F), -164.0 (d, 2F, Ar-F).
ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 597.1; found: 596.8 $[M+H]^+$.

(9) Compound **8** (2.73 g, 4.57 mmol) was dissolved in 70 mL THF. To this solution *N-Boc*-butane-1,4-diamine (0.88 mL, 4.57 mmol), PyBOP (2.38 g, 4.57 mmol) and DIPEA (2.27 mL, 13.72 mmol) were added consecutively. The mixture was stirred at room temperature for 12 h. The reaction mixture was then diluted with 150 mL ethyl acetate

and the organic layer washed with 1 N $KHSO_4$ (2 x 150 mL), sat. $NaHCO_3$ (2 x 150 mL) and brine (2 x 150 mL). After evaporation, the crude product was purified by column chromatography (silica, linear gradient of 0% - 100% ethyl acetate in DCM) to yield the pure title compound as a white solid material (2.8 g, 3.65 mmol, 80%)
^{1}H NMR (δ [ppm], DMSO-d_6, 400 MHz): 10.19 (s, 1H, -NH), 8.33 (s, 1H, -NH), 7.98 (d, 2H, Ar-H), 7.89 (d, 2H, Ar-H), 7.32-7.78 (m, 8H, Ar-H), 6.79 (t, 1H, -NH), 4.48 (q, 1H, -CH), 4.24 (m, 3H, -CH + -CH_2), 3.22-3.38 (m, 2H, -CH_2), 3.08 (m, 2H, -CH_2), 2.93 (q, 2H, -CH_2), 1.45 (d, 4H, -CH_2), 1.36 (s, 9H, -CH_3).
ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 767.3; found: 667.3 [M-*Boc*+H]$^+$, 766.9 $[M+H]^+$, 789.3 $[M+Na]^+$, 1433.3 [2M-*Boc*+H]$^+$, 1533.3 $[2M+H]^+$, 1555.0 $[2M+Na]^+$.

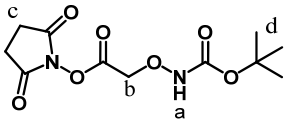
(10) Compound **9** (2.65 g, 3.46 mmol) was dissolved in 100 mL piperidine in acetonitrile (20 vol%) and stirred for 80 min. The mixture was then evaporated, but not to complete dryness, and 100 mL of acetonitrile added. The solution was evaporated straight away, but also not to complete dryness and this was repeated twice more. After evaporation, the crude product was purified by flash chromatography (silica, with a gradient of 0% - 10% methanol in ethyl acetate) to yield the pure title compound as a white solid material (960 mg, 1.76 mmol, 53%).
^{1}H NMR (δ [ppm], DMSO-d_6, 400 MHz): 8.30 (s, 1H, -NH), 7.77 (d, 2H, Ar-H), 7.62 (d, 2H, Ar-H), 6.77 (s, 1H, -NH), 3.54 (t, 1H, -CH), 3.20-3.37 (m, 2H, -CH_2), 3.04 (m, 2H, -CH_2), 2.90 (m, 2H, -CH_2), 1.41 (d + d, 4H, -CH_2), 1.35 (s, 9H, -CH_3).
ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 545.2; found: 445.3 [M-*Boc*+H]$^+$, 545.0 $[M+H]^+$, 567.3 $[M+Na]^+$, 1089.1 $[2M+H]^+$, 1111.2 $[2M+Na]^+$.

(11) A magnetically stirred solution of compound **10** (854 mg, 1.568 mmol) and dry DIPEA (0.52 mL, 3.137 mmol) dissolved in 20 mL dry dimethylformamide in dry THF (10 vol%), under argon, was cooled to 0 °C. After 15 min 1,3,5-benzenetricarbonyl trichloride (104 mg, 0.392 mmol) was added. Stirring was continued for 48 h at room temperature. After evaporation of the solvents, the crude product was purified by flash chromatography (silica, linear gradient of 30% - 50% THF in DCM) to yield the pure title compound as a white solid material (508 mg, 0.288 mmol, 72%).
^{1}H NMR (δ [ppm], DMSO-d_6, 400 MHz): 10.31 (s, 3H, -NH), 9.22 (d, 3H, -NH), 8.49 (d, 3H, Ar-H), 8.33 (t, 3H, -NH), 7.78 (d, 6H, Ar-H), 7.59 (d, 6H, Ar-H), 6.78 (m, 3H, -NH), 4.95 (q, 3H, -CH), 3.20-3.45 (m, 12H, -CH + -CH_2), 2.92 (m, 6H, -CH_2), 1.34-1.76 (m, 39H, -CH_2 + -CH_3).
ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1789.6; found: 894.7 $[M+2H]^{2+}$, 1193.3 $[2M+3H]^{3+}$, 1789.8 $[M+H]^+$.

(12) Compound **11** (364 mg, 0.203mmol) was dissolved in 10 mL DCM and 10 mL of TFA was slowly added. The reaction mixture was stirred for 80 min and then the solvent was evaporated. The resulting oil was again dissolved in 10 mL DCM and reacted with 10 mL of TFA for 75 min, before it was evaporated to dryness. LC-MS analysis showed the the protecting group was completely cleaved. The crude product was precipitated from THF (10 mL) in 240 mL diethyl ether to yield the trifluoroacetate salt of amine **12** as an off white solid material (217 mg, 0.146 mmol, 72%)
^{1}H NMR (δ [ppm], DMSO-d_6, 400 MHz): 10.32 (s, 3H, -NH), 9.20 (d, 3H, -NH), 8.49 (s, 3H, Ar-H), 8.41 (t, 3H, -NH), 7.79 (d, 6H, Ar-H), 7.69 (brs, -NH_3), 7.59 (d, 6H, Ar-H), 4.93 (q, 3H, -CH), 3.20-3.41 (m, 12H, -CH_2), 2.81 (m, 6H, -CH_2), 1.56 (m, 12H, -CH_2).
^{19}F NMR (δ [ppm], DMSO-d_6, 188 MHz): -73.6 (s, 3F, TFA), -141.9 (d, 2F, Ar-F), -157.0 (t, 1F, Ar-F), -163.8 (d, 2F, Ar-F).

ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1489.5; found: 497.5 $[M+3H]^{3+}$, 745.6 $[M+2H]^{2+}$, 993.3 $[2M+3H]^{3+}$, 1490.3 $[M+H]^+$.
MALDI-TOF-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1489.46; found: 1489.43 $[M+H]^+$, 1511.41 $[M+Na]^+$.

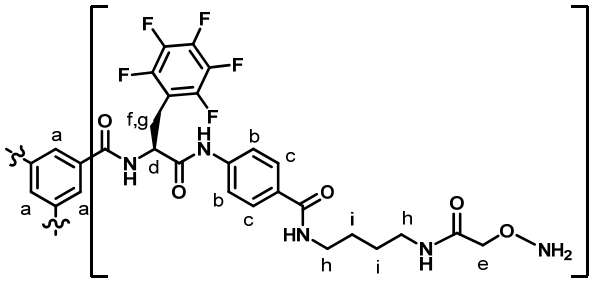
(14)



This compound was kindly provided by Dr. Freek Hoeben (SyMO-Chem, Eindhoven, The Netherlands).
^{1}H NMR (δ [ppm], $CDCl_3$, 400 MHz):7.64 (s, 1H, **a**), 4.76 (s, 2H, **b**), 2.86 (s, 4H, **c**), 1.46 (s, 9H, **d**).

(13) Compound **12** (190 mg, 0.1276 mmol) was dissolved in dry DMF (12 mL) and dry DIPEA (0.24 mL, 1.5312 mmol) was added. After stirring for 10 min under an argon atmosphere, compound **14** (121.4 mg, 0.4210 mmol) was added. The resulting solution was stirred at room temperature for 2 h. After completion, the mixture was dried by blowing off the DMF under a high-flow of air. Purification was achieved by flash chromatography (silica, linear gradient of 0% - 30% methanol in ethyl acetate) to yield the pure title compound (125 mg, 0.0622 mmol, 49%).
ESI-MS (positive mode): *m/z* calcd. for 1004.8 $[M+2H]^{2+}$; found: 1005.0 $[M+2H]^{2+}$, 1339.8 $[2M+3H]^{3+}$.

(15) Compound **13** (125 mg, 0.0622 mmol) was dissolved in 10 mL DCM and stirred at 0°C. After 15 min 10 mL of TFA was slowly added and this mixture was stirred for another 1 h at 0°C, and monitored by LC-MS. After completion the mixture was evaporated to dryness (without heat). Residual TFA was removed under high vacuum (quantitative yield).
^{1}H NMR (δ [ppm], CD_3OD-d_4, 400 MHz): 8.43 (s, 3H, **a**), 7.79 (d, 6H, **b**), 7.64 (d, 6H, **c**), 5.04 (t, 3H, **d**), 4.48 (s, 6H, **e**), 3.47 (m, 3H, **f**/**g**), 3.35 (m, 3H, **g**/**f**), 2.68 (m, 12H, **h**), 1.63 (m, 12H, **i**).



15

ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1708.4; found: 570.7 $[M+2H]^{2+}$, 855.2 $[M+2H]^{2+}$, 1139.6 $[2M+3H]^{3+}$, 1281.5 $[3M+4H]^{4+}$, 1709.1 $[M+H]^+$.
MALDI-TOF-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1708.44; found: 1708.39 $[M+H]^+$, 1730.40 $[M+Na]^+$.

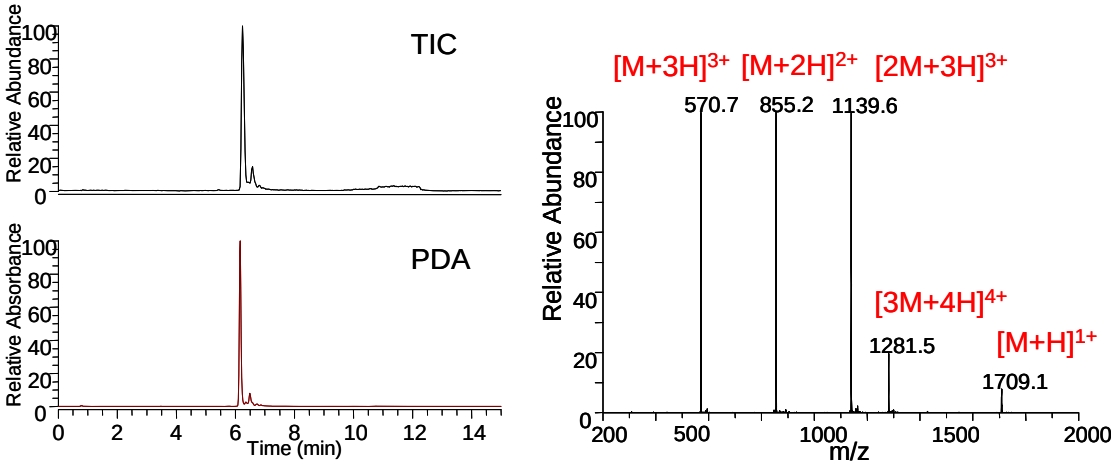


Figure S3 LC-MS trace of compound **15** including total ion current (left top), UV/Vis detection (left bottom) and positive mode ESI-MS (right).

SGGGGRGDS (**16**) & SGGGPHSRN (**17**): Both peptides were prepared by automated solid phase peptide synthesis on a Prelude synthesizer (Protein Technologies) on a 100 μmol scale on a Rink Amide resin using standard Fmoc-based protocols. Before attaching the first amino acid, the resin was swelled for 20 minutes in NMP. Each synthetic cycle consisted of removal of the Fmoc-group by 20% (v/v) piperidine in NMP (2 mL, 2 x 5 min) followed by a NMP wash (6 x 30 sec). Activation of the Fmoc amino acid (2 mL, 200 mM) by HBTU (1 mL, 400 mM) in the presence of *N,N'*-diisopropylethylamine (DIPEA, 1 mL, 600 mM) in NMP. All activated amino acids were coupled for 2 x 20 minutes. Unbound amino acids were removed by an NMP wash (6 x 30 s). The Fmoc protecting group from the last amino acid was removed before cleavage from the resin. Cleavage of the peptide from the resin was accomplished with 5 mL of 96% TFA containing 2% triisopropylsilane (TIS) and 2% H_2O for 2 h. After filtration, the deprotected peptides were precipitated with 45 mL diethyl ether and stored in the freezer for ~30 min. After centrifugation (2500 rpm, 10 min) the ethereal layer was decanted off and the pellet dissolved in water before lyophilisation. The peptides were characterised using LC-MS and used without further purification.

16: ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 748.7; found: 374.9 $[M+2H]^{2+}$, 748.5 $[M+H]^+$.
17: ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 867.87; found: 290.1 $[M+3H]^{3+}$, 434.4 $[M+2H]^{2+}$, 867.5 $[M+H]^+$.

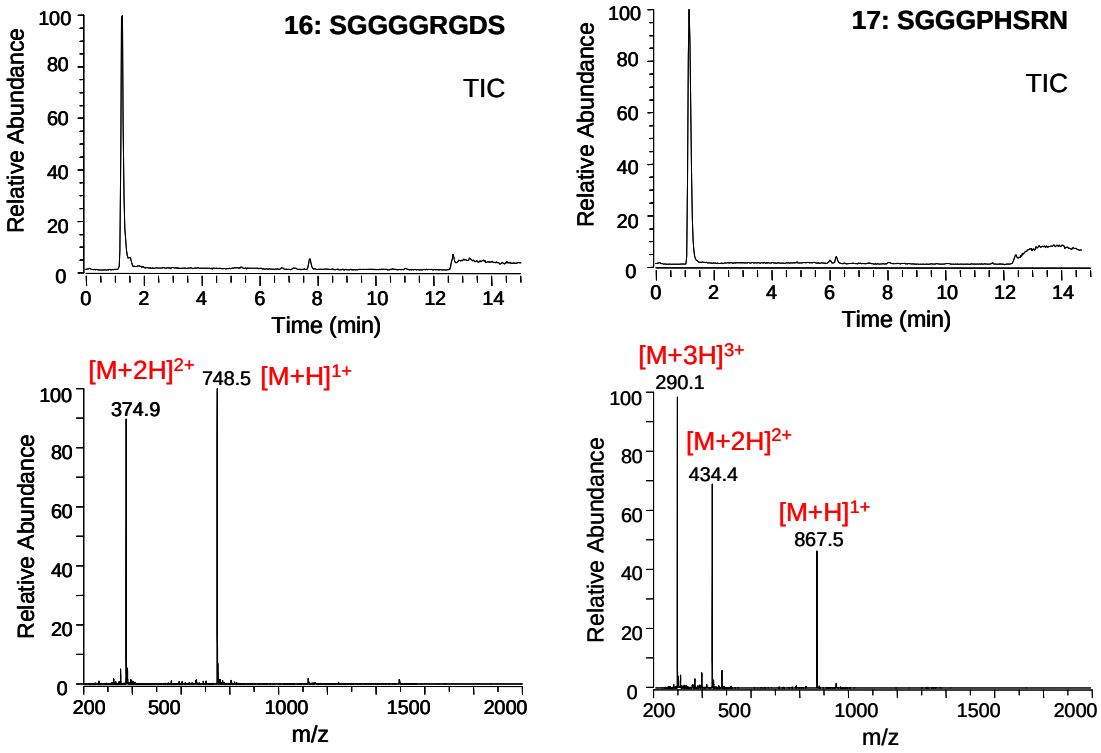


Figure S4 LC-MS traces of compounds **16** (left) and **17** (right) including total ion current (top) and positive mode ESI-MS (bottom).

(18 & 19) To a 10 mL solution of peptide **16** or **17** (8 mM) in sodium phosphate buffer (0.01 M, pH 7), 1 mL of a freshly prepared $NaIO_4$ solution (20 mM in sodium phosphate buffer 0.01 M, pH 7) was added. The reactions were complete within 20 min according to LC-MS. The mixtures were stored in the freezer and purified within a day by isocratic reversed-phase HPLC using 2% CH_3CN in H_2O containing 0.1 vol% formic acid.
18: ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 717.3; found: 717.7 $[M+H]^+$, 735.5 $[M^*+H]^+$.
19: ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 836.4; found: 418.8 $[M+2H]^{2+}$, 427.8 $[M^*+2H]^{2+}$, 836.4 $[M+H]^+$, 854.4 $[M^*+H]^+$.

M*= hydrate of M.

(1) Compound **15** (4.23 mg, 2.48 ⌈mol) and **18** (16 mg peptide containing ~50 wt% salt, 22 ⌈mol) were dissolved in 1.5 mL anilinium acetate buffer (0.1 M, pH4.5). The solution was stirred for 3 days and monitored by LC-MS. After completion the cloudy brownish mixture was purified by reversed-phase HPLC with H_2O/CH_3CN (0.1 vol% formic acid) as eluent to yield a white fluffy solid (~0.5 mg, 0.13 ⌈mol, 5%).
ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 3806.3; found: 951.9 $[M+4H]^{4+}$, 1269.0 $[M+3H]^{3+}$.

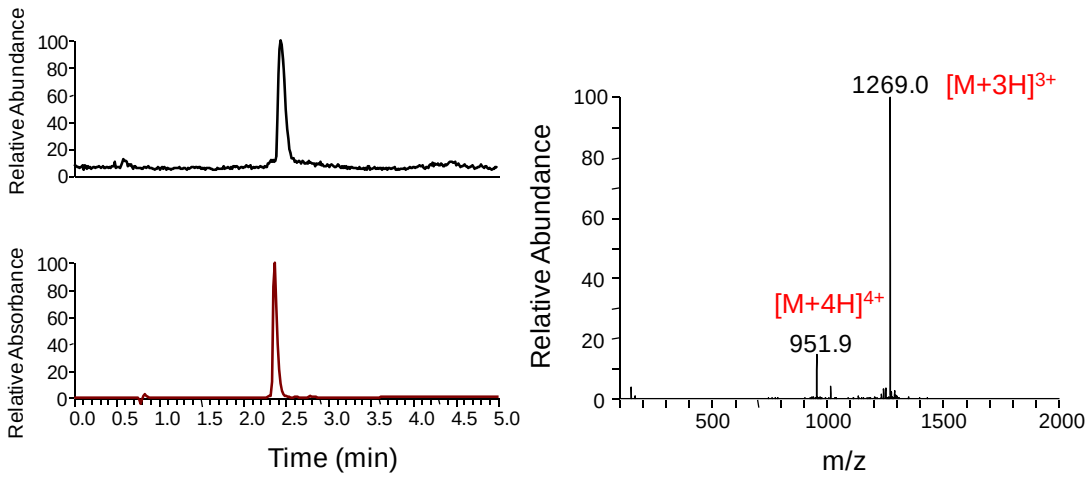


Figure S5 LC-MS trace of compound **1** including total ion current (left top), UV/Vis detection (left bottom) and positive mode ESI-MS (right).

(2) Compound **15** (3.63 mg, 2.12 ⌈mol) and **19** (16 mg peptide containing ~50 wt% salt, 19.1 ⌈mol) were dissolved in 1.5 mL anilinium acetate buffer (0.1 M, pH4.5). The solution was stirred for 3 days and monitored by LC-MS. After completion the cloudy brownish mixture was purified by reversed-phase HPLC with H_2O/CH_3CN (0.1 vol% formic acid) as eluent to yield a white fluffy solid (~0.5 mg, 0.13 ⌈mol, 5%).
ESI-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 4164.5; found: 694.6 $[M+6H]^{6+}$, 833.3 $[M+5H]^{5+}$, 1041.4 $[M+4H]^{4+}$, 1388.3 $[M+3H]^{3+}$.

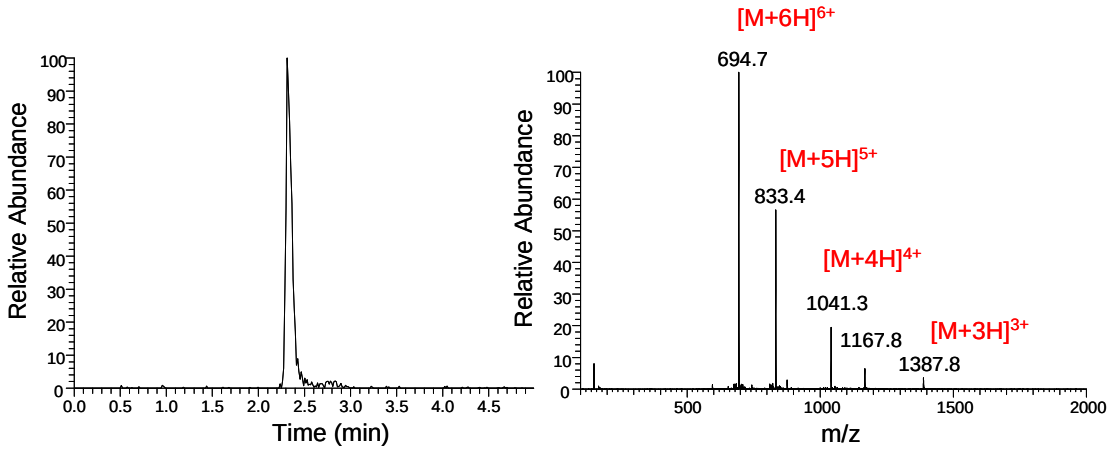


Figure S6 LC-MS trace of compound **2** including total ion current (left) and positive mode ESI-MS (right).

(3) Compound **12** (9.8 mg, 0.0066 mmol) was dissolved in dry DMF (5 mL) and dry DIPEA (0.5 mL, 3.025 mmol) was added. The mixture was stirred for 10 min under an argon atmosphere. To this solution, 5,6-carboxyfluorescein succinimidyl ester (14 mg, 0.029 mmol, mixture of isomers) was added. After stirring the reaction mixture for 24 h in an atmosphere of argon, the mixture was dried by blowing of the DMF with a high flow of air. Purification was achieved with flash chromatography (SiO_2, ethyl acetate

(containing 0.1 vol% TFA)/ methanol gradient, from 90:10 to 70:30). The pure title compound **3** was isolated as a yellow solid material (13.1 mg, 0.0051 mmol, 77 %)
ESI-MS (positive mode): *m/z* calcd. for $[M+2H]^{2+}$ 1281.8; found: 855.6 $[M+3H]^{3+}$, 1282.6 $[M+2H]^{2+}$, 1710.0 $[2M+3H]^{3+}$.
MALDI-TOF-MS (positive mode): *m/z* calcd. for $[M+Na]^{+}$ 2585.59; found 2586.93 $[M+Na]^{+}$.

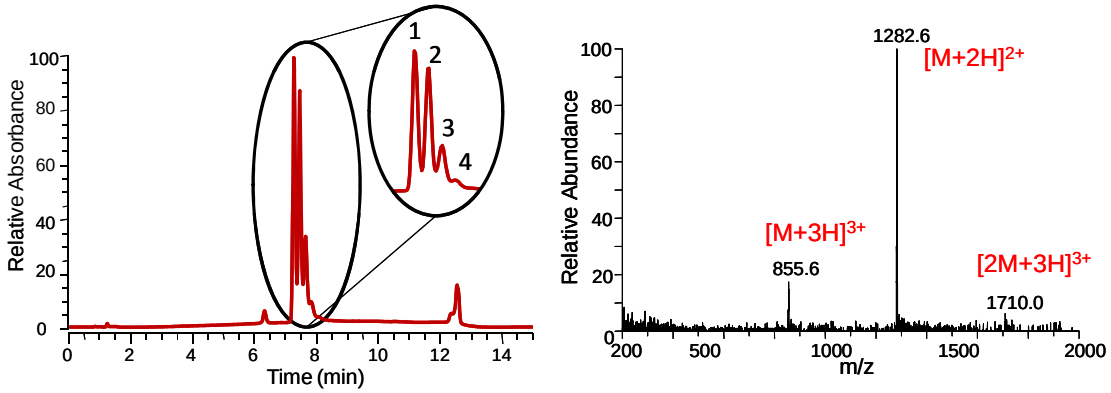


Figure S7 LC-MS trace of compound **3** including UV/Vis detection (left) and positive mode ESI-MS (right). Ligation to the mixture of 5,6-carboxyfluorescein isomers leads to four isomeric discotic products (as indicated with 1, 2, 3 and 4).

(20) To a solution of **12** (215 mg, 0.144 mmol) in DMF (5 ml) and DIPEA (0.7 ml, 3.8 mmol), was added DOTA-NHS xTFA (55% pure, 474 mg, 0.520 mmol) [synthesised and provided by Dr. Henk Keizer, SyMO-Chem, Eindhoven, The Netherlands] and the mixture stirred for 24 h. Conversion of starting material **12** was confirmed by LC-MS. Product **20** was dried by blowing off the DMF with air and was used for complexation with Gd(III) without further purification.
ESI-MS (positive mode): *m/z* calcd. for $[M+2H]^{2+}$ 1325.0; found: 530.8 $[M+5H]^{5+}$, 663.2 $[M+4H]^{4+}$, 883.9 $[M+3H]^{3+}$, 1325.2 $[M+2H]^{2+}$.

(4) 20 (270 mg, ~0.102 mmol) was dissolved in H_2O, the pH adjusted close to neutral with pyridine and $Gd(OAc)_3$ xH_2O (163 mg, 0.382 mmol, 426.26 g/mol) added in excess to cover for free remaining DOTA ligand from the previous synthetic step. The solution was stirred for 12 h, lyophilised to obtain a sticky yellow oil. The final product purified by reversed phase chromatography on a semi-preparative C18 column with H_2O/CH_3CN (0.1vol% formic acid) as eluent to yield a white fluffy solid (156 mg, 49%).
ESI-MS (positive mode): *m/z* calcd. for $[M+2H]^{2+}$ 1557.4; found: 779.0 $[M+4H]^{4+}$, 1038.3 $[M+3H]^{3+}$, 1245.4 $[2M+5H]^{5+}$, 1556.9 $[M+2H]^{2+}$.
MALDI-TOF MS: *m/z* calcd. for 3110.71 $[M+H]^{+}$; found 3110.71 $[M+H]^{+}$, 3133.66 $[M+Na]^{+}$, 3153.63 $[M-H+2Na]^{+}$.
ICP-AES (Gd^{III}): calcd. 15.2 wt%, found 12.6 wt%.

References

1. K. P. van den Hout, R. Martín-Rapún, J. A. J. M. Vekemans and E. W. Meijer, *Chem. Eur. J.*, 2007, **13**, 8111-8123.
2. P. Besenius, G. Portale, P. H. H. Bomans, H. M. Janssen, A. R. A. Palmans and E. W. Meijer, *Proc. Natl. Acad. Sci. U.S.A.*, 2010, **107**, 17888-17893.
3. P. Besenius, K. P. van den Hout, H. M. H. G. Albers, T. F. A. d. Greef, L. L. C. Olijve, T. M. Hermans, B. F. M. de Waal, P. H. H. Bomans, N. A. J. M. Sommerdijk, G. Portale, A. R. A. Palmans, M. H. P. van Genderen, J. A. J. M. Vekemans and E. W. Meijer, *Chem. Eur. J.*, 2011, **17**, 5193–5203.