Electronic Supplementary Material (ESI) for Chemical Science.

New self-assembling peptide nanotubes of large diameter using δ-amino acids

Alejandro Lamas, Arcadio Guerra, Manuel Amorín,* and Juan R. Granja*

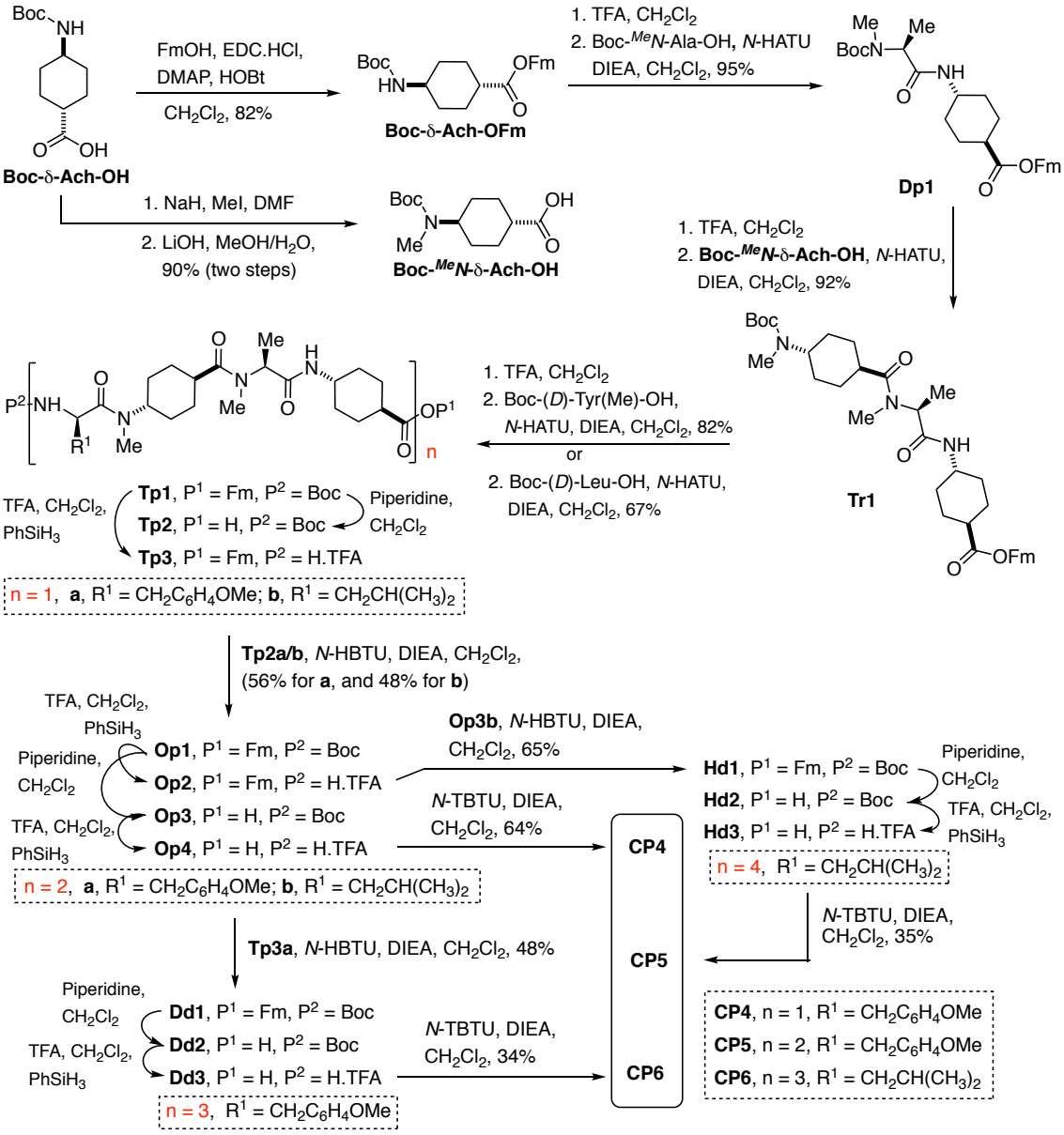
Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS) and Departamento de Química Orgánica, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

juanr.granja@usc.es and manuel.amorin@usc.es

SUPPORTING INFORMATION

Index:

Scheme 1S (Synthesis of **CP4**, **CP5** and **CP6**):



Scheme 2S (Synthesis of **CP7**):

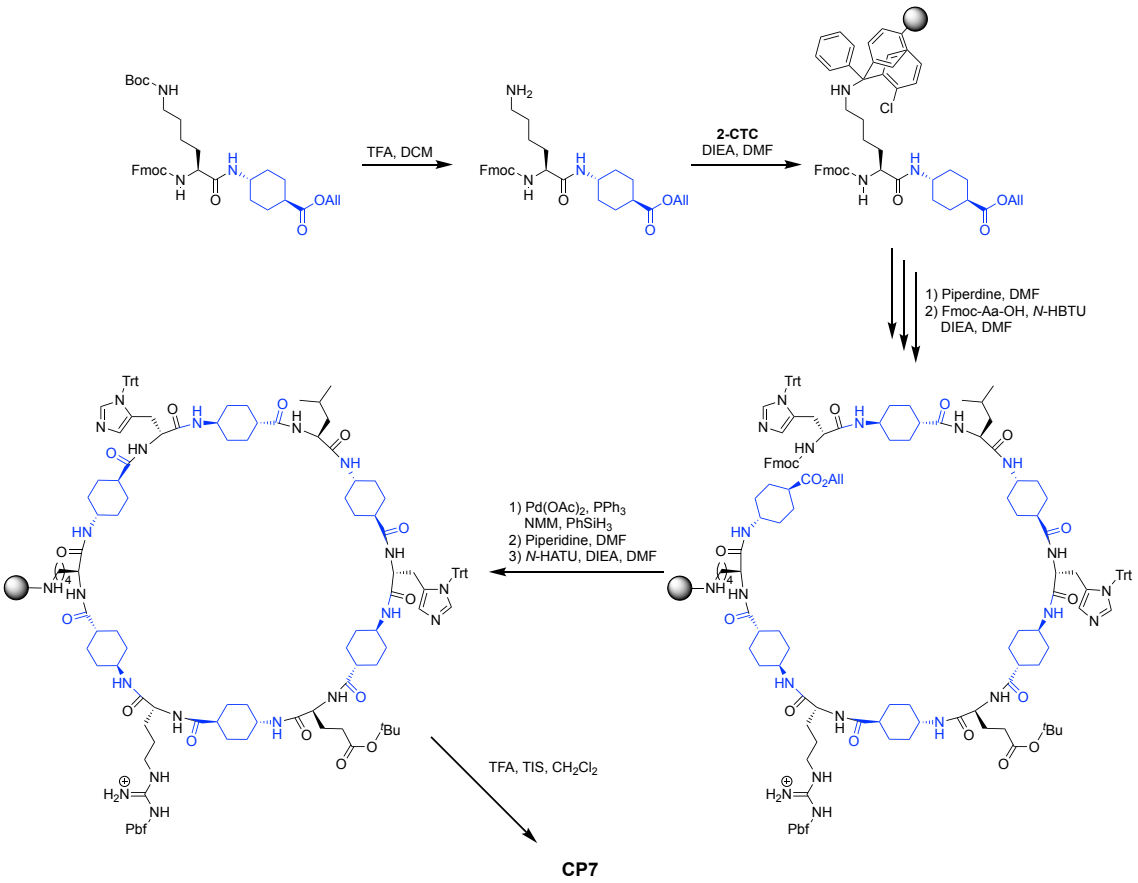
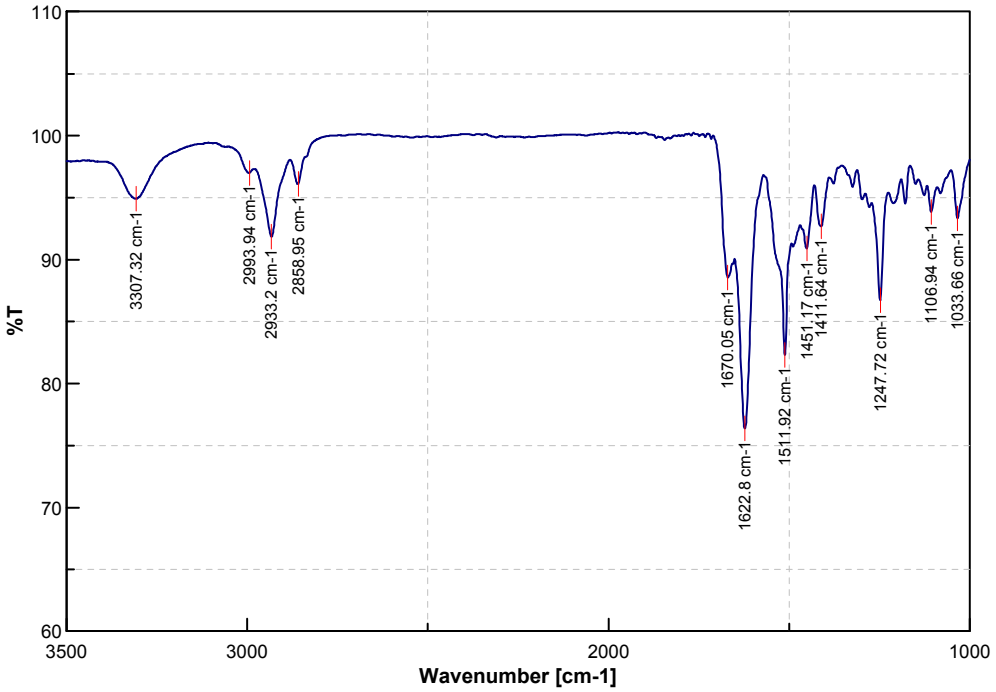
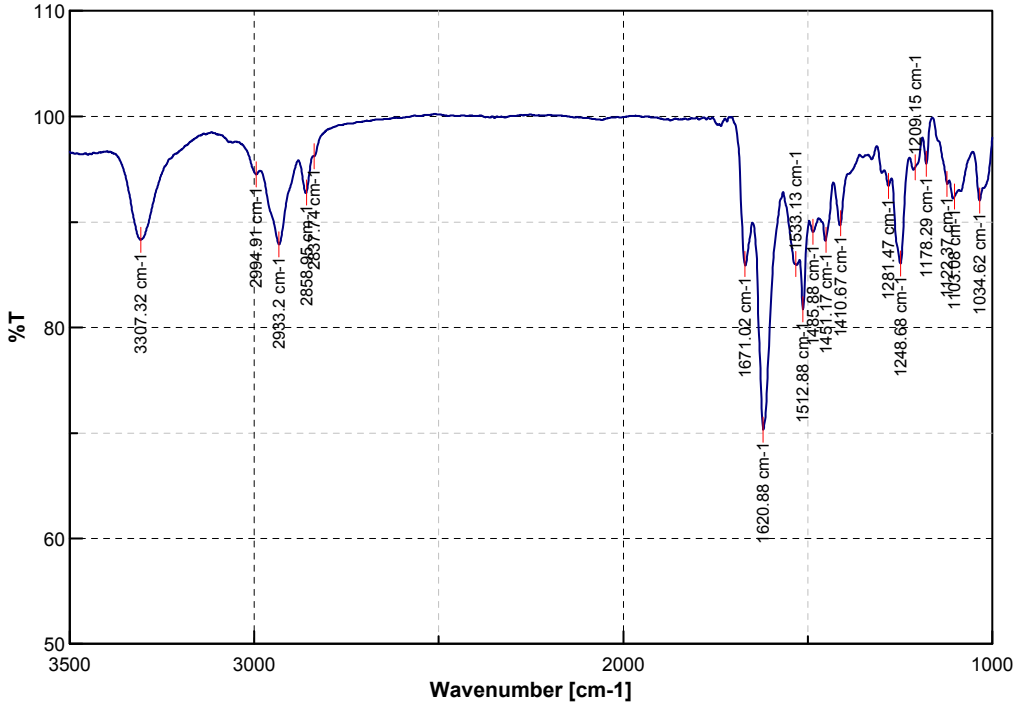


Figure 1S (FT-IR spectra of a) **CP4**, b) **CP5** and c) **CP6**):

a) **CP4** ($CHCl_3$, 298 K)



b) **CP5** ($CHCl_3$, 298 K)



c) **CP6** ($CHCl_3$, 298 K)

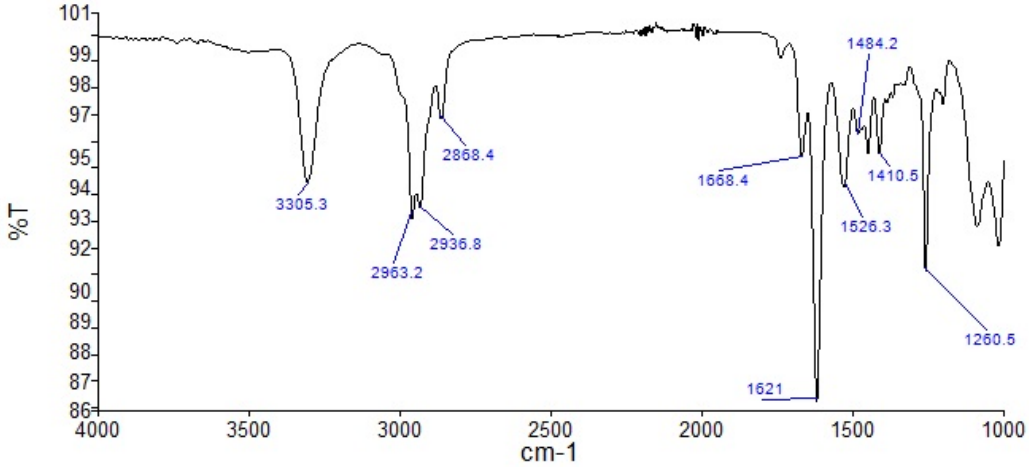
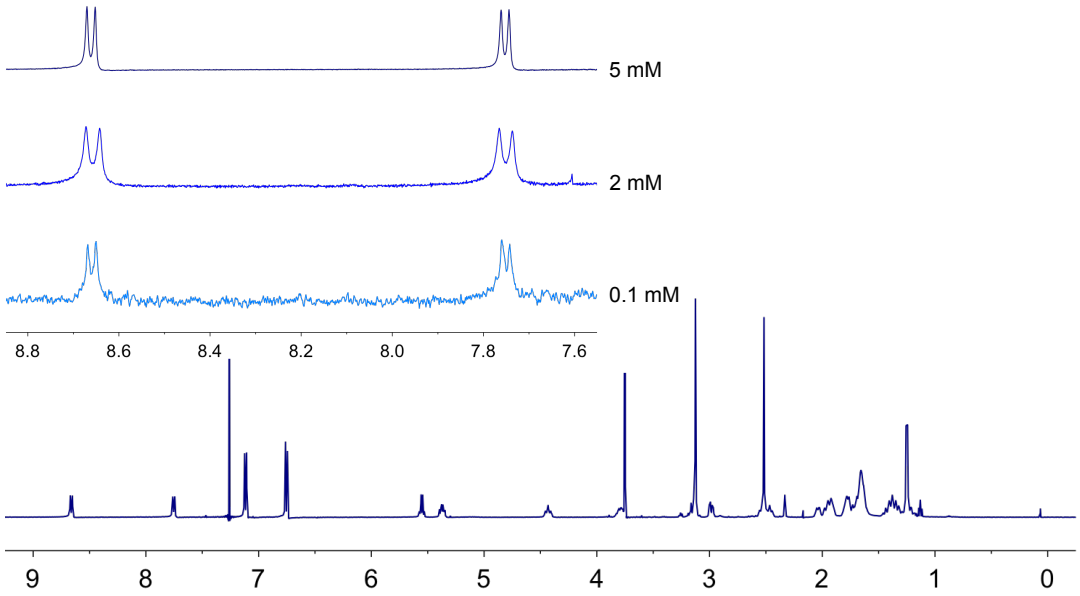


Figure 2S (^{1}H NMR experiments of a) **CP5** and b) **CP6**): Variable concentration ^{1}H NMR experiments for cyclic peptides

a) **CP5** ($CDCl_3$, 500 MHz, 298 K)



b) **CP6** ($CDCl_3$, 500 MHz, 298 K)

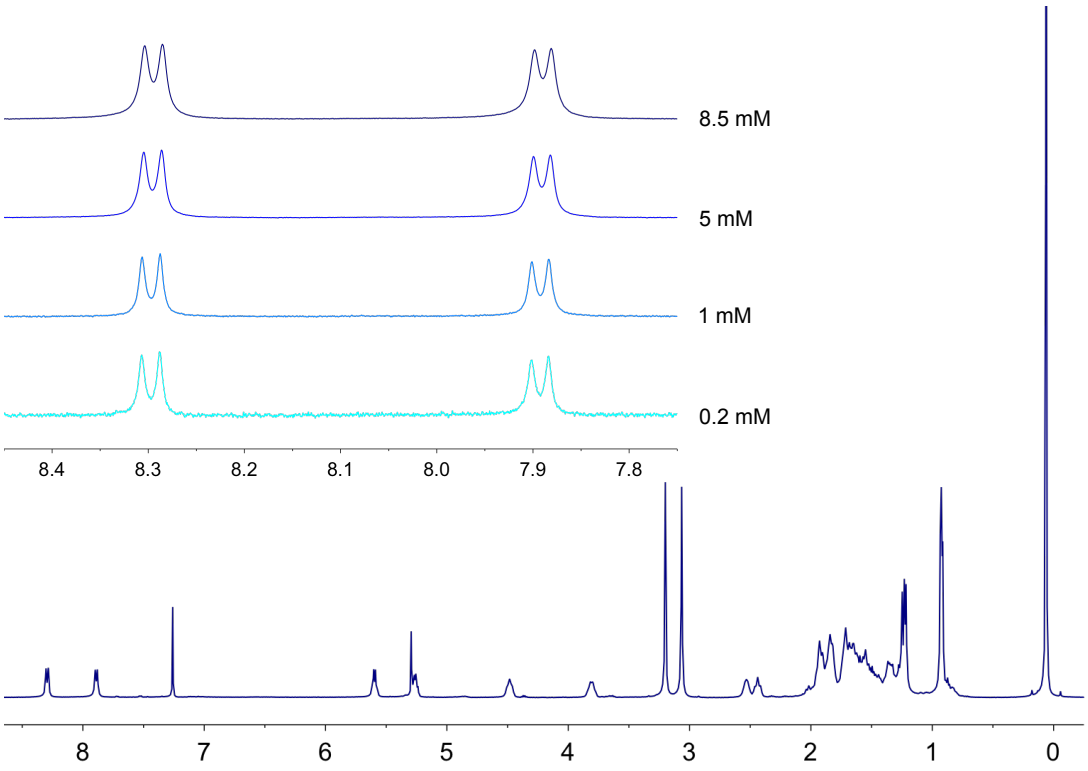


Figure 3S (nOe for dimeric structure of **CP6**): **CP6** and **D6** structures and NOE experiments in $CDCl_3$ at *rt*.

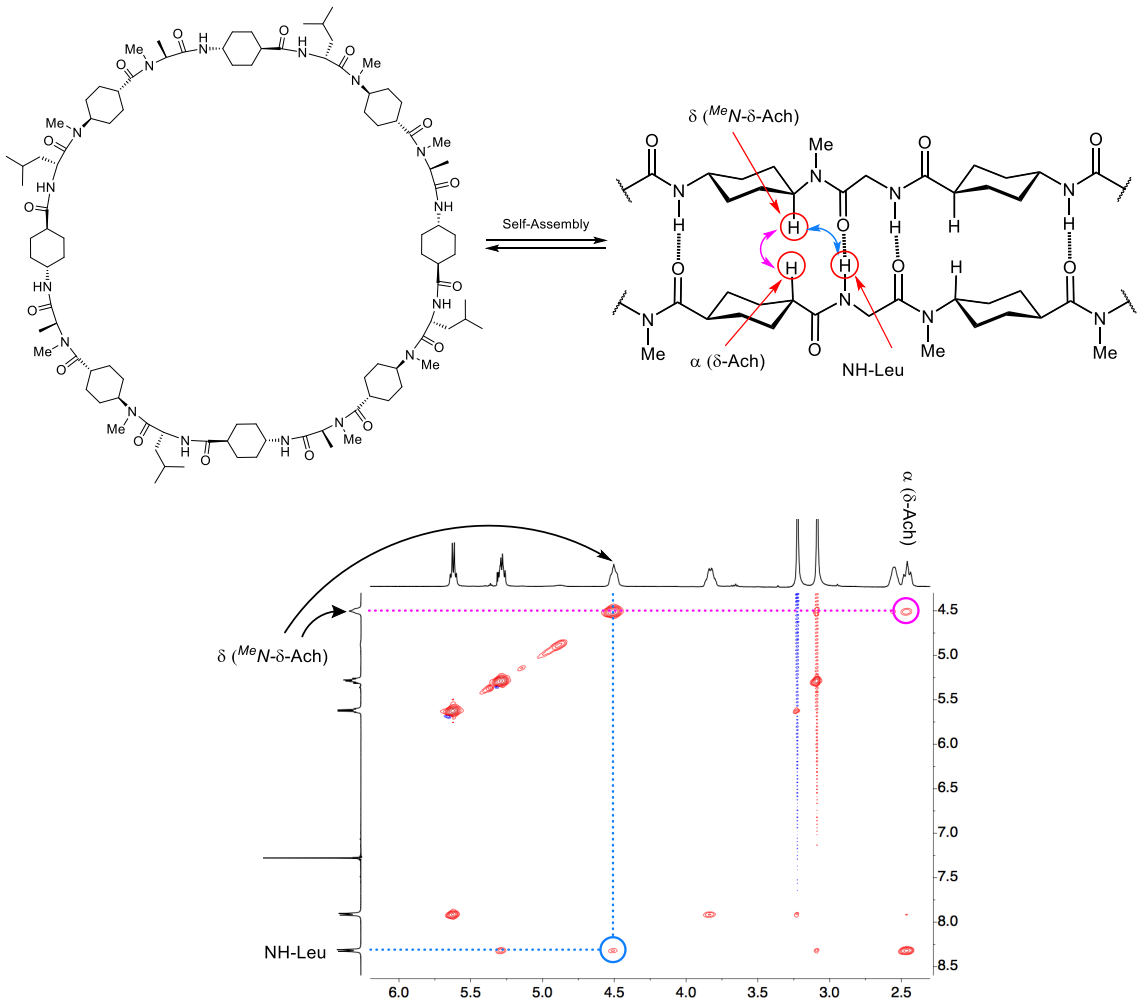
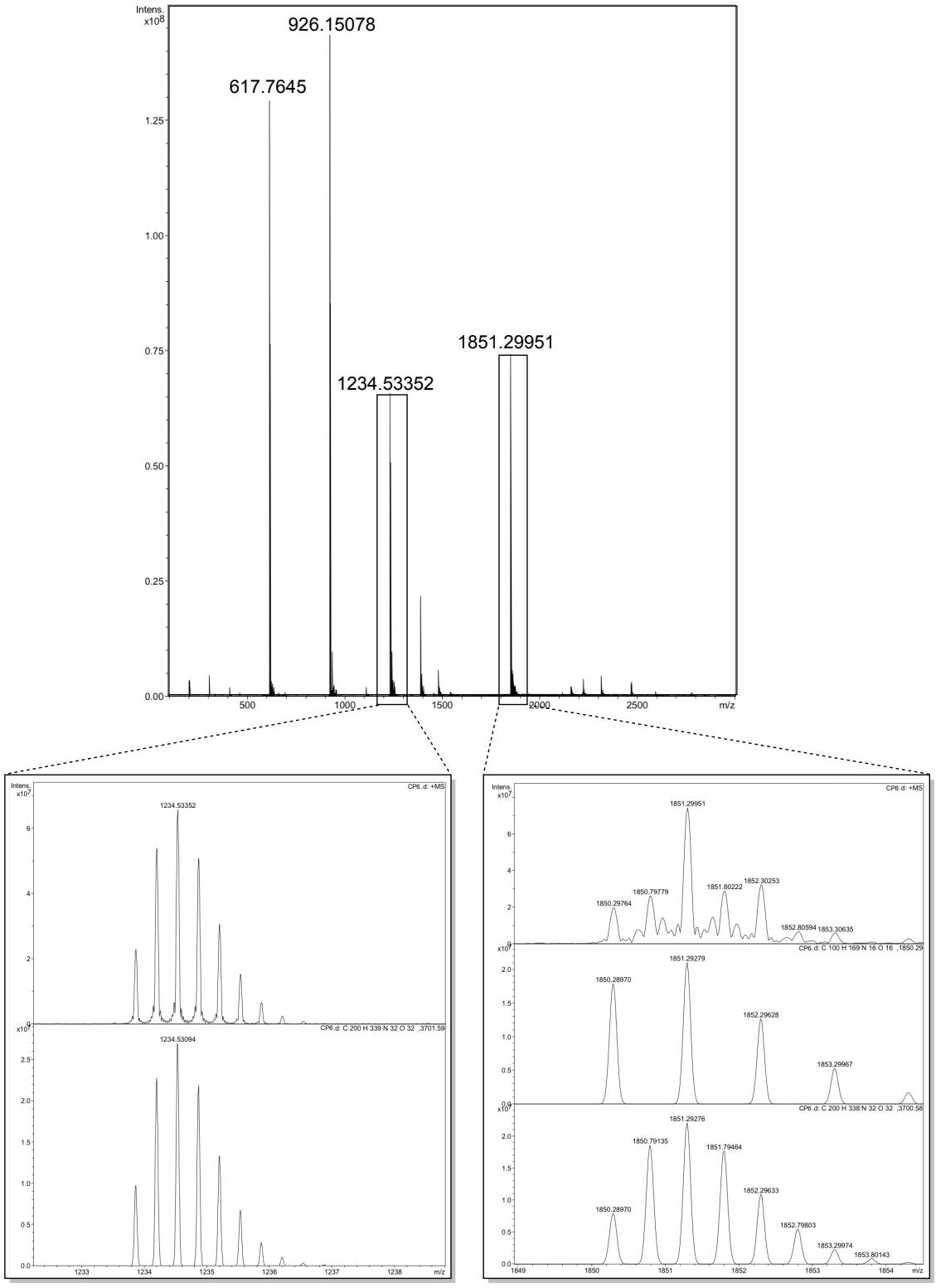


Figure 4S (ESI-TOF mass spectra of a) **CP6** and b) **CP5**): ESI-TOF mass spectra with the corresponding estimated and found isotopic distribution of the dimers with two ($[M_2H_2]^{2+}$) and three chargers ($[M_2H_3]^{3+}$) for cyclic peptides

a) **CP6**



b) **CP5**

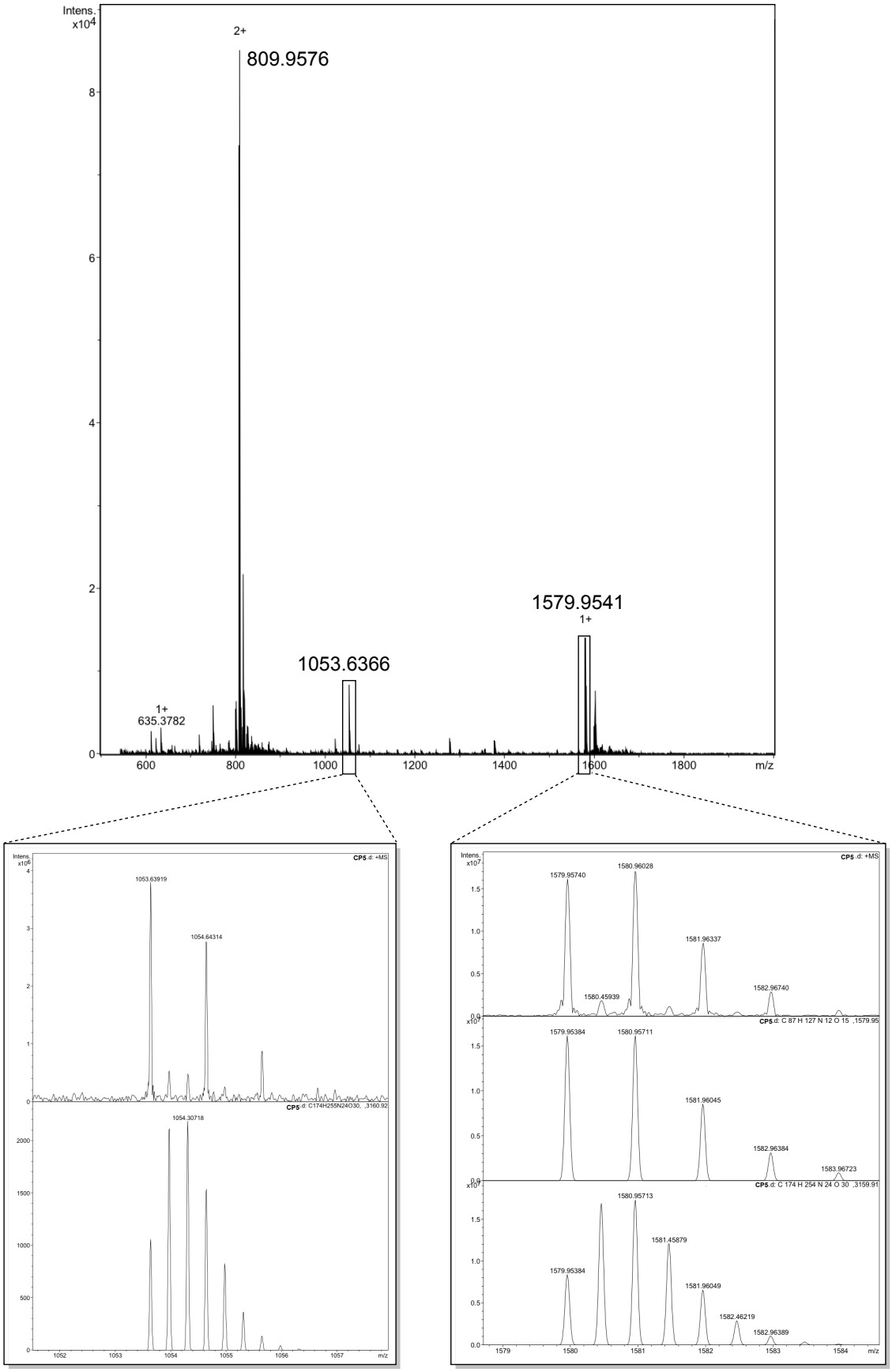
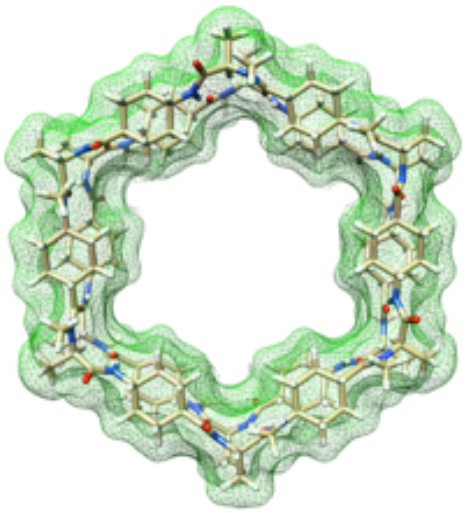

Intens.
x10^4
2+
809.9576
1579.9541
1+
1053.6366
1+
635.3782
m/z
1053.63919
1054.64314
1054.30718
1579.95740
1580.96028
1581.96337
1580.45939
1582.96740
1579.95384
1580.95711
1581.96045
1582.96384
1583.96723
1580.95713
1581.45879
1579.95384
1581.96049
1582.46219
1582.96389

Figure 5S (Computer-generated model of **CP5**): a) Top and b) side view of a computer-generated model of a cyclic dodecapeptide **CP5** in which all the side chains were substituted by methyl groups

a)



b)

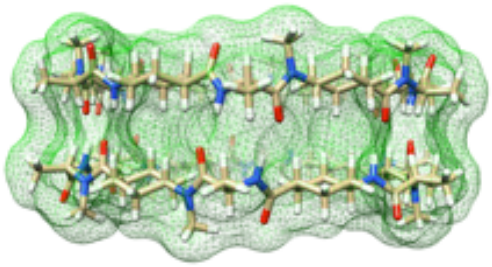
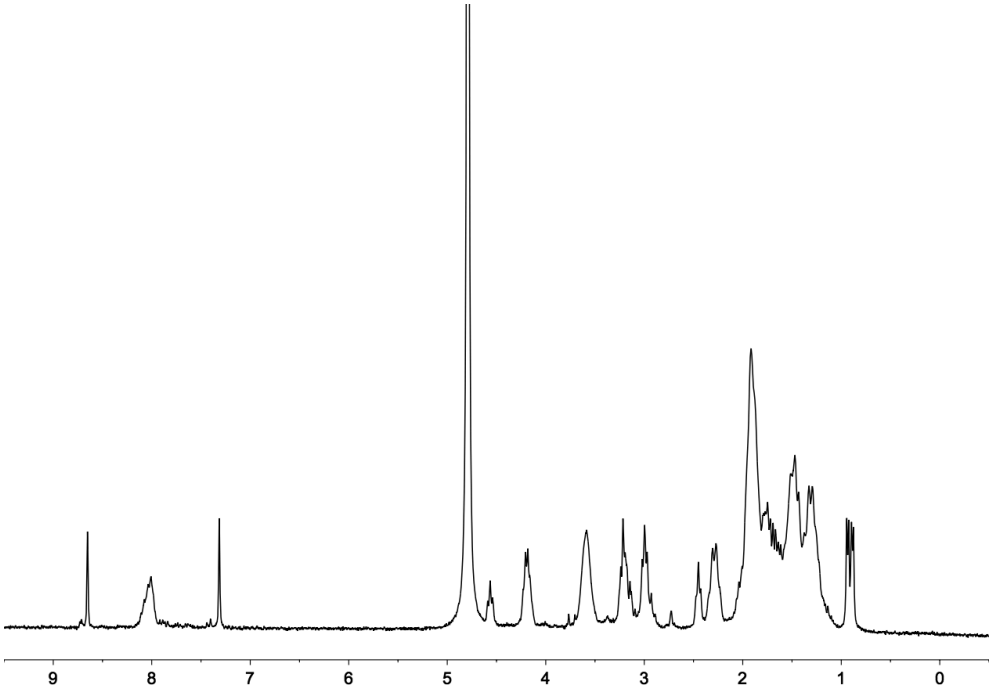


Figure 6S (**CP7** a) ^{1}H NMR and b) Mass spectrum):

a) **CP7** (D_2O, 300 MHz, 298 K)



b) **CP7** Exact mass spectrum

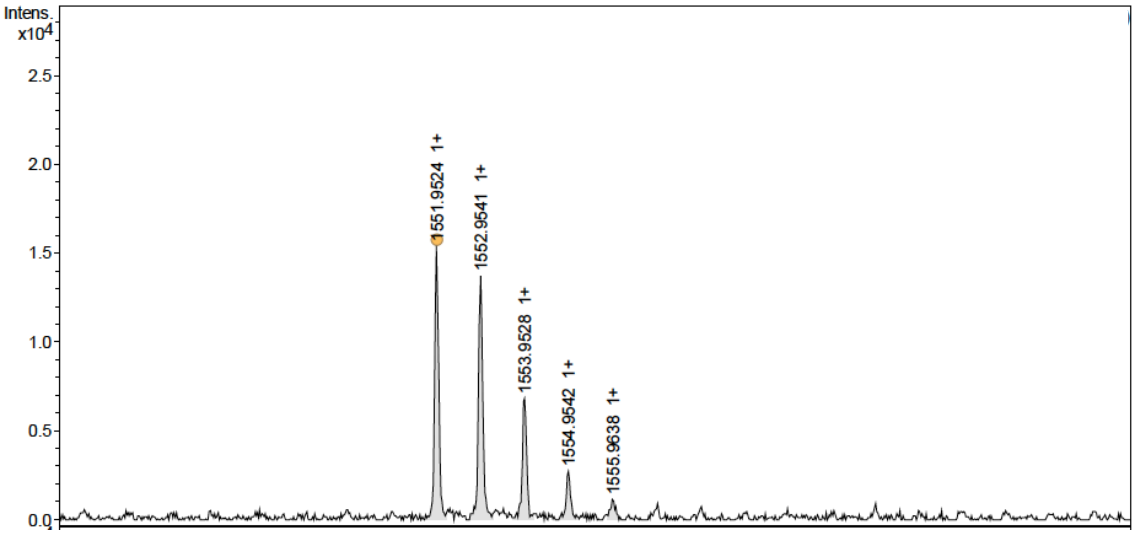


Figure 7S (FT-IR spectrum of **CP7**):

CP7 (H_2O, 298 K)

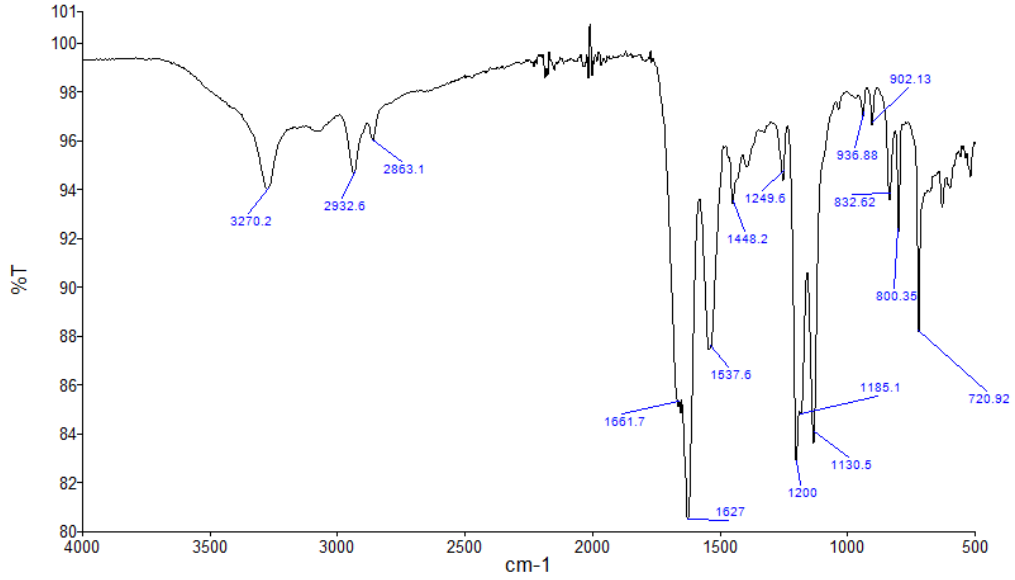


Figure 8S (Thioflavin T assays of **CP7**): Fluorescence emission spectrum of **CP7** at different concetrations in the presence of Thioflavin T (20 µM), at (a) pH 3.3 (Mili-Q water) and (b) pH 8.1 (TRIS buffer) containing 20 µM of Thioflavin T. Excitation wavelength at 420 nm and 5 mm slit.

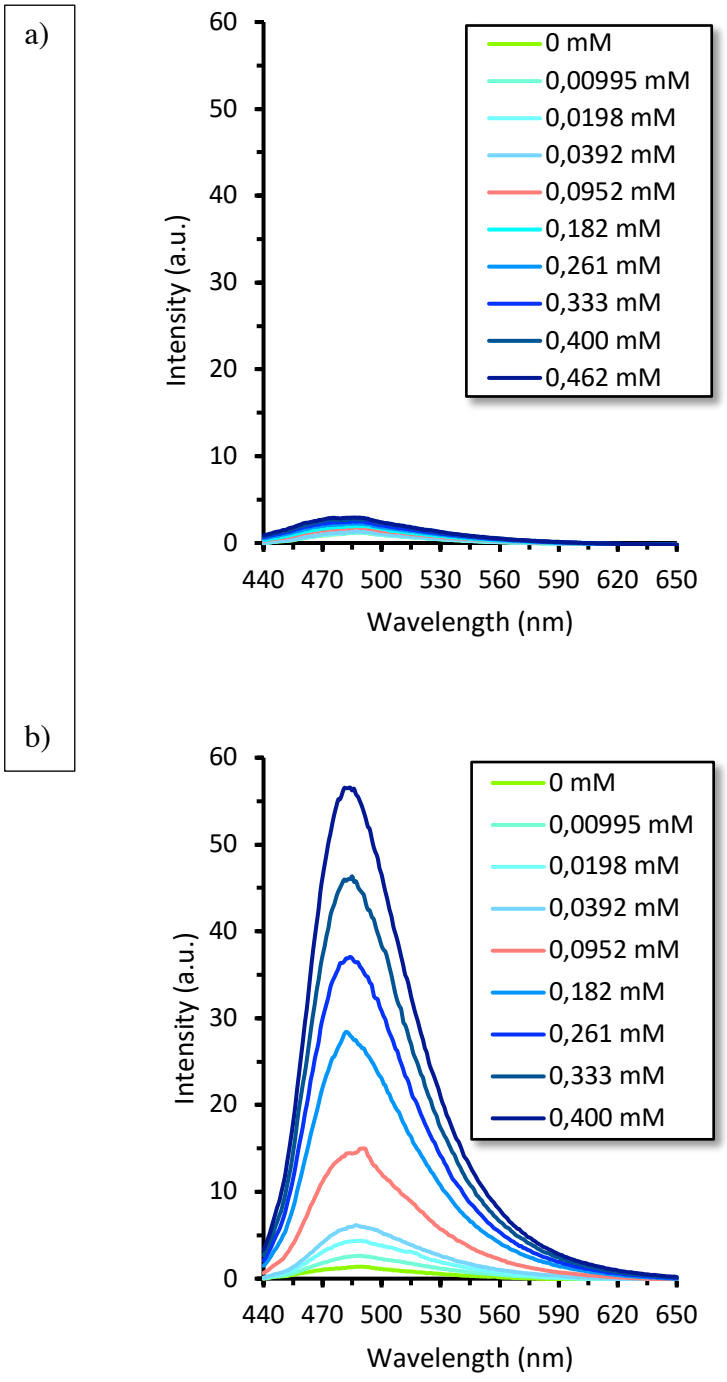


Figure 9S (Atomic Force Microscopy images of **CP7**): AFM Topography images of **CP7** from a 2 mM solution in TRIS buffer at pH 8.1 on anionic mica surface.

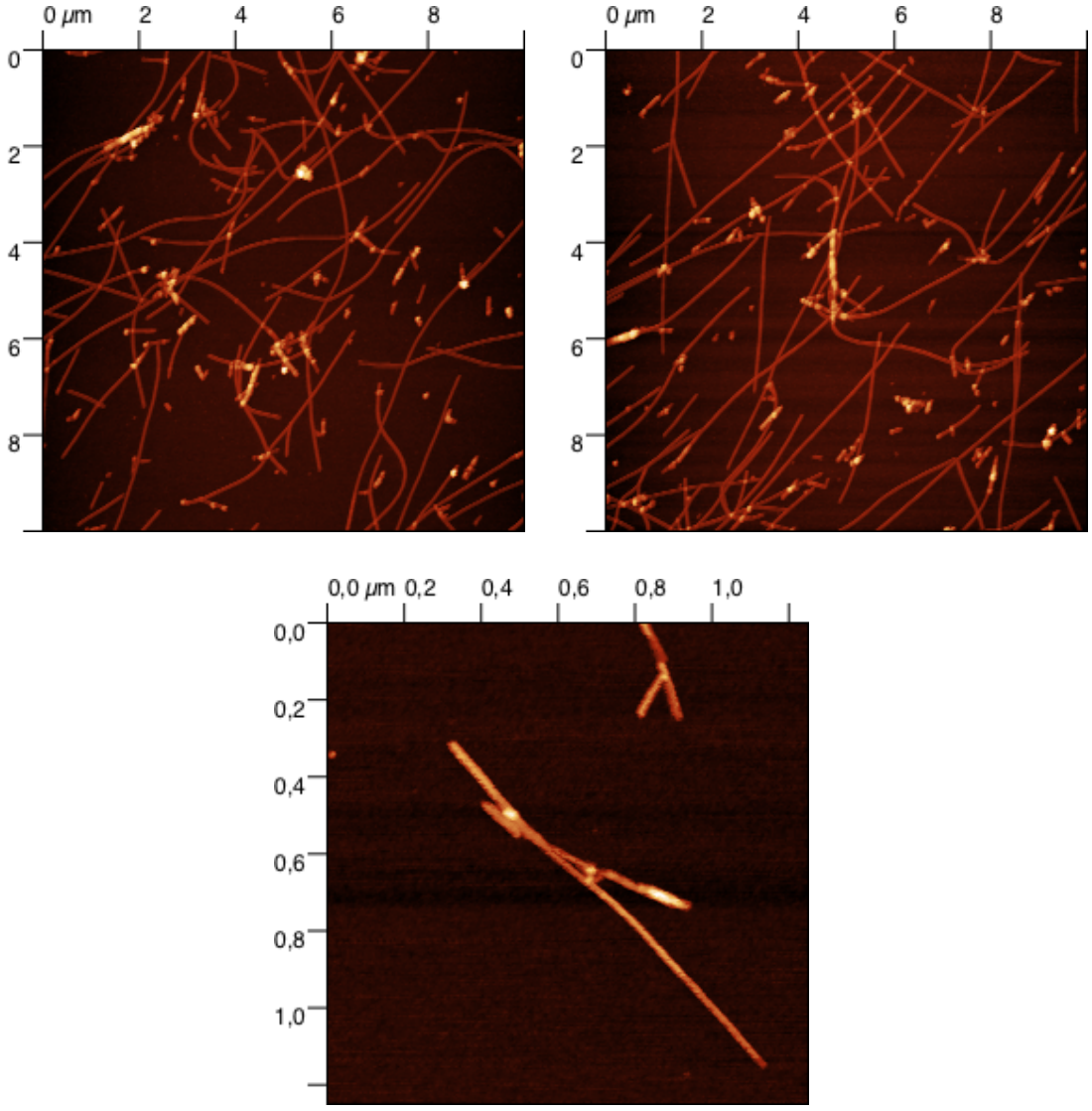


Figure 10S (UV/VIS experiments of **CP7+C_{60}**): UV/VIS spectrum of C_{60} fullerene dispersion in basic Mili-Q water at pH 8.1 (blue line), 1 mM solution of **CP7** in Mili-Q water at basic pH 8.1 (green line), C_{60} fullerene solution in 1 mM solution of **CP7** in Mili-Q water at basic pH 8.1 (brown line) and ten-fold diluted sample of **CP7+C_{60}** (orange line).

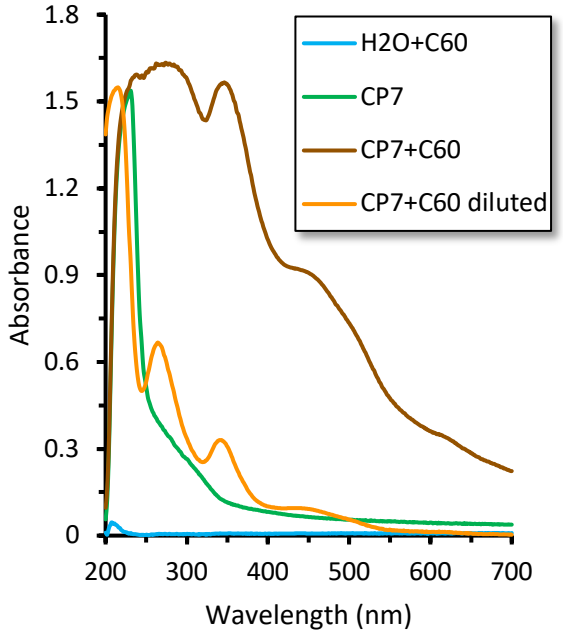


Figure 11S (Thioflavin T assays of **CP7+C_{60}**): Fluorescence emission spectrum of **CP7 + C_{60}** solution (in a ratio of 100 :7 for CP / C_{60}) at different final concetrations (pH 8.1 in Milli-Q water) added over a Thioflavin T solution (20 µM). Excitation wavelength at 420 nm and 5 mm slit.

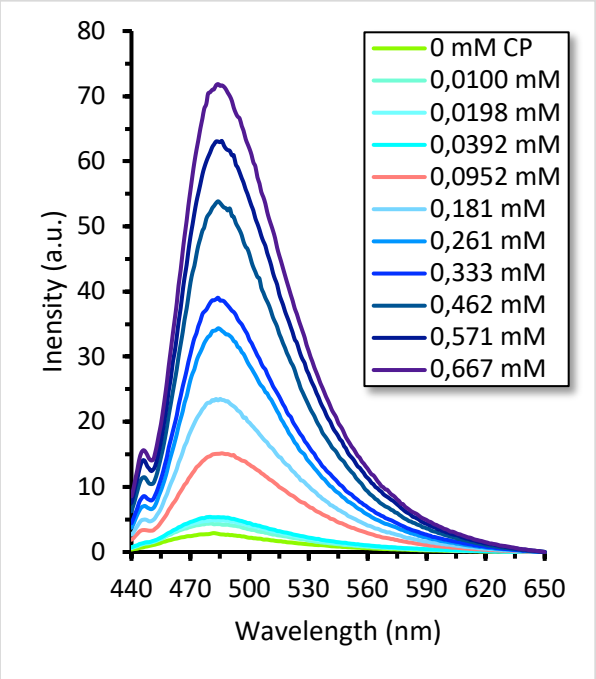


Figure 12S (FT-IR spectra of **CP7+C_{60}**): **CP7+C_{60}** (H_2O, 298 K)

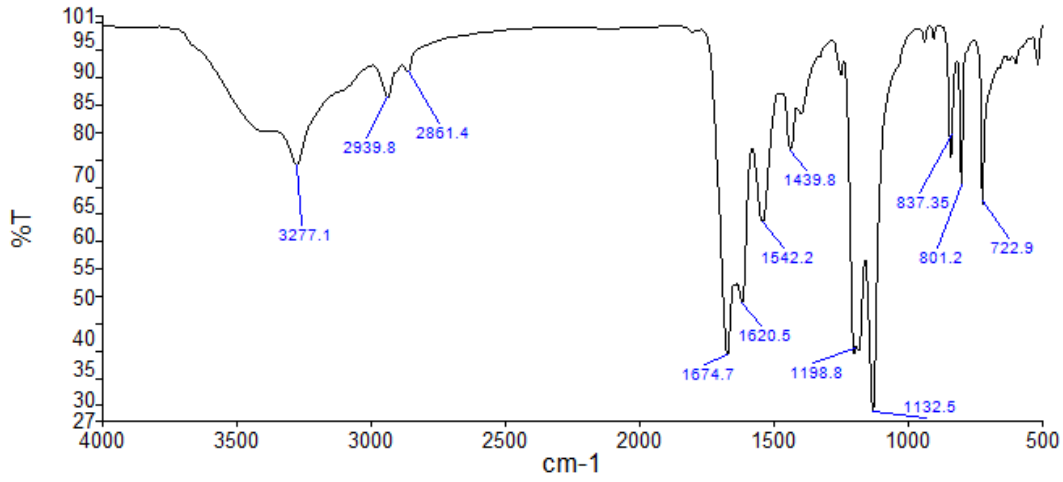
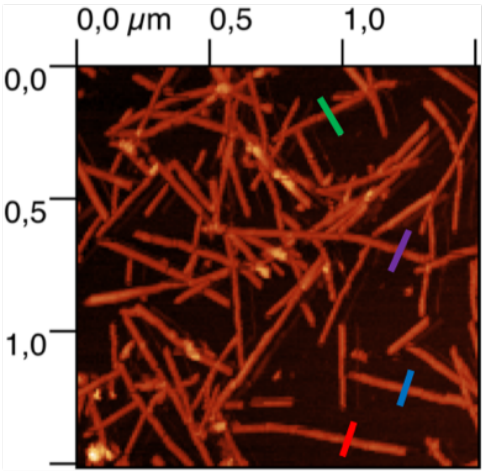


Figure 13S (Atomic Force Microscopy images and heights of **CP7+C_{60}**): AFM Topography images of **CP7** from a 1 mM peptide that contain 70 μM of C_{60} fullerene (Mili-Q water at pH 8.1) on anionic mica surface.



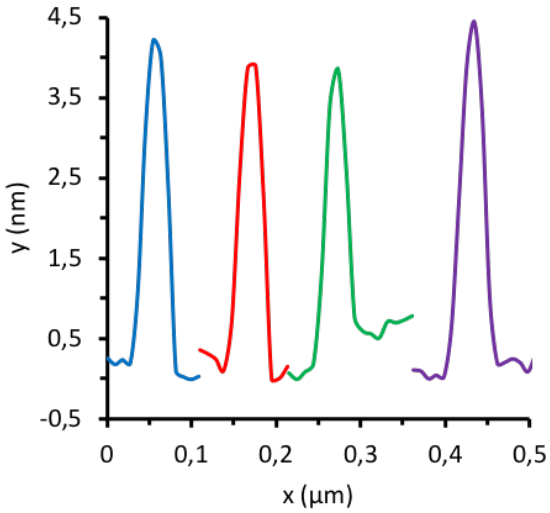


Figure 14S (Scanning Transmission Electron Microscopy images of **CP7+C_{60}**): STEM images of **CP7** from a 1 mM peptide that contain 70 μM of C_{60} fullerene (Mili-Q water at pH 8.1) drop casted onto a carbon type B film on mesh cooper grid. The sample was stained with a 2% uranyl acetate solution. (Bar scale: 100 nm).

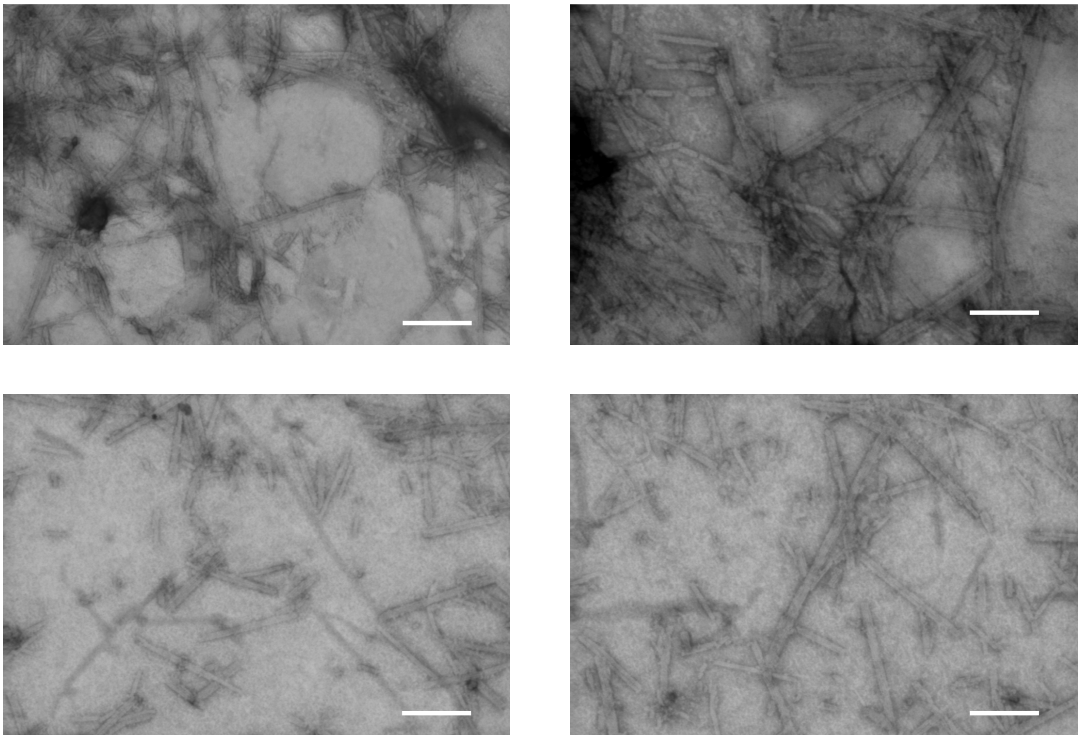


Figure 15S (Atomic Force Microscopy images and heights of sonicated **CP7**): a) AFM topography micrographs of **SCPN7** samples after sonication for 40 min and then deposited over mica (Grade V-I muscovite) from aqueous solutions (1 mM, pH ~ 8.1). b) AFM height profiles along the transects in different colors shown in a).

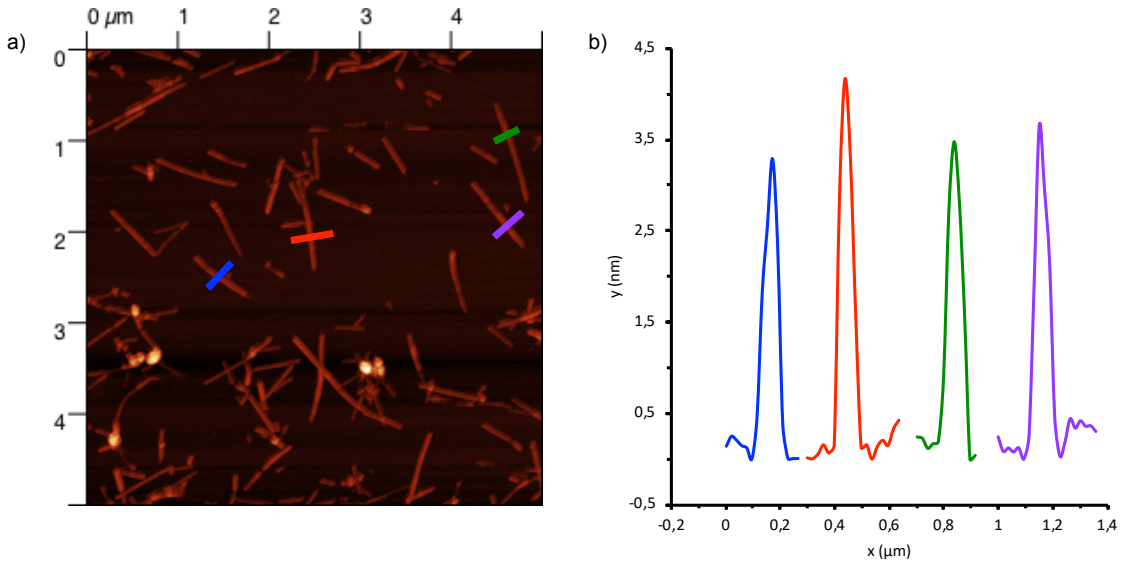
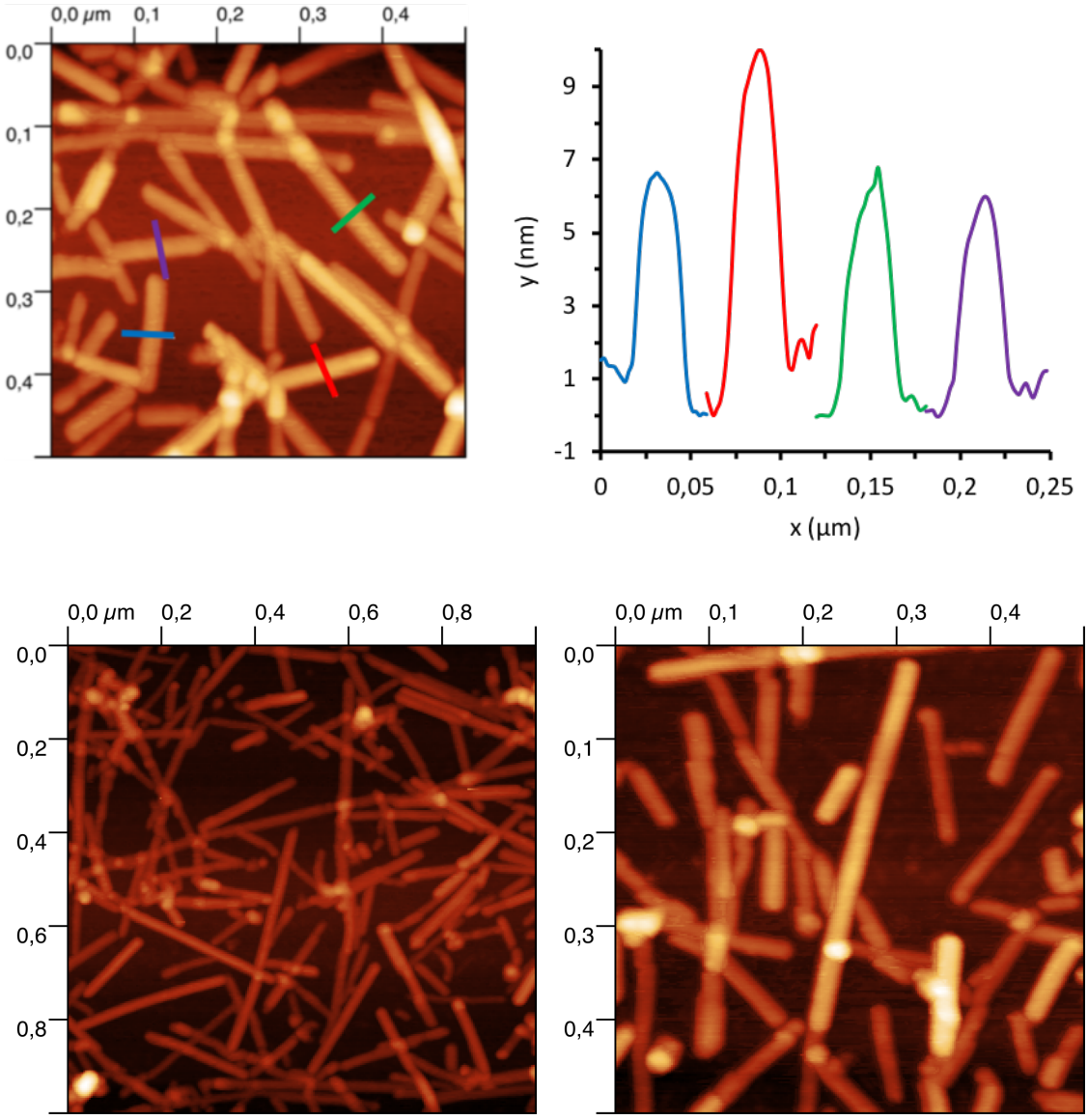


Figure 16S (Atomic Force Microscopy images of aggregated **CP7+C_{60}**): AFM Topography images of **CP7** from a 1 mM peptide that contain 70 μM of C_{60} fullerene (Mili-Q water at pH 8.1 with 1 week old) on anionic mica surface.



Materials and Methods

General. Commercially available *N*-Boc-α-amino acids, *N*-HATU, *N*-HBTU, *N*-TBTU, DIC, DMAP, EDC·HCl and 2-CTC resin (2-chlorotritryl chloride resin) were all used as obtained from Sigma-Aldrich, Novabiochem, Applied Biosystems, Bachem or Iris Biotech. Deuterated solvents ($CDCl_3$, CD_2Cl_2, DMSO-*d6*, MeOH-*d3* and MeOH-*d4*) were obtained from Aldrich. All other reagents obtained from commercial suppliers were used without further purification unless otherwise noted. Dichloromethane and piperidine were dried and distilled over calcium hydride.[1,2] *N*,*N*-Diisopropylethylamine (DIEA) was dried and distilled over calcium hydride, and then redistilled over ninhydrin[1,2].

Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates. Compounds which were not UV active were visualized by dipping the plates in a ninhydrin solution and heating. Silica gel flash chromatography was performed using E. Merck silica gel (type 60SDS, 230-400 mesh). Solvent mixtures for chromatography are reported as v/v ratios. HPLC purification was carried out on Phenomenex Luna 5 µm silica column with CH_2Cl_2/MeOH gradients between 100 and 85:15 and Luna 5 µm C-18 column with H_2O/MeCN (0.1% TFA) gradients between 0 and 95% of MeCN. Purifications using HPLC systems were carried out using Hitachi D-7000, Agilent 1100 and Jasco 4000 instruments.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian-Inova 500 Mhz, Varian-Mercury 300 MHz or Varian-VNMRS 300 MHz spectrometers. Chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethylsilane, and the coupling constants (*J*) were reported in Hz. ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q) or pentuplet (p). All first-order splitting patterns were assigned based on the appearance of the multiplet. Splitting patterns that could not be easily interpreted are designated as multiplet (m) or broad (br). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Varian-Inova 500 MHz, Varian-Mercury 300 MHz or Varian-VNMRS 300 MHz spectrometers. Carbon resonances were assigned using distortionless enhancement by polarization transfer (DEPT) spectra obtained with phase angles of 135°.

Electrospray (ESI) mass spectra were recorded on a Bruker BIOTOF II mass spectrometer. FT-IR measurements were made on a JASCO FT/IR-400 and PerkinElmer FT-IR Spectrum Two spectrophotometers using 5-10 mM in $CHCl_3$ and placed in a CaF_2 solution IR cell.

► **^{1}H NMR Assignments of Cyclic Peptides (CPs)**. The signals of the ^{1}H NMR spectra of the peptides in $CDCl_3$ were identified from the corresponding double-quantum-filled 2D COSY (2QF-COSY), TOCSY and/or NOESY and ROESY spectra acquired at concentration and temperature indicated. Mixing times (~250 ms or 400 ms) were not optimized. Spectra were typically acquired using Bruker standard pulse sequences on 300 and 500 MHz apparatuses, and were referenced relative to residual proton resonances in $CDCl_3$ (at 7.26 ppm).

[1] Brown, H. C. "*Organic Synthesis via Boranes"*, Ed. John Wiley & Sons, **1975**.
[2] Perrin, D. D.; Armarego, W. I. F. *"Purification of Laboratory Chemicals"*, Ed. Pergamon Press, **1988**.

Amino acid and peptide synthesis:

Boc-^{Me}*N*-δ-Ach-OMe: A solution of **Boc-δ-Ach-OH** (1.0 g, 4.11 mmol) in dry DMF (30 mL) was treated with NaH (490 mg, 60% in mineral oil, 12.3 mmol) and stirred at 0 °C for 30 min. Then, iodomethane (780 µL, 12.6 mmol) was added and the resulting mixture was stirred at *rt* for 24 h. After quenching with water, the mixture was washed with diethyl ether, and the organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated under reduced pressure providing a yellow oil that was purified by flash chromatography (20% EtOAc in hexanes) to obtaining 1.07 g of **Boc-^{Me}*N*-δ-Ach-OMe** as a colourless oil. [96%, R_f = 0.55 (25% EtOAc in hexanes)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 3.84 (m, 1H, $H\delta_{\delta\text{-Ach}}$), 3.58 (s, 3H, OMe), 2.64 (s, 3H, NMe), 2.12 (m, 1H, $H\alpha_{\delta\text{-Ach}}$), 2.03–1.3 (m, 8H), 1.37 (s, 9H, Boc). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): 175.6 (C), 155.4 (C), 79.1 (CH_3), 53.1 (CH), 51.4 (CH_3), 42.3 (C), 28.9 (CH_3), 28.3 (CH_3 and CH_2), 28.2 (CH_2). **MS (ESI)** [m/z, (%)]: 294.2 ([MNa]$^+$, 100), 238.1 ([MH-OMe]$^+$, 60). **HRMS (ESI) calculated** for $C_{14}H_{25}NNaO_4$ ([MNa]$^+$): 294.1676, **found**: 294.1676.

Boc-^{Me}*N*-δ-Ach-OH: A solution of **Boc-^{Me}*N*-δ-Ach-OMe** (600 mg, 2.21 mmol) in MeOH/H_2O (3:1, 30 mL) was treated with LiOH (258 mg, 11.1 mmol) and stirred at *rt* for 2 h. Then, the MeOH was concentrated under reduced pressure and the aqueous solution was acidified to pH 3 with an aqueous solution of HCl (10%). The resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic layers were dried over Na_2SO_4 anhydrous, filtered and concentrated to afford the wished product as white solid. [98%, R_f = 0.4 (5% MeOH in CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 3.93 (m, 1H, $H\delta_{\delta\text{-Ach}}$), 2.72 (s, 3H, NMe), 2.22 (m, 1H, $H\alpha_{\delta\text{-Ach}}$), 2.14–1.4 (m, 8H), 1.45 (s, 9H, Boc). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): 181.2 (C), 155.6 (C), 79.5 (C), 53.0 (CH), 42.2 (CH), 28.9 (CH_2), 28.2 (CH_3), 28.1 (CH_2). **MS (ESI)** [m/z, (%)]: 280.2 ([MNa]$^+$, 100), 156.1 ([MH-Boc]$^+$, 54). **HRMS (ESI) calculated** for $C_{13}H_{23}NNaO_4$ ([MNa]$^+$): 280.1519, **found**: 280.1522.

Boc-δ-Ach-OFm: A solution of **Boc-δ-Ach-OH** (1.0 g, 4.11 mmol) in dry CH_2Cl_2 (40 mL) was successively treated with EDC·HCl (1.18 g, 6.17 mmol), HOBt (835 mg, 6.17 mmol), 9-fluorenylmethanol (966 mg, 4.9 mmol) and DMAP (755 mg, 6.17 mmol). The resulting mixture was stirred at *rt* for 3 h, then poured into a separatory funnel and the organic layer was washed with aqueous solutions of HCl (5%, 3 x 20 mL) and $NaHCO_3$ (dil. sat., 3 x 20 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated under reduced pressure, providing a yellow residue that was purified by flash chromatography (25% EtOAc in hexanes) to give 450 mg of the **Boc**-δ-**Ach-OFm** as white solid [82%, R_f = 0.7 (50% AcOEt in hexanes)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 7.75 (d, *J* = 7.5 Hz, 2H, Ar), 7.57 (d, *J* = 7.4 Hz, 2H, Ar), 7.43 (t, *J* = 7.5 Hz, 2H, Ar), 7.34 (t, *J* = 7.4 Hz, 2H, Ar), 4.42 (m, 3H, CH_{2Fm} and $H\delta_{\delta Ach}$), 4.18 (t, *J* = 6.7 Hz, 1H, CH_{Fm}), 3.40 (m, 1H, $H\delta_{\delta Ach}$), 2.24 (tt, *J* = 12.0 and 3.5 Hz, 1H, $H\alpha_{\delta Ach}$), 1.45 (s, 9H, Boc), 2.11–1.89 (m, 4H), 1.57–1.1 (m, 4H). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): 175.0 (C), 155.6 (C), 143.8 (C), 141.3 (C), 127.8 (CH), 127.1 (CH), 124.9 (CH), 120.0 (CH), 79.2 (C), 66.1 (CH_2), 48.9 (CH), 47.0 (CH), 42.4 (CH), 32.4 (CH_2), 28.4 (CH_3), 27.7 (CH_2). **MS (ESI)** [m/z, (%)]: 444.2 ([MH]$^+$, 100), 388.1 ([MH-^{t}Bu]$^+$, 10). **HRMS (ESI) calculated** for $C_{26}H_{31}NNaO_4$ ([MNa]$^+$): 444.2145, **found**: 444.2147.

Boc-δ-Ach-OAll: A solution of **Boc-δ-Ach-OH** (1.0 g, 4.11 mmol) in dry CH_2Cl_2 (40 mL) was treated with EDC·HCl (1.58 g, 8.20 mmol), AllOH (800 µL, 12.3 mmol) and DMAP (100 mg, 0.820 mmol). The resulting mixture was stirred at *rt* for 2 h, then poured into a separatory funnel and the organic layer was washed with aqueous solutions of HCl (5%, 3 x 20 mL) and $NaHCO_3$ (dil. sat., 3 x 20 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated under reduced pressure, providing a yellow residue that was purified by flash chromatography (0.5% MeOH in CH_2Cl_2) to obtain 1.04 g of the **Boc-δ-Ach-OAll** as white solid [87%, R_f = 0.8 (5% MeOH in CH_2Cl_2)]. **^{1}H NMR** (300 MHz, $CDCl_3$, δ): 5.90 (ddt, *J* = 17.2, 10.4, 5.7 Hz, 1H, Allyl), 5.30 (dq, *J* = 17.2 and 1.4 Hz, 1H, Allyl), 5.22 (dq, *J* = 10.4 and 1.1 Hz, 1H, Allyl), 4.56 (dt, *J* = 5.7 Hz and 1.4 Hz, 2H, Allyl), 4.37 (br, 1H, $NH_{δ\text{-}Ach}$), 3.41 (br, 1H, $Hδ_{δ\text{-}Ach}$), 2.25 (tt, *J* = 12.1 and 3.4 Hz, 1H, $Hα_{δ\text{-}Ach}$), 2.15–1.95 (m, 4H), 1.58–1.49 (m, 2H), 1.43 (s, 9H, Boc), 1.19–1.02 (m, 2H). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): δ 174.7 (C), 155.0 (C), 132.0 (CH), 117.7 (CH_2), 78.6 (C), 64.6 (CH_2), 48.7 (CH), 42.1 (CH), 32.1 (CH_2), 28.2 (CH_3), 27.6 (CH_2). **MS (ESI)** [m/z, (%)]: 306.2 ([MNa]$^+$, 100), 250.1 ([MNa-^{t}Bu]$^+$, 52). **HRMS (ESI) calculated** for $C_{26}H_{31}NNaO_4$ ([MNa]$^+$) 306.1678, **found**: 306.1677.

Boc-^{Me}*N*-*L*-Ala-δ-Ach-OFm (Dp1): A solution of **Boc-δ-Ach-OFm** (400 mg, 0.95 mmol) in a mixture of TFA/CH_2Cl_2 (1:1, 10 mL) was stirred at *rt* for 15 min and then the solvent was removed under reduced pressure. The residue was dried under high vacuum for 3h. The resulting TFA salt was dissolved in dry CH_2Cl_2 (10mL) and then DIEA (980 µL, 5.7 mmol), Boc-^{Me}*N*-*L*-Ala-OH (190 mg, 0.95 mmol) and *N*-HATU (400 mg, 1.05 mmol) were successively added. The resulting solution was stirred at *rt* for 40 min and then poured into a separatory funnel. The solution was washed with aqueous solutions of HCl (5%, 3 x 10 mL) and $NaHCO_3$ (dil. sat., 3 x 10 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated under reduced pressure. The resulting yellow oil was purified by flash chromatography (25%-40% EtOAc in hexanes) to give 386 mg of **Dp1** as white foam. [80%, R_f = 0.28 (40% EtOAc in hexanes)]. **^{1}H NMR** ($CDCl_3$, 500 MHz, δ): 7.78 (d, *J* = 7.6 Hz, 2H, Ar), 7.60 (d, *J* = 7.3 Hz, 2H, Ar), 7.43 (t, *J* = 7.5 Hz, 2H, Ar), 7.34 (td, *J* = 7.5 and 1.1 Hz, 2H, Ar), 4.65 (m, 1H, $Hα_{Ala}$), 4.44 (d, *J* = 6.8 Hz, 2H, CH_{2OFm}), 4.21 (t, *J* = 6.7 Hz, 1H, CH_{OFm}), 3.73 (m, 1H, $Hδ_{δ\text{-}Ach}$), 2.78 (s, 3H, MeN_{Ala}), 2.28 (tt, *J* = 12.0 and 3.5 Hz, 1H, $Hα_{δ\text{-}Ach}$), 2.06–1.90 (m, 4H), 1.59–1.49 (m, 2H), 1.48 (s, 9H, Boc), 1.34 (d, *J* = 7.1 Hz, 3H, Me Ala), 1.22–1.06 (m, 2H). **^{13}C NMR** ($CDCl_3$, 126 MHz, δ): 175.1 (C), 170.7 (C), 143.8 (C) 141.3 (C), 127.8 (CH), 127.1 (CH), 124.9 (CH), 120.0 (CH), 80.6 (C), 66.1 (CH_2), 53.4 (CH), 47.5 (CH), 46.7 (CH), 42.4 (CH), 32.1 (CH_2), 32.0 (CH_2), 28.4 (CH_3), 27.6 (CH_2). **MS (ESI)** [m/z, (%)]: 529.3 ([MNa]$^+$, 79), 507.3 ([MH]$^+$, 33), 407.3 ([MH-Boc]$^+$, 100). **HRMS (ESI) calculated** for $C_{30}H_{38}N_2O_5$ ([MNa]$^+$): 507.2853, **found**: 507.2875.

Fmoc-*L*-Lys(Boc)-δ-Ach-OAll (Dp2): A solution of **Boc-δ-Ach-OAll** (450 mg, 1.58 mmol) in a mixture of TFA/CH_2Cl_2 (1:1, 10 mL) was stirred at *rt* for 15 min and then the solvent was removed under reduced pressure. The residue was dried under high vacuum for 3 h. The resulting TFA salt was dissolved in dry CH_2Cl_2 (9 mL) and then DIEA (1.8 mL, 10.6 mmol), Fmoc-*L*-Lys(Boc)-OH (827 mg, 1.80 mmol) and *N*-HATU (839 mg, 2.20 mmol) were successively added. The resulting solution was stirred at *rt* for 40 min and then poured into a separatory funnel. The solution was washed with aqueous solutions of HCl

(5%, 3 x 10 mL) and $NaHCO_3$ (dil. sat., 3 x 10 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated under reduced pressure. The resulting yellow oil was purified by flash chromatography (1% MeOH in CH_2Cl_2) to give 939 mg of **Dp2** as white solid. [93.5%, R_f = 0.40 (5% MeOH in CH_2Cl_2)]. **^{1}H NMR** (300 MHz, 2% MeOD-d_4/CD_2Cl_2, δ): 7.78 (d, *J* = 7.5, 2H, Ar), 7.61 (d, *J* = 7.4 Hz, 2H, Ar), 7.41 (t, *J* = 7.3 Hz, 2H, Ar), 7.32 (t, *J* = 7.5 Hz, 2H, Ar), 5.90 (ddt, *J* = 18.0, 10.4 and 5.5 Hz,1H, Allyl), 5.29 (dq, *J* = 18.0 and 1.3 Hz, 1H, Allyl), 5.21 (dq, *J* = 10.4 and 1.3 Hz, 1H, Allyl), 4.55 (dt, *J* = 5.5 and 1.3 Hz, 2H, Allyl), 4.38 (d, *J* = 6.9 Hz, 2H, CH_{2OFm}), 4.22 (t, *J* = 6.8 Hz, 1H, CH_{Fm}), 3.99 (s, 1H, $H\alpha_{\alpha\text{-Lys}}$), 3.66 (m, 1H, $H\delta_{\delta\text{-Ach}}$), 3.05 (m, 2H), 2.25 (tt, *J* = 12.2, 3.4 Hz, 1H, $H\alpha_{\delta\text{-Ach}}$), 2.06–1.81 (m, 6H), 1.65–1.43 (m, 6H), 1.41 (s, 9H, Boc), 1.36–1.07 (m, 2H). **^{13}C NMR** (75 MHz, 2% MeOH-d_4/CD_2Cl_2, δ): 175.6 (C), 171.9 (C), 157.0 (C), 144.5 (C), 141.8 (C), 133.0 (CH), 128.2 (CH), 127.6 (CH), 125.6 (CH), 120.5 (CH), 118.0 (CH_2), 79.6 (C), 67.3 (CH_2), 65.4 (CH_2), 55.3 (CH), 48.3 (CH), 47.7(CH), 42.9 (CH), 38.9 (CH), 32.8 (CH_2), 32.2 (CH_2), 32.1 (CH_2), 30.0 (CH_2), 28.6 (CH_3), 28.3 (CH_2), 23.0 (CH_2). **MS (ESI)** [m/z, (%)]: 656.3 ($[MNa]^+$, 100), 600.2 ($[MNa\text{-}^t Bu]^+$, 70), 407.3 ($[MNa\text{-Boc}]^+$, 35). **HRMS (ESI) calculated** for $C_{26}H_{31}NNaO_4$ ($[1MNa]^+$): 656.3306, **found**: 656.3306.

Boc-*^{Me}N*-δ-Ach-*^{Me}N-L*-Ala-δ-Ach-OFm (Tr1): A solution of **Boc-*^{Me}N-D*-Ala-δ-Ach-OFm** (**dp1**, 221 mg, 0.43 mmol) in a mixture of TFA/ CH_2Cl_2 (1:1, 5 mL) was stirred at *rt* for 30 min and then the solvent was removed under reduced pressure. The residue was dried under high vacuum for 3 h. The resulting TFA salt was dissolved in dry CH_2Cl_2 (3 mL) and then DIEA (290 µL, 1.72 mmol) was added. After this, a solution of DIEA (150 µL, 0.86 mmol), **Boc-*^{Me}N*-δ-Ach-OH** (122 mg, 0.473 mmol) and *N*-HATU (196 mg, 0.473 mmol) in dry CH_2Cl_2 (3 mL) was added drop wise. The resulting solution was stirred at *rt* for 60 min and then and poured into a separatory funnel. The solution was washed with aqueous solutions of HCl (5%, 3 x 10 mL) and $NaHCO_3$ (dil. sat, 3 x 10 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated under reduced pressure, providing white foam that was purified by flash chromatography (0%-2% MeOH in CH_2Cl_2), obtaining 275 mg of **Tr1** as white foam. [99%, R_f = 0.4 (5% MeOH in CH_2Cl_2)]. **^{1}H NMR:** ($CDCl_3$, 300 MHz, δ): 7.70 (d, *J* = 7.5 Hz, 2H, Ar), 7.52 (d, *J* = 7.8 Hz, 2H, Ar), 7.34 (t, *J* = 7.5 Hz, 2H, Ar), 7.25 (m, 2H, Ar), 6.17 (d, *J* = 8.2 Hz, 1H, NH), 5.06 (q, *J* = 7.1 Hz, 1H, $H\alpha_{Ala}$), 4.35 (d, *J* = 6.7 Hz, 2H, CH_{2OFm}), 4.12 (t, *J* = 6.7 Hz, 1H, CH_{Fm}), 4.10–3.80 (m, 1H, $H\delta_{MeN\text{-}\delta\text{-}Ach}$), 3.65 (m, 1H, $H\delta_{\delta\text{-}Ach}$), 2.93 (s, 3H, $NMe_{MeN\text{-}\delta\text{-}Ach}$), 2.74 (s, 3H, NMe_{Ala}), 2.42 (m, 1H, $H\alpha_{MeN\text{-}\delta\text{-}Ach}$), 2.26 (m, 1H, $H\alpha_{\delta\text{-}Ach}$), 2.06–1.50 (m, 10H), 1.46 (s, 9H, Boc), 1.29 (d, *J* = 7.1 Hz, 3H, Me_{Ala}), 1.20–1.00 (m, 2H). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): 176.3 (C), 175.0 (C), 170.2 (C), 155.5 (C), 143.7 (C), 141.3 (C), 127.7 (CH), 127.0 (CH), 124.9 (CH), 119.9 (CH), 79.3 (C), 66.0 (CH_2), 53.5 (CH), 51.3 (CH), 47.3 (CH), 46.9 (CH), 42.3 (CH), 40.1 (CH), 38.5 (CH_3), 31.9 (CH_2), 31.8 (CH_2), 30.1 (CH_3), 29.0 (CH_2), 28.5 (CH_3), 28.3 (CH_2), 28.2 (CH_2), 27.5 (CH_2), 13.1 (CH_3). **MS (ESI)** [m/z, (%)]: 646.4 ($[MH]^+$, 100). **HRMS (ESI) calculated for** $C_{38}H_{52}N_3O_6$ ($[MH]^+$): 646.3851, **found**: 646.3851.

Boc-*D*-Tyr(Me)-*^{Me}N*-δ-Ach-*^{Me}N-L*-Ala-δ-Ach-OFm (Tp1a): A solution of **Boc-*^{Me}N*-δ-Ach-*^{Me}N-D*-Ala-δ-Ach-OFm** (142 mg, 0.22 mmol) in a mixture of TFA/CH_2Cl_2 (1:1, 4 mL) was stirred at *rt* for 30 min and then the solvent was removed under reduced pressure. The residue was dried under high vacuum for 3 h. The resulting TFA salt was dissolved in dry

CH_2Cl_2 (2 mL) and DIEA (150 µL, 0.86 mmol) was added. After this, a solution of DIEA (750 µL, 0.44 mmol), Boc-*L*-Tyr(Me)-OH (72 mg, 0.242 mmol) and *N*-HATU (100.4 mg, 0.264 mmol) in dry CH_2Cl_2 (2 mL) was added drop wise. The resulting solution was stirred at *rt* for 45 min and then poured into a separatory funnel. The solution was washed with aqueous solutions of HCl (10%, 3 x 10 mL) and $NaHCO_3$ (dil. sat., 3 x 10 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated under reduced pressure. The resulting yellow residue was purified by flash chromatography (1-3% MeOH in CH_2Cl_2) to give 180 mg of **Tp1a** as white foam. [82%, R_f = 0.33 (5% MeOH in CH_2Cl_2)]. **MS (ESI)** [m/z, (%)]: 845.5 ([MNa]$^+$, 100) 823.5 ([MH]$^+$, 88), 723.4 ([MH-Boc]$^+$, 41). **HRMS (ESI) calculated** for $C_{48}H_{63}N_4O_8$ ([MH]$^+$): 823.4640, **found**: 823.4642.

Boc-*D*-Leu-^{Me}*N*-δ-Ach-^{Me}*N*-*L*-Ala-δ-Ach-OFm (Tp1b): A solution of **Tr1** (580 mg, 0.88 mmol) in a mixture of TFA/CH_2Cl_2 (1:1, 8 mL) was stirred at *rt* for 30 min and then the solvent was removed under reduced pressure. The residue was dried under high vacuum for 3 h. The resulting TFA salt was dissolved in dry CH_2Cl_2 (8 mL) and then DIEA (900 µL, 5.28 mmol), Boc-*D*-Leu-OH (220 mg, 0.88 mmol) and *N*-HATU (368 mg, 0.96 mmol) were successively added. The resulting solution was stirred at *rt* for 45 min and then poured into a separatory funnel. The solution was washed with aqueous solutions of HCl (10%, 3 x 10 mL) and $NaHCO_3$ (dil. sat., 3 x 10 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated under reduced pressure. The yellow residue was purified by flash chromatography (1-3% MeOH in CH_2Cl_2) to provide 180 mg of the **Tp1b** as white foam. [67%, R_f = 0.4 (5% MeOH in CH_2Cl_2)]. **MS (ESI)** [m/z, (%)]: 781.4 ([MNa]$^+$, 62), 759.4 ([MH]$^+$, 25), 659.4 ([MH-Boc]$^+$, 100). **HRMS (ESI) calculated** for $C_{44}H_{63}N_4O_7$ ([MH]$^+$): 759.4691, **found**: 759.4708.

Boc-[(*D*-Tyr(Me)-^{Me}*N*-δ-Ach-^{Me}*N*-*L*-Ala-δ-Ach-)$_2$]-OFm (Op1a): A solution of **Tp1a** (60 mg, 0.072 mmol) in a mixture of piperidine/CH_2Cl_2 (1:4, 2 mL) and was stirred at *rt* for 30 min. The resulting mixture was poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 10 mL), dried over Na_2SO_4 anhydrous, filtered and concentrated to give **Tp2a** as white foam, which was used without further purification.

A solution of tetrapeptide **Tp1a** (57 mg, 0.069 mmol) in a mixture of TFA/$PhSiH_3$/CH_2Cl_2 (25:10:65, 2 mL) was stirred at *rt* for 10 min and then the solvent was removed under reduced pressure. The resulting residue was dried under high vacuum for 3 h. This residue was dissolved in dry CH_2Cl_2 (1 mL), treated with DIEA (25 µL, 0.12 mmol) and a solution of previously prepared **Tp2a**, DIEA (40 µL, 0.24 mmol) and *N*-HBTU (28 mg, 0.073 mmol) in dry CH_2Cl_2 (1.5 mL) was added. The resulting reaction mixture was stirred at *rt* for 60 min. Then, the solution was poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 5 mL) and $NaHCO_3$ (dil. sat., 3 x 5 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated. The resulting yellow oil was purified by flash chromatography (2-5% MeOH in CH_2Cl_2) to give 50 mg of **Op1a** as white foam. [61%, R_f = 0.28 (5% MeOH in CH_2Cl_2)]. **MS (ESI)** [m/z, (%)]: 1371.8 ([MNa]$^+$, 60), 1349.8 ([MH]$^+$, 100), 1249.7 ([MH-Boc]$^+$, 51). **HRMS (ESI) calculated** for $C_{77}H_{105}N_8O_{13}$ ([MH]$^+$): 1349.7796, **found**: 1349.7818.

Boc-[(*D*-Leu-^{Me}*N*-δ-Ach-^{Me}*N*-*L*-Ala-δ-Ach-)$_2$]-OFm (Op1b): This peptide was prepared following the same protocol used for the synthesis of **Op1a**, starting from the tetrapeptide **Tp1b** (149 mg, 196 mmol) to afford, after the purification by flash chromatography (1%-7% MeOH in CH_2Cl_2), 120 mg of **Op1b** [49%, R_f = 0.26, 5% MeOH in CH_2Cl_2]. **MS (ESI)** [m/z, (%)]: 1243.8 ([MNa]$^+$, 63), 1221.8 ([MH]$^+$, 100), 1121.7 ([MH-Boc]$^+$, 65). **HRMS (ESI) calculated** for $C_{69}H_{105}N_8O_{12}$ ([MH]$^+$): 1221.7897, **found**: 1221.7838.

***c*-[(*D*-Tyr(Me)-^{Me}*N*-δ-Ach-^{Me}*N*-*L*-Ala-δ-Ach-)$_2$] (CP4)**: The linear octapeptide **Op1a** (175 mg, 0.129 mmol) was dissolved in a mixture piperidine/CH_2Cl_2 (1:4, 4 mL) and the solution was stirred at *rt* for 30 min. The mixture was poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 10 mL), dried over Na_2SO_4 anhydrous, filtered and concentrated giving octapeptide **Op3a**, which was used without further purification.

Op3a was dissolved in mixture of TFA/$PhSiH_3$/CH_2Cl_2 (25:10:65, 3 mL) and stirred at *rt* for 10 min. Then, the solvent was removed under reduced pressure and the resulting solid was dried under high vacuum for 3 h, providing the octapeptide **Op4b**. This compound was dissolved in dry CH_2Cl_2 (130 mL) under Ar and then sequentially treated with DIEA (135 µL, 0.774 mmol) and *N*-TBTU (46 mg, 0.142 mmol). The reaction mixture was stirred at *rt* for 12 h and then the solution was concentrated to dryness. The residue was dissolved in CH_2Cl_2 (10-15 mL) and washed with aqueous solutions of HCl (5%, 3 x 5 mL), dried over Na_2SO_4 anhydrous, filtered and concentrated. The resulting yellow oil was purified by flash chromatography (2-6% MeOH in CH_2Cl_2) to give 57 mg of **CP4** as white solid. [42%, R_f = 0.22 (5% MeOH in CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 500 MHz, δ): 7.09 (d, *J* = 8.4 Hz, 2H, Ar_{Tyr}), 6.79 (d, *J* = 8.2 Hz, 2H, Ar_{Tyr}), 6.47 (d, *J* = 8.0 Hz, 1H, NH_{Tyr}), 6.22 (d, *J* = 9.1 Hz, 1H, $NH_{δ\text{-}Ach}$), 5.12 (q, *J* = 7.0 Hz, 1H, $Hα_{MeN\text{-}Ala}$), 4.97 (td, *J* = 8.4 and 5.3 Hz, 1H, $Hα_{Tyr}$), 4.49 (m, 1H, $Hδ_{MeN\text{-}δ\text{-}Ach}$), 3.77 (s, 3H, $MeN_{MeN\text{-}Ala}$), 3.69 (m, 1H, $Hδ_{δ\text{-}Ach}$), 3.00–2.80 (m, 2H, $Hβ_{Tyr}$), 2.41 (s, 3H, $MeN_{MeN\text{-}δ\text{-}Ach}$), 2.38 (m, 1H, $Hα_{MeN\text{-}δ\text{-}Ach}$), 2.10–1.40 (m, 14H), 1.27 (d, *J* = 7.1 Hz, 3H, Me_{Ala}), 1.13 (m, 1H), 0.81 (m, 1H). **MS (ESI)** [m/z, (%)]: 1072.6 ([MNa]$^+$, 49), 1053.6 ([MH]$^+$, 100), 723.4 ([MH]$^{2+}$, 41). **HRMS (ESI) calculated** for $C_{58}H_{85}N_8O_{10}$ ([MH]$^+$): 1053.6383, **found**: 1053.6398. **FT-IR** (293K, $CHCl_3$): 3307 (amide A), 1670, 1622 (amide I), 1511 cm^{-1} (amide II_{II}).

Boc-[(*D*-Tyr(Me)-^{Me}*N*-δ-Ach-^{Me}*N*-*L*-Ala-δ-Ach-)$_3$]-OFm (Dd1a): The tetrapeptide **Tp1a** (27.1 mg, 0.033 mmol) was dissolved in a mixture of TFA/$PhSiH_3$/CH_2Cl_2 (25:10:65, 2 mL) and stirred at *rt* for 10 min. Then, the solvent was removed under reduced pressure and the resulting solid was dried under high vacuum for 3 h to obtain **Tp3a**, which was used without further purification.

A solution of Op1a (45 mg, 0.035 mmol) in a mixture of piperidine/CH_2Cl_2 (1:4, 2 mL) was stirred at *rt* for 30 min. Then, the reaction mixture was poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 10 mL), dried over Na_2SO_4 anhydrous, filtered and concentrated to obtain octapeptide **Op3a** as white foam, which was used without any further purification.

A solution of tetrapeptide **Tp3a** in dry CH_2Cl_2 (850 µL) was treated with DIEA (12 µL 0.07 mmol) and then a solution of previously prepared **Op3a**, DIEA (24 µL, 0.14 mmol) and *N*-HATU (15 mg, 0.039 mmol) in dry CH_2Cl_2 (1 mL) was added. The resulting reaction mixture was stirred at *rt* for 2 h, and then poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 3 mL) and $NaHCO_3$ (dil. sat., 3 x 3 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated. The yellow oil was purified by flash chromatography (2-7% MeOH in CH_2Cl_2) to give 32 mg of **Dd1a** as white solid [48%, R_f = 0.25 (5% MeOH in CH_2Cl_2)]. **MS (ESI)** [m/z, (%)]: 1877.1 ($[MH]^+$, 64), 1777.0 ($[MH\text{-}Boc]^+$, 100). **HRMS (ESI) calculated** for $C_{106}H_{146}N_{12}O_{18}$ ($[MH]^{2+}$): 1876.0912, **found**: 1876.0898.

***c*-[(*D*-Tyr(Me)-^{Me}*N*-δ-Ach-^{Me}*N*-*L*-Ala-δ-Ach-)$_3$] (CP5)**: A solution of linear dodecapeptide **Dd1a** (30 mg, 0.016 mmol) in a mixture of piperidine/CH_2Cl_2 (1:4, 2 mL) was stirred at *rt* for 30 min and then poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 5 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated to obtain the *C*-terminus unprotected dodecapeptide **Dd2**. Then, this peptide **Dd2** was dissolved in a mixture of TFA/$PhSiH_3$/CH_2Cl_2 (25:10:65, 2 mL) and stirred at *rt* for 5 min. The solvent was removed under reduced pressure and the resulting residue was dried under high vacuum for 3 h to provide the dodecapeptide **Dd3**, which was used without further purification.

The unprotected dodecapeptide **Dd3** was dissolved in dry CH_2Cl_2 (16 mL) under Ar and treated with DIEA (17 µL, 0.096 mmol) and *N*-TBTU (5.6 mg, 0.018 mmol). The reaction mixture was stirred at *rt* for 12 h and then poured into a separation funnel and washed with aqueous solutions of HCl (5%, 3 x 5 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated. The resulting yellow oil was purified by flash chromatography (2%-10% MeOH in CH_2Cl_2) obtaining 9 mg of **CP5** as white solid. [30%, R_f = 0.45 (10% MeOH in CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 500 MHz, δ): 8.68 (d, *J* = 9.0 Hz, 1H, NH_{Tyr}), 7.78 (d, *J* = 8.7 Hz, 1H, $NH_{δ\text{-}Ach}$), 7.14 (d, *J* = 8.7 Hz, 2H, Ar_{Tyr}), 6.78 (d, *J* = 8.7 Hz, 2H, Ar_{Tyr}), 5.57 (q, *J* = 7.1 Hz, 1H, $Hα_{MeN\text{-}Ala}$), 5.39 (td, *J* = 9.5 and 5.5 Hz, 1H, $Hα_{Tyr}$), 4.45 (t, *J* = 12.0 Hz, 1H, $Hδ_{MeN\text{-}δ\text{-}Ach}$), 3.81 (m, 1H, $Hδ_{δ\text{-}Ach}$), 3.77 (s, 3H, O-Me), 3.17 (m, 1H, $Hβ_{Tyr}$), 3.15 (s, 3H, $MeN_{MeN\text{-}Ala}$), 3.01 (dd, *J* = 12.8 and 5.4 Hz, 1H, $Hβ_{Tyr}$), 2.6–2.45 (m, 2H, $Hα_{MeN\text{-}δ\text{-}Ach}$ y $Hα_{δ\text{-}Ach}$), 2.54 (s, 3H, $MeN_{MeN\text{-}Ach}$), 2.1–1.3 (m, 14H), 1.27 (d, *J* = 7.1 Hz, 3H, CH_{3Ala}). **MS (ESI)** [m/z, (%)]: 1580.9 ($[MH]^+$, 4), 791.0 ($[MH]^{2+}$, 100). **HRMS (ESI) calculated** for $C_{87}H_{126}N_{12}O_{15}$ ($[MH]^+$): 1579.9538, **found**: 1579.9520. **FT-IR** (293K, $CHCl_3$): 3307 (amide A), 1669, 1620 (amide I), 1512 cm^{-1} (amide II_{II}).

Boc-([*D*-Leu-^{Me}*N*-δ-Ach-^{Me}*N*-*L*-Ala-δ-Ach-)$_4$]-OFm, (Hd1): A solution of octapeptide **Op1b** (50 mg, 0.041 mmol) in a mixture of TFA/$PhSiH_3$/CH_2Cl_2 (25:10:65, 2 mL) was stirred at *rt* for 5 min. The solvent was removed under reduced pressure and the resulting solid was dried under high vacuum for 3 h to give octapeptide **Op3b**, which was used without further purification.

A solution of octapeptide **Op1b** (50 mg, 0.041 mmol) in a mixture of piperidine/CH_2Cl_2 (1:4, 2 mL) was stirred at *rt* for 30 min. The reaction mixture was poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 5 mL). The organic

layer was dried over Na_2SO_4 anhydrous, filtered and concentrated to provide the *C*-terminus unprotected octapeptide **Op2b** as white foam, which was used without further purification.

A solution of the previously prepared **Op3b** in dry CH_2Cl_2 (1.0 mL) was treated with DIEA (14 µL 0.082 mmol) and then a solution of **Op2b**, DIEA (28 µL, 0.164 mmol) and *N*-HBTU (19 mg, 0.049 mmol) in dry CH_2Cl_2 (1.0 mL) was added. The resulting reaction mixture was stirred at *rt* for 2 h and then poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 5 mL) and $NaHCO_3$ (dil. sat., 3 x 3 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated. The resulting yellow oil was purified by flash chromatography (3-8% MeOH in CH_2Cl_2) to give 58 mg of **Hd1** as white solid [65%, R_f = 0.38 (10% MeOH in CH_2Cl_2)]. **MS (ESI)** [m/z, (%)]: 1074.2 ($[MH]^{2+}$, 100), 1024.2 ($[MH\text{-}Boc]^{2+}$, 33). **HRMS (ESI) calculated** for $C_{119}H_{190}N_{16}O_{19}$ ($[MH]^{2+}$): 1073.7191, **found**: 1073.7152**.**

***c*-[(*D*-Leu-^{Me}*N*-δ-Ach-^{Me}*N*-*L*-Ala-δ-Ach-)$_4$] (CP6):** A solution of linear hexadecapeptide **Hd1** (50 mg, 0.023 mmol) in a mixture of piperidine/CH_2Cl_2 (1:4, 1 mL) was stirred at *rt* for 30 min. Then, the resulting solution was poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 5 mL), dried over Na_2SO_4 anhydrous, filtered and concentrated to provide **Hd2**, which was used without further purification.

A solution of hexadecapeptide **Hd2** in a mixture of TFA/$PhSiH_3$/CH_2Cl_2 (25:10:65, 1 mL) was stirred at *rt* for 10 min. Then, the solvent was removed under reduced pressure and the residue was dried under high vacuum for 3 h to obtain the unprotected peptide **Hd3**, which was used without further purification.

A solution of the peptide **Hd3** in dry CH_2Cl_2 (23 mL) was treated with DIEA (24 µL, 0.14 mmol) and *N*-HATU (11 mg, 0.028 mmol). The reaction mixture was stirred at *rt* for 12 h and then poured into a separatory funnel and washed with aqueous solutions of HCl (5%, 3 x 10 mL). The organic layer was dried over Na_2SO_4 anhydrous, filtered and concentrated. The yellow residue was purified by flash chromatography (4-9% MeOH in CH_2Cl_2) followed by HPLC purification (7-14% MeOH in CH_2Cl_2 in 35 min, rt = 33.6 min, Phenomenex Luna 5 µm silica column) to give 15 mg of **CP6** as white solid. [35%, R_f = 0.35 (10% MeOH en CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 500 MHz, δ): 8.32 (d, *J* = 9.3 Hz, 1H, NH_{Tyr}), 7.91 (d, *J* = 8.8 Hz, 1H, $NH_{δ\text{-}Ach}$), 5.62 (q, *J* = 7.1 Hz, 1H, $Hα_{MeN\text{-}Ala}$), 5.28 (td, *J* = 9.0 and 5.6 Hz, 1H, $Hα_{Leu}$), 4.50 (m, 1H, $Hδ_{MeN\text{-}δ\text{-}Ach}$), 3.83 (m, 1H, $Hδ_{δ\text{-}Ach}$), 3.22 (s, 3H, $MeN_{MeN\text{-}Ala}$), 3.09 (s, 3H, $MeN_{MeN\text{-}δ\text{-}Ach}$), 2.55 (m, 1H, $Hα_{MeN\text{-}δ\text{-}Ach}$), 2.46 (m, 1H, $Hα_{δ\text{-}Ach}$), 2.00–1.30 (m, 18H), 1.24 (d, *J* = 7.0 Hz, 3H, Me_{Ala}), 0.94 (dd, *J* = 6.4 and 4.4 Hz, 6H, Me_{Leu}). **MS (ESI)** [m/z, (%)]: 926.2 ($[MH]^{2+}$, 100), 617.8 ($[MH]^{3+}$, 6). **HRMS (ESI) calculated** for $C_{100}H_{170}N_{16}O_{16}$ ($[MH]^{2+}$): 925.6485, **found**: 925.6472. **FT-IR** (293K, $CHCl_3$): 3305 (amide A), 1658, 1621 (amide I), 1526 cm^{-1} (amide II_{II}).

Solid phase Synthesis:

Attachment of Fmoc-*L*-Lys-δ-Ach-OAll to 2-CTC-resin.

Fmoc-*L*-Lys-δ-Ach-OAll (Dp3): A solution of **Fmoc-*L*-Lys(Boc)-δ-Ach-OAll** (450 mg, 0.71 mmol) in a mixture of TFA/CH_2Cl_2 (1:1, 5 mL) was stirred at *rt* for 15 min. After this time, the solvent was removed under reduced pressure and the resulting residue was dried under high vacuum for 4h to give **Dp3a** as white solid, which was used without further purification.

2-CTC-resin (300 mg, 0.150 mmol) was swelled in dry CH_2Cl_2 (3 mL) for 2 h and then washed with dry CH_2Cl_2 (3 x 2 mL, 2 min). A solution of **Fmoc-*L*-Lys-δ-Ach-OAll** (200 mg, 0.375 mmol) and DIEA (128 µl, 0.75 mmol) in CH_2Cl_2 (2 mL) was stirred at rt for 5 min. The mixture was added over a peptide synthesis vessel that contains the previously swelled **2-CTC-resin** (300 mg, 0.150 mmol). The corresponding suspension was mechanically stirred for 2 h and then filtered and washed with dry CH_2Cl_2 (3 x 2 ml, 2 min) and Et_2O (3 x 2 mL, 2 min). The loading of the resulting resin (0.324 mmol/g) was calculated using Fmoc test. [3] Finally, the resin was washed with a mixture of CH_2Cl_2/MeOH/DIEA (8.5:1:0.5, 2 x 2 ml, 10 min), CH_2Cl_2 (3 x 2 mL, 2 min), DMF (3 x 2 mL, 2 min) and finally CH_2Cl_2 (3 x 2 ml, 2 min) to afford the desired **Fmoc-*L*-Lys(CTC-resin)-δ-Ach-OAll**.

Solid phase synthesis: CP7 was synthesized by manual Fmoc solid-phase peptide synthesis from **Fmoc-*L*-Lys(CTC-resin)-δ-Ach-OAll**, following the standard protocol. [4] Typically, Fmoc-*L*-Lys(CTC-resin)-δ-Ach-OAll (0.324 mmol/g) was weighed in a peptide synthesis vessel. Coupling cycle consisted of Fmoc group deprotection with a mixture of piperidine/DMF (1:4, 3 mL, 20 min) and DMF wash (3 x 2 mL, 1 min) followed by amino acid coupling with α-amino acids (3 equiv), *N*-HBTU (3 equiv) and DIEA (6 equiv) for 30 min or with Fmoc-δ-Ach-OH (2 equiv), *N*-HBTU (2 equiv) and DIEA (3 equiv) for 60 min. Finally, the resin was washed with DMF (3 x 2 mL, 1 min). Each peptide coupling and deprotection was followed employing the TNBS test.[3] Fmoc-*D*-His(Trt)-δ-Ach-*L*-Leu-δ-Ach-*D*-His(Trt)-δ-Ach-*L*-Glu(^{t}Bu)-δ-Ach-*D*-Arg(Pbf)-δ-Ach-*L*-Lys(CTC-resin)-δ-Ach-OAll was treated at *rt* for 5 h with a deoxygenated mixture of $Pd(OAc)_2$ (0.25 equiv), PPh_3 (1.25 equiv), NMM (10 equiv) and phenylsilane (10 equiv) in dry CH_2Cl_2 (3 mL). Finally, the resin was filtered and washed with CH_2Cl_2 (3 x 2 mL, 1 min), DMF (3 x 2 mL, 1 min), sodium diethyldithiocarbamate solution in DMF (10%, 2 x 3 mL, 30 min), DIEA solution in DMF (10%, 3 x 4 mL, 3 min) and DMF (3 x 2 mL, 1 min) to give the corresponding C-terminal free Fmoc-*D*-His(Trt)-δ-Ach-*L*-Leu-δ-Ach-*D*-His(Trt)-δ-Ach-*L*-Glu(^{t}Bu)-δ-Ach-*D*-Arg(Pbf)-δ-Ach-*L*-Lys(CTC-resin)-δ-Ach-OH. The Fmoc protecting group was removed by treatment with piperidine in DMF (1:4, 3 ml, 45 min) and then washed with DMF (3 x 2 mL, 3 min), DIEA solution in DMF (5%, 3 x 2 mL, 3 min), lithium chloride solution in DMF

[3] Kay, C.; Lorthioir, O. E.; Parr, N. J.; Congreve, M.; McKeown, S. C.; Scicinski, J. J.; Ley, S. V. *Biotechnol. Bioeng.* **2000**, *71*, 110-118.
[4] Behrendt, R.; White, P.; Offer, J. *J. Pept. Sci.* **2016**, *22*, 4-27.

(0.8 M, 3 x 2 mL, 3 min) and DMF (3 x 2 mL, 1 min). The resulting resin was treated with *N*-TBTU (3 equiv) and DIEA (12 equiv) and then it was shaked at *rt* for 12 h. After filtration, the resin was washed with DMF (3 x 2 mL, 3 min) and CH_2Cl_2 (3 x 3 mL, 1 min). The peptide was deprotected and cleaved from the resin by treatment with a mixture of TFA/CH_2Cl_2/H_2O/TIS (9:5:2.5:2.5, 2 mL/ 300 mg of resin) for 2 h. The mixture was filtered, and the resin was washed with TFA (2 mL) and the resulting solution was treated with Et_2O (10 mL/mL of peptide solution). The resulting mixture was centrifuged (6000 rpm) and then decanted. The resulting residue was washed with Et_2O (2 x 5 mL) and with a mixture of $CHCl_3$/Et_2O (1:9, 2 x 5 mL). The solid was dissolved in water (10 mL) and purified RP-HPLC [Nucleosil 100-7 C18, H_2O (0,1% TFA)/CH_3CN (0,1% TFA) 100:0 (0→5 min), 100:0→88:12 (5→10 min), 88:12→78:22 (10→40 min)] to provide 30 mg of **CP7**. **^{1}H NMR** (D_2O, 300 MHz, δ) 8.65 (s, 1H, Ar_{His}), 8.14–7.93 (m, 5H, NH), 7.32 (s, 1H, Ar_{His}), 4.62–4.47 (m, 3H), 4.27–4.09 (m, 5H, $H\alpha_{\alpha\text{-aa}}$), 3.69–3.44 (m, 6H, $H\delta_{\delta\text{-Aca}}$), 3.27–3.05 (m, 6H, $H\delta_{Arg}$ and $H\beta_{His}$), 3.05–2.85 (m, 6H, $H\alpha_{\delta\text{-Aca}}$), 2.45 (t, *J* = 6.1 Hz, 2H, $H\varepsilon_{Lys}$), 2.37–2.17 (m, 6H, $H\gamma_{Glu}$), 2.03–1.79 (m, 20H), 1.59–1.38 (m, 14H), 1.37–1.13 (m, 11H), 0.91 (Overlapped doublets, *J* = 5.9 and 5.7 Hz, 6H, Me_{Leu}). **MS (ESI)** [m/z, (%)]: 926.2 ($[MH]^{2+}$, 100), 518.8 ($[MH]^{3+}$, 6). **HRMS (ESI) calculated** for $C_{77}H_{123}N_{20}O_{14}$ ($[MH]^+$): 1551.9524, **found**: 1551.9522. **FT-IR** (293K, H_2O): 3277 (amide A), 1674, 1620 (amide I), 1542 cm^{-1} (amide II_{II}).

Thioflavin T fluorescence emission assays:

Fluorescence emission experiments were carried out using a Cary Eclipse Fluorescence Spectrophotometer (Agilent) with an excitation wavelength of 420 nm and recording the spectra between 440-650 nm with 5 nm slit. Emission spectra were measured adding different volumes of 2 mM stock solution of **CP7** in Mili-Q water (pH 3.3) or TRIS buffer at pH 8.1 over a 20 µM solution of ThT (Thioflavin T, Acros Organics).

Solubilization of C_{60} in aqueous media:

C_{60} fullerene (1 mg, Sigma Aldrich) was suspended in a 1 mM solution of **CP7** in Mili-Q water at basic pH 8.1. pH was adjusted by addition of small aliquots of NaOH (0.1 M in Mili-Q water). The suspension was sonicated for 40 min in a Branson 2800 Ultrasonic Cleanear. Then, 3 cycles of 5 min in a Branson 450 SSE-1 tip horn ultrasonicator (20 cycles per min, 20% power) and another 40 min in the Branson 2800 Ultrasonic Cleaner. Finally, the suspension was centrifuged at 7500 rpm for 10 min and the supernatant of **CP7+C_{60}** was carefully decanted. UV/vis spectroscopy experiments were carried out to estimate the amount of dissolved fullerene. The UV/vis spectra were recorded in a Biochrom Libra S60 Double Beam UV/vis spectrophotometer between 200 nm and 700 nm.

Atomic Force Microscopy (AFM):

AFM measurements were conducted in ambient atmosphere at room temperature using a XE-100 instrument (Park Systems Corporation) and AFM-Nanoscope V (Bruker) in non-contact mode. The high-resonance non-contact AFM cantilever (ACTA probe, ν = 330 kHz). For AFM **CP7** imaging, 20 µL of 2 mM solution in TRIS buffer at pH 8.1 were dropped onto freshly exfoliated mica disc (Ted Pella Inc. PELCO® Mica Discs, 9.9 mm Diameter) and after 1 min mica was dried under nitrogen flow. For AFM **CP7+C_{60}** imaging, 20 µL of sample described above were dropped onto freshly exfoliated mica disc (Ted Pella Inc. PELCO® Mica Discs, 9.9 mm Diameter) and after 1 min mica was thoroughly washed with Mili-Q water and dried under nitrogen flow.

Scanning Transmission Electron Microscopy (STEM):

CP7+C_{60} sample were drop casted in commercial microscopy grids (carbon type B film on 300 mesh cooper grid). After 10 min the sample excess was removed by placing the grid onto a filter paper and left to dry at room temperature overnight. The sample was stained with a 2% uranyl acetate solution by depositing a droplet onto the grid. The excess was removed with filer paper and left to dry at room temperature for 1 h. Micrographs were recorded with Zeiss Fesem Ultra Plus with EDX operating at 0.2 kV.