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Supporting Information

Optimization and Automation of Helical Aromatic Oligoamide Foldamer Solid-Phase Synthesis

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1. Materials and Methods

Chemicals and reagents were used as commercially supplied without any further purification unless otherwise stated. Low loading Wang resin (0.41 mmol/g) was purchased from Novabiochem. Ghosez reagent (1-chloro-*N*,*N*,2-trimethyl-1 propenylamine) was purchased from Sigma Aldrich. *N,N*-diisopropylethylamine (DIPEA) was distilled over calcium hydride. Reactions requiring anhydrous conditions were performed under nitrogen. Quinoline monomers (O), (D, D'), and (L) were synthesized following procedures reported previously by our group.[1]

Analytical grade organic solvents were used for solid phase synthesis. Anhydrous THF and CH_2Cl_2 for solid phase synthesis were dispensed from an MBRAUN SPS-800 solvent purification system. Peptide synthesis-grade DMF was purchased from Carlo Erba and NMP from IRIS Biotech. HPLC grade acetonitrile and MilliQ water were used for RP-HPLC analyses and purification. RP-HPLC analyses were performed at 1.0 mL.min^{-1} by using a Macherey–Nagel Nucleodur C18 HTec column (4 x 100 mm, 5 µm). The mobile phase was composed of 0.1% (v/v) TFA/H_2O (solvent A) and 0.1% TFA/CH_3CN (solvent B). Monitoring was performed by UV detection at 214, 254 and 300 nm with a diode array detector. Semi-preparative RP-HPLC purifications of oligomers were performed at 5 mL.min^{-1} by using a Macherey-Nagel Nucleodur C18 HTEC column (10 x 125 mm, 5 µm). The mobile phase was the same as for the analytic inJections. Monitoring was performed by UV detection at 300 nm.

NMR spectra were recorded on Avance III NMR spectrometer (Bruker Biospin) with a vertical 16.45 T narrowbore/ultrashield magnet operating at 700 MHz for ^1H observation by means of a 5-mm TXI $^1H/^{13}C/^{15}N$ probe with single axis Z-gradient capabilities. Chemical shifts are reported in ppm and are calibrated against residual solvent signal of DMSO-d_6 (δ 2.50). Data processing was performed with Bruker TOPSPIN 2.1 software.

LC-ESI-MS spectra were recorded on Agilent Technologies 6230 Time of Flight LC/MS for foldamers **1**-**7.** The analyses were performed at 0.4 mL.min^{-1} by using a Macherey–Nagel Nucleoshell C18 RP 18plus column (2 x 50 mm, 2.7 µm). 0.1% formic acid was added to the aqueous mobile phase (solvent A) and acetonitrile (solvent B). The column eluent was monitored by UV detection at 214, 254, and 300 nm with a diode array detector. LC-ESI-MS spectra of foldamers **8** and **9** were recorded on a Bruker microTOF II in positive ionization mode. The HPLC line was an Ultimate 3000 RP-HPLC System (ThermoFisher Scientific) equipped with a Nucleodur C18 Gravity column (2 x 50 mm, 1.8 µm, Macherey-Nagel) at a flow rate of 0.33 ml.min^{-1} with the same set of solvents and UV detection mode to the previous analyses.

1.1. Methods for RP-HPLC analysis

Solutions of foldamers **1**-**9** (100 µM) were prepared in a mixture of 0.1% (v/v) TFA/H_2O and 0.1% TFA/CH_3CN. HPLC chromatograms of crude and pure oligomers were recorded at r.t.

1.2. Methods for NMR analysis

Solutions of foldamers **1-7** (1 mM) were prepared in DMSO-d_6 (99.9%). ^1H NMR spectra were recorded at 333 K using a water suppression sequence (watergate).

1.3. Methods for LC-ESI-MS analysis

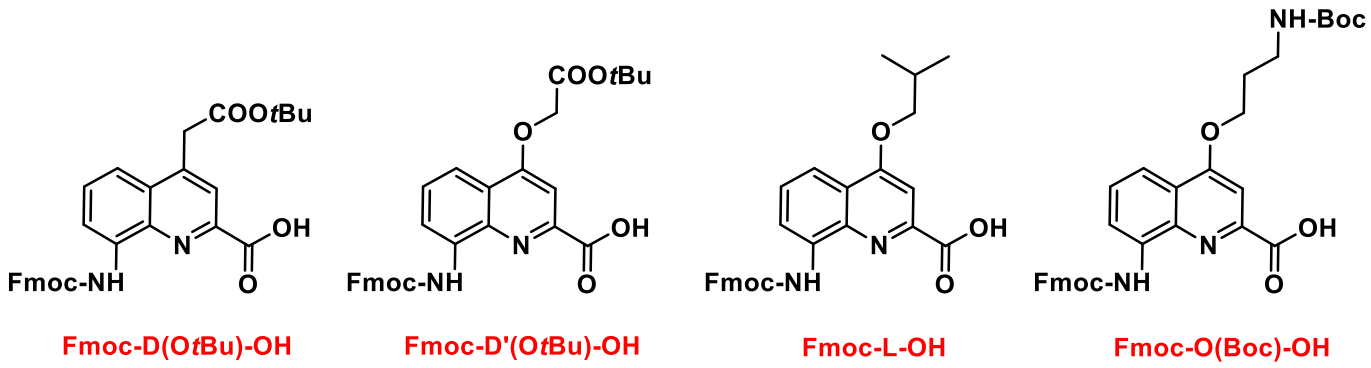
LC-ESI-MS experiments were performed in positive ion mode. Solutions of oligomers **1**-**9** were prepared in a mixture of 0.1% (v/v) formic acid in H_2O (solvent A) and 0.1% formic acid in CH_3CN (solvent B). Foldamers **1**-**4** were analyzed by using a flow rate of 0.4 ml.min^{-1} and a gradient from 10% B to 100% B in 5 min.

Foldamers **5**-**7** were analyzed by using a flow rate of 0.4 ml.min^{-1} and a gradient from 20% B to 100% B in 5 min.

Foldamers **8** and **9** were analyzed by using a flow rate of 0.33 ml.min^{-1} and a gradient from 20% B to 100% B in 8 min.

2. Experimental section

2.1. Manual microwave-assisted SPFS of foldamers 1-4



Bromination of low loading Wang resin: Low loading Wang resin (150 mg, 0.0615 mmol) was swollen in 2 mL anhydrous DMF for 1 h under N_2. PPh_3 (81 mg, 0.3075 mmol, 5.0 equiv.) and CBr_4 (103 mg, 0.3075 mmol, 5 equiv.) were then added in that order and the resin was stirred overnight under N_2 at room temperature. The resin was filtered off, and washed with anhydrous DMF (3 x 3 mL), DCM (3 x 3 mL) and isopropanol (3 x 3 mL, sequence repeated three times) and then dried under high vacuum.

Loading of first monomer unit: Low loading bromo-Wang resin (150 mg, 0.0615 mmol) was swollen in anhydrous DMF (2 mL) for 1 h under N_2. Fmoc-O(Boc)-OH (178 mg, 0.1845 mmol, 3.0 equiv.) and CsI (48 mg, 0.1845 mmol, 3.0 equiv.) were then added, followed by DIEA (64 μL, 0.369 mmol, 6.0 equiv.). The reaction vessel was then placed under microwave irradiation (50 W, ramp to 50 ºC over 5 min, then hold at 50 ºC for 5 min). The resin was washed with anhydrous DMF (3 x 3 mL) and the process was repeated once. The resin was then washed thoroughly with DMF (6 x 3 mL).

General procedure for Fmoc deprotection: To Fmoc- O(Boc)-Wang resin was added a 20% solution of piperidine in DMF (4 mL) and the resin was stirred for 3 min at room temperature. The resin was washed with DMF (3 x 3 mL) and the deprotection step was repeated once for 7 min. After filtration, the resin was then washed thoroughly with DMF (3 x 3 mL) and anhydrous THF (3 x 3 mL) prior to coupling reaction.

General procedure for acid chloride activation exemplified by preparation of Fmoc-D(O*t*Bu)-Cl: Fmoc-D(O*t*Bu)-OH (200 mg, 0.369 mmol) was dissolved in anhydrous DCM (3 mL) under N_2, and 1-chloro-*N,N*,2-trimethyl-1-propenylamine (100 µL, 0.718 mmol) was added. The mixture was stirred for 1 h before solvent removal and drying under high vacuum.

General procedure of Fmoc-Q-Cl coupling on Wang resin-bound oligoquinoline, exemplified with Fmoc-D(O*t*Bu)-Cl: To a H-$(Q)_n$-Wang resin (150 mg, 0.0615 mmol) swollen in anhydrous THF (1.5 mL), was added DIEA (64 µL, 0.369 mmol, 6.0 equiv.). Next, freshly prepared Fmoc-D(O*t*Bu)-Cl was dissolved in anhydrous THF (3 mL) and 1.5 mL of this solution (0.184 mmol, 3 equiv.) was added to the resin. The reaction vessel was then placed under microwave irradiation (50 W, ramp to 50° C over 5 min, then hold at 50 °C for

15 min). The resin was filtered off and washed with anhydrous THF (3 x 3 mL) and the coupling was repeated once. The resin was filtered off before and washed thoroughly with DMF (6 x 3 mL).
The same deprotection/coupling cycles were performed until 8-mer **O-D-O-L-D-O-D-O-Wang**. This sequence is common for the SPFS of all the oligomers. The resin was then divided into several batches to prepare foldamers **1**-**7**.
General procedure for resin cleavage and side chain deprotection: The resin-bound foldamer was washed with DMF (3 x 3 mL), DCM (3 x 3 mL) before to be dried under desiccator. The resin was then suspended in a solution of TFA/TIS/H_2O (95:2.5:2.5, v/v/v, 4-5 ml) and next was shaken for 2 h at room temperature. The resin was then filtered off and washed once with the TFA solution. The combined filtrate was evaporated under reduced pressure. The resulting oily solid was precipitated with cold Et_2O, triturated, washed with Et_2O, centrifuged and lyophilized to obtain the crude foldamer.

Compound 1: Foldamer **1** was purified by RP-HPLC semi-preparative chromatography using a linear gradient from 5% B to 40% B in 20 min (C_{18} column; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; HPLC purity >99%). **^{1}H NMR** (700 MHz, DMSO-d_6): **amide NHs** δ 10.90 (s, 1H), 10.84 (s, 1H), 10.74 (s, 2H), 10.27 (d, *J* = 17.8 Hz, 2H), 10.14 (d, *J* = 15.6 Hz, 2H), 10.05 (s, 1H), 9.82 – 9.77 (m, 5H); **aromatic CHs** δ 8.20 (s), 7.95 (t, *J* = 7.1 Hz), 7.82 (d, *J* = 6.8 Hz), 7.62 – 7.46 (m), 7.45 – 7.31 (m), 7.29 – 7.09 (m), 7.05 (t, *J* = 6.9 Hz), 7.02 – 6.89 (m), 6.85 (d, *J* = 6.5 Hz), 6.81 – 6.78 (m), 6.75 (d, *J* = 6.5 Hz), 6.71 (s), 6.67 (s), 6.56 (s), 6.44 (s), 6.34 (s), 6.28 (s), 6.23 (s), 6.18 (d, *J* = 5.8 Hz), 5.88 (s), 5.66 (s), 5.54 (d, *J* = 13.9 Hz), 5.41 (s); **aliphatic CHs** δ 4.47 – 3.73 (m), 3.05 – 2.96 (m), 2.32 – 1.96 (m), 1.91 (s), 1.65 – 1.47 (m), 1.31 – 1.19 (m), 1.15 (d, *J* = 6.6 Hz). **HRMS** (ESI^+) m/z calcd. (most abundant mass peak) for $C_{194}H_{181}N_{38}O_{37}$: 726.8684 $(M+5H)^{5+}$; found: 726.9652.

Compound 2: Foldamer **2** was purified by RP-HPLC semi-preparative chromatography using a linear gradient from 5% B to 40% B in 20 min (C_{18} column; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; HPLC purity >99%). **^{1}H NMR** (700 MHz, DMSO-d_6): **amide NHs** δ 10.89 (s, 1H), 10.83 (d, *J* = 17.8 Hz, 2H), 10.74 (s, 1H), 10.31 (d, *J* = 12.4 Hz, 2H), 10.27 (s, 1H), 10.10 (s, 1H), 10.06 (s, 1H), 9.92 (d, *J* = 14.4 Hz, 2H), 9.88 (s, 1H), 9.85 (s, 1H); **aromatic CHs** δ 8.30 (s), 7.96 (d, *J* = 7.0 Hz), 7.82 (d, *J* = 6.9 Hz), 7.63 – 7.43 (m), 7.41 – 7.33 (m), 7.29 – 7.20 (m), 7.20 (d, *J* = 7.5 Hz), 7.18 – 7.13 (m), 7.12 (dd, *J* = 13.3, 6.0 Hz), 7.05 (dt, *J* = 17.9, 8.7 Hz), 7.03 – 6.94 (m), 6.94 – 6.85 (m), 6.81 (d, *J* = 6.5 Hz), 6.75 (t, *J* = 6.1 Hz), 6.70 (s), 6.57 (s), 6.45 (s), 6.36 (d, *J* = 9.5 Hz), 6.18 (d, *J* = 6.1 Hz), 5.89 (s), 5.65 – 5.57 (m), 5.56 (s); **aliphatic CHs** δ 4.24 – 3.80 (m), 3.26 – 3.13 (m), 3.09 – 2.90 (m), 2.36 – 2.11 (m), 2.09 – 2.0 (m), 1.30 – 1.20 (m). **HRMS** (ESI^+) m/z calcd. (most abundant mass peaks) for $C_{181}H_{168}N_{35}O_{35}$: 678.4490 $(M+5H)^{5+}$; for $C_{181}H_{167}N_{35}O_{35}$: 848.0602 $(M+4H)^{4+}$; for $C_{181}H_{166}N_{35}O_{35}$: 1130.0768 $(M+3H)^{3+}$; found: 678.7491; 848.4468; 1130.2835.

Compound 3: Foldamer **3** was purified by RP-HPLC semi-preparative chromatography using a linear gradient from 5% B to 40% B in 20 min (C_{18} column; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; HPLC purity >99%). **^{1}H NMR** (700 MHz, DMSO-d_6): **amide NHs** δ 10.93 (s, 1H), 10.84 (s, 1H), 10.75 (s, 2H), 10.33 (s, 1H), 10.13 (s, 4H), 9.95 (s, 1H), 9.93 (s, 1H), 9.87 (s, 1H), 9.82 (s, 1H); **aromatic CHs** δ 8.21 (s), 7.97 (s), 7.82 – 7.79 (m), 7.60 – 6.65 (m), 6.44 (s), 6.39 (s), 6.33 (s), 6.23 – 6.16 (m, 5H), 5.91 (s), 5.76 (s), 5.65 (d, *J* = 17.3 Hz), 5.45 (s); **aliphatic CHs** δ 4.35 – 3.94 (m), 3.11 – 2.90 (m), 2.37 – 1.94 (m), 1.31 – 1.19 (m). **HRMS** (ESI^+) m/z calcd. (most abundant mass peaks) for $C_{181}H_{168}N_{35}O_{35}$: 678.4490 $(M+5H)^{5+}$; for $C_{181}H_{167}N_{35}O_{35}$: 847.8090 $(M+4H)^{4+}$; for $C_{181}H_{166}N_{35}O_{35}$: 1130.0768 $(M+3H)^{3+}$; found: 678.5468; 847.9452; 1130.2755.

Compound 4: Foldamer **4** was purified by RP-HPLC semi-preparative chromatography using a linear gradient from 5% B to 40% B in 20 min (C_{18} column; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; HPLC purity

>99%). **^{1}H NMR** (700 MHz, DMSO-d_6): **amide NHs** δ 10.97 (s, 1H), 10.81 (d, *J* = 13.3 Hz, 2H), 10.72 (s, 1H), 10.32 (s, 1H), 10.27 (s, 1H), 10.22 (d, *J* = 12.5 Hz, 2H), 10.12 (s, 1H), 9.95 (s, 2H), 9.91 (s, 1H), 9.87 (s, 1H); **aromatic CHs** δ 8.21 (s), 7.97 (d, *J* = 6.9 Hz), 7.82 (d, *J* = 6.9 Hz), 7.65 – 7.45 (m), 7.44 – 7.33 (m), 7.29 (t, *J* = 7.4 Hz), 7.26 – 7.19 (m), 7.14 – 7.05 (m), 7.02 – 6.88 (m), 6.80 (d, *J* = 6.4 Hz), 6.76 (s), 6.69 (d, *J* = 8.2 Hz), 6.43 (s), 6.35 (d, *J* = 14.9 Hz), 6.20 (d, *J* = 6.8 Hz), 6.15 (s), 5.92 (s), 5.78 (s), 5.67 (s), 5.59 (s), 5.49 (s); **aliphatic CHs** δ 4.35 – 3.77 (m), 3.26 – 3.16 (m), 3.09 – 2.90 (m), 2.38 – 2.21 (m), 2.19 – 2.10 (m), 2.07 – 1.97 (m), 1.28 (t, *J* = 6.8 Hz, 14H), 1.25 – 1.21 (m). **HRMS** (ESI$^+$) m/z calcd. (most abundant mass peaks) for $C_{180}H_{167}N_{36}O_{35}$: 678.6480 $(M+5H)^{5+}$; for $C_{180}H_{166}N_{36}O_{35}$: 848.0582 $(M+4H)^{4+}$; for $C_{180}H_{165}N_{36}O_{35}$: 1130.4085 $(M+3H)^{3+}$; found: 678.7487; 847.9458; 1130.6132.

2.2. Manual microwave-assisted SPFS of foldamers 5-7

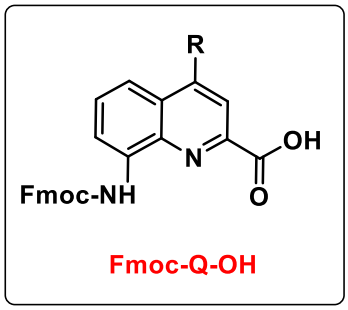
The SPFS of oligomers **5**-**7** was performed as described before.[10a] The only difference was the introduction of a capping step after each quinoline monomer coupling. Resin-bound foldamer (35 mg, 0.0143 mmol) was suspended in anhydrous THF (1 mL), to which was added DIEA (30 µL, 0.1722 mmol, 12.0 equiv.) followed by Ac-Cl (6 µL, 0.0861 mmol, 6.0 equiv.). The resin was shaken at r.t. for 5 min, washed with anhydrous THF (3 x 3 mL), and the capping step was repeated twice. The resin was then washed thoroughly with THF (6 x 3 mL) and DMF (6 x 3 mL).

Compound 5: Foldamer **5** was purified by RP-HPLC semi-preparative chromatography using a linear gradient from 20% B to 40% B in 35 min (C_{18} column; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; HPLC purity >99%). **^{1}H NMR** (700 MHz, DMSO-d_6): **amide NHs** δ 10.64 (s, 1H), 10.61 (s, 1H), 10.53 (s, 2H), 10.08 (s, 1H), 10.04 (s, 1H), 9.95 (s, 1H), 9.88 (s, 1H), 9.78 (s, 1H), 9.70 (s, 1H), 9.51 – 9.46 (m, 4H), 9.42 (s, 1H), 9.37 (s, 1H), 9.34 – 9.07 (m, 15H); **aromatic CHs** δ 8.01 (s), 7.78 (d, *J* = 6.3 Hz), 7.65 (d, *J* = 6.4 Hz), 7.44 – 7.26 (m), 7.23 – 7.19 (m), 7.17 – 7.12 (m), 7.13 – 7.04 (m), 7.06 – 6.98 (m), 6.98 – 6.88 (m), 6.83 (t, *J* = 6.9 Hz), 6.79 (t, *J* = 6.3 Hz), 6.77 – 6.70 (m), 6.67 (m, *J* = 4.8 Hz), 6.61 – 6.45 (m), 6.42 – 6.29 (m), 6.23 (s), 6.07 (s), 5.96 (d, *J* = 6.9 Hz), 5.83 (d, *J* = 12.6 H), 5.79 (s), 5.74 (s), 5.71 (s), 5.67 (s), 5.49 (s), 5.47 (s), 5.40 (s), 5.33 (s), 5.30 (s), 5.26 (s), 5.24 – 5.20 (m), 5.17 (s), 5.16 – 5.11 (m), 5.09 (d, *J* = 7.1 Hz); **aliphatic CHs** δ 4.13 – 3.31 (m), 2.18 – 1.88 (m, 1H), 1.60 – 1.55 (m), 1.50 – 1.46 (m), 1.34 (d, *J* = 4.5 Hz), 1.19 – 1.14 (m), 1.09 (q), 1.00 – 0.89 (m, 31H). **HRMS** (ESI$^+$) m/z calcd. (most abundant mass peaks) for $C_{413}H_{379}N_{79}O_{77}$: 959.9783 $(M+8H)^{8+}$; for $C_{413}H_{378}N_{79}O_{77}$: 1096.9741 $(M+7H)^{7+}$; found: 959.9868; 1096.9943.

Compound 6: Foldamer **6** was purified by RP-HPLC semi-preparative chromatography using a linear gradient from 20% B to 40% B in 35 min (C_{18} column; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; HPLC purity >99%). **^{1}H NMR** (700 MHz, DMSO-d_6): **amide NHs** δ 10.73 (s, 1H), 10.64 (d, *J* = 15.1 Hz, 3H), 10.16 (s, 1H), 10.12 (s, 1H), 10.07 (s, 1H), 10.00 (s, 1H), 9.87 (s, 1H), 9.72 (s, 1H), 9.65 (s, 1H), 9.57 (s, 3H), 9.47 – 9.42 (m, 3H), 9.38 (d, *J* = 16.3 Hz, 2H), 9.33 – 9.18 (m, 18H), 9.13 (s, 1H), 9.10 (s, 1H); **aromatic CHs** δ 8.09 (s), 7.87 (d, *J* = 6.1 Hz), 7.74 (d, *J* = 6.4 Hz), 7.58 (d, *J* = 5.8 Hz), 7.52 – 7.38 (m), 7.36 – 7.28 (m), 7.27 – 7.16 (m), 7.16 – 7.06 (m), 7.06 – 6.97 (m), 6.96 – 6.87 (m), 6.86 – 6.31 (m), 6.16 (s), 6.11 (s), 6.05 (s), 5.98 (s), 5.91 (d, *J* = 7.0 Hz), 5.86 (d, *J* = 11.7 Hz), 5.79 (d, *J* = 9.4 Hz), 5.75 (d, *J* = 3.8 Hz), 5.68 (s), 5.57 (s), 5.49 (d, *J* = 6.8 Hz), 5.39 (d, *J* = 7.5 Hz), 5.32 (s), 5.28 (s), 5.25 (d, *J* = 10.6 Hz), 5.23 – 5.15 (m), 5.11 (s); **aliphatic CHs** δ 4.10 – 3.43 (m), 2.28 – 1.97 (m), 1.68 (m), 1.61 – 1.56 (m), 1.53 – 1.47 (m), 1.29 – 1.24 (m), 1.20 – 1.15 (m), 1.13 – 1.08 (m), 1.07 – 0.93 (m). **HRMS** (ESI$^+$) m/z calcd. (most abundant mass peaks) for $C_{516}H_{471}N_{98}O_{96}$: 1064.3901 $(M+9H)^{9+}$; for $C_{516}H_{470}N_{98}O_{96}$: 1197.3129 $(M+8H)^{8+}$; found: 1064.3789; 1197.3117.

Compound 7: Foldamer **7** was purified by RP-HPLC semi-preparative chromatography using a linear gradient from 20% B to 40% B in 35 min (C_{18} column; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; HPLC purity >99%). **^{1}H NMR** (700 MHz, DMSO-d_6): **amide NHs** δ 10.72 (s, 1H), 10.67 (s, 1H), 10.63 (d, *J* = 9.1 Hz, 2H), 10.15 (s, 1H), 10.11 (s, 1H), 9.96 (d, *J* = 11.6 Hz, 2H), 9.86 (s, 1H), 9.74 (s, 1H), 9.58 – 9.52 (m, 4H), 9.49 (s, 1H), 9.45 (s, 1H), 9.41 (s, 1H), 9.36 (d, *J* = 13.2 Hz, 3H), 9.31 – 9.16 (m, 18H), 9.12 (s, 1H), 9.09 (s, 1H); **aromatic CHs** δ 8.07 (s), 7.86 (d, *J* = 6.3 Hz), 7.73 (d, *J* = 6.3 Hz), 7.61 (d, *J* = 5.8 Hz), 7.55 – 7.35 (m), 7.34 – 7.26 (m), 7.23 (t, *J* = 8.2 Hz), 7.19 – 6.95 (m), 6.93 – 6.72 (m), 6.70 – 6.56 (m), 6.54 – 6.30 (m), 6.21 (s), 6.15 (s), 6.04 (s), 6.01 (s), 5.95 (s), 5.89 (d, *J* = 6.9 Hz), 5.85 (d, *J* = 14.3 Hz), 5.80 – 5.75 (m), 5.73 (s), 5.60 (s), 5.56 (s), 5.48 (s), 5.38 (s), 5.31 (s), 5.27 (s), 5.25 (s), 5.23 (s), 5.21 – 5.14 (m), 5.10 (s); **aliphatic CHs** δ 4.17 (s), 4.12 (s), 4.0 – 3.75 (m, 1H), 3.73 – 3.42 (m), 2.25 – 1.95 (m), 1.69 – 1.65 (m), 1.59 – 1.55 (m), 1.29 – 1.24 (m), 1.23 – 1.14 (m), 1.13 – 1.08 (m), 1.10 – 1.06 (m), 1.06 – 0.96 (m), 0.98 – 0.93 (m). **HRMS** (ESI$^+$) m/z calcd. (most abundant mass peaks) for $C_{529}H_{484}N_{101}O_{98}$: 1091.4013 $(M+9H)^{9+}$; for $C_{529}H_{483}N_{101}O_{98}$: 1227.7005 $(M+8H)^{8+}$; found: 1091.3884; 1227.6965.

2.3. Automation of the SPFS of foldamers 8-9



Construction of the library of Fmoc-Q-OH

In order to automate the SPFS of aromatic oligoamides on Chorus PurePep synthesizer, the first step was to build a library of aromatic monomers and to assign them a specific and unique location of amino acid bottle line. To do so an excel file was generated and each aromatic building block was named in a similar manner to the one or three letters code used for α-amino acid residues (Figure S1).

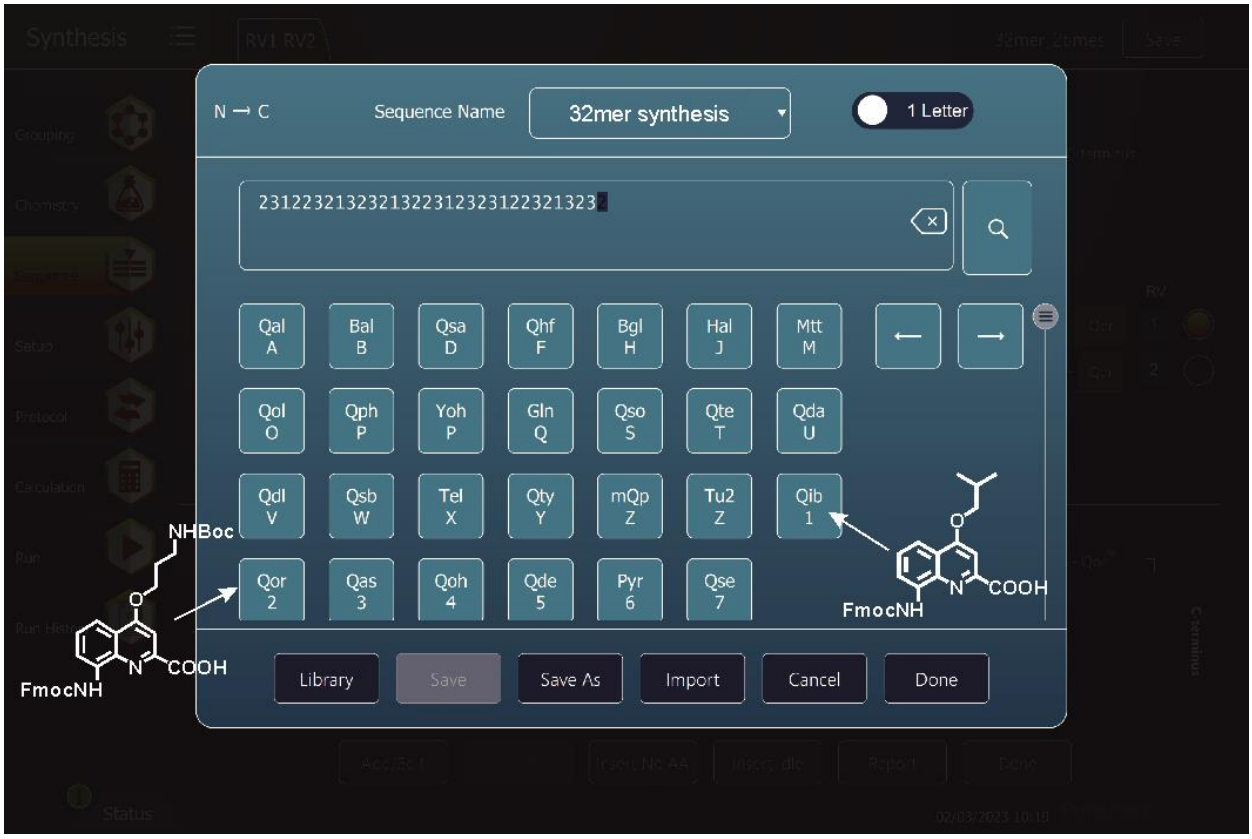


Figure S1. Illustration of the sequence building and the library of aromatic monomers with their one and three letter code, respectively.

2.4. Setting up the Chorus PurePep ® synthesizer for SPFS

Solvent lines and solvent distribution

Each bottle is always filled-up with the same solvent or reagent solution so that there will never be any contamination.

The two first solvent lines are meant for top and timed delivery. Peptide grade DMF was assigned in position 1 because it is the most used solvent for resin and system washings.

Anhydrous THF is in solvent line 2, 2% DBU in NMP in solvent 3 and when capping is required the line 4 is filled up with AC_2O in DCM (50/50, v/v).

For metered delivery, we use the solvent lines 5-7. Solvent line 5 for PPh_3 in anhydrous THF, line 6 TCAN in anhydrous THF and line 7 for 2,4,6-collidine in anhydrous THF.

If solvent line 1 and 2 deliver the solvent from the top of the RV, all the remaining lines perform solution dispensing from the bottom of the RV. The set-up of solvent is recapitulated in following table S1.

Table S1. Description of solvent set-up for SPFS

Solvent line	Solvent
1	DMF
2	Anhydrous THF
3	2% DBU in NMP
4	Ac_2O in DCM (50:50, v/v)
5	PPh_3 in anhydrous THF
6	TCAN in anhydrous THF
7	Collidine in anhydrous THF
8	DCM

The implementation of the coupling protocol for quinoline-type monomers on solid support follows the same series of steps for protocols earlier developed by Protein Technologies for SPPS. In other words, a coupling cycle in SPFS is composed of three main tasks: Deprotection, washings and coupling. A cycle begins always with the Fmoc-deprotection and ends with resin washings.

In the context of aromatic oligoamides, the deprotection is performed twice for 10 min with 2% DBU in NMP (step 1 in table S2). At the contrary to what has been reported for Fmoc deprotection in peptide chemistry, we have never observed any difficulty in removing the Fmoc protecting group on quinoline monomers. This deprotection step has not yet been optimized. However, because we favour high purity of the crude material with respect of synthesis time, we have not yet spent efforts in optimizing this specific step. If performed, it will be reported in due course.

Once the deprotection steps are completed, the resin is filtered off and washed (see steps 2 and 3 in table S2). The extensive washings with THF are meant for preparing the resin to the coupling in anhydrous THF. For the same reason although empty, the PV is rinsed twice with anhydrous THF (see line 4, PV box crossed) for pre-conditioning before the in situ activation process in this vessel.

Next, start the coupling phase. At first, like performed manually, the solution of collidine in anhydrous THF is added to the RV. The solution of aromatic monomer is then delivered to the PV and subsequently, PPh_3 solution in THF and at last TCAN solution in THF (steps 6-8 in table S2). The PV is then shaken for one minute and N_2 bubbling is performed for Fmoc-Q-Cl solution homogenisation. Because, we performed the acid chloride activation in pure THF, an eye-visible precipitate forms spontaneously upon TCAN addition. This precipitate has been characterized and does correspond to the phosphonium salt: $Cl^-,{}^+PPh_3\text{-}CH_2CN$. It is washed away at the end of the cycle by rinsing the PV with DMF (see step 25 in table S2).

After one minute of activation, the freshly made acid chloride solution is transferred to the RV by using the "*PV to RV operation*" (row 9 in table S2).

The coupling then takes place in the RV by heating it at 50 °C for 15 min. The monitoring of the temperature shows a fast ramping time and a stable target temperature over the course of the coupling step. At the end of the coupling cycle, the resin is filtered off and the steps 3 to 11 are repeated once.

Final washings of the RV and PV with DMF allow the resin to be placed in the conditions of the next cycle.

If a capping step is required, it is performed after the coupling steps (see steps 22 and 23 in table S2).

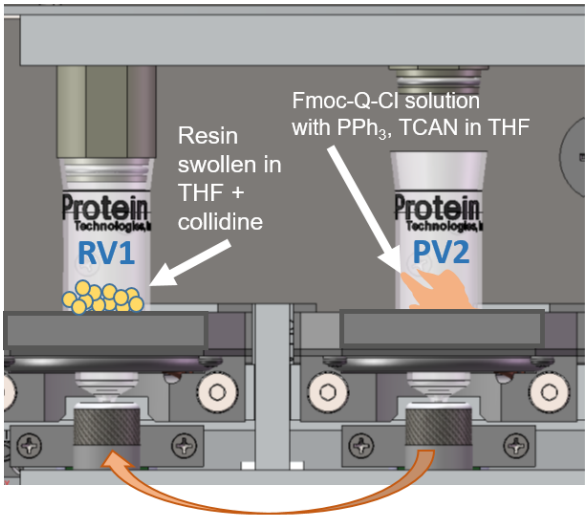


Figure S2. Cartoon illustrating the formation of the Fmoc-Q-Cl in the pre-activation vessel (PV) followed by the step called PV to RV.

Table S2. General protocol developed for SPFS automation on Chorus PurePep ® synthesizer

Step	operation	Solvent	Vol(mL)	Mix Time(HH:MM:SS)	Drain	PV	Reps	N_2	Shake/ RPM	Heat (depro*)
1	Bottom Delivery	DBU/NMP	3.00	00:10:00	✓		2	✓	250	
2	Top Delivery	DMF	3.00	00:00:15	✓		1	✓		
3	Top Delivery	THF	3.00	00:00:20	✓		3	✓		
4	Top Delivery	THF	2.00	00:00:10	✓		2	✓		
5	Bottom Delivery	Coll	0.50	00:00:01			1			
6	AA Building Block	None	1.00	00:00:01		✓	1	✓		
7	Bottom Delivery	PPh_3	1.00	00:00:01		✓	1	✓		
8	Bottom Delivery	TCAN	0.50	00:01:00		✓	1	✓	150	
9	PV to RV	None					1			
10	Mix	None		00:15:00	✓		1		250	✓ 50°C
11	Drain	None					1			
Repeat steps 3 to 11 one time										
21	Top Delivery	DMF	3.00	00:00:15	✓		3	✓		
22	Bottom Delivery	DCM	3.00	00:00:10	✓		3	✓		
23	Bottom Delivery	Ac_2O	3.00	00:00:10	✓		1	✓		✓ 40°C
24	Top Delivery	DMF	3.00	00:00:15	✓		3	✓		
25	Top Delivery	DMF	2.00	00:00:10	✓		2	✓		

*When a capping step is included in the protocol to heat it at a different temperature to the coupling one, the deprotection box is selected, thus offering the possibility to apply two different temperatures per coupling cycle. The steps 22 & 23 were introduced after the 8th coupling; i.e. the eight first coupling cycles were done with no capping.

Figure S3. Views of the protocol sub-window where the protocol for a given cycle is selected and the target temperatures for coupling and capping are saved.

2.5. Synthesis of Ylide 10 in solution

Fmoc-L-Cl: In a 25 mL flask, Fmoc-L-OH (24.2 mg, 0.05 mmol, 1.0 equiv) was suspended in 2 mL of dry DCM. Oxalyl chloride (5 μL, 0.06 mmol, 1.2 equiv.) was then added. The reaction mixture instantly became homogenous and turned yellow. After 1 h, solvent and excess of oxalyl chloride were removed under high vacuum.

Ylide 10: In a NMR tube (5 mm diameter), freshly formed Fmoc-L-Cl was dissolved in 0.5 mL of $CDCl_3$, previously passed through activated alumina. Commercially available (Cyanomethylene)-triphenylphosphorane **11** (15.1 mg, 0.05 mmol, 1 equiv.) was then added to the reaction mixture. After 15 min, a ^{31}P NMR spectrum of the mixture was recorded at 298 K and showed the formation of ylide **10** (see Figure S20d).

2.6. Regeneration of Fmoc-L-OH from 10

In a 10 mL flask, **10** (38.5 mg, 0.05 mmol, 1.0 equiv.) was dissolved in a mixture of 2 mL 1:1 DMF/H_2O. N-chlorosuccinimide (NCS) (67.5 mg, 0.25 mmol, 5 equiv.) was then added. The reaction was then monitored by RP-HPLC until complete disappearance of the starting material (see Figure S22).

3. Characterization of foldamers

3.1. HPLC chromatograms of crude foldamers

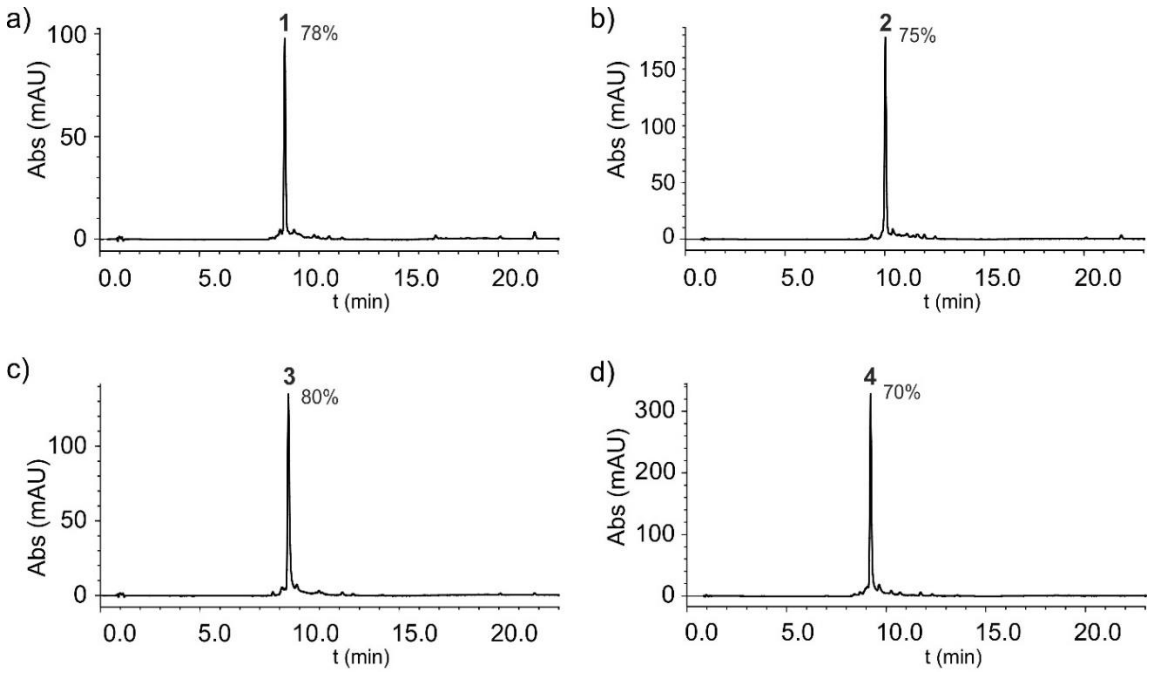


Figure S4. RP-HPLC chromatograms of crude foldamers **1** (a), **2** (b), **3** (c), and **4** (d) using a linear gradient from 5% B to 40% B in 10 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; UV detection at λ = 300 nm.

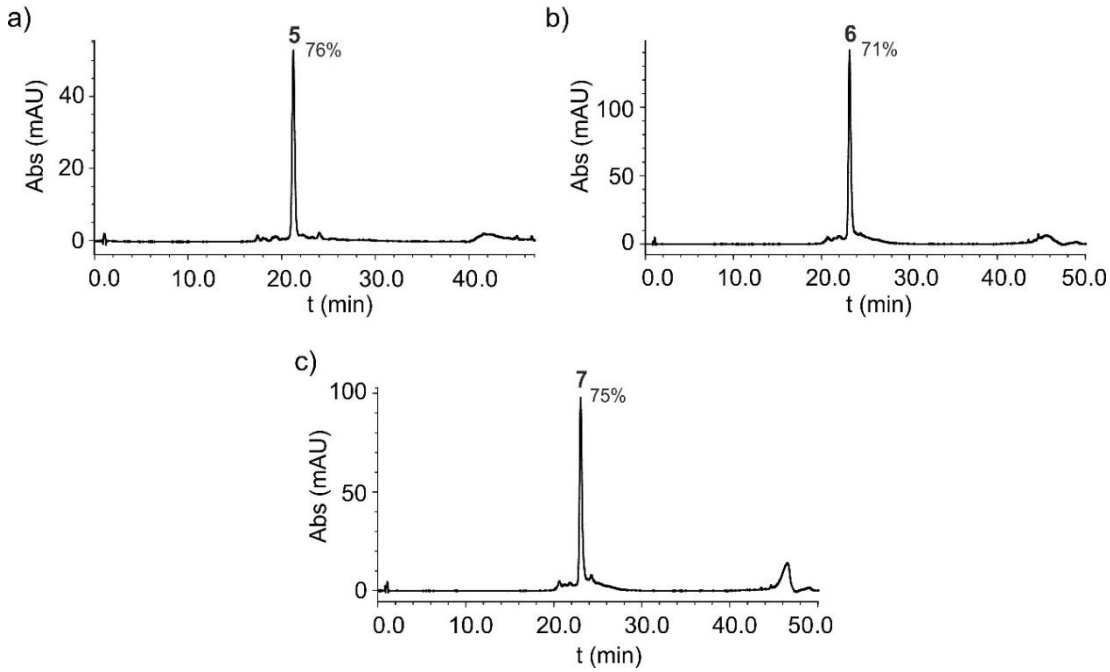


Figure S5. RP-HPLC chromatograms of crude foldamers **5** (a), **6** (b), and **7** (c) using a linear gradient from 20% B to 40% B in 35 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; UV detection at λ = 300 nm.

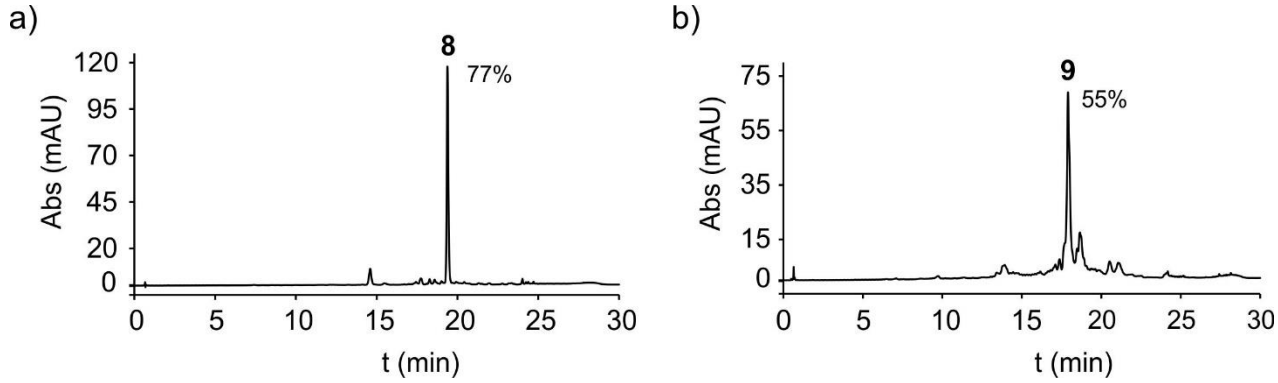


Figure S6. RP-HPLC chromatograms of crude foldamers **8** (a), and **9** (d) using a linear gradient from 20% B to 40% B in 23 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; UV detection at λ = 300 nm.

3.2. HPLC chromatograms of pure foldamers

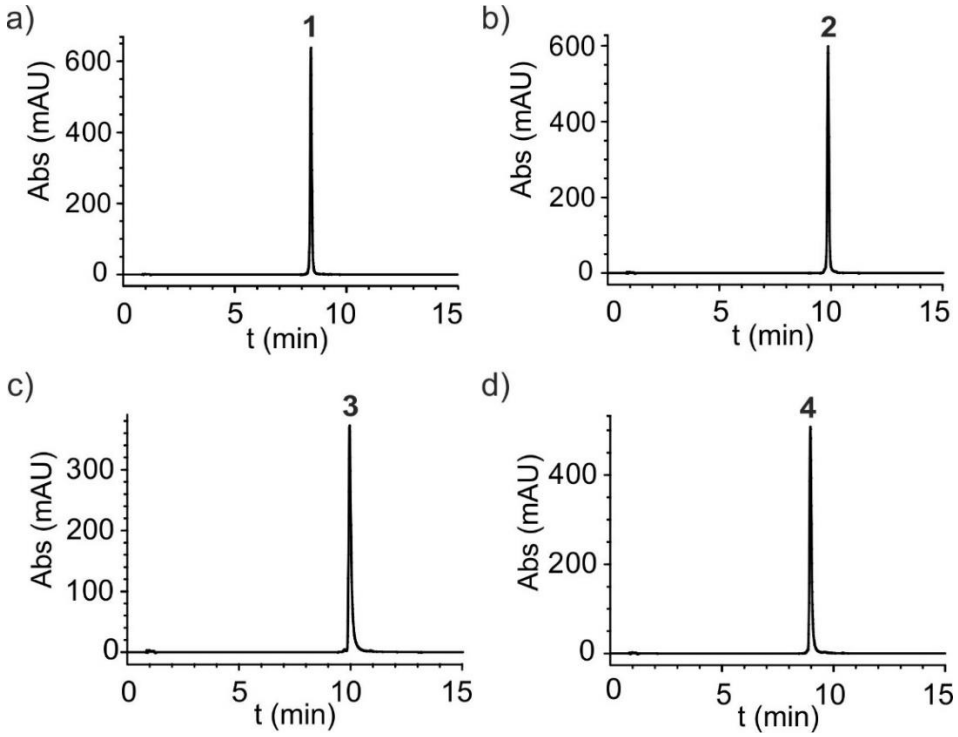


Figure S7. RP-HPLC chromatograms of pure foldamers **1** (a), **2** (b), **3** (c), and **4** (d) using a linear gradient from 5% B to 40% B in 10 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; UV detection at λ = 300 nm.

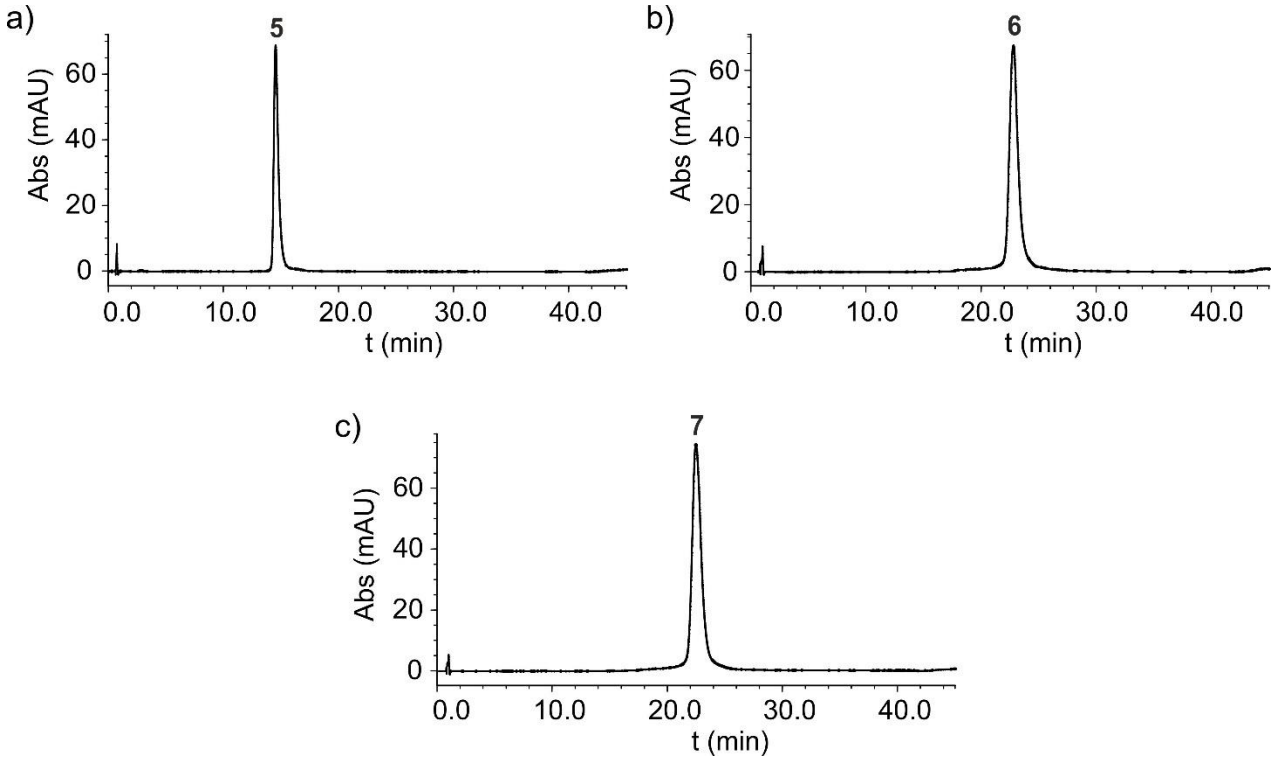


Figure S8. RP-HPLC chromatograms of pure foldamers **5** (a), **6** (b), and **7** (c) using a linear gradient from 20% B to 40% B in 35 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; UV detection at λ = 300 nm.

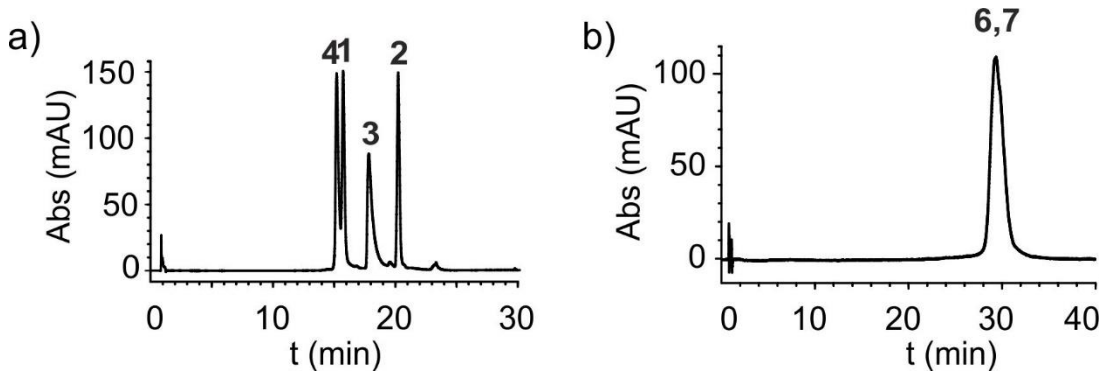


Figure S9. RP-HPLC chromatograms of the co-inJection of foldamers **1**, **2**, **3**, and **4** (a) using a linear gradient from 5% B to 20% B in 23 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA, and foldamers **6** and **7** (b) using a linear gradient from 20% B to 40% B in 35 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; UV detection at λ = 300 nm.

3.3. ^{1}H NMR spectra of pure foldamers

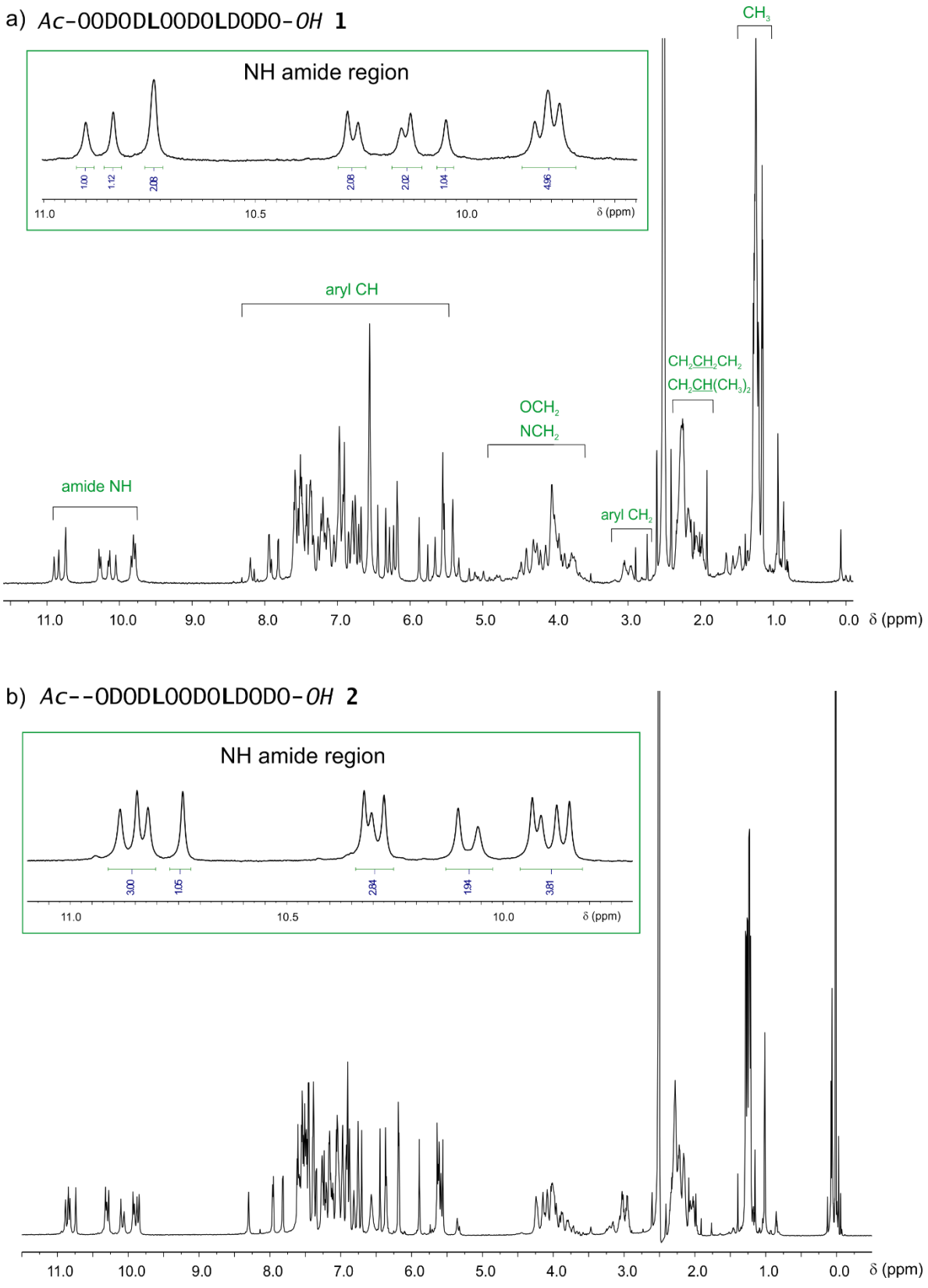


Figure S10. ^{1}H NMR spectra (700 MHz) at 333 K of foldamers **1** (a), and **2** (b), (1 mM) in DMSO-d_6.

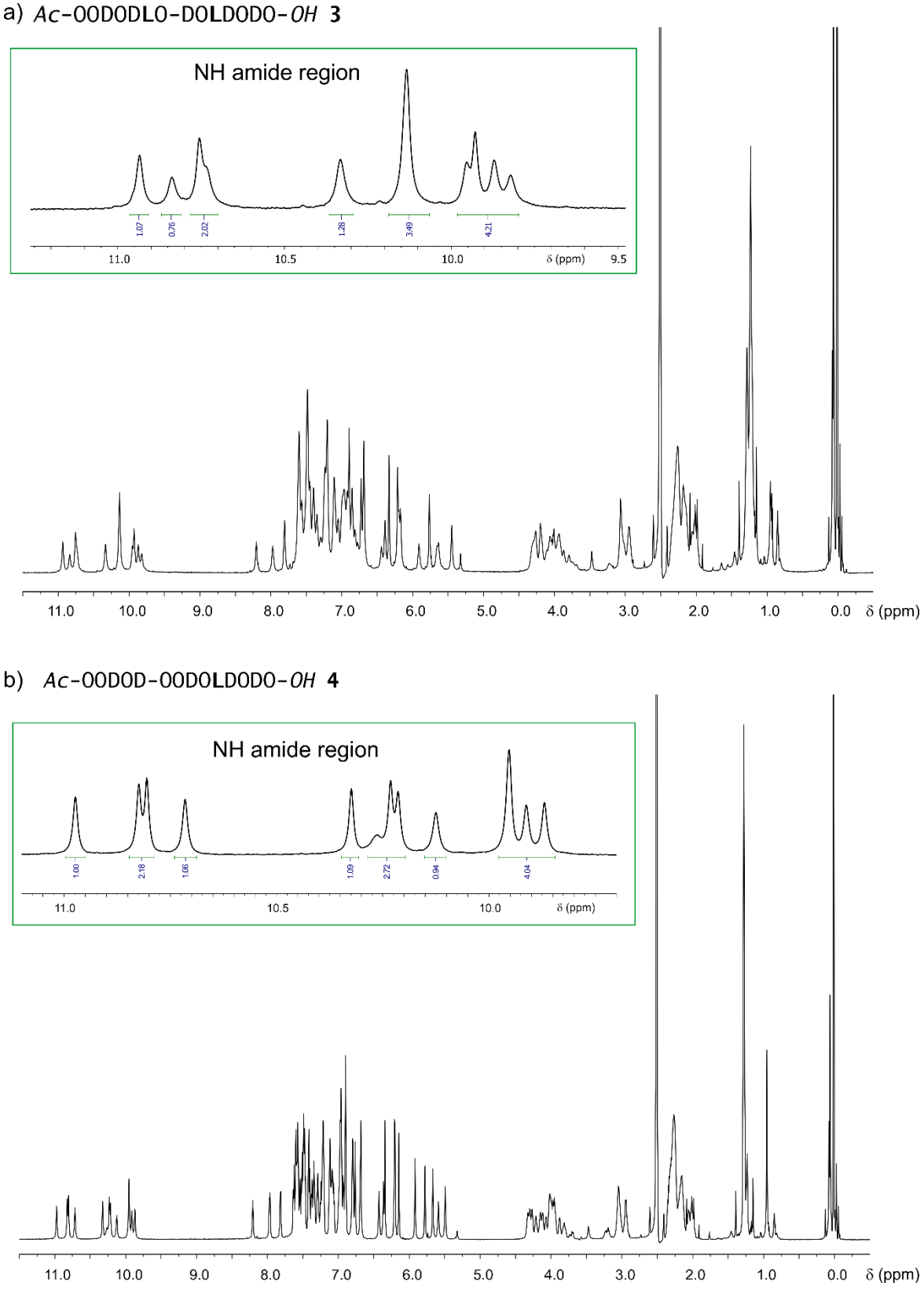
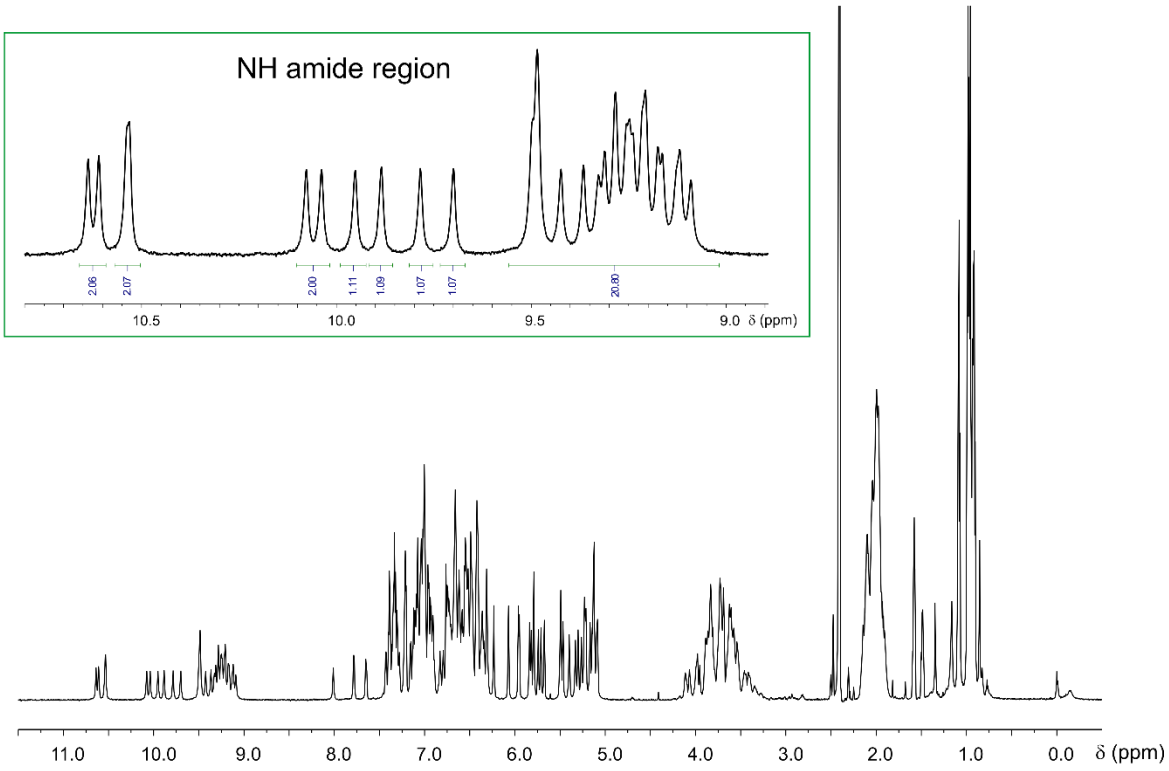


Figure S11. ^{1}H NMR spectra (700 MHz) at 333 K of foldamers **3** (a), and **4** (b), (1 mM) in DMSO-d_6.

a) *Ac*-ODL**O**OD**O**L**D**O**D**O**L**D**O**O**D**L**O**D**O**D**L**O**O**D**O**L**D**O**D**O-*OH* **5**



b) *Ac*--L**D**O**O**D**L**O**D**O**D**L**O**O**D**O**L**D**O**D**O**L**D**O**O**D**L**O**D**O**D**L**O**O**D**O**L**D**O**D**O-*OH* **6**

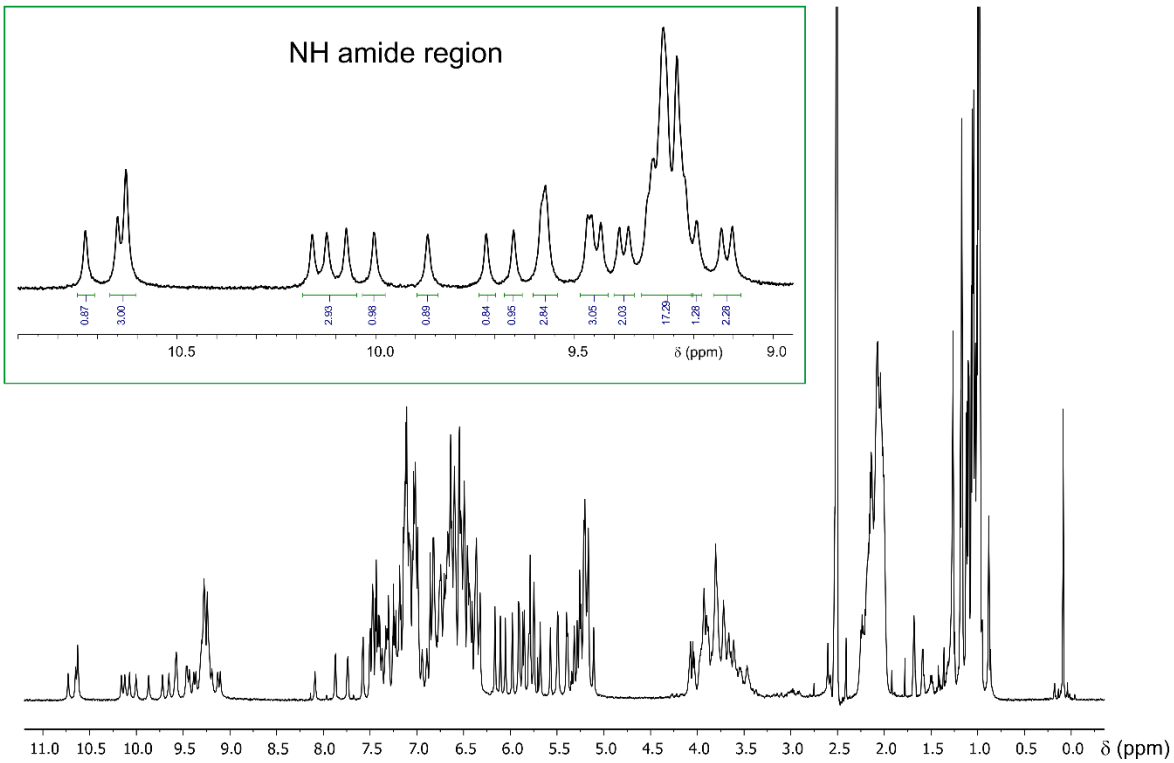


Figure S12. ^{1}H NMR spectra (700 MHz) at 333 K of oligomers **5** (a), and **6** (b), (1 mM) in DMSO-d_6.

Ac-0L**D**00**D**L0**D**0**D**L00**D**0L**D**0**D**0L**D**00**D**L0**D**0**D**L00**D**0L**D**0**D**0-*OH* **7**

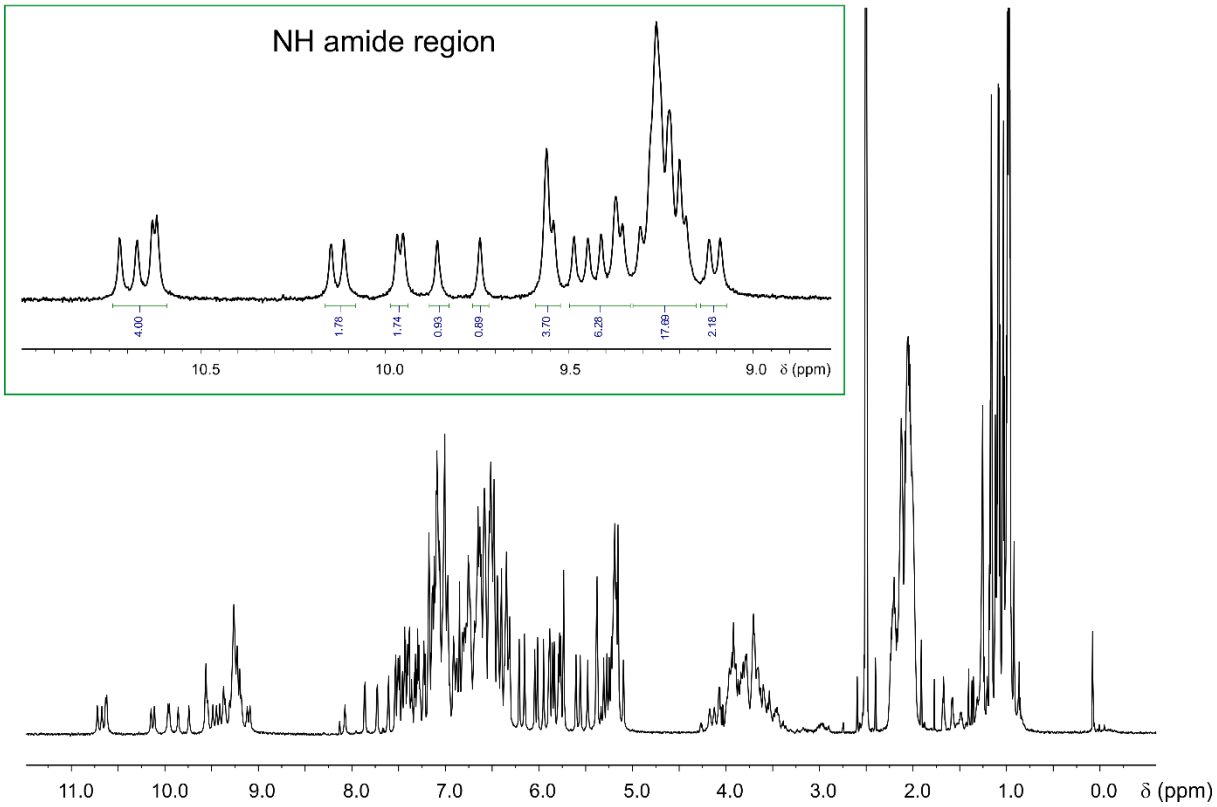


Figure S13. ^{1}H NMR spectrum (700 MHz) at 333 K of oligomer **7**, (1 mM) in DMSO-d_6.

3.4. Mass spectrometry analysis of pure foldamers

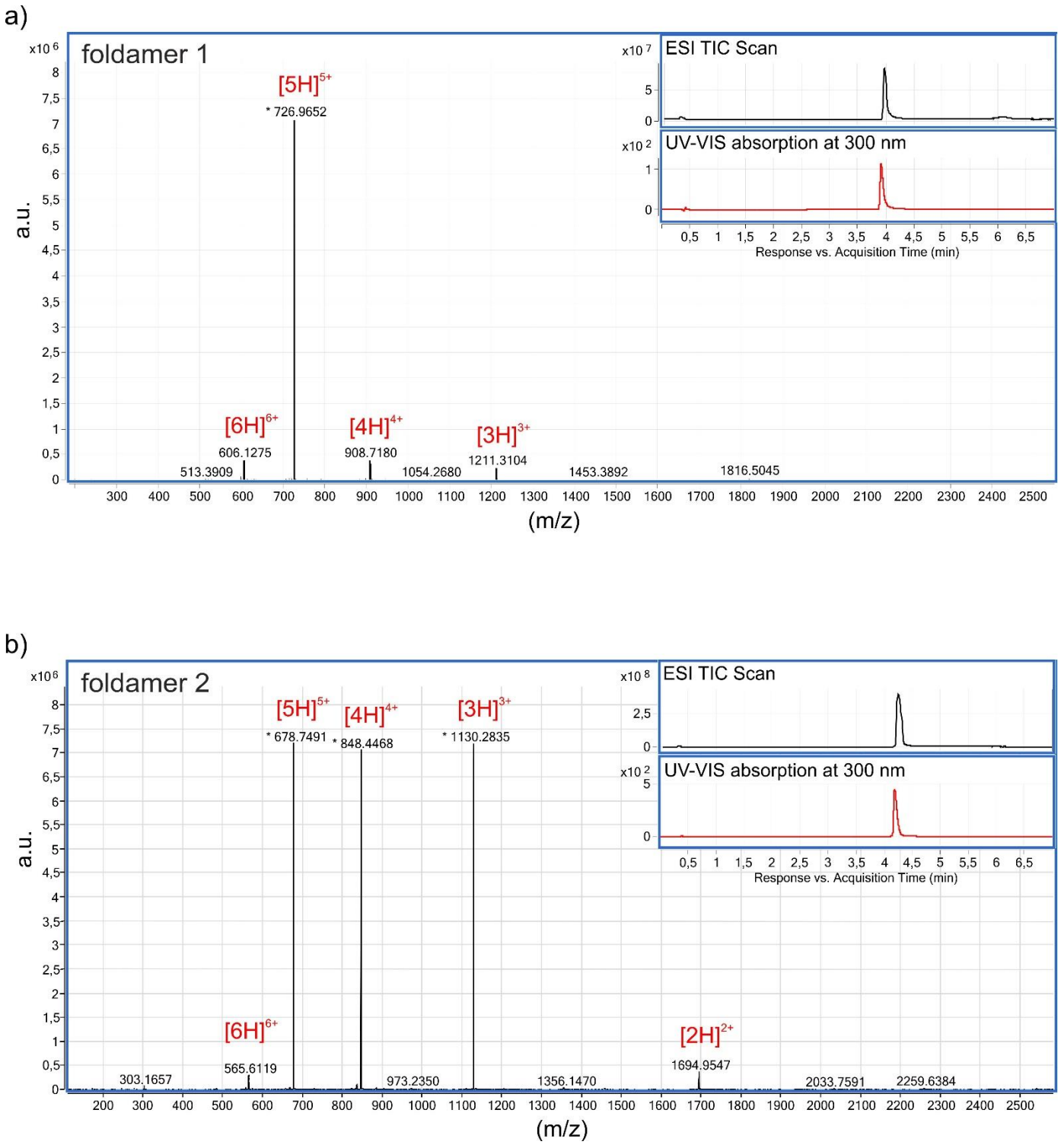


Figure S14. Multicharged species observed by LC-ESI-MS of foldamers **1** (a), and **2** (b).

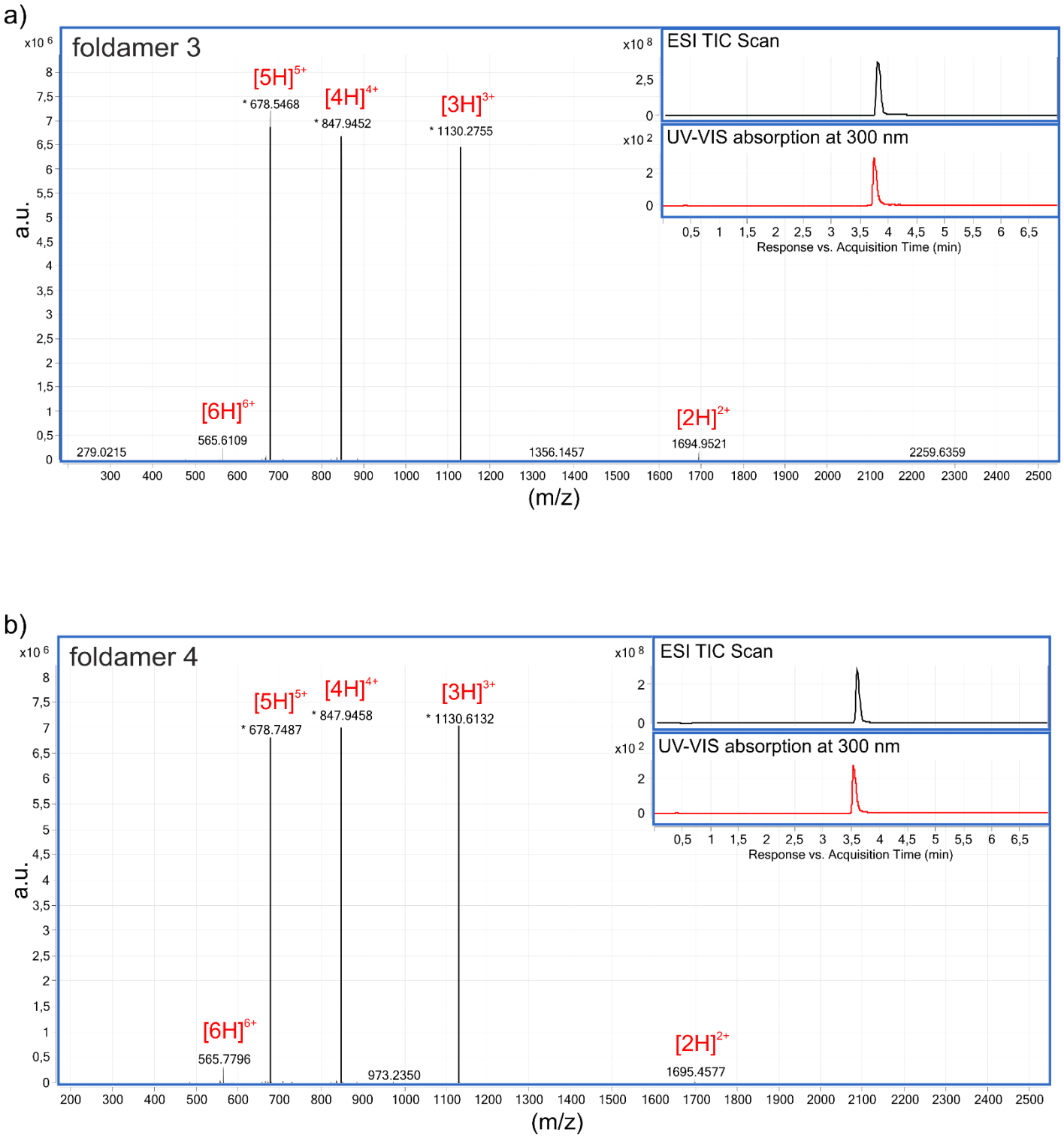


Figure S15. Multicharged species observed by LC-ESI-MS of foldamers **3** (a), and **4** (b).

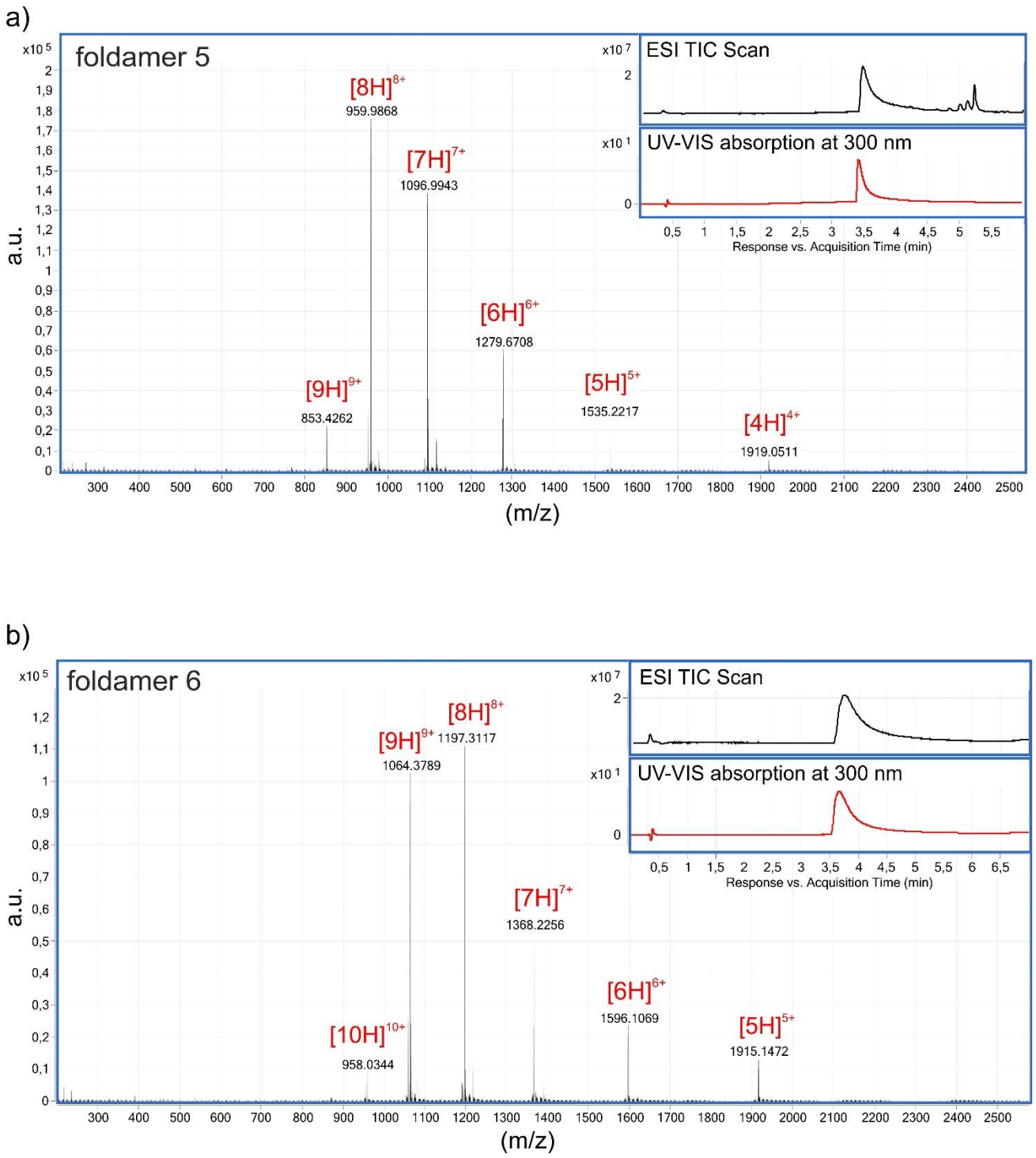
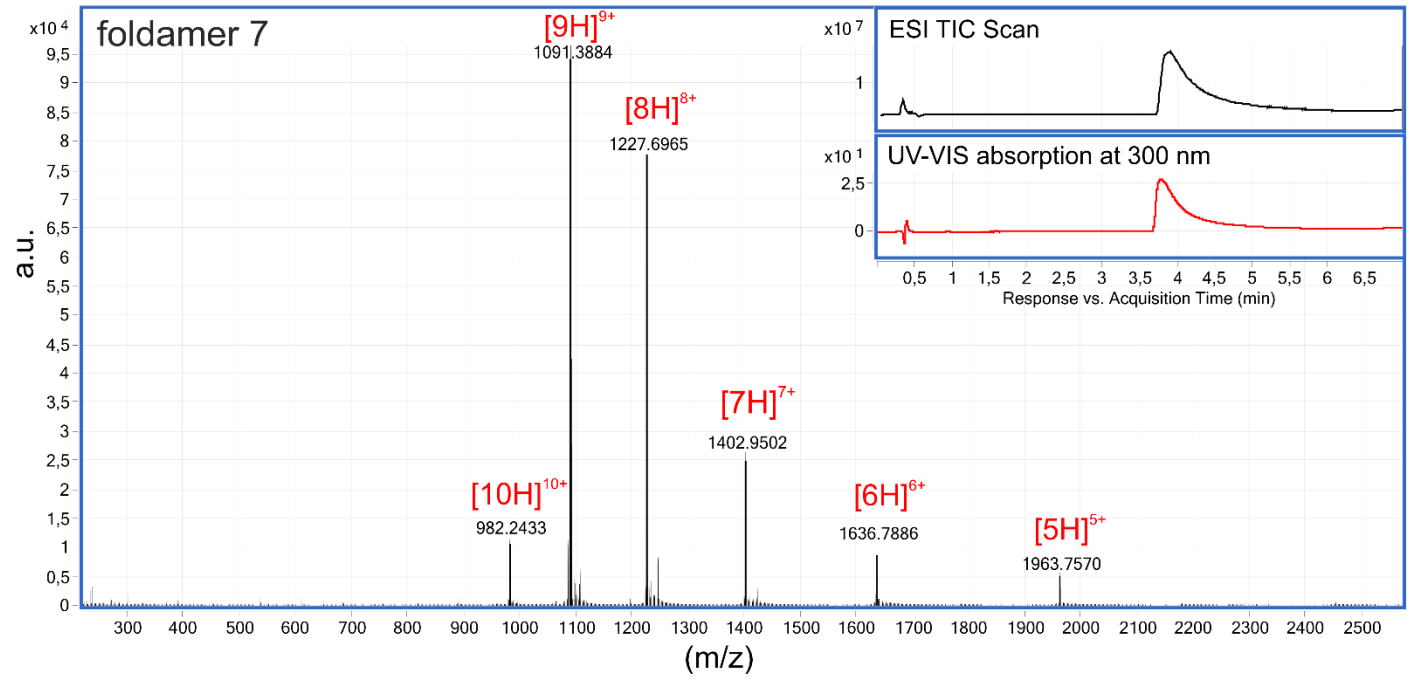


Figure S16. Multicharged species observed by LC-ESI-MS of foldamers **5** (a), and **6** (b).

a)



b)

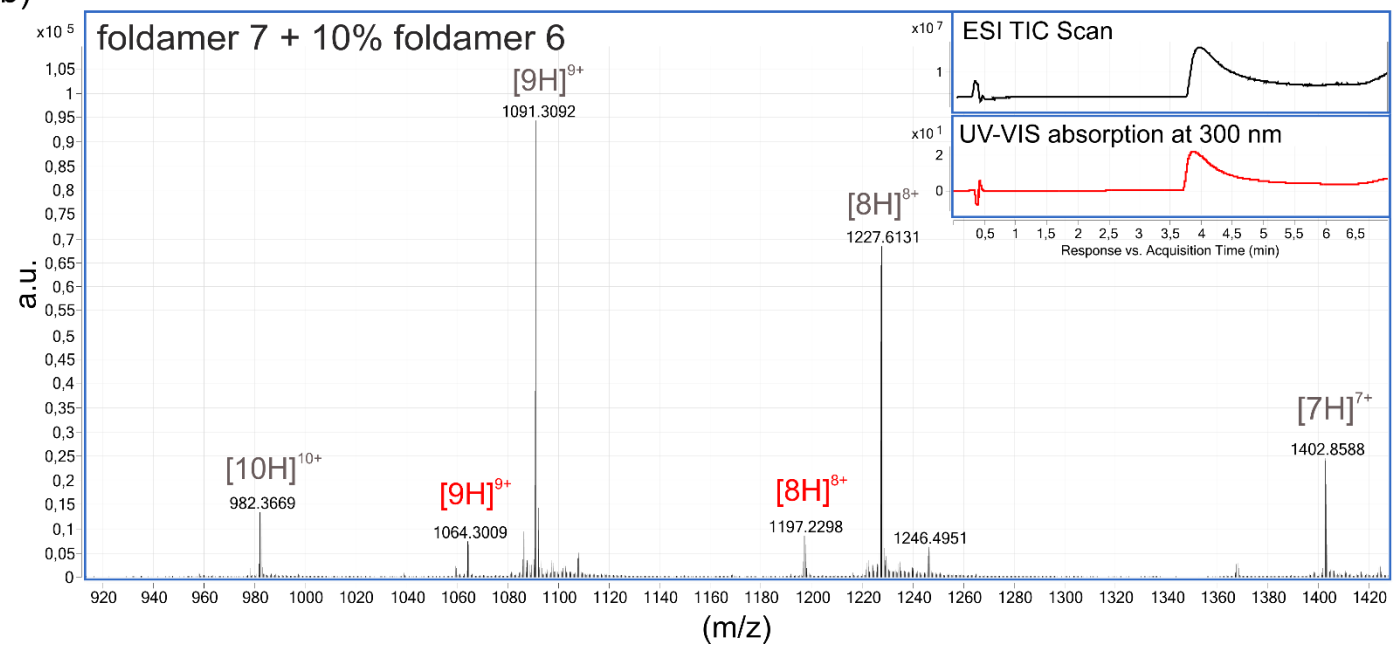


Figure S17. Multicharged species observed by LC-ESI-MS of foldamer **7** (a), and a mixture of **6** (10%) and **7** (b). Red and grey multicharged species correspond to foldamers **6** and **7**, respectively.

3.5. Mass spectrometry analysis of crude foldamers 8-9

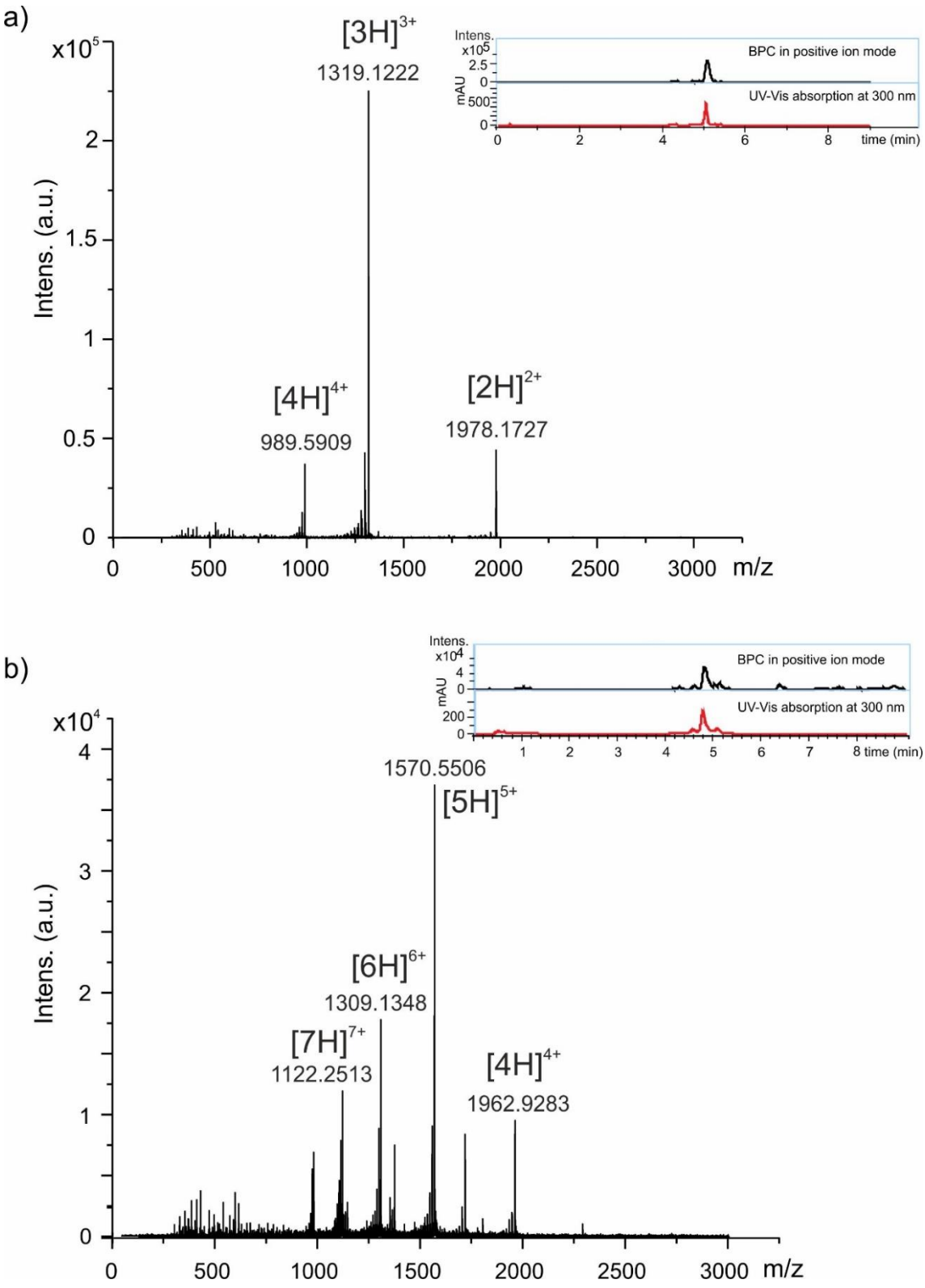


Figure S18. Multicharged species observed by LC-ESI-MS of crude foldamers **8** (a), and **9** (b).

4. Synthesis and Characterization of ylide 10

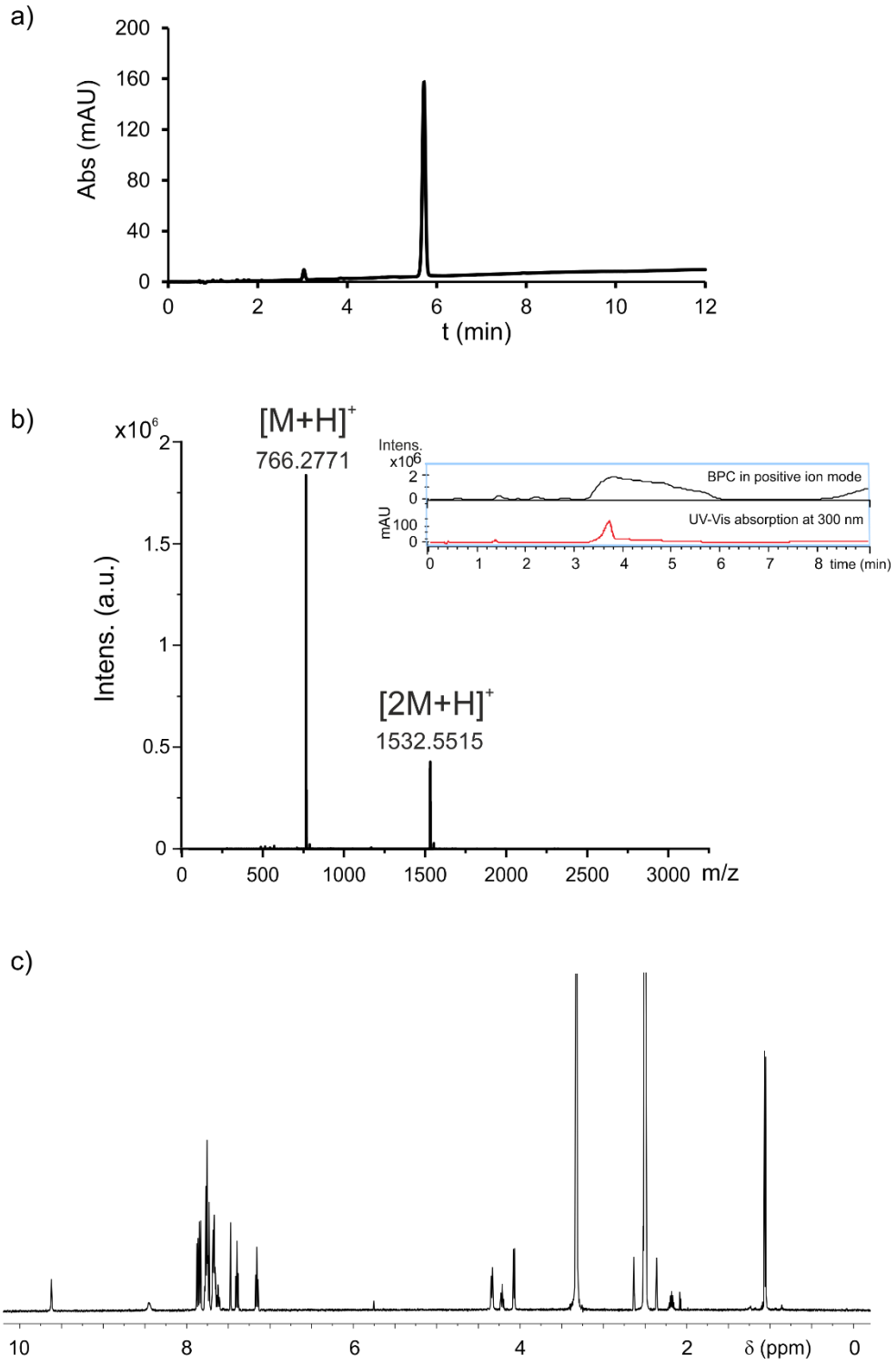


Figure S19. (a) RP-HPLC chromatogram (linear gradient from 80% B to 100% B in 10 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; UV detection at λ = 300 nm), (b) multicharged species observed by LC-ESI-MS, and (c) ^1H NMR spectrum (500 MHz at 298 K in DMSO-d_6) of ylide **10**. **^1H NMR**: δ 9.62 (s, 1H), 8.45 (s, 1H), 7.85 (dd, *J* = 15.7, 7.8 Hz, 5H), 7.80 – 7.71 (m, 9H), 7.71 – 7.59 (m, 7H), 7.47 (s, 1H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.16 (td, *J* = 7.5, 1.1 Hz, 2H), 4.34 (d, *J* = 7.8 Hz, 2H), 4.21 (t, *J* = 7.6 Hz, 1H), 4.07 (d, *J* = 6.5 Hz, 2H), 2.18 (dt, *J* = 13.3, 6.7 Hz, 1H), 1.06 (d, *J* = 6.7 Hz, 6H).

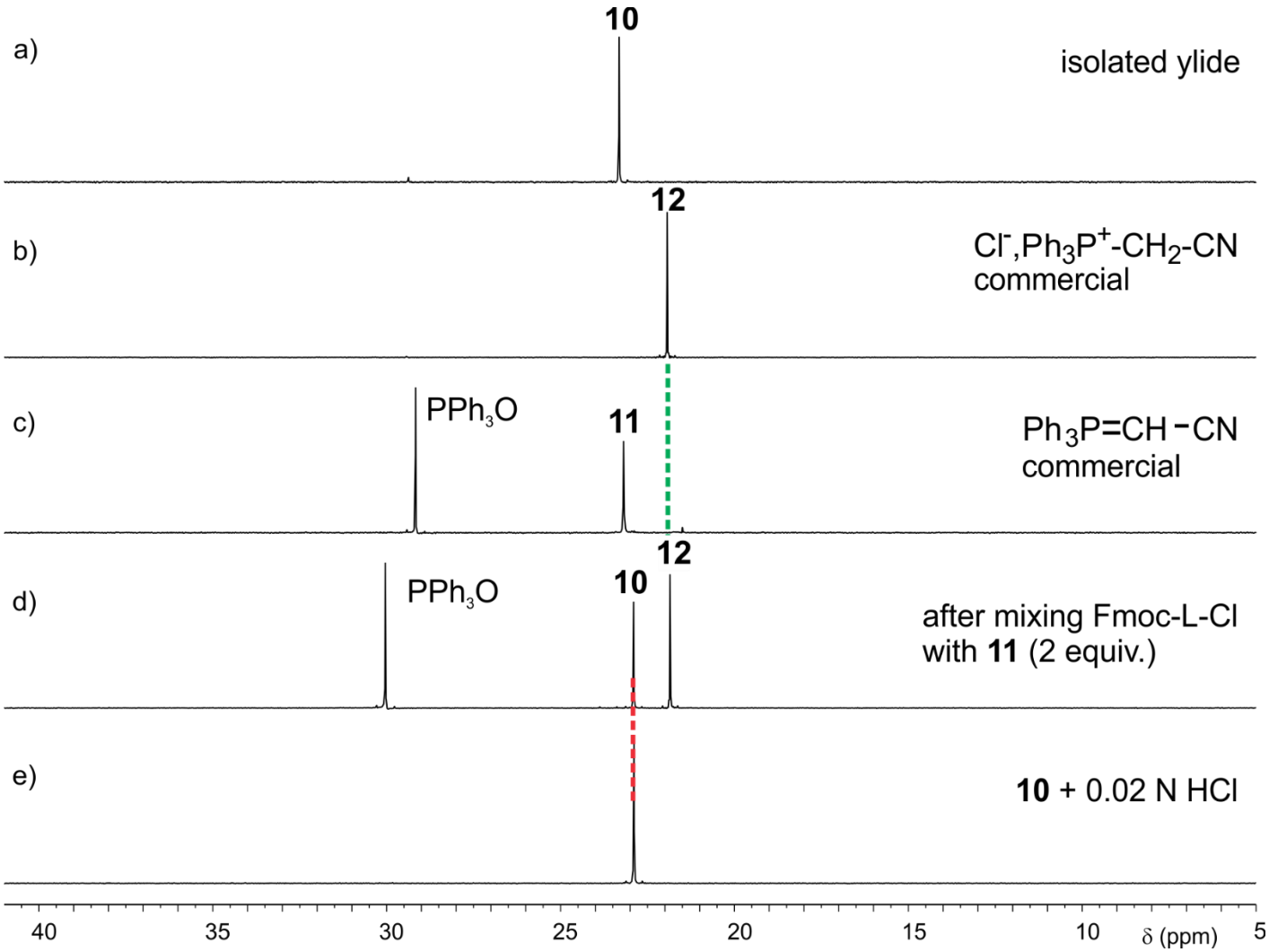


Figure S20. ^{31}P NMR spectra (162 MHz at 298K in $CDCl_3$) showing the formation of ylide **10** using commercial (cyanomethylene)-triphenylphosphorane **11**.

To confirm that **11** formed during the coupling step, thus leading to the formation of ylide **10** (a), the latter was synthesized on purpose. The acyl chloride of the monomer Fmoc-L-OH (Fmoc-L-Cl) was first synthesized in the presence of oxalyl chloride. After isolation, it was mixed with commercial phosphorane **11** (2 equiv.) (c). The ^{31}P NMR of the mixture was next recorded in $CDCl_3$ (d) (see section 2.5). Since no base was introduced during this reaction, the phosphonium salt **12** quickly formed and its identity (d) was further confirmed by overlaying the ^{31}P NMR spectrum of the mixture (d) with the one of the commercial compound (b). The formation of ylide **10** was then proven by ^{31}P NMR. Due to the acidic environment in the mixture, a visible difference in the chemical shift value was observed (spectra in a, and d). A fast addition of 0.02 N HCl solution in the NMR tube of the isolated ylide **10** showed the good alignment of the signals at 23 ppm (spectra in a, and e).

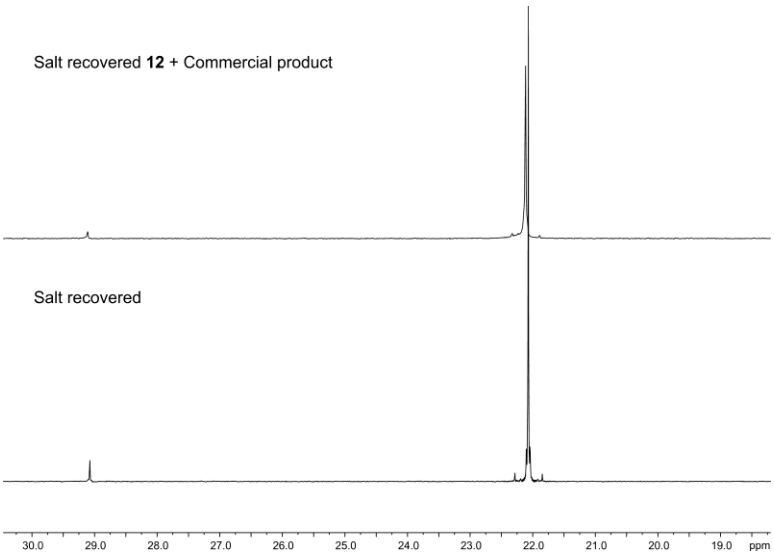


Figure S21. ^{31}P NMR spectra (162 MHz at 298K in $CDCl_3$) of the phosphonium salt **12** isolated as a precipitate during in situ acid chloride activation with TCAN/PPh_3 (bottom) and of a mixture of that salt and a genune commercial sample of **12** (top).

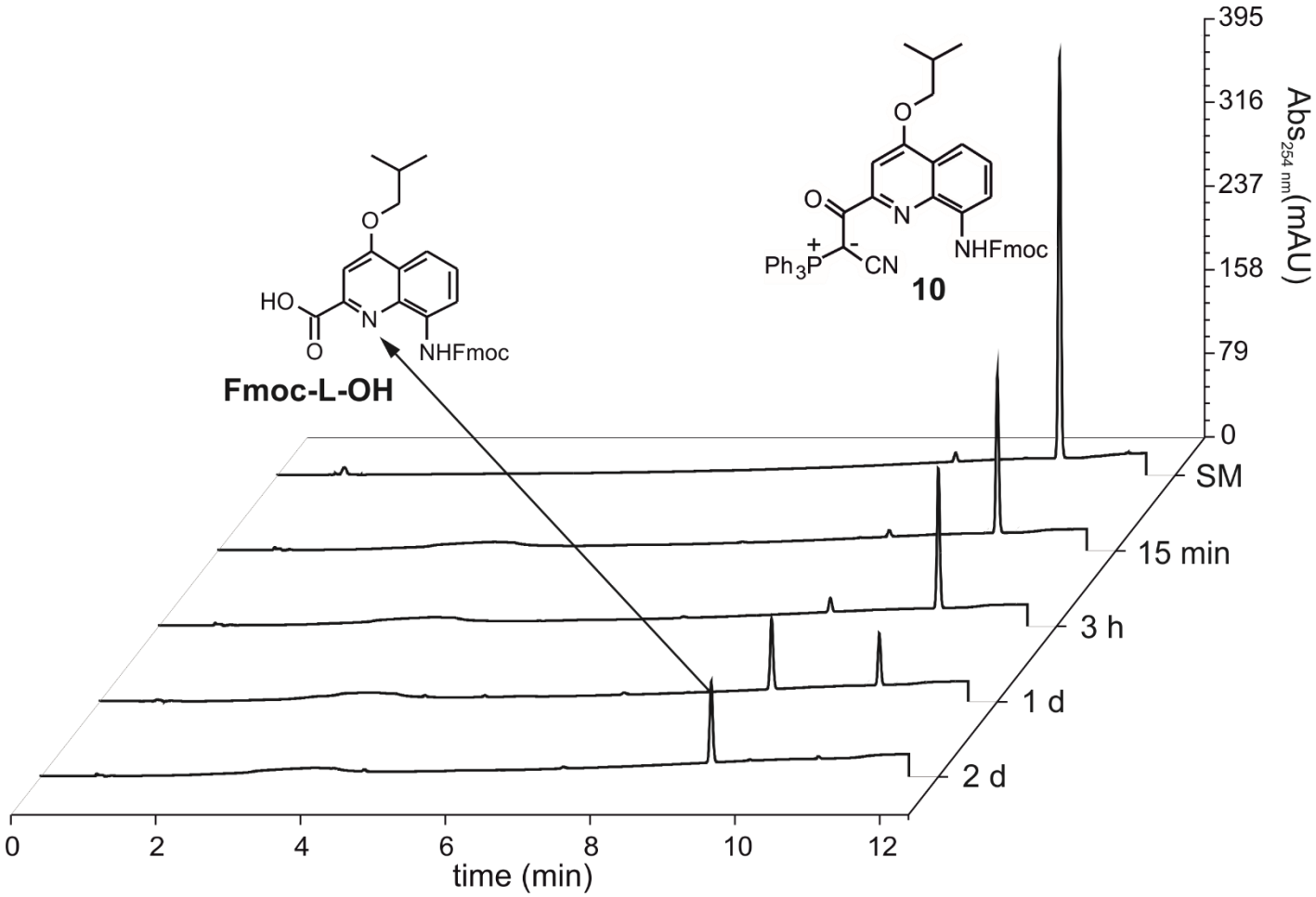


Figure S22. RP-HPLC monitoring of the degradation of ylide **10** into **Fmoc-L-OH** in the presence of NCS (linear gradient from 30% B to 100% B in 10 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; UV detection at λ = 300 nm).

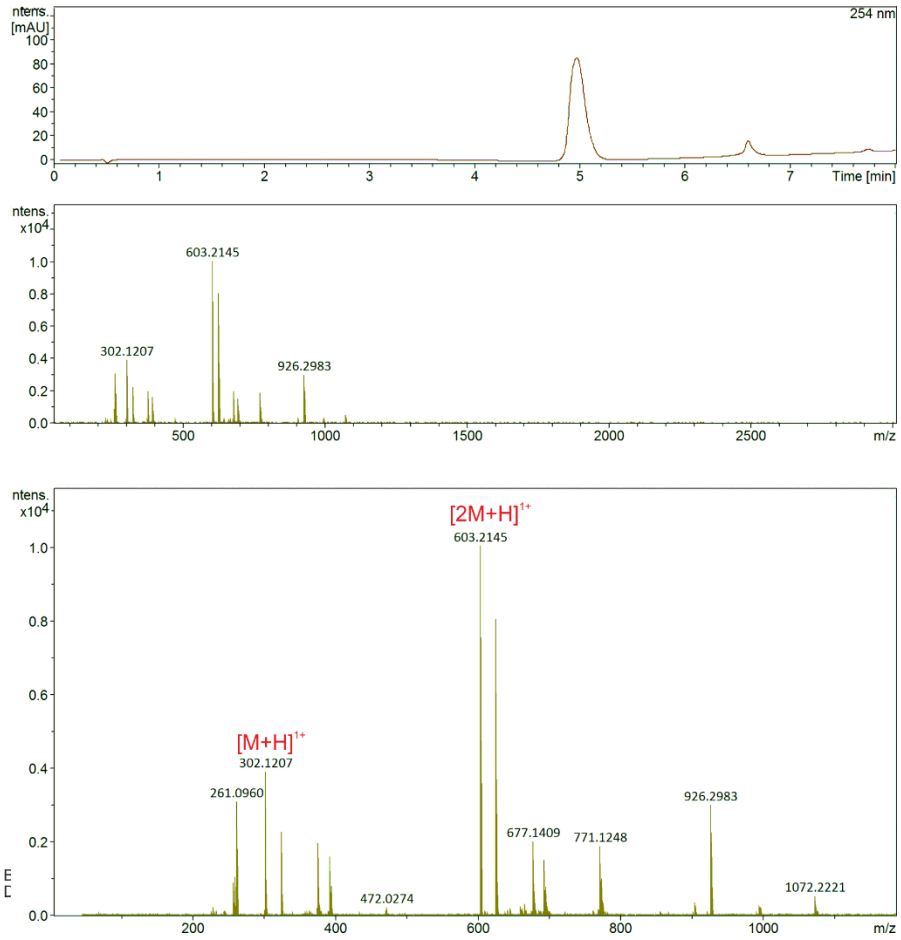


Figure S23. LC-ESI-MS analysis of the recovered precipitate from the RV after acid chloride formation.

5. Crystal structure of isolated ylide 10

The X-ray intensity data of compound **10** were measured on a Bruker D8 Venture TXS system equipped with a multilayer mirror monochromator and a Mo Kα rotating anode X-ray tube (λ = 0.71073 Å). The frames were integrated with the Bruker SAINT software package.[32] Data were corrected for absorption effects using the Multi-Scan method (SADABS).[33] The structure was solved and refined using the Bruker SHELXTL Software Package.[34] All C-bound hydrogen atoms have been calculated in ideal geometry riding on their parent atoms, the N-bound hydrogen atom has been refined freely. The PLATON SQUEEZE[35] program has been applied in order to squeeze-out solvent contents which could not be modelled properly. The voids contain 60 electrons, which would fit to CH_2Cl_2 (42 electrons) and CH_3OH (18 electrons).

Table S3. Crystallographic data of **10**

	10
net formula	$C_{49}H_{40}N_3O_4P$
M_r/g mol^{-1}	765.81
crystal size/mm	0.080 × 0.060 × 0.040
T/K	102.(2)
radiation	MoKα
diffractometer	'Bruker D8 Venture TXS'
crystal system	monoclinic
space group	'P 1 21/c 1'
a/Å	14.3498(6)
b/Å	9.0342(3)
c/Å	34.3552(14)
α/°	90
β/°	98.325(2)
γ/°	90
V/Å^3	4406.8(3)
Z	4
calc. density/g cm^{-3}	1.154
μ/mm^{-1}	0.108
absorption correction	Multi-Scan
transmission factor range	0.94–1.00
refls. measured	75978
R_{int}	0.0733
mean σ(*I*)/*I*	0.0369
θ range	2.553–25.345
observed refls.	6985
x, y (weighting scheme)	0.0457, 15.3618
hydrogen refinement	mixed
refls in refinement	8048
parameters	520
restraints	0
$R(F_{obs})$	0.0915
$R_w(F^2)$	0.2192
S	1.263
shift/error$_{max}$	0.001
max electron density/e Å^{-3}	0.744
min electron density/e Å^{-3}	−0.363
CCDC#	2243294

6. Characterization of the *N*,*N*-dimethyl-*N*'-(quinolin-8-yl)isobutyramidine

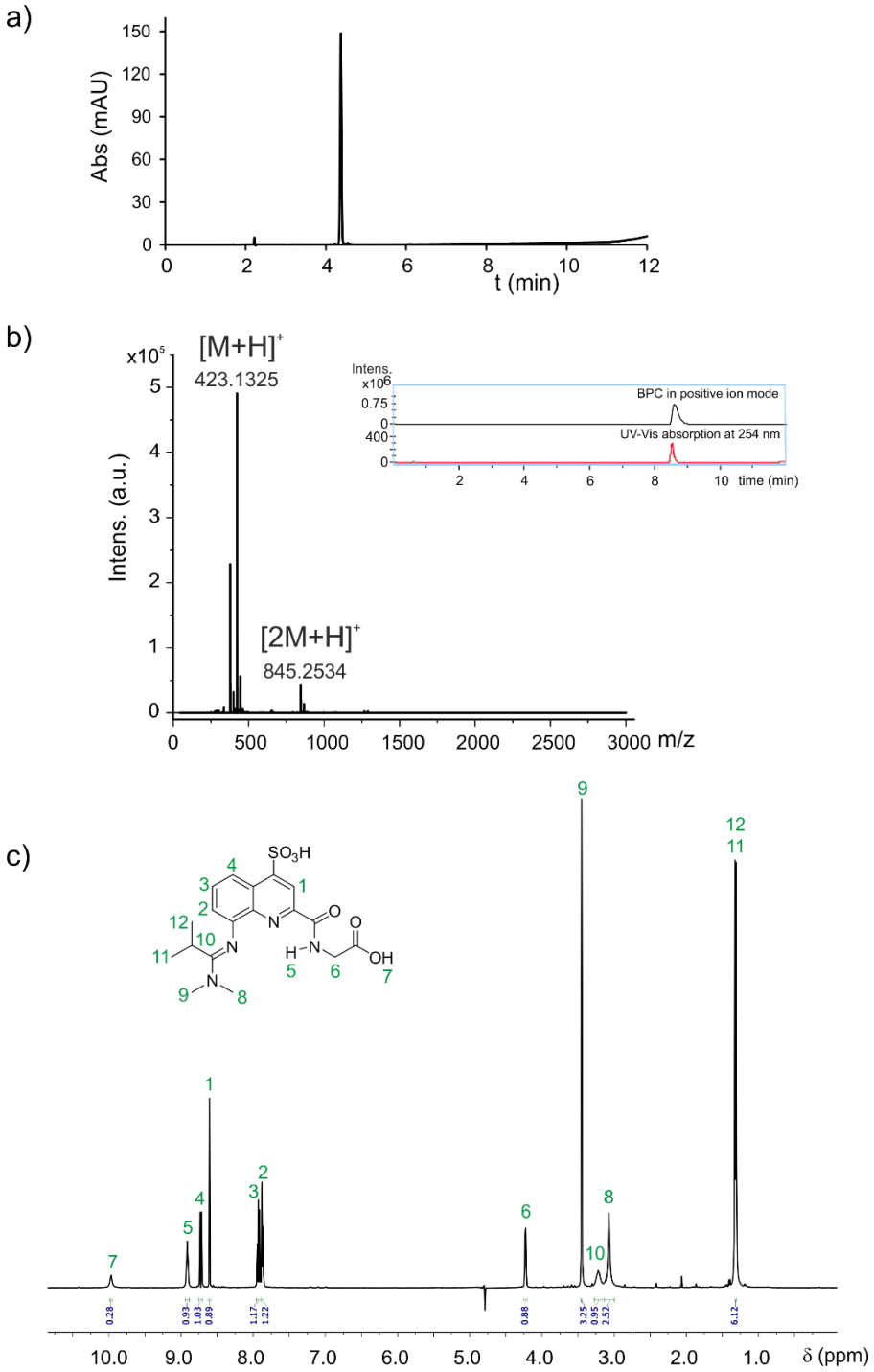


Figure 24. Characterization of the *N*,*N*-dimethyl-*N*'-(quinolin-8-yl)isobutyramidine isolated from the side reaction of 8-aminoquinoline bearing sulfonic acid side chain with Ghosez's reagent**.** (a) RP-HPLC chromatogram (linear gradient from 0% B to 50% B in 10 min; A: H_2O + 0.1% TFA and B: CH_3CN + 0.1% TFA; UV detection at λ = 254 nm), (b) multicharged species observed by LC-ESI-MS, and (c) ^{1}H NMR spectrum (500 MHz at 298 K in H_2O/D_2O 9:1 with water suppression). **^{1}H NMR**: δ 9.97 (s_{br}, 1H), 8.91 (t, *J* = 5.7 Hz, 1H), 8.72 (d_{br}, *J* = 9.2 Hz, 1H), 8.60 (s, 1H), 7.93 (t_{br}, *J* = 7.9 Hz, 1H), 7.87 (d_{br}, *J* = 7.1 Hz, 1H), 4.23 (d, *J* = 5.5 Hz, 2H (reduced integration due to water suppression)), 3.45 (s, 3H), 3.25 – 3.16 ($sept_{br}$, 1H), 3.07 (s, 3H), 1.32 (d, *J* = 7.5 Hz, 6H). Assignment of CH_3 (8) and CH_3 (9) has been performed based on ^{1}H NOESY spectrum. **HRMS** (ESI^+) m/z calcd. (most abundant mass peak) for $C_{18}H_{23}N_4O_6S$: 423.1333 $(M+H)^+$; found: 423.1325.