Supplementary Information

Display selection of macrocyclic peptides as diastereoselective ligands of helical aromatic foldamers

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This PDF file includes:

1. Supplementary figures

1.1. Figure S1

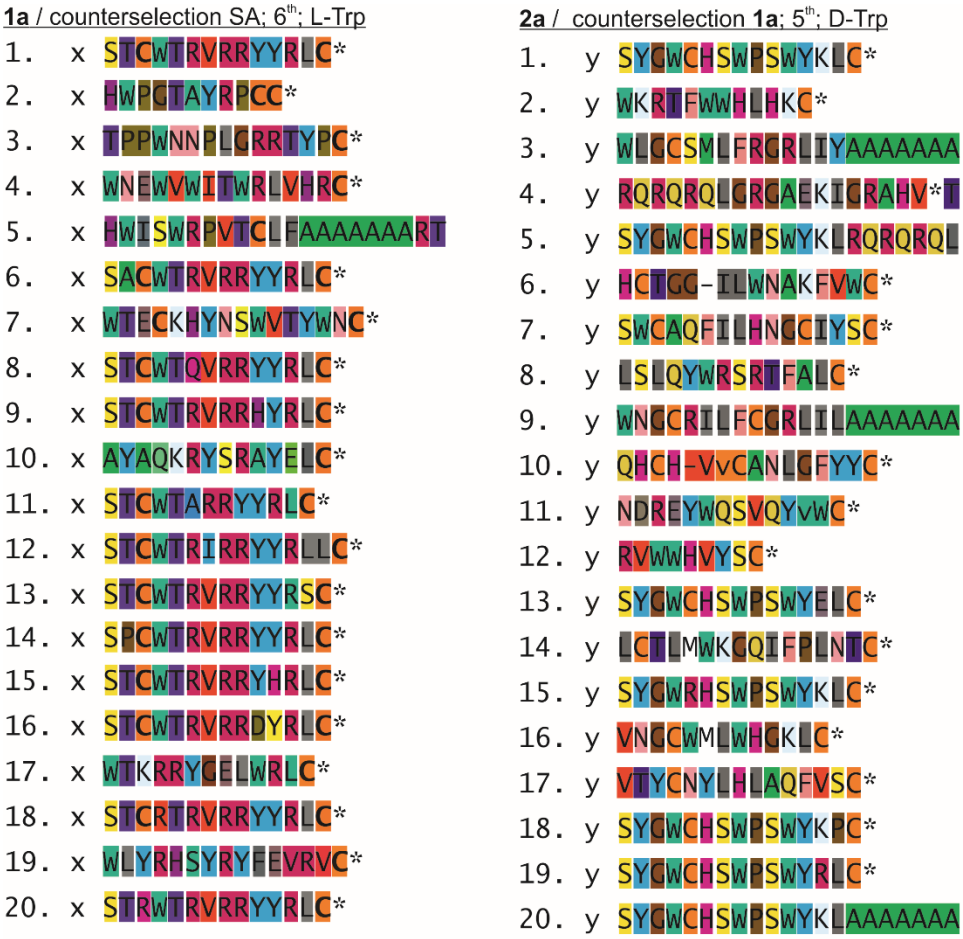


Figure S1. Table results of the two selections performed with **1a** (left) with SA as a counterselection and **2a** (right) with **1a** as a counterselection. The 20 most abundant peptide sequences for both libraries, in which: (x) stands for the CH_2CO-Trp, (y) for the CH_2CO-(D)-Trp (*) for the GSGSGS linker followed by the amber stop codon (TAG) and (-) in the middle of the sequence (sequence 6 in the right library) indicates the appearance of the amber stop codon (TAG). The GSGSGS linker in some cases was mutated to AAAAAA and RQRQRQ due to indels that occurred during the initial oligo synthesis, or during PCR, transcription or reverse transcription. Codons for methionine (M) cannot be translated as methionine as this amino acid was not added to the translation system, likely this was translated as Isoleucine.

1.2. Figure S2

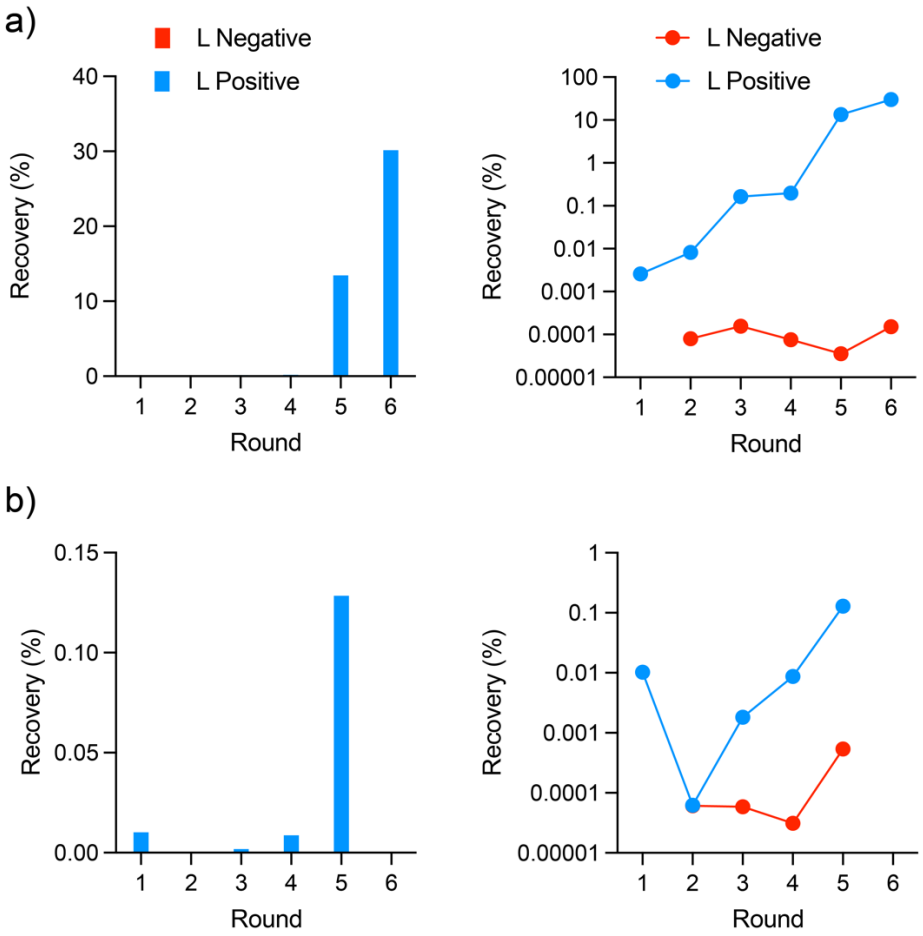


Figure S2. RaPID selection against aromatic oligoamides **1a** and **2a** resulted in an increase in DNA recovery suggesting an increase in the binding affinity of the library as particular peptides become enriched. a) DNA recovery, as measured by qPCR, for RaPID against **1a** (positive) with streptavidin as a counterselection (negative). Higher recovery is observed for positive, suggesting binders to the solid support are not being recovered. b) DNA recovery for RaPID against **2a** (positive) with **1a** as a counterselection (negative).

1.3. Figure S3

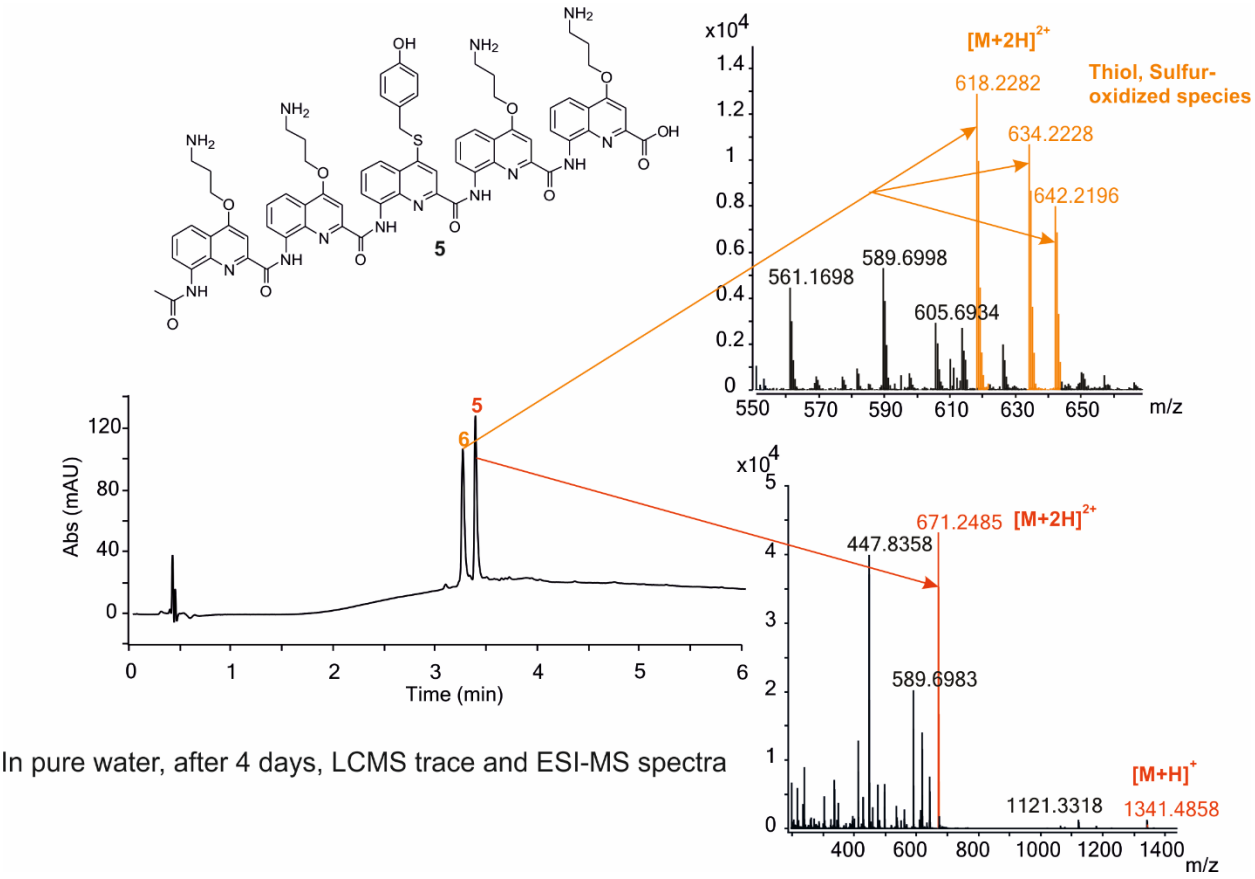


Figure S3. Monitoring of the degradation of model Ac-Q_5-OH pentamer **5** in pure water.

1.4. Figure S4

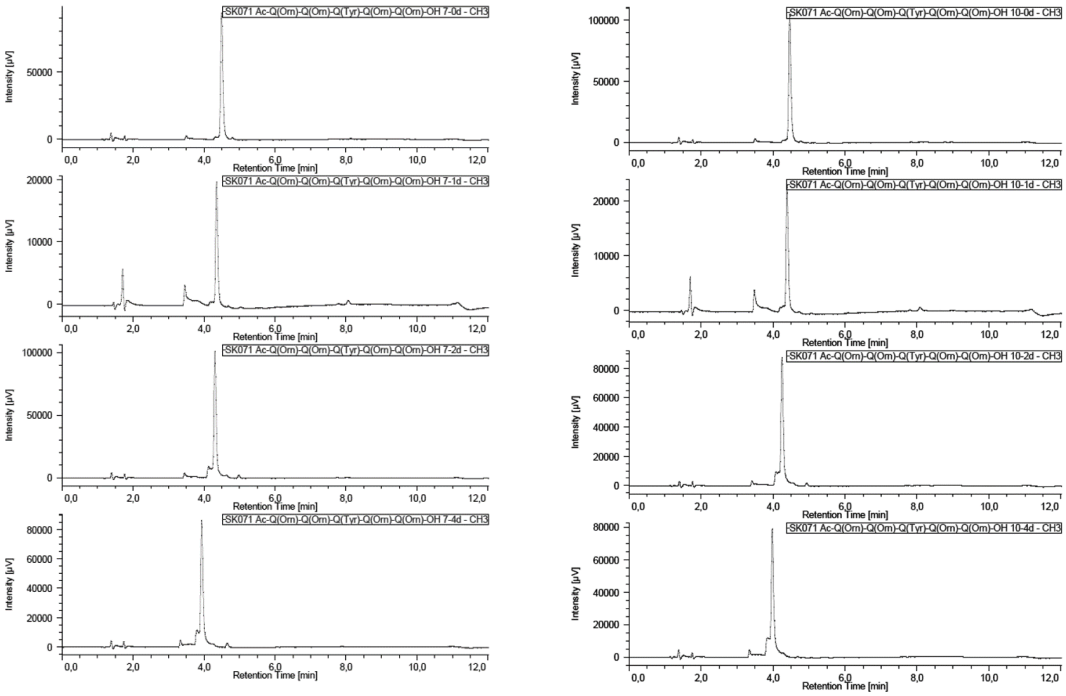


Figure S4. Examples of the monitoring of the stability of Ac-Q_5-OH (**5**) at two different pH values of phosphate buffer over 4 days: pH 6.4 (left) and pH 7 (right).

1.5. Figure S5

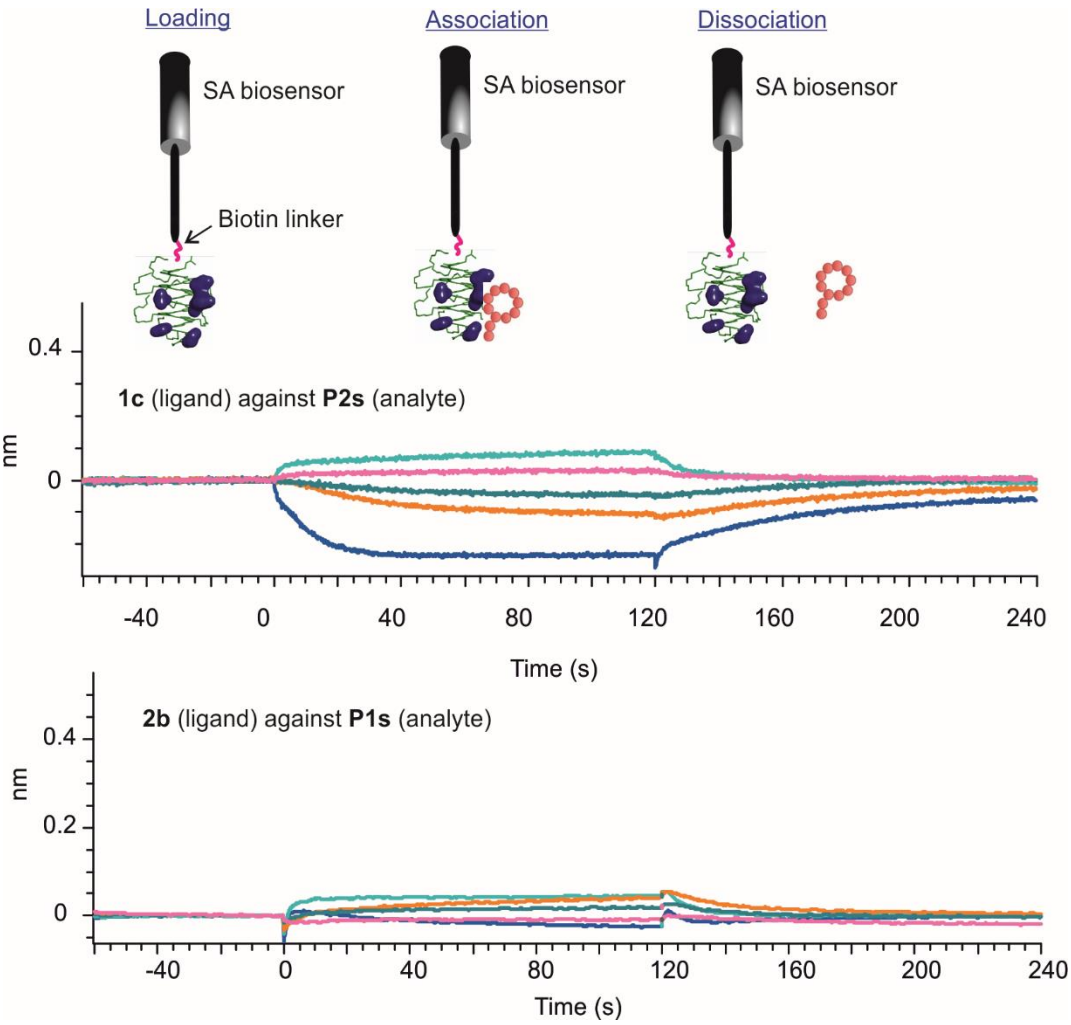


Figure S5. BLI control experiments to confirm the selective and specific binding of each teMP **3a** and **4a** to their corresponding foldamer sequence **1c** and **2b** respectively. For each experiment, five concentrations of peptide were used, ranging from 39-2.5 μM for **3a** and **4a**. In both assays, a double referencing was performed to remove any nonspecific binding of the peptide macrocycle to the SA-surface.

1.6. Figure S6

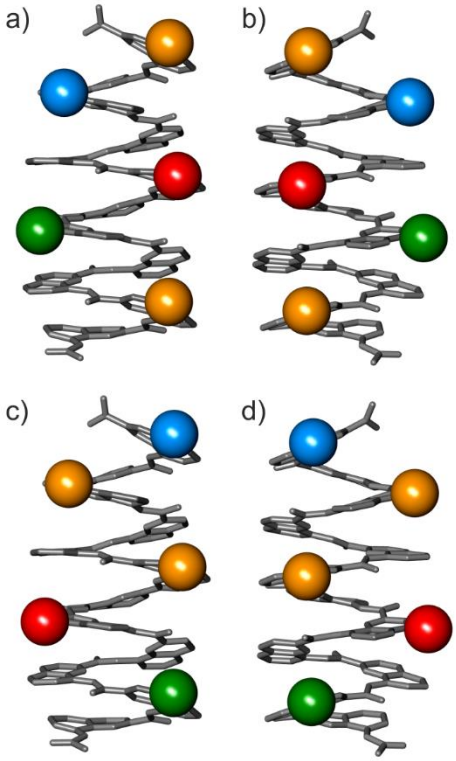


Figure S6. Biogenic side chain presentation at the surface of the *M* helix (a) and the *P* helix (b) of **1a**, and at the surface of the *M* helix (c) and the *P* helix (d) of **1b**. The biogenic side chains are represented by a colored sphere. The color code is the same as in Fig. 1 (orange = Q^O, red = Q^U, green = Q^L, blue = Q^D). The helix backbone is shown in gray tube representation. Other side chains and hydrogen atoms have been removed for clarity.

1.7. Figure S7

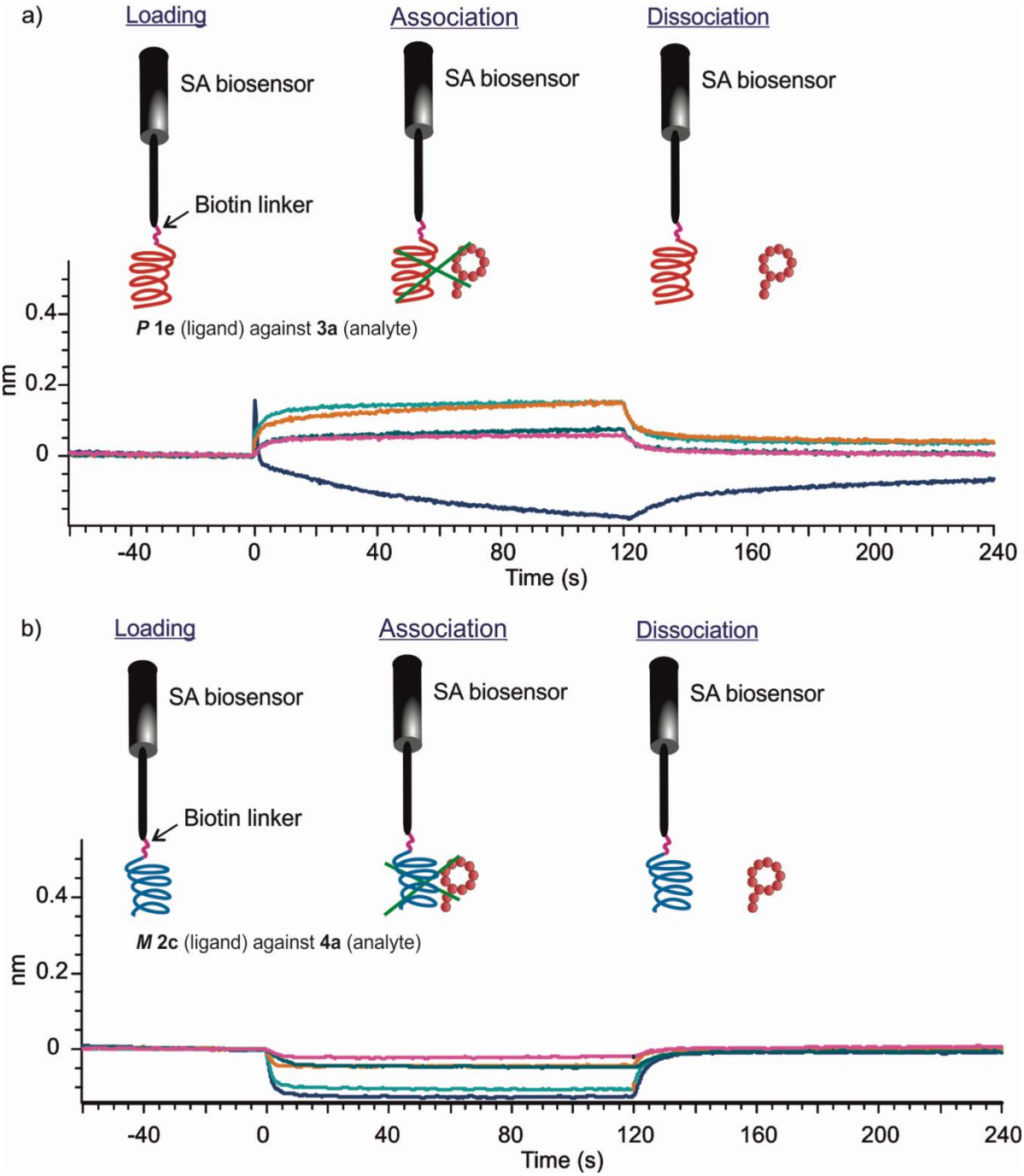


Figure S7. Negative control BLI experiments to confirm the selective binding of one aromatic helix handedness for the selected peptides: a) the *P* helix of foldamer **1** (**1e**) does not bind to **3a** and b) the *M* helix of foldamer **2** (**2c**) does not recognize the **4a**. For each experiment, five concentrations of peptide were used, ranging from 39-2.5 μM for **3a** and from 35-2.2 μM for **4a**. In both assays, a double referencing was performed to remove any nonspecific binding of the macrocyclic peptides to the SA-biosensors.

1.8. Figure S8

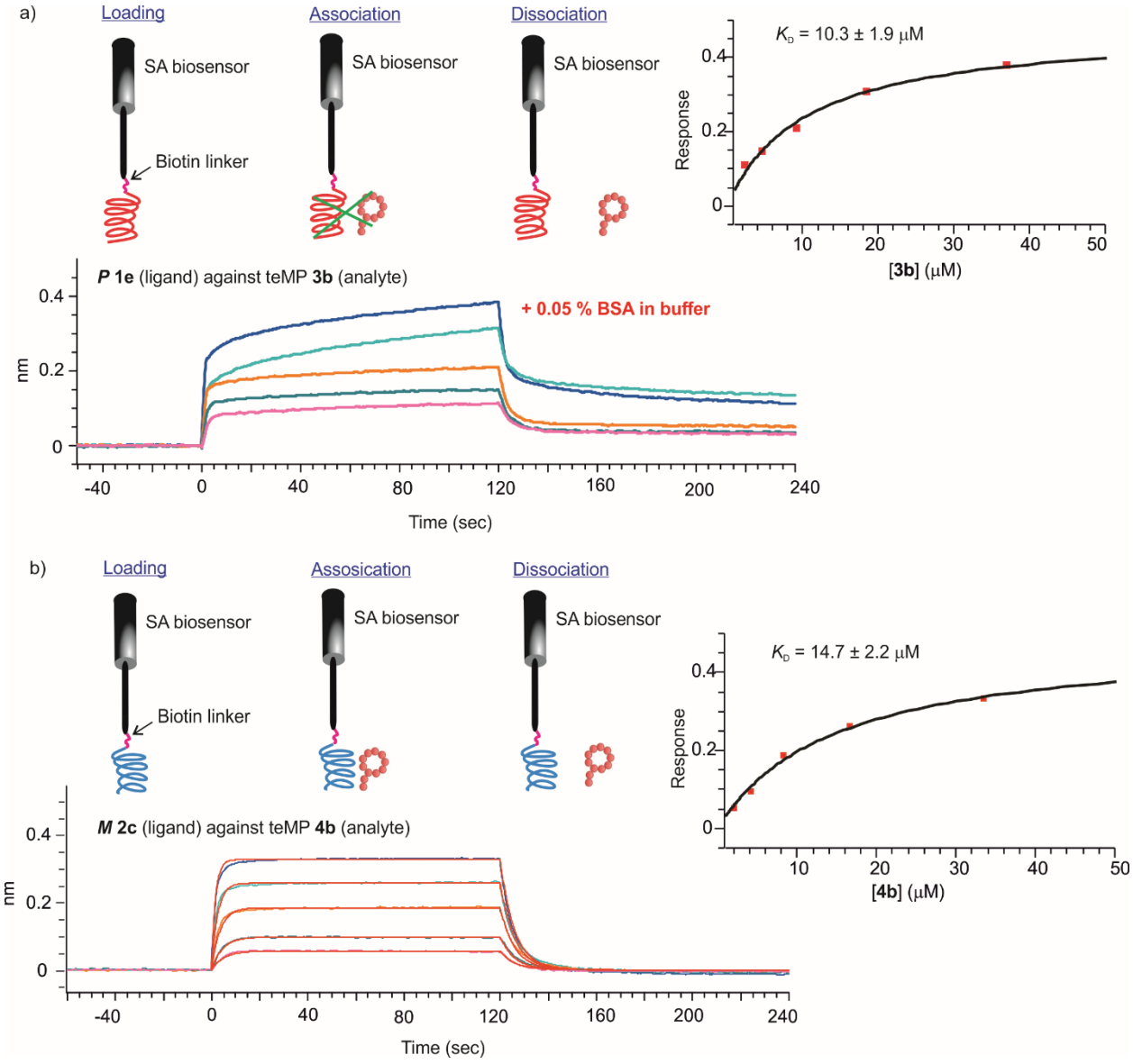


Figure S8. BLI experiments to confirm the selective binding of the opposite aromatic helix handedness for the other peptide enantiomer (**3b**) or (**4b**): a) the *P* helix of foldamer **1** (**1e**) binds to **3b** and b) the *M* helix of foldamer **2** (**2c**) does recognize **4b**. For each experiment, five concentrations of peptide were used ranging, from 35-2.2 μM for **3b** and from 33-2.1 μM for **4b**. In both assays, a double referencing was performed to remove any nonspecific binding of the peptide macrocycle to the SA- surface.

2. Experimental section

2.1. Experimental procedures for RaPID system

Two RNA libraries, consisting of 8–15 NNK codons, were prepared as previously described.[1] Briefly, RNA molecules were synthesized by T7 RNA polymerase reactions from DNA templates assembled by PCR and purified by PAGE. Furthermore, puromycin-linked mRNA was prepared by incubation with puromycin-linked oligonucleotide and T4 RNA ligase and was purified by phenol/chloroform extraction and ethanol precipitation. Oligonucleotides for both libraries and puromycin-linked oligonucleotide were previously reported.[2]

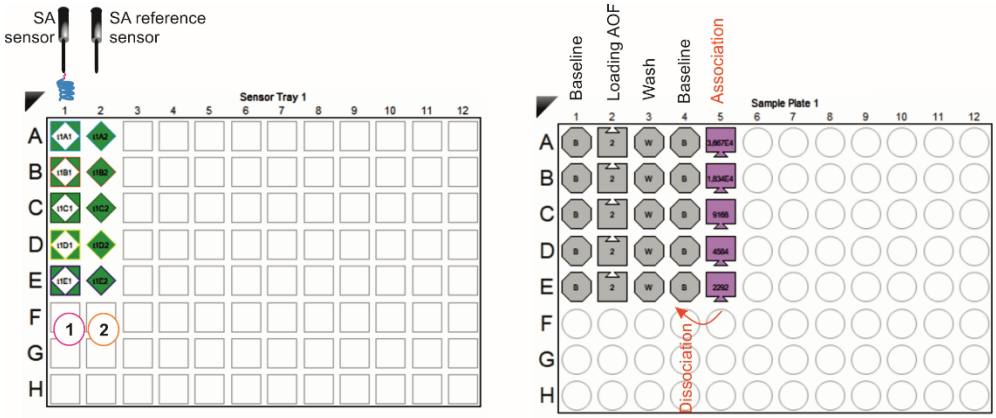
Ribosomal synthesis of the macrocyclic peptide libraries was performed as previously described.[3] In brief, for the initial selection, 1.2 μM puromycin-linked mRNA library was translated in a Met-deficient translation system reaction containing 25 μM of **ClAc$^{L(D)}$Trp**-tRNA^{fMet} for 30 min at 37 °C (150 μL scale for first round, 5 μL scale for subsequent rounds). The reaction was incubated at 25 °C for 12 min before disruption of the ribosome–mRNA complex by incubation at 37 °C for 30 min in the presence of 20 mM EDTA. The resulting peptide-linked mRNAs were then reverse-transcribed using RNase H-reverse transcriptase (Promega) for 1 h at 42 °C. For the first selection, affinity screening was performed by three serial passages (counterselections, 30 min each at 4 °C) of the library over Streptavidin Dynabeads (Life Technologies) followed by affinity selection against 200 nM **1a** immobilized on the same beads for 30 min at 4 °C. For the second selection, affinity screening was performed by three serial passages (counterselections, 30 min each at 4 °C) of the library over Streptavidin Dynabeads loaded with **1a** (Life Technologies) followed by affinity selection against 200 nM **2a** immobilized on the same beads for 30 min at 4 °C. cDNA was eluted from the beads by heating to 95 °C for 5 min and fractional recovery from the final counterselection (negative control) and affinity selection step were assessed by quantitative PCR using Sybr Green I on a LightCycler thermal cycler (Roche) (Figure **S8)**. Enriched DNA libraries were recovered by PCR and used as input for transcription reactions to generate the mRNA library for the subsequent round of screening. After five iterative rounds of library synthesis, affinity selection, and recovery, the final DNA library was sequenced to identify putative binders (Figure 2 and S2).

2.2. Binding characterization by BioLayer Interferometry (BLI)

BLI experiments were performed on an Octet R8 instrument from Sartorius, following Sartorius recommendations. Prior to an assay, streptadvidin (SA) sensor tips were soaked for at least 10 min in phosphate buffer saline (1 × PBS). The kinetic experiment always starts with a baseline step over 60 s in 1 × TBST 0.1% DMSO (TBST-D) buffer, followed by the loading Bt-foldamer at 2 ug/mL over 60 s in TBST-D. After foldamer ligand immobilization, the biosensors were washed for 60 s in the same buffer, before to record a second baseline for 120 s, again in TBST-D. Serial column dilutions (× 2) of the teMPs in TBST-D were analysed (five concentrations in total). Association lasted 120 s, followed by dissociation for 240 s.

The curves were fitted to binding models using the Octet analysis studio 13.0 software. A double referencing was performed for each foldamer/teMP kinetic experiment. A second set of five sensors were hence used following the same series of kinetic steps for the kinetic curve at the exception of the loading step, which was replaced with pure TBST-D buffer. Each teMPs concentration was subtracted with its reference well/unloaded SA-sensor tip (see figure below). Of note, this double referencing was set-up to subtract any nonspecific binding of the teMPs to streptavidin. For all foldamer/teMPs binary complex, the dissociation constants (K_D) were calculated with the Langmuir's equation assuming, a 1:1 binding model (see equation below).

$$R = Rmax \times [teMP]/(KD + [teMP])$$



Assay Steps

N	Step Name	Type	Time	Assay	Sample Location
1	Wash	Custom	60	1	p1A3
2	Baseline	Baseline	60	1	p1A4
3	Association	Association	120	1	p1A5
4	Dissociation	Dissociation	200	1	p1A4
5	Wash	Custom	60	2	p1A3
6	Baseline	Baseline	60	2	p1A4
7	Association	Association	120	2	p1A5
8	Dissociation	Dissociation	200	2	p1A4

2.3. Fluorescence titrations of foldamers **2e** and **2f** with **4c**

In a F-bottom, black 96-well plate (Greiner, 738-0026), a serial dilution in triplicate of foldamer was prepared in RaPID selection TBST buffer (Tris-HCl (20 mM) pH 7.4, NaCl (150 mM), and Tween-20 (0.05% (v/v)). teMP **4c** or 5(6)-carboxyfluorescein (Sigma-Aldrich, 21877) was added to each well to a final concentration of 20 nM and a final well volume of 200 µL. Buffer-only and fluorophore-only (no foldamer) controls were also included on the plate. The plates were incubated at 4 °C for a minimum of 30 min before measurement. Fluorescence data were measured on a TECAN Infinite M1000 Pro at ambient temperature (24°C) with the following parameters: λ_{ex} = 470 nm (bandwidth = 5 nm), λ_{em} = 525 nm (bandwidth = 5 nm), gain/z-position calculated from well with highest protein concentration, flashes = 12, settle time = 50 ms, G-factor (calibrated from 1 nM fluorescein standard in 10 mM NaOH) = 1.086. For the calculation of K_D values, dose-response data were fit in OriginPro 2019b software to the quadratic 1:1 binding model using the following equation.

$$Polarization = BOTTOM + (TOP - BOTTOM)\left(\frac{([\mathbf{4c}] + K_D + [AOF]) - \sqrt{(A_0 + K_{dD} + [AOF])^2 - 4[\mathbf{4c}][AOF]}}{2[\mathbf{4c}]}\right)$$

Where $[AOF]$ is total foldamer concentration (μM), *BOTTOM* is minimum polarization (mP, unbound), *TOP* is maximum polarization (mP, fully bound), K_D is the dissociation constant (μM), and $[\mathbf{4c}]$ is total **4c** concentration, constrained to 0.02 μM.

To note, the concentration of foldamer was determined by UV on a NanoDrop instrument (Thermo Fisher) at λ=375 nm with an extinction coefficient (ε) of 32124 L/mol/cm.

2.4. Materials and methods for chemical synthesis and characterizations

Fmoc-Q^{-}-OH, Fmoc-Q^{D}-OH, Fmoc-Q^{L}-OH, Fmoc-Q^{O}-OH, Fmoc-Q^{U}-OH, Fmoc-Q^{F}-OH were synthesized by following the reported protocols.[4] The synthesis of Fmoc-Q$^{Y(t\text{Bu})}$-OH and FmocQ^{F}-OH are introduced in chapter 3.1. If not otherwise mentioned, chemical reagents were purchased from Sigma-Aldrich, and solvents from Fisher Scientific and used without further purification. Anhydrous tetrahydrofuran (THF) and anhydrous dichloromethane (DCM) were obtained from MBRAUN SPS-800 solvent purification system. Anhydrous chloroform ($CHCl_3$) and *N,N*-diisopropylethylamine (DIPEA) were distilled over CaH_2 prior to use. Exclusively ultrapure water was used. DMF and NMP (peptide grade) were purchased from Carlo Erba. Cl-MPA ProTide®, and low-loading Wang resins were purchased from CEM. Fmoc-N-protected amino acids, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) were purchased from IRIS. ^{1}H NMR spectra were recorded on Avance III HD 400 MHz Bruker BioSpin and Avance III HD 500 MHz Bruker BioSpin spectrometers. All chemical shifts (δ) are reported in ppm and calibrated against residual solvent signals of DMSO-d_6 (δ 2.50 ppm) and $CDCl_3$ (δ 7.26 ppm). High-resolution electrospray mass spectra for compounds **3-8** were recorded on a Thermo Finnigan LTQ FT Ultra FourierTransform Ion Cyclotron Resonance Mass Spectrometer by direct infusion of the analyte dissolved in either DCM or aqueous media in positive or negative ionization mode. Mass spectra for foldamer **1a**-**1e**, **2a**-**2f** were recorded on a Bruker microTOF II from aqueous media in positive ionization mode. RP-HPLC analyses, as well as semi-preparative purification, were performed on an Ultimate 3000 HPLC System (ThermoFisher Scientific). Preparative RP-HPLC purification was performed on a Waters system with a 2707 Autosampler, a 2489 UV/Visible detector, a 2545 Quaternary Gradient Module and a Fraction Collector III. For analytical analysis, a Nucleodur C18 Gravity column (4 × 100 mm, 5 µm, Macherey-Nagel) was used, and semi-preparative purifications were performed on a Nucleodur C18 EC column (10 × 250 mm, 5 µm, Macherey-Nagel). When using acidic conditions 0.1% (v/v) TFA was added to the aqueous mobile phase (referred to as mobile phase A) and to acetonitrile (referred to as mobile phase B). For analytical RP-HPLC analysis, a flow rate of 1.0 mL.min^{-1} was applied, semipreparative purification on RP-HPLC was performed at a flow

rate of either 5.0 or 25 mL.min^{-1} (on the Waters preparative instrument). The column eluent was monitored by UV detection at 214, 254, and/or 300 nm with a diode array detector.

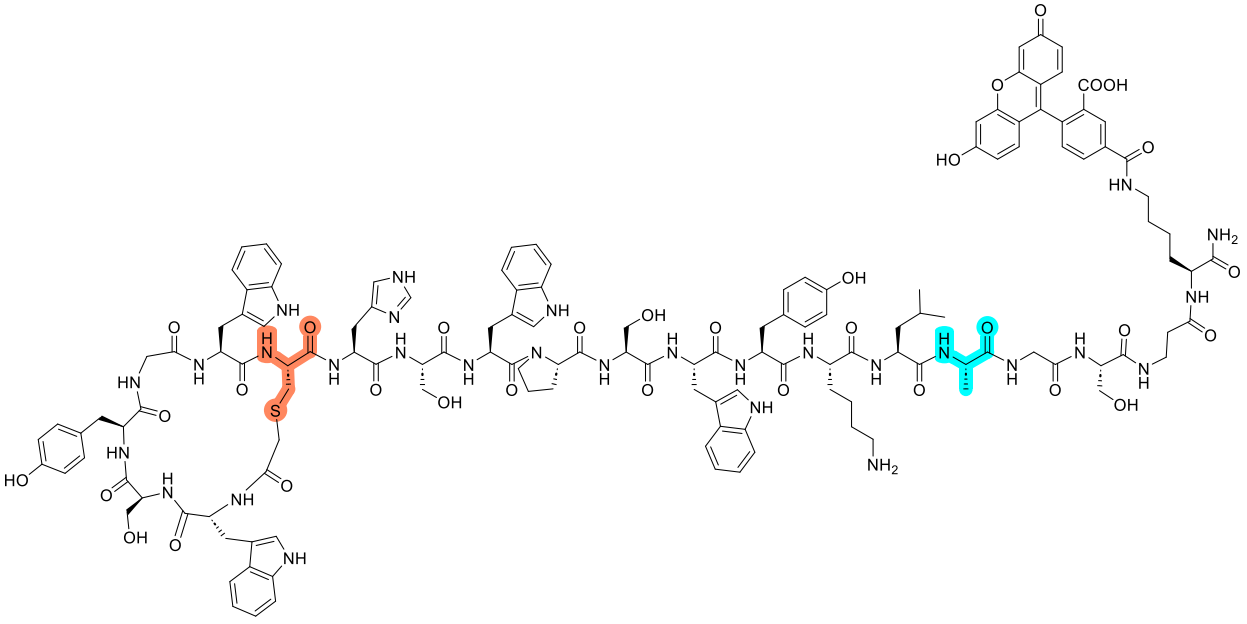
The CD spectra of foldamers were recorded on a Jasco J-1500 spectrometer with 2 mm quartz cuvette. The following parameters were used: wavelength range from 450 to 250 nm. Scan speed: 200 nm/min; accumulation: 2; response time: 1.0 s; bandwidth: 2; temperature: 25°C; sensitivity: standard (100 mdeg); data pitch: 0.5 nm; nitrogen gas flow rate: 500 L/h. Δε values (in M^{-1}.cm^{-1}) were obtained by using the formula: $\Delta\varepsilon = m°/(C.l.32980)$ where m° = CD value in millidegrees; l = cuvette pathlength in cm; C = sample concentration in mol/L

2.4.1. Solid phase synthesis

2.4.1.1. Solid Phase Peptide Synthesis

The peptides were assembled by using a Liberty Blue CEM® synthesizer at a scale of 50 μmol, using Fmoc-Gly-Wang PS resin. Microwave couplings were performed twice at 50 °C for 10 min with N-Fmoc-amino acid (6 equiv. relative to the resin loading), HCTU (6 equiv.), and NMM (12 equiv.) in DMF. Fmoc deprotection was performed twice with 20% piperidine in DMF at 75 °C (1 × 30 sec. and 1 × 180 sec.). The resin was washed with DMF (2 × 2 mL) after each deprotection step and one time 3 mL after each coupling step. The procedure of N-terminal chloroacetylation, TFA cleavage and cyclization follow recently reported protocols.[5] The crude peptide macrocycles were purified by semi-prep RP-HPLC to yield the pure compound.

2.4.1.2. SPPS of fluorescently-labelled **4c**



The resin-bound **Fmoc-4a-Ser-βAla-Lys(Alloc)** was synthesized on the Liberty Blue CEM® synthesizer at a scale of 50 μmol using a low-loading Rink amide MBHA resin. Fmoc-Lys(Alloc)-OH amino acid was first

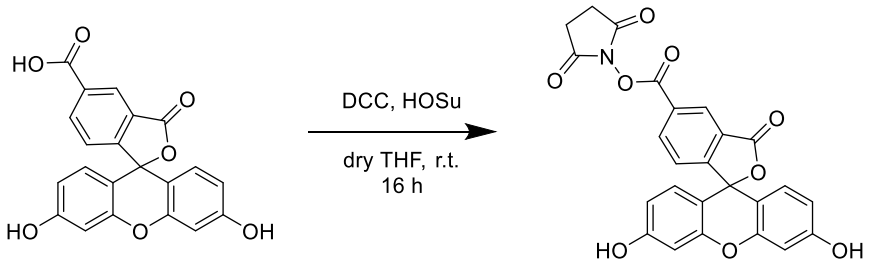
loaded using the same HCTU/NMM procedure as reported above. After the Lys(Alloc) coupling, a Fmoc-βAla-OH was coupled and served as a spacer. All the remaining amino acid residues, including the Fmoc-(*D*)-Trp(Boc)-OH were installed using the same coupling/deprotection cycle procedure.

Half of the resin (25 μmol) was then transferred in a syringe equipped with a filter, the Fmoc deprotection was performed manually at room temperature in the presence of 20% piperidine in DMF (1 × 3 min and 1 × 7 min). After several rounds of washings with DMF (3 × 3 mL) and then DCM (3 × 3 mL), chloroacetic anhydride (10 equiv.) was dissolved together with DIPEA (20 equiv.) in DCM (3 mL). The reaction mixture was directly added to the resin and shaken for 15 minutes at r.t. This coupling step was repeated once without any washing in between. The resin was then filtered off, washed with DCM (3 × 3 mL) and dried briefly under a nitrogen stream.

In a second time, the ε-Alloc protecting group was removed in the presence of $Pd(PPh_3)_4$ (0.1 equiv.) and Ph_3SiH (20 equiv.) in dry DCM (2mL) for 30 min under Ar atmosphere. This step was repeated once with washings with dry DCM (3 × 3 mL) in between. The deprotection of the Lys side-chain was qualitatively controlled with the TNBST test.

At last, the fluorescein was installed on the ε-NH_2 of the Lys residue. Freshly prepared NHS-carboxyfluorescein (3 equiv., see below) was added to the resin swollen in DMF (1.5 mL). The resin was next shaken for 16 hours. After washings with DMF (3 × 3 mL), the efficiency of the fluorescein coupling was monitored with the TNBS test, which proved to be negative.

After final TFA cleavage (TFA, TIS, H_2O, EDT / 92.5, 2.5, 2.5, 2.5, v/v/v), half of the crude **4c** (42.67 mg, 60%) was purified by using semi-preparative RP-HPLC to give **4c** as a yellow solid after lyophilization (1.34 mg, 3.7%). HRMS (ESI+): m/z calculated for $C_{139}H_{161}N_{29}O_{33}S$ $[M+2H]^{2+}$ 1399.0839 found 1399.1524.



NHS-carboxyfluorescein

NHS-carboxyfluorescein: 5-Carboxyfluorescein (250 mg, 0.66 mmmol) was suspended in dry THF (2.5 mL) and a solution of *N*-hydroxysuccinimide (1.3 equiv., 99 mg, 0.86 mmol) dissolved in dry THF (0.5 mL) was added, followed by the addition of a solution of *N*,*N'*-dicyclohexylcarbodiimide (1.3 equiv., 177 mg, 0.86 mmol) in dry THF (0.5 mL). After 16 hours stirring at r.t. the mixture was filtered and the precipitate washed with Et_2O (2 × 10 mL) and EtOAc (1 x 10 mL). The filtrate was concentrated under reduced pressure and remaining solvents azeotroped with toluene (3 x 5 mL) providing **NHS-carboxyfluorescein** as an orange solid (270 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 2H), 8.54 (d, *J* = 1.7 Hz, 1H), 8.43 (dd, *J* =

8.1, 1.6 Hz, 1H), 7.56 (d, *J* = 8.1 Hz, 1H), 6.71 (s, 1H), 6.70–6.68 (m, 3H), 6.55 (d, *J* = 2.3 Hz, 1H), 6.53 (d, *J* = 2.3 Hz, 1H), 2.93 (s, 4H). HRMS (ESI$^+$): *m/z* calculated for $C_{25}H_{15}NO_9$ $[M+H]^+$ 474.0820 found 474.1079.

2.4.2. Solid Phase Foldamer Synthesis (SPFS)

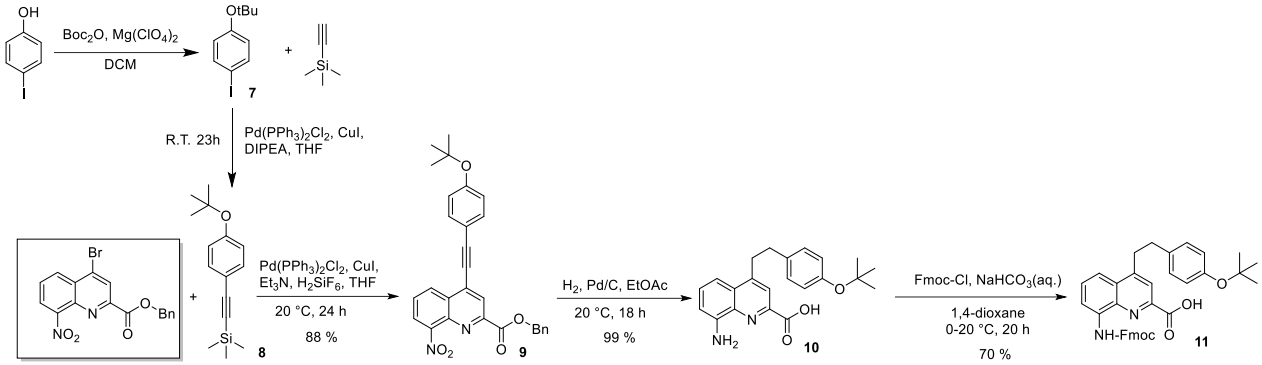
The microwave-assisted solid phase synthesis of aromatic oligoamide foldamers **1a**, **2a** and **5** was carried out on a Discover Bio CEM® microwave oven in an opened vessel mode manually. The temperature of the reaction mixture within the reactor vessel was monitored with an optical fiber probe. The LL-Wang resin was first brominated, and the Fmoc-Q-OH unit was loaded using the CsI assisted reaction. The efficiency of the first quinoline monomer loading was determined by UV-dosing the dibenzofulvene-piperidine adduct at 301 nm with an ε= 7800 L/mol/cm: 80% (30.4 mmol/g, 60 µmol).[6] The following Fmoc-Q/B units and Fmoc-3-Aminobenzoic acid were coupled with the *in-situ* activation protocol.[7] N-terminal biotinylation was performed on the resin-bound H-Amb-Q_{12}mer. The resin was suspended in 1mL DMF (15 µmol scale) in a syringe equipped with a filter. Biotin-Peg-OH (2 equiv.), PyBOP (2.1 equiv.) were dissolved in another 1 mL DMF, followed by DIPEA (4 equiv.), then the solution was transferred to the resin and shaken overnight (reaction can be monitored by TNBS test to check whether all free amines have been consumed). The introduction of the PEG tail in **2e** and **2f** followed the same procedure.

Foldamers **1b-1e** and **2b-2f** were prepared using the PurePep® Chorus synthesizer from Protein Technologies with LL-Wang resin or Cl-MPA ProTide® resin. The first loading on Cl-MPA ProTide® resin followed the recently published procedure[8]: Fmoc-$Q^{D(O\mathit{t}Bu)}$-OH or Fmoc-$Q^{O(Boc)}$-OH monomer (3.0 equiv.) was dissolved dry DMF and added to the resin together with a solution containing CsI (3.0 equiv.) and DIPEA (7.65 equiv.). The resin suspension was shaken overnight before to be washed first with DMF then with DCM, followed by loading determination.[Error! Bookmark not defined.] After automated SPFS[7] and biotin moiety coupling, the foldamer was cleaved from the resin and deprotected with a solution of TFA/H_2O/TIS (95:2.5:2.5, v/v/v). The crude was then lyophilised before to be purified by semi-prep RP-HPLC to furnish the desired foldamer in high purity (> 95%).

Of particular note, some RP-HPLC chromatogram show an additional peak, upfront which corresponds to the oxidized-biotin foldamer conjugates. This biotin oxidation and its percentage varies with the foldamer sequence. In our hands, this biotin oxidation appeared to be inconsequential on the foldamer ligand loading to SA sensor tips. The oxidized-biotin foldamer conjugate is annotated with a red star on the RP-HPLC chromatograms.

3. Experimental procedures for chemical synthesis

3.1. Scheme S1. Synthetic route of Fmoc-Q$^{Y(t\mathrm{Bu})}$-OH (**11**)



Compound 7: This compound was prepared from the reported method.[9] 4-Iodophenol (6.0 g, 27.3 mmol, 1.0 equiv.) was dissolved in anhydrous CH_2Cl_2 (60 mL) and $Mg(ClO_4)_2$ (1.22 g, 5.5 mmol, 0.2 equiv.) was added. Boc_2O (13.7 g, 62.7 mmol, 2.3 equiv.) was dissolved in CH_2Cl_2 (8 mL) and added dropwise to the first solution. The reaction was stirred under nitrogen atmosphere at room temperature for 16 hours. The reaction mixture was then washed with water (2 × 100 mL) followed by aqueous NaOH (2M, 2 × 100 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to yield compound **4** as oil (4.9 g, 60%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.61-7.58 (m, 2H), 6.82-6.78 (m, 2H), 1.28 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.0, 137.7, 126.1, 86.9, 78.5, 28.4. Spectral data matched literature data.[10]

Compound 8: This compound was prepared from the reported method.[11] To a dry nitrogen-flushed Schlenk flask, **7** (2.0 g, 7.24 mmol, 1.0 equiv.), $Pd(PPh_3)_2Cl_2$ (51 mg, 72.40 µmol, 1.0 mol%), CuI (28 mg, 144.80 µmol, 2.0 mol%), anhydrous THF (40 mL), and anhydrous DIPEA (2.5 mL, 14.5 mmol, 2.0 equiv.) were added and degassed using the freezepump-thaw method of three cycles and finally back-flushed with nitrogen. Trimethylsilylacetylene (1.24 mL, 8.69 mmol, 1.2 equiv.) was added, and the reaction was stirred under nitrogen atmosphere for 23 hours. CH_2Cl_2 (50 mL) was added to the reaction mixture and the mixture was washed with water (2 × 100 mL) and brine (2 × 100 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by filtration over a plug of silica using CH_2Cl_2 as the eluent, which furnished compound **8** as an oil after concentration of the filtrate (1.6 g, 90%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.39-7.32 (m, 2H), 6.99-6.92 (m, 2H), 1.31 (s, 9H), 0.21 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.9, 132.6, 123.3, 116.4, 105.2, 93.1, 78.8, 28.5, -0.03. Spectral data matched literature data.[11]

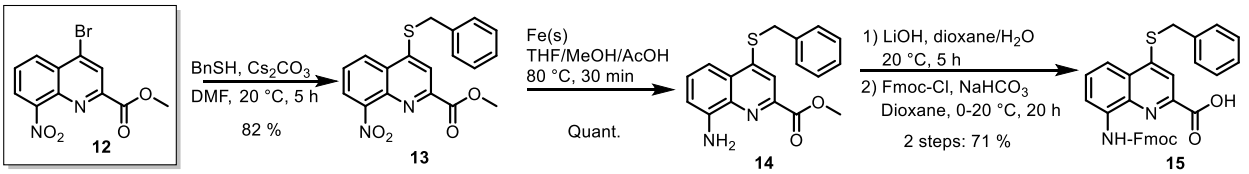
Compound 9: To a dry nitrogen-flushed Schlenk flask, compound **8** (2.09 g, 5.41 mmol, 1.0 equiv.), $Pd(PPh_3)_2Cl_2$ (57 mg, 54.1 µmol, 1.0 mol%), CuI (31 mg, 0.11 mmol, 2.0 mol%) and 4-bromo-8-nitro-phenylmethyl ester (1.6 g, 6.49 mmol, 1.2 equiv.)[4c] were dissolved in anhydrous THF (100 mL), and Et_3N (16.21 mL, 116 mmol, 20 equiv,) was added. The reaction mixture was immediately degassed using the freeze-pump-thaw method of three cycles and finally back-flushed with nitrogen. Hexafluorosilicic acid (32%

aq., 0.91 mL, 2.7 mmol, 0.5 equiv.) was added, and the reaction was stirred under nitrogen atmosphere for 24 hours. CH_2Cl_2 (80 mL) was added to the reaction mixture, and it was washed with citric acid (5% aq., 3 × 75 mL) and brine (2 × 100 mL) and the organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by flash column chromatography using CH_2Cl_2 as the eluent. Compound **9** was obtained as a yellow solid (2.3 g, 88.5%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.70 (dd, *J* = 8.5, 1.3 Hz, 1H), 8.46 (dd, *J* = 7.5, 1.3 Hz, 1H), 8.36 (s, 1H), 8.01 (dd, *J* = 8.5, 7.5, Hz, 1H), 7.80-7.72 (m, 2H), 7.57-7.49 (m, 2H), 7.49-7.31 (m, 3H), 7.17-7.08 (m, 2H), 5.48 (s, 2H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, DMSO-*d*$_6$), δ 163.4, 157.4, 149.2, 148.7, 138.0, 135.6, 133.6, 131.3, 129.6, 129.0, 128.5, 128.3, 128.3, 128.1, 124.9, 124.4, 123.0, 114.4, 101.8, 83.5, 79.3, 67.2, 28.5. HRMS (ESI$^+$): calcd. for $C_{29}H_{25}N_2O_5$ $[M+H]^+$ 481.1758 found 481.1878.

Compound 10: Compound **9** (1.46 g, 3.04 mmol, 1.0 equiv.) was dissolved in EtOAc (210 mL) containing DMF (10 mL). Pd/C (140 mg, 10% w/w) was added to the solution and the mixture was degassed for 15 min (with nitrogen balloon in an ultra sound bath), and finally the flask was backflushed with H_2. The reaction mixture was stirred under H_2-atmosphere at room temperature for 17 hours. The reaction mixture was then filtered over a pad of celite, which was washed several times with EtOAc. The filtrate was concentrated *in vacuo* (co-evaporation with toluene to remove DMF) to furnish **10** as a yellow solid (1.04 g, 95%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.72 (s, 1H), 7.85 (s, 1H), 7.43 (dd, *J* = 8.4, 7.7 Hz, 1H), 7.26 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.15-7.12 (m, 2H), 6.88 (dd, *J* = 7.7, 1.2 Hz, 1H), 6.87-6.83 (m, 2H), 6.57 (s, 2H), 3.32 (dd, *J* = 9.4, 6.5 Hz, 2H), 2.95 (dd, *J* = 9.4, 6.5 Hz, 2H), 1.25 (s, 9H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 165.7, 153.1, 149.0, 147.2, 142.4, 135.7, 135.6, 130.3, 129.1, 128.9, 123.7, 119.5, 108.8, 108.7, 77.6, 34.7, 33.9, 28.5. HRMS (ESI$^+$): calcd for $C_{22}H_{25}N_2O_3$ $[M+H]^+$ 365.1859 found 365.1979.

Compound 11: Compound **10** (2.4 g, 6.59 mmol, 1.0 equiv.) was dissolved in 1,4-dioxane (80 mL) and aqueous $NaHCO_3$ (10%, 116 mL) was added. The solution was cooled down to 0 °C and a solution of Fmoc-Cl (2.21 g, 8.56 mmol, 1.3 equiv.) in 1,4-dioxane (90 mL) was added dropwise over 1 hour. The reaction mixture was stirred at 0 °C for another hour, and then at room temperature for 16 hours. The reaction was quenched with 1 M HCl to pH around 2. CH_2Cl_2 (150 mL) was added, and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL), and the organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. Final purification was done by silica gel column chromatography twice with an eluent of CH_2Cl_2/MeOH 100:0 to 95:5. Compound **11** was isolated as a green foam (2.7 g, 70%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.58 (bs, 1H), 10.39 (s, 1H), 8.32 (bs, 1H), 8.01 (s, 1H), 7.93 (d, *J* = 7.5 Hz, 2H), 7.86 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.64 (t, *J* = 8.5, 1H), 7.49-7.40 (m, 2H), 7.38-7.34 (m, 2H), 7.19-7.08 (m, 2H), 6.88-6.79 (m, 2H), 4.61 (d, *J* = 6.8 Hz, 2H), 4.44 (t, *J* = 6.8 Hz, 1H), 3.43 (dd, *J* = 9.2, 6.6 Hz, 2H), 2.96 (dd, *J* = 9.2, 6.6 Hz, 2H), 1.25 (s, 9H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 166.1, 153.3, 153.2, 149.9, 143.7, 140.8, 136.7, 136.0, 135.4, 128.9, 128.8, 128.1, 127.8, 127.2, 125.1, 123.7, 120.7, 120.2, 117.0, 115.4, 77.6, 66.4, 46.6, 35.0, 33.6, 28.5. HRMS (ESI$^+$): calcd for $C_{37}H_{35}N_2O_5$ $[M+H]^+$ 587.2540 found 587.2547.

3.2. Scheme S2. Synthesis route of Fmoc-Q^F-OH (**15**)



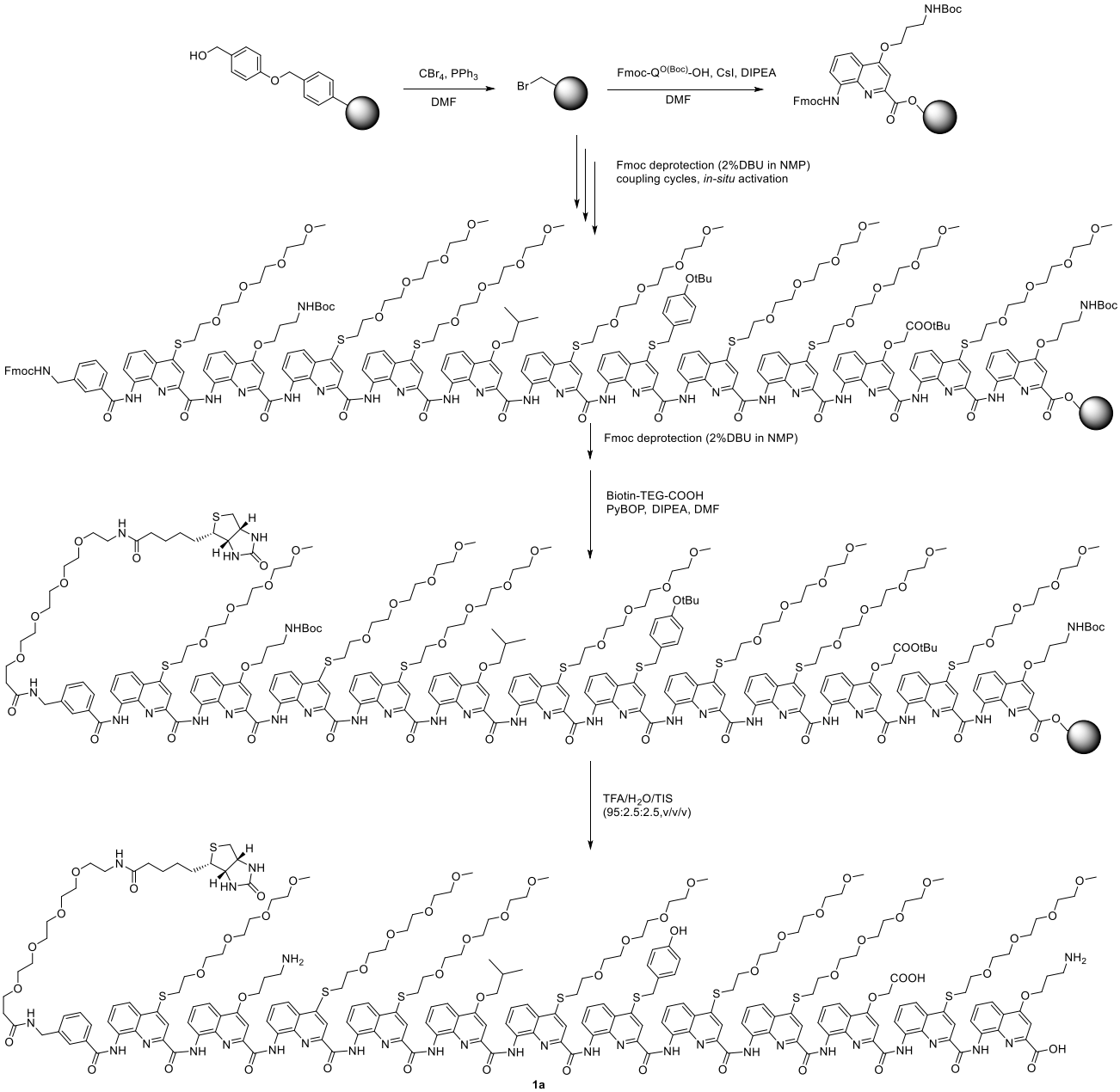
Compound 13: Compound **12** was synthesized according to the protocol reported in ref. 4b. Compound **12** (6.0 g, 19.3 mmol, 1 equiv.) was then suspended in dry DMF (90 mL) and Cs_2CO_3 (9.43 g, 8.9 mmol, 0.5 equiv.) were added. While stirring under N_2, benzylthiol was added dropwise (2.15 mL, 18.3 mmol, 1 equiv.). The reaction mixture was heated to 55 °C for 5 h and cooled down to room temperature. EtOAc (150 mL) was added to the reaction mixture and the solution was washed with brine (3 × 100 mL). The organic layer was dried over $MgSO_4$, and concentrated under vacuum. The remaining solid was recrystallized from CH_2Cl_2/Et_2O and **13** was isolated by filtration and washed with cold ether. Yield: 5.3 g (82%) ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (dd, *J* = 8.6, 1.3 Hz, 1H), 8.16 (s, 1H), 8.07 (dd, *J* = 7.5, 1.3 Hz, 1H), 7.68 (dd, *J* = 8.5, 7.5 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.44 – 7.34 (m, 2H), 7.36 – 7.29 (m, 1H), 4.45 (s, 2H), 4.03 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.42, 150.48, 149.15, 148.71, 138.54, 134.12, 129.11, 129.03, 128.26, 127.79, 127.40, 126.77, 124.62, 117.34, 53.42, 36.50. HRMS (ESI+): calcd for $C_{18}H_{15}N_2O_4S$ $[M+H]^+$ 355.0747, found 355.0737.

Compound 14: Compound **13** (3.24 g, 9.14 mmol, 1 equiv.) was suspended in a solvent mixture composed of THF (100 mL), MeOH (95 mL), and AcOH (61 mL). The reaction mixture was heated up to 80 °C and Fe (2.55 g, 45.7 mmol, 5 equiv.) was added portionwise. After stirring at 80 °C for 30 min, the reaction mixture was let to cool down to r.t., and the yellow precipitate ($Fe(CH_3CO_2)_2$) was removed by filtration and washed with CH_2Cl_2. The washing and filtrate were combined and concentrated *in vacuo*. The crude product was purified by filtration over a plug of silica (eluent: CH_2Cl_2/MeOH increasing from 0 to 10 %) and **14** was recovered quantitatively (3 g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.96 (s, 1H), 7.58 – 7.46 (m, 2H), 7.41 (dd, *J* = 8.3, 7.7 Hz, 1H), 7.40 – 7.31 (m, 2H), 7.34 – 7.26 (m, 1H), 7.14 (dd, *J* = 8.3, 1.2 Hz, 1H), 6.94 (dd, *J* = 7.7, 1.2 Hz, 1H), 6.16 (s, 2H), 4.54 (s, 2H), 3.94 (s, 3H), 1.22 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.75, 148.10, 147.38, 143.29, 136.19, 135.52, 130.68, 129.61, 129.11, 128.03, 127.40, 116.03, 110.21, 108.79, 53.07, 35.03. HRMS (ESI$^+$): calcd. for $C_{18}H_{17}N_2O_2S$ $[M+H]^+$ 325.1005, found 325.1005.

Compound 15: Compound **14** (3.4 g, 10.6 mmol, 1 equiv.) was dissolved in 1,4-dioxane (280 mL) and a solution of LiOH (0.40 g, 15.9 mmol, 1.5 equiv.) in water (70 mL) was added and the mixture was stirred for 5 hours. The reaction mixture was neutralized by dropwise addition of HCl (1M). Aqueous $NaHCO_3$ (10% v/v, 187 mL) was added to the reaction mixture, and the solution was cooled to 0 °C. A solution of Fmoc-Cl (3.6 g, 13.8 mmol, 1.3 equiv.) in 1,4-dioxane (75 mL) was prepared and added dropwise over 1 h. Afterwards the reaction was stirred at 0 °C for another 1 hour, and then at r.t. for 20 hours. The mixture was acidified by slow addition of aqueous HCl (1M, approx. 270 mL) and afterwards extracted with CH_2Cl_2 (2 x 300 mL). The organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by

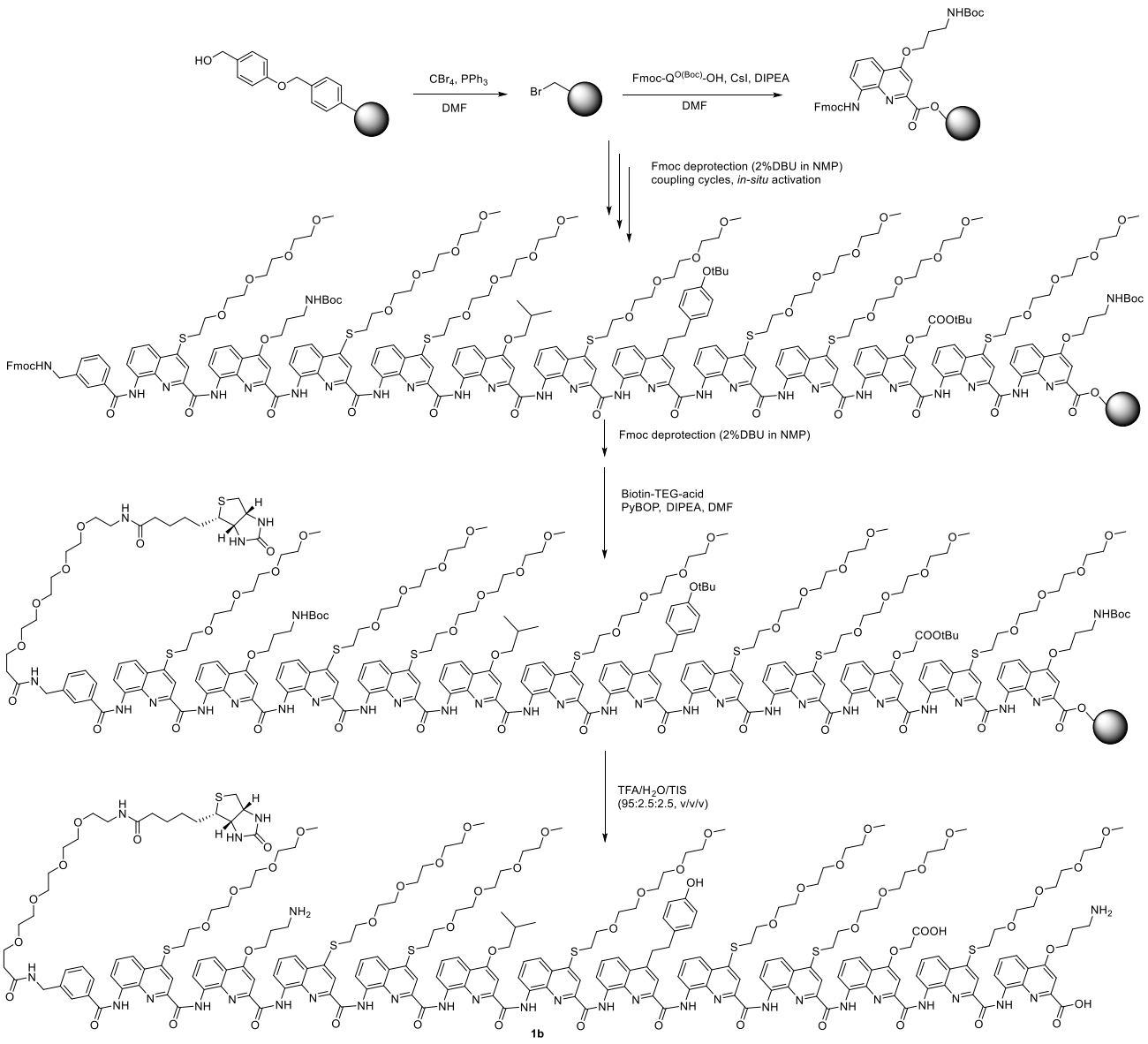
flash column chromatography (gradient: CH_2Cl_2: MeOH 100:0 to CH_2Cl_2: MeOH 90:10). The fractions containing **13** were collected and concentrated and recrystallized from CH_2Cl_2/Et_2O, to furnish 13 in 71 % yield (4.0 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.51 (s, 1H), 10.43 (s, 1H), 8.34 (s, 1H), 8.12 (d, *J* = 1.2 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 2H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.72 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.65 (t, *J* = 8.1 Hz, 1H), 7.53 (dd, *J* = 7.9, 1.6 Hz, 2H), 7.47 – 7.26 (m, 8H), 4.64 – 4.58 (m, 4H), 4.44 (t, *J* = 6.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.76, 153.92, 150.28, 144.16, 141.29, 136.81, 136.09, 135.84, 129.89, 129.64, 129.16, 128.26, 128.13, 127.70, 126.86, 125.62, 120.72, 116.97, 116.50, 115.82, 66.93, 47.05, 35.12. HRMS (ESI+): calcd. for $C_{32}H_{25}N_2O_4S$ $[M+H]^+$ 533.1530 found 533.1531.

3.3. SPFS of compounds **1**-**5**



Compound 1a: Foldamer **1a** was synthesized on a low loading LL-Wang resin (19 µmol scale). After TFA cleavage and side chain deprotection, the crude foldamer was purified by semi prep RP-HPLC to furnish **1a** as yellow solid (1.7 mg, 1.9%). HRMS (ESI$^+$): m/z calcd. for $C_{231}H_{272}N_{30}O_{56}S_9$ $[M+3H]^{3+}$1551.9055, found 1551.9388.

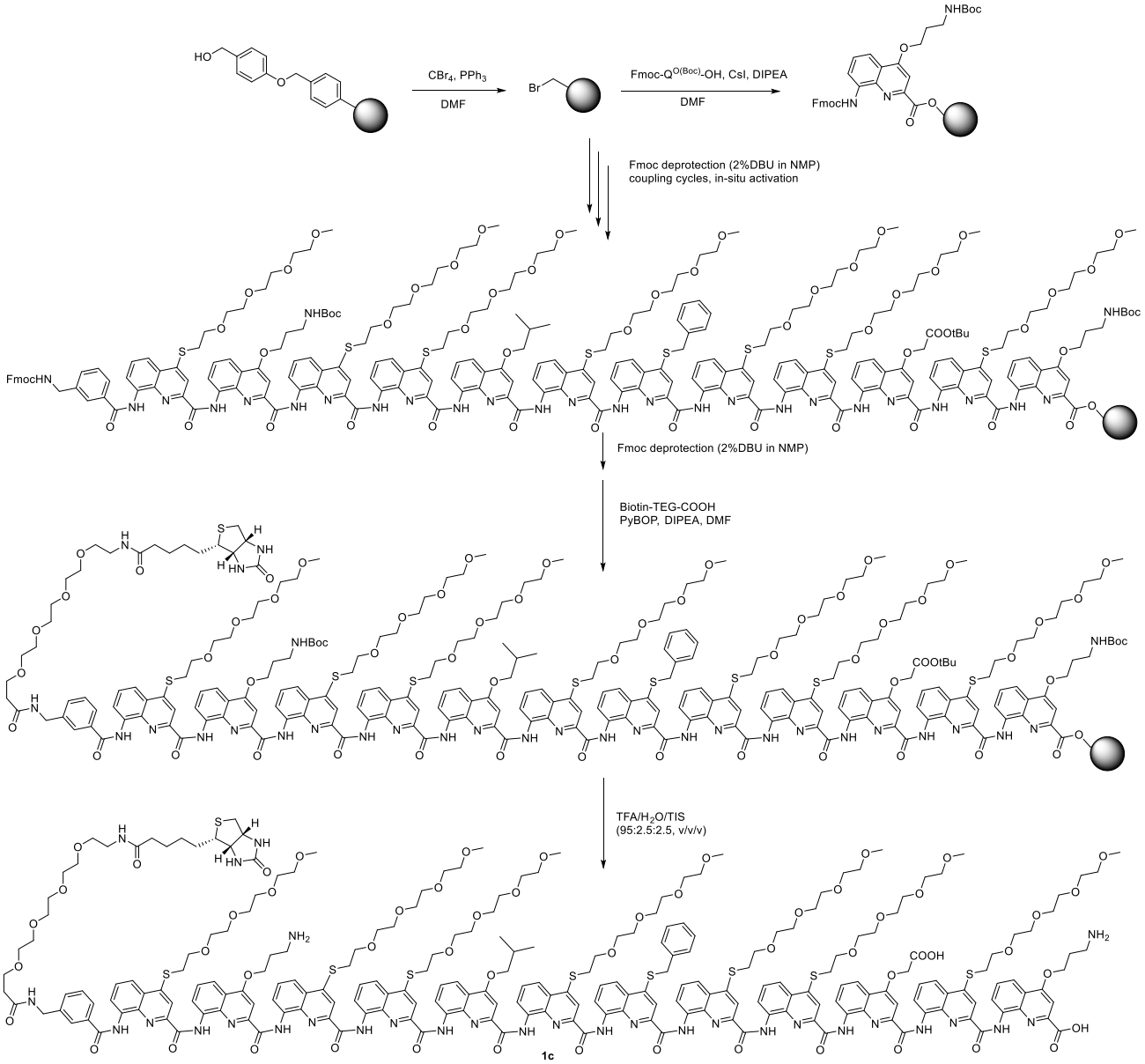
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.89 (s, 2H), 10.61 (s, 1H), 10.36 (s, 1H), 10.28 (s, 2H), 10.19 (s, 1H), 10.17 (s, 1H), 10.14 (s, 1H), 10.11 (s, 1H), 10.06 (s, 1H), 9.69 (s, 1H), 9.04 (s, 1H), 7.81 (d, *J* = 6.1 Hz, 7H), 7.77 (d, *J* = 7.2 Hz, 2H), 7.71 (q, *J* = 5.8 Hz, 2H), 7.63 (dd, *J* = 15.0, 7.8 Hz, 6H), 7.57 (d, *J* = 7.2 Hz, 1H), 7.54 – 7.29 (m, 18H), 7.26 (t, *J* = 7.6 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 4H), 7.13 – 7.07 (m, 6H), 7.07 – 7.00 (m, 5H), 6.99 (d, *J* = 7.3 Hz, 2H), 6.96 – 6.82 (m, 11H), 6.73 (d, *J* = 6.8 Hz, 2H), 6.69 (s, 1H), 6.66 (s, 1H), 6.54 (s, 1H), 6.45 (dd, *J* = 15.0, 7.6 Hz, 2H), 6.32 (d, *J* = 7.5 Hz, 2H), 6.27 (d, *J* = 8.2 Hz, 2H), 6.23 (s, 1H), 6.18 (s, 1H), 6.12 (s, 1H), 6.08 (d, *J* = 8.6 Hz, 3H), 6.03 (s, 1H), 5.56 (s, 1H), 4.53 (d, *J* = 15.1 Hz, 1H), 4.28 (s, 3H), 4.23 – 4.16 (m, 2H), 4.06 (d, *J* = 11.6 Hz, 2H), 4.04 – 3.99 (m, 2H), 3.97 – 3.80 (m, 19H), 3.82 – 3.69 (m, 40H), 3.69 – 3.55 (m, 52H), 3.55 – 3.36 (m, 53H), 3.19 (s, 5H), 3.13 (s, 5H), 3.10 – 3.01 (m, 15H), 3.00 – 2.90 (m, 5H), 2.71 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.23 – 2.17 (m, 2H), 2.08 – 1.97 (m, 6H), 1.93 (t, *J* = 7.4 Hz, 3H), 1.41 – 1.30 (m, 4H), 1.16 (d, *J* = 5.2 Hz, 7H), 1.14 (d, *J* = 3.9 Hz, 4H), 1.12 (d, *J* = 1.2 Hz, 2H).



Compound 1b: Foldamer **1b** was synthesized on a low loading LL-Wang resin (15 µmol scale). After TFA cleavage and side chain deprotection, 14 mg of crude foldamer were recovered. The crude was then purified by semi prep RP-HPLC to furnish **1b** as yellow solid (5 mg, 12%). HRMS (ESI$^+$): m/z calcd. for $C_{232}H_{274}N_{30}O_{56}S_8$ $[M+2H]^{2+}$ 2318.3322, found 2318.3337.

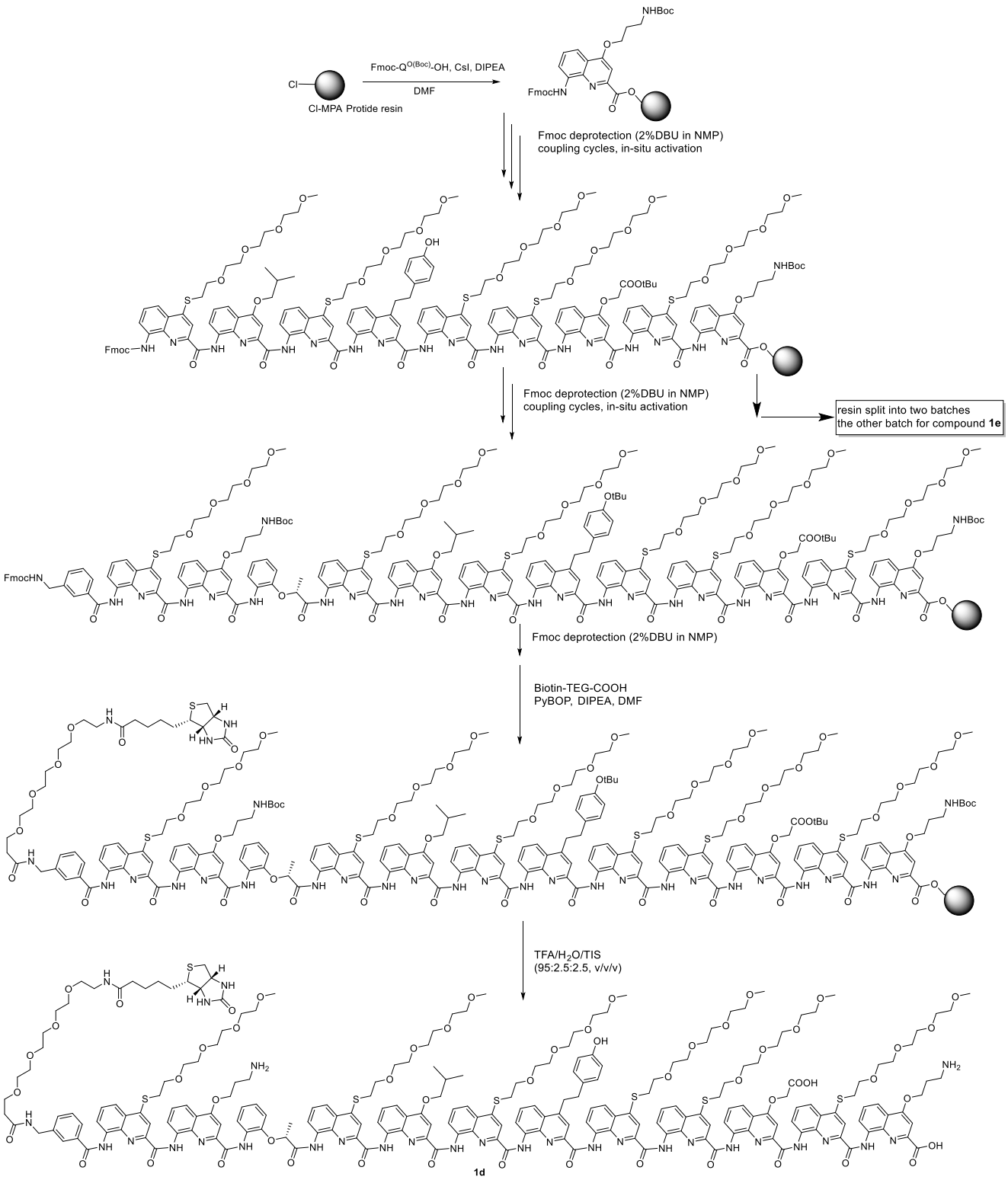
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.92 (s, 1H), 10.70 (s, 1H), 10.43 (s, 2H), 10.37 (s, 1H), 10.34 (s, 1H), 10.28 (s, 1H), 10.25 (s, 2H), 10.21 (s, 1H), 9.38 (s, 1H), 9.12 (s, 1H), 7.97 – 7.82 (m, 5H), 7.82 – 7.72 (m, 6H), 7.65 (ddt, *J* = 23.9, 16.6, 7.7 Hz, 10H), 7.58 – 7.46 (m, 14H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.38 (d, *J* = 7.9 Hz, 3H), 7.31 – 7.20 (m, 6H), 7.21 – 7.03 (m, 14H), 7.01 (d, *J* = 7.2 Hz, 2H), 6.97 (d, *J* = 7.3 Hz, 2H), 6.92 (dd, *J* = 8.1, 6.1 Hz, 3H), 6.80 (dd, *J* = 11.7, 7.5 Hz, 2H), 6.74 (d, *J* = 7.8 Hz, 2H), 6.59 (s, 1H), 6.51 (t, *J* = 7.3 Hz, 1H), 6.41 (s, 1H), 6.37 (s, 1H), 6.35 – 6.27 (m, 3H), 6.22 – 6.13 (m, 4H), 5.67 (s, 1H), 4.59 (d, *J* = 15.5 Hz, 1H), 4.42 (t, *J* = 7.7 Hz, 1H), 4.39 – 4.20 (m, 3H), 4.08 (d, *J* = 6.6 Hz, 1H), 4.03 – 3.89 (m, 13H),

3.89 – 3.81 (m, 22H), 3.81 – 3.76 (m, 16H), 3.76 – 3.72 (m, 12H), 3.72 – 3.68 (m, 18H), 3.68 – 3.62 (m, 23H), 3.62 – 3.58 (m, 13H), 3.58 – 3.52 (m, 21H), 3.52 – 3.49 (m, 6H), 3.49 – 3.44 (m, 11H), 3.41 – 3.36 (m, 17H), 3.26 (s, 6H), 3.20 (s, 5H), 3.13 – 3.06 (m, 11H), 2.96 (s, 5H), 2.85 (dd, *J* = 12.4, 7.0 Hz, 4H), 2.78 (dd, *J* = 12.5, 5.0 Hz, 1H), 2.29 – 2.22 (m, 3H), 2.11 – 1.96 (m, 9H), 1.74 – 1.65 (m, 1H), 1.52 – 1.41 (m, 3H), 1.39 (s, 1H), 1.28 (s, 1H), 1.27 – 1.15 (m, 13H), 0.89 – 0.80 (m, 1H).



Compound 1c: Foldamer **1c** was synthesized on a LL-Wang resin (15 µmol scale). After TFA cleavage and side chain deprotection, 21 mg of crude foldamer was obtained. The crude was purified by semi prep RP-HPLC to furnish **1c** as yellow solid (2 mg, 4.3%). HRMS (ESI+): m/z calcd. for $C_{231}H_{272}N_{30}O_{55}S_9$ $[M+2H]^{2+}$ 2319.3554, found 2319.3642.

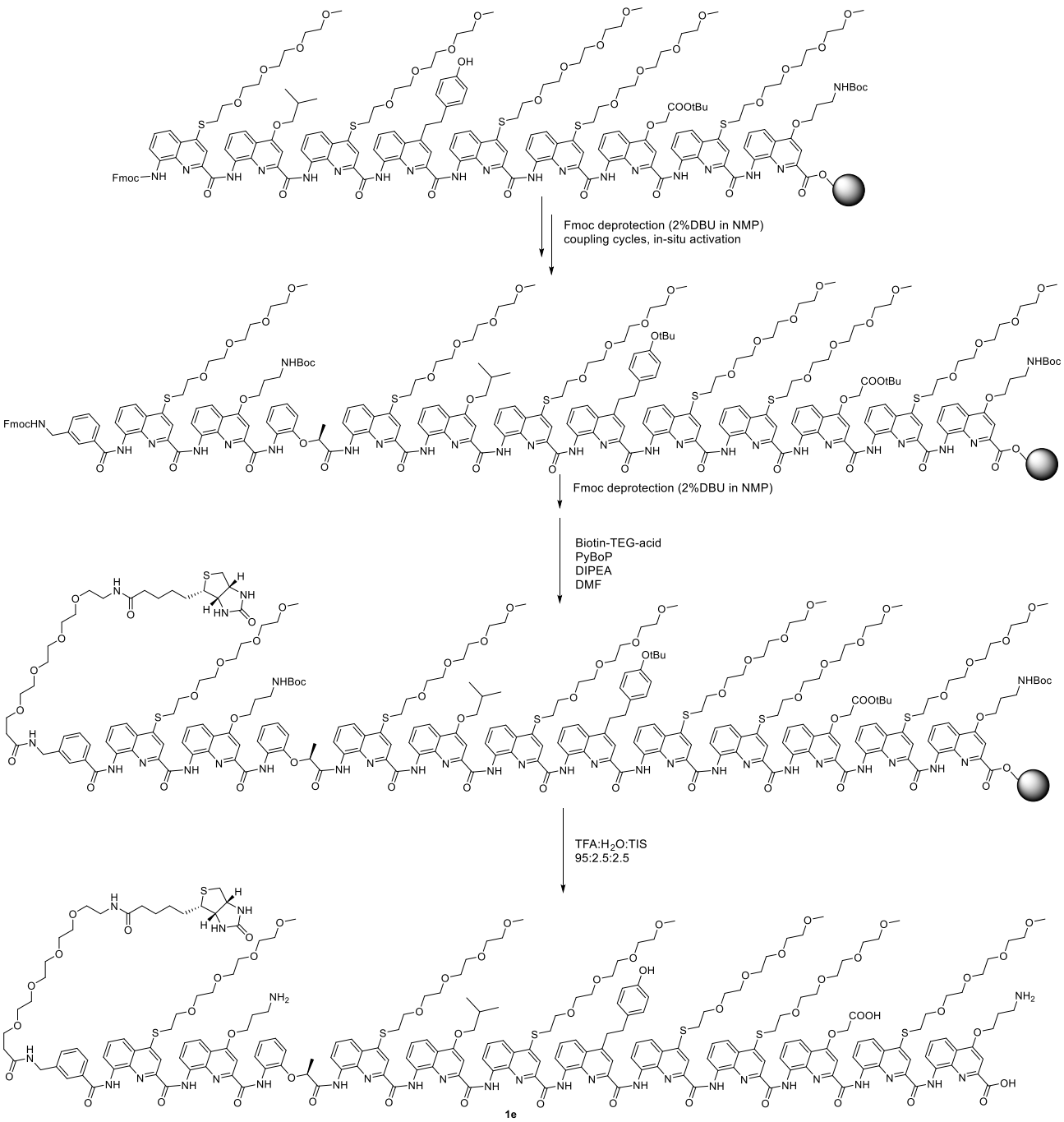
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.93 (s, 1H), 10.89 (s, 1H), 10.69 (s, 1H), 10.43 (s, 1H), 10.40 (s, 1H), 10.30 (s, 1H), 10.27 (s, 1H), 10.23 (s, 1H), 10.21 (s, 2H), 9.12 (s, 1H), 7.99 – 7.81 (m, 8H), 7.81 – 7.74 (m, 2H), 7.74 – 7.39 (m, 33H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.24 (q, *J* = 7.5 Hz, 4H), 7.21 – 7.08 (m, 11H), 7.06 (d, *J* = 7.3 Hz, 2H), 7.04 – 6.97 (m, 4H), 6.94 (dd, *J* = 17.5, 7.8 Hz, 3H), 6.81 (t, *J* = 7.1 Hz, 4H), 6.75 (d, *J* = 10.2 Hz, 4H), 6.67 (s, 1H), 6.62 (s, 1H), 6.52 (t, *J* = 7.4 Hz, 1H), 6.41 (s, 1H), 6.39 – 6.29 (m, 3H), 6.23 (s, 1H), 6.17 (d, *J* = 8.0 Hz, 3H), 6.11 (s, 2H), 5.65 (s, 1H), 4.59 (d, *J* = 15.5 Hz, 1H), 4.43 (t, *J* = 7.5 Hz, 1H), 4.35 (s, 2H), 4.32 – 4.26 (m, 2H), 4.24 (s, 1H), 4.17 (d, *J* = 12.9 Hz, 1H), 4.09 (d, *J* = 7.2 Hz, 1H), 4.07 – 3.89 (m, 17H), 3.89 – 3.77 (m, 41H), 3.77 – 3.63 (m, 58H), 3.63 – 3.58 (m, 15H), 3.58 – 3.53 (m, 21H), 3.53 – 3.43 (m, 23H), 3.26 (s, 8H), 3.21 (s, 6H), 3.18 – 3.06 (m, 19H), 3.01 (s, 3H), 2.91 – 2.74 (m, 3H), 2.31 – 2.22 (m, 3H), 2.16 – 1.96 (m, 10H), 1.74 – 1.68 (m, 1H), 1.59 – 1.40 (m, 5H), 1.30 – 1.14 (m, 15H), 0.90 – 0.81 (m, 1H).



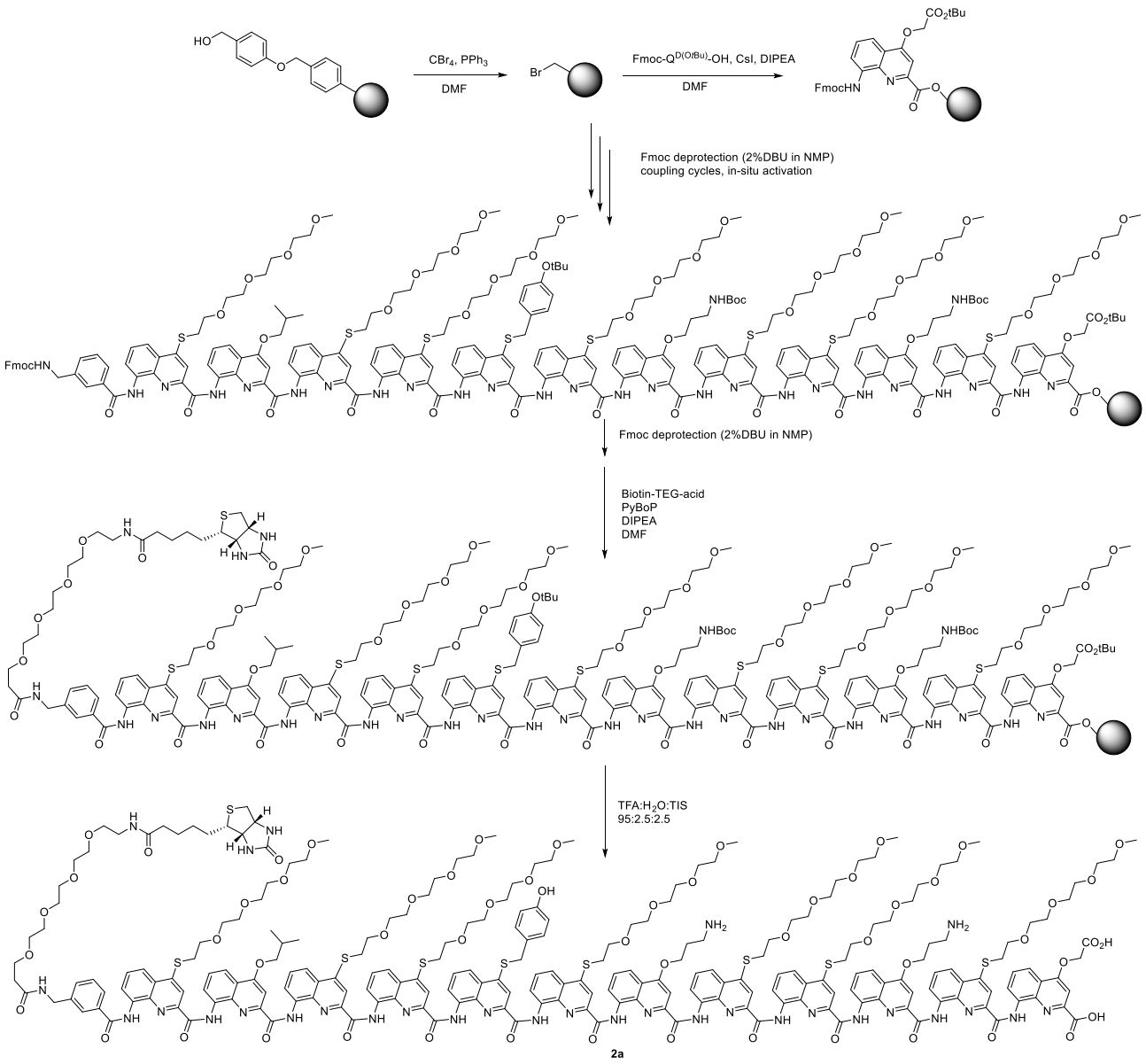
Compound 1d: Foldamer **1d** was synthesized on a Cl-MPA ProTide® resin (20 µmol scale). After the 9th quinoline coupling, the resin was divided into two batches. The other 7.5 µmol was used for synthesizing **1e**. After TFA cleavage and side chain deprotection, 30 mg of crude foldamer was obtained. The crude was

purified by semi prep RP-HPLC to furnish **1d** as yellow solid (7.4 mg, 22%). HRMS (ESI^+): calcd. for $C_{222}H_{259}N_{29}O_{53}S_7$ $[M+3H]^{3+}$ 1468.5575 found 1468.5728.

^{1}H NMR (500 MHz, DMF-d_7) δ 11.11 (s, 1H), 11.03 (s, 1H), 10.80 (s, 1H), 10.60 (s, 3H), 10.48 (s, 1H), 10.40 (s, 1H), 10.26 (s, 1H), 9.90 (s, 1H), 9.65 (s, 1H), 9.08 (s, 1H), 8.84 (s, 1H), 8.46 (d, *J* = 7.4 Hz, 2H), 8.37 (s, 6H), 7.86 (s, 1H), 7.85 – 7.80 (m, 2H), 7.80 – 7.71 (m, 6H), 7.69 (d, *J* = 8.1 Hz, 3H), 7.62 (s, 3H), 7.55 (dt, *J* = 16.0, 8.0 Hz, 5H), 7.49 (d, *J* = 7.1 Hz, 3H), 7.44 (d, *J* = 7.7 Hz, 4H), 7.39 (d, *J* = 7.2 Hz, 2H), 7.37 – 7.20 (m, 11H), 7.17 (s, 2H), 7.09 (t, *J* = 9.4 Hz, 5H), 7.04 (d, *J* = 7.7 Hz, 3H), 6.91 (d, *J* = 7.6 Hz, 2H), 6.84 (s, 2H), 6.66 (t, *J* = 7.5 Hz, 1H), 6.56 (s, 3H), 6.49 (d, *J* = 14.4 Hz, 3H), 6.39 (s, 4H), 6.34 (s, 1H), 6.31 (s, 1H), 6.25 (s, 1H), 6.05 (d, *J* = 8.2 Hz, 1H), 5.97 (t, *J* = 7.7 Hz, 1H), 4.81 (d, *J* = 15.3 Hz, 1H), 4.73 (s, 1H), 4.45 (dd, *J* = 7.8, 5.1 Hz, 3H), 4.30 – 4.22 (m, 1H), 4.12 (s, 8H), 4.08 – 3.99 (m, 7H), 3.97 – 3.88 (m, 20H), 3.87 – 3.81 (m, 15H), 3.81 – 3.76 (m, 17H), 3.76 – 3.73 (m, 10H), 3.73 – 3.70 (m, 7H), 3.70 (s, 3H), 3.68 (td, *J* = 5.8, 3.4 Hz, 12H), 3.66 – 3.61 (m, 8H), 3.61 – 3.54 (m, 20H), around 3.5 (broad water solvent peak), 3.45 (d, *J* = 3.2 Hz, 10H), 3.43 – 3.40 (m, 12H), 3.40 (s, 5H), 3.37 (d, *J* = 2.8 Hz, 8H), 3.30 – 3.24 (m, 10H), 3.10 – 3.04 (m, 2H), 2.79 – 2.76 (m, 5H), 2.71 (q, *J* = 2.1 Hz, 1H), 2.45 (s, 2H), 2.39 – 2.29 (m, 4H), 2.26 – 2.17 (m, 3H), 2.15 (s, 2H), 2.13 (d, *J* = 7.5 Hz, 2H), 1.76 – 1.67 (m, 1H), 1.61 – 1.50 (m, 4H), 1.42 – 1.33 (m, 3H), 1.31 – 1.23 (m, 11H), 0.88 (t, *J* = 6.8 Hz, 1H), -0.31 (s, 3H).



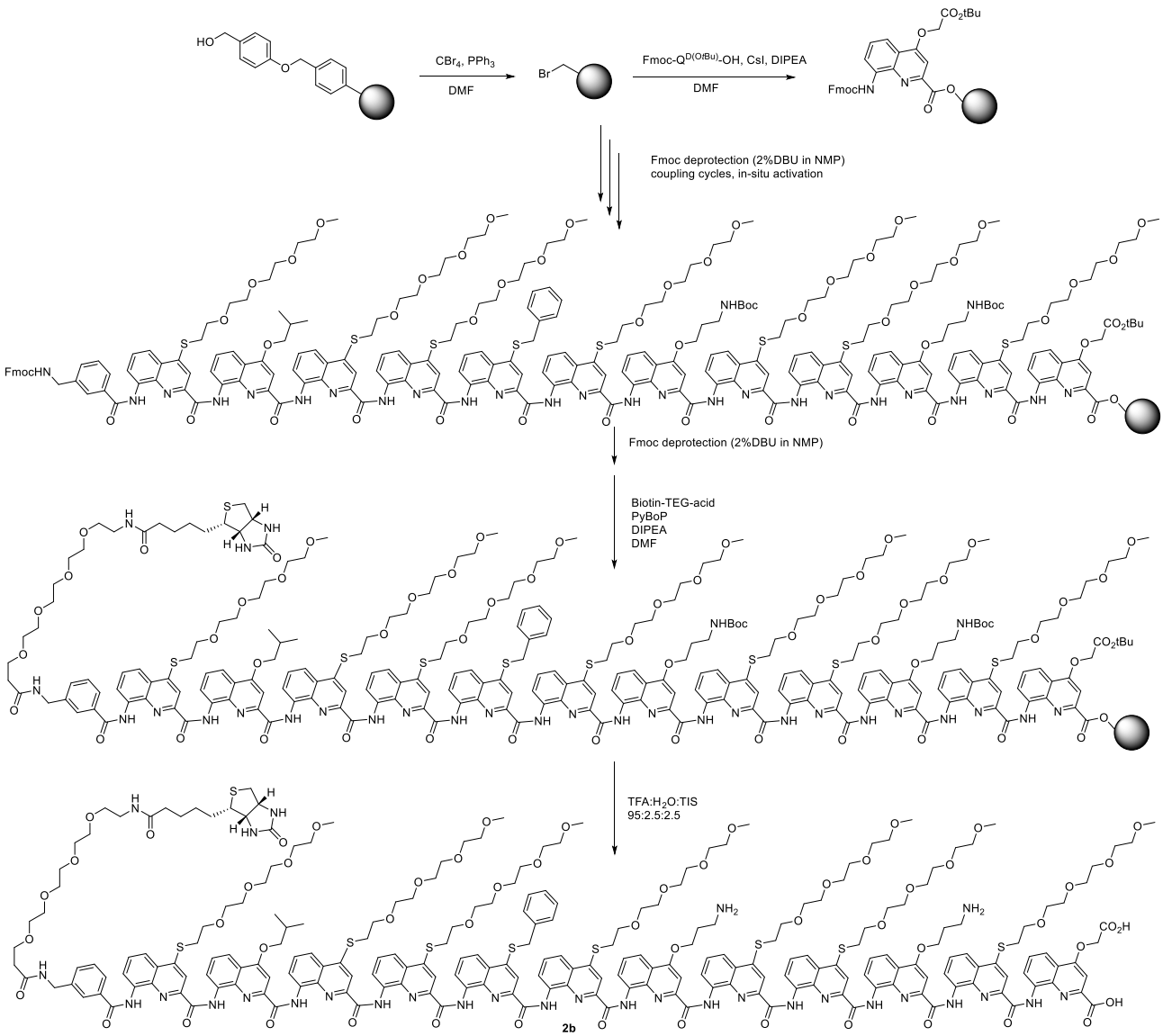
Compound 1e: The crude foldamer was purified by semi prep RP-HPLC to furnish **1e** as yellow solid (1.6 mg, 5%). HRMS (ESI$^+$): calcd. for $C_{222}H_{259}N_{29}O_{53}S_7$ $[M+3H]^{3+}$: 1468.5575 found 1468.5850.



Compound 2a: Foldamer **2a** was synthesized on a LL-Wang resin (15 µmol scale). After TFA cleavage and side chain deprotection, 15.2 mg of crude foldamer was obtained. The crude was purified by semi prep RP-HPLC to furnish **2a** as a yellow solid (5.7 mg, 12%). HRMS (ESI$^+$): m/z calcd. for $C_{231}H_{272}N_{30}O_{56}S_9$ $[M+3H]^{3+}$ 1551.9055, found 1551.9253.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 10.86 (s, 1H), 10.68 (s, 1H), 10.49 (s, 1H), 10.45 (s, 1H), 10.42 (s, 1H), 10.30 (s, 1H), 10.26 (s, 1H), 10.20 (s, 1H), 10.11 (s, 1H), 10.04 (s, 1H), 9.74 (s, 1H), 9.12 (s, 1H), 7.97 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 7.3 Hz, 1H), 7.82 (s, 3H), 7.79 – 7.74 (m, 2H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.63 (t, *J* = 8.5 Hz, 2H), 7.58 – 7.33 (m, 17H), 7.28 – 6.89 (m, 18H), 6.85 – 6.78 (m, 2H), 6.76 (s, 2H), 6.54 (s, 1H), 6.39 (s, 2H), 6.33 (d, *J* = 9.6 Hz, 2H), 6.28 (s, 1H), 6.18 – 6.08 (m, 5H), 4.14 – 4.01 (m, 2H), 3.98 – 3.90 (m, 2H), 3.86 – 3.43 (m, 62H), 3.40 – 3.35 (m, 9H), 3.25 (s, 3H), 3.19 (s, 3H), 3.13 – 3.03

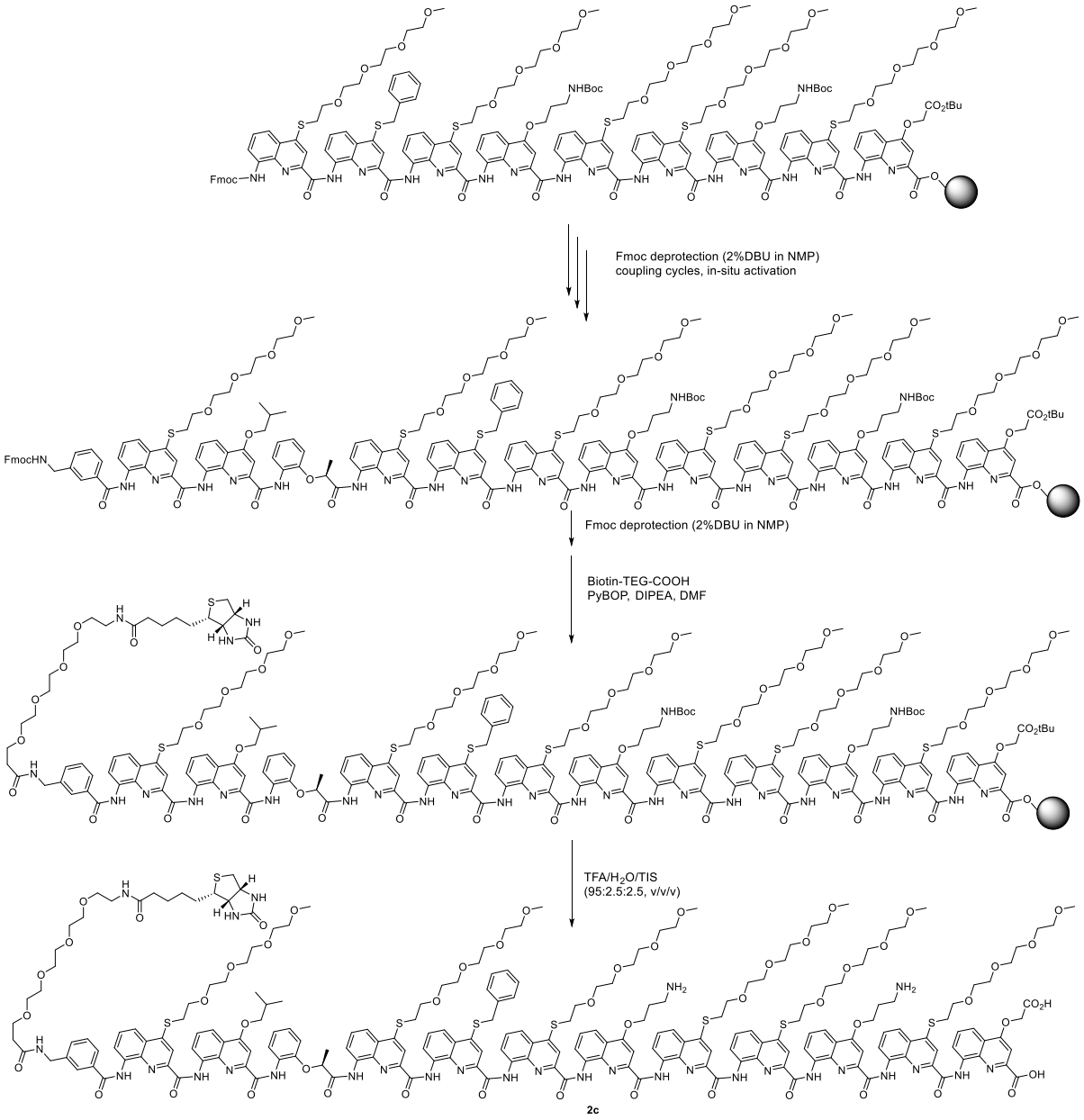
(m, 2H), 2.78 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.34 – 2.27 (m, 4H), 2.23 – 2.15 (m, 3H), 2.11 – 2.06 (m, 2H), 2.06 – 1.96 (m, 2H), 1.42 (m, 3H), 1.23 (s, 4H), 1.15 (t, *J* = 6.2 Hz, 7H).



Compound 2b: Foldamer **2b** was synthesized on a LL-Wang resin (17 µmol scale). After TFA cleavage and side chain deprotection, 50 mg crude was obtained. The crude was purified by semi prep RP-HPLC to furnish **2b** as yellow solid (5 mg, 8%). HRMS (ESI$^+$): m/z calcd. for $C_{231}H_{272}N_{30}O_{55}S_9$ $[M+2H]^{2+}$ 2319.3554, found 2319.3724.

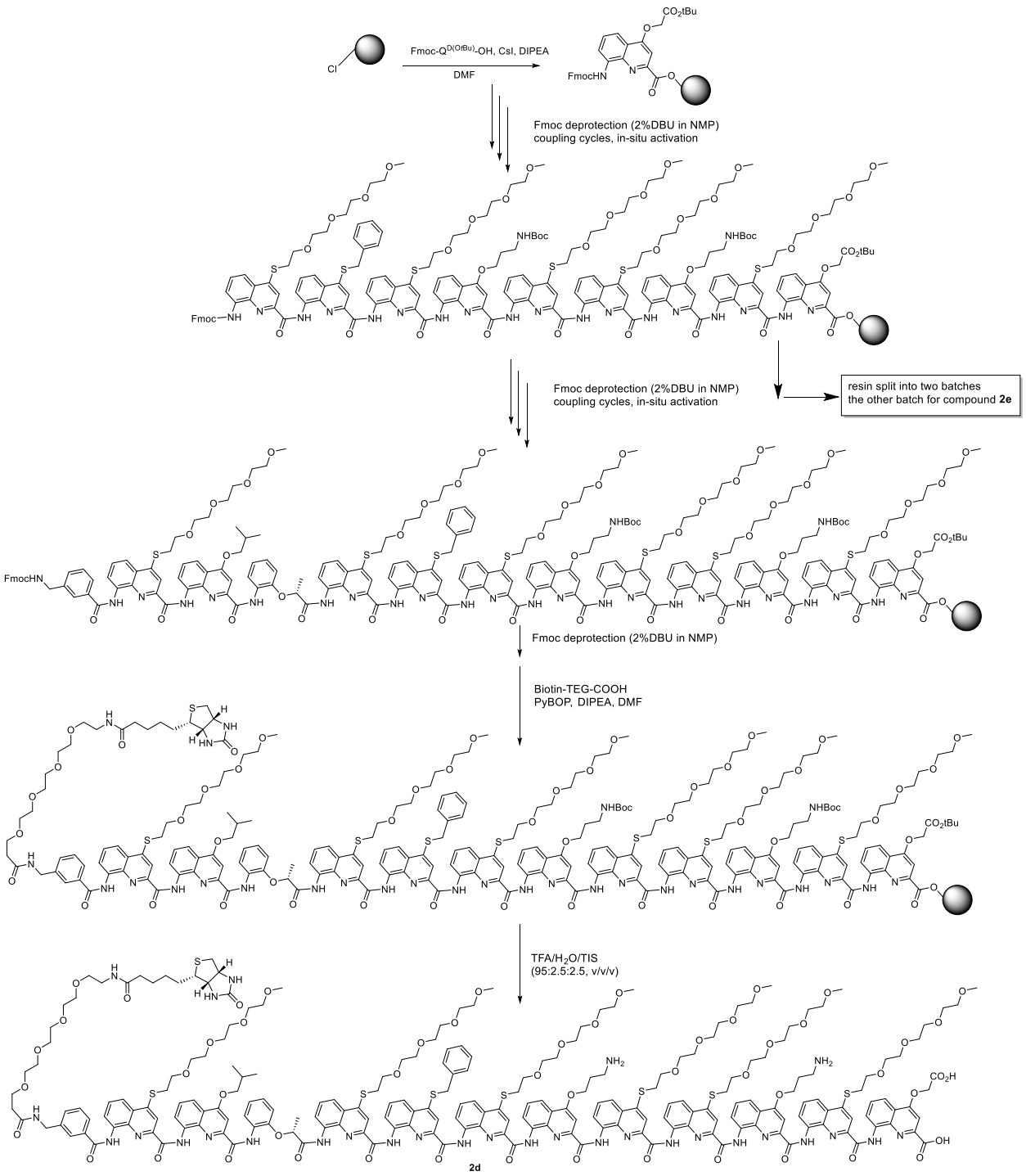
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.89 (s, 1H), 10.68 (s, 1H), 10.62 (s, 1H), 10.49 (s, 1H), 10.45 (s, 1H), 10.35 (s, 1H), 10.29 (s, 1H), 10.24 (s, 1H), 10.22 (s, 1H), 10.12 (s, 2H), 9.11 (s, 1H), 7.98 (dd, *J* = 22.5, 7.3 Hz, 2H), 7.75 – 7.57 (m, 13H), 7.57 – 7.36 (m, 17H), 7.23 (q, *J* = 7.2 Hz, 6H), 7.17 (s, 4H), 7.10 (d, *J* = 7.4 Hz, 6H), 7.05 (s, 5H), 7.00 (s, 3H), 6.96 (d, *J* = 7.3 Hz, 2H), 6.92 (d, *J* = 7.2 Hz, 3H), 6.89 (s, 1H), 6.86 (d, *J* = 7.4 Hz, 2H), 6.80 (s, 1H), 6.76 (d, *J* = 7.6 Hz, 2H), 6.55 (s, 1H), 6.42 – 6.26 (m, 5H), 6.24 – 6.03 (m,

6H), 5.75 (s, 1H), 4.42 (s, 1H), 4.31 – 4.13 (m, 8H), 4.13 – 4.05 (m, 5H), 4.00 – 3.00 (peaks were overlapped with water solvent peak), 2.05 – 1.89 (m, 3H), 1.23 (s, 11H), 1.14 (t, *J* = 6.3 Hz, 9H), 0.85 (t, *J* = 6.7 Hz, 2H).

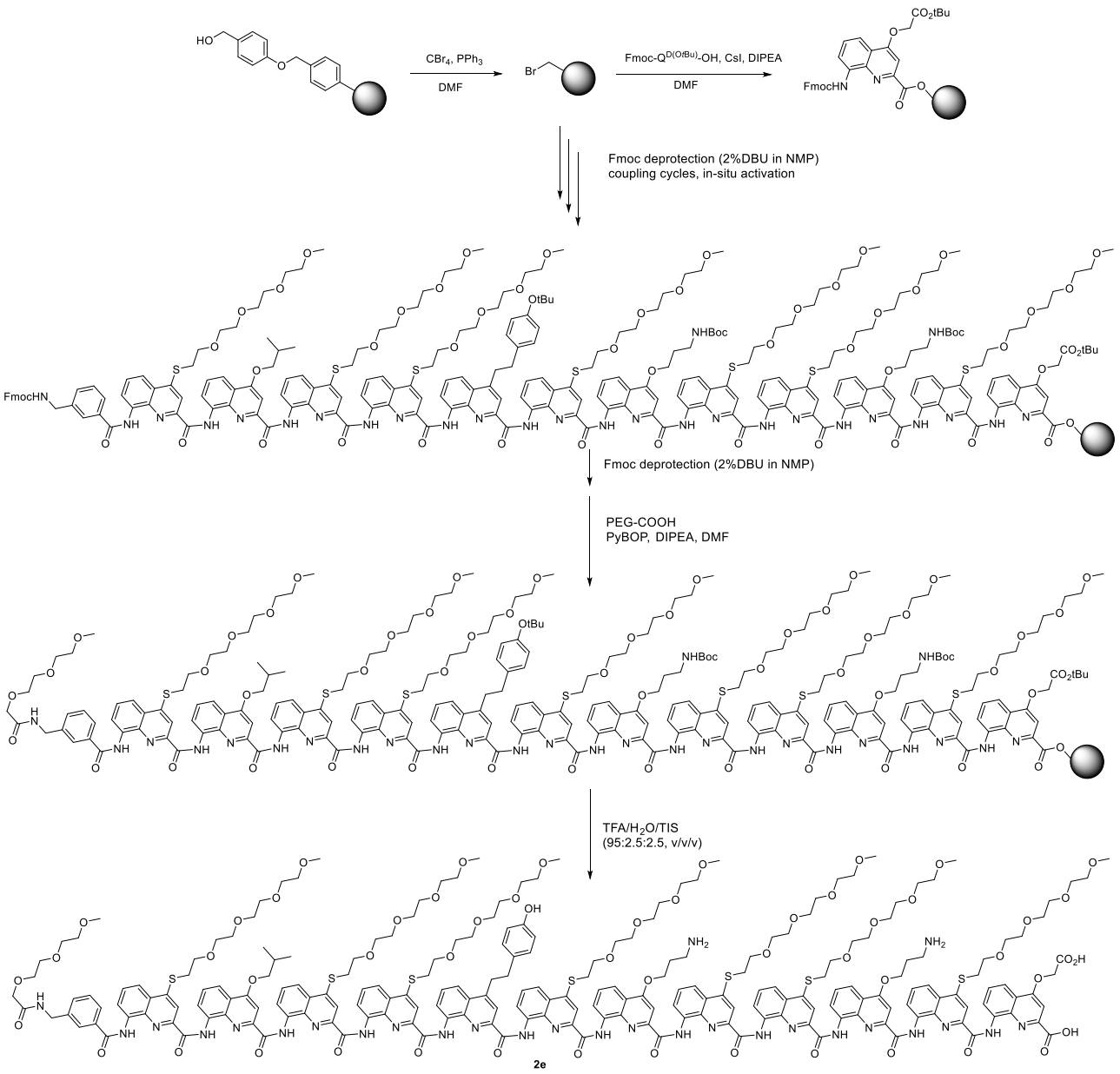


Compound 2c: Foldamer **2c** was synthesized on a Cl-MPA ProTide® resin (30 µmol scale). After the 9th Q coupling, the resin was divided into two batches. The other 15 µmol was used for **2d** synthesis (see below). After TFA cleavage and side chain deprotection, 30 mg of crude foldamer was obtained. The crude was purified by semi prep RP-HPLC to furnish **2c** as yellow solid (9.2 mg, 14%). HRMS (ESI$^+$): calcd. for $C_{221}H_{259}N_{29}O_{52}S_8$ $[M+2H]^{2+}$: 2204.8198, measured: 2204.9361.

^{1}H NMR (500 MHz, DMF-d_7) δ 11.18 (s, 1H), 10.91 (s, 1H), 10.71 (s, 1H), 10.67 (s, 1H), 10.61 (s, 1H), 10.53 (s, 1H), 10.42 (s, 1H), 10.24 (s, 1H), 10.22 (s, 1H), 9.90 (s, 1H), 9.14 (s, 1H), 8.87 (s, 1H), 8.47 (d, *J* = 7.4 Hz, 1H), 8.15 (dd, *J* = 19.4, 7.1 Hz, 2H), 7.86 (s, 1H), 7.80 (t, *J* = 6.7 Hz, 5H), 7.76 (s, 1H), 7.75 (d, *J* = 1.1 Hz, 1H), 7.74 – 7.67 (m, 5H), 7.67 – 7.60 (m, 6H), 7.60 – 7.51 (m, 6H), 7.51 – 7.43 (m, 5H), 7.41 (d, *J* = 7.4 Hz, 1H), 7.39 – 7.19 (m, 12H), 7.19 – 7.12 (m, 2H), 7.06 (d, *J* = 7.5 Hz, 1H), 7.01 (s, 1H), 6.96 (d, *J* = 7.5 Hz, 1H), 6.87 (d, *J* = 5.5 Hz, 2H), 6.77 (d, *J* = 7.9 Hz, 1H), 6.62 (d, *J* = 16.2 Hz, 2H), 6.49 (s, 1H), 6.47 (s, 1H), 6.45 – 6.40 (m, 2H), 6.39 (s, 1H), 6.34 (s, 1H), 6.22 (s, 1H), 6.05 (s, 1H), 5.95 (s, 2H), 5.91 (t, *J* = 7.3 Hz, 1H), 4.87 – 4.69 (m, 2H), 4.62 (d, *J* = 13.5 Hz, 1H), 4.54 – 4.36 (m, 5H), 4.35 – 4.22 (m, 2H), 4.20 – 3.97 (m, 13H), 3.97 – 3.88 (m, 18H), 3.88 – 3.81 (m, 16H), 3.81 – 3.53 (m, 80H), around 3.5 (broad water solvent peak), 3.47 – 3.44 (m, 14H), 3.42 (d, *J* = 3.5 Hz, 12H), 3.39 (d, *J* = 3.9 Hz, 9H), 3.36 (s, 5H), 3.31 – 3.23 (m, 13H), 3.21 – 3.15 (m, 3H), 2.59 (d, *J* = 9.9 Hz, 5H), 2.31 (dt, *J* = 14.1, 7.2 Hz, 1H), 2.24 – 2.18 (m, 2H), 2.16 (d, *J* = 1.6 Hz, 4H), 1.77 – 1.69 (m, 1H), 1.62 – 1.51 (m, 4H), 1.39 (d, *J* = 7.0 Hz, 3H), 1.29 (d, *J* = 9.2 Hz, 4H), 1.23 – 1.17 (m, 7H), -0.33 (d, *J* = 6.3 Hz, 3H).



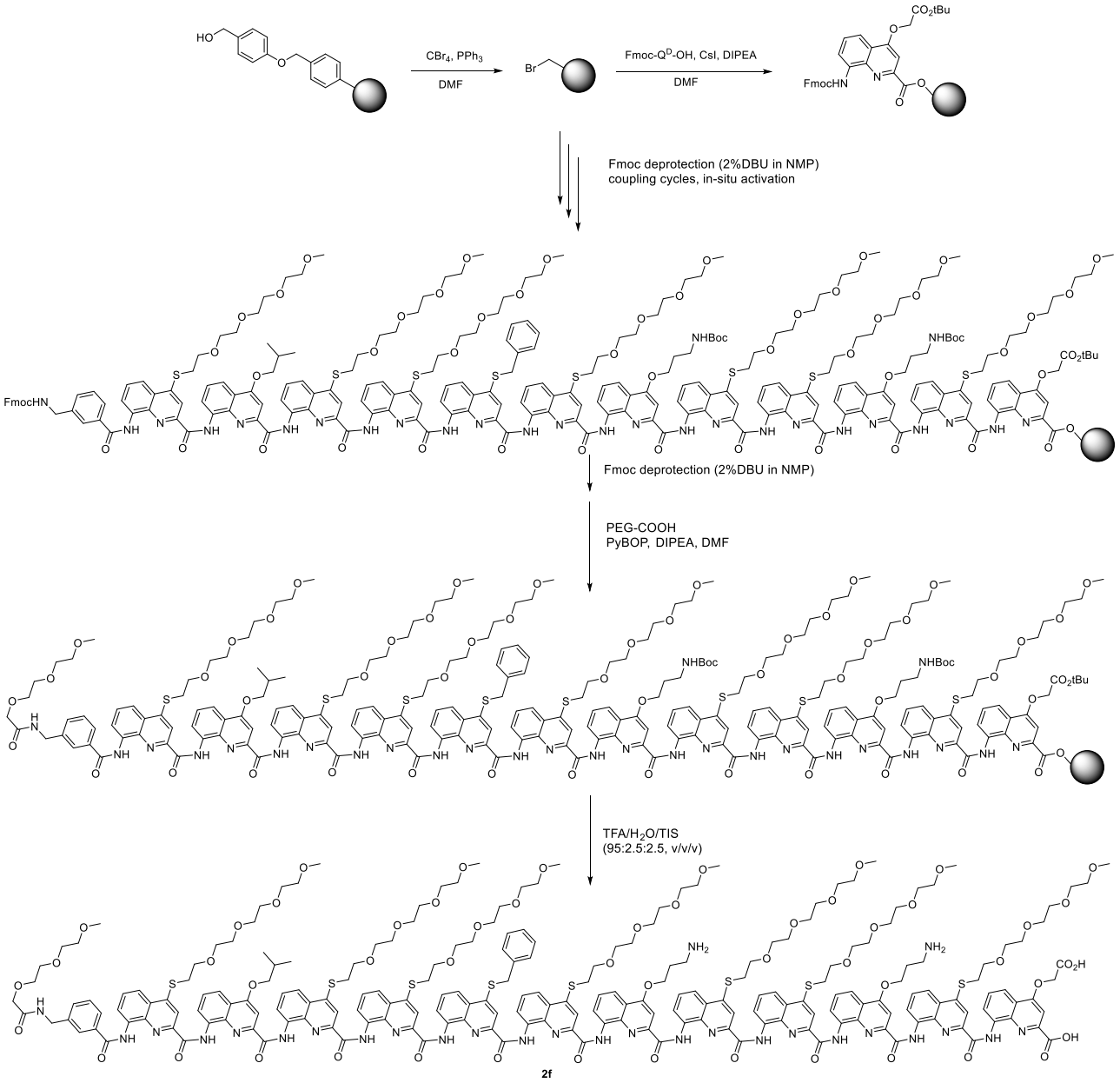
Compound 2d: The synthesis started from the resin described above (9mer, 15 µmol). After TFA/H_2O/TIS cleavage, 28 mg crude was obtained. The crude was purified by semi prep RP-HPLC to furnish **2d** as yellow solid (4.1 mg, 6%). HRMS (ESI+): calcd for $C_{221}H_{259}N_{29}O_{52}S_8$ $[M+2H]^{2+}$ 2204.8198, found 2204.9232. NMR of **2d** was omitted since **2c** and **2d** are enantiomers.



Compound 2e: Foldamer **2e** was synthesized on a LL-Wang resin (150 µmol scale). After TFA cleavage and side chain deprotection, the crude was purified by semi prep RP-HPLC to furnish **2e** as an yellow solid (85 mg, 62%). HRMS (ESI$^+$) calcd. for $C_{218}H_{252}N_{27}O_5S_7$ $[M+2H]^{2+}$ 2161.8030, found 2161.8397.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.98 (s, 1H), 10.80 (s, 1H), 10.69 (s, 1H), 10.47 (s, 2H), 10.41 (s, 1H), 10.29 (s, 1H), 10.25 (s, 1H), 10.24 (s, 1H), 10.15 (s, 1H), 10.03 (s, 1H), 9.38 (s, 1H), 9.11 (s, 1H), 7.96 (d, *J* = 7.2 Hz, 1H), 7.90 (d, *J* = 7.2 Hz, 1H), 7.79 (s, 2H), 7.71 (d, *J* = 7.2 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.60 (dd, *J* = 13.1, 6.6 Hz, 4H), 7.56 (d, *J* = 9.3 Hz, 2H), 7.54 – 7.41 (m, 9H), 7.33 (d, *J* = 8.2 Hz, 3H), 7.29 – 7.21 (m, 5H), 7.20 – 6.96 (m, 16H), 6.90 (q, *J* = 7.5, 6.1 Hz, 5H), 6.80 (td, *J* = 16.2, 15.7, 7.4 Hz, 5H), 6.57 (s, 1H), 6.41 – 6.31 (m, 3H), 6.26 (s, 1H), 6.23 (d, *J* = 4.2 Hz, 2H), 6.20 (s, 1H), 6.15 (s, 1H), 6.10 (s,

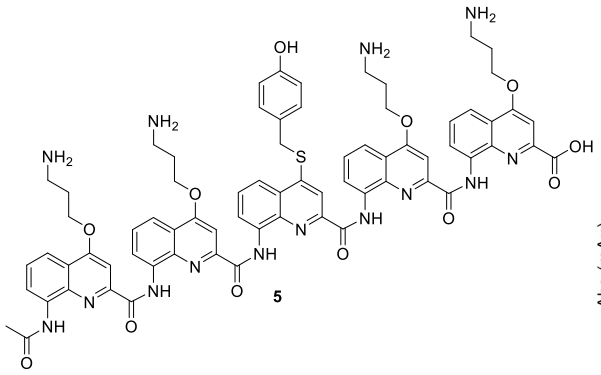
1H), 5.74 (s, 1H), 4.21 – 4.09 (m, 1H), 4.09 – 3.88 (m, 9H), 3.89 – 3.43 (m, 116H), 3.40 – 3.36 (m, 8H), 3.25 (s, 7H), 3.19 – 3.04 (m, 20H), 3.01 (s, 5H), 2.35 – 2.27 (m, 5H), 2.16 (d, *J* = 7.7 Hz, 3H), 1.23 (s, 1H), 1.19 – 1.13 (m, 8H).

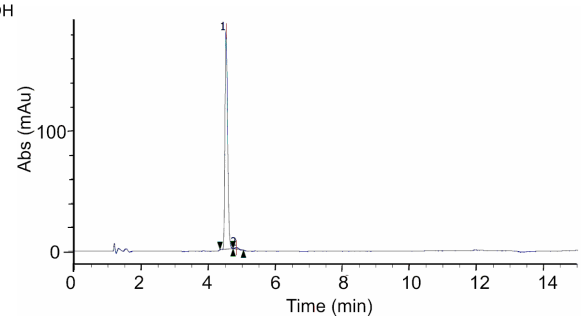


Compound 2f: Foldamer **2f** was synthesized on a LL-Wang resin (150 µmol scale). After TFA cleavage and side chain deprotection, the crude was purified by semi prep RP-HPLC to furnish **2f** as yellow solid (52 mg, 38%). HRMS (ESI+): m/z calcd for $C_{217}H_{250}N_{27}O_{52}S_8$ $[M+2H]^{2+}$ 2162.7838, found 2162.8219.

^{1}H NMR (500 MHz, DMSO-*d*$_6$) ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 13.34 (s, 1H), 11.81 (s, 1H), 11.02 (s, 1H), 10.89 (s, 1H), 10.66 (s, 1H), 10.49 (s, 1H), 10.44 (s, 2H), 10.33 (s, 1H), 10.24 (s, 1H), 10.17 (s, 1H), 10.11 (s, 1H), 10.08 (s, 1H), 9.10 (s, 1H), 8.04 (s, 4H), 7.96 (d, *J* = 6.9 Hz, 2H), 7.91 (d, *J* = 7.3 Hz, 2H), 7.83 (s,

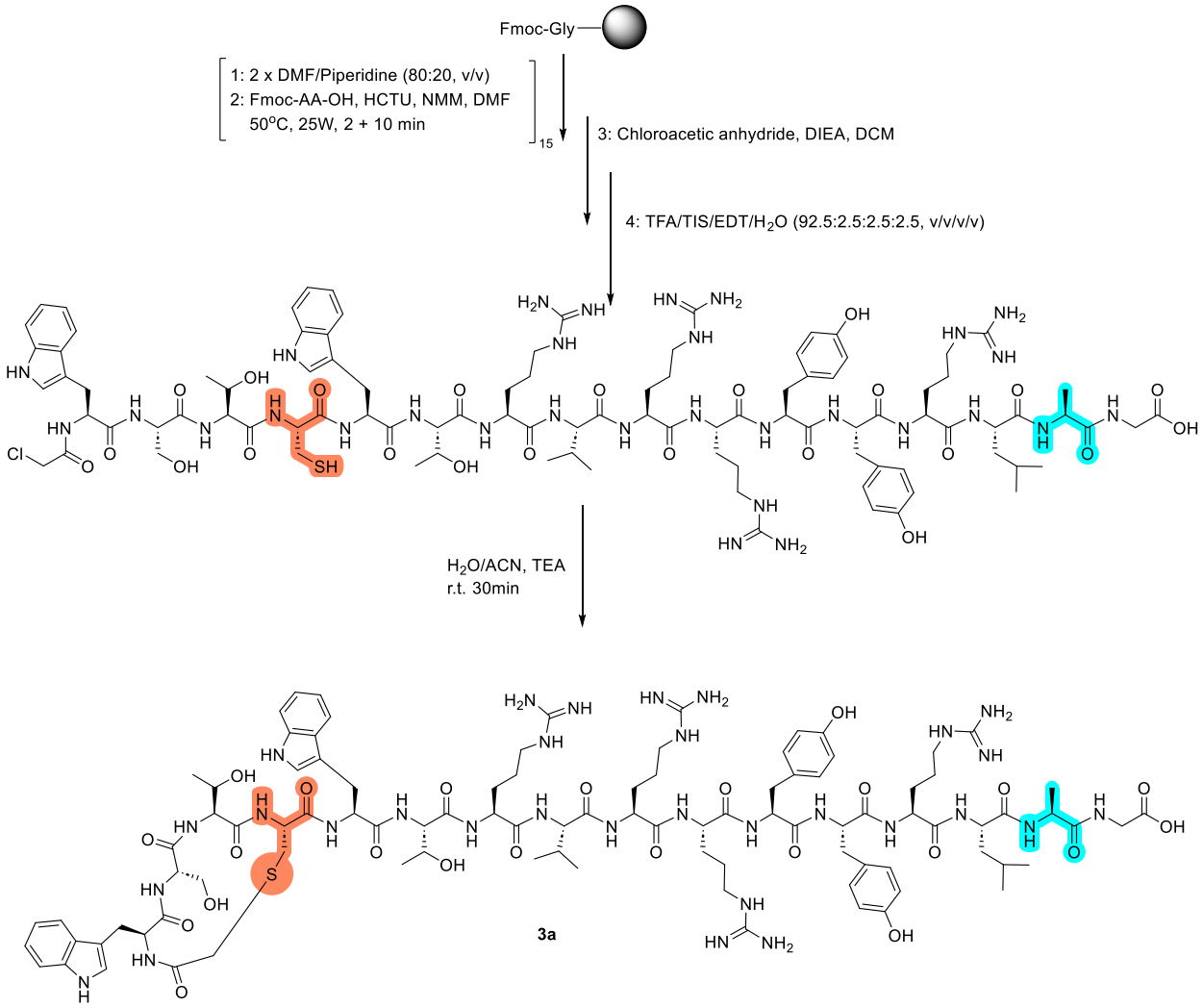
4H), 7.76 (d, *J* = 7.6 Hz, 3H), 7.70 (d, *J* = 7.2 Hz, 2H), 7.64 (t, *J* = 7.4 Hz, 6H), 7.61 – 7.32 (m, 28H), 7.33 – 7.21 (m, 9H), 7.21 – 6.99 (m, 20H), 6.99 – 6.91 (m, 5H), 6.88 (d, *J* = 4.5 Hz, 3H), 6.79 (s, 5H), 6.54 (s, 1H), 6.43 (d, *J* = 7.7 Hz, 1H), 6.38 (s, 1H), 6.30 (d, *J* = 4.6 Hz, 2H), 6.21 – 6.09 (m, 6H), 5.73 (s, 1H), 4.71 (d, *J* = 16.0 Hz, 1H), 4.57 (d, *J* = 16.0 Hz, 1H), 4.19 (d, *J* = 13.3 Hz, 3H), 4.15 – 4.05 (m, 7H), 4.03 – 3.89 (m, 17H), 3.88 – 3.80 (m, 25H), 3.80 – 3.76 (m, 14H), 3.76 – 3.67 (m, 42H), 3.66 – 3.57 (m, 47H), 3.57 – 3.48 (m, 45H), 3.46 (m 18H), 3.37 (d, *J* = 3.5 Hz, 10H), 3.34 (s, 5H), 3.33 – 3.30 (m, 15H), 3.25 (s, 6H), 3.19 (s, 6H), 2.03 – 1.94 (m, 2H), 1.23 (s, 13H), 1.14 (t, *J* = 6.4 Hz, 8H), 1.06 – 1.01 (m, 2H), 0.98 (d, *J* = 6.4 Hz, 6H), 0.85 (t, *J* = 6.7 Hz, 2H).



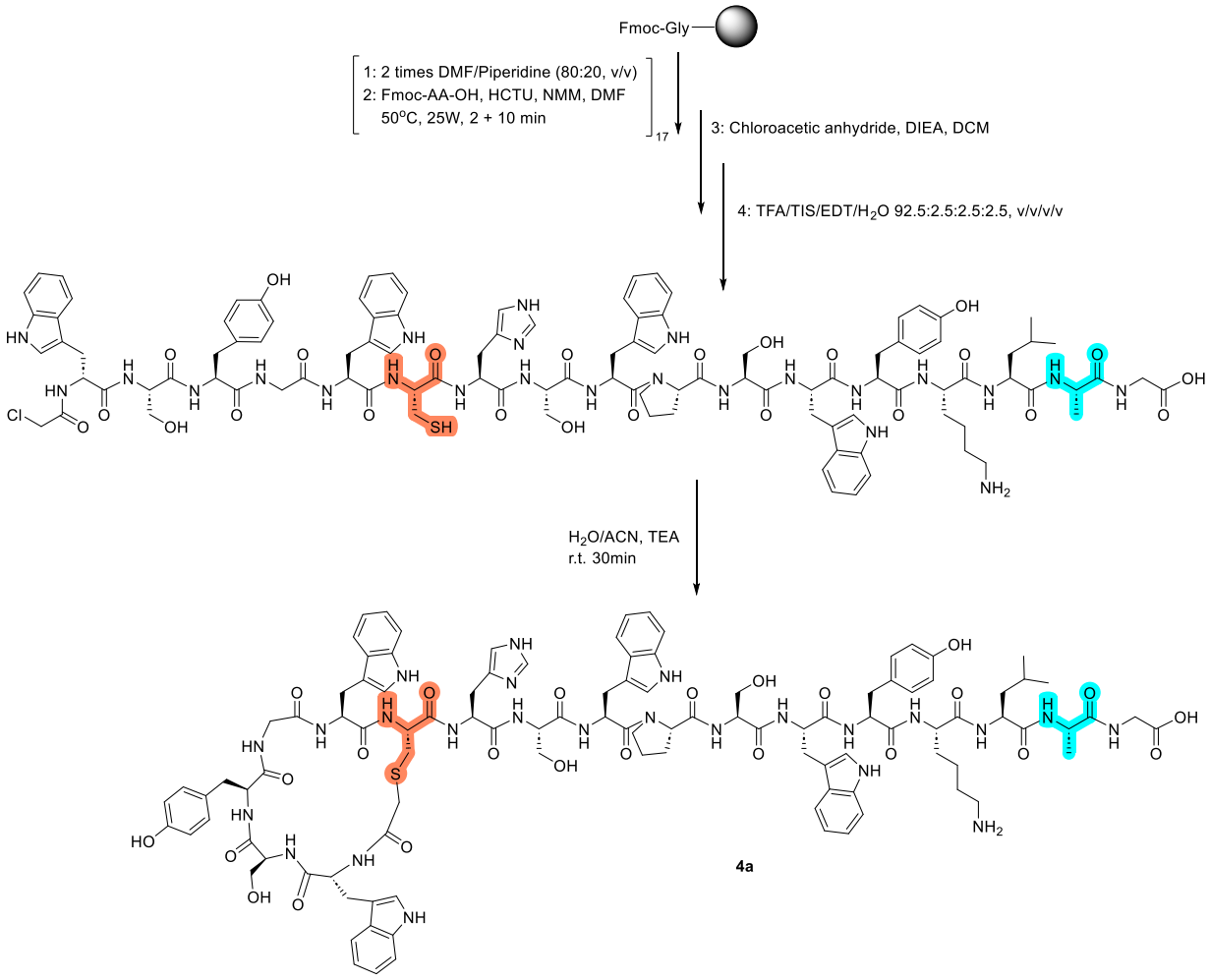


Compound 5: Pentamer **5** was synthesized on a LL-Wang resin (0.41 mmol/g, 10.25 µmol scale). After TFA cleavage and RP-HPLC purification, **5** was recovered in 62 % yield (3 mg). HRMS (ESI$^+$): calcd. for $C_{71}H_{68}N_{14}O_{12}S$ $[M+2H]^{2+}$ 671.3355, found 671.2494.

3.4. Synthesis of peptide macrocycles



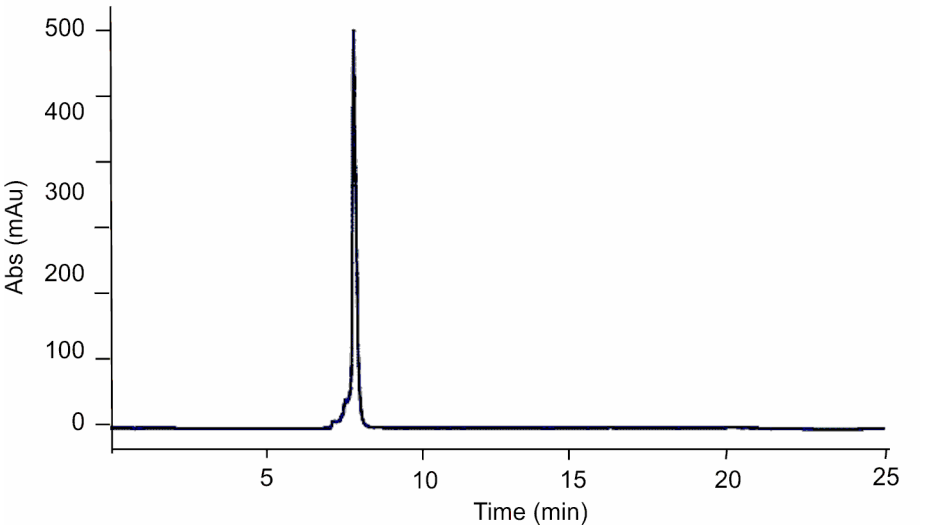
Peptide macrocycle 3a: The SPPS of linear peptide **3a** was performed Fmoc-Gly-Wang resin on a 50 µmol scale. 73 mg of crude peptide was next dissolved in 5 mL of a CH_3CN/water mixture and TEA (375 µL, 0.5 M) was added. The completion of cyclization was monitored by RP-HPLC, and after 30 min, the reaction was quenched by diluting the reaction mixture with water/ 0.1% TFA. After lyophilisation, the crude macrocyclic peptide was purified by semi-prep HPLC to furnish **3a** as a white powder (21 mg, 20%). HRMS (ESI$^+$): m/z calcd. for $C_{96}H_{141}N_{30}O_{23}S$ $[M+H]^+$ 2114.0507, found 2114.0133.



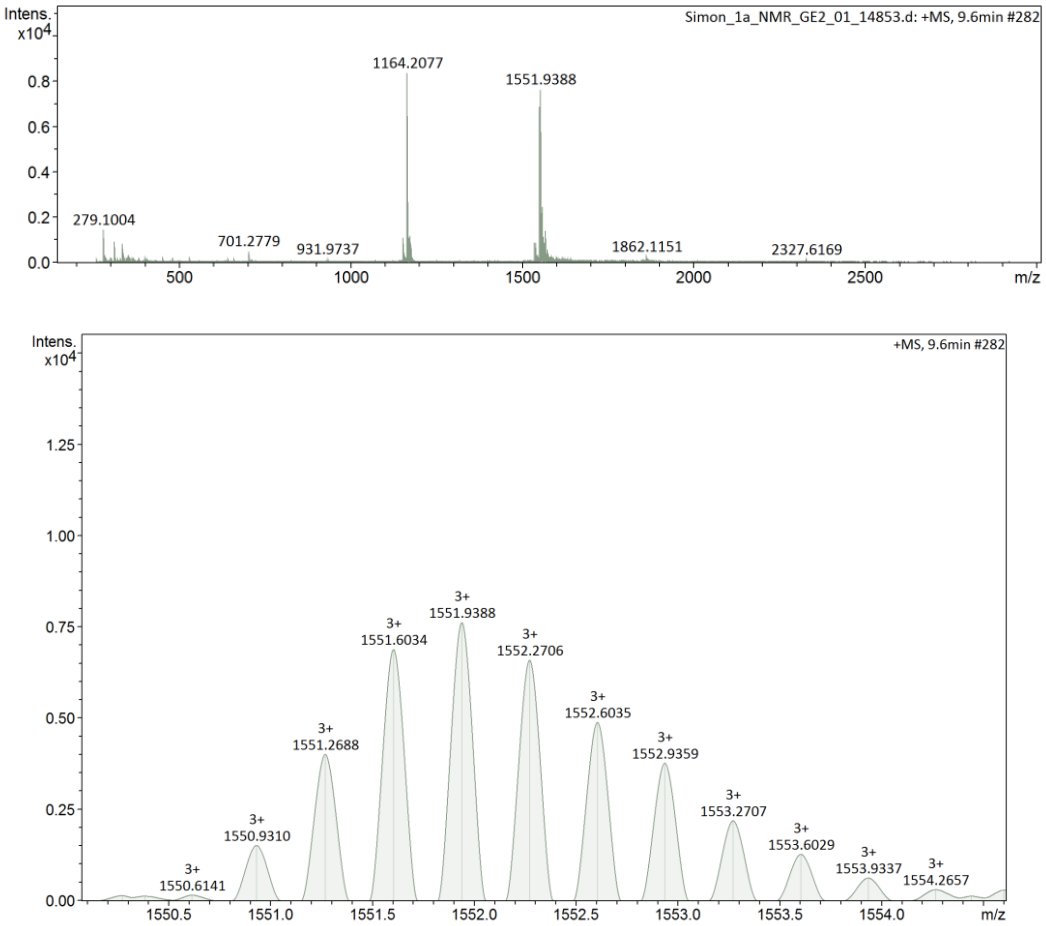
Peptide macrocycle 4a: The SPPS of linear peptide **4a** was performed on Fmoc-Gly-Wang resin on a 50 µmol scale. 81 mg of crude peptide was next dissolved in 5 mL of a CH_3CN/water mixture, and TEA (375 µL, 0.5 M) was added. The completion of cyclization was monitored by RP-HPLC, and after 30 min, the reaction mixture was quenched by pouring water/ 0.1% TFA. After RP-HPLC purification, **4a** was recovered as a white powder (29 mg, 27%). HRMS (ESI$^+$): m/z calcd. for $C_{106}H_{129}N_{24}O_{24}S$ $[M+H]^+$ 20153.9332, found 2153.9327.

3b and **4b** were synthesized following the same procedure using commercially available Fmoc-(*D*)-amino acid. The corresponding macrocyclic peptides were produced, and purified in a similar manner. To note in **4b**, the *N*-term (*D*)-Trp was replaced by a (*L*)-Trp.

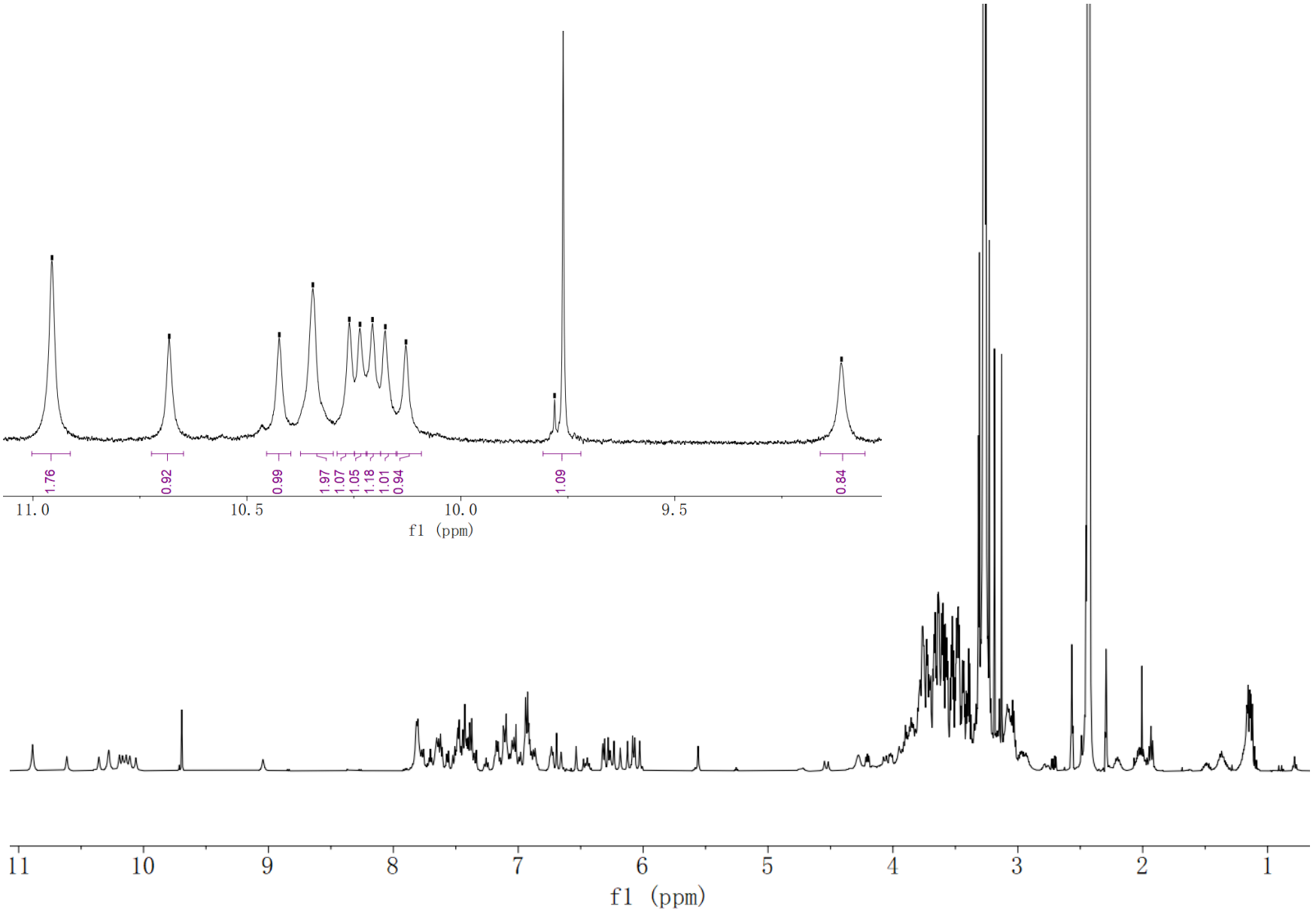
4. HPLC profiles, HRMS (ESI$^+$) and ^{1}H NMR spectra



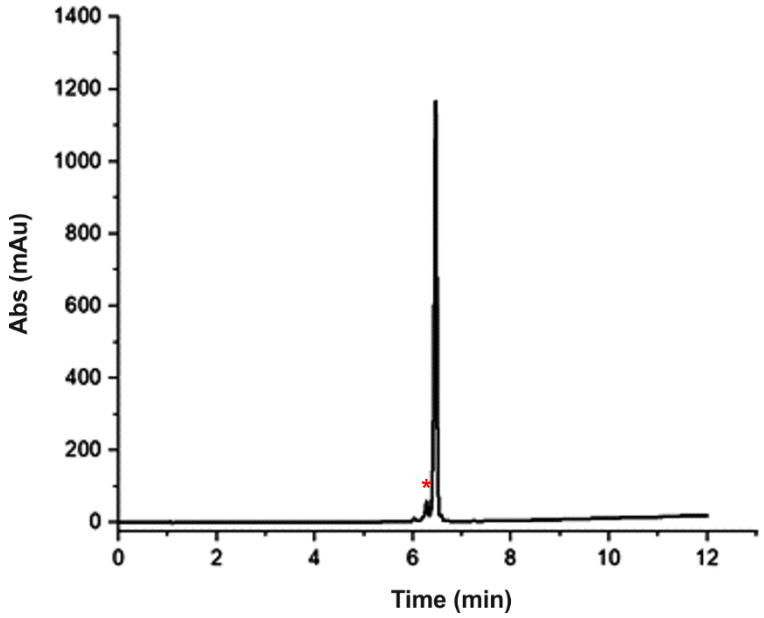
Analytical RP-HPLC profile of foldamer **1a** (Gradient: 5-100% B over 15 min, then 100% B for 10min, λ= 254 nm).



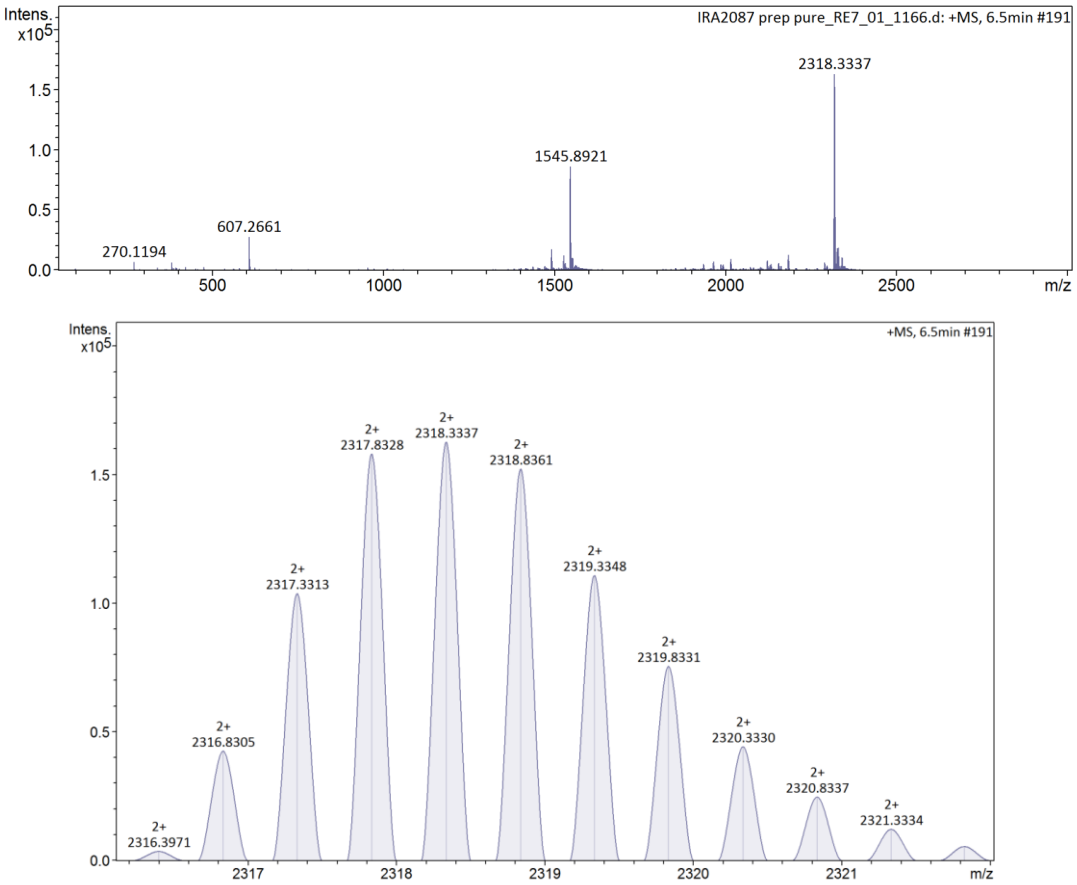
HRMS (ESI$^+$) spectra of foldamer **1a**.



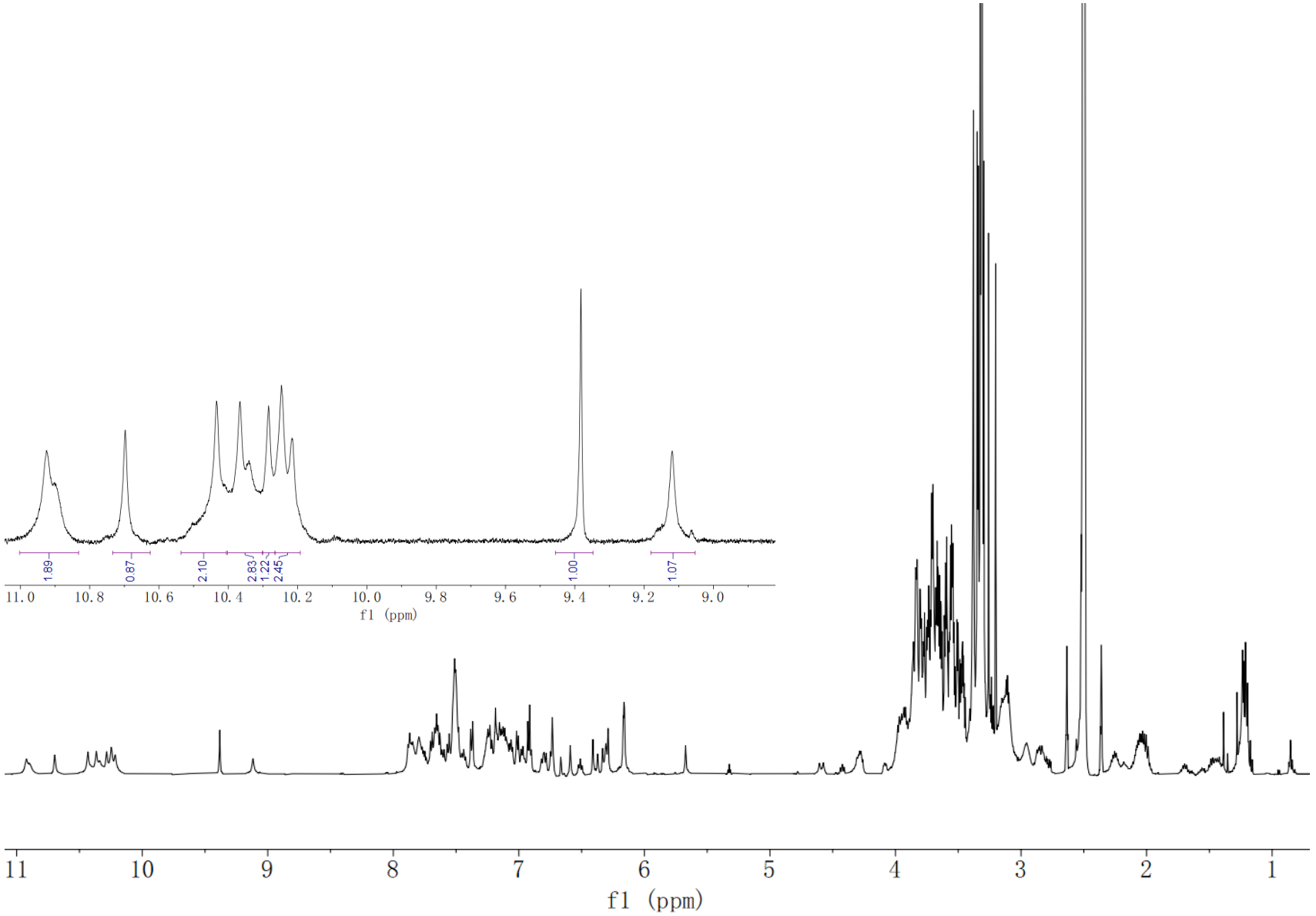
^{1}H NMR spectrum of foldamer **1a** and zoomed region (500 MHz, DMSO-d_6), 25 °C.



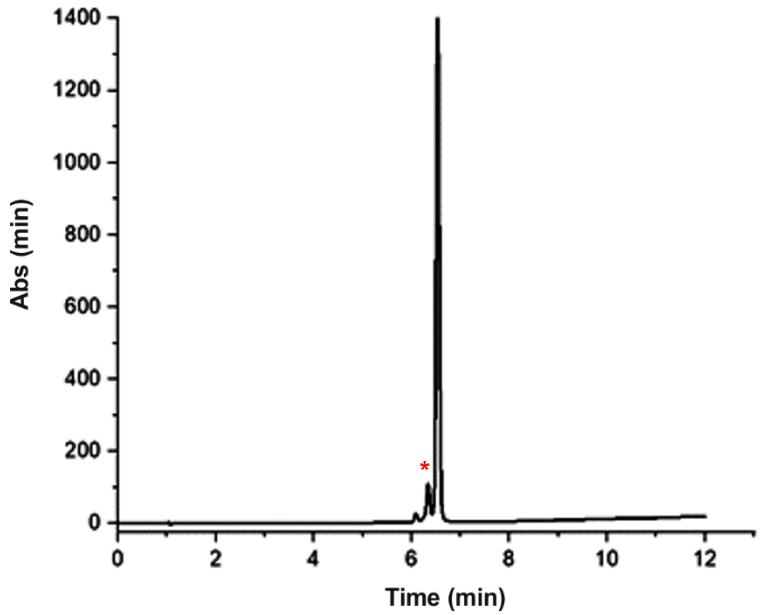
Analytical RP-HPLC profile of pure foldamer **1b** (Gradient: 10-100% B over 10 min, λ= 254 nm).



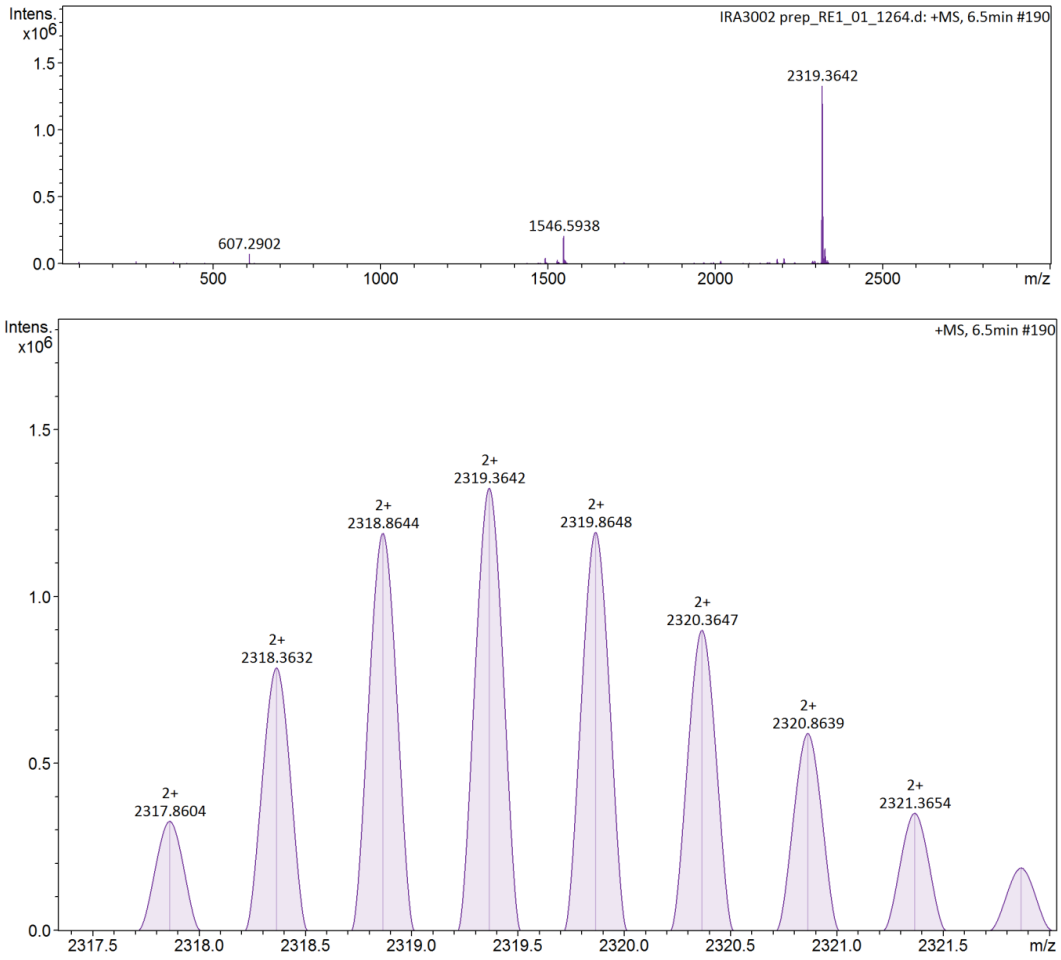
HRMS (ESI⁺) spectra of foldamer **1b**



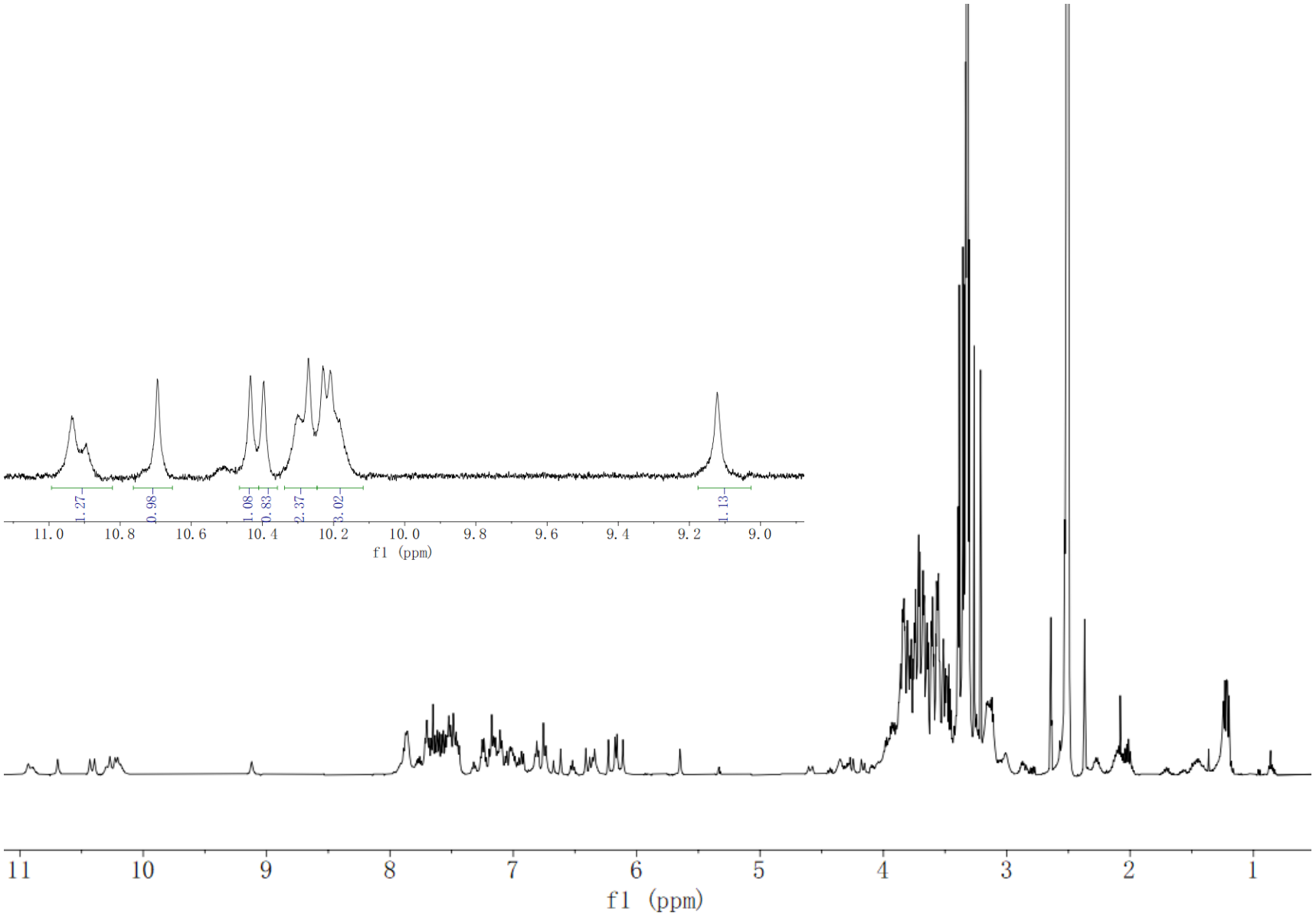
^{1}H NMR spectrum of foldamer **1b** and zoomed region (500 MHz, DMSO-d_6), 25 °C



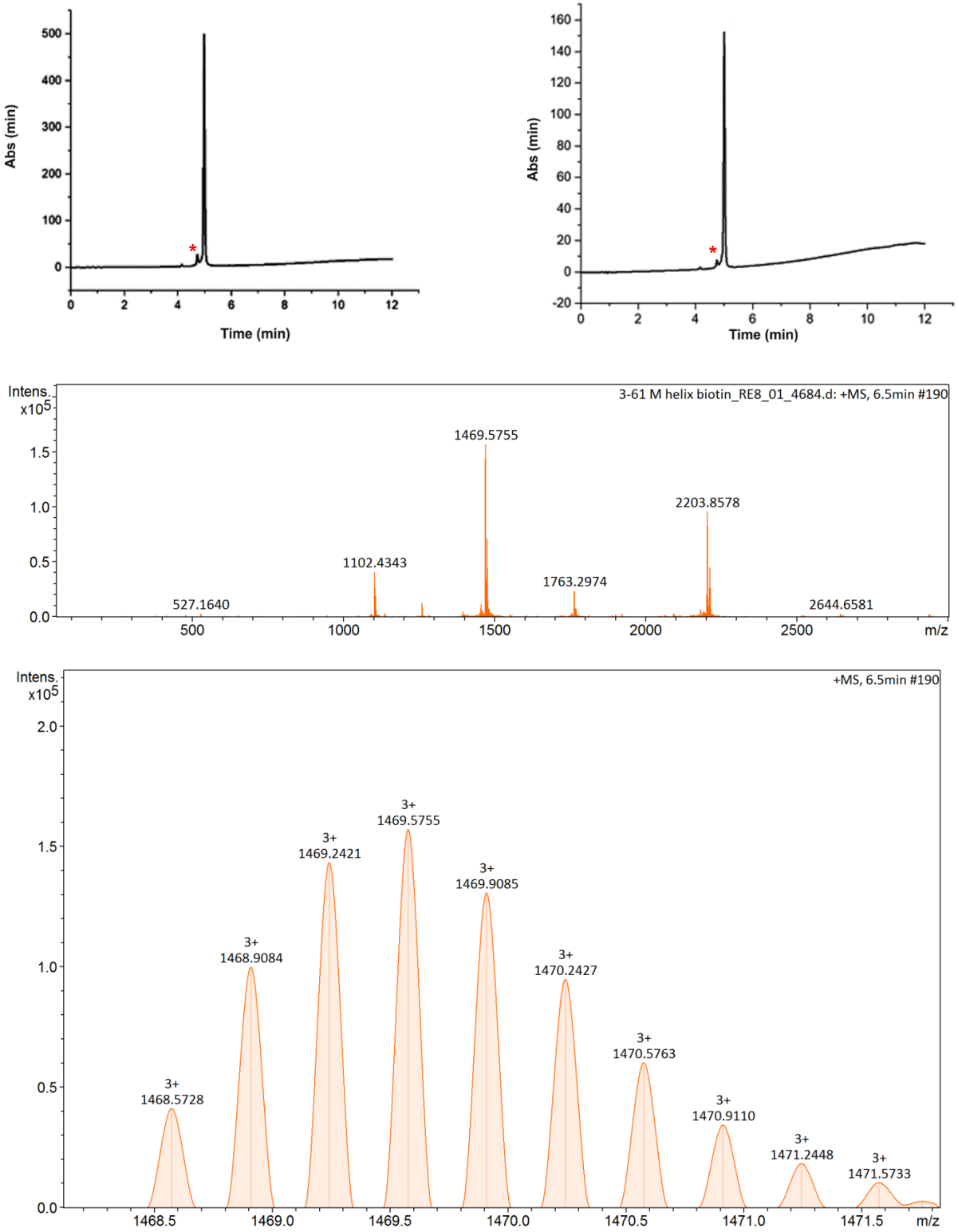
Analytical RP-HPLC profile of pure foldamer **1c** (Gradient: 10-100% B over 10 min, λ= 254 nm).



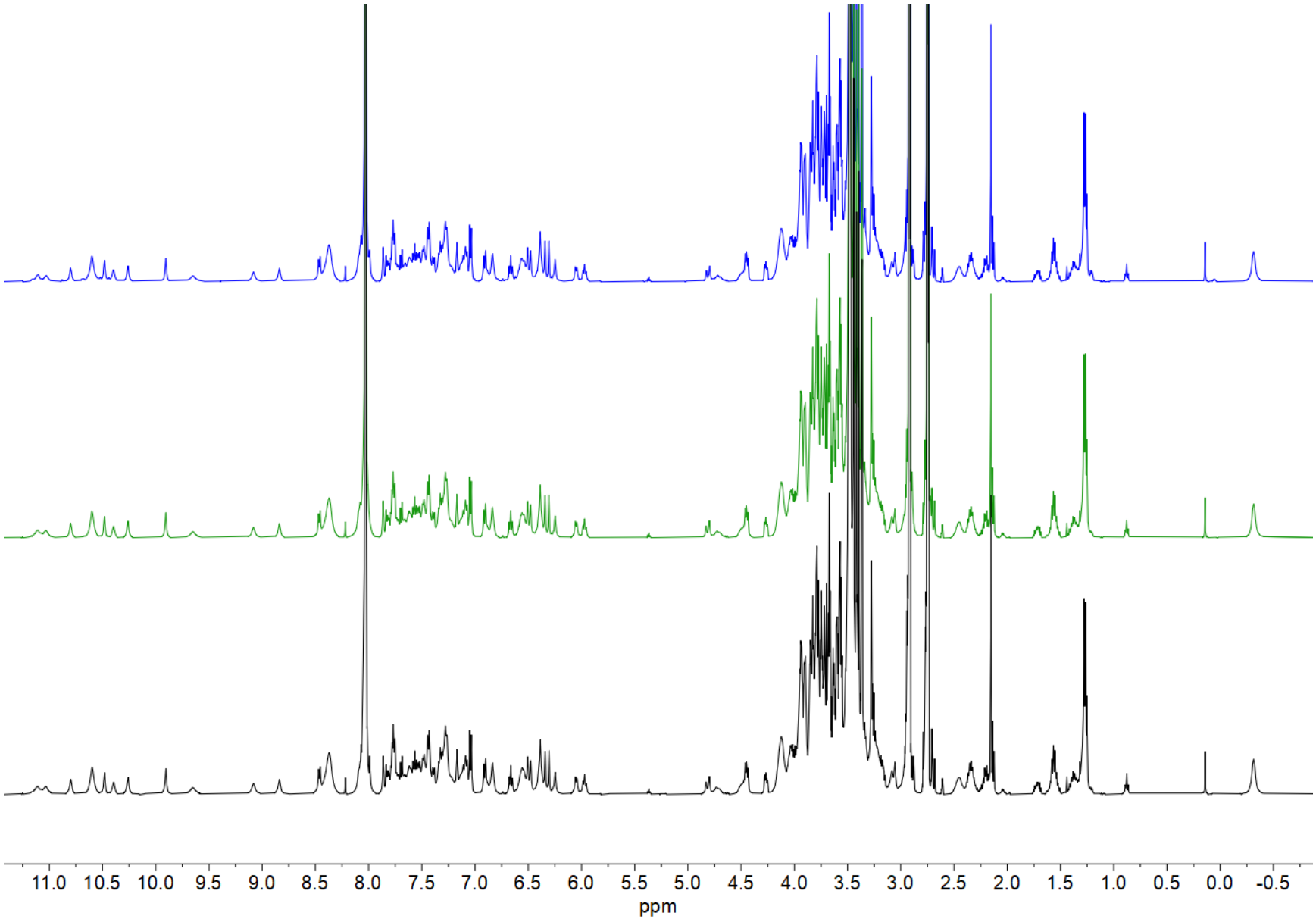
HRMS (ESI$^+$) spectrum of foldamer **1c**.

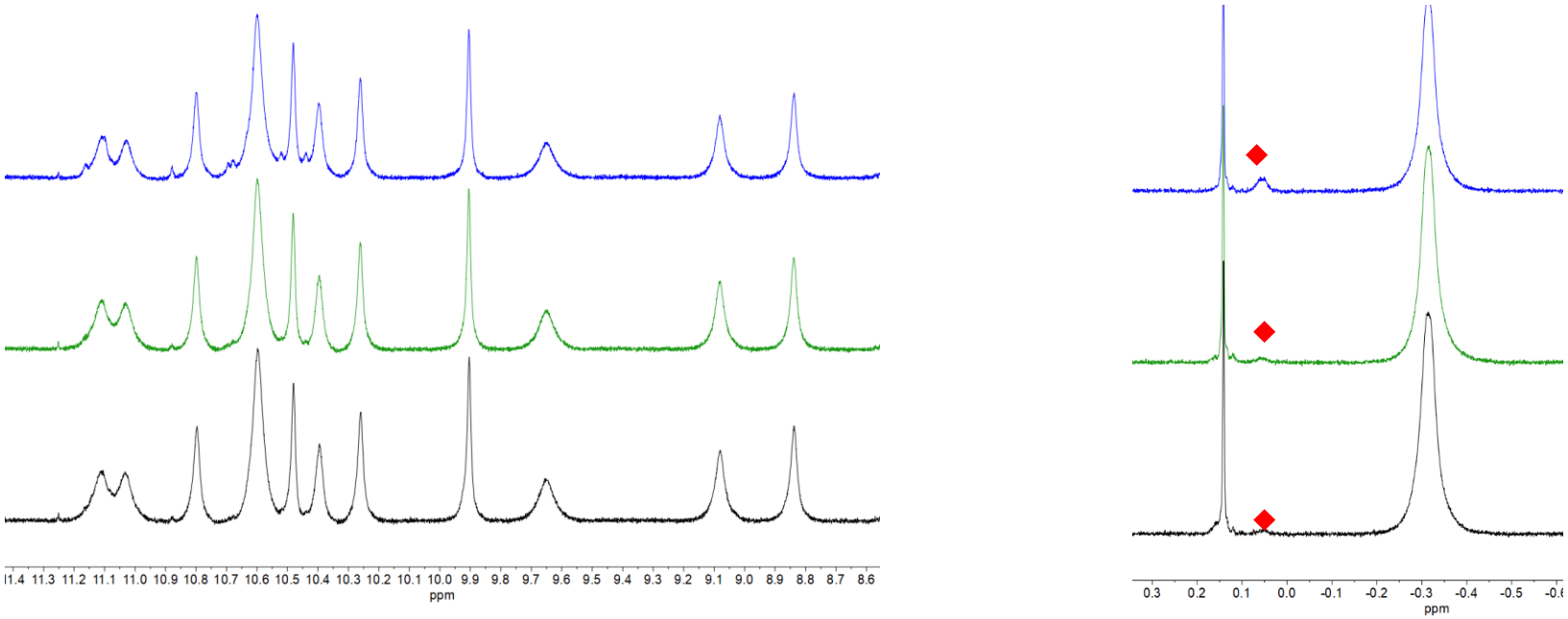


^{1}H NMR spectrum of foldamer **1c** and zoomed region (500 MHz, DMSO-d_6), 25 °C

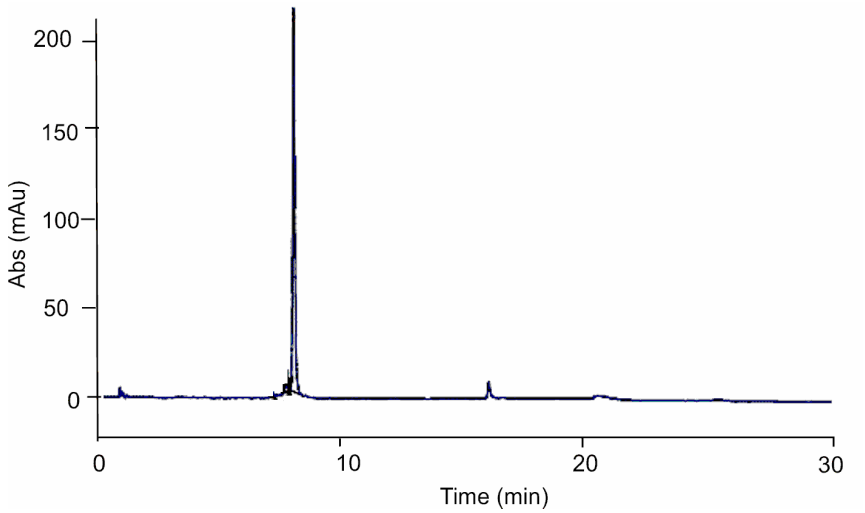


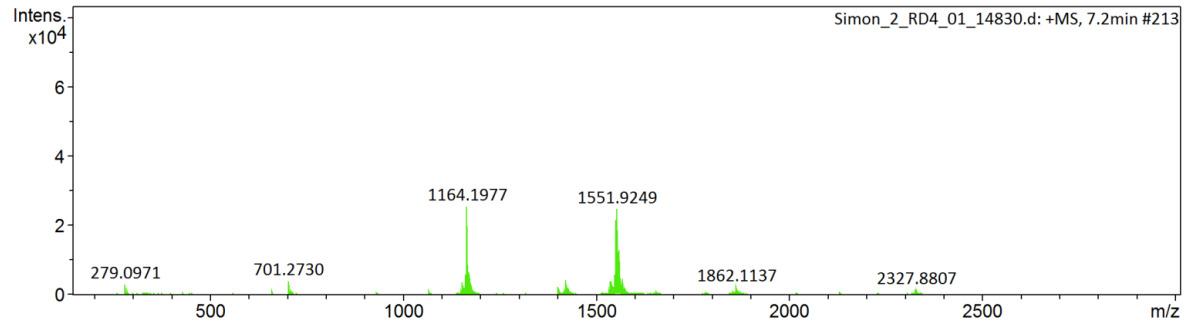
Top left: RP-HPLC profile of compound **1d** (Gradient: 10-100% B over 10 min, λ= 254 nm). Top right RP-HPLC profile of compound **1e**. The * annotation on the RP-HPLC profiles corresponds to the biotin-oxidized derivative. HRMS (ESI+) spectrum of foldamer **1d** (ESI-HRMS spectra for **1e** was omitted since they are enantiomers).

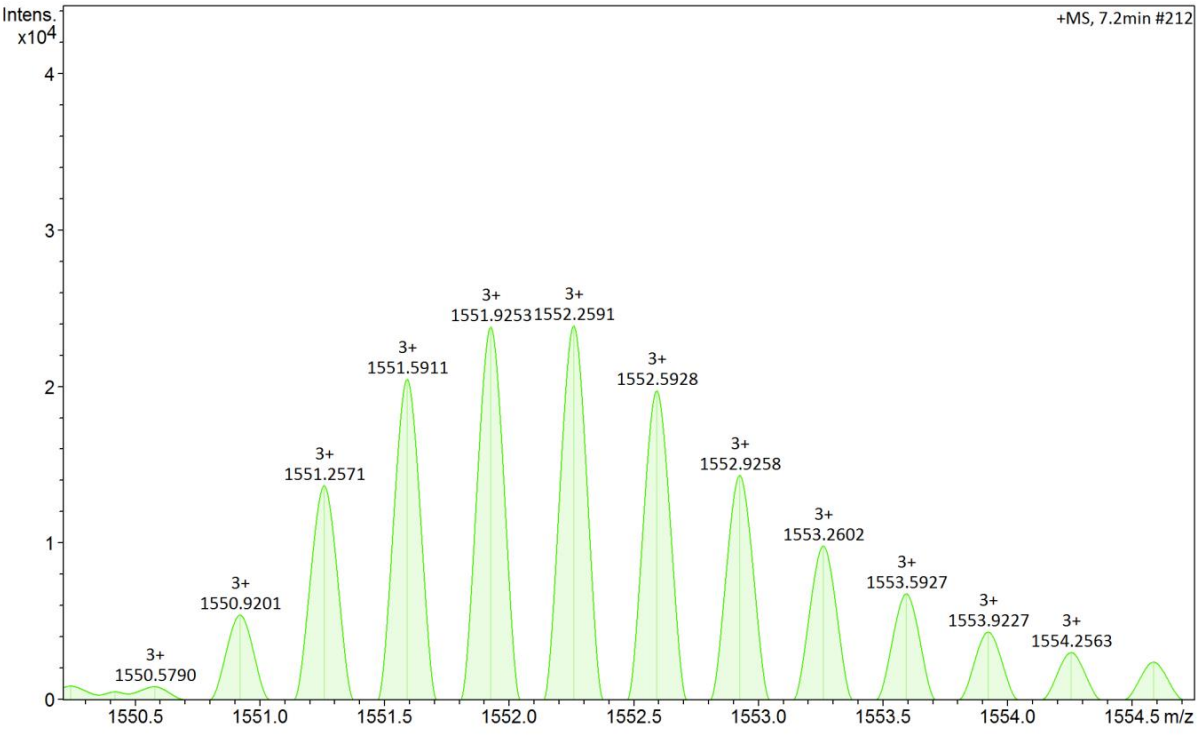

11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm



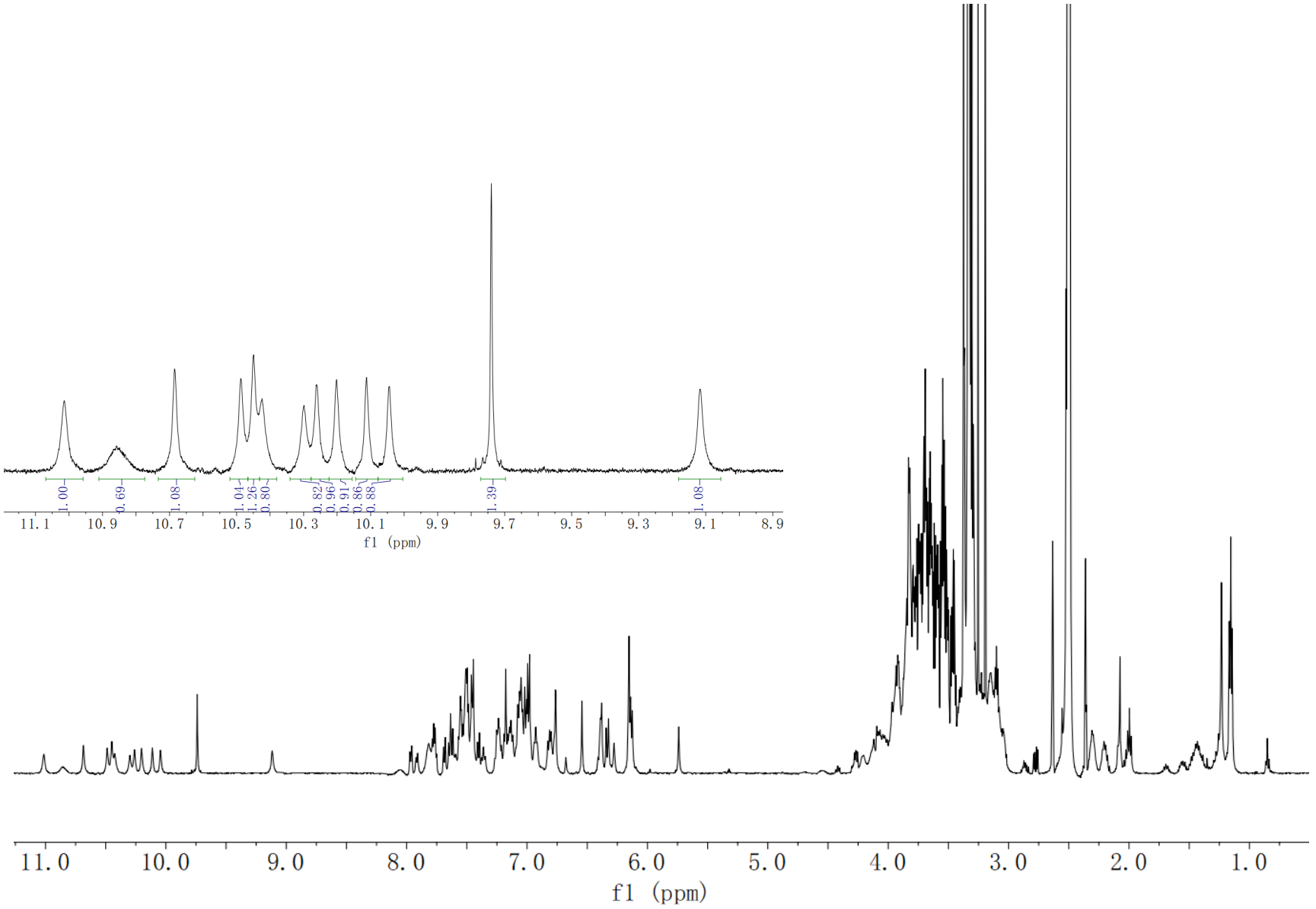
Full ^{1}H NMR spectra (500 MHz, DMF-d_7) of **1d** and zoomed region, monitoring at time point 0 h (blue), 18 h (green), 42 h (black), 25 °C. The red diamond indicates signal of the CH_3 group of chiral **B** unit from diastereomeric conformer. The same process was followed for **1e.**



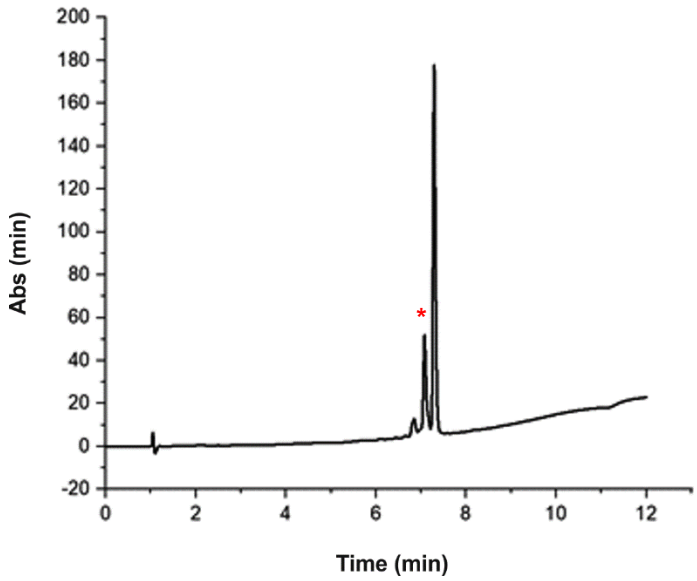


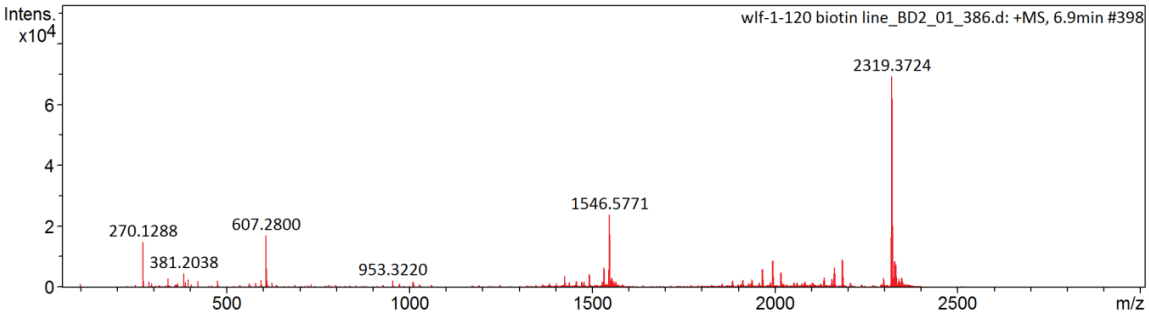


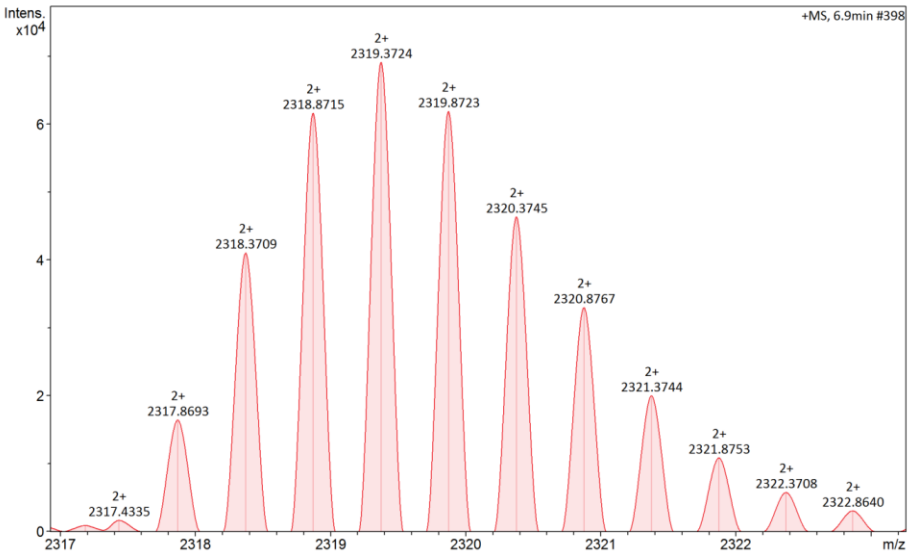
Analytical HPLC profile of pure foldamer **2a** (Gradient: 0 – 20% B over 17 min, then 20 – 100% B over 3 min, then 100% B for 5 min., λ= 254 nm) and ESI$^+$-HRMS spectrum of pure foldamer **2a**



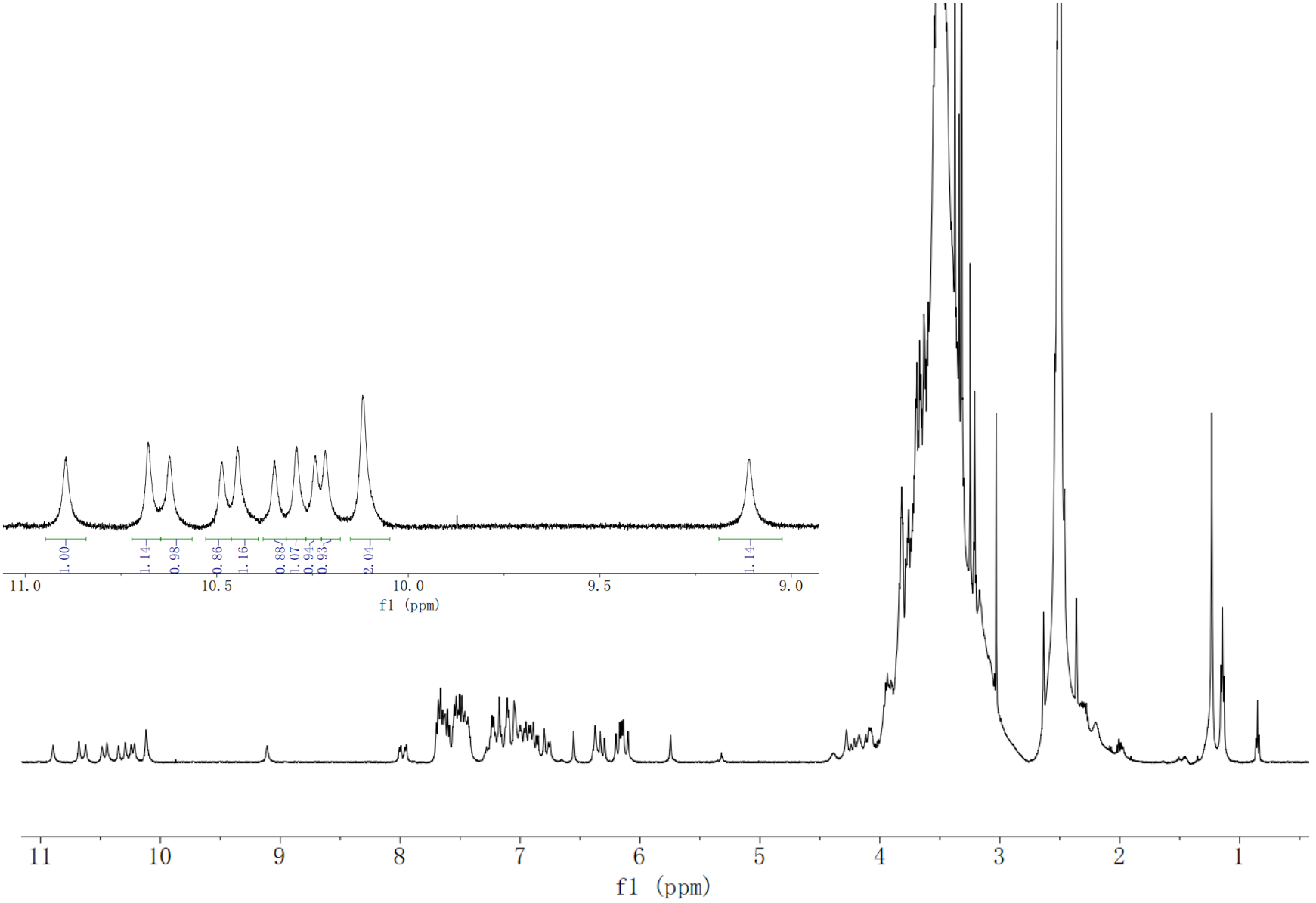
^{1}H NMR spectrum of foldamer **2a** and zoomed region (500 MHz, DMSO-d_6), 25 °C.



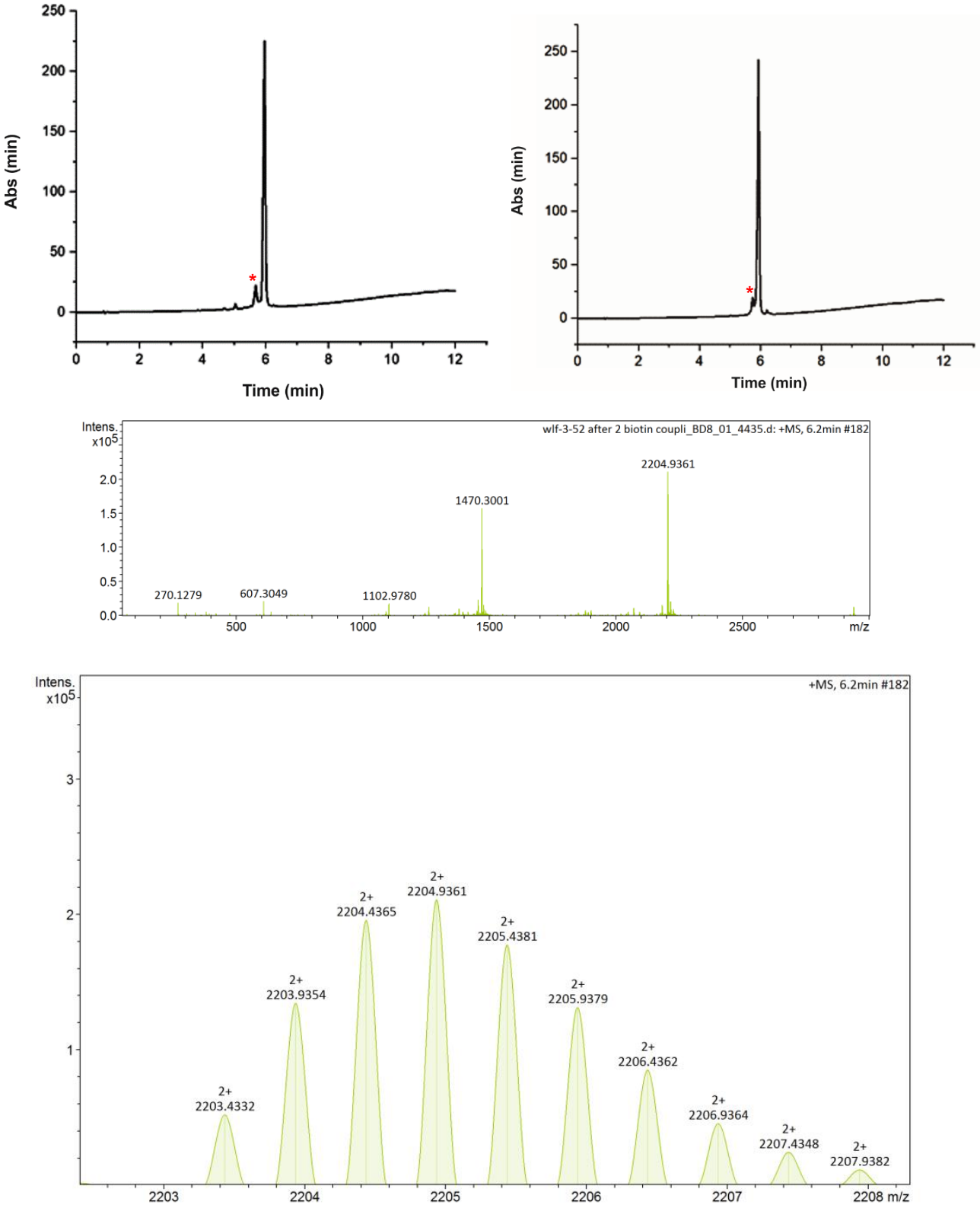




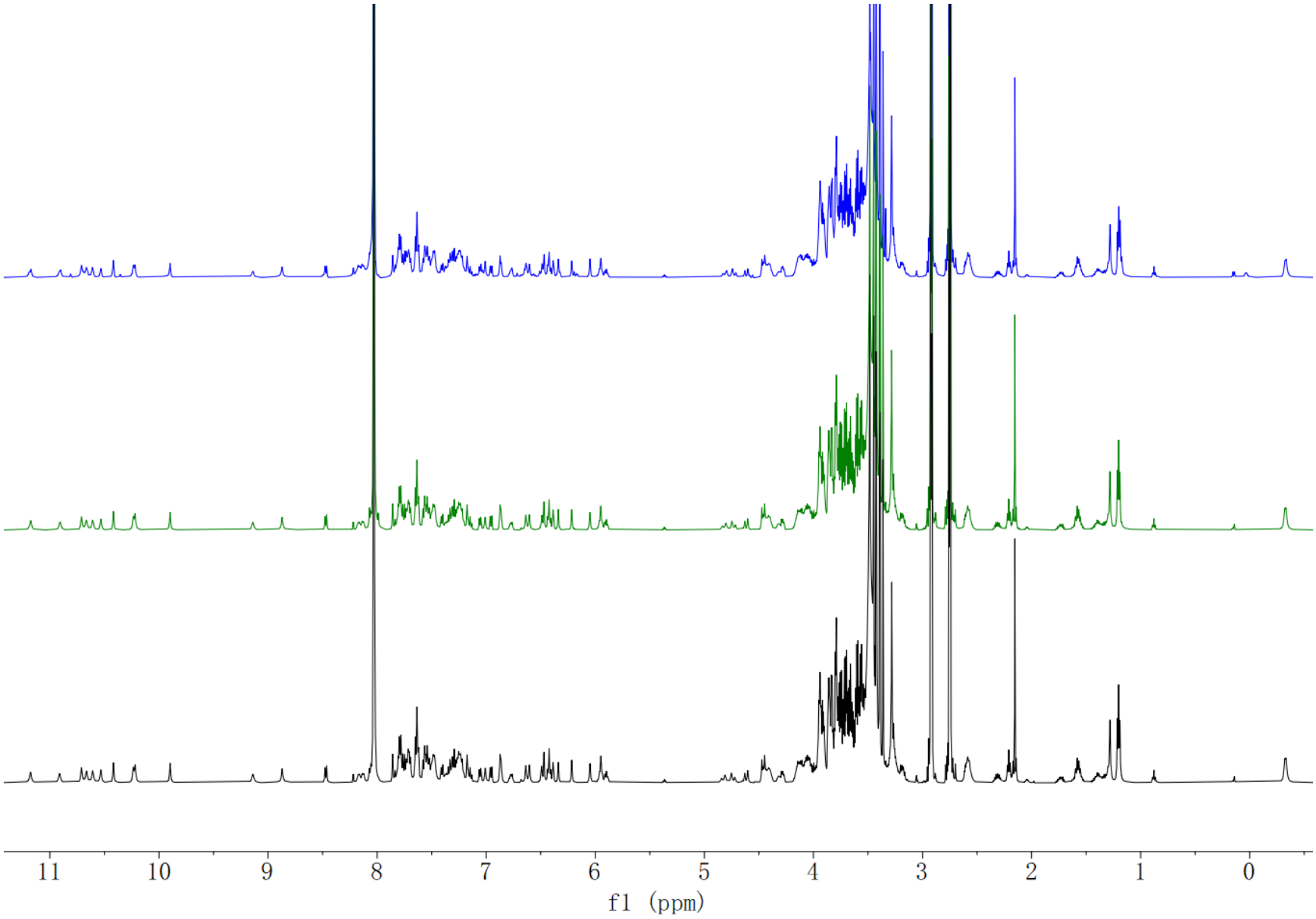
Analytical HPLC (10-100% B over 10 min, 254 nm) profile of foldamer **2b**. HRMS (ESI+) profile of foldamer **2b.** The * annotation corresponds to the biotin-oxidized derivative.

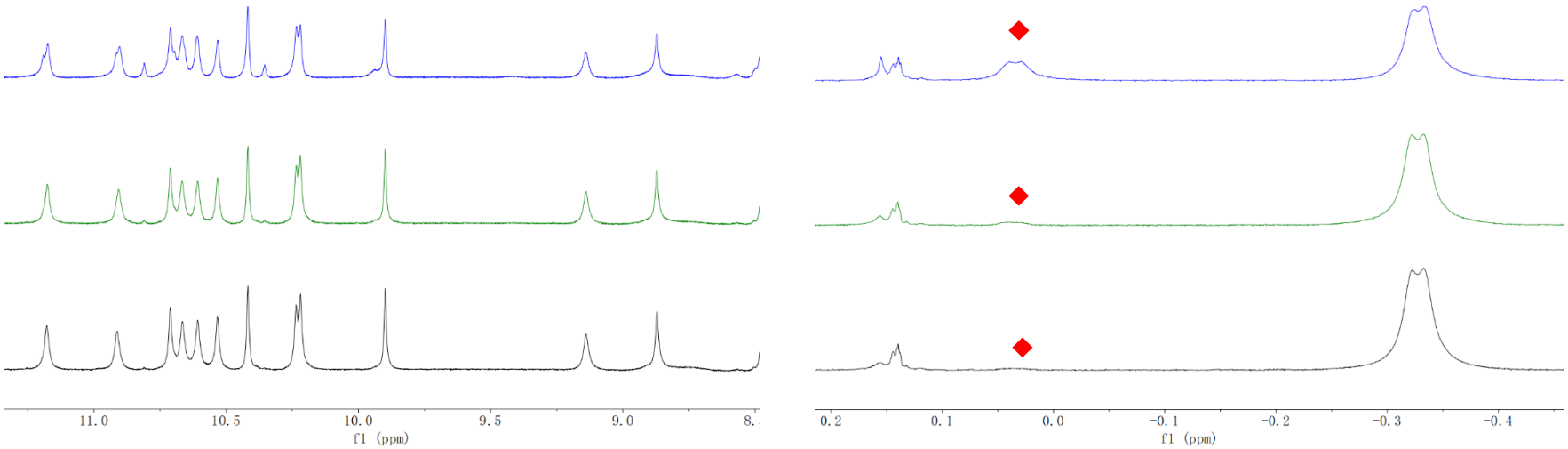


^{1}H NMR Spectrum of foldamer **2b** and zoomed region (500 MHz, DMSO-d_6), 25 °C.

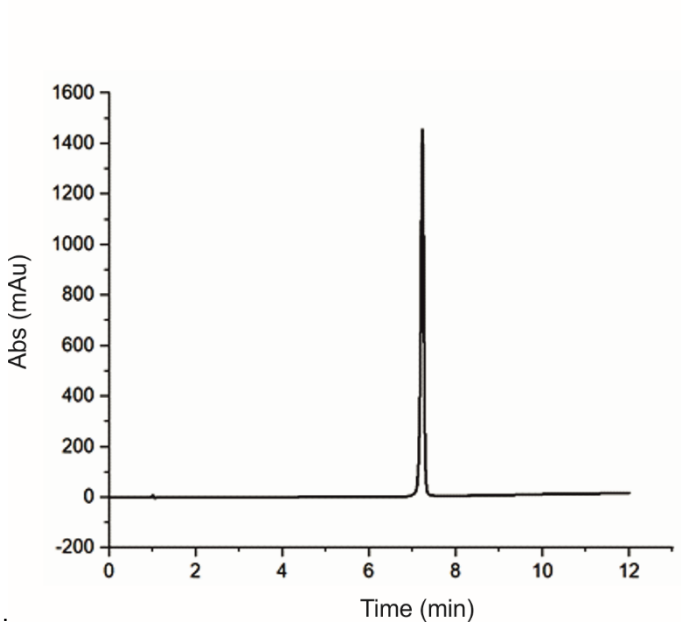


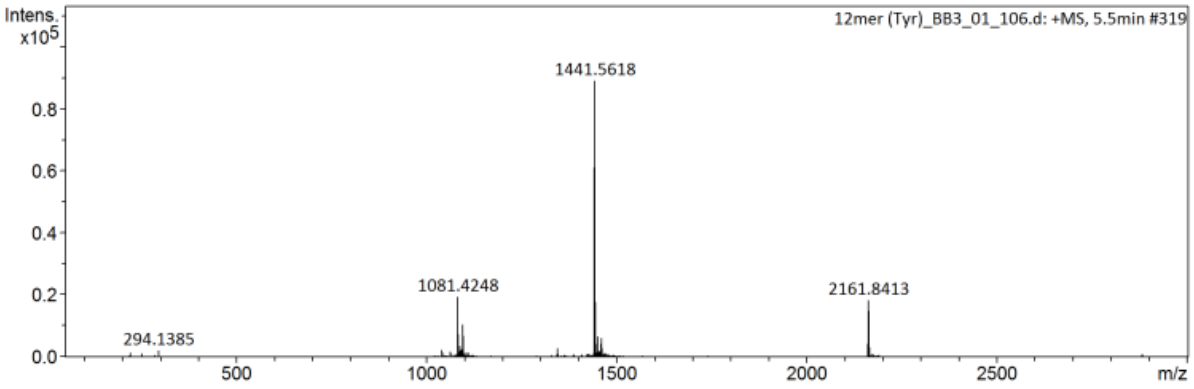
Analytical RP-HPLC profile of pure **2c** (top left) and **2d** (top right) (10-100% B over 10 min). ^{1}H NMR Spectrum (500 MHz, DMF-d_7, 25 °C), monitoring at time point 0 h (blue), 18 h (green), 42 h (black), 25 °C) of **2c.** HRMS (ESI+) profile of foldamer **2c** (ESI-HRMS spectra for **2d** was omitted since they are enantiomers)..

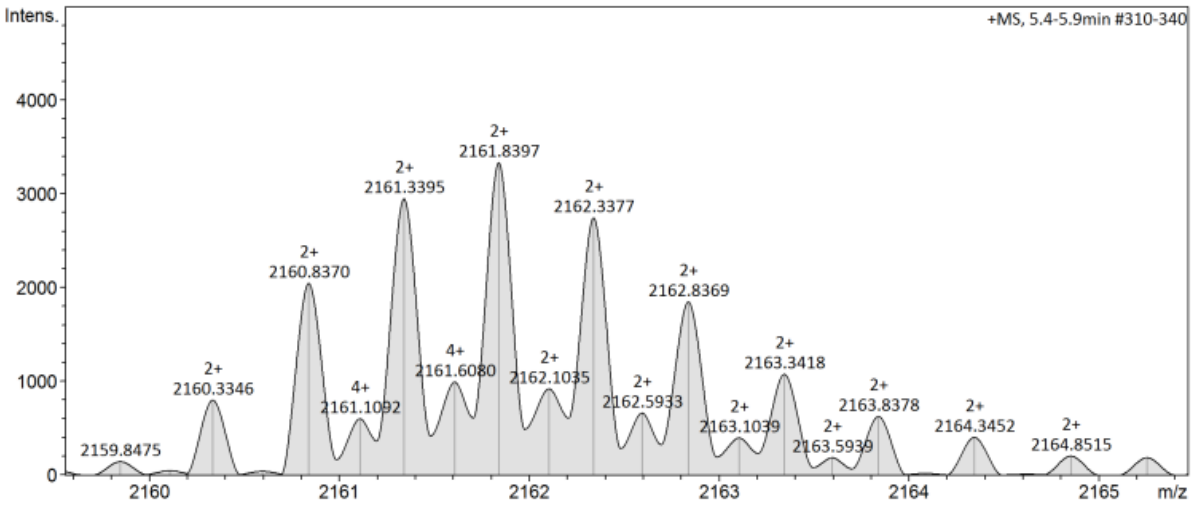

11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)



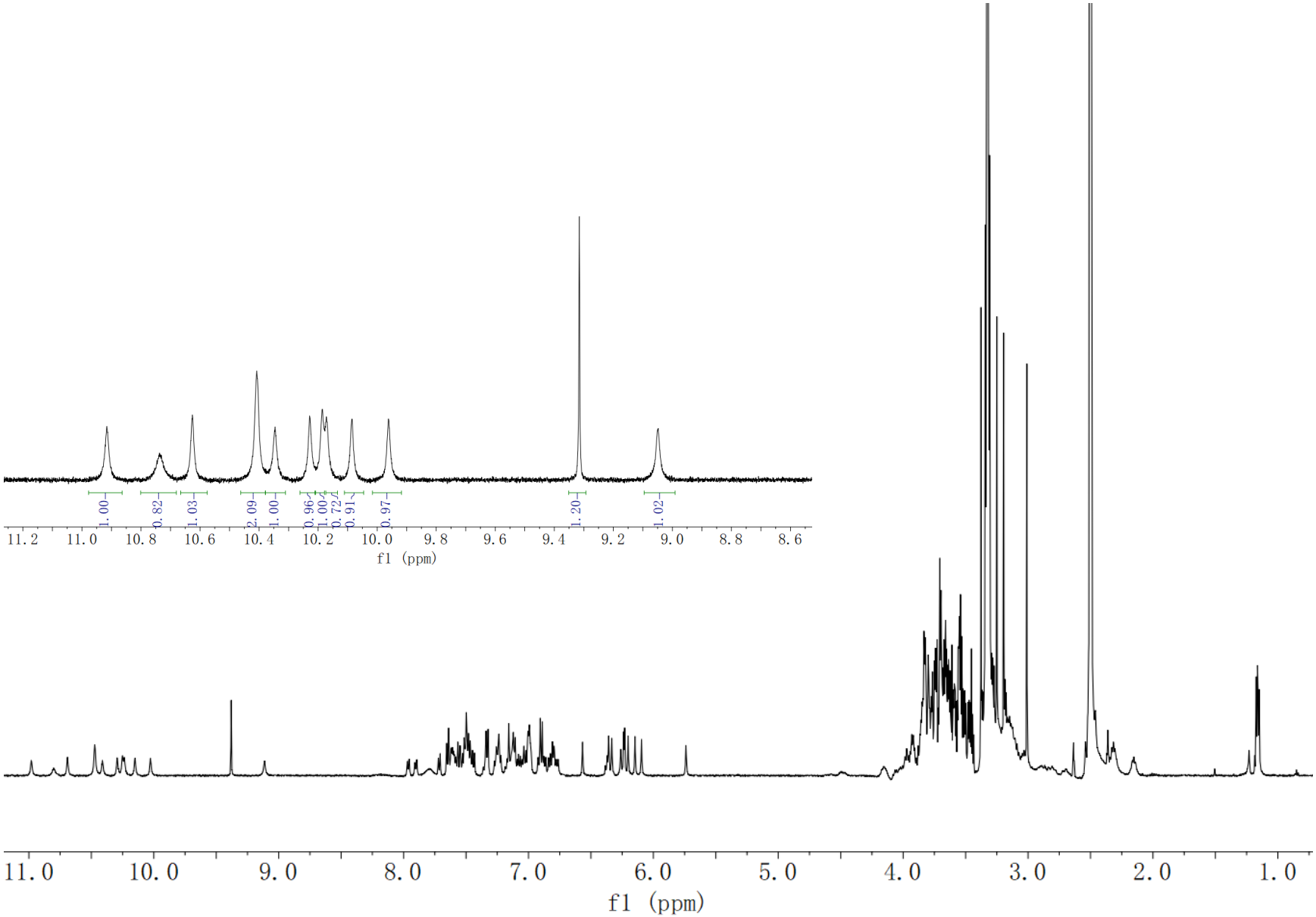
Full ^{1}H NMR spectra (500 MHz, DMF-d_7) of **2c** and zoomed region, monitoring at time point 0 h (blue), 18 h (green), 42 h (black), 25 °C. The red diamond indicates signal of the CH_3 group of chiral **B** unit from diastereomeric conformer. The same process was followed for **2d.**



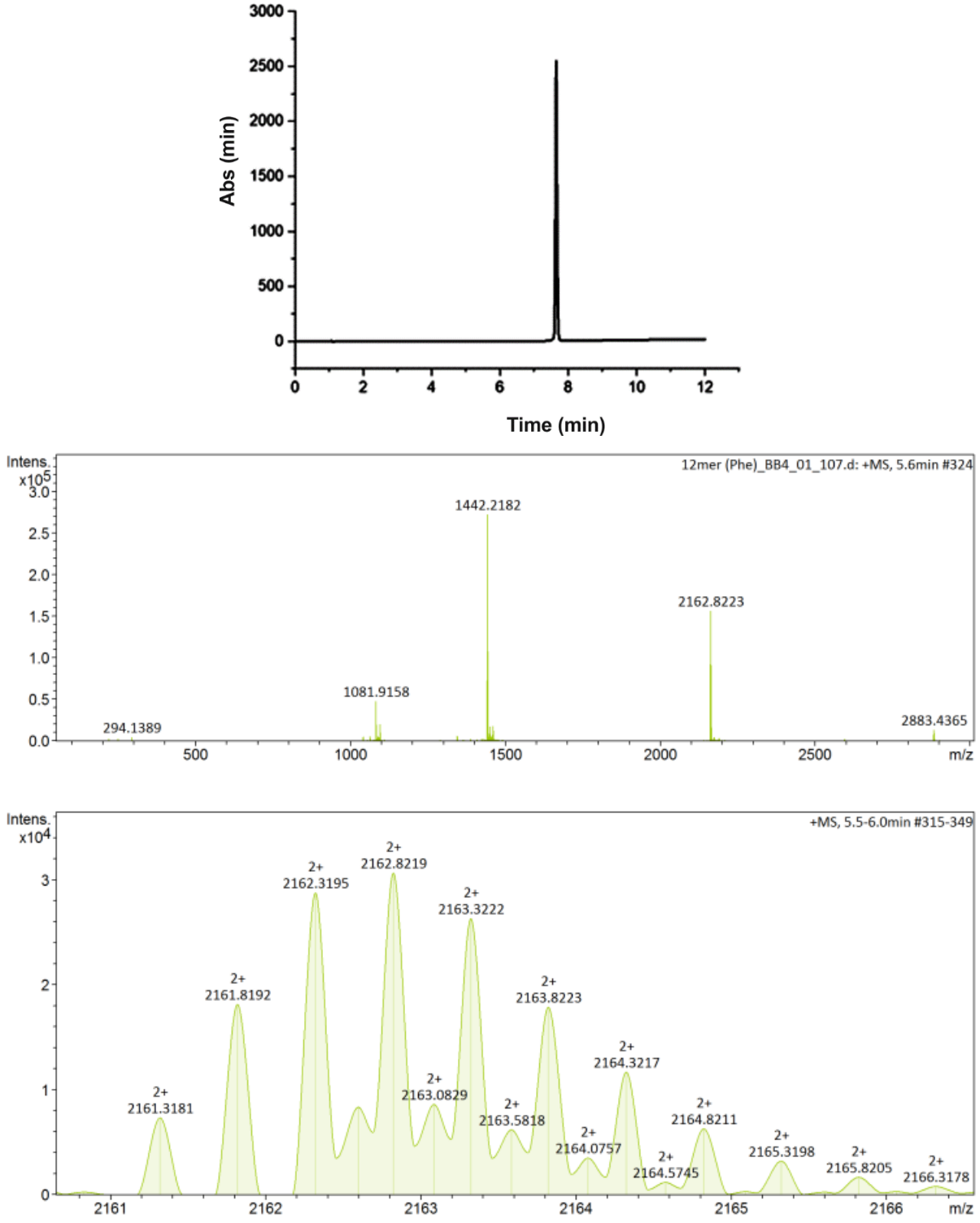




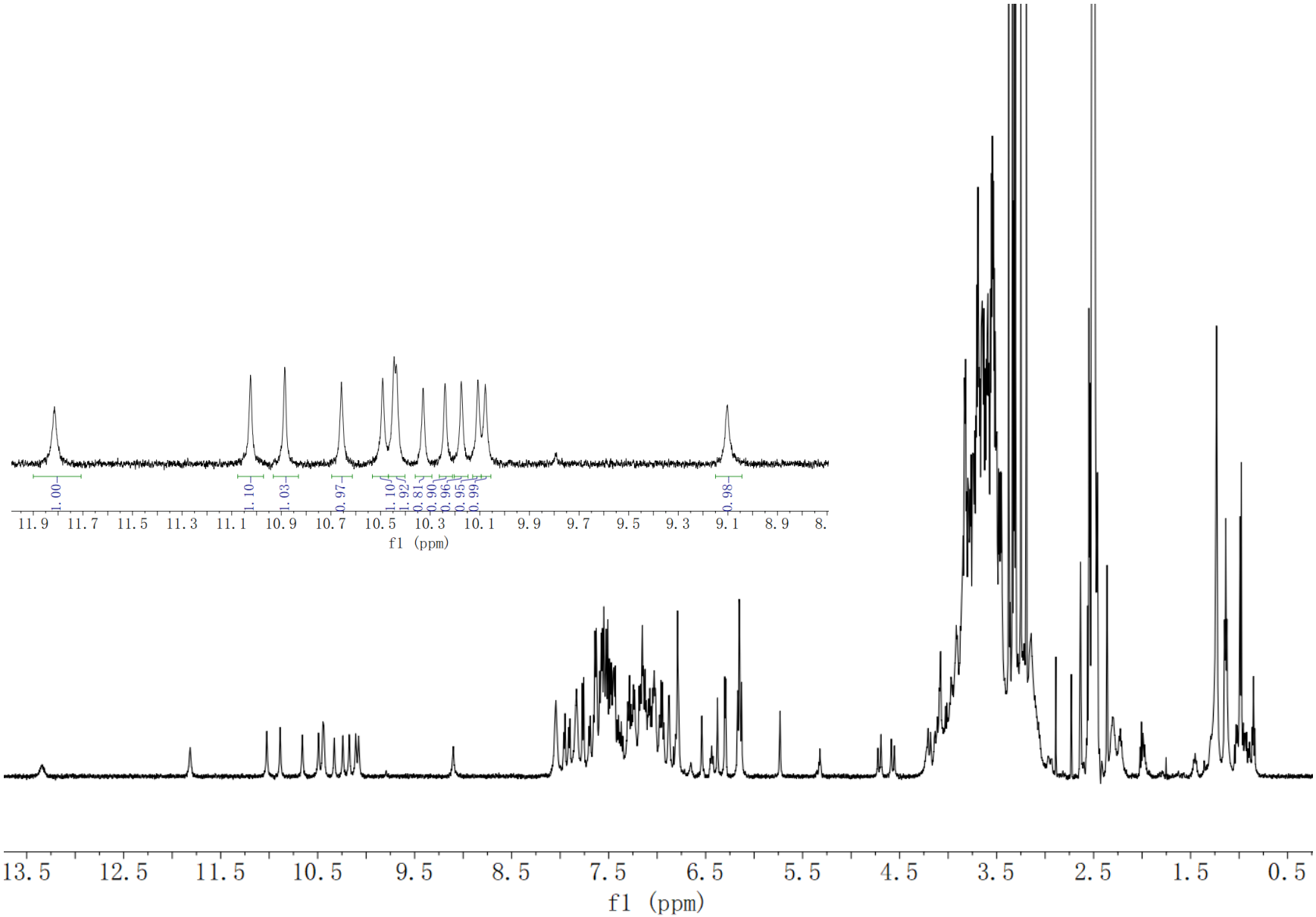
Analytical RP-HPLC (10-100% B over 10 min, 254 nm) profile of **2e**. HRMS (ESI$^+$) spectrum of foldamer **2e**.



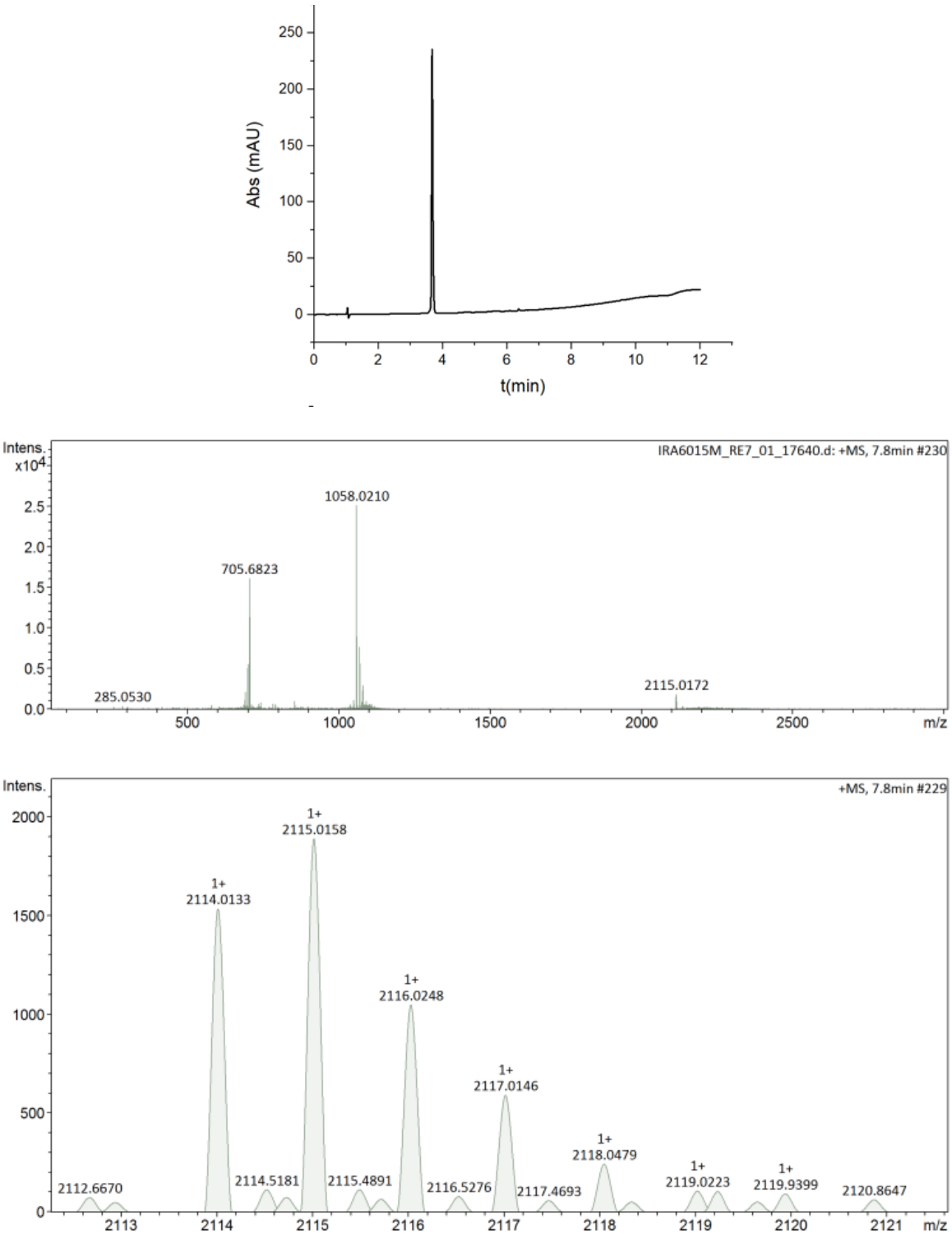
^{1}H NMR Spectrum of foldamer **2e** (500 MHz, DMSO-d_6) and zoomed region, 25 °C.



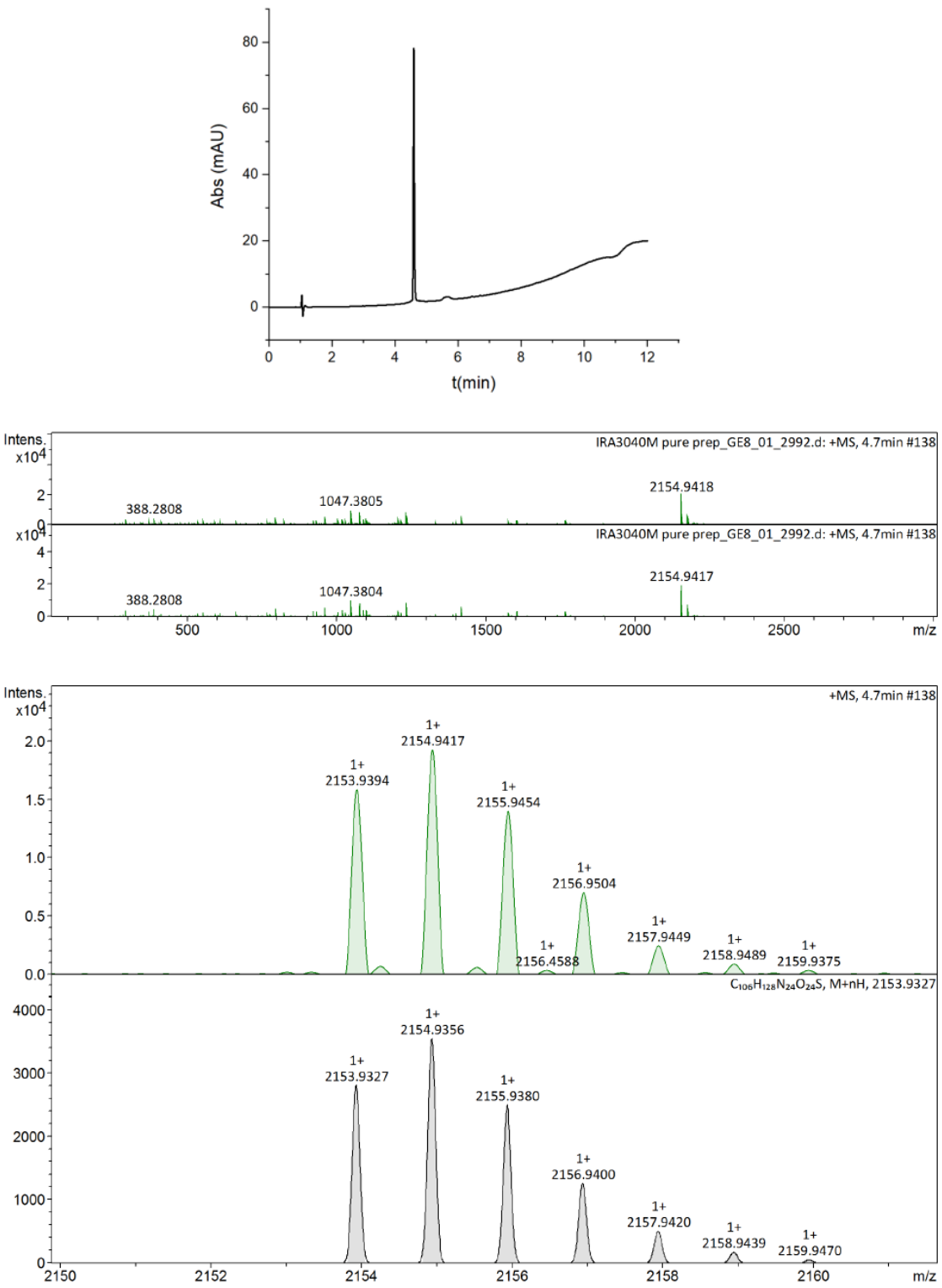
Analytical HPLC (10-100% B over 10 min, 254 nm) profile of foldamer **2f.** HRMS (ESI+) profile of foldamer **2f**.



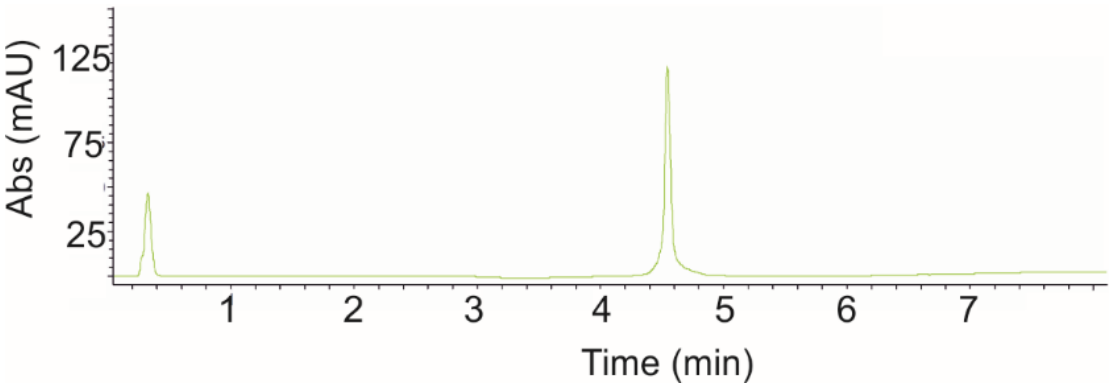
^{1}H NMR Spectrum of foldamer **2f** (500 MHz, DMSO-d_6) and zoomed region 25 °C.

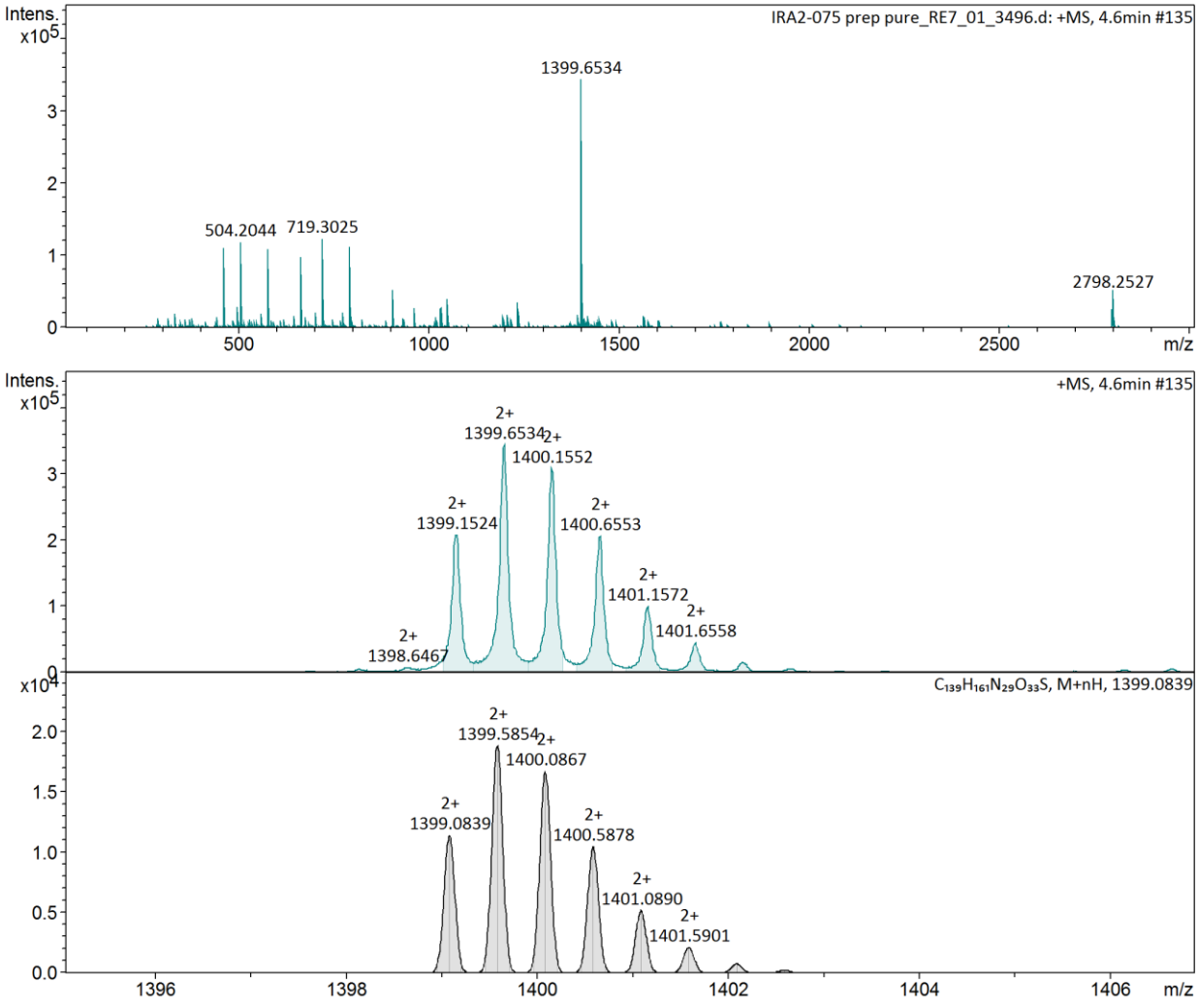


RP-HPLC profile and HRMS (ESI+) spectra of peptide **3a**

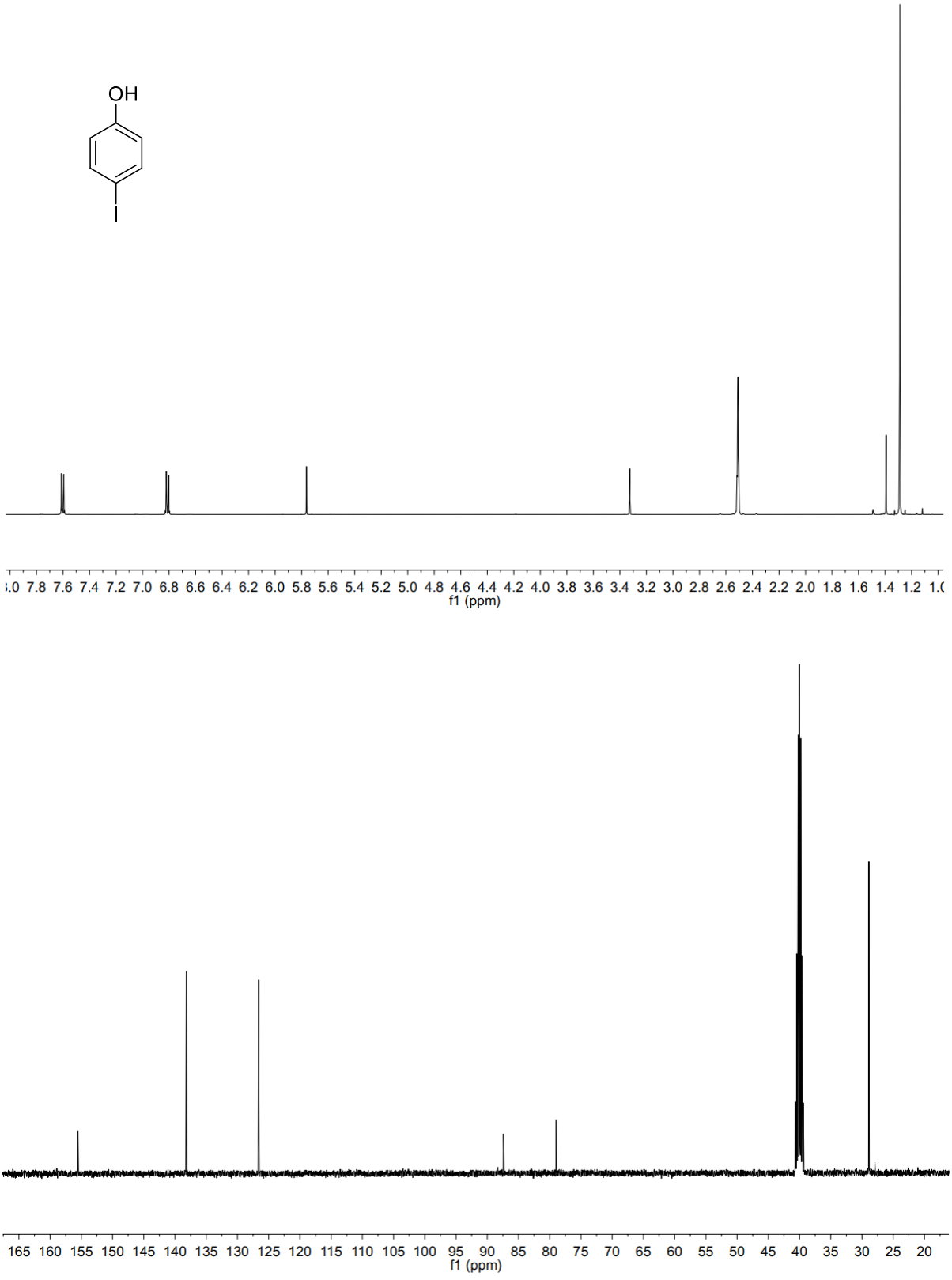


RP-HPLC profile and HRMS (ESI+) spectra of peptide **4a**

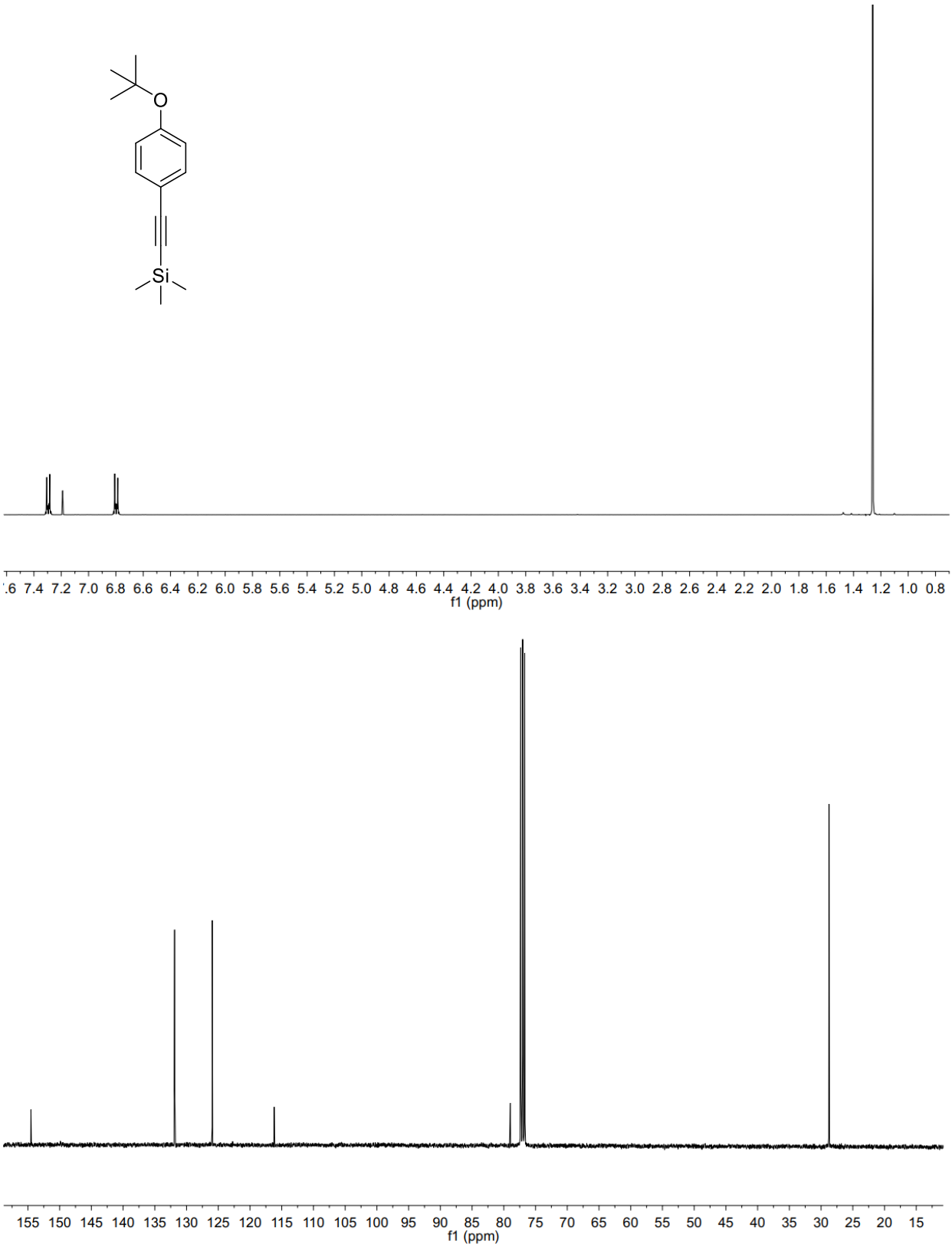




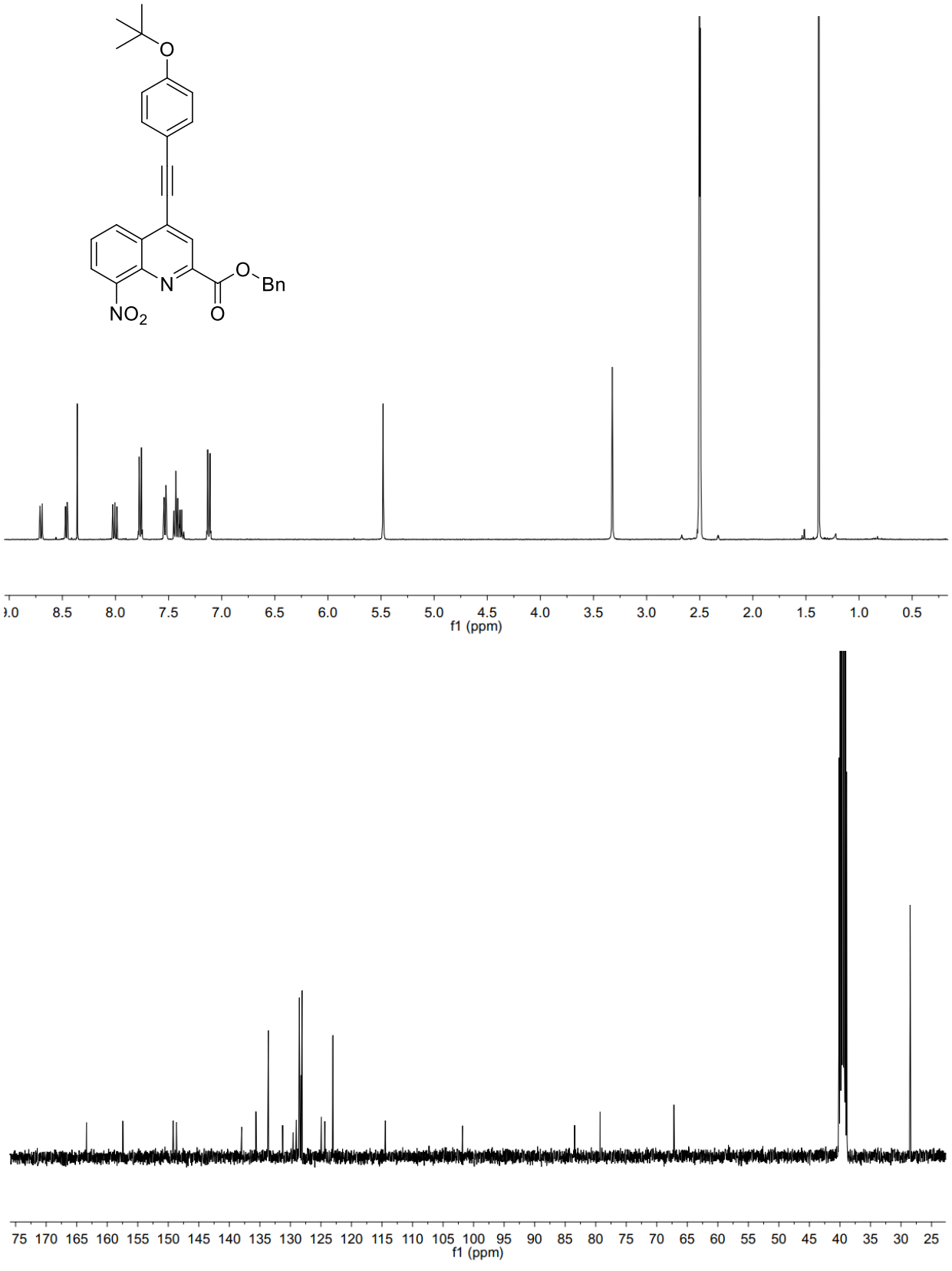
RP-HPLC profile and HRMS (ESI+) spectra of peptide **4c**



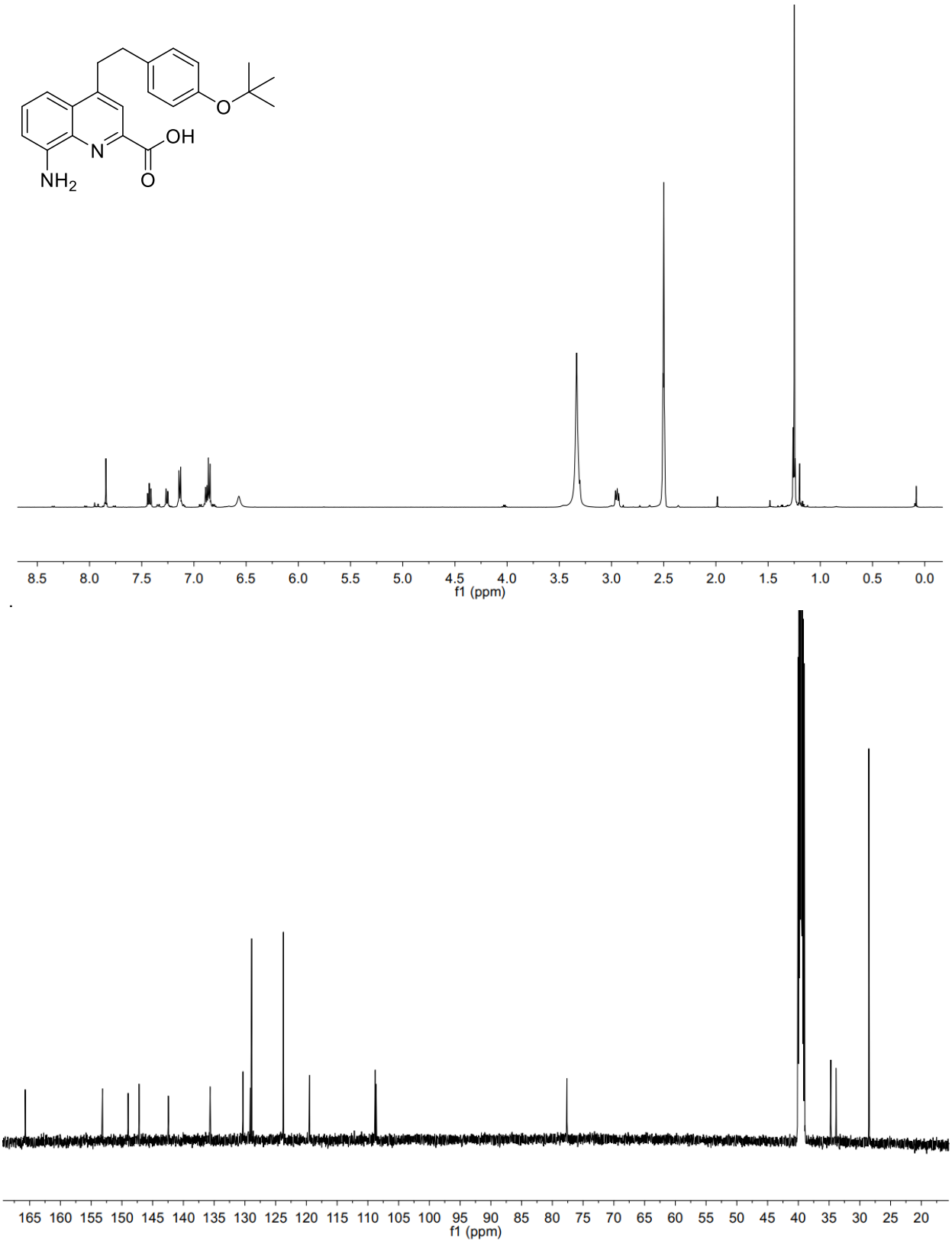
NMR spectra of compound **7**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR Spectrum (126 MHz, DMSO-d_6)



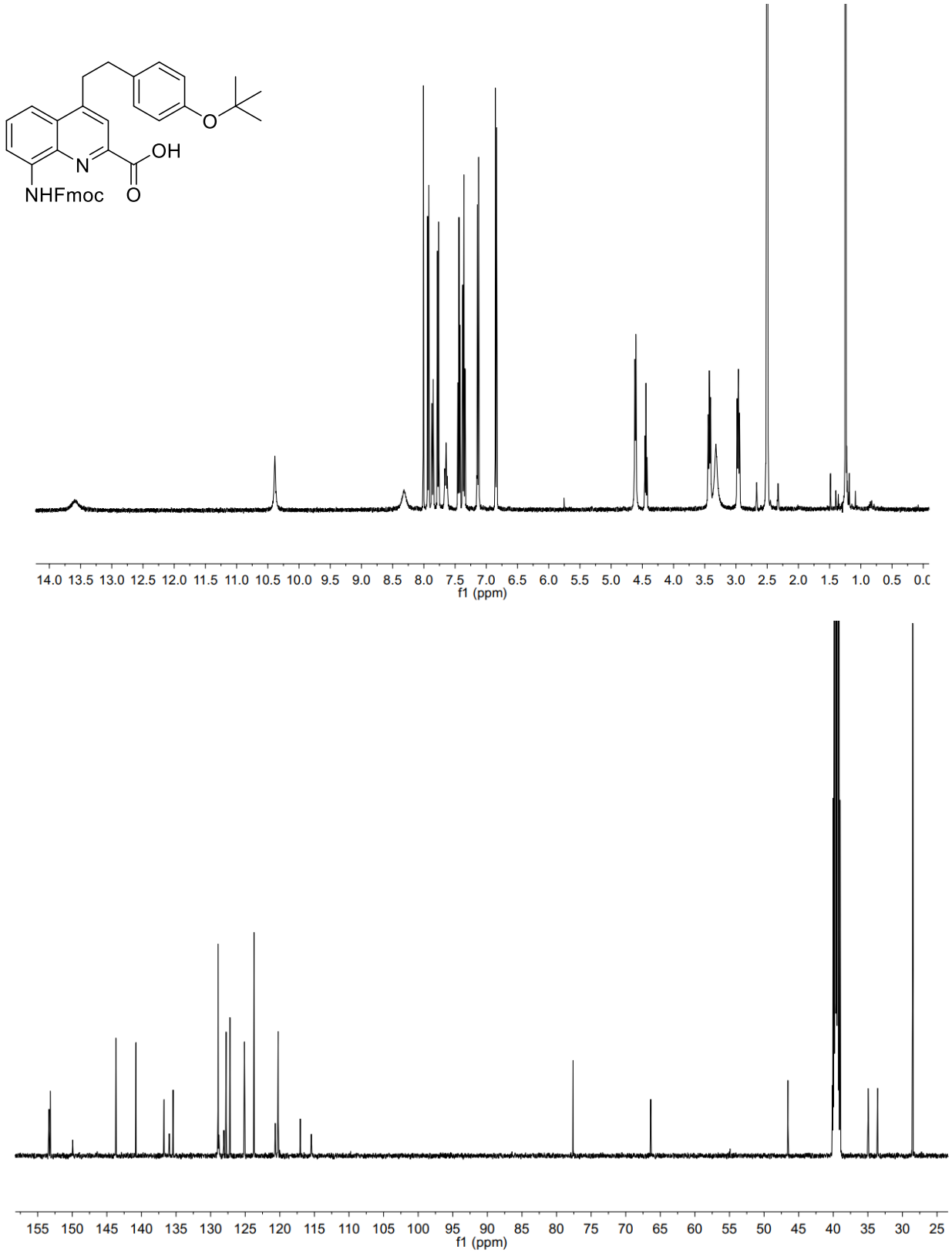
NMR spectra of compound **8**: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) and ^{13}C NMR Spectrum (126 MHz, $CDCl_3$)



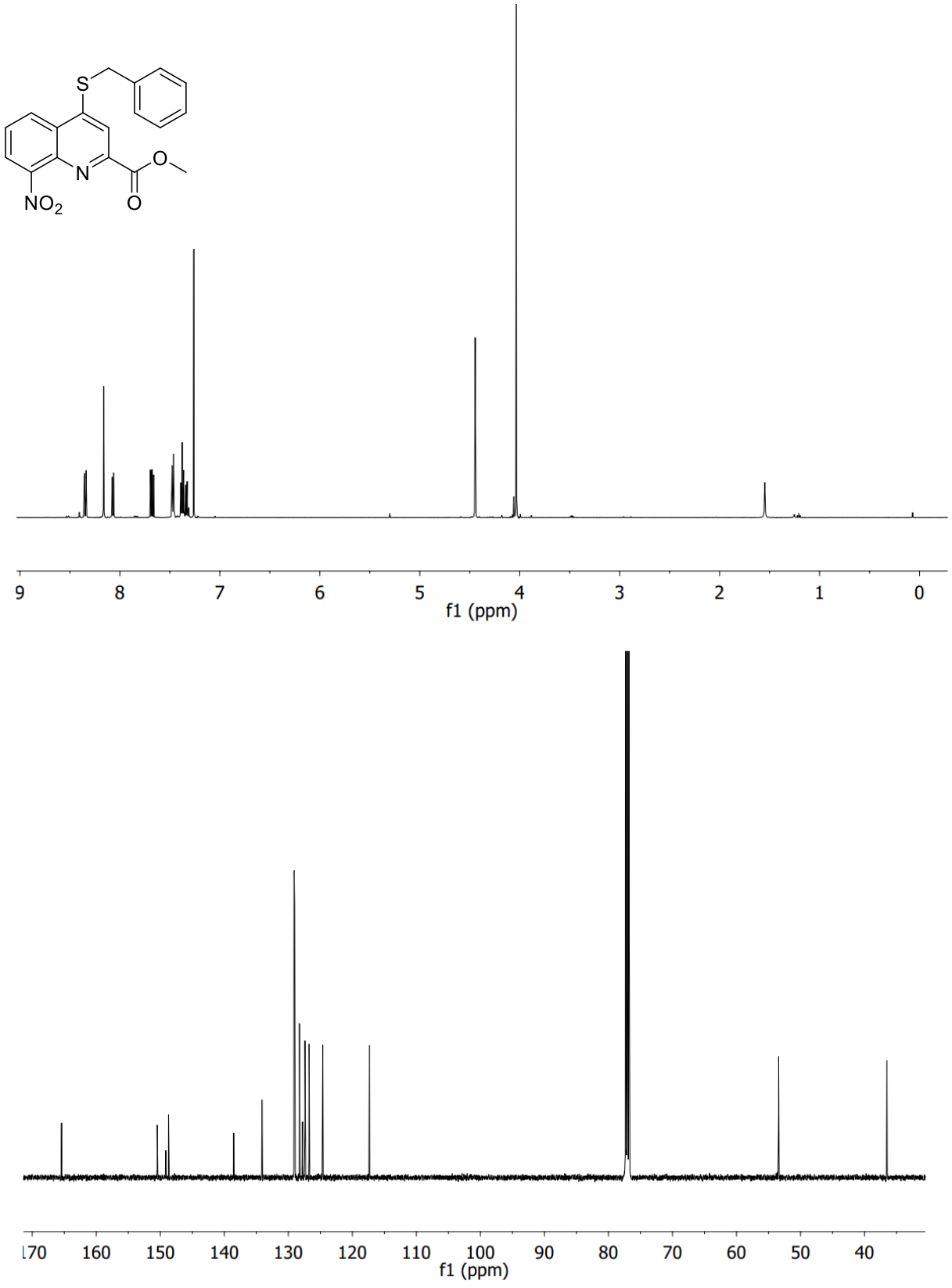
NMR spectra of compound **9**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR Spectrum (126 MHz, DMSO-d_6)



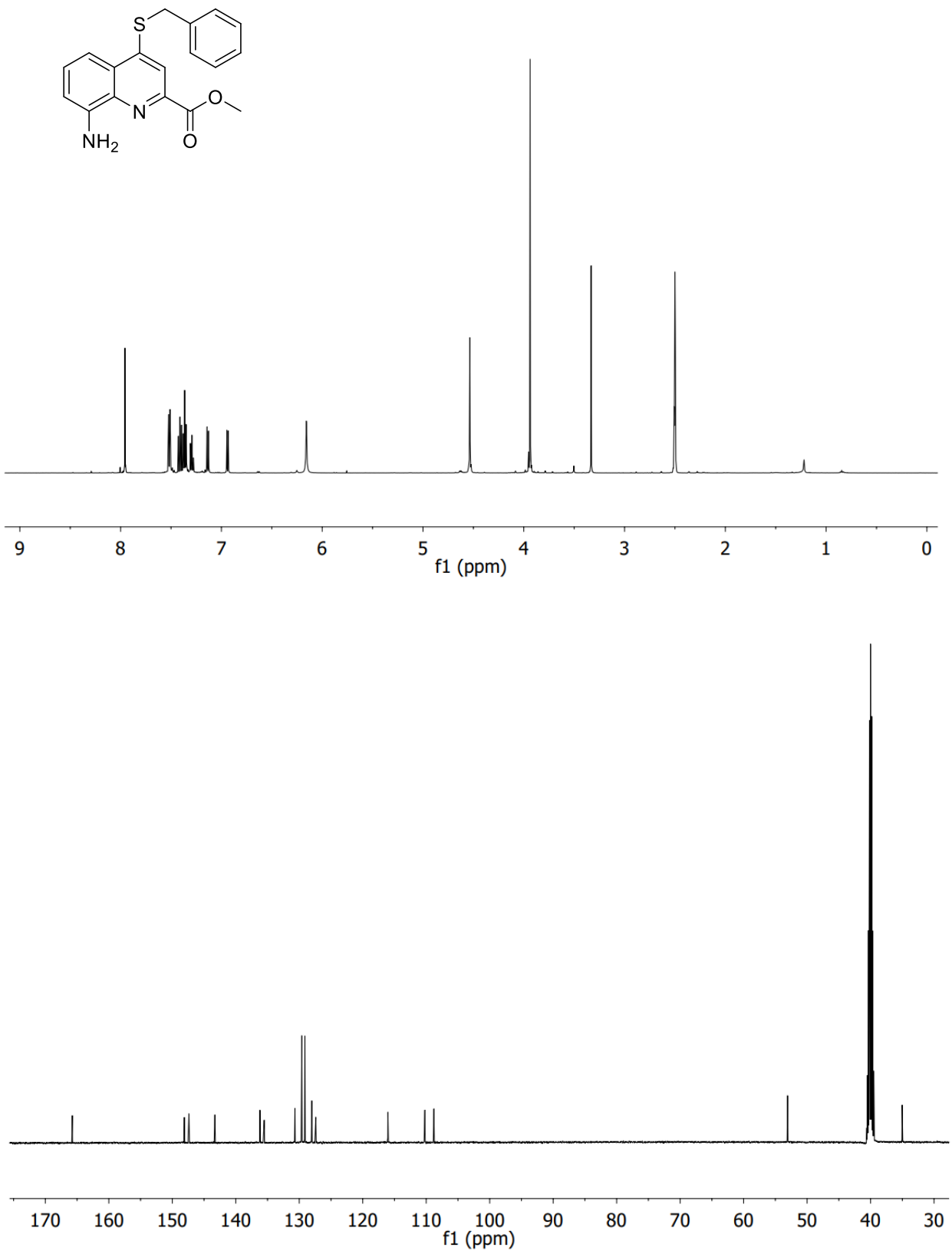
NMR spectra of compound **10**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR Spectrum (126 MHz, DMSO-d_6)



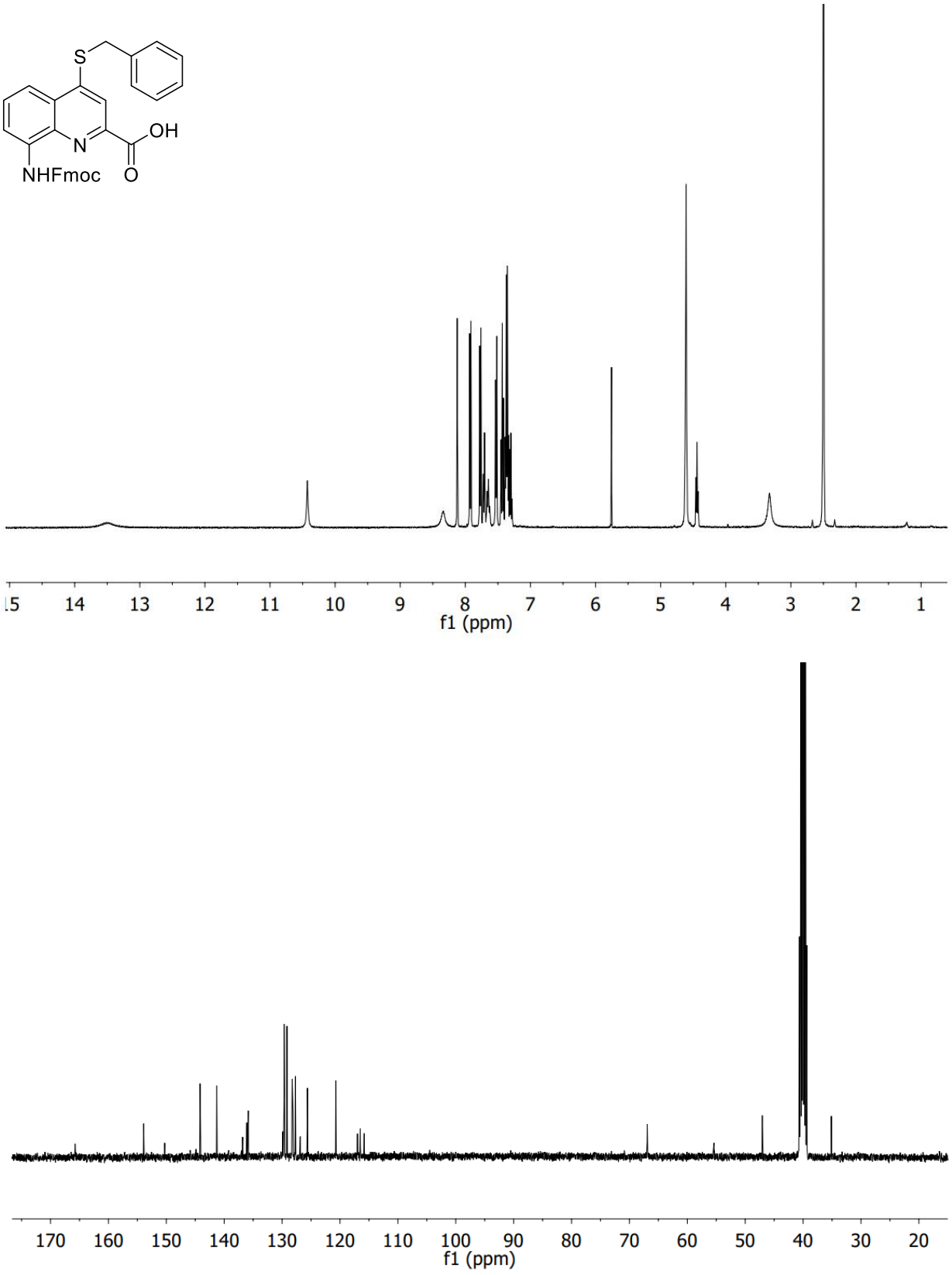
NMR spectra of compound **11**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR Spectrum (126 MHz, DMSO-d_6)



NMR spectra of compound **13**: ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR Spectrum (126 MHz, $CDCl_3$)

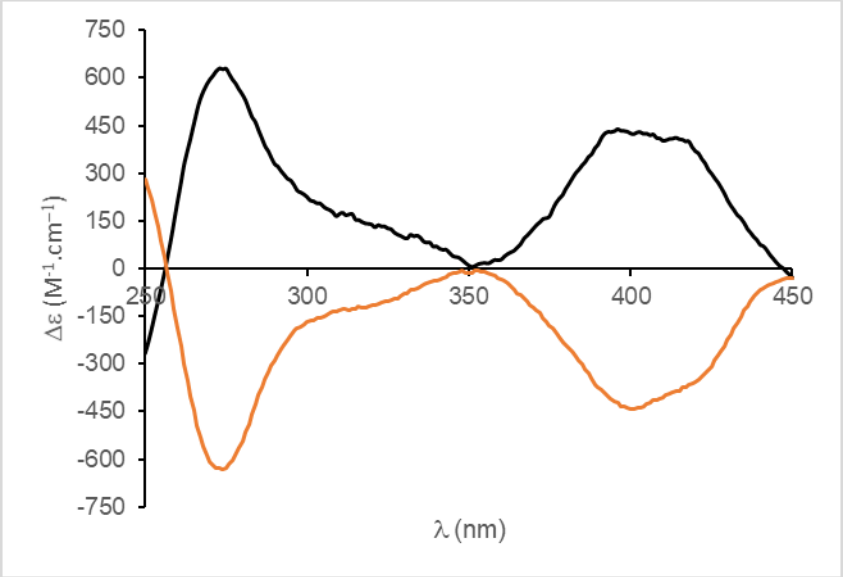


NMR spectra of compound **14**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR Spectrum (126 MHz, DMSO-d_6)

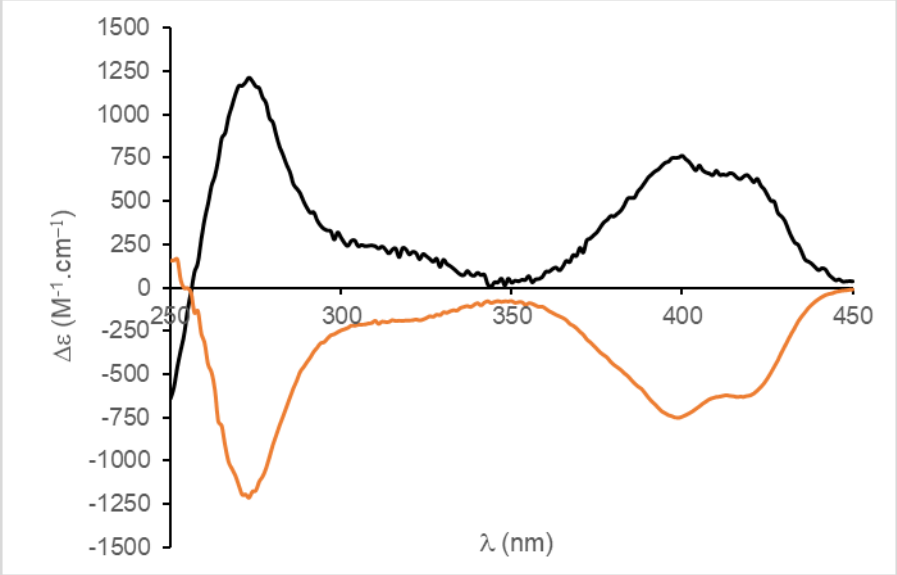


NMR spectra of compound **15**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR Spectrum (126 MHz, DMSO-d_6)

5. CD spectra of foldamers 1d-1e, 2c-2d



CD spectra of **2c** (orange curve) and **2d** (black curve) recorded in TBST-D buffer at 25°C.



CD spectra of **1d** (orange curve) and **1e** (black curve) recorded in TBST-D buffer at 25°C.

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