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Self-Assembled Micro-Fibres by Oxime Connection of Linear Peptide Amphiphiles

Richard Booth, Ignacio Insua, Ghibom Bhak and Javier Montenegro*

*Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain. *E-mail: javier.montenegro@usc.es*

1. Materials and methods

Reagents were acquired from Sigma Aldrich, TCI, Fluorochem, Iris Biotech or Fisher. Purified water was obtained from a Millipore Milli-Q integral 5 water purification system. Analytical HPLC was carried out using an Agilent 1260 Infinity II equipped with an Agilent SB-C18 column and connected to a 6120 Quadrupole LCMS. HR-MS was acquired using a Bruker MicroTOF instrument. ^{1}H NMR spectra were acquired using a Varian 300 MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to D_2O (δ = 4.79 ppm). Epifluorescence microscopy was carried out using a Nikon eclipse T*i* microscope equipped with an Andor Zyla sCMOS camera with images taken at either a magnification of 10x or 60x.

2. Characterisation of peptide heads (P1-6): ^{1}H-NMR, HR-MS, HPLC-MS

P1 (RRRGAVV-ONH_2): **^{1}H-NMR** (300 MHz, D_2O) δ = 4.68 (s, 2H), 4.33-4.24 (m, 4H), 4.15 (d, *J* = 7.8 Hz, 1H), 4.08 (d, *J* = 8.3 Hz, 1H), 3.90 (s, 2H), 3.20-3.14 (m, 6H), 2.10-1.95 (m, 2H), 1.89-1.54 (m, 12H), 1.37 (d, *J* = 7.1 Hz, 3H), 0.93-0.88 (m, 12H) ppm. **HR-MS** (ESI, +eV) m/z calculated for $[C_{35}H_{69}N_{18}O_9]^+$ = 885.5489; m/z found = 885.5485. ***R*$_t$ 8.5 min (Fig. S13) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)]. Yield = 42.3 mg (52%).

P2 (RRRGAVVV-ONH_2): **^{1}H-NMR** (300 MHz, D_2O) δ = 4.70 (s, 2H), 4.36-4.26 (m, 4H), 4.17 (d, *J* = 7.7 Hz, 1H), 4.13 (d, *J* = 8.9 Hz, 1H), 4.08 (d, *J* = 8.3 Hz, 1H), 3.93 (s, 2H), 3.22-3.17 (m, 6H), 2.10-1.94 (m, 2H), 1.92-1.58 (m, 12H), 1.39 (d, *J* = 7.2 Hz, 3H), 0.96-0.88 (m, 18H) ppm. **HR-MS** (ESI, +eV) m/z calculated for $[C_{40}H_{78}N_{19}O_{10}]^+$ = 984.6174; m/z found = 984.6176. ***R*$_t$ 9.5**

min (Fig. S14) RP-HPLC [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)]. Yield = 55.7 mg (61%).

P3 (RRRGAAVV-ONH_2): **^{1}H-NMR** (300 MHz, D_2O) δ = 4.70 (s, 2H), 4.35-4.23 (m, 5H), 4.17 (d, *J* = 7.7 Hz, 1H), 4.09 (d, *J* = 8.2 Hz, 1H), 3.93 (s, 2H), 3.22-3.17 (m, 6H), 2.11-1.97 (m, 2H), 1.92-1.57 (m, 12H), 1.38 (t, *J* = 7.4 Hz, 6H), 0.95-0.90 (m, 12H) ppm. **HR-MS** (ESI, +eV) m/z calculated for $[C_{38}H_{74}N_{19}O_{10}]^+$ = 956.5861; m/z found = 956.5859. ***R_t* 9 min (Fig. S15) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)]. Yield = 41.2 mg (47%).

P4 (EEGAVV-ONH_2): **^{1}H-NMR** (300 MHz, D_2O) δ = 4.66 (s, 2H), 4.44-4.28 (m, 3H), 4.21 (d, *J* = 7.6 Hz, 1H), 4.15 (d, *J* = 8.1 Hz, 1H), 4.04-3.90 (m, 2H), 2.54-2.47 (m, 4H), 2.23-1.96 (m, 6H), 1.41 (d, *J* = 7.2 Hz, 3H), 0.97-0.93 (m, 12H) ppm. **HR-MS** (ESI, +eV) m/z calculated for $[C_{27}H_{47}N_8O_{12}]^+$ = 675.3308; m/z found = 675.3311. ***R_t* 9 min (Fig. S16) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. Yield = 22.1 mg (32%).

P5 (EEAAVV-ONH_2): **^{1}H-NMR** (300 MHz, D_2O) δ = 4.56 (s, 2H), 4.40-4.26 (m, 4H), 4.20 (d, *J* = 7.8 Hz, 1H), 4.13 (d, *J* = 8.2 Hz, 1H), 2.54-2.48 (m, 4H), 2.23-1.95 (m, 6H), 1.41 (d, *J* = 7.2 Hz, 3H), 1.40 (d, *J* = 7.2 Hz, 3H), 0.98-0.94 (m, 12H) ppm. **HR-MS** (ESI, +eV) m/z calculated for $[C_{28}H_{48}N_8NaO_{12}]^+$ = 711.3284; m/z found = 711.3288. ***R_t* 9.5 min (Fig. S17) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. Yield = 29.6 mg (49%).

P6 (EEAAAVV-ONH_2): **^{1}H-NMR** (300 MHz, D_2O) δ = 4.55 (s, 2H), 4.40-4.24 (m, 5H), 4.20 (d, *J* = 8.0 Hz, 1H), 4.13 (d, *J* = 8.1 Hz, 1H), 2.51 (t, *J* = 7.3 Hz, 4H), 2.23-1.97 (m, 6H), 1.41 (d, *J* = 6.9 Hz, 3H), 1.40 (d, *J* = 7.2 Hz, 3H), 1.40 (d, *J* = 6.9 Hz, 3H), 0.98-0.93 (m, 12H) ppm. **HR-MS** (ESI, +eV) m/z calculated for $[C_{31}H_{53}N_9NaO_{13}]^+$ = 782.3655; m/z found = 782.3646. ***R_t* 10 min (Fig. S18) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. Yield = 24.6 mg (40%).

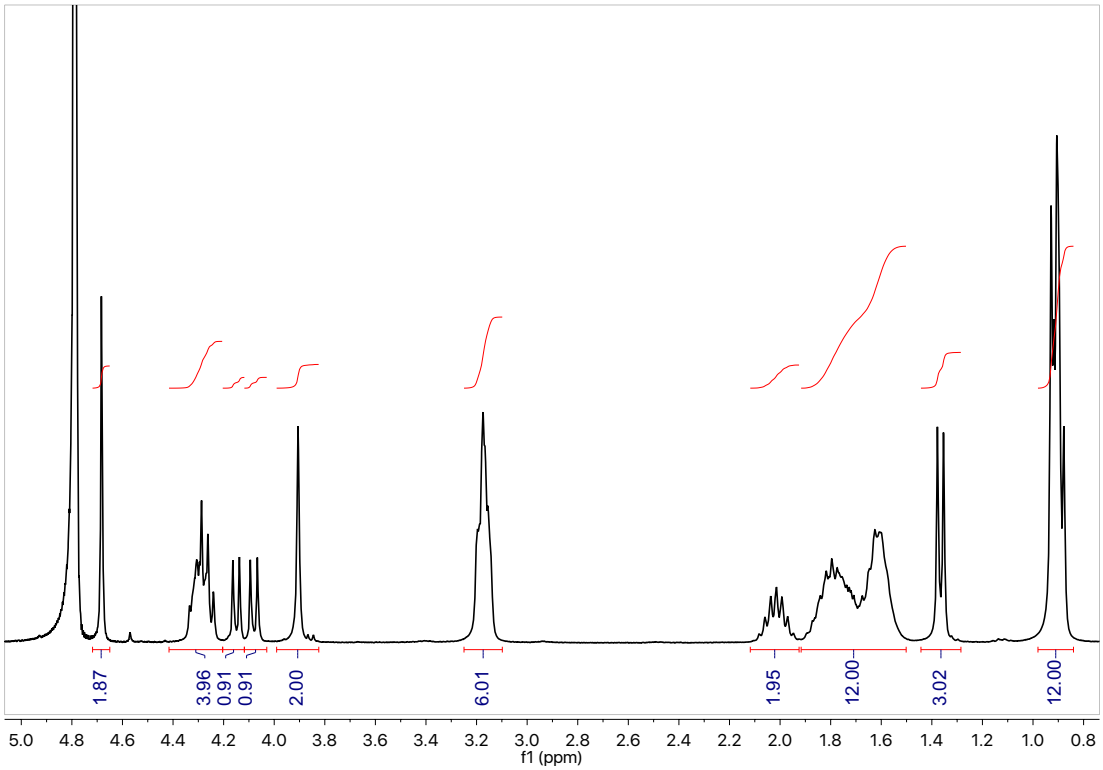

Figure S1 *^{1}H-NMR (300 MHz, D_2O) of* ***P1*** *(RRRGAVV-ONH$_2$).*

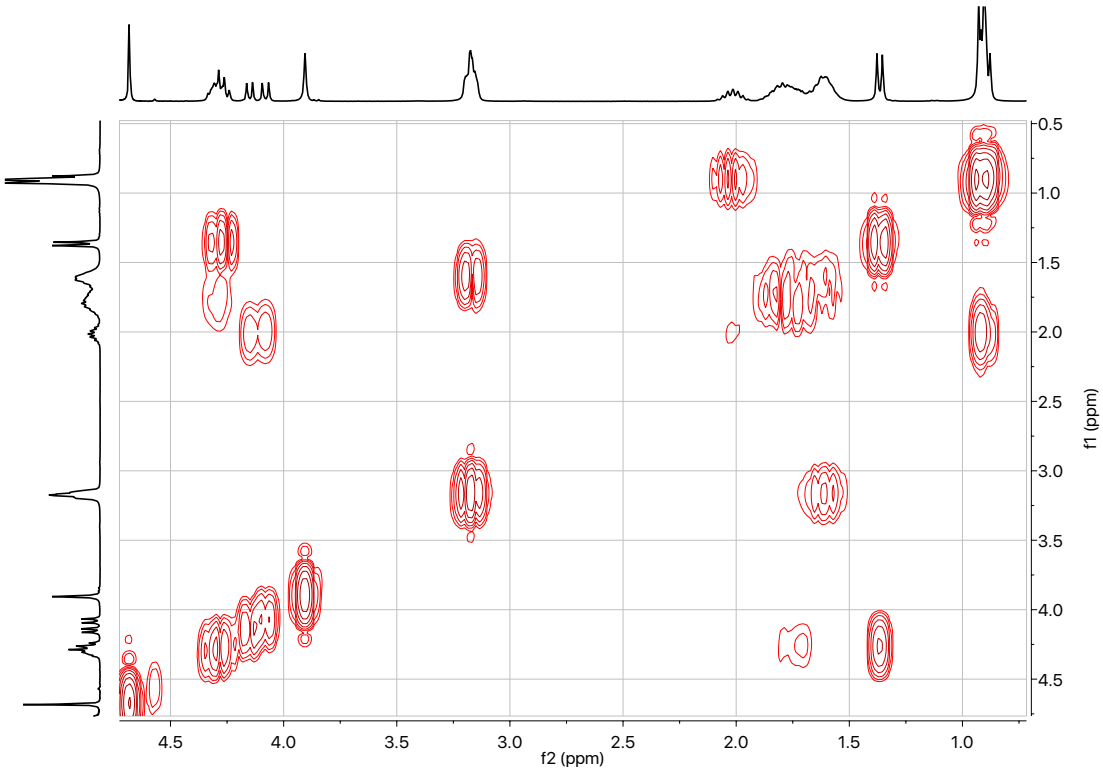

Figure S2 *^{1}H-COSY NMR (300 MHz, D_2O) of* ***P1*** *(RRRGAVV-ONH$_2$).*

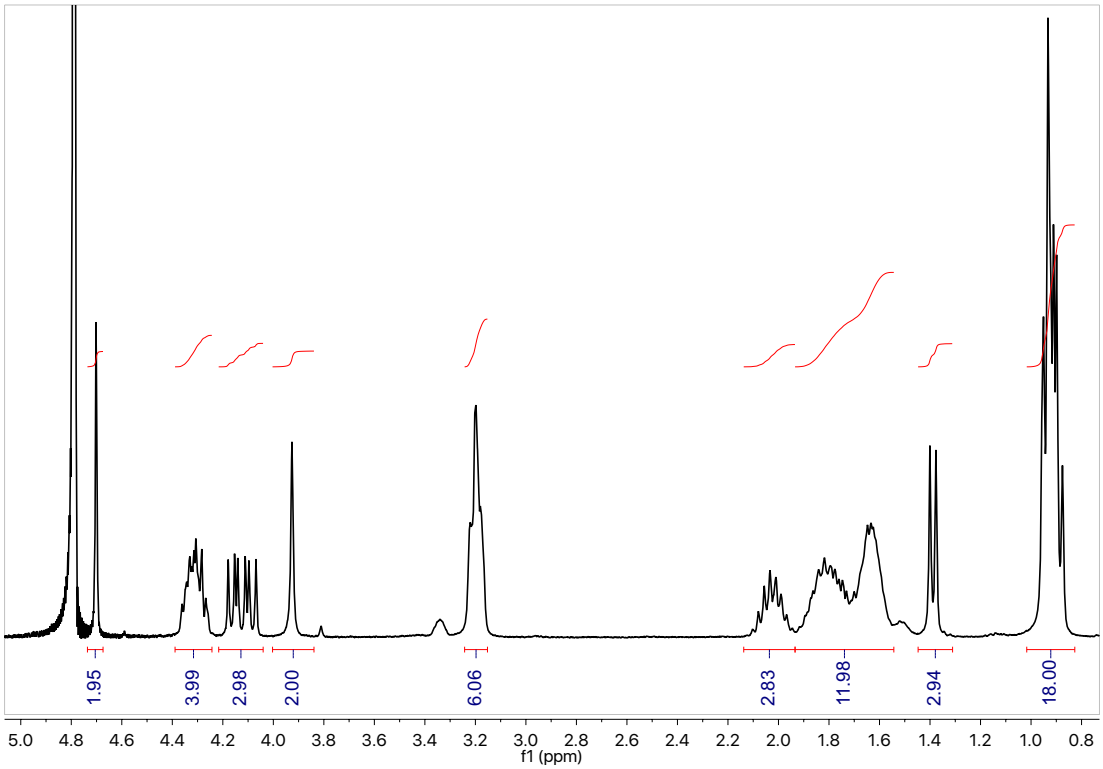

Figure S3 *^{1}H-NMR (300 MHz, D_2O) of **P2** (RRRGAVVV-ONH$_2$).*

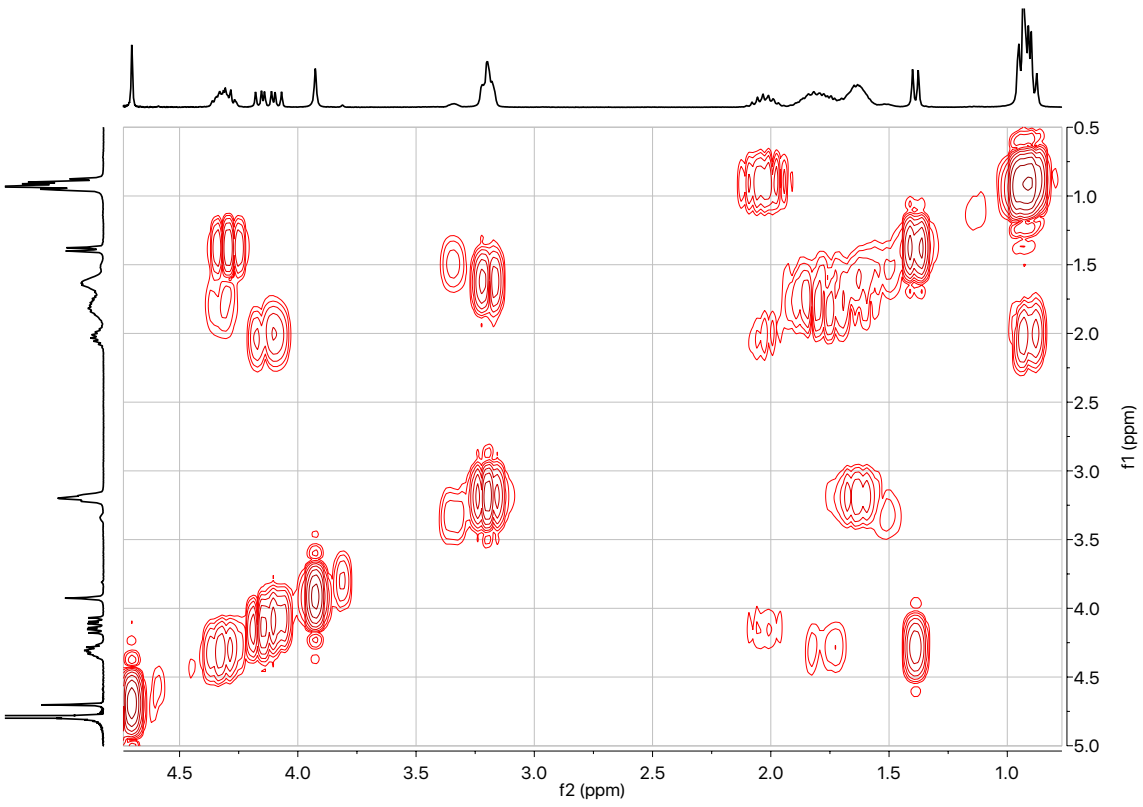

Figure S4 *^{1}H-COSY NMR (300 MHz, D_2O) of **P2** (RRRGAVVV-ONH$_2$).*

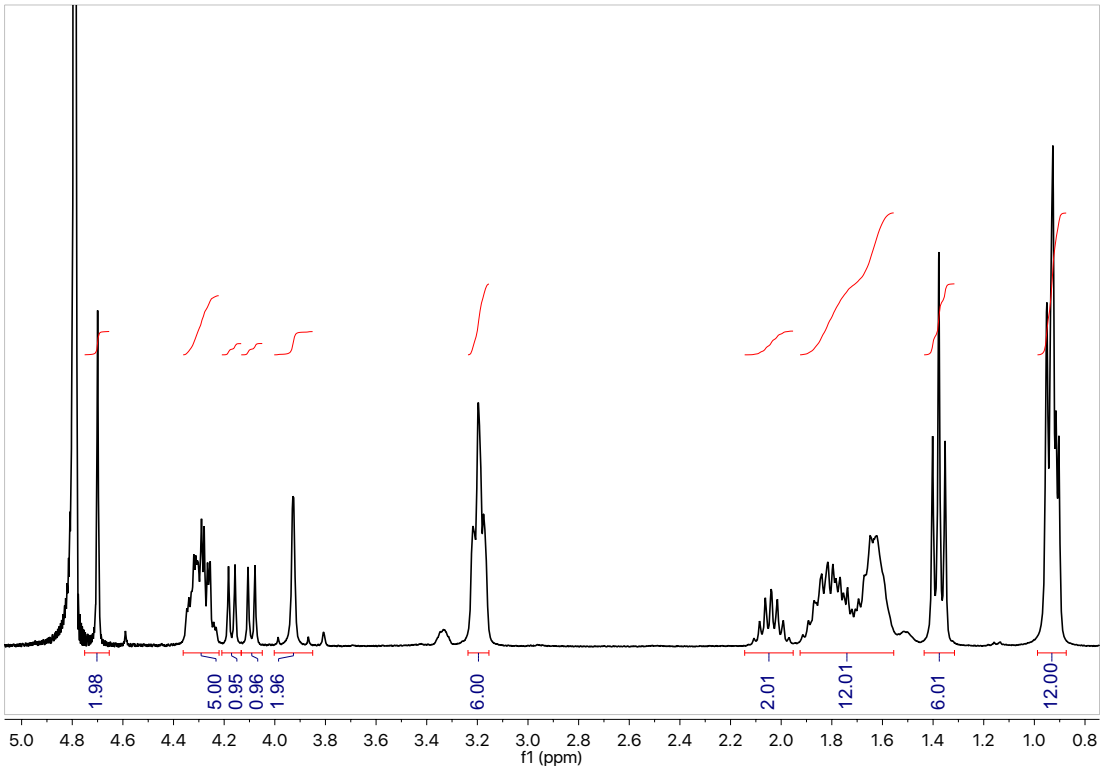

Figure S5 *^{1}H-NMR (300 MHz, D_2O) of* ***P3*** *(RRRGAAVV-ONH$_2$).*

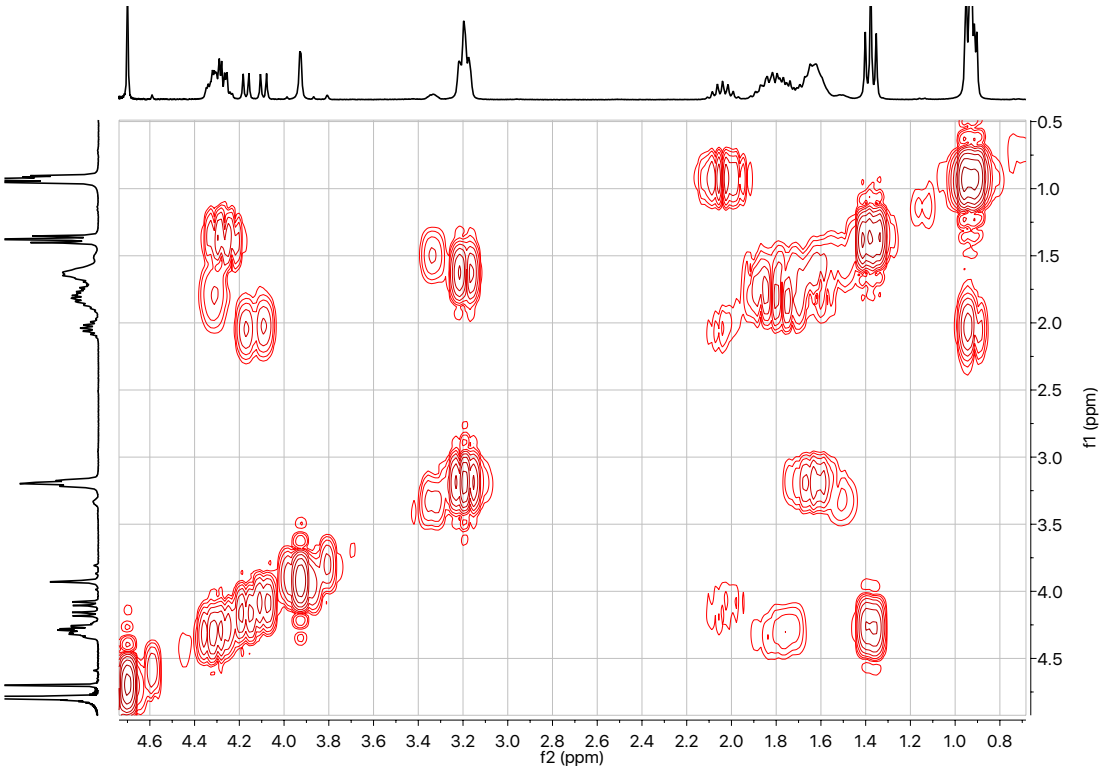

Figure S6 *^{1}H-COSY NMR (300 MHz, D_2O) of* ***P3*** *(RRRGAAVV-ONH$_2$).*

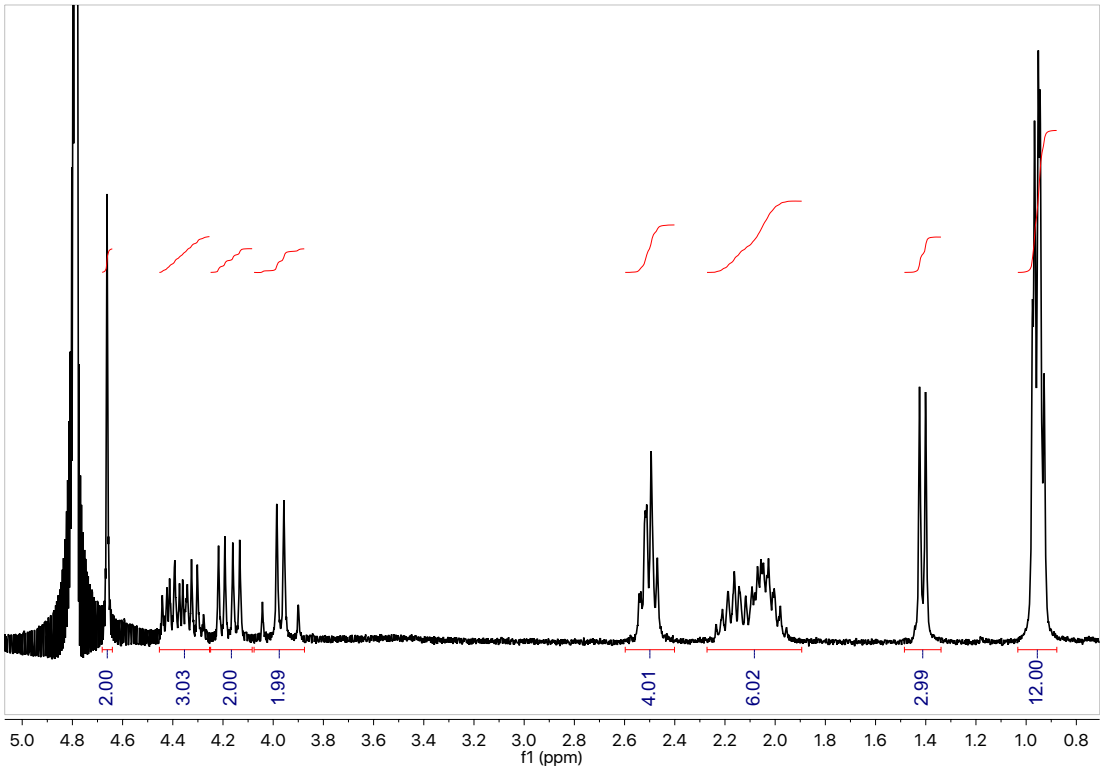

Figure S7 *^{1}H-NMR (300 MHz, D_2O) of* ***P4*** *(EEGAVV-ONH$_2$).*

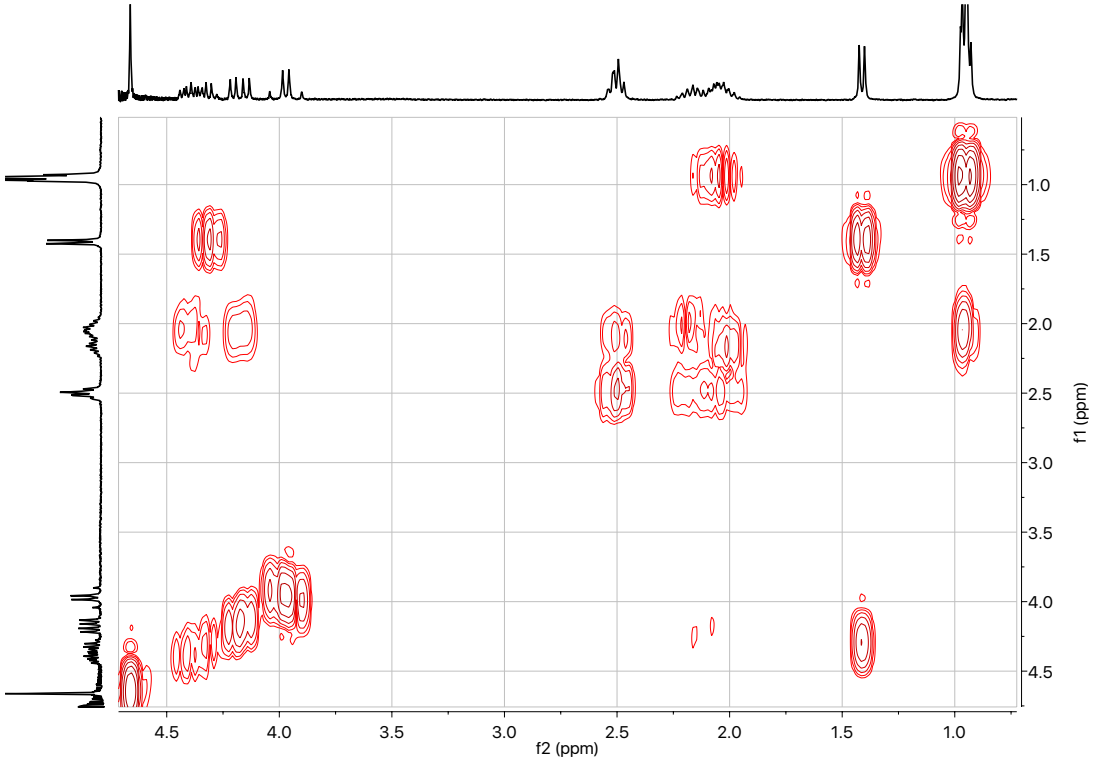

Figure S8 *^{1}H-COSY NMR (300 MHz, D_2O) of* ***P4*** *(EEGAVV-ONH$_2$).*

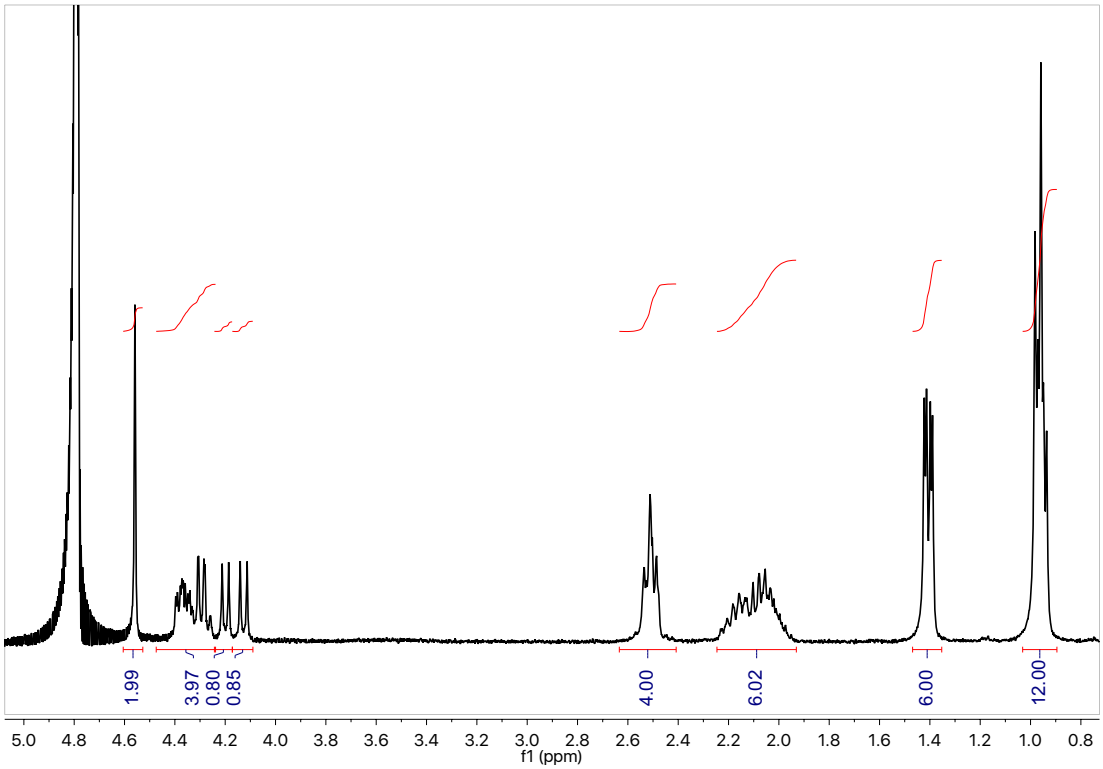

Figure S9 *^{1}H-NMR (300 MHz, D_2O) of* ***P5*** *(EEAAVV-ONH$_2$).*

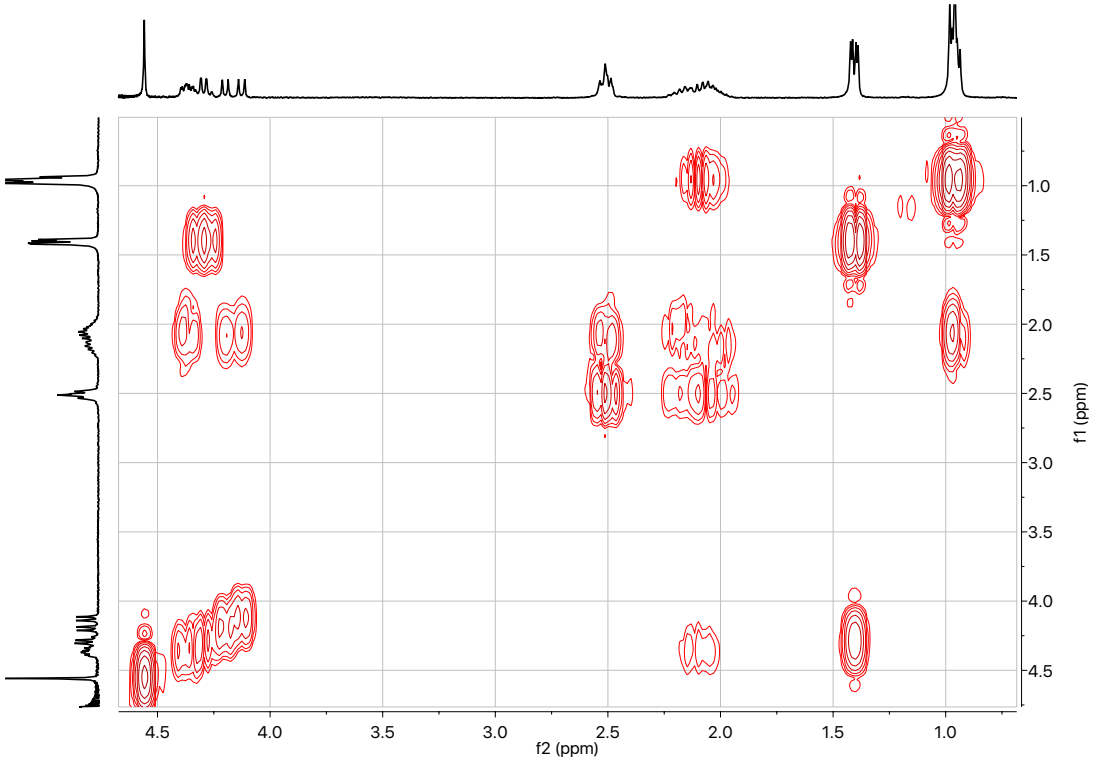

Figure S10 *^{1}H-COSY NMR (300 MHz, D_2O) of* ***P5*** *(EEAAVV-ONH$_2$).*

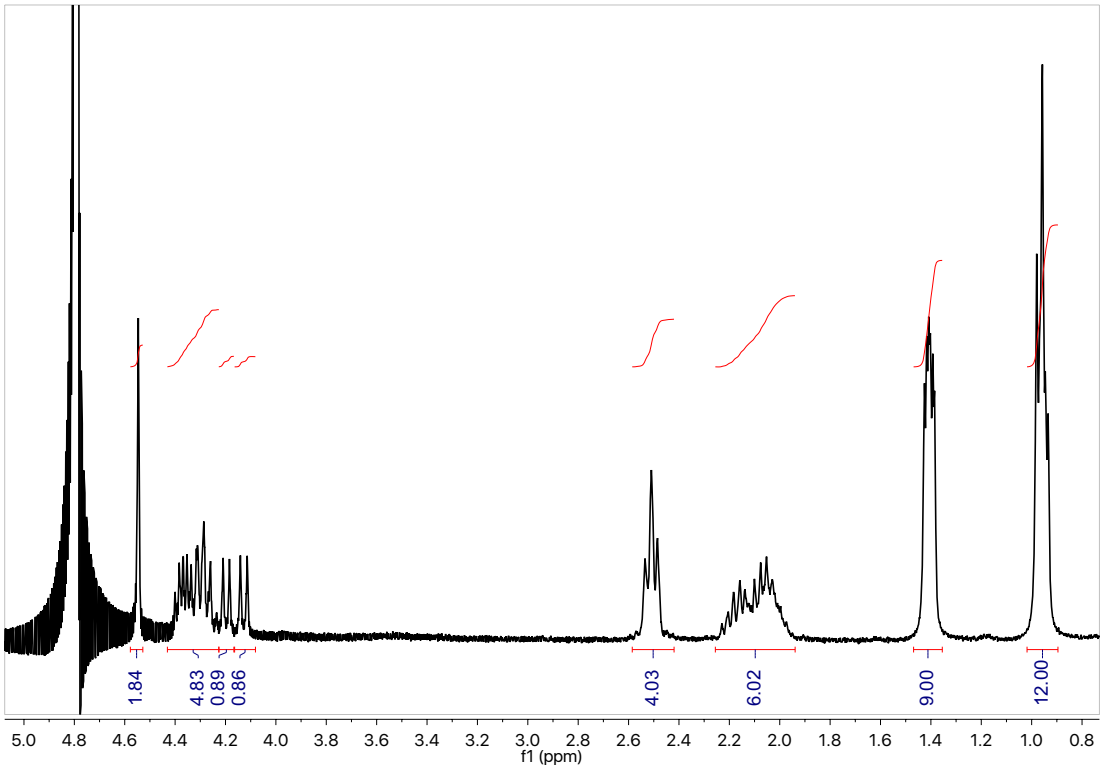

Figure S11 *^{1}H-NMR (300 MHz, D_2O) of* ***P6*** *(EEAAAVV-ONH$_2$).*

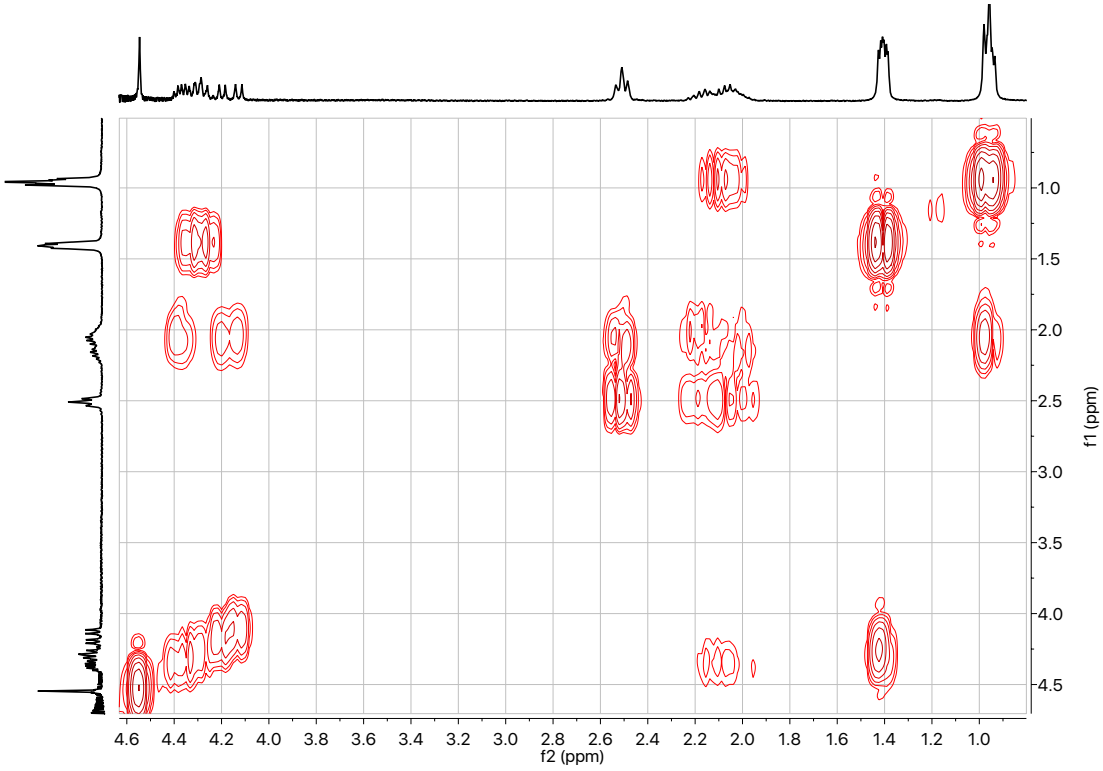

Figure S12 *^{1}H-COSY NMR (300 MHz, D_2O) of* ***P6*** *(EEAAAVV-ONH$_2$).*

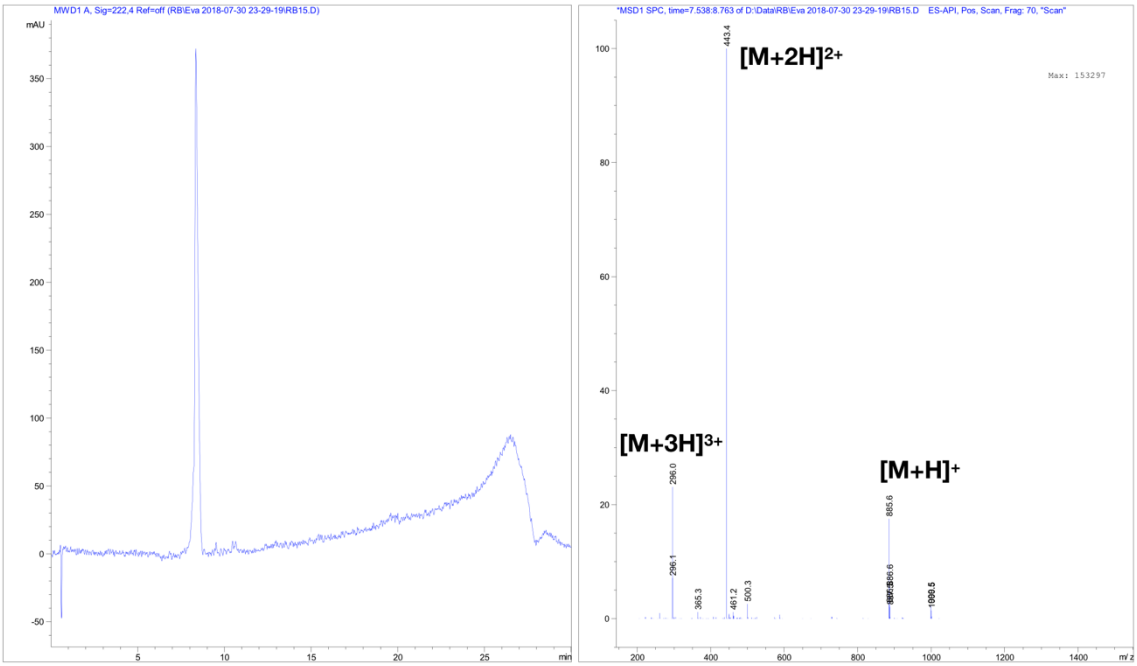

Figure S13 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of* ***P1*** *(RRRGAVV-ONH$_2$).*

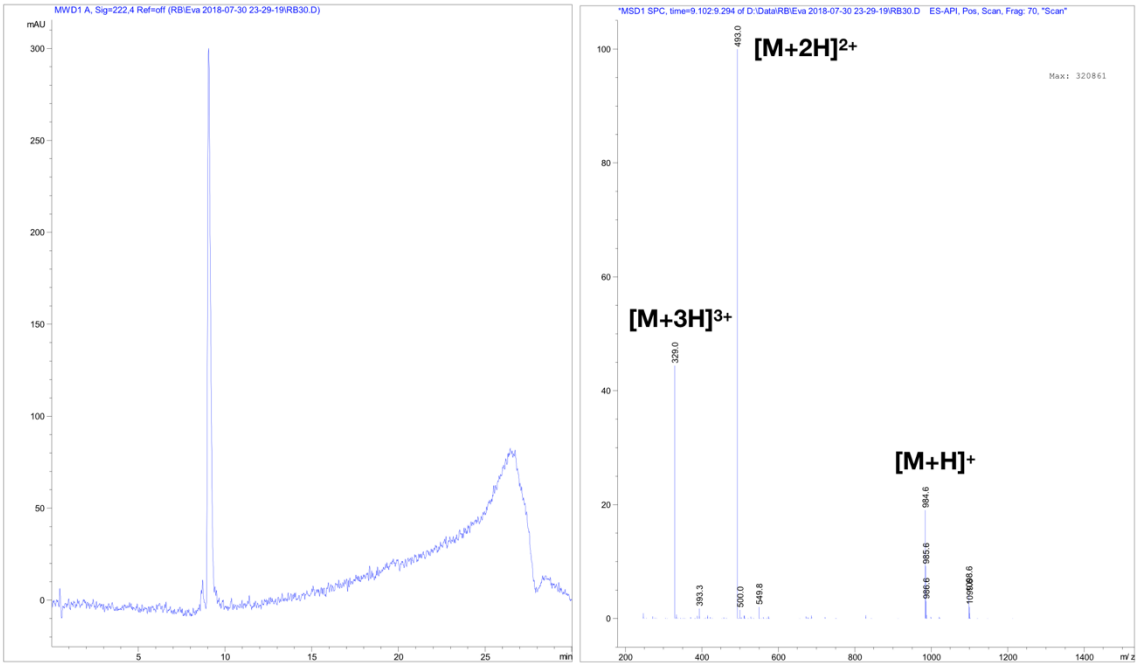

Figure S14 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of* ***P2*** *(RRRGAVVV-ONH$_2$).*

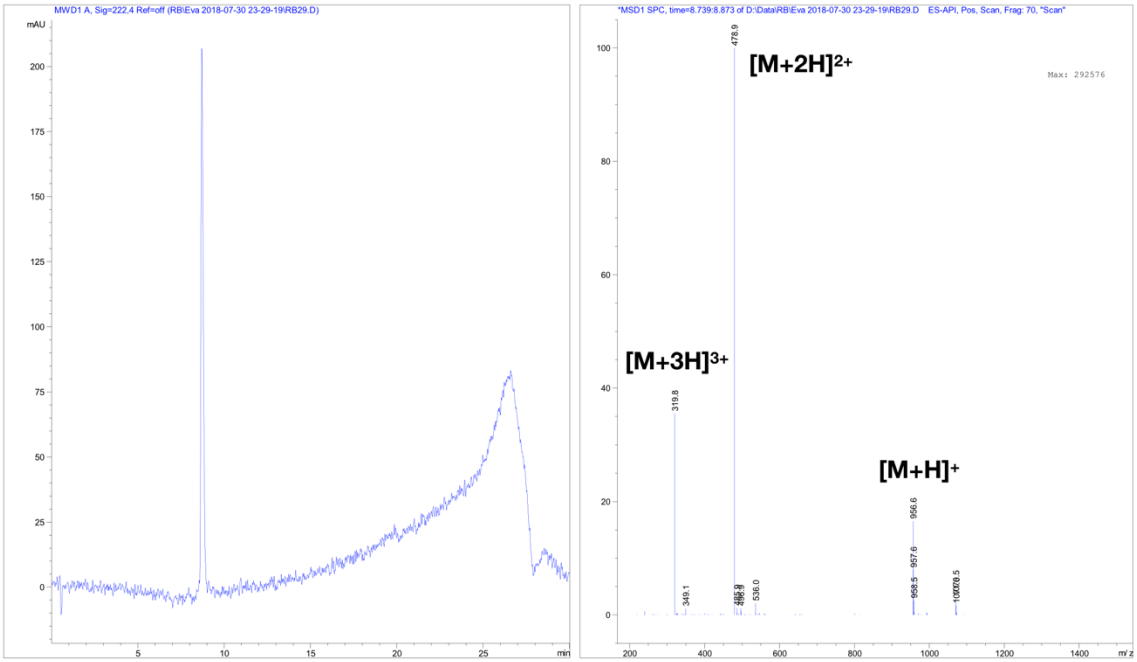

Figure S15 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of* ***P3*** *(RRRGAAVV-ONH$_2$).*

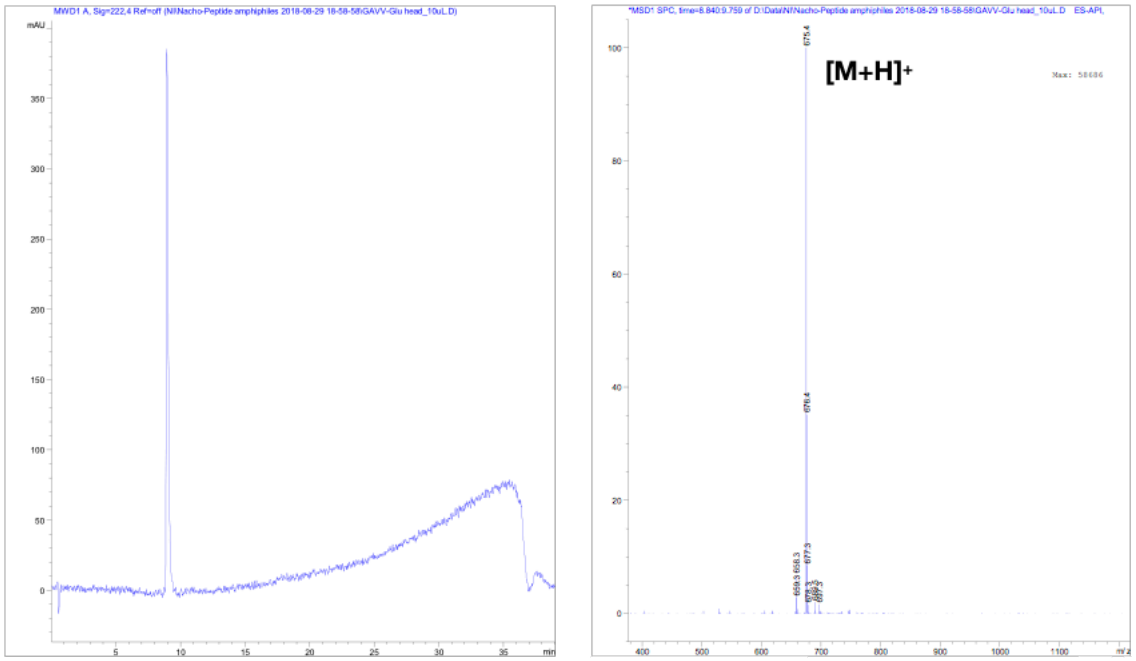

Figure S16 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of* ***P4*** *(EEGAVV-ONH$_2$).*

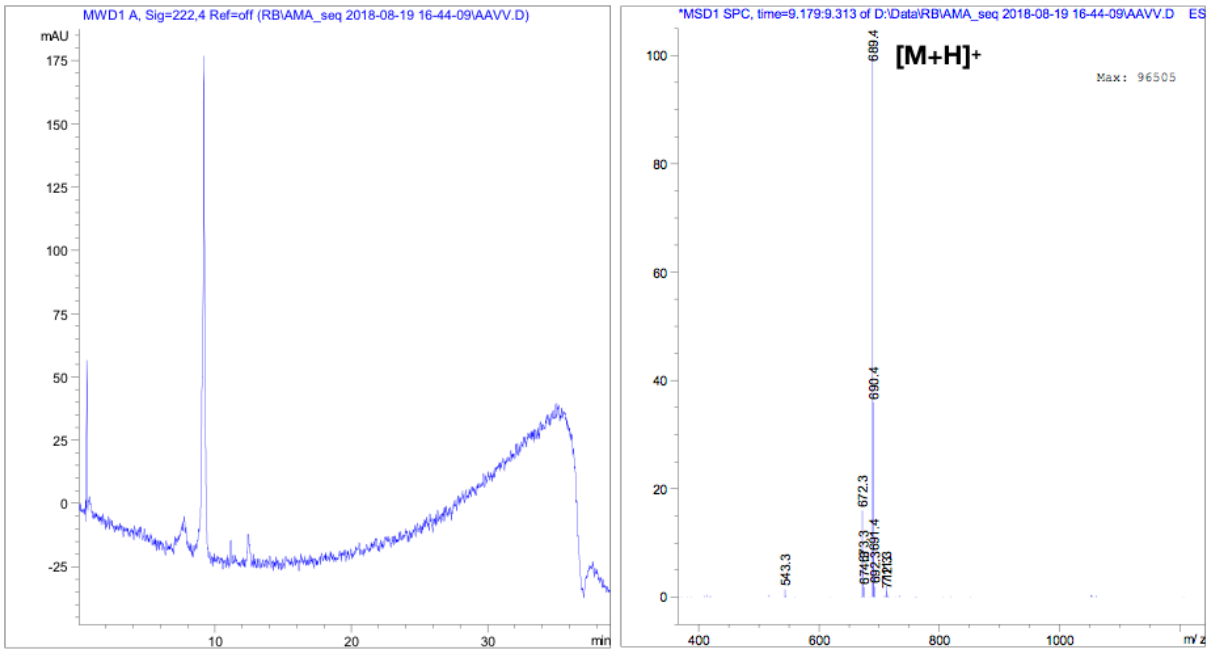

Figure S17 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of* ***P5*** *(EEAAVV-ONH_2).*

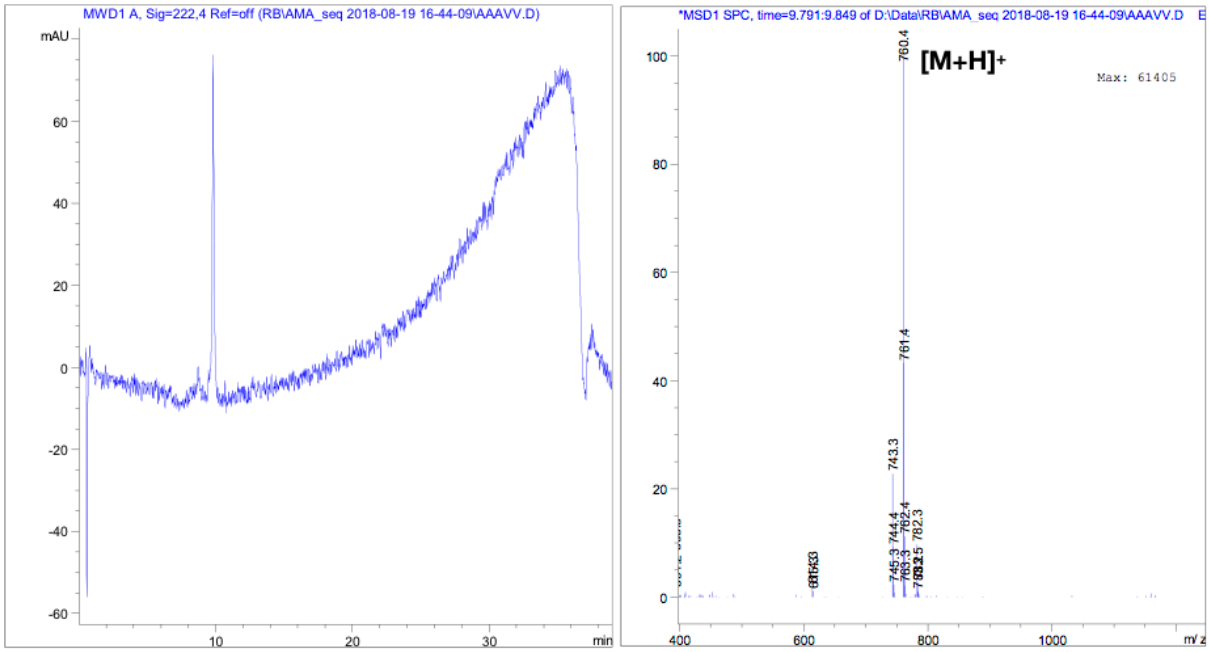

Figure S18 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of* ***P6*** *(EEAAAVV-ONH_2).*

3. Characterisation of peptide amphiphiles: HR-MS, HPLC-MS

P1T8 (RRRGAVV-ON=C_8H_{15}): R_t **14.5 min (Fig. S19) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)].
HR-MS (ESI, +eV) m/z calculated for $[C_{43}H_{83}N_{18}O_9]^+$ = 995.6585; m/z found = 995.6583.

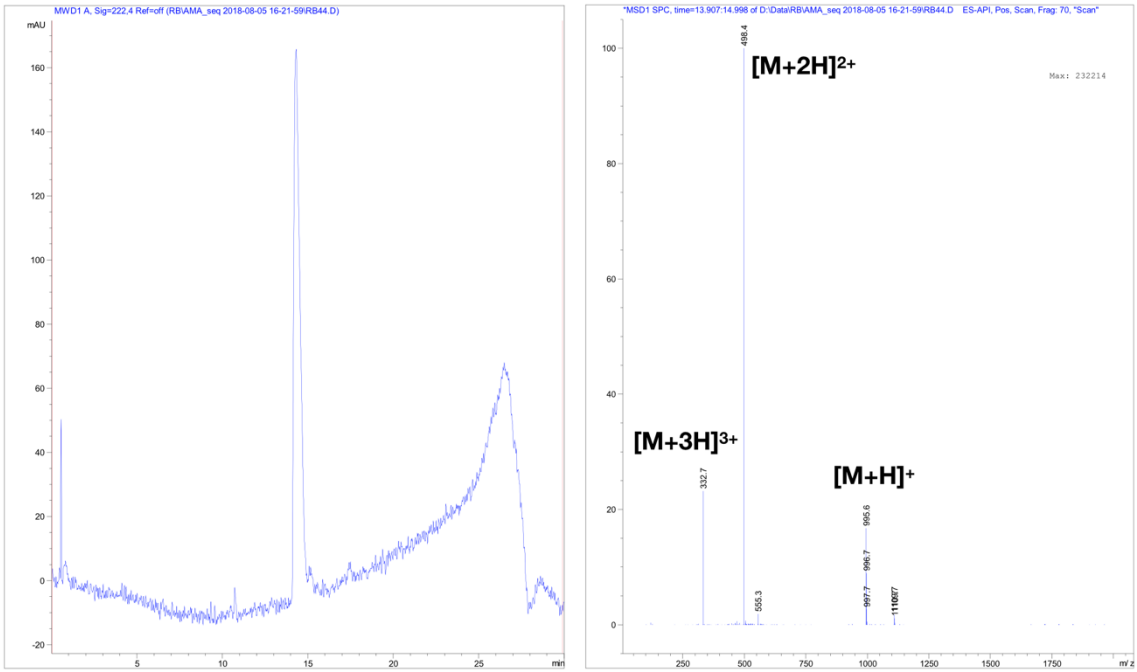

Figure S19 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P1T8****.*

P1T10 (RRRGAVV-ON=$C_{10}H_{19}$): ***R_t* 16.5 min (Fig. S20) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)]. **HR-MS** (ESI, +eV) m/z calculated for $[C_{45}H_{87}N_{18}O_9]^+$ = 1023.6898; m/z found = 1023.6891.

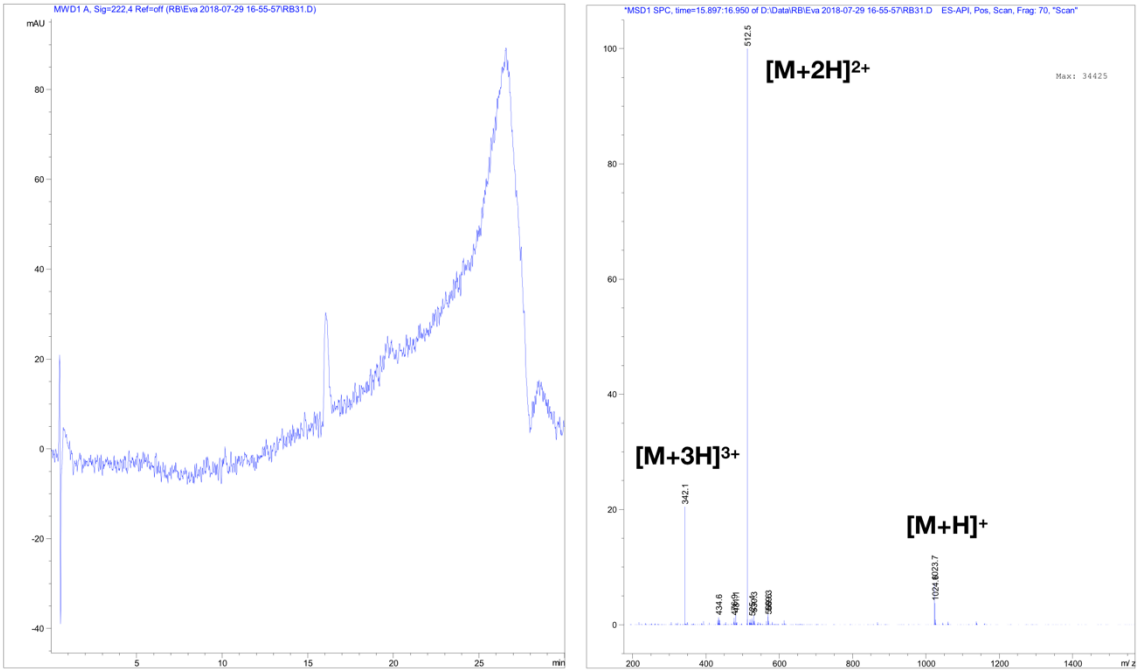

Figure S20 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P1T10****.*

P1T12 (RRRGAVV-ON=$C_{12}H_{23}$): ***R_t* 17.5 min (Fig. S21) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)].
HR-MS (ESI, +eV) m/z calculated for $[C_{47}H_{91}N_{18}O_9]^+$ = 1051.7211; m/z found = 1051.7204.

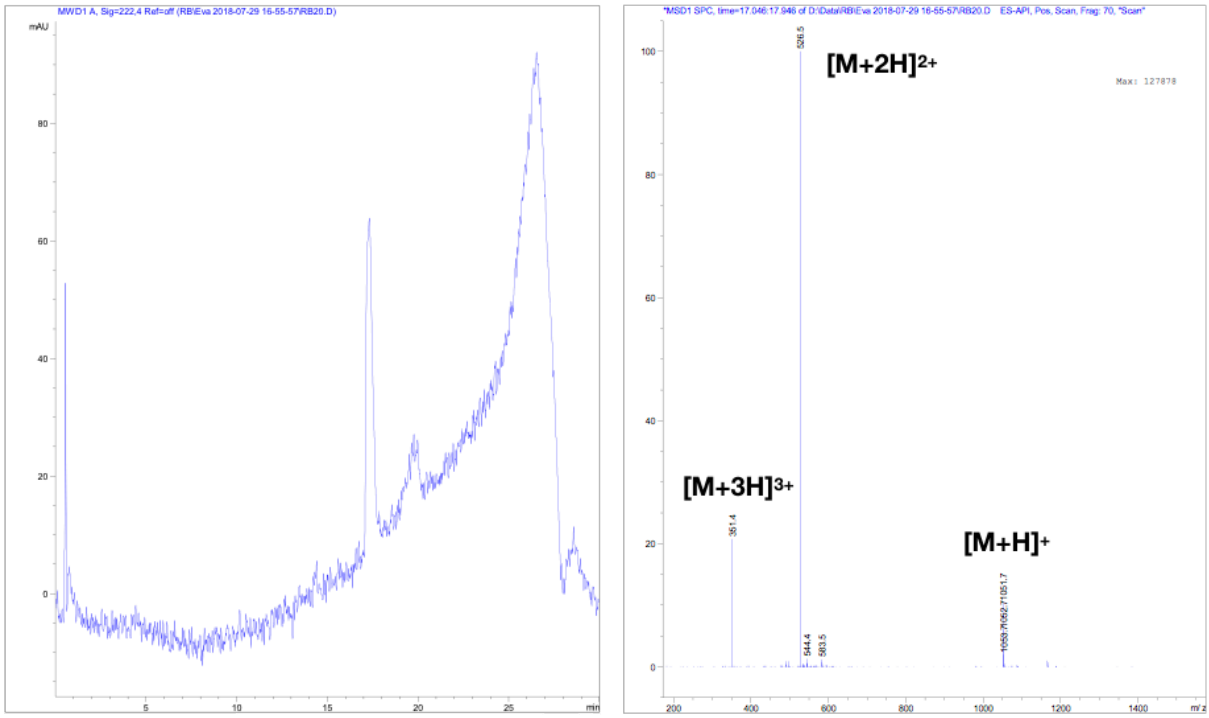

Figure S21 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P1T12****.*

P2T8 (RRRAVVV-ON=C_8H_{15}): ***R_t*** **15 min (Fig. S22) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{48}H_{92}N_{19}O_{10}]^+$ = 1094.7269; m/z found = 1094.7266.

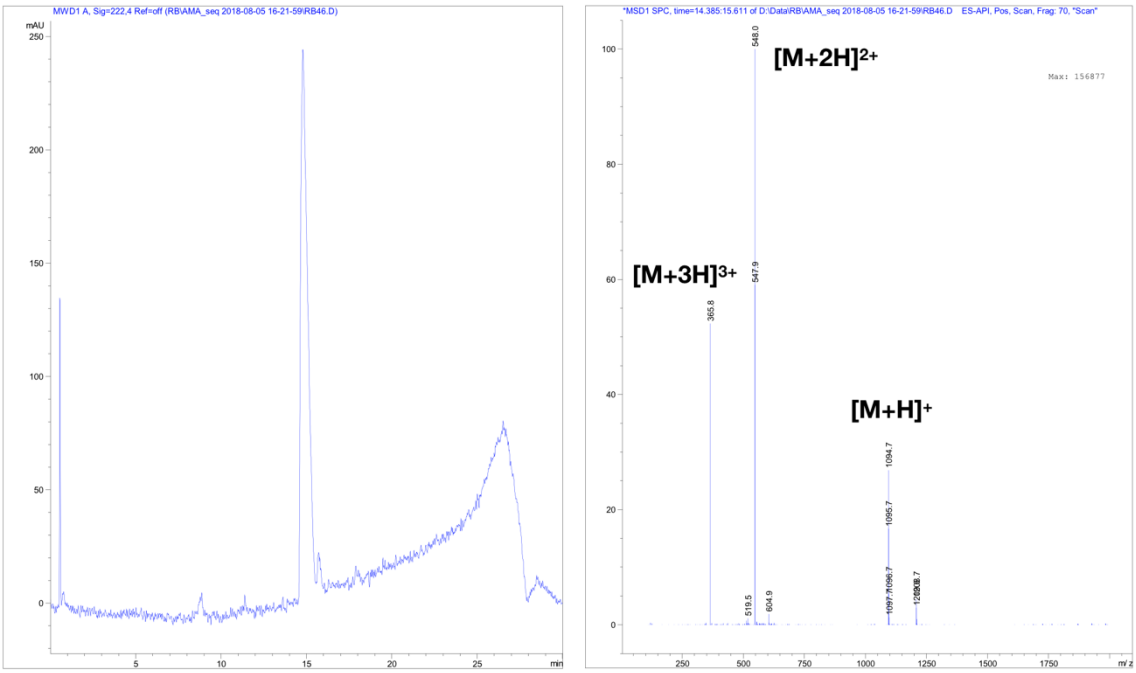

Figure S22 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P2T8****.*

P2T10 (RRRAVVV-ON=$C_{10}H_{19}$): ***R_t* 16.5 min (Fig. S23) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{50}H_{96}N_{19}O_{10}]^+$ = 1122.7582; m/z found = 1122.7580.

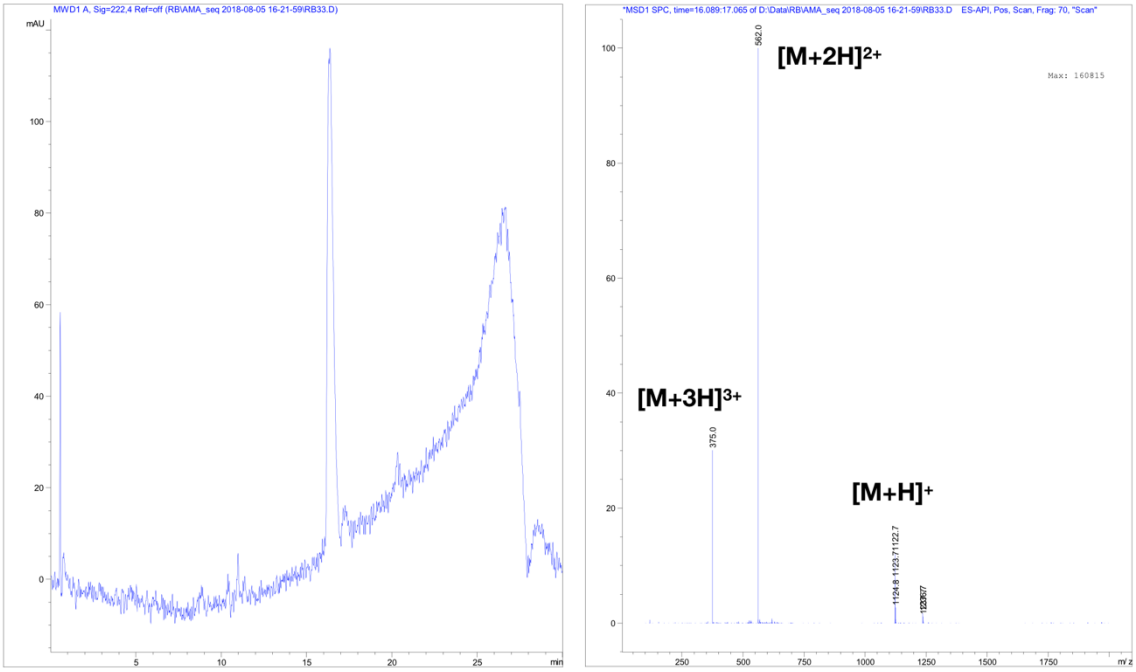

Figure S23 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P2T10****.*

P2T12 (RRRAVVV-ON=$C_{12}H_{23}$): ***R*$_t$ 18 min (Fig. S24) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{52}H_{100}N_{19}O_{10}]^+$ = 1150.7895; m/z found = 1150.7890.

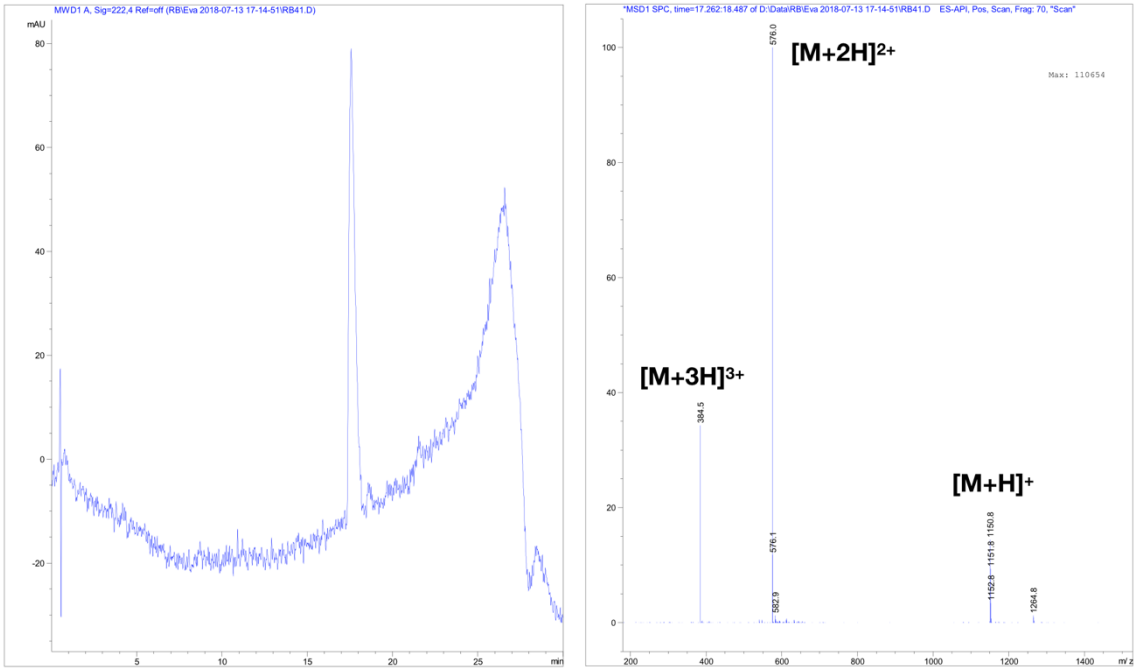

Figure S24 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P2T12.***

P3T8 (RRRAAVV-ON=C_8H_{15}): R_t **15 min (Fig. S25) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{46}H_{88}N_{19}O_{10}]^+$ = 1066.6956; m/z found = 1066.6958.

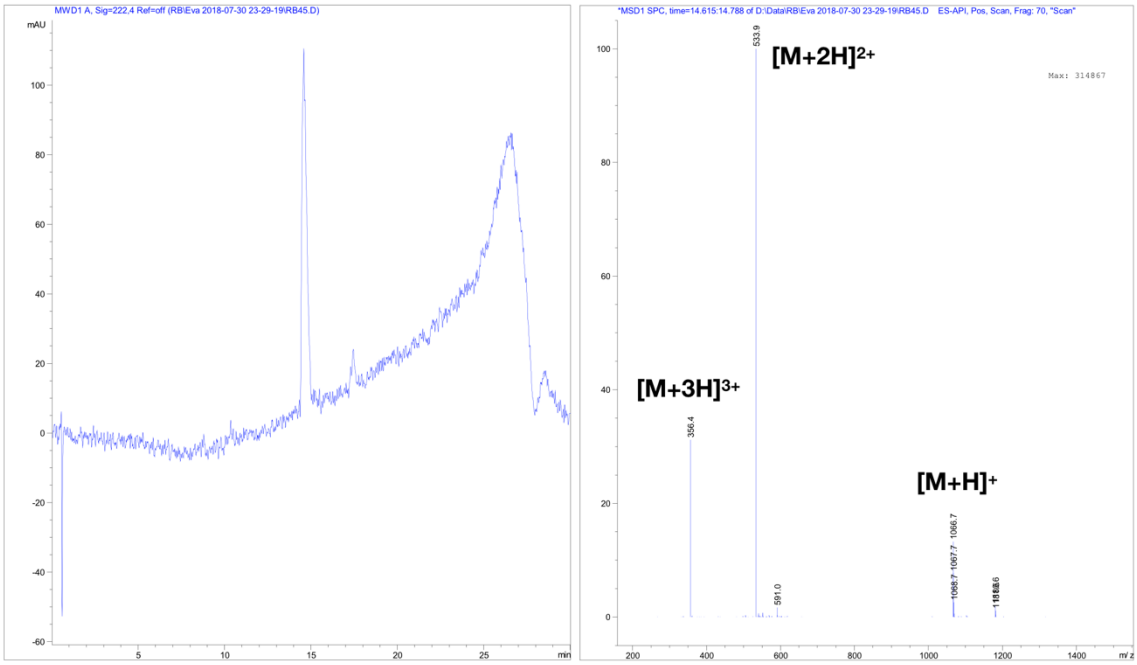

Figure S25 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P3T8.***

P3T10 (RRRAAVV-ON=$C_{10}H_{19}$): ***R*$_t$ 16 min (Fig. S26) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{48}H_{92}N_{19}O_{10}]^+$ = 1094.7269; m/z found = 1094.7271.

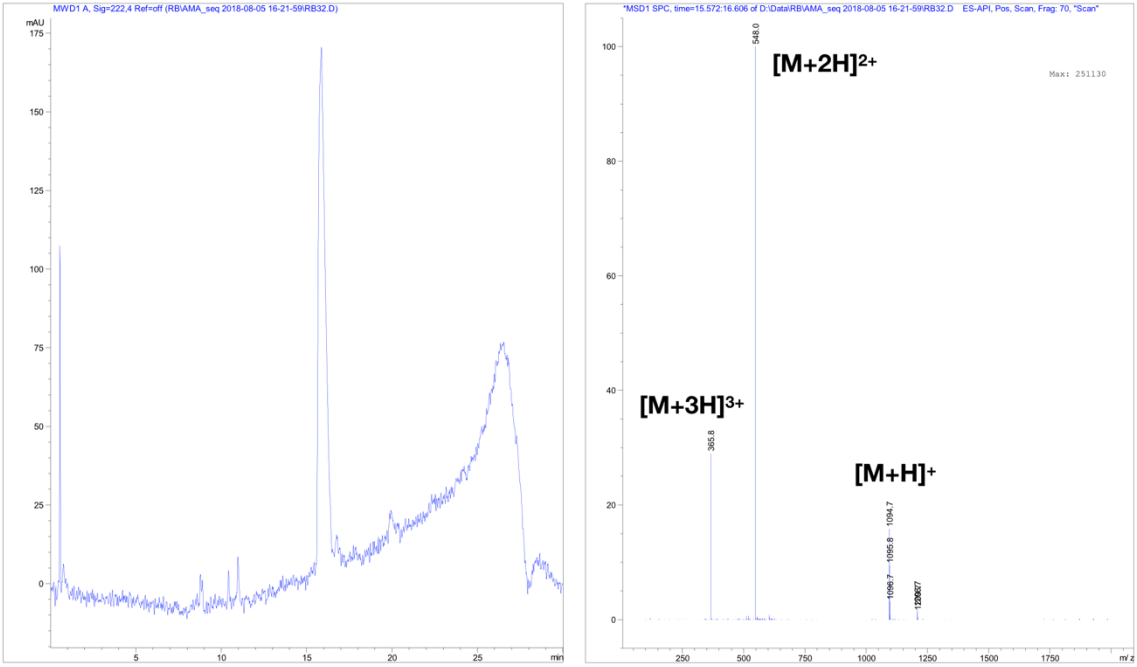

Figure S26 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P3T10****.*

P3T12 (RRRAAVV-ON=$C_{12}H_{23}$): ***R_t* 17.5 min (Fig. S27) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→22 min), 0:100 (>22 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{50}H_{96}N_{19}O_{10}]^+$ = 1122.7582; m/z found = 1122.7586.

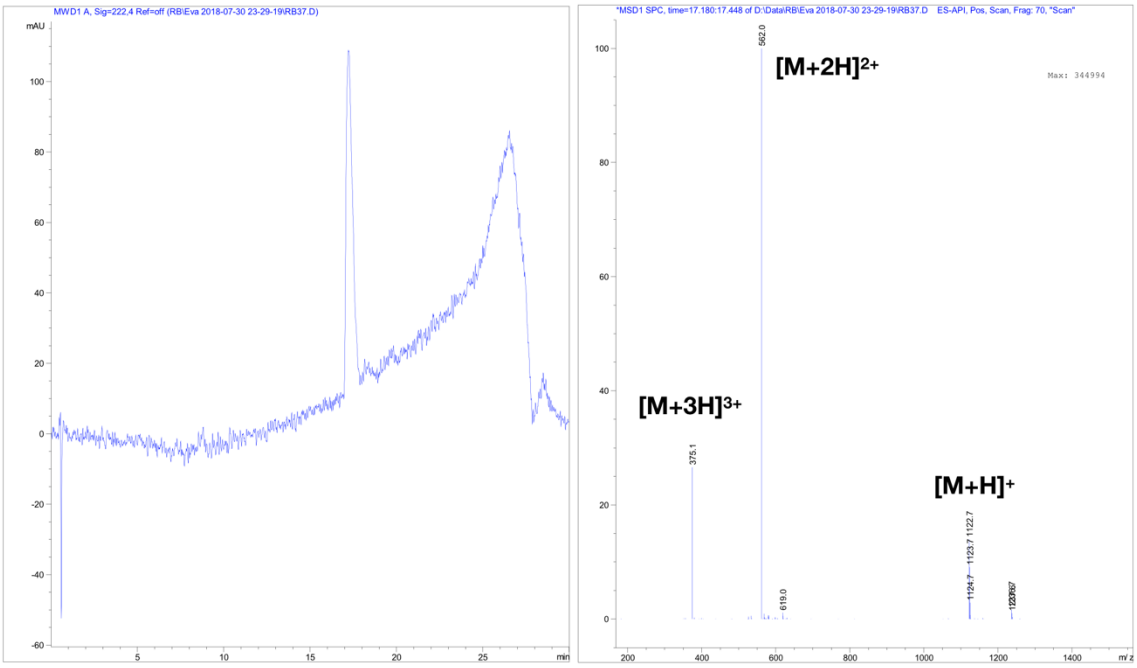

Figure S27 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P3T12****.*

P4T8 (EEGAVV-ON=C_8H_{15}): ***R*$_t$ 18.5 min (Fig. S28) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{35}H_{60}N_8NaO_{12}]^+$ = 807.4223; m/z found = 807.4226.

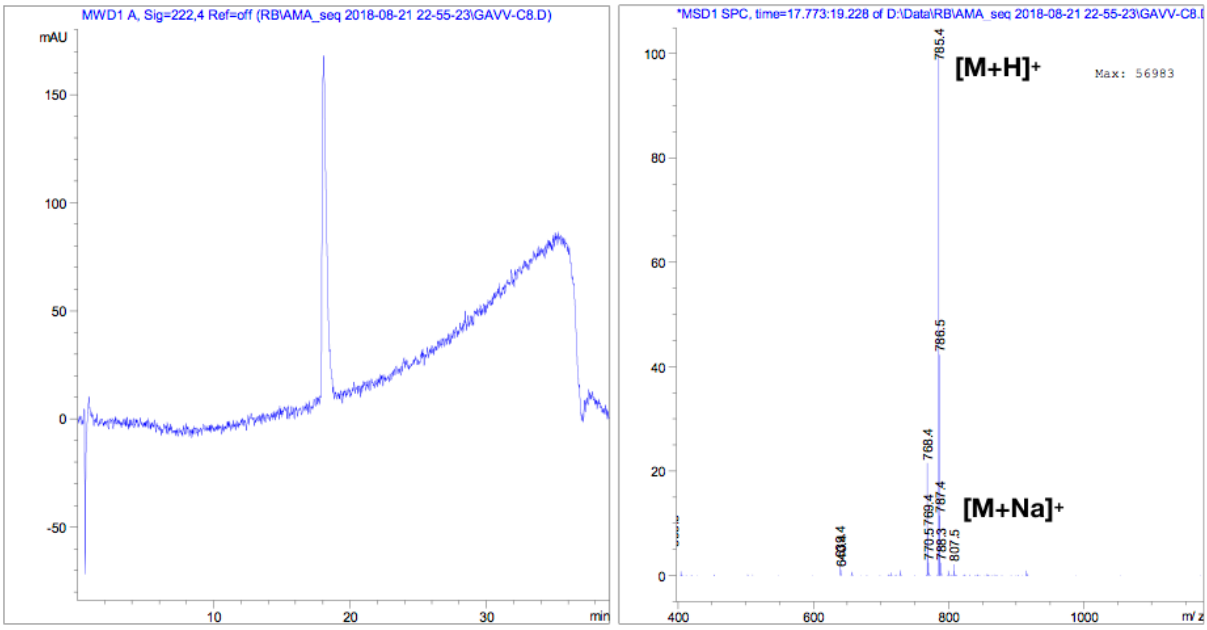

Figure S28 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P4T8****.*

P4T10 (EEGAVV-ON=$C_{10}H_{19}$): R_t **21 min (Fig. S29) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{37}H_{65}N_8O_{12}]^+$ = 813.4716; m/z found = 813.4718.

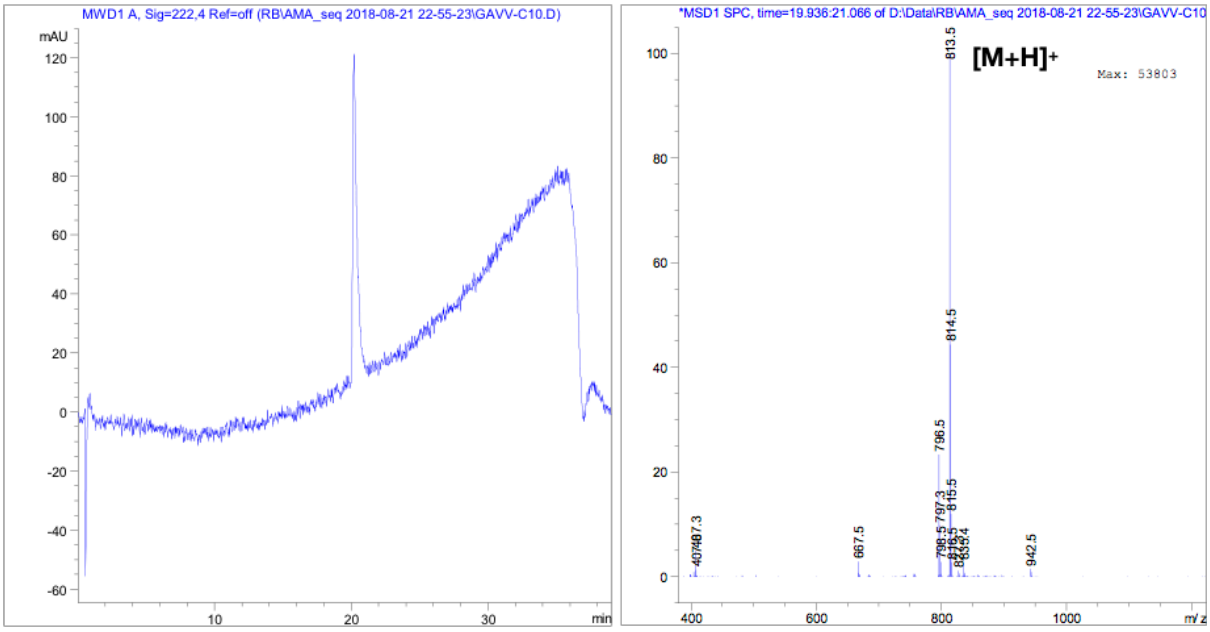

Figure S29 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P4T10****.*

P4T12 (EEGAVV-ON=$C_{12}H_{23}$): ***R_t* 23 min (Fig. S30) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{39}H_{68}N_8NaO_{12}]^+$ = 863.4849; m/z found = 863.4850.

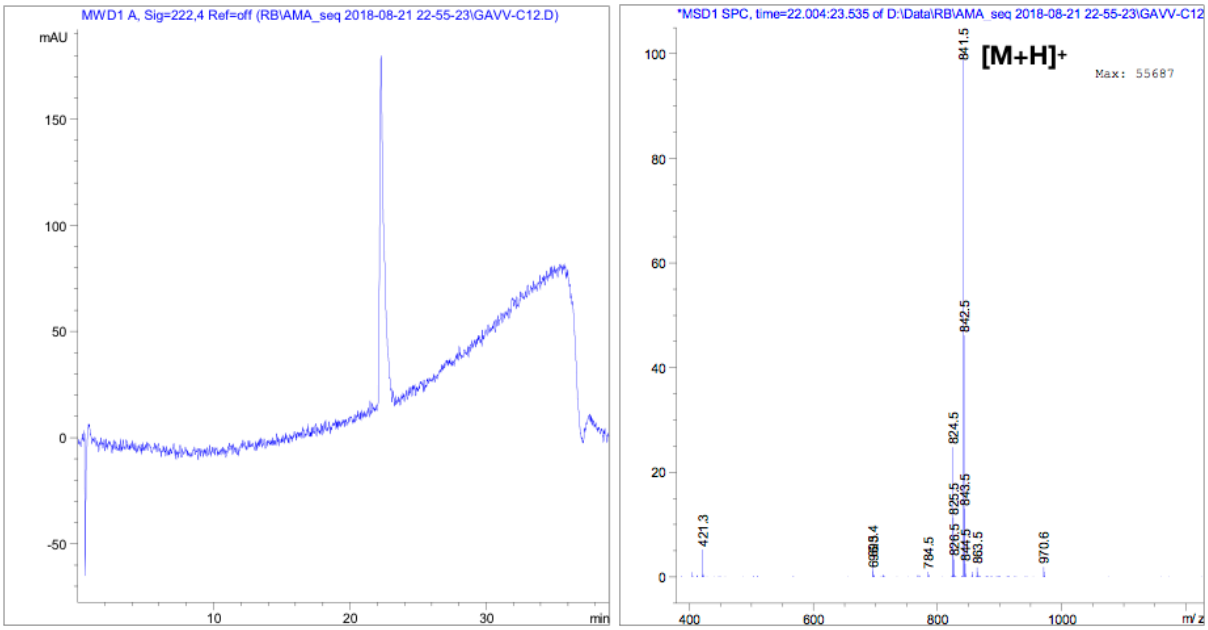

Figure S30 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P4T12****.*

P4T14 (EEGAVV-ON=$C_{14}H_{27}$): ***R_t* 25.5 min (Fig. S31) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{41}H_{72}N_8NaO_{12}]^+$ = 891.5162; m/z found = 891.5164.

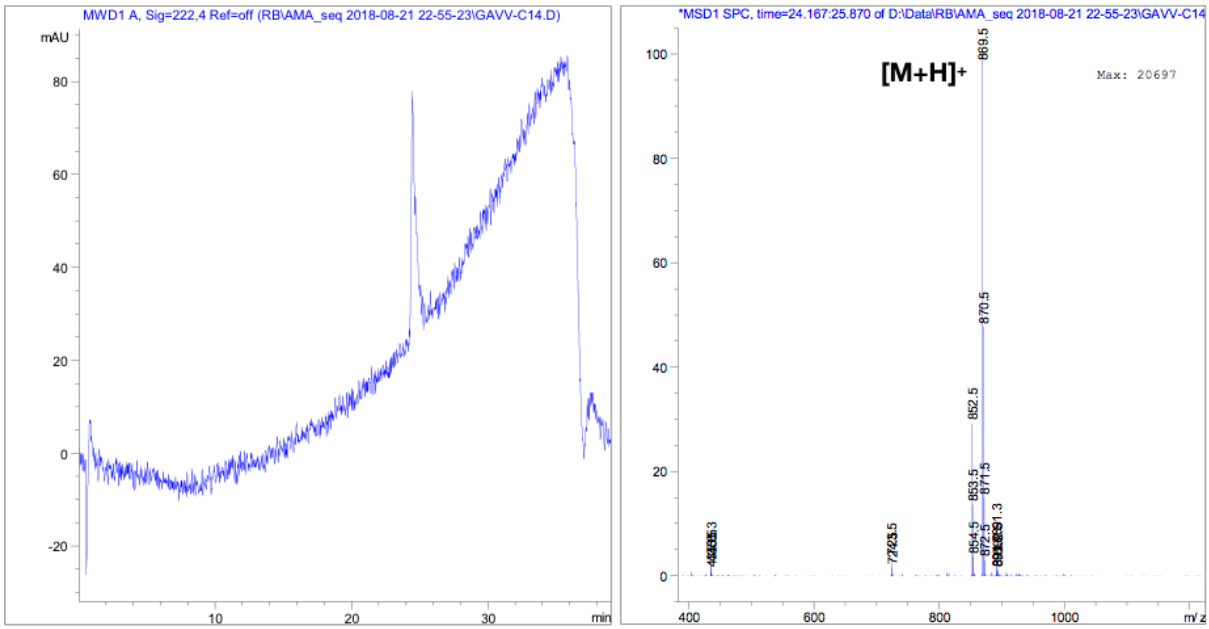

Figure S31 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P4T14****.*

P5T8 (EEAAVV-ON=C_8H_{15}): ***R*$_t$ 19 min (Fig. S32) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. **HR-MS** (ESI, +eV) m/z calculated for $[C_{36}H_{63}N_8O_{12}]^+$ = 799.4560; m/z found = 799.4558.

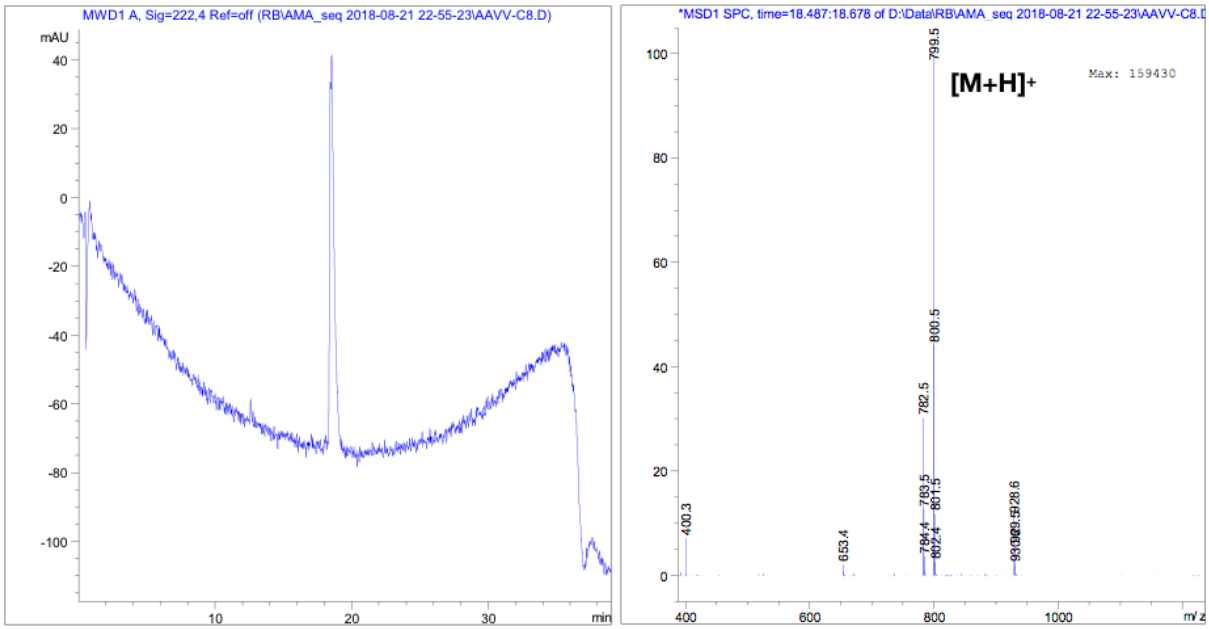

Figure S32 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P5T8****.*

P5T10 (EEAAVV-ON=$C_{12}H_{23}$): ***R_t* 23 min (Fig. S33) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. **HR-MS** (ESI, +eV) m/z calculated for $[C_{38}H_{67}N_8O_{12}]^+$ = 827.4873; m/z found = 827.4878.

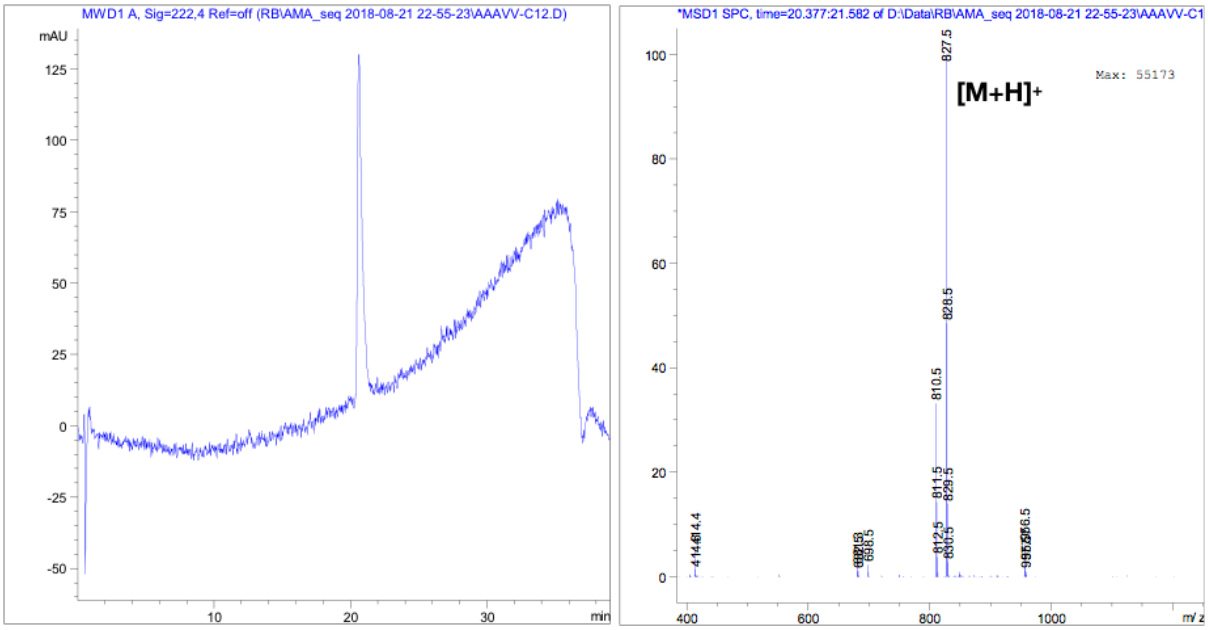

Figure S33 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P5T10****.*

P5T12 (EEAAVV-ON=$C_{12}H_{23}$): ***R_t* 23.5 min (Fig. S34) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. **HR-MS** (ESI, +eV) m/z calculated for $[C_{40}H_{71}N_8O_{12}]^+$ = 855.5186; m/z found = 855.5187.

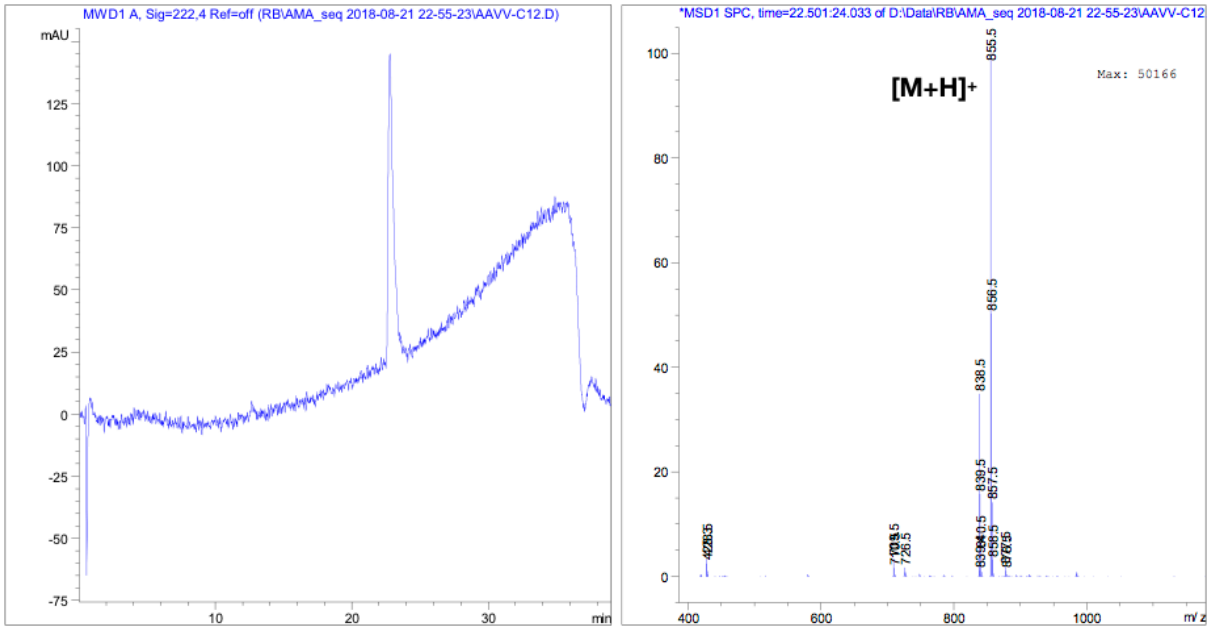

Figure S34 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P5T12****.*

P5T14 (EEAAVV-ON=$C_{14}H_{27}$): ***R_t* 25.5 min (Fig. S35) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. **HR-MS** (ESI, +eV) m/z calculated for $[C_{42}H_{75}N_8O_{12}]^+$ = 883.5499; m/z found = 883.5497.

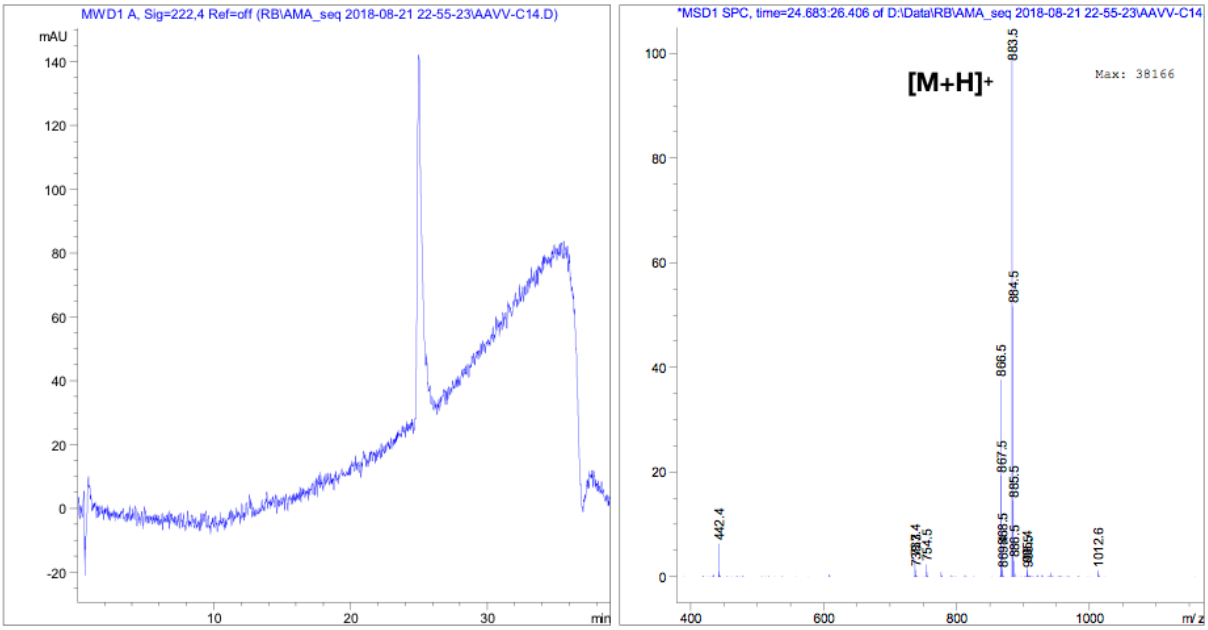

Figure S35 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P5T14****.*

P6T8 (EEAAAVV-ON=C_8H_{15}): ***R*$_t$ 19.5 min (Fig. S36) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. **HR-MS** (ESI, +eV) m/z calculated for $[C_{39}H_{68}N_9O_{13}]^+$ = 870.4931; m/z found = 870.4930.

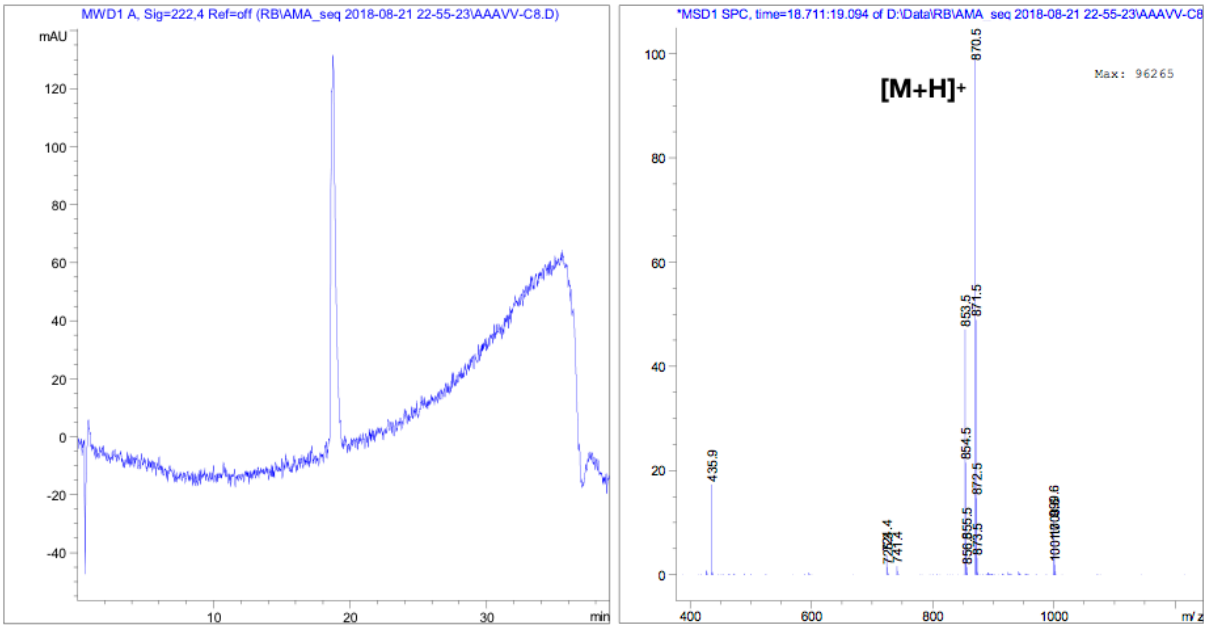

Figure S36 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P6T8****.*

P6T10 (EEAAAVV-ON=$C_{10}H_{19}$): ***R_t* 21.5 min (Fig. S37) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)].

HR-MS (ESI, +eV) m/z calculated for $[C_{41}H_{72}N_9O_{13}]^+$ = 898.5244; m/z found = 898.5244.

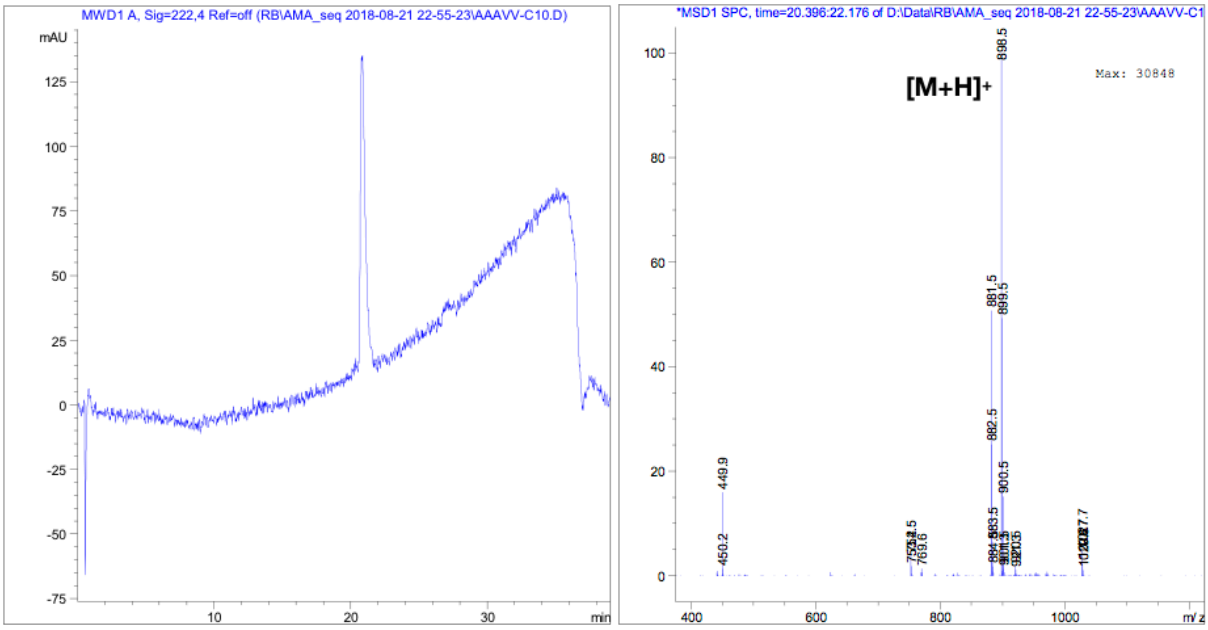

Figure S37 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P6T10****.*

P6T12 (EEAAAVV-ON=$C_{12}H_{23}$): ***R*$_t$ 24 min (Fig. S38) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. **HR-MS** (ESI, +eV) m/z calculated for $[C_{43}H_{76}N_9O_{13}]^+$ = 926.5557; m/z found = 926.5554.

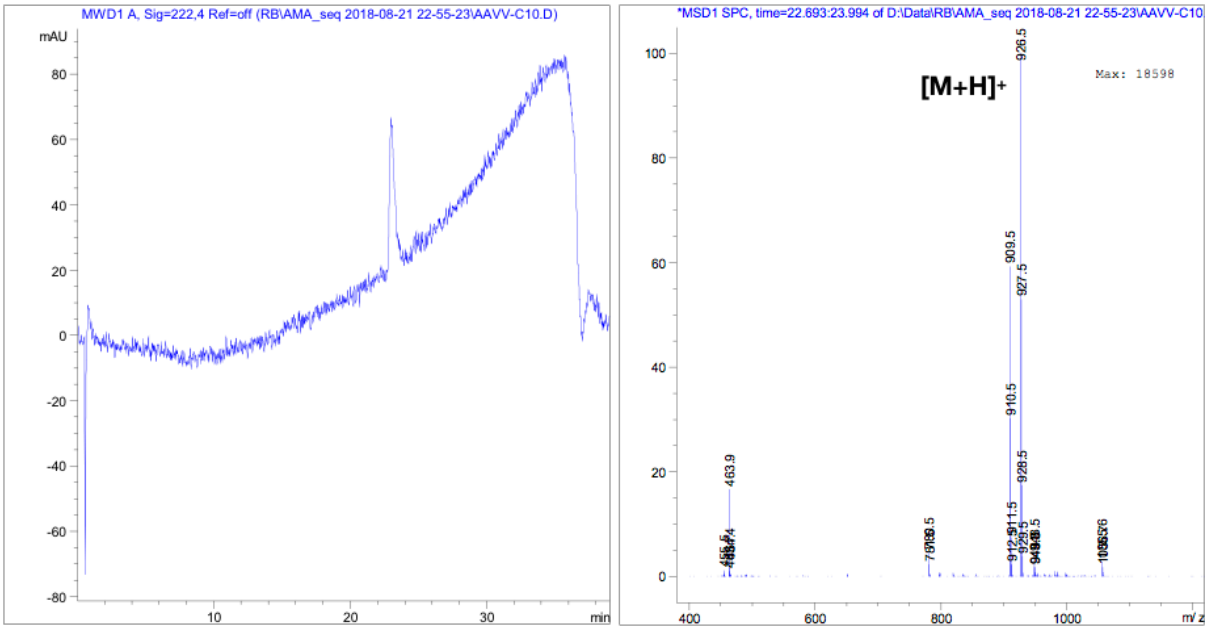

Figure S38 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P6T12****.*

P6T14 (EEAAAVV-ON=$C_{14}H_{27}$): ***R*$_t$ 26 min (Fig. S39) RP-HPLC** [Agilent SB-C18, H_2O (0.1% TFA)/MeCN (0.1% TFA) 100:0 (0→2 min), 100:0→5:95 (2→32 min), 0:100 (>32 min)]. **HR-MS** (ESI, +eV) m/z calculated for $[C_{45}H_{80}N_9O_{13}]^+$ = 954.5870; m/z found = 954.5874.

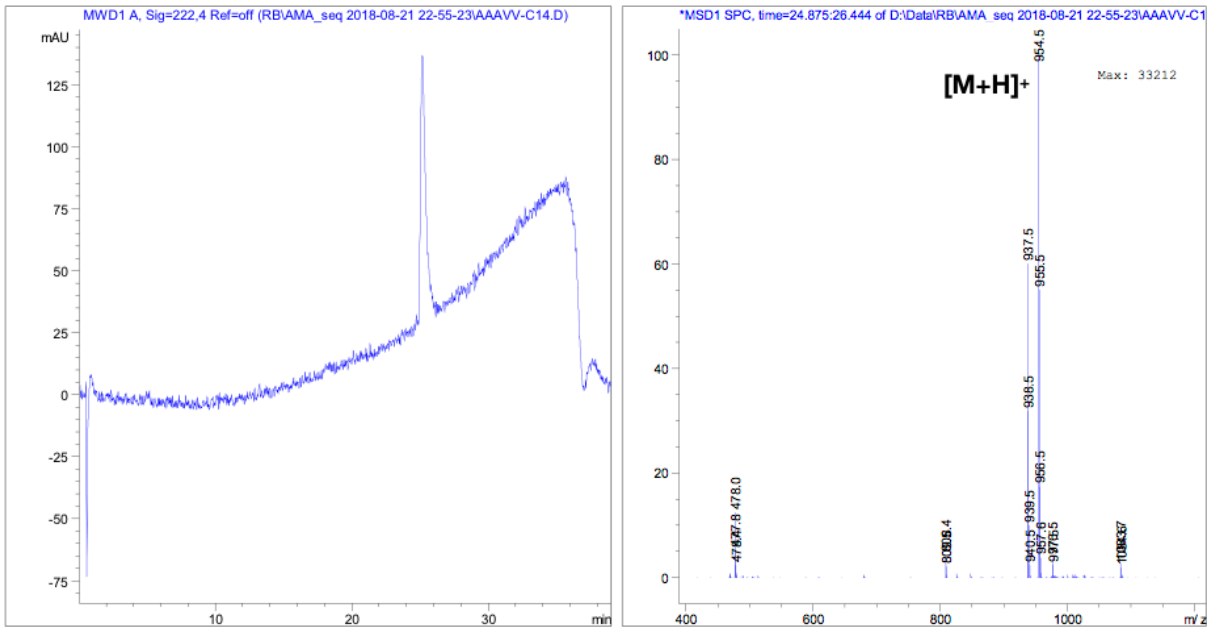

Figure S39 *HPLC (UV-Vis detection at 222 nm; left) and MS (ESI +eV; right) of amphiphile* ***P6T14****.*

4. Microscopy images (fluorescence/STEM)

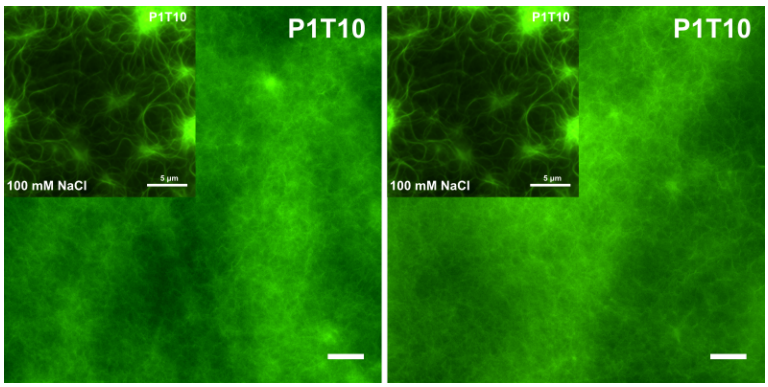

Figure S40 Fluorescence microscopy images of **P1T10** peptide amphiphile (10 mg·mL^{-1}, 50 mM Tris-HCL, pH 7.5) after self-assembly overnight. For comparison, inset pictures represent **P1T10** in the same buffer with 100 mM NaCl (**Figure 2**). All samples self-assembled on a glass slide, main image scale bars = 20 µm, inset scale bars = 5 µm.

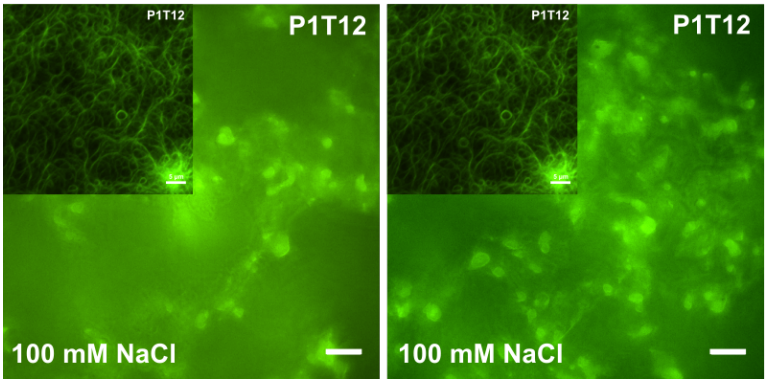

Figure S41 Fluorescence microscopy images of **P1T12** peptide amphiphile (10 mg·mL^{-1}, 50 mM Tris-HCL, pH 7.5) after self-assembly overnight in the presence of NaCl (100 mM). For comparison, inset pictures represent **P1T12** in the same buffer without NaCl (**Figure 2**). All samples self-assembled on a glass slide, main image scale bars = 20 μm, inset scale bars = 5 μm.

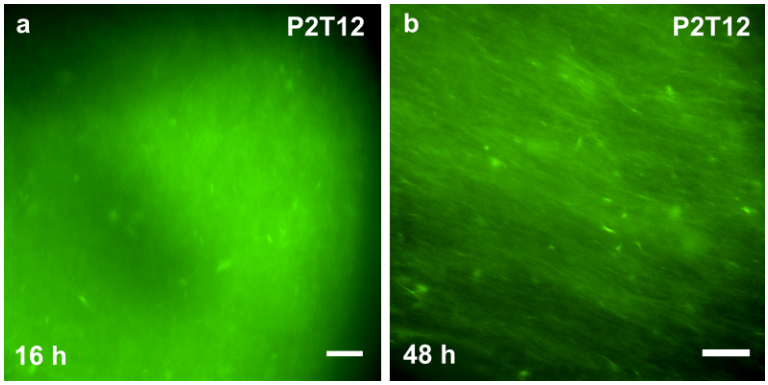

Figure S42 Fluorescence microscopy images of **P2T12** peptide amphiphile (10 mg·mL^{-1}, 50 mM Tris-HCl, pH 7.5) after self-assembly overnight (**a**) and after a period of 48 h (**b**), showing more defined fibres at longer times. All samples self-assembled on a glass slide, all scale bars = 20 μm.

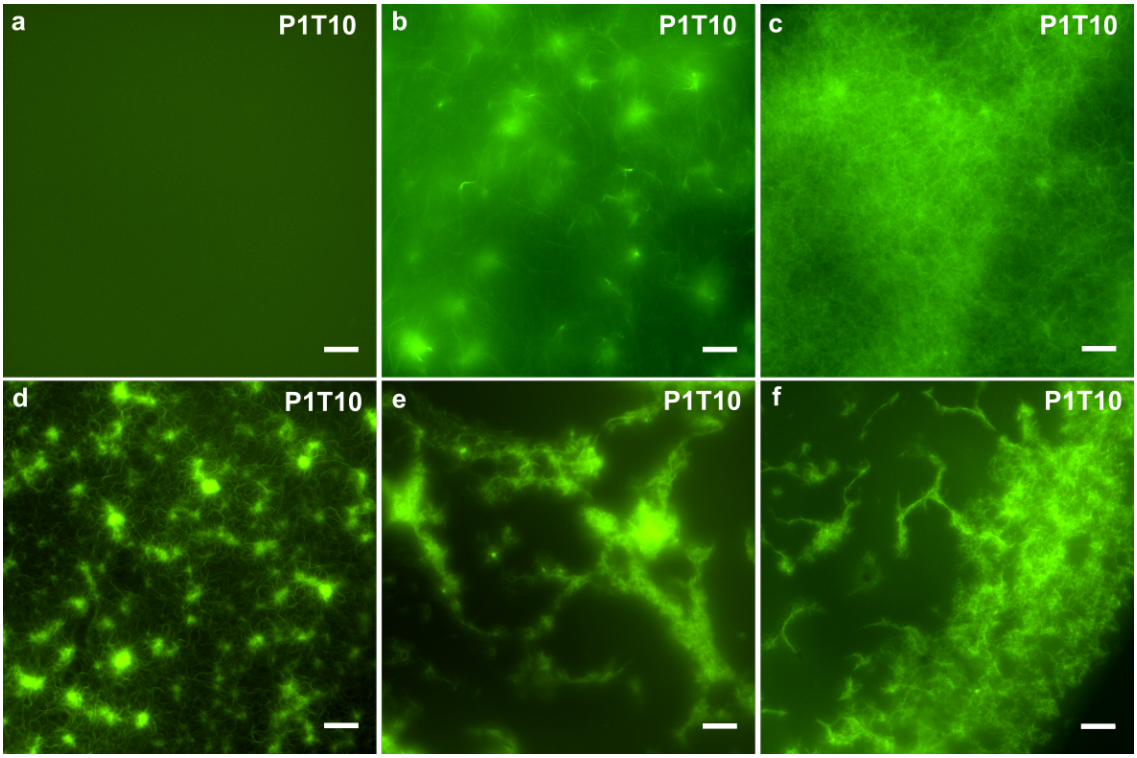

Figure S43 Fluorescence microscopy images of **P1T10** self-assembled under different experimental conditions: (**a**) in an Eppendorf tube at a concentration of 10 mg·mL^{-1} over a period of 2 weeks (50 mM Tris-HCl, pH 7.5, 100 mM NaCl), (**b**) in an Eppendorf tube at a concentration of 50 mg·mL^{-1} over a period of 2 weeks (50 mM Tris-HCl, pH 7.5, 100 mM NaCl), (**c**) on a glass slide over a period of 16 h, (50 mM Tris-HCl, pH 7.5) (**d**) on a glass slide over a period of 16 h after the addition of 100 mM NaCl (50 mM Tris-HCl, pH 7.5) and (**e,f**) on a hydrophobic glass slide (coated with (1*H*,1*H*,2*H*,2*H*-perfluorooctyl)silane) at a concentration of 10 mg·mL^{-1} (50 mM Tris-HCl, pH 7.5, 100 mM NaCl): (**e**) centre of droplet and (**f**) left hand side of droplet. All scale bars = 20 μm.

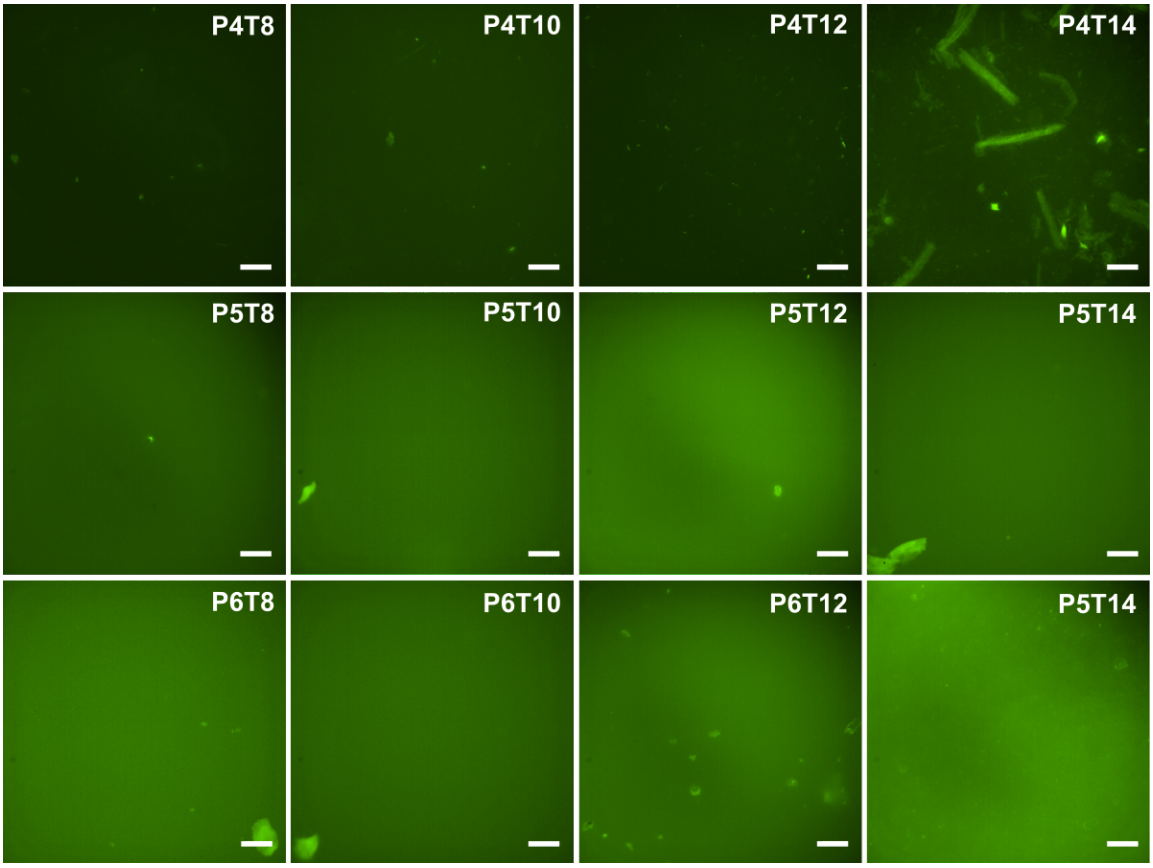

Figure S44 Fluorescence microscopy example images showing the lack of ordered self-assembly on the micron scale in anionic peptide amphiphiles at 10 mg·mL^{-1} (100 mM HEPES, pH 7.5, 100 mM NaCl). All samples self-assembled on a glass slide, all scale bars = 20 μm.

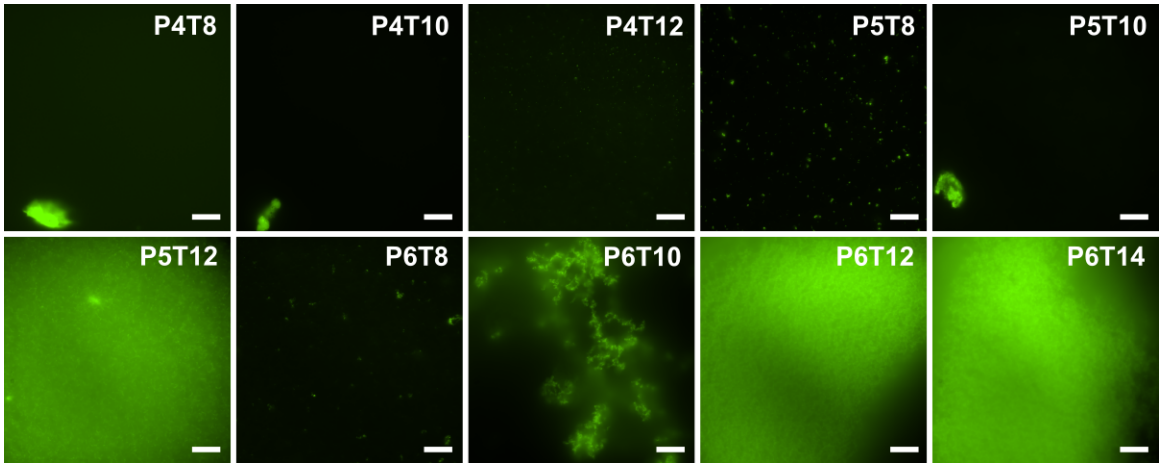

Figure S45 Fluorescence microscopy images of anionic peptide amphiphiles showing the lack of self-assembly or disordered aggregation at 0.5 mg·mL^{-1} (100 mM HEPES, pH 7.5, 100 mM NaCl). All samples self-assembled on a glass slide, all scale bars = 20 μm.

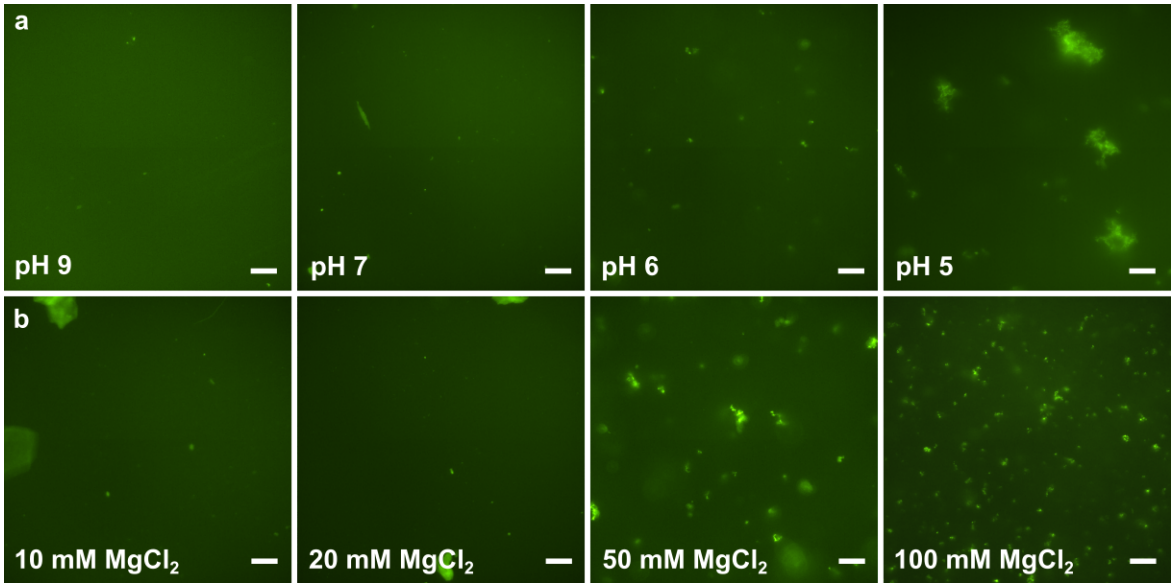

Figure S46 Fluorescence microscopy of **P5T8** self-assembly under different conditions after 1 h at a peptide concentration of 0.5 mg·mL^{-1} (**a**) range of pH (100 mM HEPES, 100 mM NaCl and 10 mM $MgCl_2$) and (**b**) range of $MgCl_2$ concentrations (100 mM HEPES, pH 7.5, 100 mM NaCl). All samples self-assembled on a glass slide, all scale bars = 20 μm.

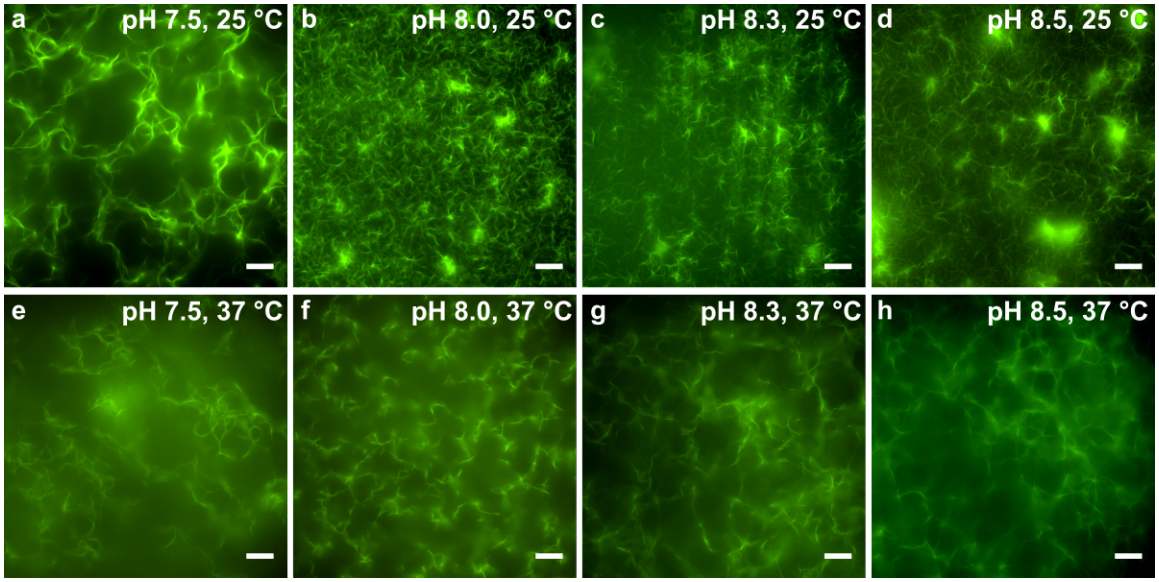

Figure S47 Fluorescence microscopy images of **P5T14** (0.5 mg·mL^{-1}) prepared in 100 mM HEPES, 100 mM NaCl, 10 mM $MgCl_2$ at different pH and temperature: (**a-d**) at 25 °C *versus* pH; (**e-h**) at 37 °C *versus* pH. All samples self-assembled on a glass slide, all scale bars = 20 μm.

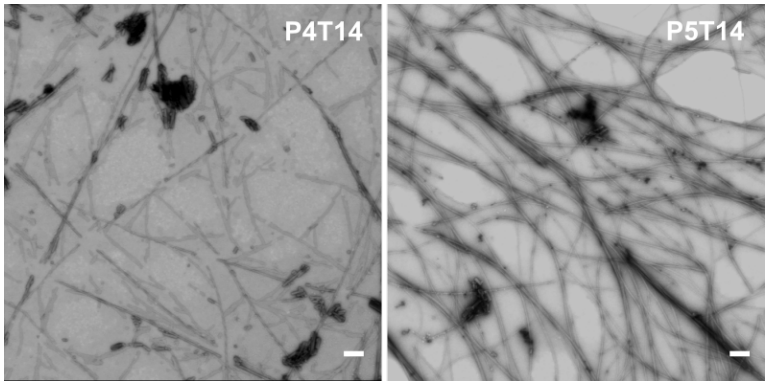

Figure S48 STEM images of **P4T14** and **P5T14** forming nanofibers at a concentration of 0.5 mg·mL^{-1} (100 mM HEPES, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$) and a self-assembly time of 10 min on a TEM copper grid. Scale bars = 200 nm.

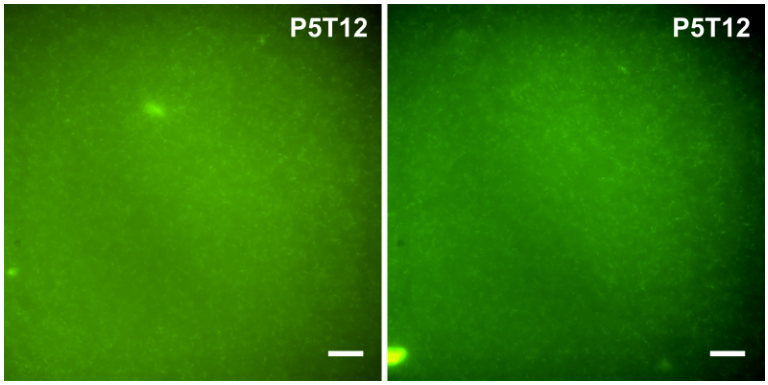

Figure S49 Fluorescence microscopy images of **P5T12** forming small fibre-like aggregates at a concentration of 0.5 mg·mL^{-1} (100 mM HEPES, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$) but no micron-sized fibres. All samples self-assembled on a glass slide, all scale bars = 20 μm.

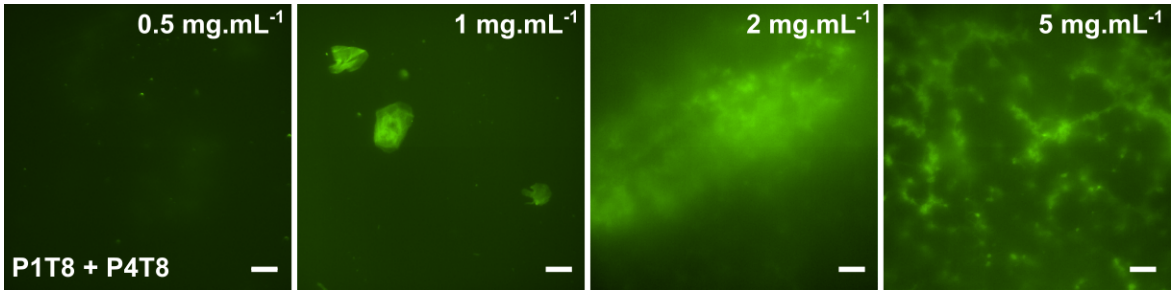

Figure S50 Fluorescence microscopy of 1:1 mixtures of cationic and anionic peptide amphiphiles (**P1T8** + **P4T8**) with matching central sequence (GAVV) and tail (T8) after 1 h (100 mM HEPES, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$). Final concentrations of each peptide between 0.5-5 mg·mL^{-1}. All samples self-assembled on a glass slide, all scale bars = 20 μm.