Electronic Supplementary Information (ESI) for:

Constitutional self-selection from dynamic combinatorial libraries in aqueous solution through supramolecular interactions

Jordi Solà, Maria Lafuente, Joan Atcher and Ignacio Alfonso**

Dr. J. Solà, M. Lafuente, J. Atcher and Dr. I. Alfonso
Department of Biological Chemistry and Molecular Modelling. Institute of Advanced Chemistry of Catalonia, IQAC-CSIC
Jordi Girona 18-26, 08034, Barcelona, Spain. Fax: (+34)932045904
E-mail: ignacio.alfonso@iqac.csic.es
jordi.sola@iqac.csic.es

Table of Contents:

GENERAL CHARACTERISTICS

General: Reagents and solvents were purchased from commercial suppliers (Aldrich, Fluka or Merck) and were used without further purification. Flash chromatographic purifications and preparative reversed-phase purifications were performed on a Biotage® Isolera Prime™ equipment. TLCs were performed using 6x3 cm SiO_2 pre-coated aluminium plates (ALUGRAM® SIL G/UV_{254}).

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) analyses were performed on a Hewlett Packard Series 1100 (UV detector 1315A) modular system using:

i) For the characterization of [**1a,b**]: a reversed-phase X-Terra C_{18} (15 x 0.46 cm, 5 µm) column. (CH_3CN + 0.07% TFA and H_2O + 0.1% TFA) mixtures at 1 mL/min were used as mobile phase and the monitoring wavelengths were set at 220 and 254 nm.

ii) For the analysis of the DCLs: a reversed-phase kromaphase C_{18} (25 x 0.46 cm, 5µm) column. (CH_3CN + 20 mM HCOOH and H_2O + 20 mM HCOOH) mixtures at 1 mL/min were used as mobile phase and the monitoring wavelength was set at 254 nm.

Nuclear Magnetic Resonance (NMR) spectroscopic experiments were carried out on a Varian INOVA 500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C) and a Varian Mercury 400 instrument (400 MHz for ^1H and 101 MHz for ^{13}C). The chemical shifts are reported in ppm relative to trimethylsilane (TMS), and coupling constants (J) are reported in Hertz (Hz).

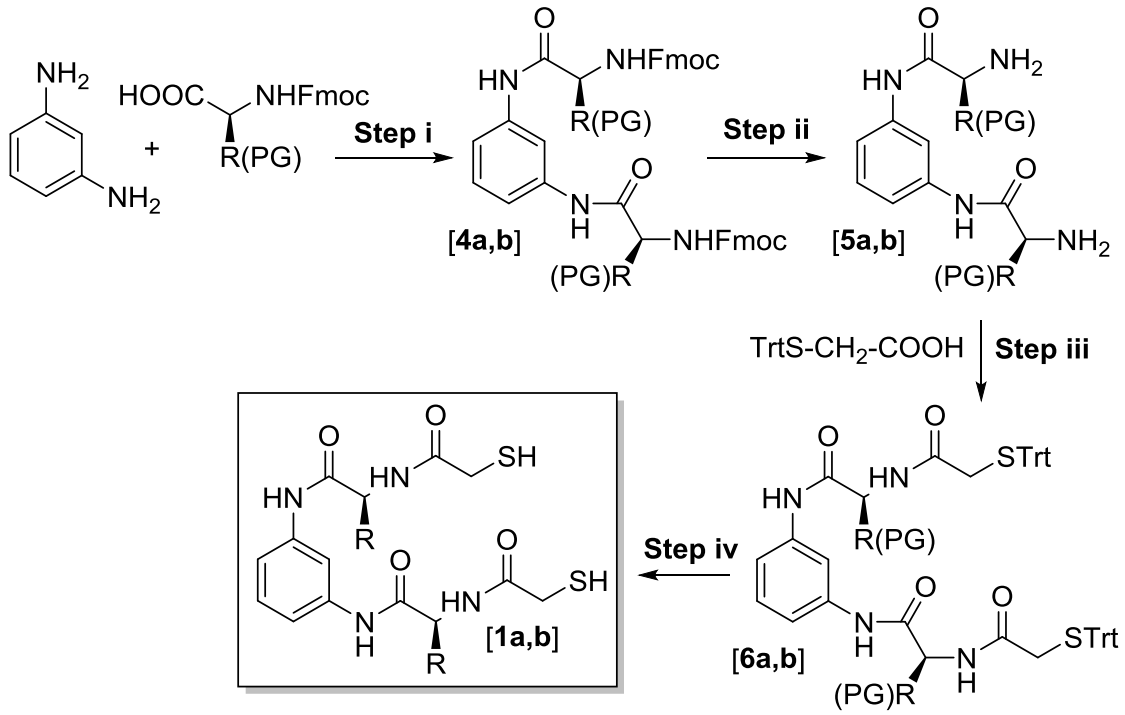
pH measurements were performed at room temperature on a Crison GLP21 pH-meter with the electrodes Crison 50 14T (≥10 mL samples) and PHR-146 Micro (<10 mL samples).

High Resolution Mass Spectrometry (HRMS) analyses were carried out at the IQAC Mass Spectrometry Facility, using a UPLC-ESI-TOF equipment: [Acquity UPLC® BEH C_{18} 1.7 mm, 2.1x100 mm, LCT Premier Xe, Waters]. (CH_3CN + 20 mM HCOOH and H_2O + 20 mM HCOOH) mixtures at 0.3 mL/min were used as mobile phase.

SYNTHESIS OF THE BUILDING BLOCKS

The tritylsulfanyl acetic acid was prepared as previously described.[1] Also the compound [**2**] was synthesized as previously reported.[2] The compounds [**3a-g**] were purchased from commercial suppliers (Sigma-Aldrich, Iris Biotech).

Synthetic scheme of [**1a,b**]

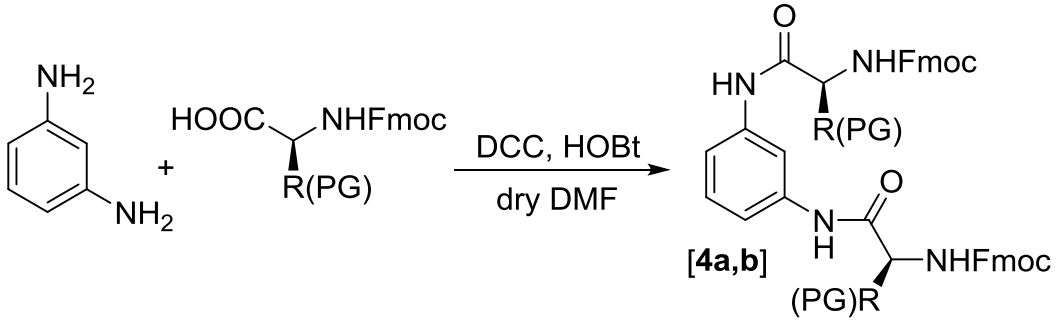


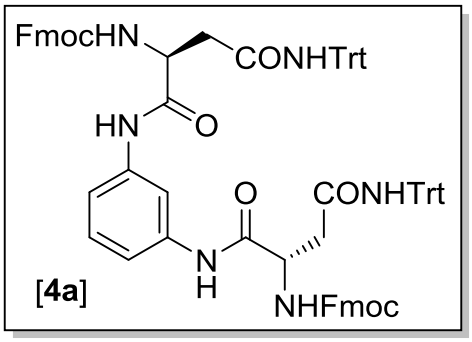
Related references:

1. A. P. Kozikowski, Y. Chen, A. Gaysin, B. Chen, M. A. D'Annibale, C. M. Suto and B. C. Langley, *J. Med. Chem.*, **2007**, *50*, 3054-3061.

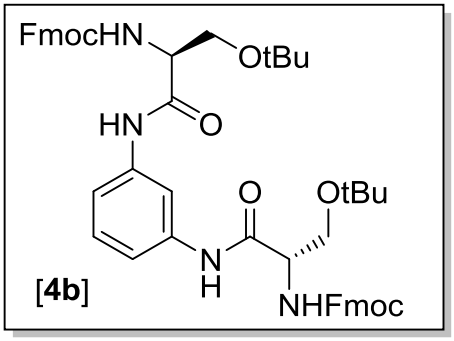
2. K. R. West, K. D. Bake and S. Otto, *Org. Lett.*, **2005**, *7*, 2615-2618.

Step i: Experimental procedure for the synthesis of [**4a,b**]





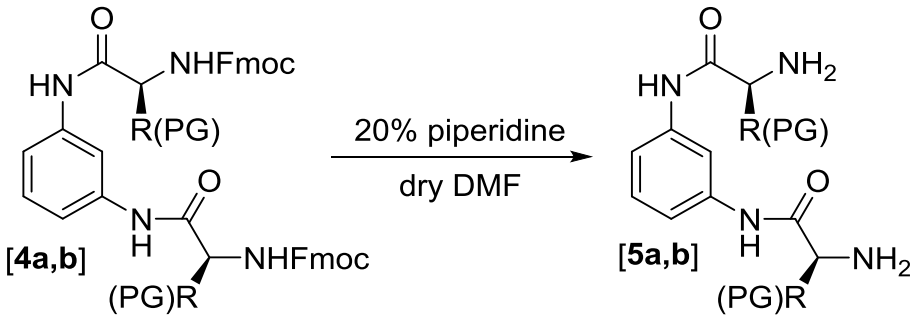
[**4a**]: Fmoc-Asn(Trt)-OH (4.24 g, 7.11 mmol) was dissolved in dry DMF (16 mL) and both dicyclohexylcarbodiimide (DCC, 2.231 g, 10.81 mmol) and 1-hydroxybenzotriazole (HOBt, 1.252 g, 9.27 mmol) were added over the solution. The reaction mixture was cooled to 0ºC. A solution of *m*-phenylenediamine (334 mg, 3.09 mmol) in dry DMF (10 mL) was added over the mixture through a cannula. The solution was stirred at room temperature for 60 hours, after which complete conversion of the starting material was observed by TLC (Rf AcOEt/Hexane, 1:1 (v:v): 0.58). The mixture was filtered, and the filtrate was diluted with DCM, washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography using hexane: AcOEt as eluent (from 30% to 50% AcOEt) to give 2.047 g of [**4a**] (52% yield) as a white solid. HRMS (ESI+) calcd. for $C_{82}H_{68}N_6O_8$ $[M+H]^+$ (m/z): 1265.5171, found: 1265.5183. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.77 (brs, 2H, NH), 7.81 – 7.66 (m, 5H, CH_{Ar}), 7.61 – 7.51 (m, 4H, CH_{Ar}), 7.38 (t, *J* = 7.5 Hz, 4H, CH_{Ar}), 7.32 – 7.04 (m, 37H, CH_{Ar}), 6.97 (s, 2H, NH), 6.54 (brs, 2H, NH), 4.68 (brs, 2H, C*H), 4.51 – 4.32 (m, 4H, CH_2), 4.20 (t, *J* = 7.0 Hz, 2H, CH), 3.16 (d, *J* = 15.7 Hz, 2H, C$\underline{H}_2$C*H), 2.66 (dd, *J* = 15.7, 6.9 Hz, 2H, C$\underline{H}_2$C*H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.8 (2 x CO), 169.0 (2 x CO), 156.4 (2 x CO), 144.2 (6 x C_{Ar}), 143.8 (2 x C_{Ar}), 143.8 (2 x C_{Ar}), 141.4 (2 x C_{Ar}), 141.4 (2 x C_{Ar}), 138.0 (2 x C_{Ar}), 129.3 (1 x CH_{Ar}), 128.7 (12 x CH_{Ar}), 128.2 (12 x CH_{Ar}), 127.9 (4 x CH_{Ar}), 127.3 (6 x CH_{Ar}), 127.3 (4 x CH_{Ar}), 125.3 (4 x CH_{Ar}), 120.1 (4 x CH_{Ar}), 116.3 (2 x CH_{Ar}), 111.7 (1 x CH_{Ar}), 71.2 (2 x C), 67.5 (2 x CH_2), 52.2 (2 x C*H), 47.2 (2 x CH), 38.9 (2 x $\underline{C}H_2$C*H).

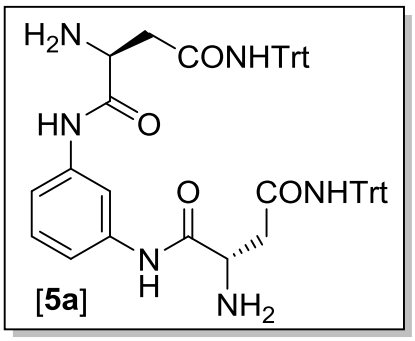


[**4b**]: this compound was obtained as described above starting from the Fmoc-Ser(*t*Bu)-OH. The residue was purified by flash chromatography using hexane: AcOEt as eluent (from 25% to 40% AcOEt, Rf AcOEt/Hexane, 3:2 (v:v): 0.83) to give 1.05 g of [**4b**] (43% yield) as a white solid. HRMS (ESI+) calcd. for $C_{50}H_{54}N_4O_8$ $[M+H]^+$ (m/z): 839.4014, found: 839.4029. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.80 (brs, 2H, NH), 7.96 (s, 1H, CH_{Ar}), 7.77 (d, *J* = 7.6 Hz, 4H, CH_{Ar}), 7.62 (d, *J* = 7.1 Hz, 4H, CH_{Ar}), 7.41 (t, *J* = 7.4 Hz, 4H, CH_{Ar}), 7.32 (t, *J* = 7.8 Hz, 4H, CH_{Ar}), 7.29–7.20 (m, 3H, CH_{Ar}), 5.87 (brs, 2H, NH), 4.44 (d, *J* = 7.0 Hz, 4H, CH_2), 4.35 (brs, 2H, C$\underline{H}_2$C*H), 4.25 (t, *J* = 6.9 Hz, 2H, CH), 3.92 (brs, 2H, C$\underline{H}_2$C*H), 3.45 (t, *J* = 8.7

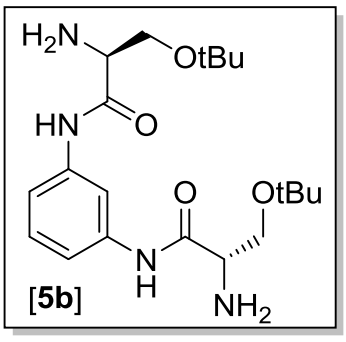
Hz, 2H: C*H), 1.28 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.5 (2 x CO), 156.2 (2 x CO), 143.9 (4 x C_{Ar}), 141.4 (4 x C_{Ar}), 138.4 (2 x C_{Ar}), 129.9 (1 x CH_{Ar}), 127.9 (4 x CH_{Ar}), 127.2 (4 x CH_{Ar}), 125.2 (4 x CH_{Ar}), 120.2 (4 x CH_{Ar}), 115.5 (2 x CH_{Ar}), 111.0 (1 x CH_{Ar}), 75.1 (2 x C), 67.3 (2 x CH_2), 61.9 (2 x $\underline{C}H_2$C*H), 54.8 (2 x C*H), 47.3 (2 x CH), 27.6 (6 x CH_3).

Step ii: Experimental procedure for the synthesis of [**5a,b**]



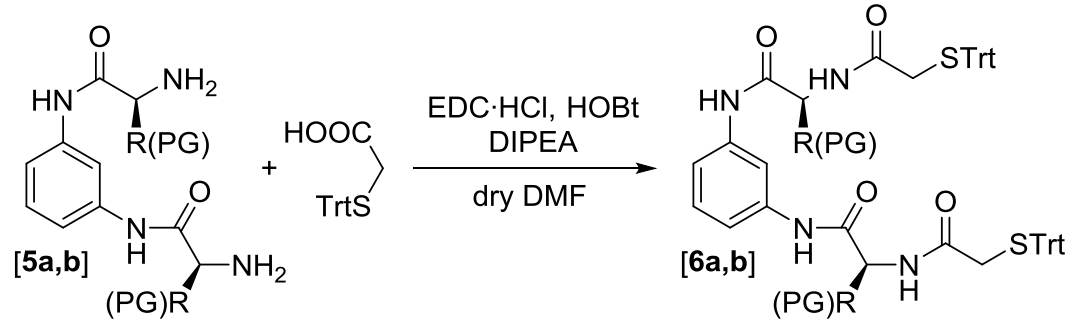


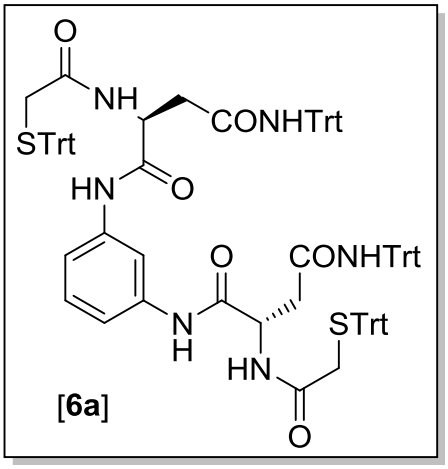
[**5a**]: [**4a**] (600 mg, 0.47 mmol) was dissolved in 4.0 mL of 20% piperidine in dry DMF. After several minutes the product precipitated as a white solid but the mixture was allowed to react at room temperature for 4 hours until complete conversion of starting material. Diethyl ether was added over the reaction mixture and the product was filtered off and washed with diethyl ether. 293 mg of [**5a**] (75% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{52}H_{48}N_6O_4$ $[M+H]^+$ (m/z): 821.3810, found: 821.3712. ^{1}H NMR (400 MHz, MeOD-d_4): δ = 7.93 (s, 1H, CH_{Ar}), 7.38 – 7.31 (m, 2H, CH_{Ar}), 7.30 – 7.10 (m, 31H, CH_{Ar}), 3.77 (dd, *J* = 7.5, 5.5 Hz, 2H, C*H), 2.77 (dd, *J* = 15.3, 5.5 Hz, 2H, CH_2), 2.68 (dd, *J* = 15.3, 7.6 Hz, 2H, CH_2). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 174.6 (2 X CO), 172.5 (2 x CO), 145.9 (6 x C_{Ar}), 140.0 (2 x C_{Ar}), 130.1 (1 x CH_{Ar}), 130.0 (12 x CH_{Ar}), 128.7 (12 x CH_{Ar}), 127.8 (6 x CH_{Ar}), 117.0 (2 x CH_{Ar}), 112.8 (1 x CH_{Ar}), 71.7 (2 x C), 54.0 (2 x C*H), 42.3 (2 x CH_2).



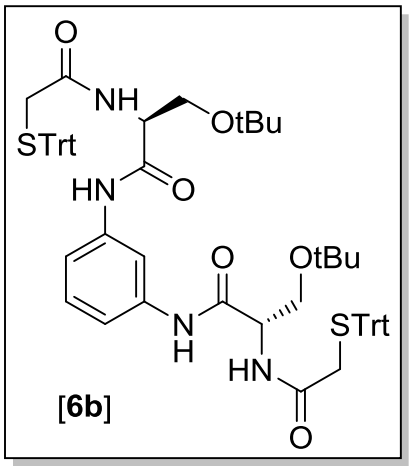
[**5b**]: 522 mg of [**5b**] (99% yield) were obtained as described above starting from the [**4b**]. HRMS (ESI+) calcd. for $C_{20}H_{34}N_4O_4$ $[M+H]^+$ (m/z): 395.2653, found: 395.2672. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.54 (s, 2H, NH), 7.91 (t, *J* = 2.1 Hz, 1H, CH_{Ar}), 7.39–7.35 (m, 2H, CH_{Ar}), 7.29–7.23 (m, 1H, CH_{Ar}), 3.67 (dd, *J* = 7.2, 3.3 Hz, 2H, CH_2), 3.62–3.55 (m, 4H, 2H x C*H + 2H x CH_2), 2.00 (brs, 4H, NH_2), 1.21 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.6 (2 x CO), 138.6 (2 x C_{Ar}), 129.6 (1 x CH_{Ar}), 115.0 (2 x CH_{Ar}), 110.4 (1 x CH_{Ar}), 73.8 (2 x C), 63.8 (2 x CH_2), 56.0 (2 x C*H), 27.7 (6 x CH_3).

Step iii: Experimental procedure for the synthesis of [**6a,b**]





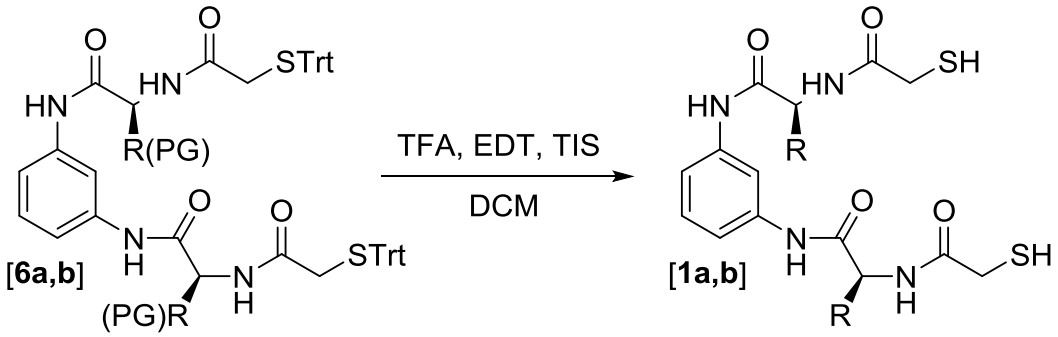
[**6a**]: tritylsulfanyl acetic acid (229 mg, 0.69 mmol) was dissolved in dry DMF (10 mL) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, 144 mg, 0.75 mmol), HOBt (101 mg, 0.75 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 240 µL, 1.38 mmol) were added over the solution. The reaction mixture was cooled to 0ºC and then, [**5a**] (267 mg, 0.33 mmol) was added over the mixture. The solution was allowed to stir at room temperature for 60 hours, after which complete conversion of the starting material was observed by TLC (Rf AcOEt/Hexane, 1:1 (v:v): 0.46). The mixture was diluted with DCM, washed with saturated aqueous $NaHCO_3$ and dried under reduced pressure. The residue was purified by flash chromatography using hexane: AcOEt as eluent (from 40% to 60% AcOEt) to give 343 mg of [**6a**] (73% yield) as a white solid. HRMS (ESI+) calcd. for $C_{94}H_{80}N_6O_6S_2$ $[M+H]^+$ (m/z): 1453.5654, found: 1453.5665. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.84 (s, 2H, NH), 7.60 (t, *J* = 2.0 Hz, 1H, CH_{Ar}), 7.54 – 7.00 (m, 65H, 2H x NH + 63H x CH_{Ar}), 6.91 (s, 2H, NH), 4.49 (td, *J* = 7.5, 3.0 Hz, 2H, C*H), 3.05 (ABq, δ_A = 3.02, δ_B = 3.08, *J* = 15.7Hz, 4H, C$\underline{H}_2$STrt), 2.96 – 2.83 (m, 2H, C$\underline{H}_2$C*H), 2.39 (dd, *J* = 15.7, 7.8 Hz, 2H, C$\underline{H}_2$C*H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.7 (2 x CO), 169.0 (2 x CO), 168.3 (2 x CO), 144.2 (6 x C_{Ar}), 144.1 (6 x C_{Ar}), 138.11 (2 x C_{Ar}), 129.7 (12 x CH_{Ar}), 129.2 (1 x CH_{Ar}), 128.7 (12 x CH_{Ar}), 128.3 (12 x CH_{Ar}), 128.2 (12 x CH_{Ar}), 127.3 (6 x CH_{Ar}), 127.1 (6 x CH_{Ar}), 116.3 (2 x CH_{Ar}), 111.8 (1 x CH_{Ar}), 71.1 (2 x C), 67.9 (2 x C), 50.7 (2 x C*H), 38.4 (2 x $\underline{C}H_2$C*H), 36.2 (2 x $\underline{C}H_2$STrt).

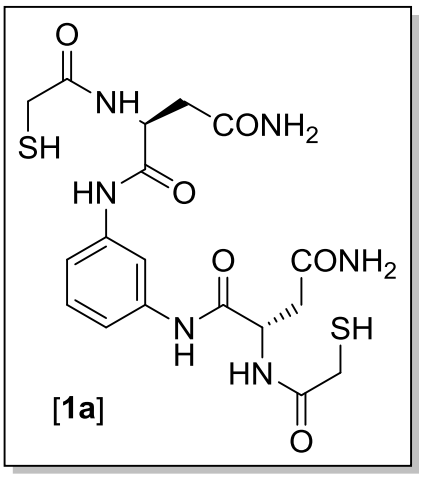


[**6b**]: this compound was obtained as described above starting from [**5b**]. The residue was purified by flash chromatography using hexane: AcOEt as eluent (from 35% to 45% AcOEt, Rf AcOEt/Hexane, 2:3 (v:v): 0.27) to give 612 mg of [**6b**] (51% yield) as a white solid. HRMS (ESI+) calcd. for $C_{62}H_{66}N_4O_6S_2$ $[M+H]^+$ (m/z): 1027.4497, found: 1027.4492. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.68 (s, 2H, NH), 7.79 (t, *J* = 1.8 Hz, 1H, CH_{Ar}), 7.43 (d, *J* = 7.3 Hz, 12H, CH_{Ar}), 7.28 (t, *J* = 7.6 Hz, 12H, CH_{Ar}), 7.25–7.18 (m, 9H, CH_{Ar}), 7.10 (d, *J* = 5.8 Hz, 2H, NH), 4.24–4.18 (m, 2H, C*H), 3.71 (dd, *J* = 8.6, 4.3 Hz, 2H, C$\underline{H}_2$O^{t}Bu), 3.20–3.08 (m, 6H, 2H x C$\underline{H}_2$O^{t}Bu + 4H x

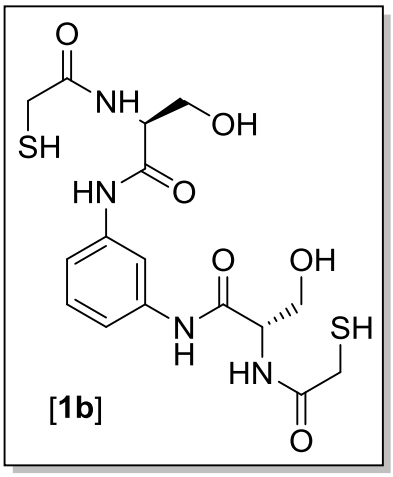
C<u>H</u>$_2$STrt), 1.22 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.7 (2 x CO), 168.2 (2 x CO), 144.1 (6 x C_{Ar}), 138.4 (2 x C_{Ar}), 129.8 (1 x CH_{Ar}), 129.7 (12 x CH_{Ar}), 128.3 (12 x CH_{Ar}), 127.1 (6 x CH_{Ar}), 115.5 (2 x CH_{Ar}), 110.9 (1 x CH_{Ar}), 75.0 (2 x C), 68.0 (2 x C), 61.0 (2 x <u>C</u>H_2C*H), 53.5 (2 x C*H), 36.2 (2 x CH_2), 27.6 (6 x CH_3).

<u>Step iv: Experimental procedure for the synthesis of [**1a,b**]</u>





[**1a**]: [**6a**] was dissolved in DCM (1 mL) and 5.5 mL of trifluoroacetic acid (TFA), 313 µL of triisobutylsilane (TIS) and 152 µL of 1,2-ethanedithiol (EDT) were added rapidly and under stirring. The reaction mixture was allowed to stir at room temperature for 40 min, after which the solvents were partially evaporated using a N_2 flow. Diethyl ether was added over the reaction mixture and the product was filtered off and washed with diethyl ether. The product was purified using reversed-phase flash chromatography (gradient: from 5% to 30% CH_3CN in H_2O) and 37.8 mg of [**1a**] (52% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{18}H_{24}N_6O_6S_2$ $[M+H]^+$ (m/z): 485.1277, found: 485.1271. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.97 (s, 2H, N<u>H</u>COC*H), 8.34 (d, *J* = 7.7 Hz, 2H, C*HN<u>H</u>CO), 7.93 (t, *J* = 2.0 Hz, 1H, CH_{Ar}), 7.36 (s, 2H, NH_2), 7.29 (dd, *J* = 7.6, 2.0 Hz, 2H, CH_{Ar}), 7.24 – 7.15 (m, 1H, CH_{Ar}), 6.91 (s, 2H, NH_2), 4.67 (app q, *J* = 7.1 Hz, 2H, C*H), 3.17 (d, *J* = 7.9 Hz, 4H, C<u>H</u>$_2$SH), 2.73 (t, *J* = 7.9 Hz, 2H, SH), 2.62 – 2.41 (m, 4H, C<u>H</u>$_2$C*H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.1 (2 x $CONH_2$), 169.5 (2 x <u>C</u>OC*H), 169.4 (2 x <u>C</u>OCH_2), 139.1 (2 x C_{Ar}), 128.6 (1 x C_{Ar}), 114.6 (2 x C_{Ar}), 110.9 (1 x C_{Ar}), 50.9 (2 x C*H), 37.1 (2 x <u>C</u>H_2C*H), 27.0 (2 x CH_2SH).



[**1b**]: 76.4 mg of [**1b**] (90% yield) were obtained as described above starting from [**6b**]. RP-HPLC (gradient: from 5% to 30% CH_3CN in H_2O). HRMS (ESI+) calcd. for $C_{16}H_{22}N_4O_6S_2$ $[M+H]^+$ (m/z): 431.1059, found: 431.1042. ^1H NMR (400 MHz, MeOD-*d*$_4$): δ = 7.92 (t, *J* = 1.8 Hz, 1H, CH_{Ar}), 7.36–7.31 (m, 2H, CH_{Ar}), 7.28–7.23 (m, 1H, CH_{Ar}), 4.56 (t, *J* = 5.3 Hz, 2H, C*H), 3.92–3.82 (m, 4H, C$\underline{H}_2$OH), 3.28 (s, 4H, C$\underline{H}_2$SH). ^{13}C NMR (101 MHz, MeOD-*d*$_4$): δ = 173.0 (2 x $\underline{C}OCH_2$), 170.2 (2 x $\underline{C}$OC*H), 139.6 (2 x C_{Ar}), 129.9 (1 x CH_{Ar}), 117.3 (2 x CH_{Ar}), 113.4 (1 x CH_{Ar}), 62.8 (2 x CH_2OH), 57.2 (2 x C*H), 27.9 (2 x CH_2SH).

NMR spectra, HRMS (ESI+) spectra and HPLC traces of [**1a,b**]

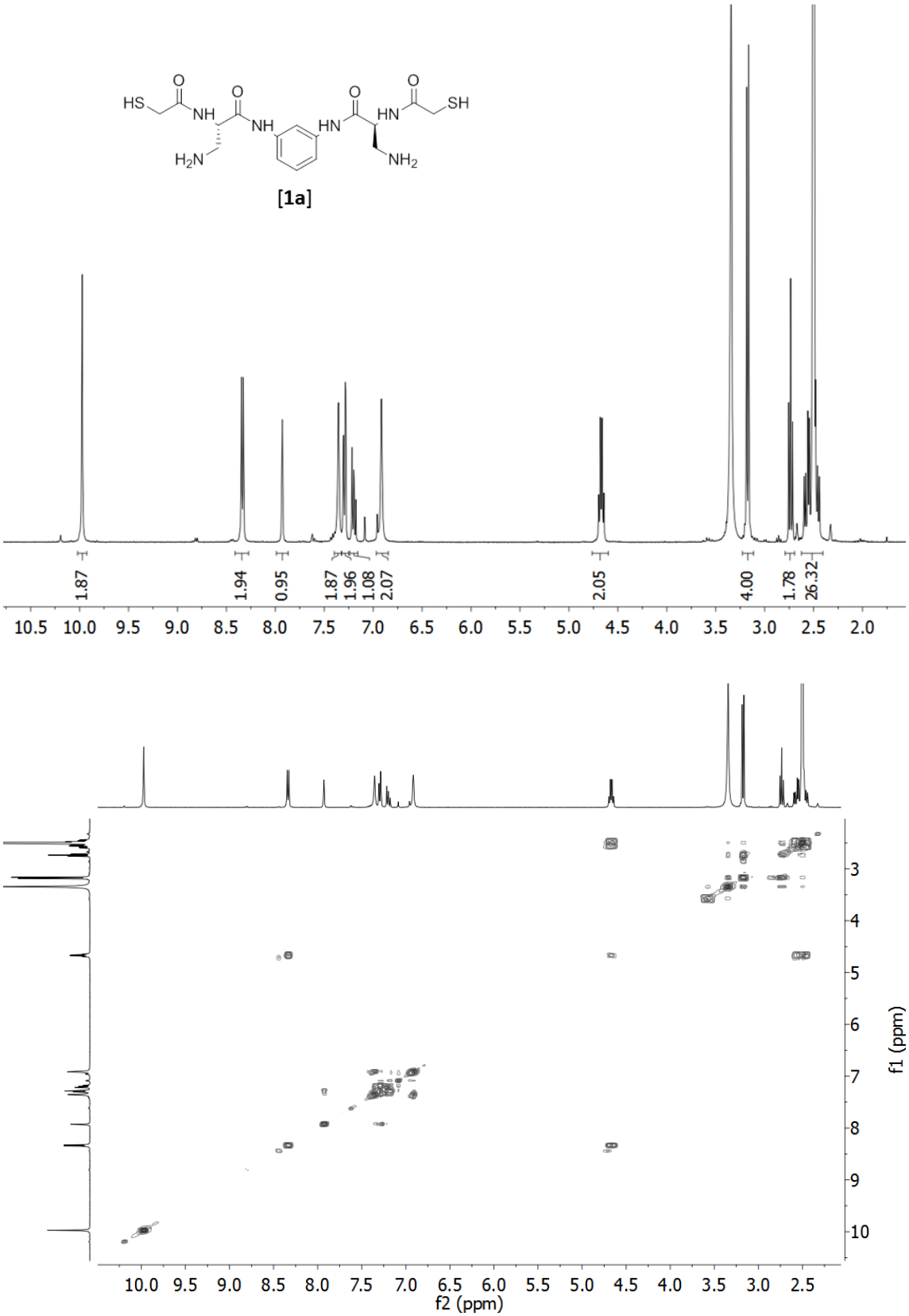


Figure S1: ^{1}H (400 MHz, 298 K in DMSO-d_6) and gCOSY (400 MHz, 298 K in DMSO-d_6) spectra of [**1a**].

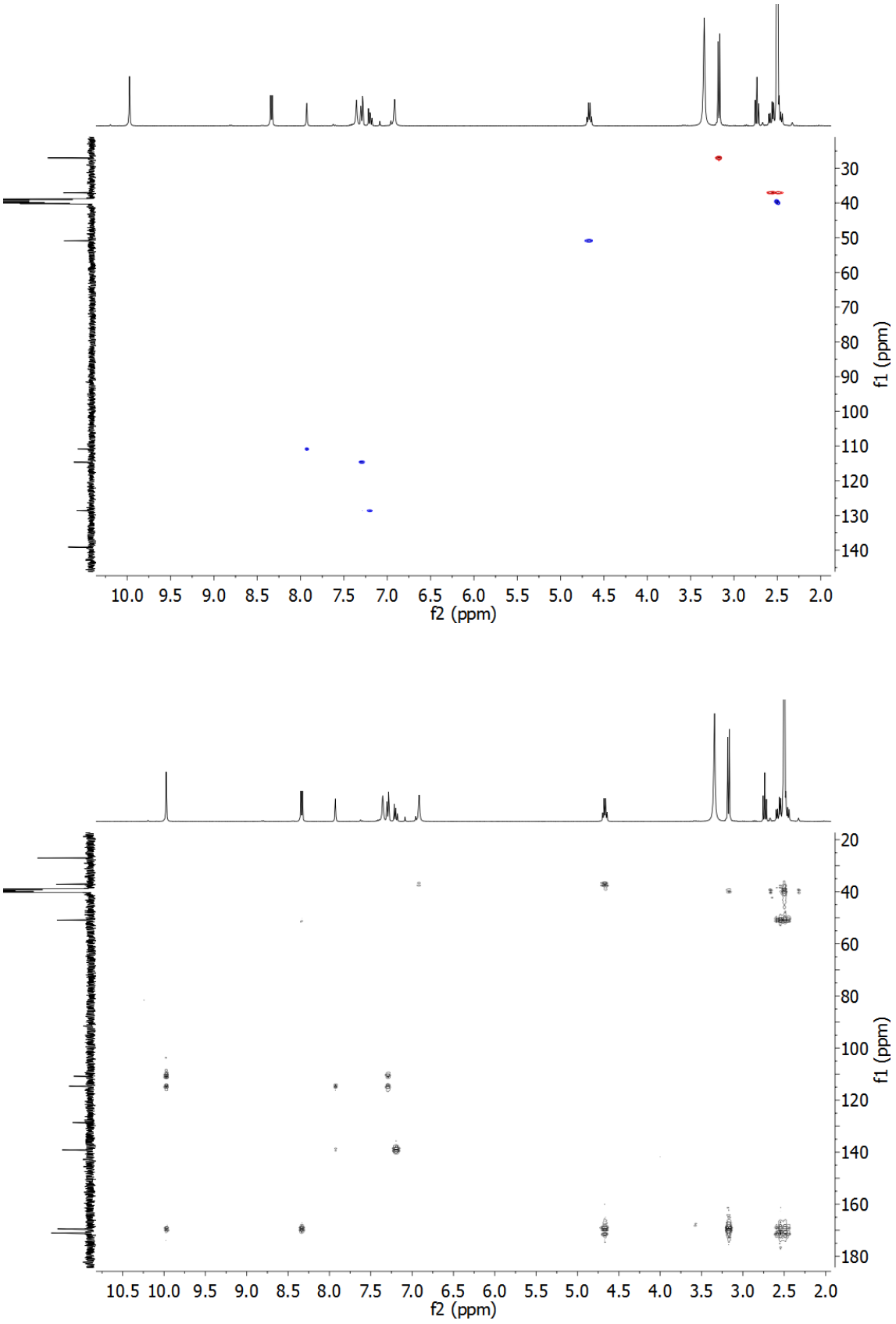


Figure S2: $^{1}H/^{13}C$ gHSQC (400 MHz, 298 K in DMSO-d_6) and $^{1}H/^{13}C$ gHMBC (400 MHz, 298 K in DMSO-d_6) spectra of [**1a**].

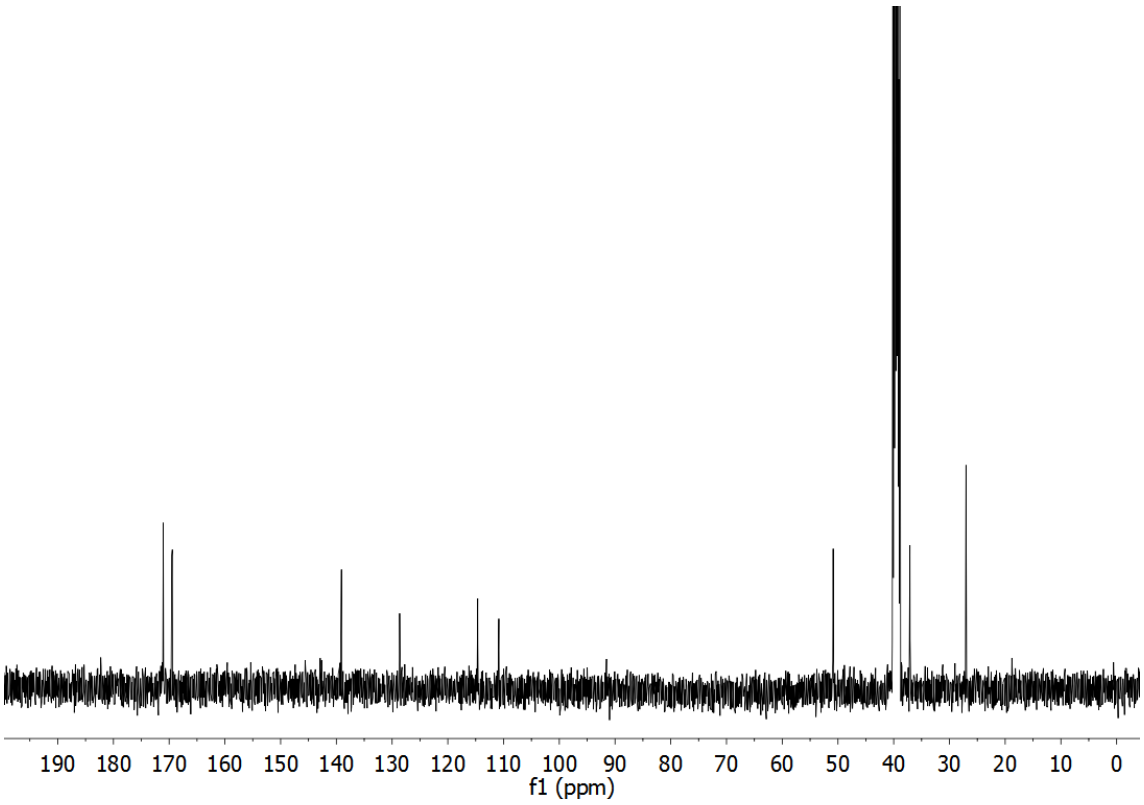


Figure S3: ^{13}C (101 MHz, 298 K in DMSO-d_6) spectrum of [**1a**].

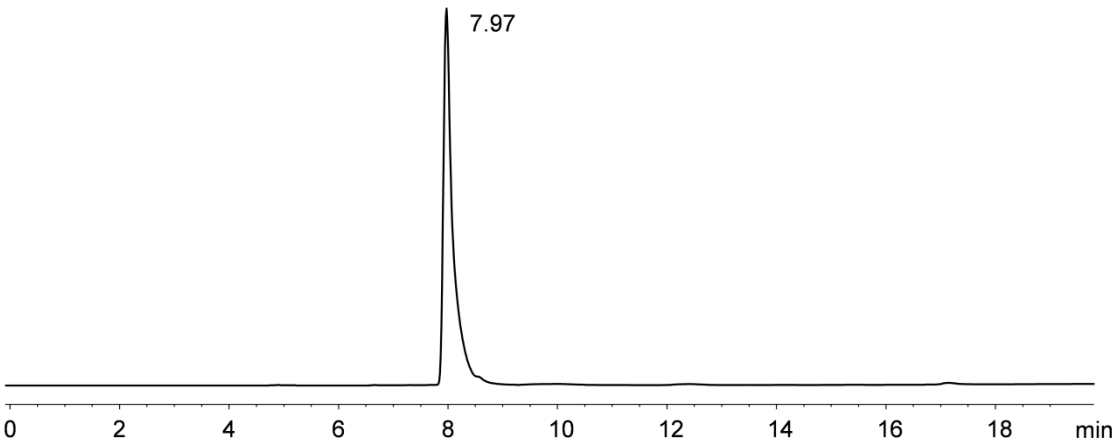


Figure S4: HPLC of [**1a**] (2 min at 5% CH_3CN in H_2O, then linear gradient from 5% to 100% CH_3CN over 18 min).

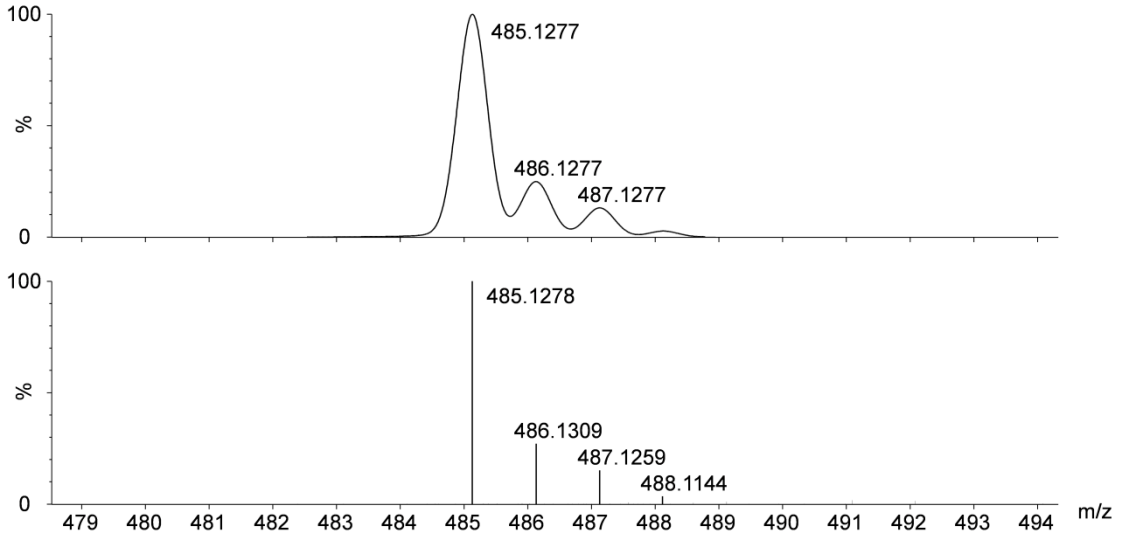


Figure S5: Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of [**1a**].

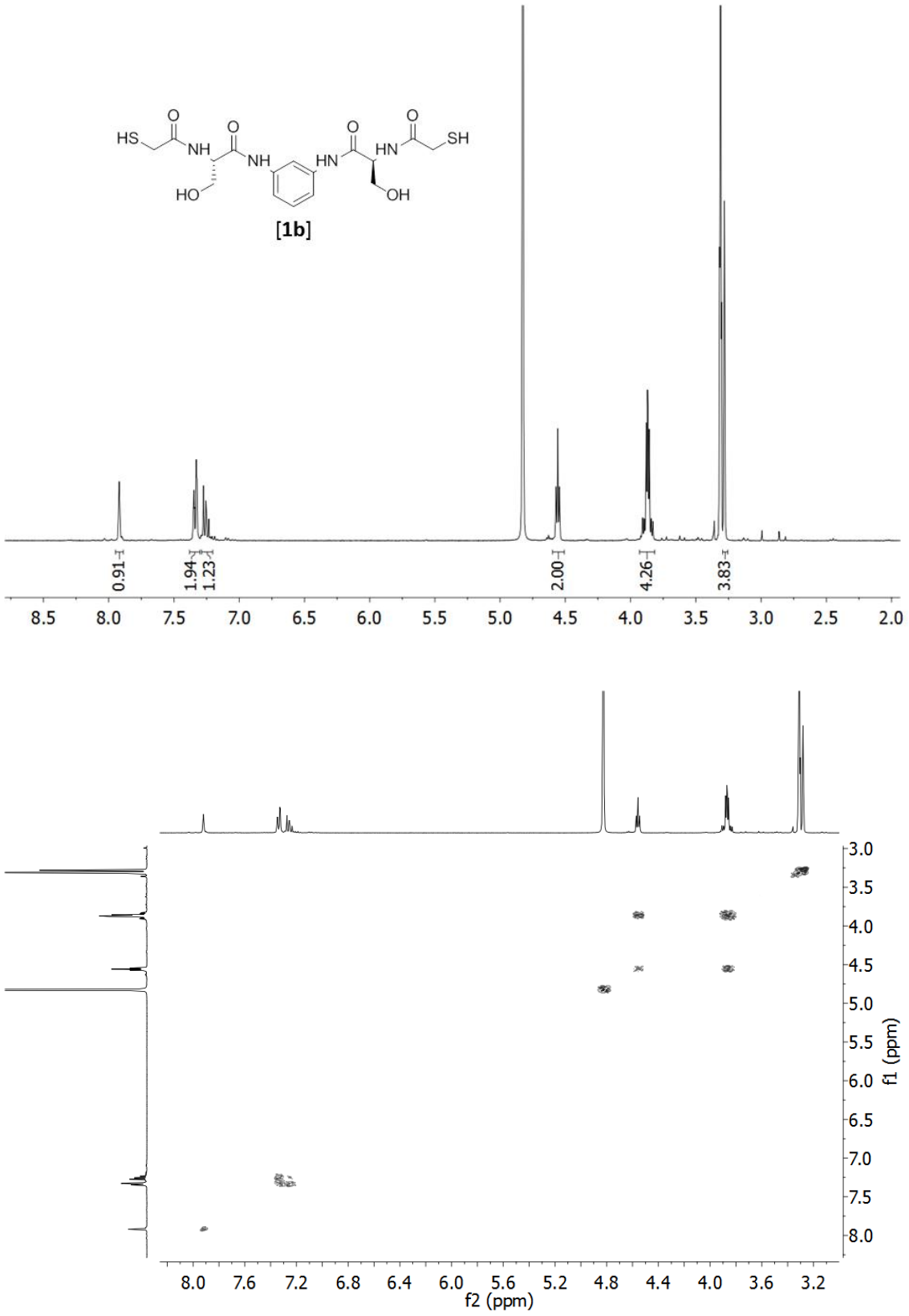


Figure S6: ^{1}H (400 MHz, 298 K in MeOD-d_4) and gCOSY (400 MHz, 298 K in MeOD-d_4) spectra of [**1b**].

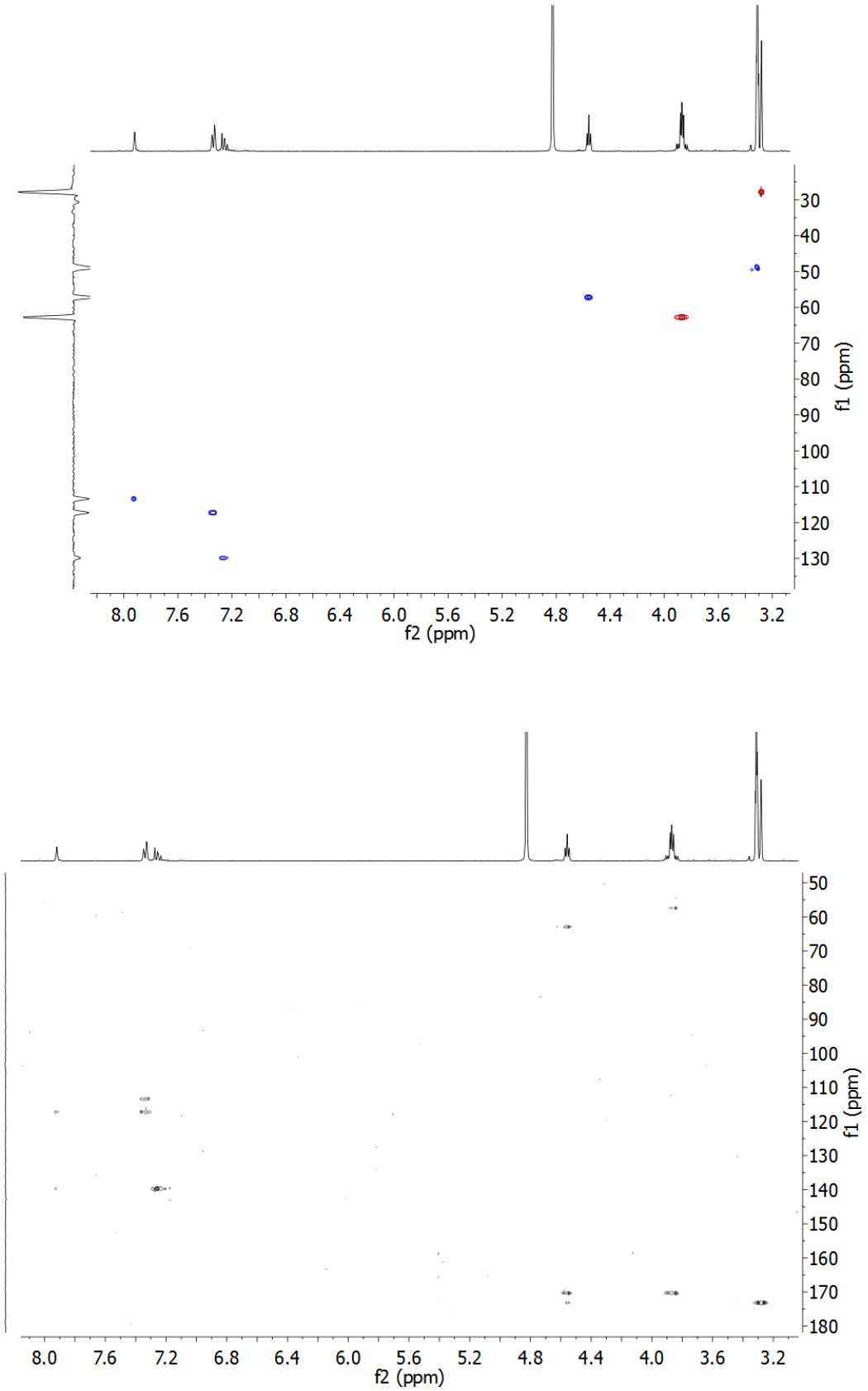


Figure S7: $^{1}H/^{13}C$ gHSQC (400 MHz, 298 K in MeOD-d_4) and $^{1}H/^{13}C$ gHMBC (400 MHz, 298 K in MeOD-d_4) spectra of [**1b**].

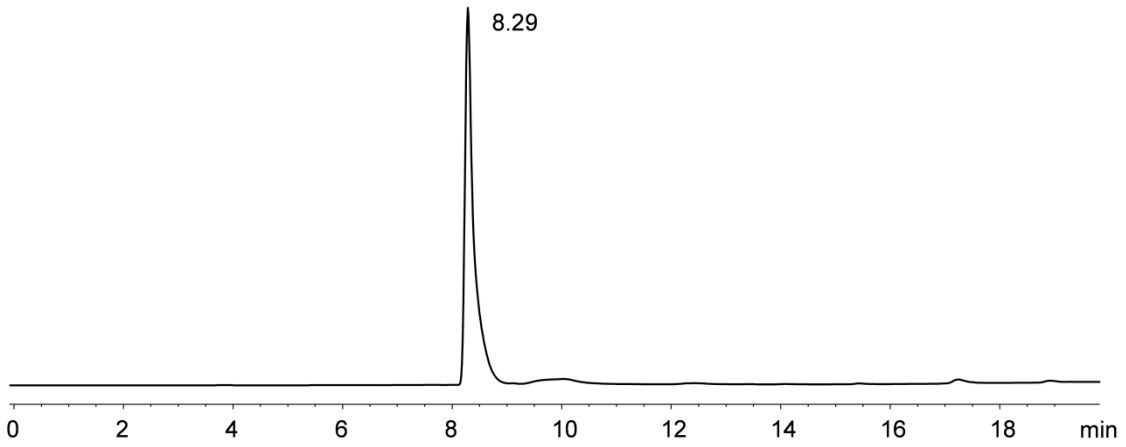


Figure S8: HPLC of [**1b**] (2 min at 5% CH_3CN in H_2O, then linear gradient from 5% to 100% CH_3CN over 18 min).

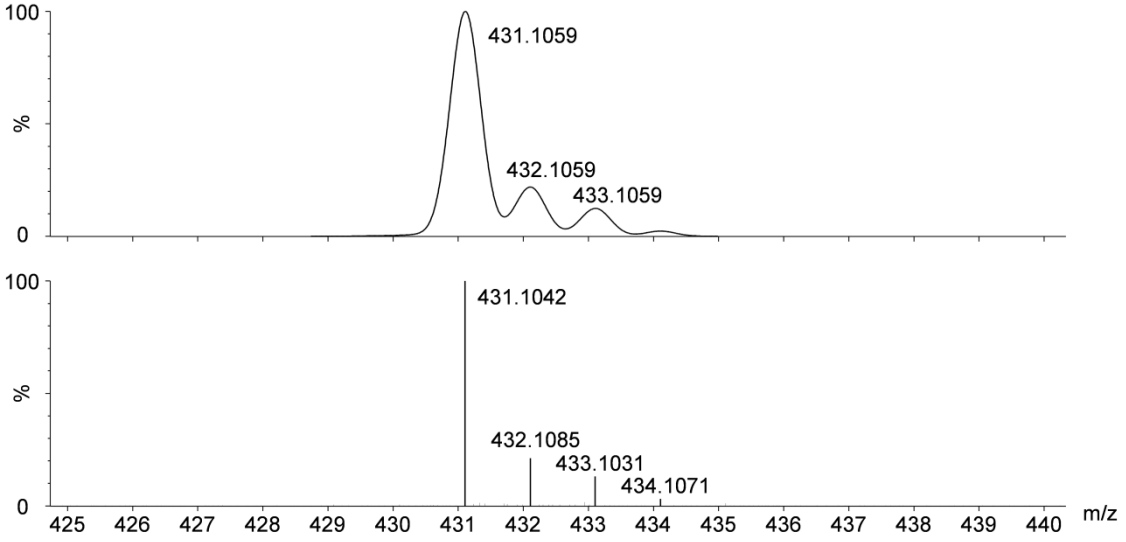


Figure S9: Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of [**1b**].

DYNAMIC COMBINATORIAL LIBRARIES

General procedure for the preparation and HPLC analysis of the DCLs

A 66.7 mM BIS-Tris methane buffer was prepared by dissolving 1.39 g of the free amine in 100 mL of milli-Q water and adjusting the pH of the solution to 6.5 by the addition of HCl (aq).

The reaction mixtures were prepared by dilution of individual stocks of the building blocks (BBs) **1a**, **1b**, **2**, **3a-g**. For those experiments to be compared, the reaction mixtures were prepared by dilution of a stock mixture of the BBs, ensuring no differences in concentration between the reaction mixtures of the same batch. Unless otherwise specified, the conditions for the generation of the DCLs were: 0.5 mM of the di- and tripodal BBs **1a**, **1b** and **2** in a 50 mM BIS-Tris methane buffer (pH 6.5) with 25% DMSO. The concentrations of the monopodal BBs **3a-g** are specified for each of the experiments.

After complete oxidation of the free thiols (24 hours at room temperature) the reaction mixtures were analysed by HPLC. The HPLC samples were prepared by adding 40 µL of the corresponding reaction mixture to 65 µL of a solution of 89% H_2O, 10% CH_3CN and 1% TFA. Eluent used: 2 min at 5% CH_3CN in H_2O, then linear gradient from 5% to 40% CH_3CN over 48 min.

Mixture of **1a**+**2** and **1a**+**2**+**3a**

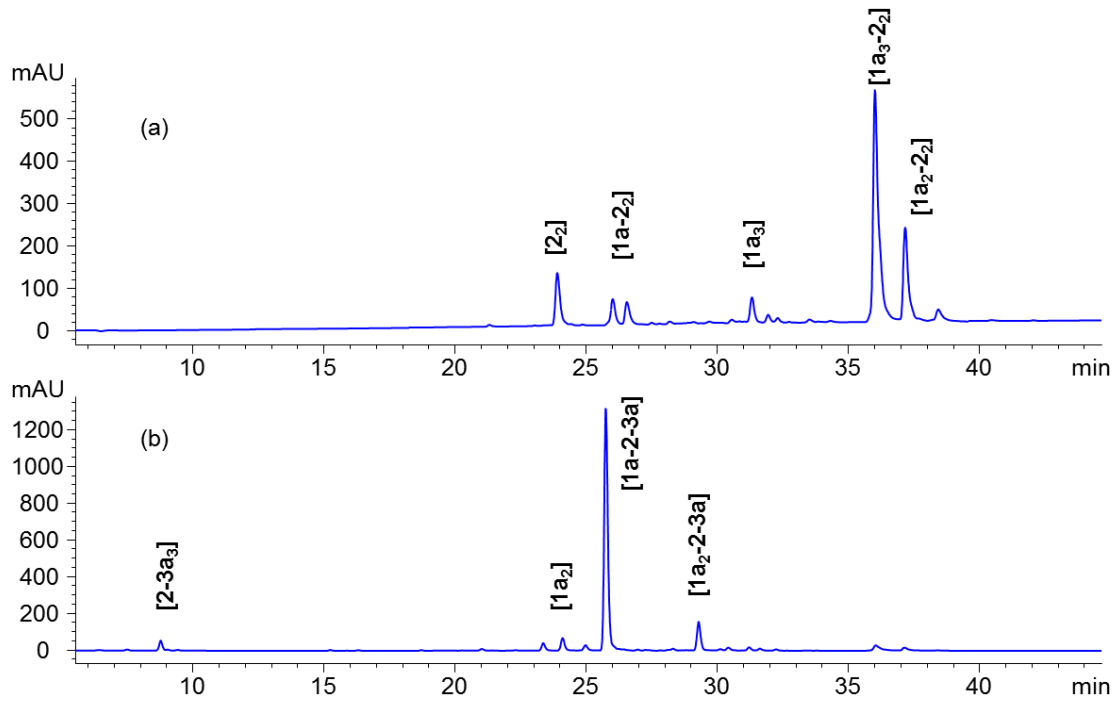


Figure S10: HPLC traces of the mixture **1a**+**2** in the absence of any monopodal BB (a), and in the presence of 2.5 mM of **3a** (b).

Mixture of **1b**+**2** and **1b**+**2**+**3a**

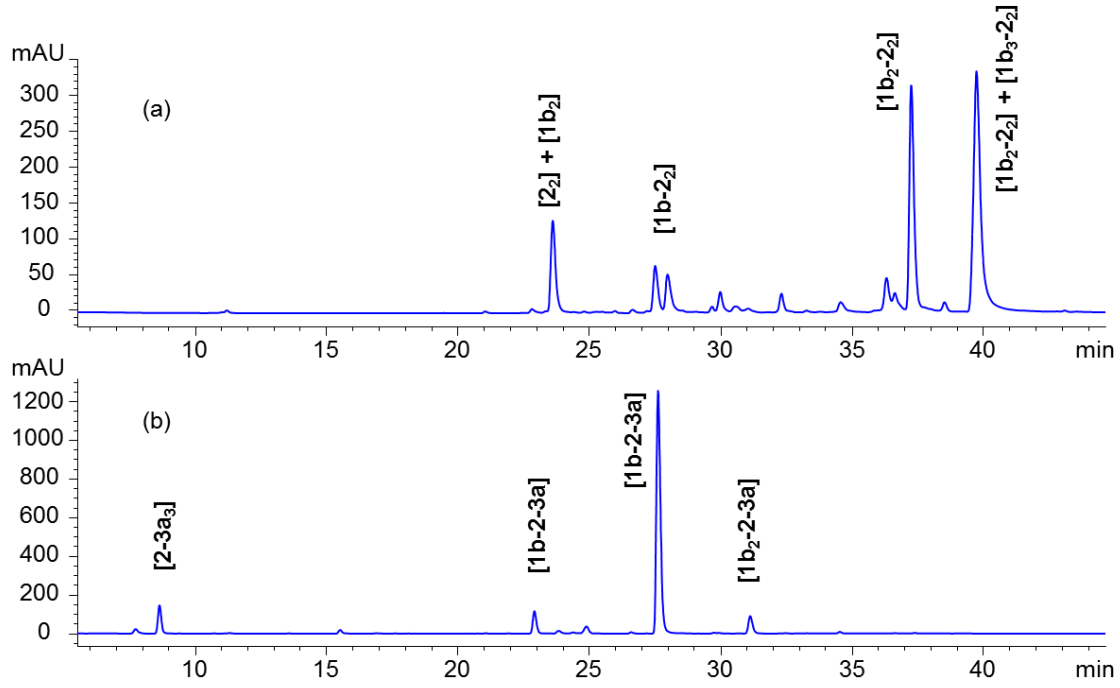


Figure S11: HPLC traces of the mixture **1b**+**2** in the absence of any monopodal BB (a), and in the presence of 2.5 mM of **3a** (b).

Mixture of **1a**+**2** with different concentrations of **3a**

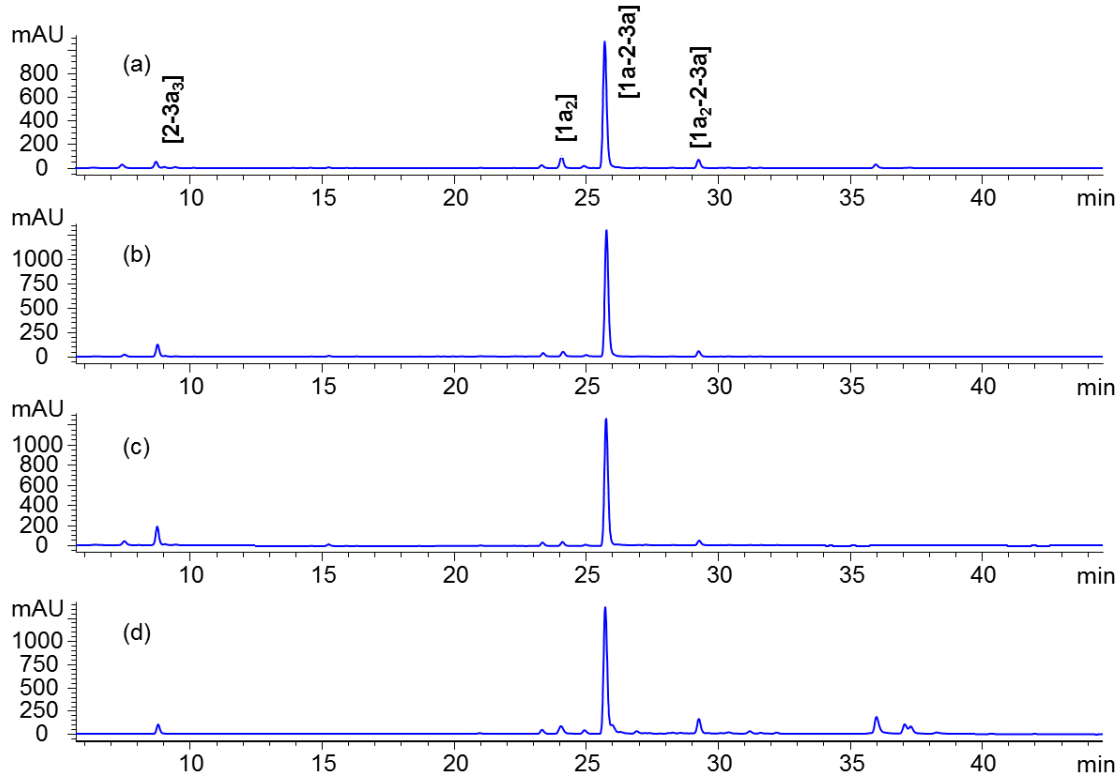


Figure S12: HPLC traces of the mixture of **1a**+**2** in the presence of 100 mM (a), 20 mM (b), 5 mM (c) and 0.5 mM (d) of **3a.**

Mixture of **1a**+**2**+**3b-g**

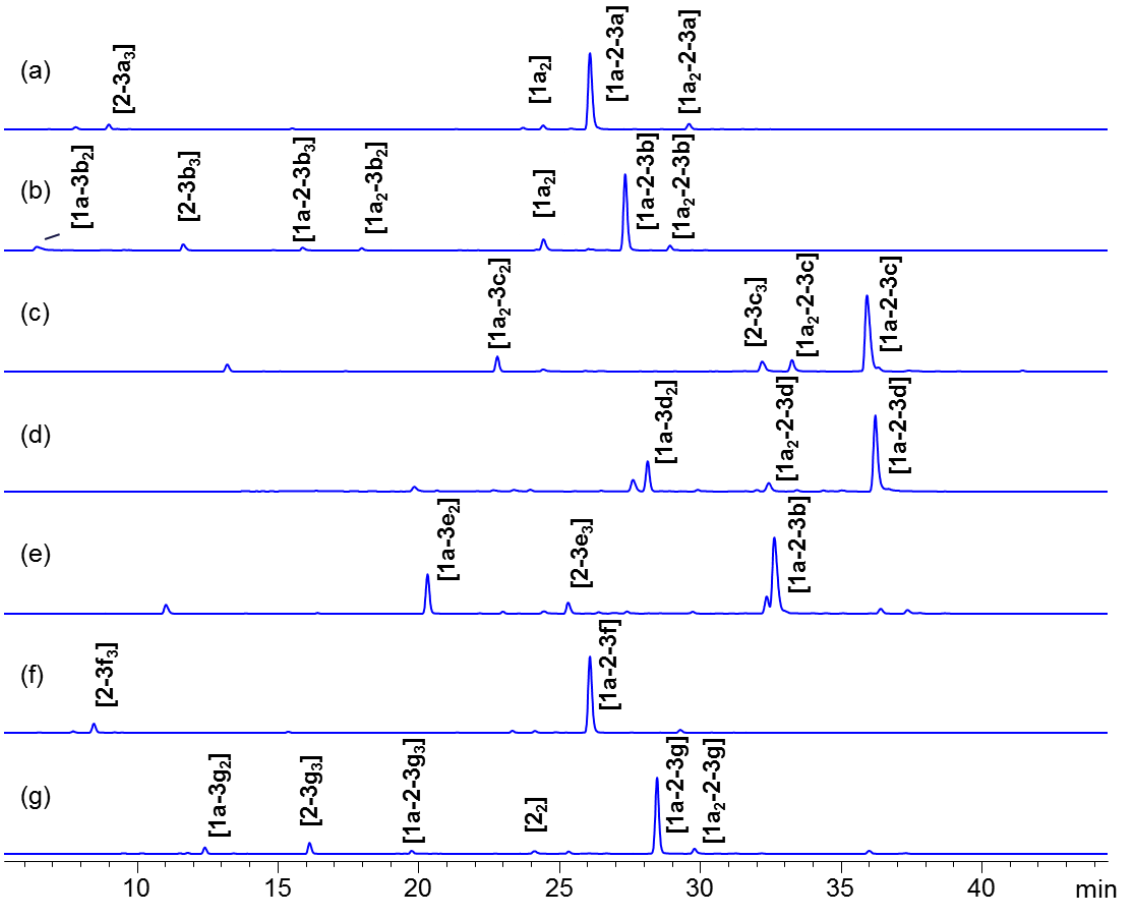


Figure S13: HPLC traces of the mixture of **1a**+**2** in the presence of 2.5 mM of **3a** (a), **3b** (b), **3c** (c), **3d** (d), **3e** (e), **3f** (f) and **3g** (g).

Mixture of **1a**+**2**+**3a**+**3f**+**3g**

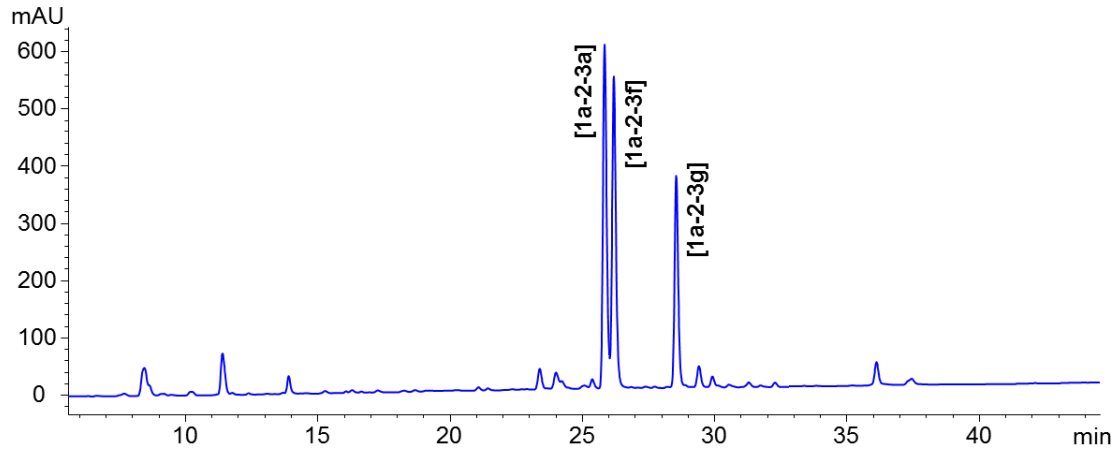


Figure S14: HPLC traces of the mixture of **1a**+**2**+**3a**+**3f**+**3g** (each monopodal BB at 1.0 mM).

Mixture of **1a**+**2**+**3a** at non-equimolar proportions of **1a** and **2**

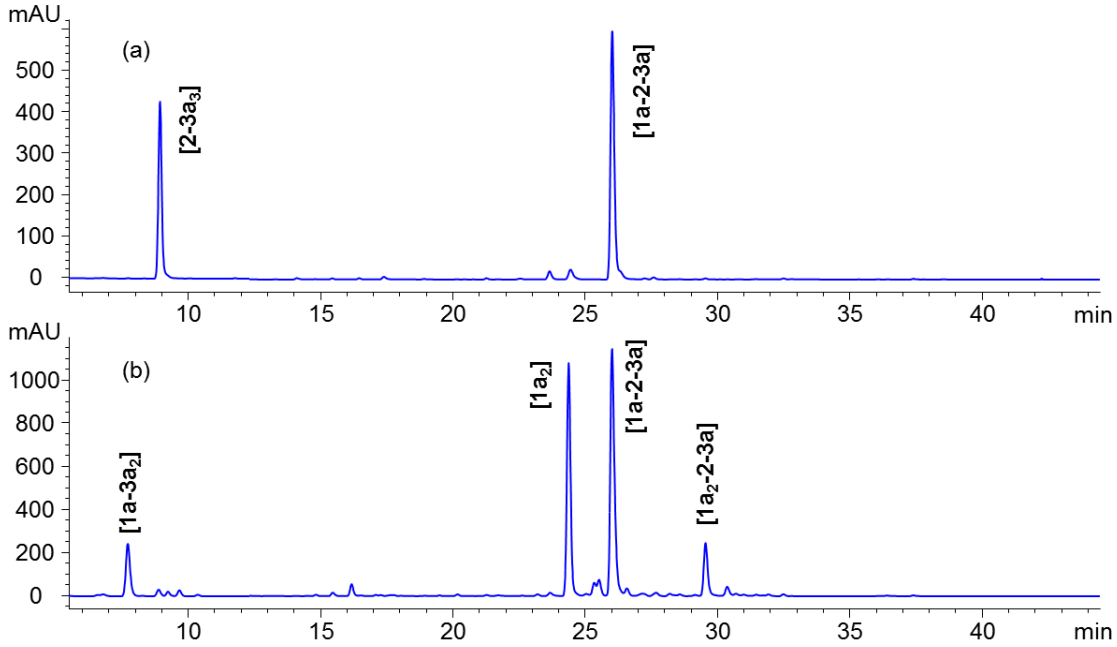


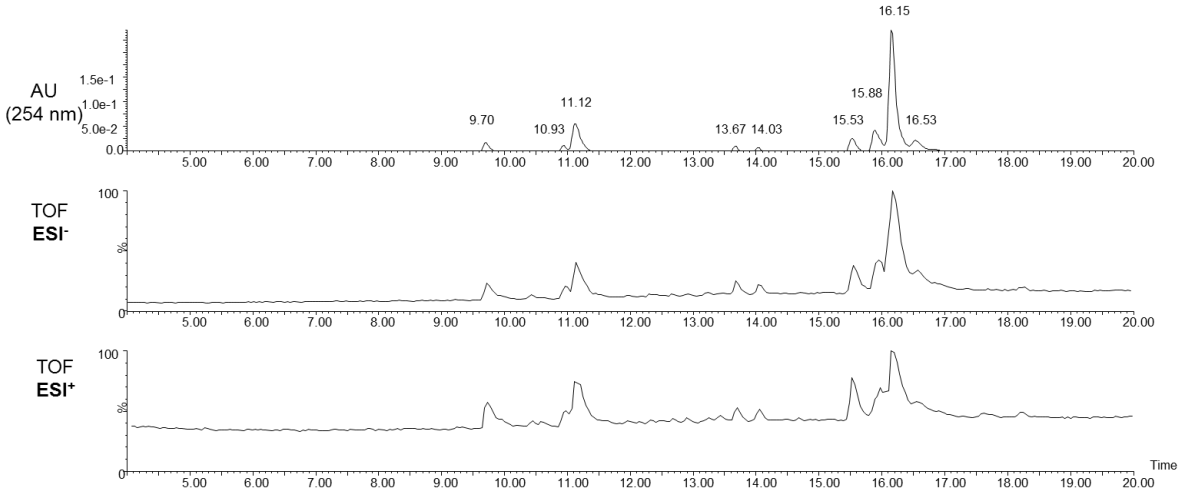
Figure S15: HPLC traces of the mixture of **1a**+**2**+**3a** at 0.5 mM, 1.0 mM and 4.0 mM respectively (a), and at 1.0 mM, 0.5 mM and 3.5 mM respectively (b).

MASS SPECTROMETRY

General procedure for the analysis of the DCLs by HRMS

The HRMS (UPLC-ESI-TOF) samples were prepared by adding 20 μL of the corresponding reaction mixture to 40 μL of a solution of 89% H_2O, 10% MeCN and 1% TFA. Eluent used: 2.5 min at 5% CH_3CN in H_2O, then linear gradient from 5% to 50% CH_3CN over 27.5 min.

Mixture of **1a**+**2** (0.5 mM each, pH.6.5)

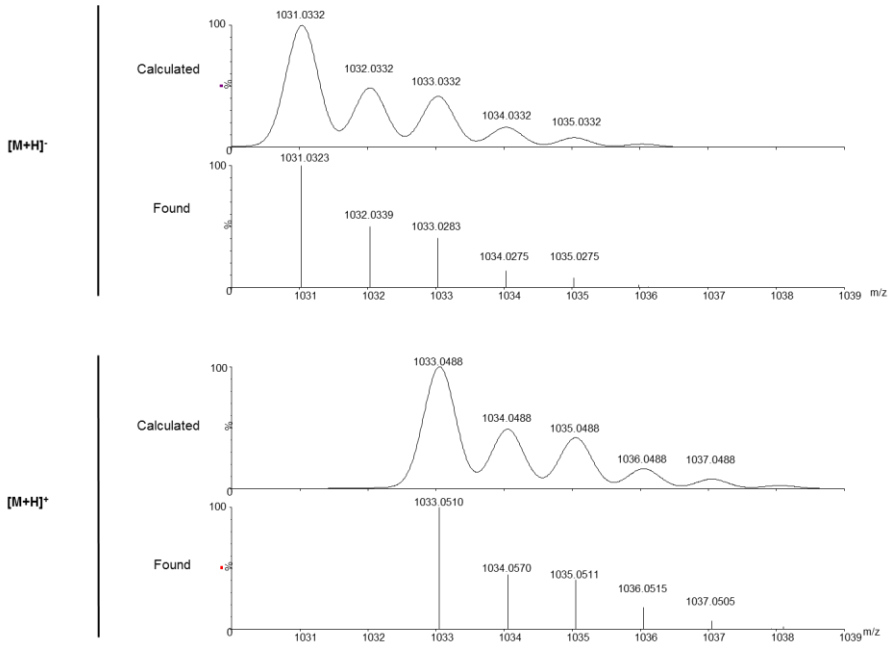


Identification of the products:

[$\mathbf{2_2}$]
Retention time: 9.70 min.

Chemical Formula:
$C_{36}H_{36}N_6O_{18}S_6$

Exact Mass:
1032.0410

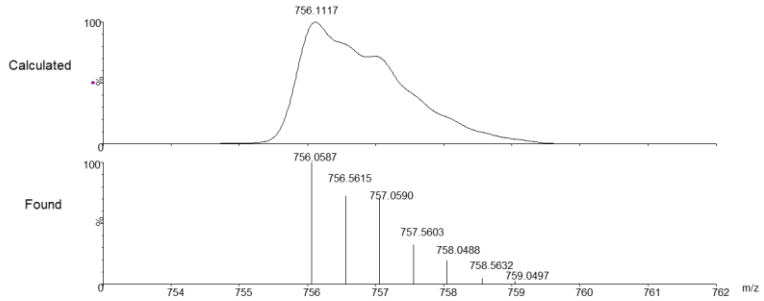


[**1a-2$_2$**]
Retention time: 11.12 min.

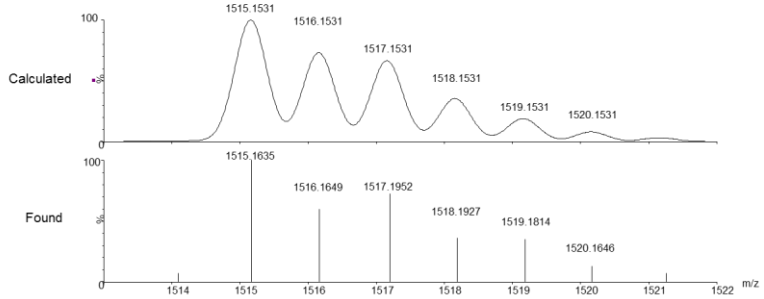
Chemical Formula:
$C_{54}H_{58}N_{12}O_{24}S_8$

Exact Mass:
1514.1453

$[M+H]^{2-}$



$[M+H]^{+}$

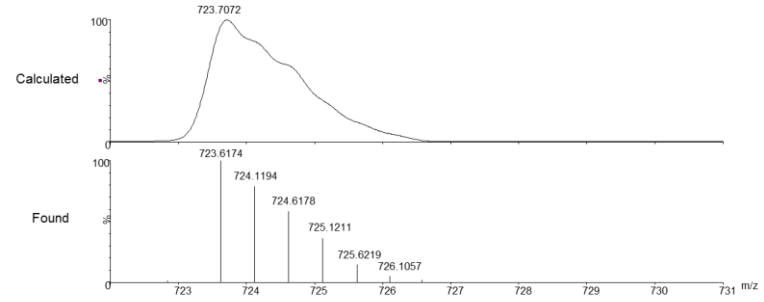


[**1a$_3$**]
Retention time: 13.67 min.

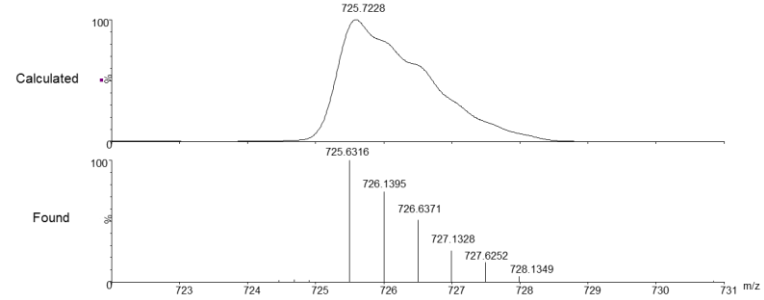
Chemical Formula:
$C_{54}H_{66}N_{18}O_{18}S_6$

Exact Mass:
1446.3127

$[M+H]^{2-}$



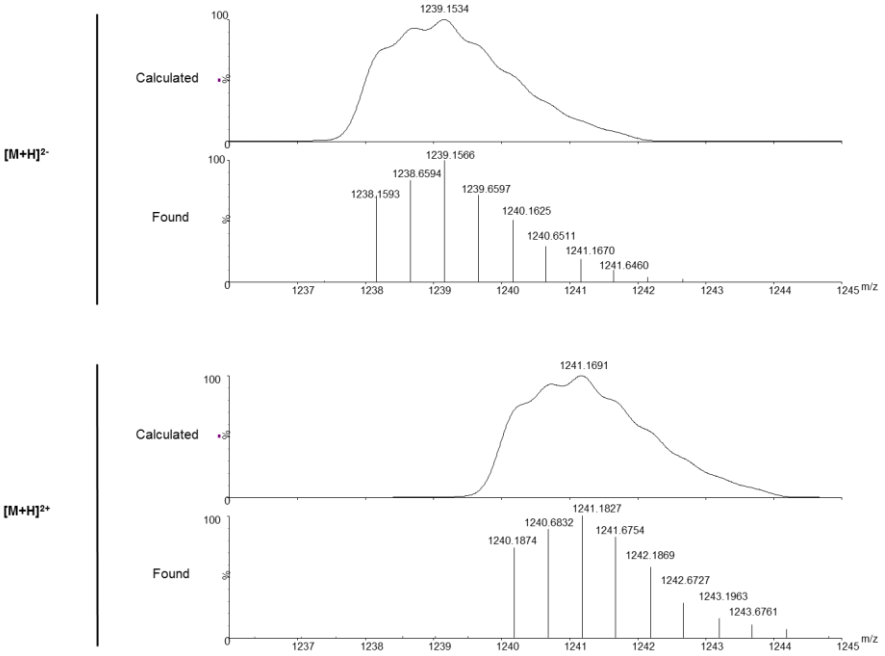
$[M+H]^{2+}$



[**1a$_3$-2$_2$**]
Retention time: 15.88 min.

Chemical Formula:
$C_{90}H_{102}N_{24}O_{36}S_{12}$

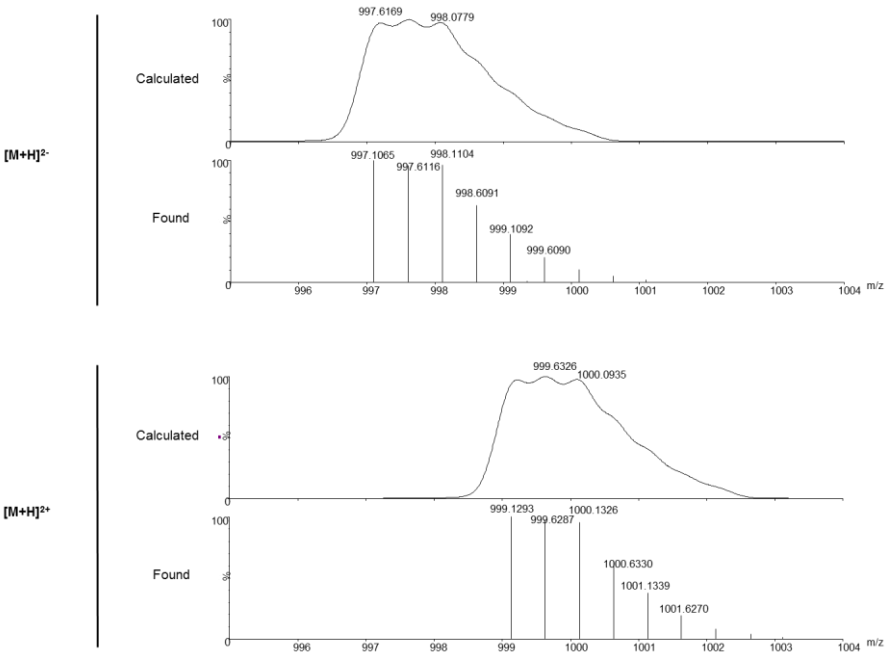
Exact Mass:
2478.3537



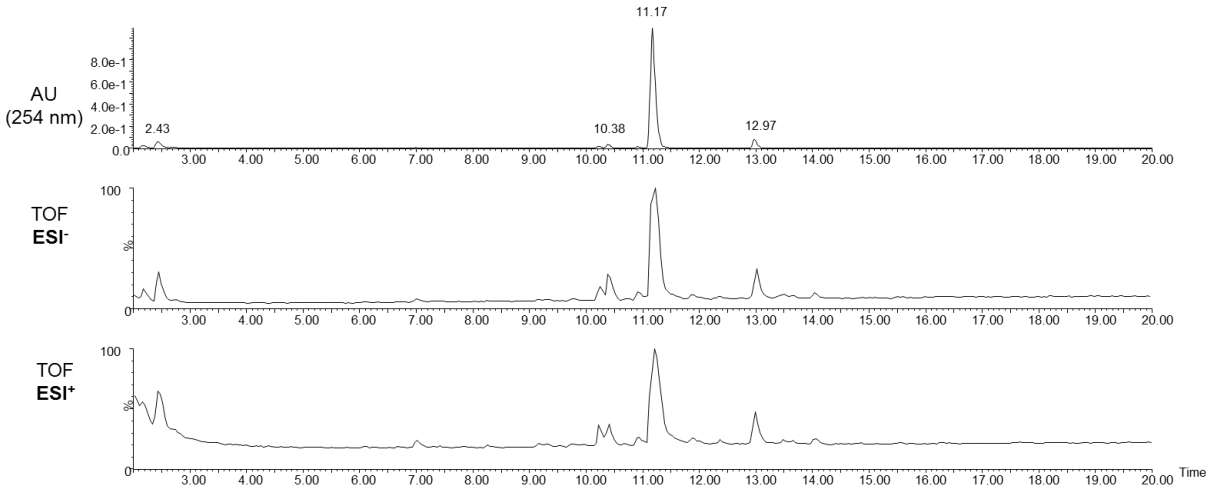
[**1a$_2$-2$_2$**]
Retention time: 16.17 min.

Chemical Formula:
$C_{72}H_{80}N_{18}O_{30}S_{10}$

Exact Mass:
1996.2495



Mixture of **1a**+**2**+**3a** (0.5, 0.5 and 2.5 mM respectively, pH.6.5)

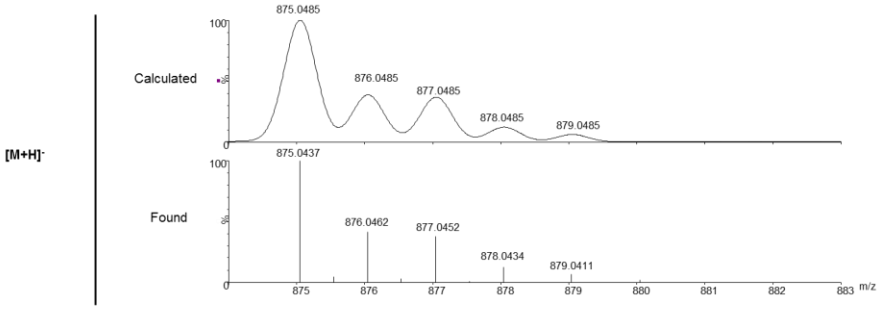


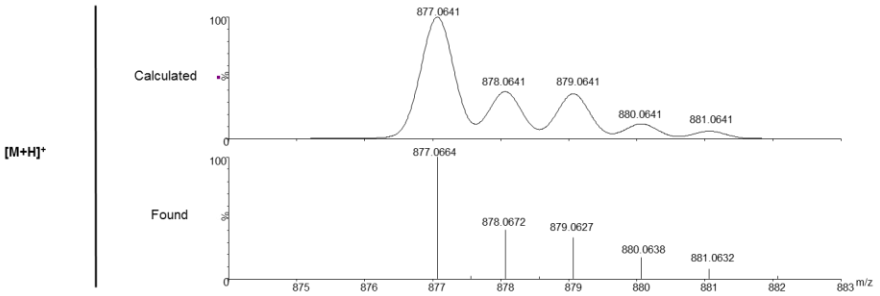
Identification of the products:

[**2-3a**$_3$]
Retention time: 2.43 min.

Chemical Formula:
$C_{27}H_{36}N_6O_{15}S_6$

Exact Mass:
876.0563



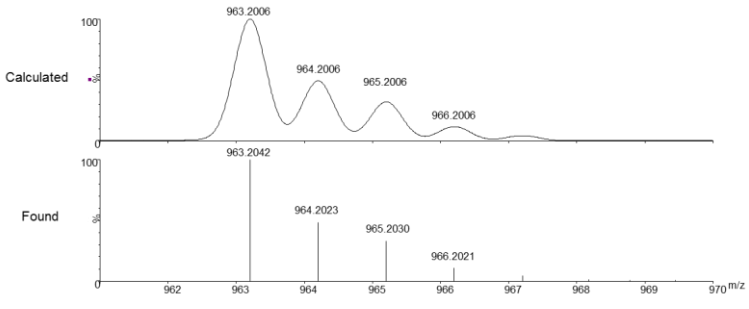


[**1a$_2$**]
Retention time: 10.38 min.

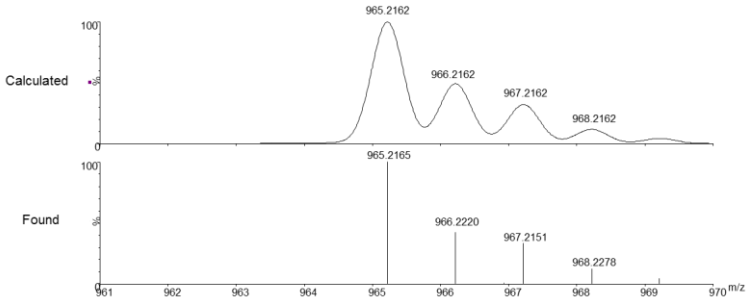
Chemical Formula:
$C_{36}H_{44}N_{12}O_{12}S_4$

Exact Mass:
964.2084

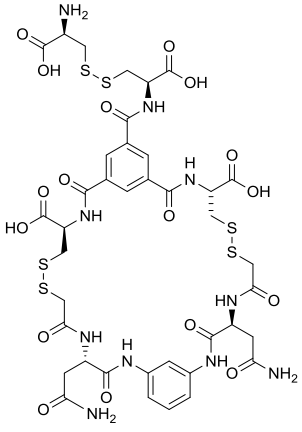
[M+H]$^-$



[M+H]$^+$



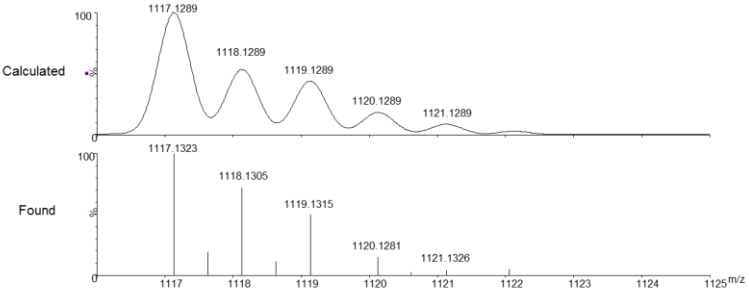
[**1a-2-3a**]
Retention time: 11.17 min.



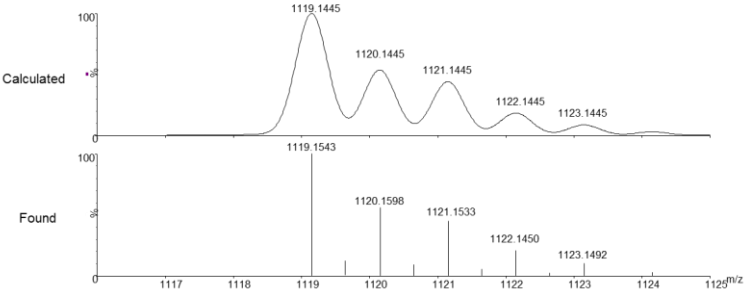
Chemical Formula:
$C_{39}H_{46}N_{10}O_{17}S_6$

Exact Mass:
1118.1367

[M+H]$^-$



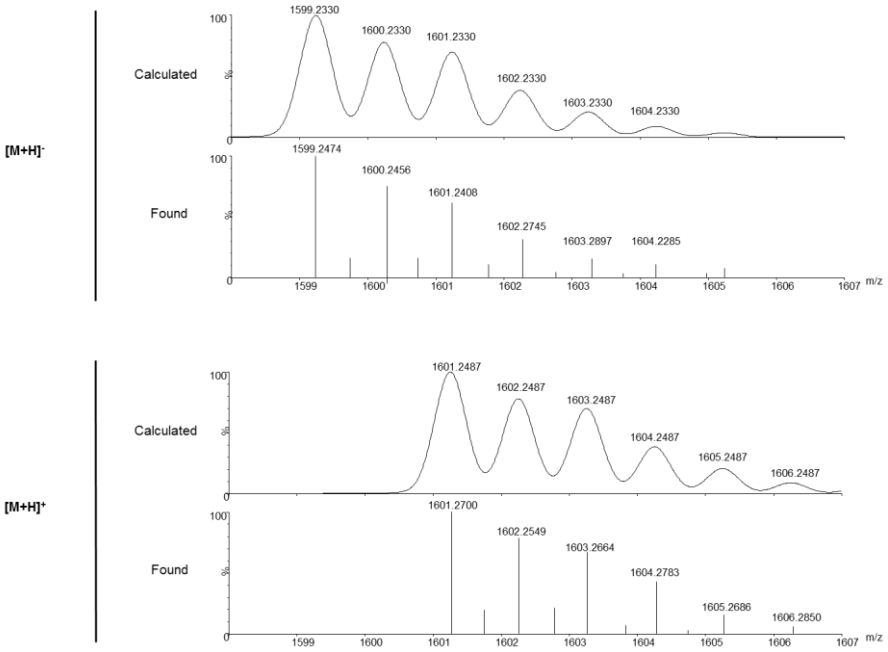
[M+H]$^+$



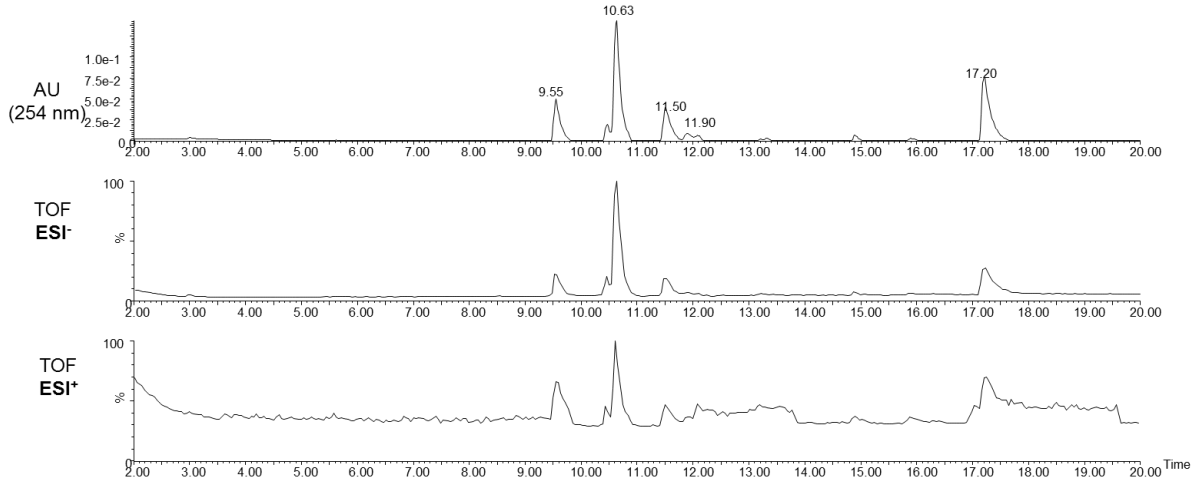
[**1a$_2$-2-3a**]
Retention time: 12.37 min.

Chemical Formula:
$C_{57}H_{68}N_{16}O_{23}S_8$

Exact Mass:
1600.2409



Mixture of **1b**+**2** (0.5 mM each, pH.6.5)



Identification of the products:

[**2$_2$**] (previously identified)
Retention time: 9.55 min.

Chemical Formula: $C_{36}H_{36}N_6O_{18}S_6$

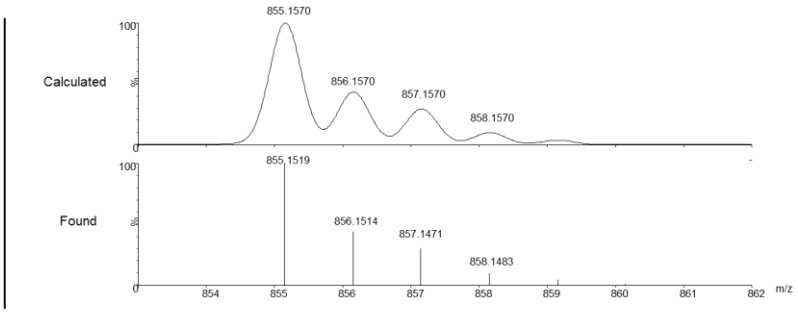
Exact Mass:
1032.0410

[**1b**$_2$]
Retention time: 10.63 min

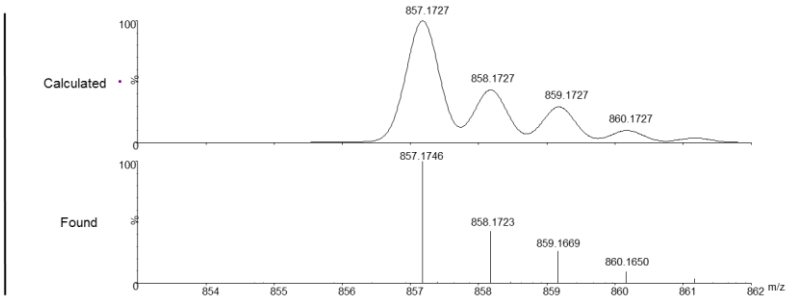
Chemical Formula:
$C_{32}H_{40}N_8O_{12}S_4$

Exact Mass:
856.1649

[M+H]$^-$



[M+H]$^+$

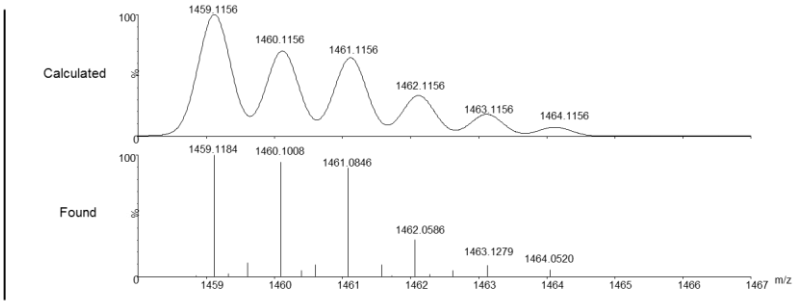


[**1b**-**2**$_2$]
Retention time: 11.50 and 11.90 min

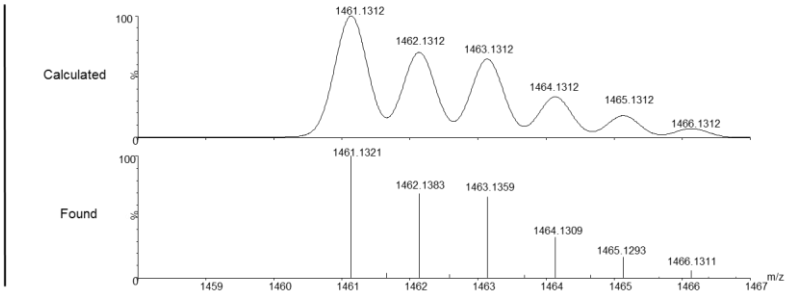
Chemical Formula:
$C_{52}H_{56}N_{10}O_{24}S_8$

Exact Mass:
1460.1235

[M+H]$^-$



[M+H]$^+$

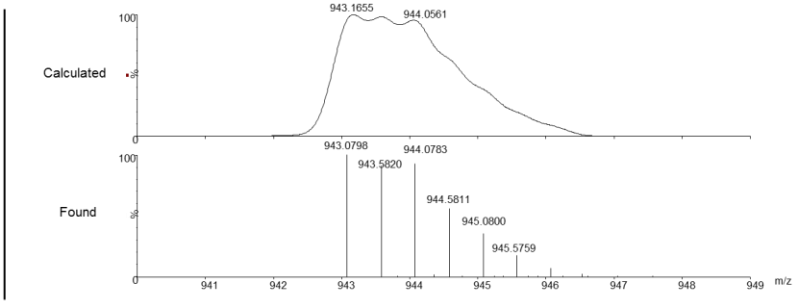


[**1b$_2$-2$_2$**]
Retention time: 15.90 and 17.22 min

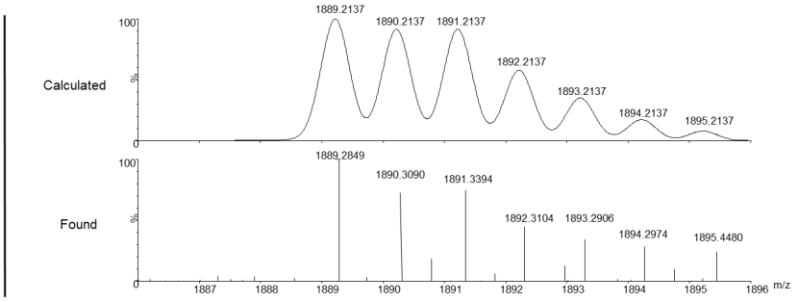
Chemical Formula:
$C_{68}H_{76}N_{14}O_{30}S_{10}$

Exact Mass:
1888.2059

[M+H]$^{2-}$



[M+H]$^{+}$

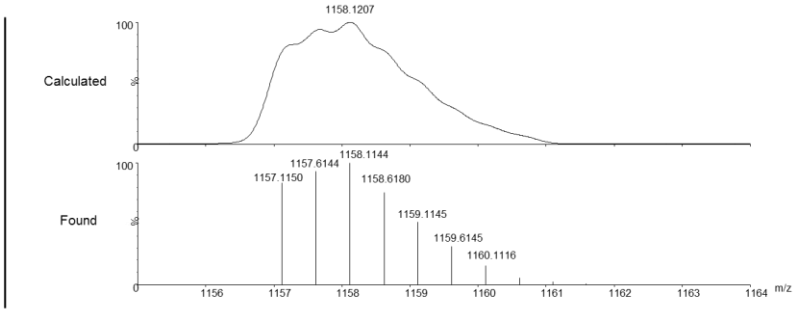


[**1b$_3$-2$_2$**]
Retention time: 17.22 min

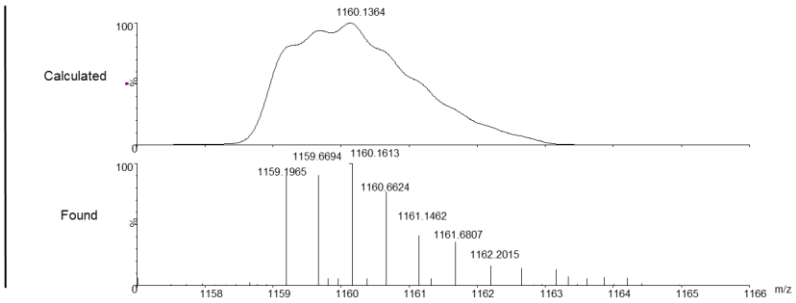
Chemical Formula:
$C_{84}H_{96}N_{18}O_{36}S_{12}$

Exact Mass:
2316.2883

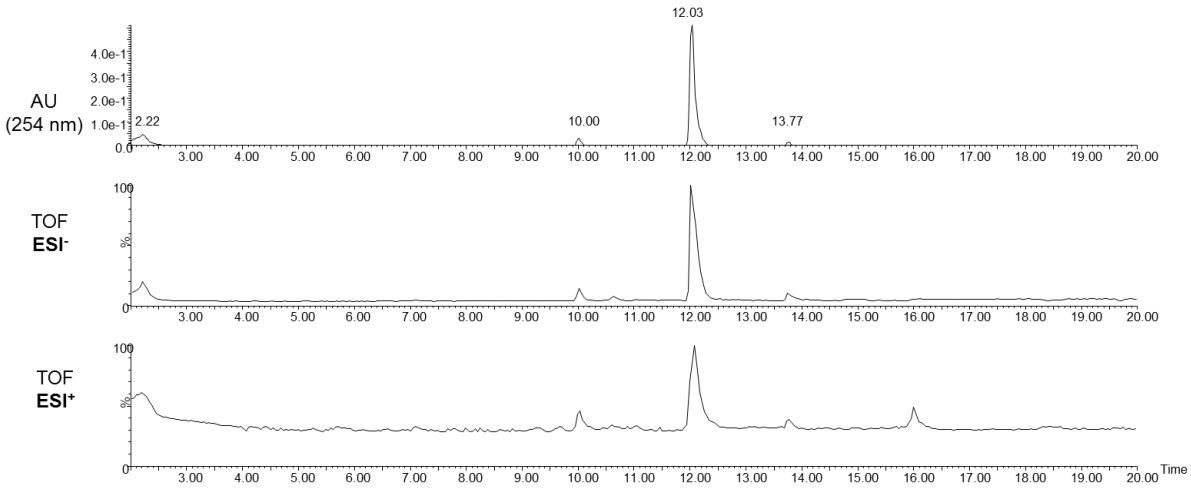
[M+H]$^{2-}$



[M+H]$^{2+}$



Mixture of **1b**+**2**+**3a** (0.5, 0.5 and 2.5 mM respectively, pH.6.5)



Identification of the products:

[**2-3a$_3$**] (previously identified)
Retention time: 2.22 min.

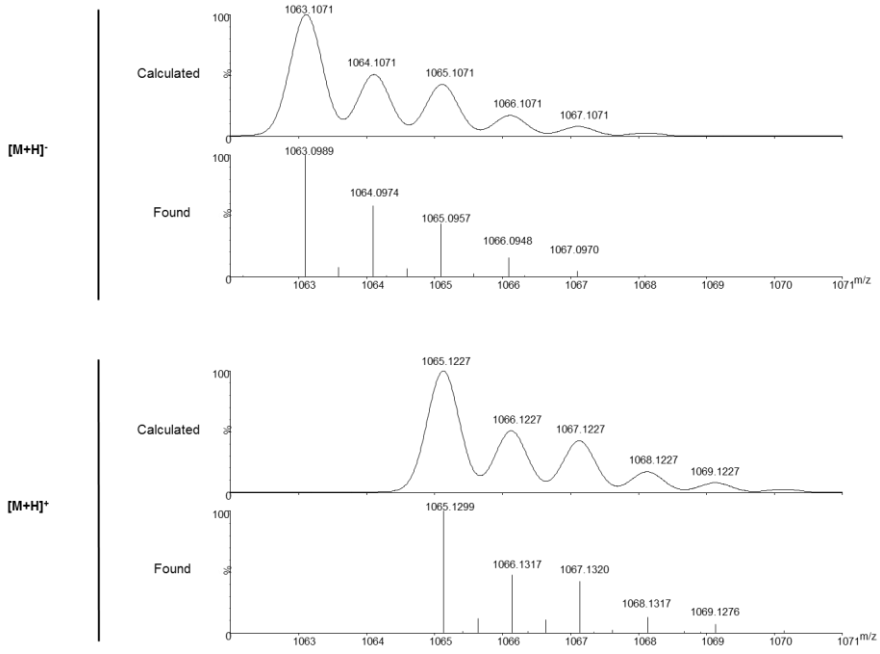
Chemical Formula:
$C_{27}H_{36}N_6O_{15}S_6$

Exact Mass:
876.0563

[**1b-2-3a**]
Retention time: 10.00 and12.03 min.

Chemical Formula:
$C_{37}H_{44}N_8O_{17}S_6$

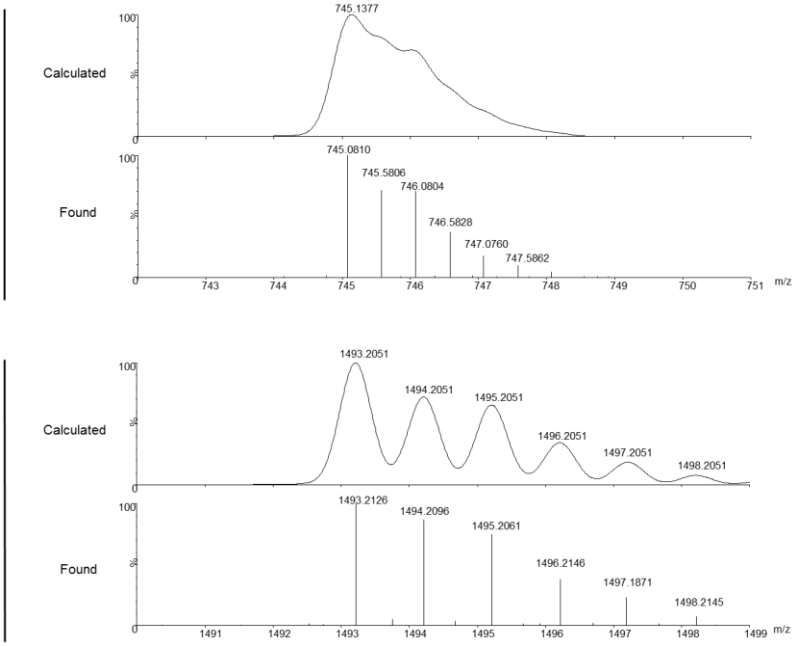
Exact Mass:
1064.1149



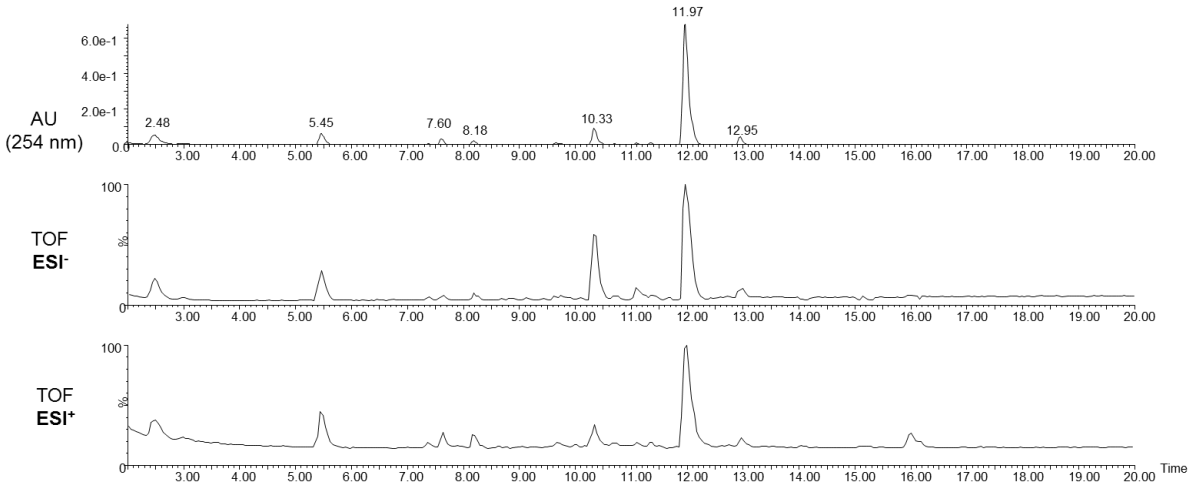
[**1b$_2$-2-3a**]
Retention time: 13.77 min.

Chemical Formula:
$C_{53}H_{64}N_{12}O_{23}S_8$

Exact Mass:
1492.1973



Mixture of **1a**+**2**+**3b** (0.5, 0.5 and 2.5 mM, pH.6.5)



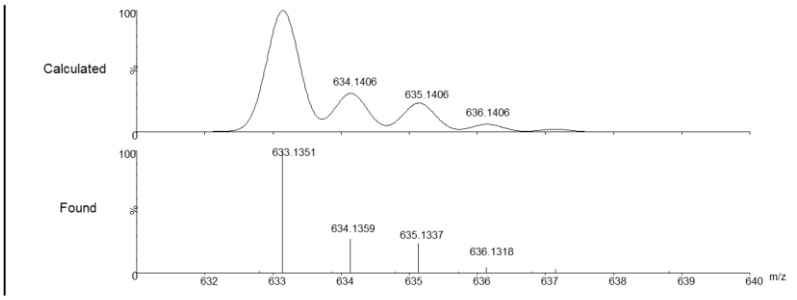
Identification of the products:

[**1a-3b$_2$**]
Retention time: 2.48 min.

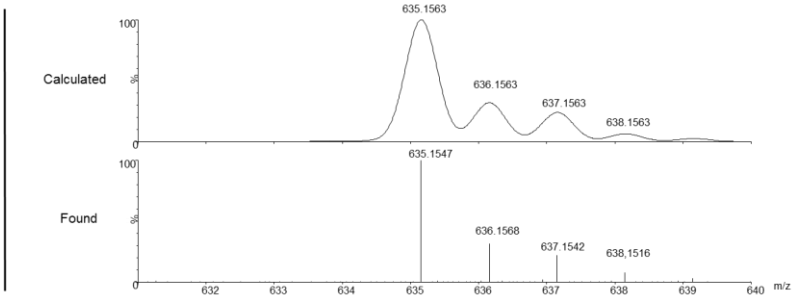
Chemical Formula:
$C_{22}H_{34}N_8O_6S_4$

Exact Mass:
634.1484

[M+H]$^-$



[M+H]$^+$

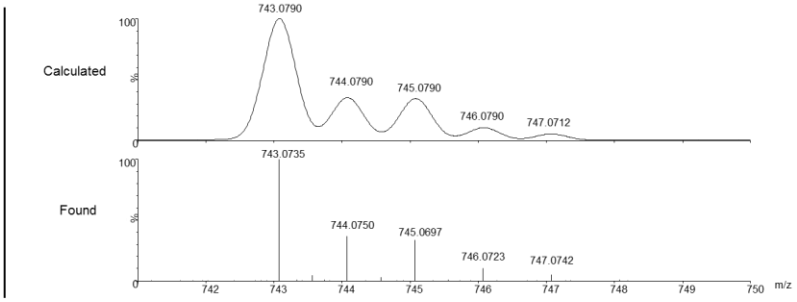


[**2-3b$_3$**]
Retention time: 5.45 min.

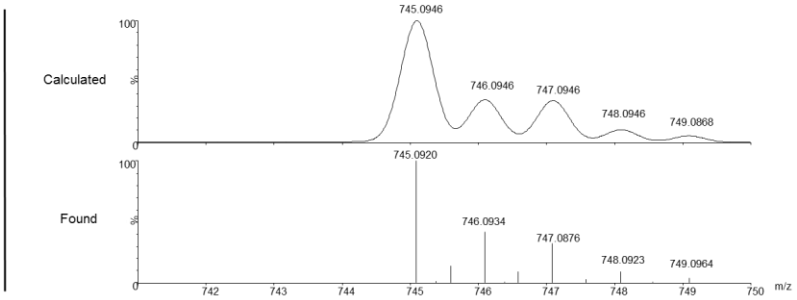
Chemical Formula:
$C_{24}H_{36}N_6O_9S_6$

Exact Mass:
744.0868

[M+H]$^-$



[M+H]$^+$

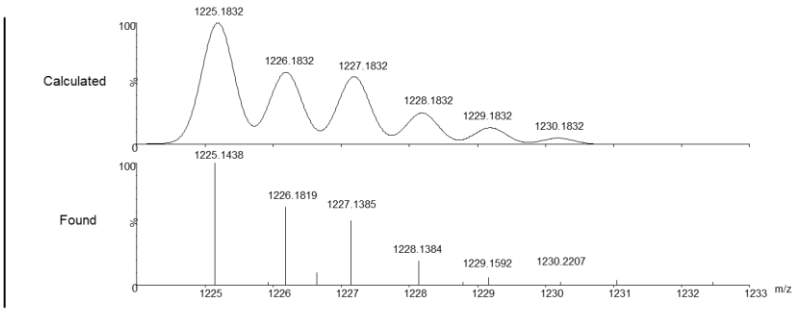


[**1a-2-3b$_3$**]
Retention time: 7.60 min.

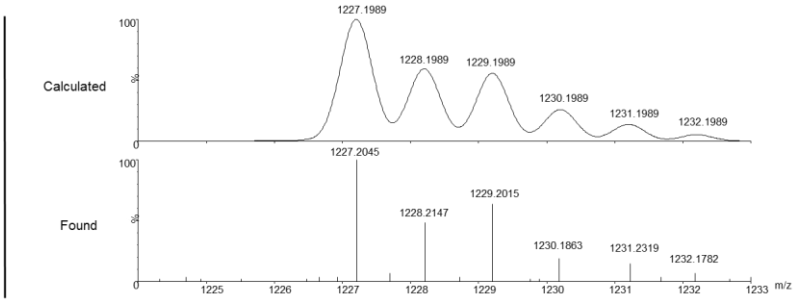
Chemical Formula:
$C_{42}H_{58}N_{12}O_{15}S_8$

Exact Mass:
1226.1910

[M+H]$^-$



[M+H]$^+$

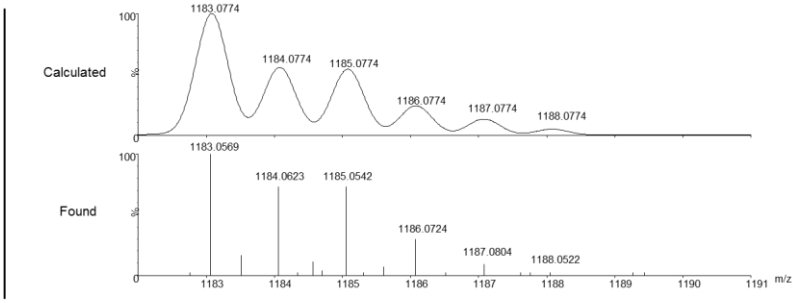


[**2$_2$-3b$_2$**]
Retention time: 8.18 min.

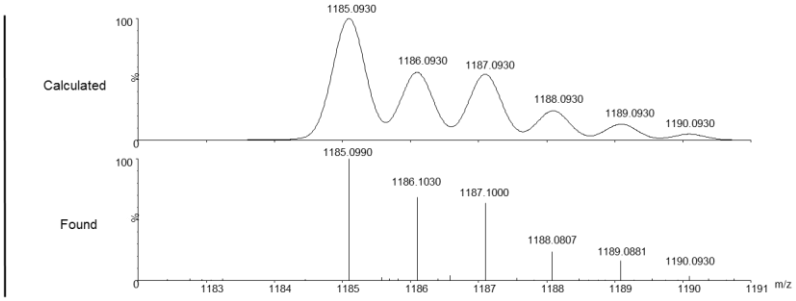
Chemical Formula:
$C_{40}H_{48}N_8O_{18}S_8$

Exact Mass:
1184.0852

[M+H]$^-$



[M+H]$^+$



[**1a$_2$**] (previously identified)
Retention time: 10.32 min.

Chemical Formula:
$C_{36}H_{44}N_{12}O_{12}S_4$

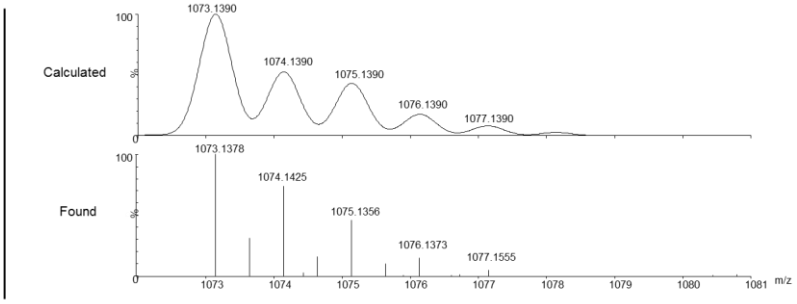
Exact Mass:
964.2084

[**1a-2-3b**]
Retention time: 11.97 min.

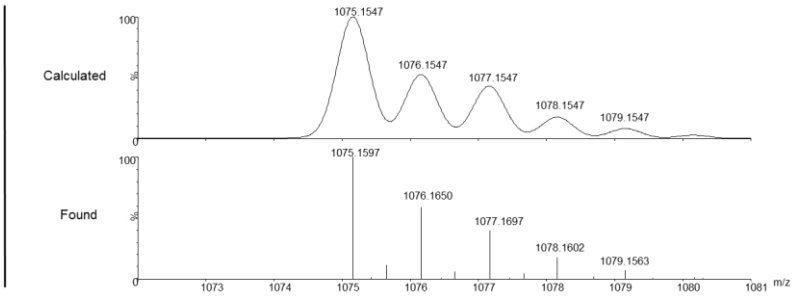
Chemical Formula:
$C_{38}H_{46}N_{10}O_{15}S_6$

Exact Mass:
1074.1468

[M+H]$^-$



[M+H]$^+$

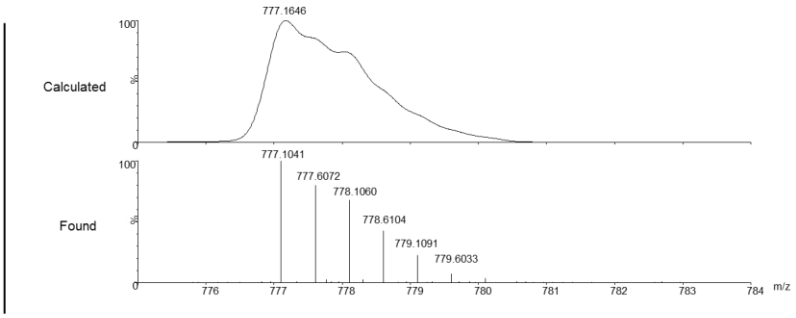


[**1a$_2$-2-3b**]
Retention time: 12.95 min.

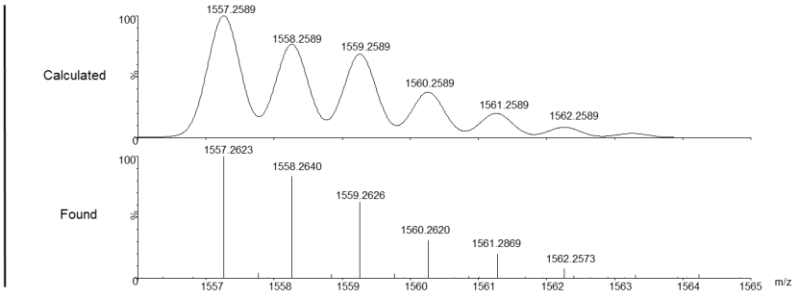
Chemical Formula:
$C_{56}H_{68}N_{16}O_{21}S_8$

Exact Mass:
1556.2511

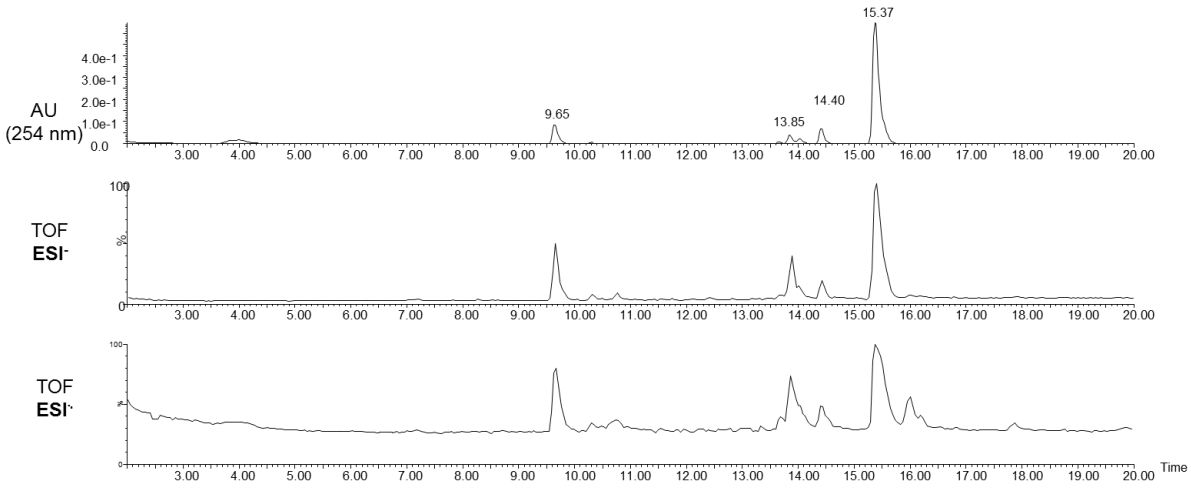
[M+H]$^{2-}$



[M+H]$^+$



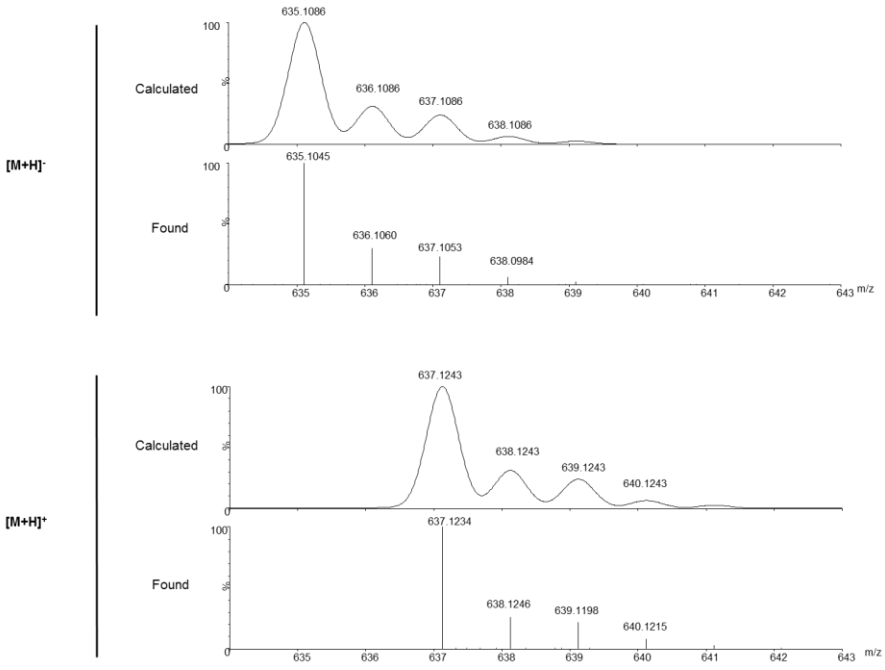
Mixture of **1a**+**2**+**3c** (0.5, 0.5 and 2.5 mM respectively, pH.6.5)



Identification of the products:

[**1a-3c$_2$**]
Retention time: 9.65 min.

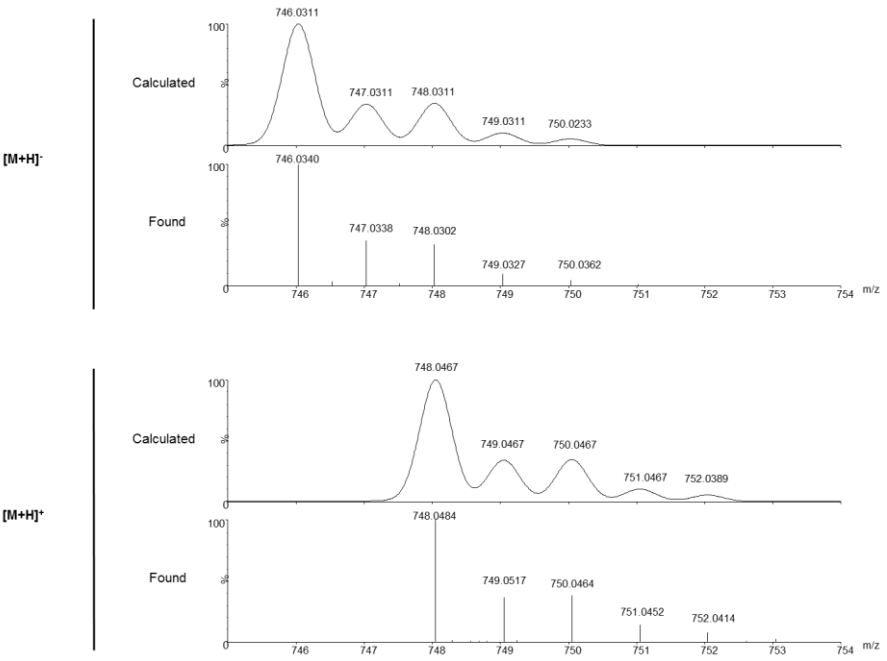
Chemical Formula:
$C_{22}H_{32}N_6O_8S_4$
Exact Mass:
636.1164



[**2-3c$_3$**]
Retention time: 13.85 min.

Chemical Formula:
$C_{24}H_{33}N_3O_{12}S_6$

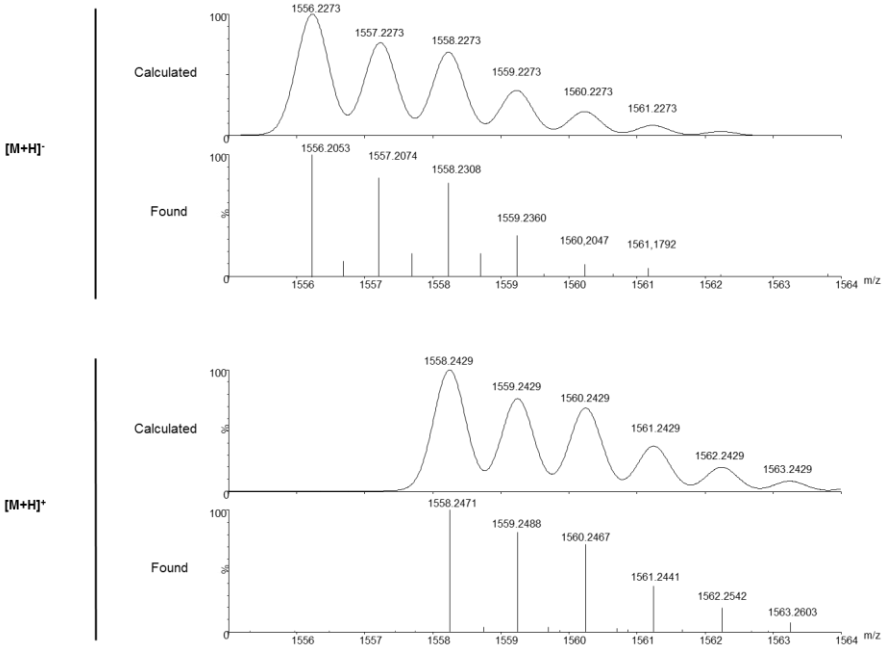
Exact Mass:
747.0388



[**1a$_2$-2-3c$_3$**]
Retention time: 14.40 min.

Chemical Formula:
$C_{56}H_{67}N_{15}O_{22}S_8$

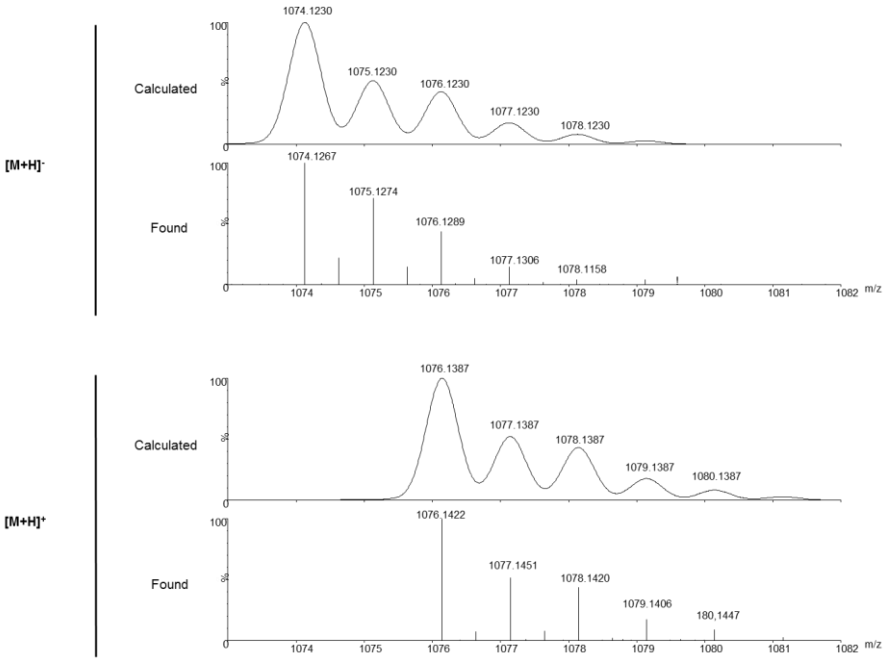
Exact Mass:
1557.2351



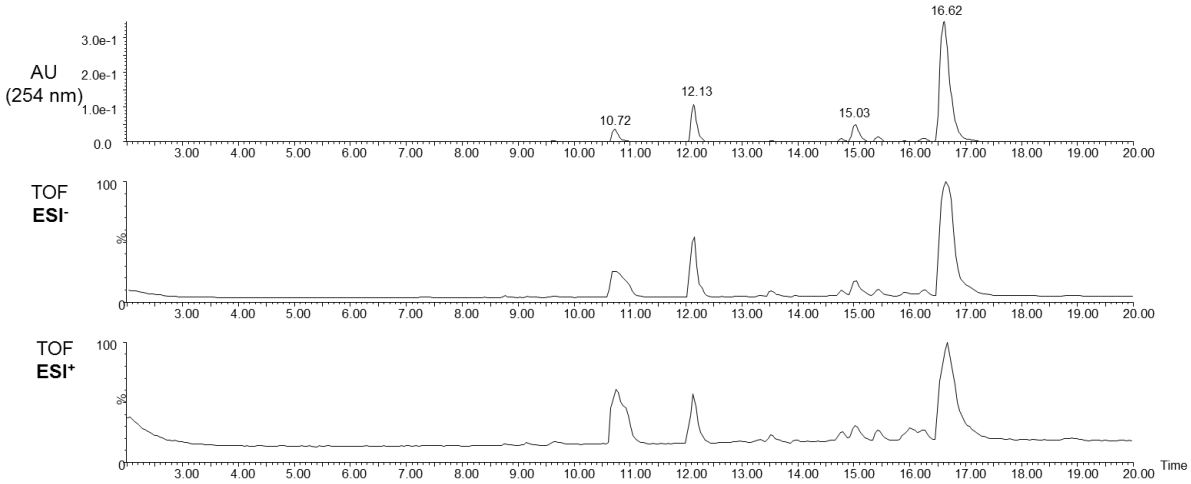
[**1a**$_2$-**2**-**3c**$_3$]
Retention time: 15.37 min.

Chemical Formula:
$C_{38}H_{45}N_9O_{16}S_6$

Exact Mass:
1075.1309



Mixture of **1a**+**2**+**3d** (0.5, 0.5 and 2.5 mM respectively, pH.6.5)



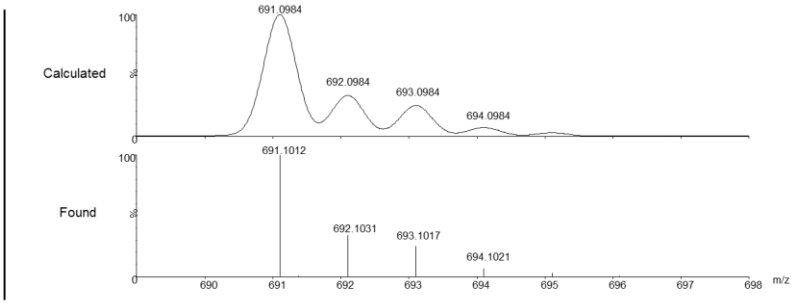
Identification of the products:

[**1a-3d$_2$**]
Retention time: 12.13 min.

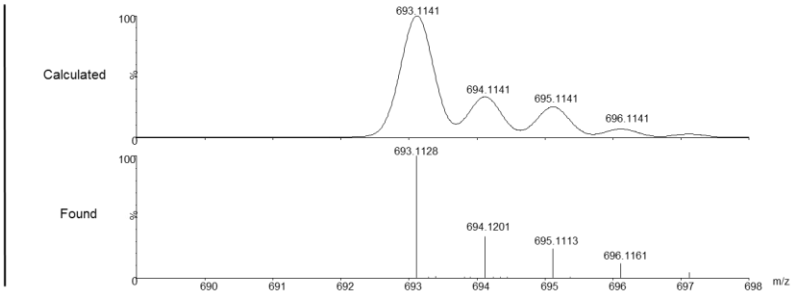
Chemical Formula:
$C_{24}H_{32}N_6O_{10}S_4$

Exact Mass:
692.1063

[M+H]$^-$



[M+H]$^+$

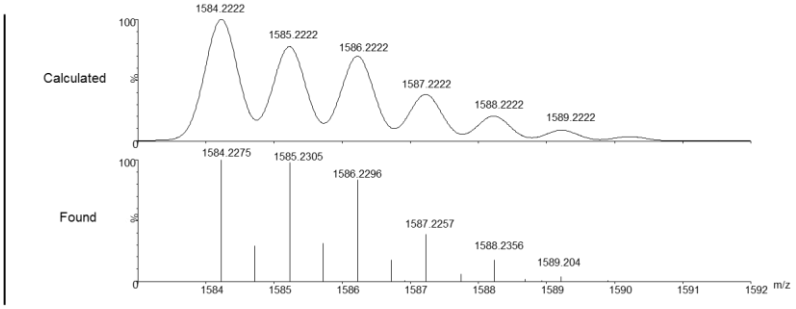


[**1a$_2$-2-3d**]
Retention time: 16.62 min.

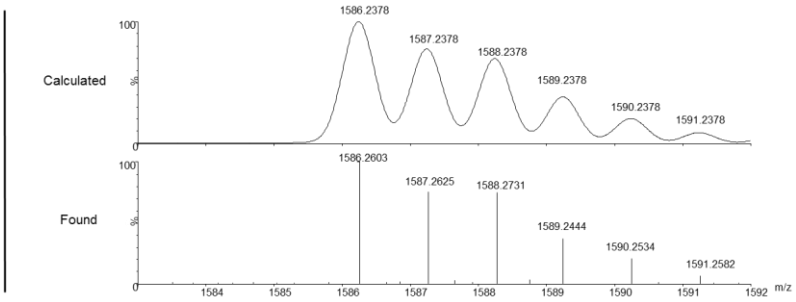
Chemical Formula:
$C_{57}H_{67}N_{15}O_{23}S_8$

Exact Mass:
1585.2300

[M+H]$^-$



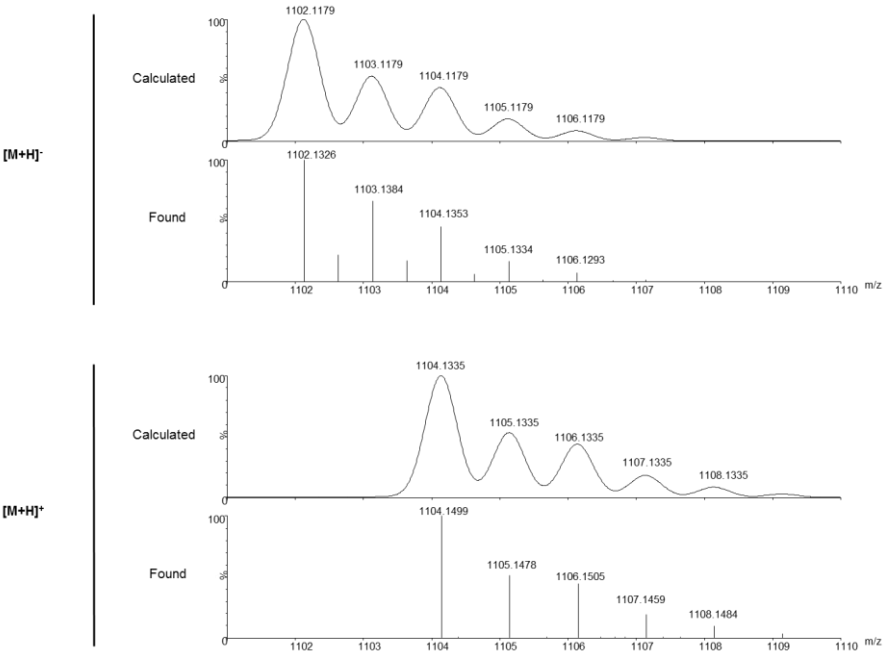
[M+H]$^+$



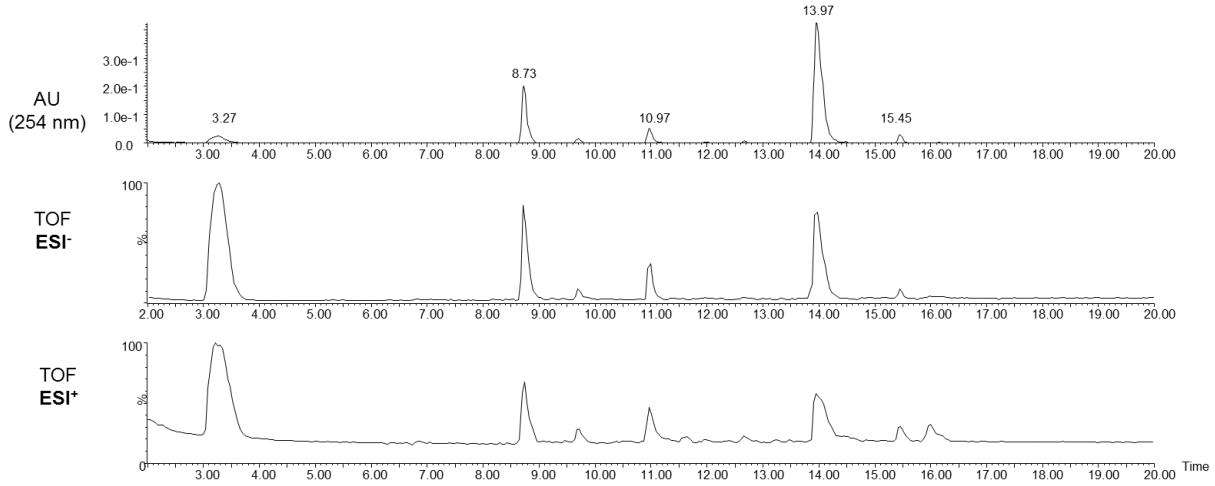
[**1a-2-3d**]
Retention time: 15.03 min.

Chemical Formula:
$C_{39}H_{45}N_9O_{17}S_6$

Exact Mass:
1103.1258



Mixture of **1a**+**2**+**3e** (0.5, 0.5 and 2.5 mM respectively, pH.6.5)



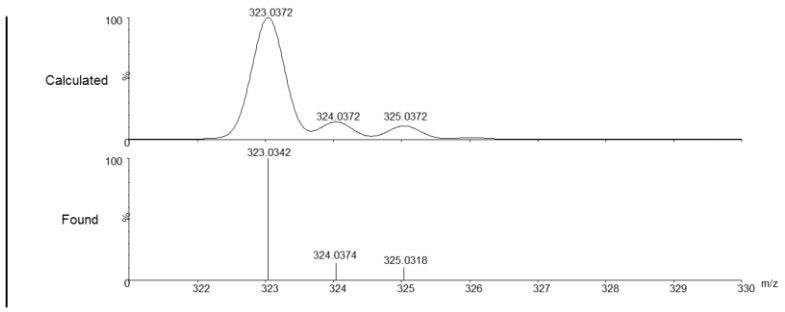
Identification of the products:

[**3e$_2$**]
Retention time: 3.27 min.

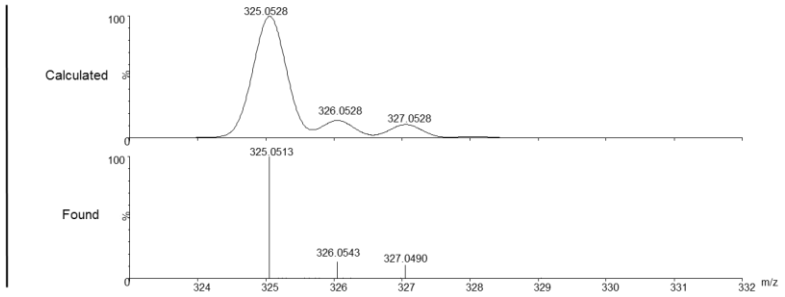
Chemical Formula:
$C_{10}H_{16}N_2O_6S_2$

Exact Mass:
324.0450

[M+H]$^-$



[M+H]$^+$

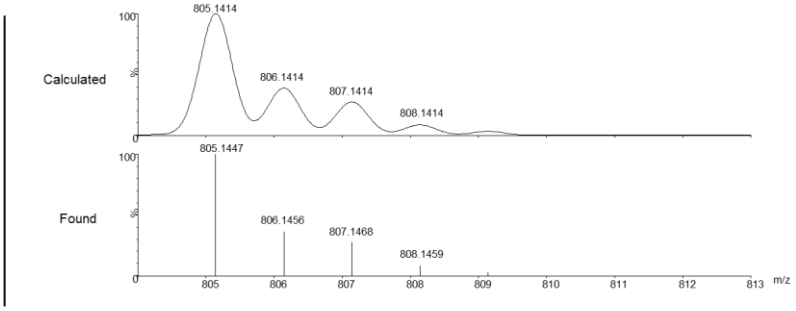


[**1a-3e$_2$**]
Retention time: 8.73 min.

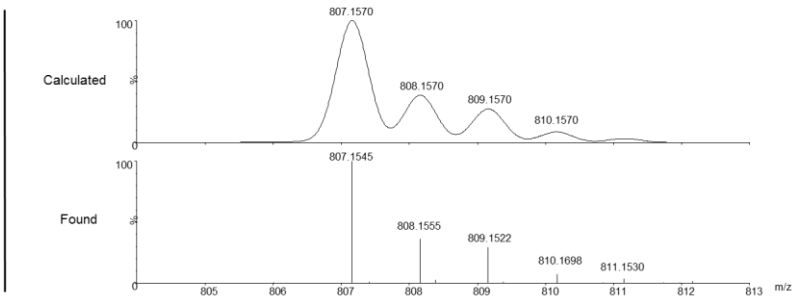
Chemical Formula:
$C_{28}H_{38}N_8O_{12}S_4$

Exact Mass:
806.1492

[M+H]$^-$



[M+H]$^+$

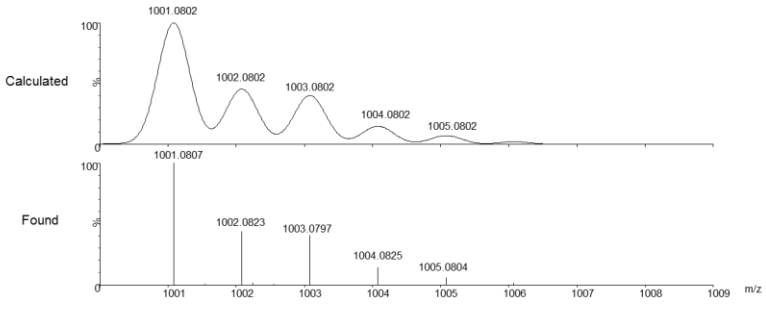


[**2-3e$_3$**]
Retention time: 10.97 min.

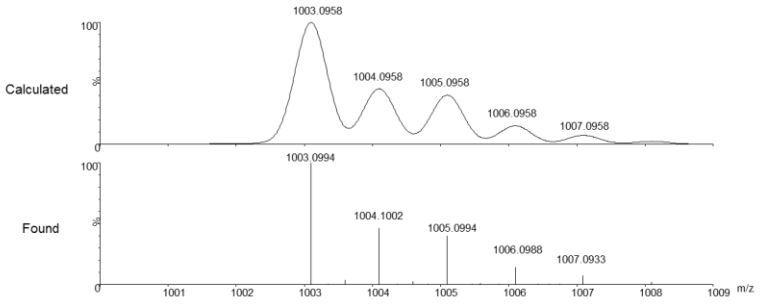
Chemical Formula:
$C_{33}H_{42}N_6O_{18}S_6$

Exact Mass:
1002.0880

[M+H]$^-$



[M+H]$^+$

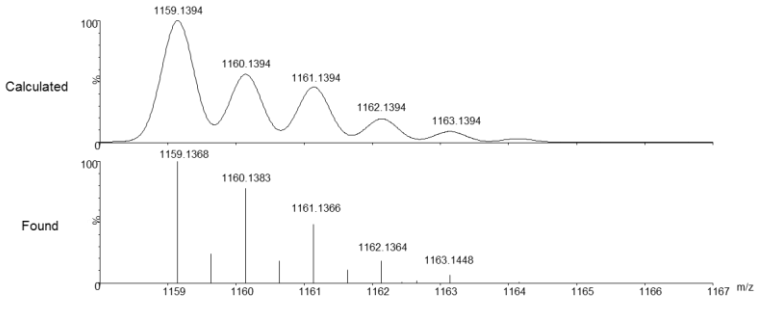


[**1a-2-3e$_3$**]
Retention time: 13.97 min.

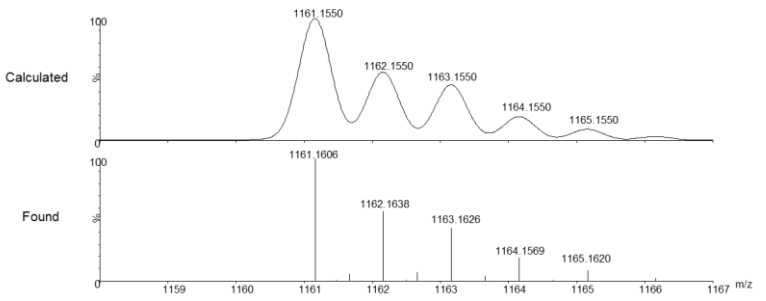
Chemical Formula:
$C_{41}H_{48}N_{10}O_{18}S_6$

Exact Mass:
1160.1472

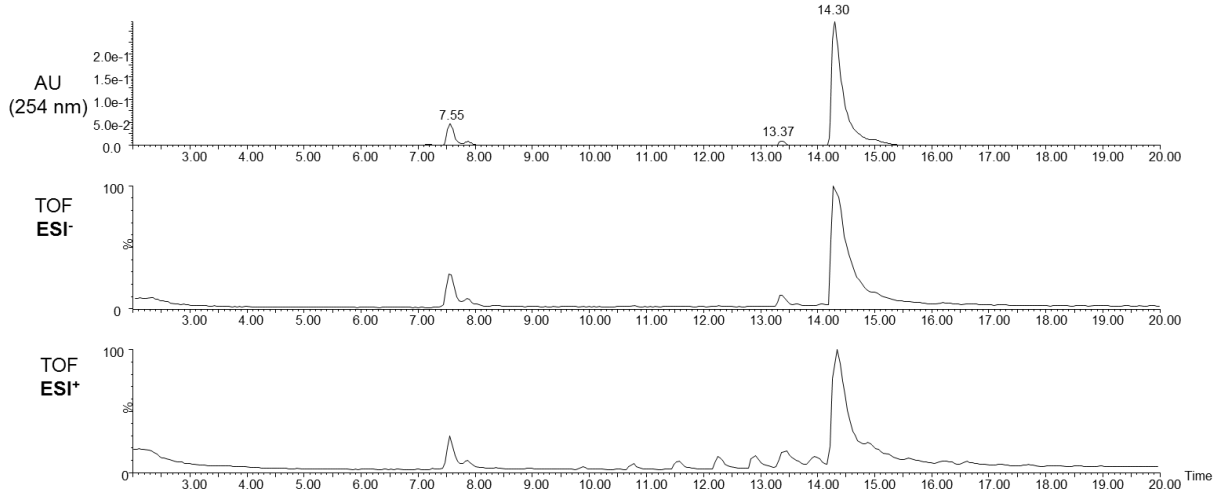
[M+H]$^-$



[M+H]$^+$



Mixture of **1a**+**2**+**3f** (0.5, 0.5 and 2.5 mM respectively, pH.6.5)

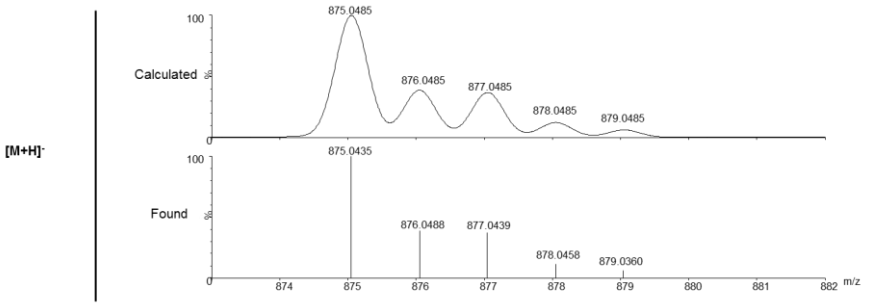


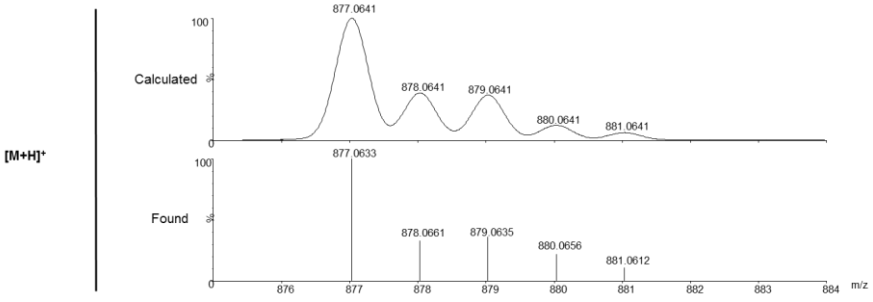
Identification of the products:

[**2-3f$_3$**]
Retention time: 7.55 min.

Chemical Formula:
$C_{27}H_{36}N_6O_{15}S_6$

Exact Mass:
876.0563





[**1a$_2$**] (previously identified)
Retention time: 13.37 min.

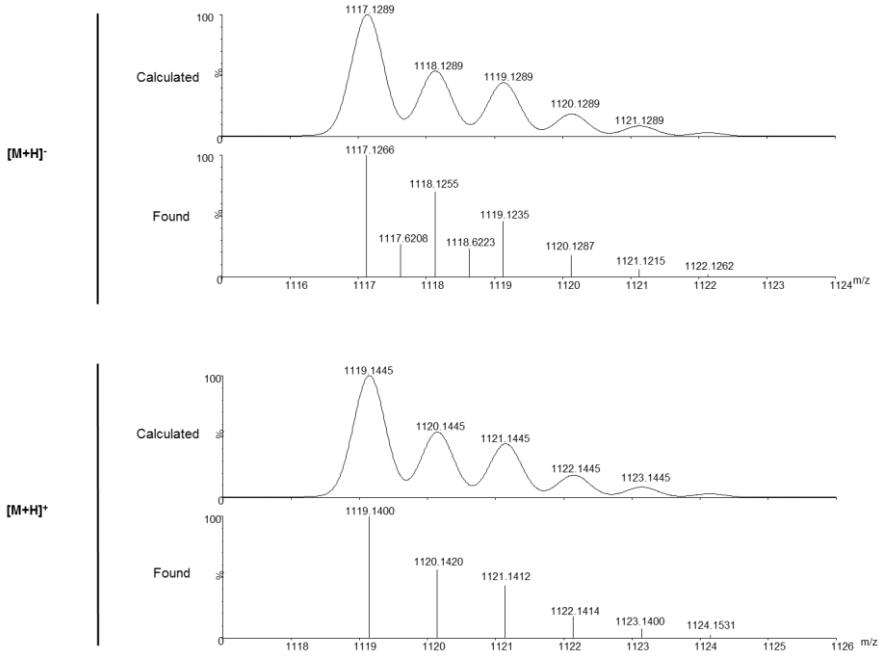
Chemical Formula: $C_{36}H_{44}N_{12}O_{12}S_4$

Exact Mass:
964.2084

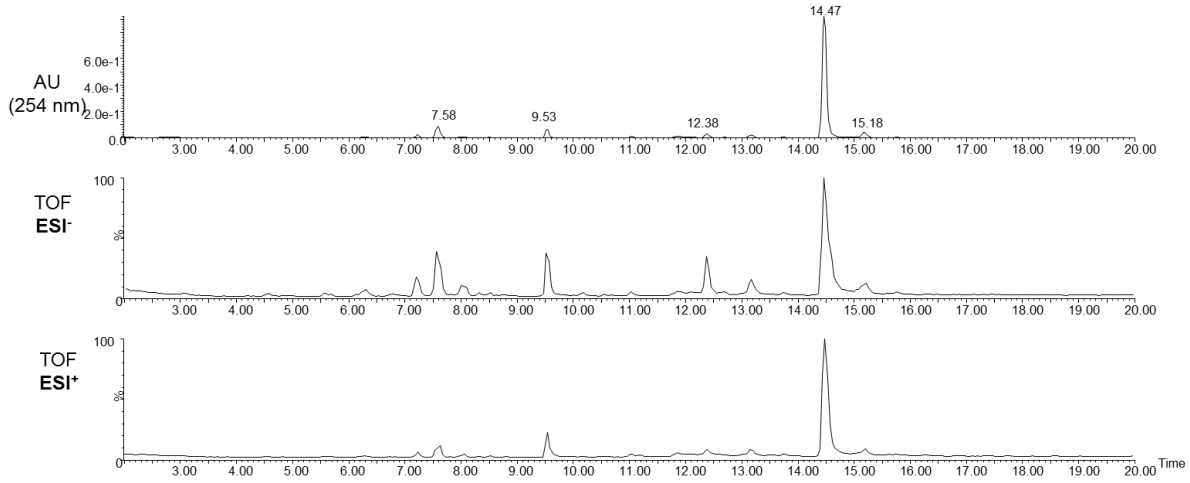
[**1a-2-3f**]
Retention time: 14.30 min.

Chemical Formula:
$C_{39}H_{46}N_{10}O_{17}S_6$

Exact Mass:
1118.1367



<u>Mixture of **1a**+**2**+**3f** (0.5, 0.5 and 2.5 mM respectively, pH.6.5)</u>



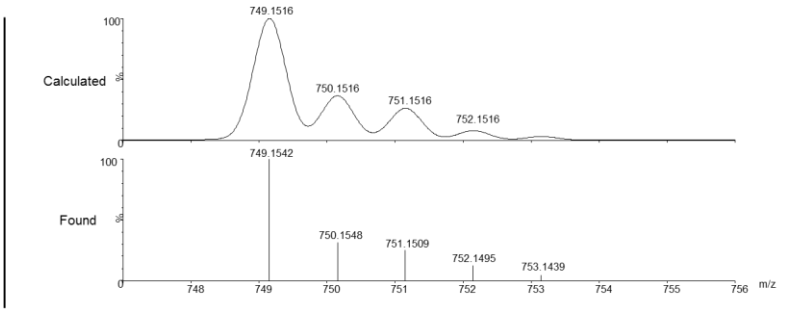
Identification of the products:

[**1a-3g$_2$**]
Retention time: 7.58 min.

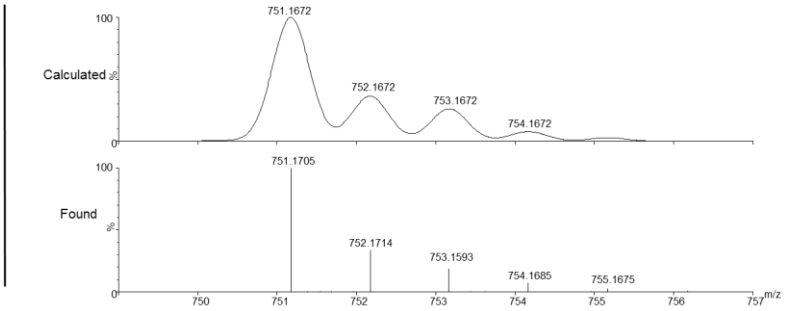
Chemical Formula:
$C_{26}H_{38}N_8O_{10}S_4$

Exact Mass:
750.1594

[M+H]$^-$



[M+H]$^+$

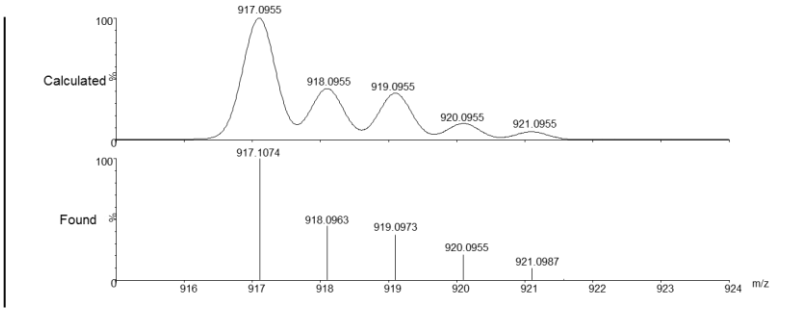


[**2-3g$_3$**]
Retention time: 9.53 min.

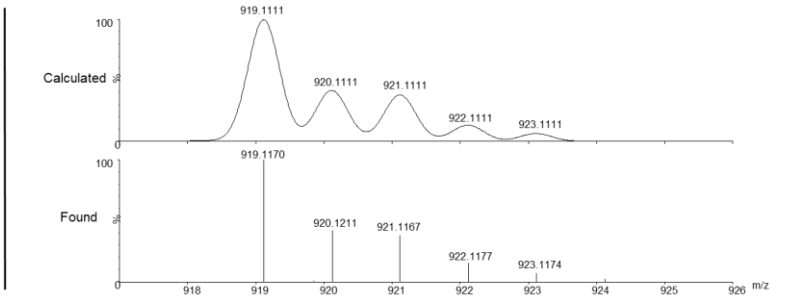
Chemical Formula:
$C_{30}H_{42}N_6O_{15}S_6$

Exact Mass:
918.1032

[M+H]$^-$



[M+H]$^+$



[**2**$_2$] (previously identified)
Retention time: 12.38 min.

Chemical Formula:
$C_{36}H_{36}N_6O_{18}S_6$

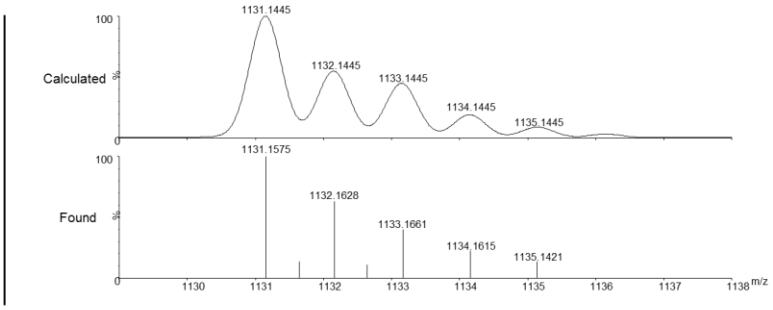
Exact Mass:
1032.0410

[1a-2-3g]
Retention time: 14.47 min.

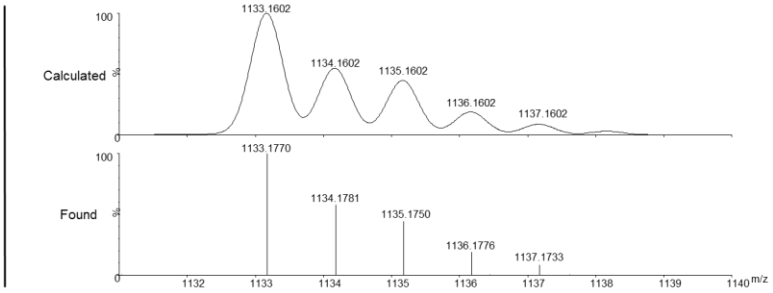
Chemical Formula:
$C_{40}H_{48}N_{10}O_{17}S_6$

Exact Mass:
1132.1523

[M+H]$^-$



[M+H]$^+$

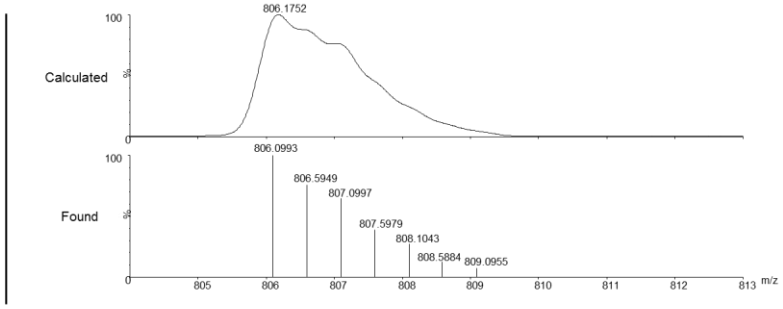


[**1a**$_2$**-2-3g**]
Retention time: 15.18 min.

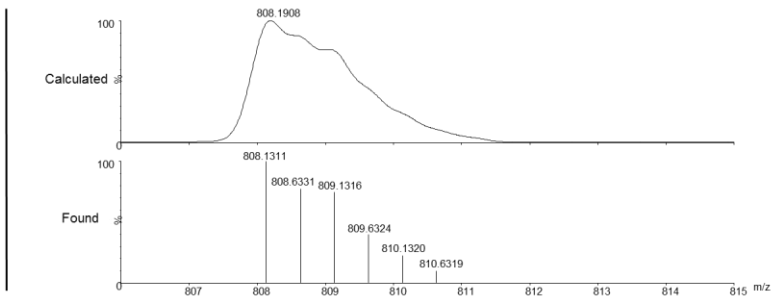
Chemical Formula:
$C_{58}H_{70}N_{16}O_{23}S_8$

Exact Mass:
1614.2565

[M+H]$^{2-}$

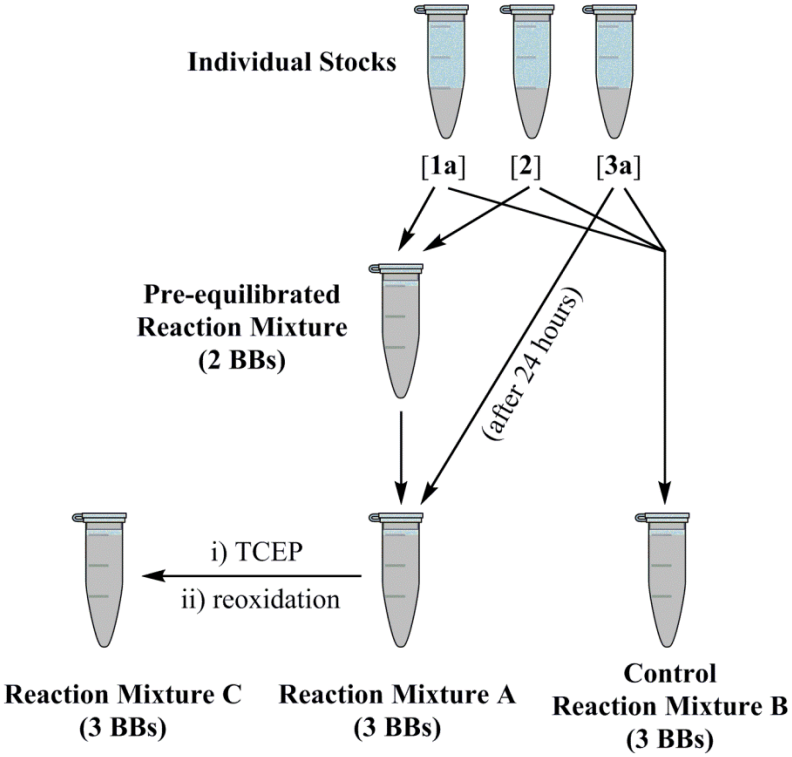


[M+H]$^{2+}$



REVERSIBILITY TEST

Individual stocks of [**1a**, **2, 3a**] were prepared (4 mM in DMSO for **1a** and **2**, and 20 mM in milli-Q water for **3a**) (see scheme S1). From these, a **pre-equilibrated reaction mixture** was prepared by adding 40 µL of [**1a**] and [**2**] to 200 µL of a 66.7 mM BIS-Tris methane buffer (pH 6.5). The **individual stock** of [**3a**] was stored at -80 ºC. After 24 hours, the **reaction mixture A** was prepared by adding 30 µL of the **individual stock** of [**3a**] to 210 µL of the **pre-equilibrated reaction mixture**.



Scheme S1: Preparation of the reaction mixtures of the reversibility test.

Simultaneously, the **control reaction mixture B** was prepared by mixing 30 µL of each **individual stock** with 150 µL of a 66.7 mM BIS-Tris methane buffer (pH 6.5). After 24 hours, the **reaction mixture A**, the **control reaction mixture B** and the **pre-equilibrated reaction mixture** were analysed by HPLC (see figure S16).

Finally, the **reaction mixture C** was prepared by adding 0.35 equivalents of Tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl)[3] to the completely oxidised **reaction mixture A**. The substoichiometric amount of TCEP allowed the partial reduction of the disulphides present in the mixture. After the reoxidation of the generated free thiols, 24 hours later, the **reaction mixture C** was analysed by HPLC (see figure S16).

Related references:

3. J. A. Burns, J. C. Butler, J. Moran, and G. M. Whitesides, *J. Org. Chem.*, **1991**, *56*, 2648-2650.

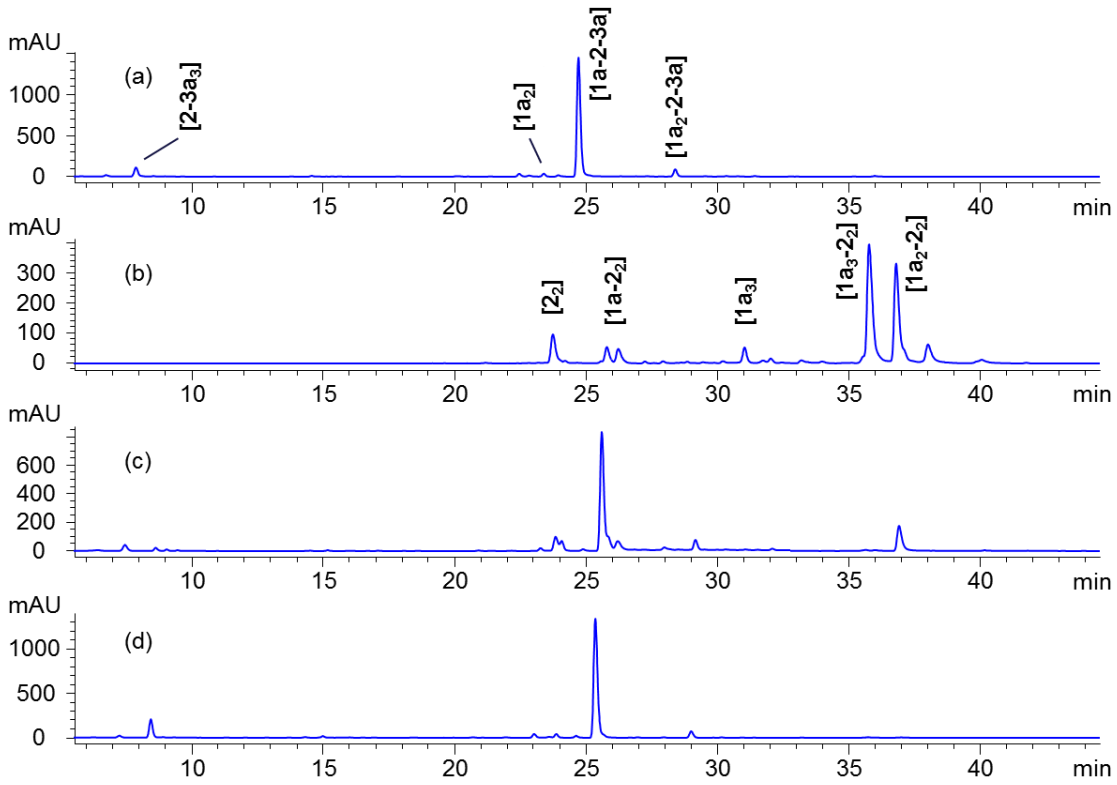
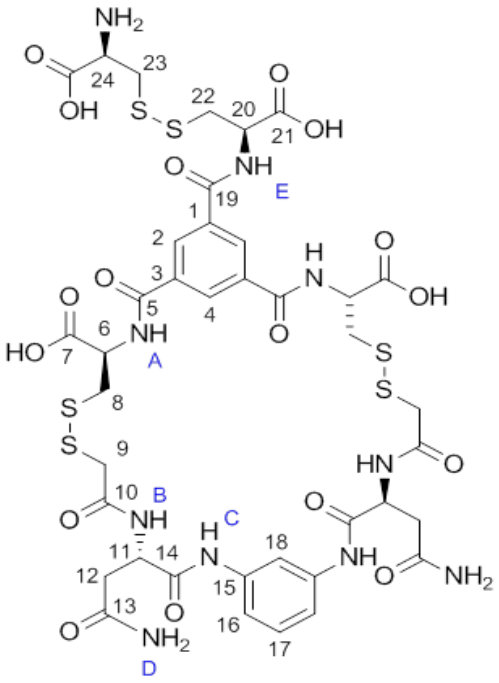


Figure S16: HPLC traces of the **control reaction mixture B** (a), the **pre-equilibrated reaction mixture** (b), the **reaction mixture A** (c) and the **reaction mixture C** (d).

SYNTHESIS AND NMR CHARACTERIZATION OF **1a23a** (**Ia**)



For the synthesis of the trimer **1a23a** (**Ia**), [**1a**] (4.8 mg, 0.010 mmol), [**2**] (5.2 mg, 0.010 mmol) and [**3a**] (2.4 mg, 0.20 mmol) were dissolved in 2 mL of MeOH and 1 mL of milli-Q water. Then, the pH of the solution was adjusted to 7.0 by the addition of NaOH (aq). After 6 days at room temperature, complete oxidation of the starting reagents was observed by HPLC. The MeOH was evaporated *in vacuo* and the residue was lyophilized. The resulting white solid was purified by reversed-face flash chromatography (gradient: from 5% to 15% CH_3CN in H_2O) and 3.8 mg of pure [**Ia**] (34% yield) were obtained as a white solid. HRMS (ESI-) calcd. for $C_{39}H_{46}N_{10}O_{17}S_6$ $[M-H]^-$ (m/z): 1117.1289, found: 1117.1323. ^{1}H NMR (500 MHz, H_2O:DMSO-d_6 (85:15)): δ = 8.69 (s, 2H, H_C), 8.62 (s, 1H, H_4), 8.54 (d, J = 8.4 Hz, 2H, H_B), 8.46 (d, J = 8.8 Hz, 2H, H_A), 8.38 (d, J = 8.2 Hz, 1H, H_E), 8.29 (d, J = 1.7 Hz, 2H, H_2), 7.58 (s, 2H, H_D), 7.20 (d, J = 1.9 Hz, 1H, H_{18}), 6.94 (t, J = 8.2 Hz, 1H, H_{17}), 6.89 (d, J = 8.2 Hz, 2H, H_{16}), 6.76 (s, 2H, $H_{D'}$), 4.05 – 4.00 (m, 1H, H_{24}), 4.01 (d, J = 14.8 Hz, 2H, H_9), 3.47 (dd, J = 15.2 Hz, J = 2.4 Hz, 1H, H_8), 3.45 (d, J = 2.2 Hz, 1H, $H_{9'}$), 3.35 (dd, J = 9.9 Hz, J = 3.9 Hz, 1H, H_{22}), 3.32 (dd, J = 10.7 Hz, J = 4.3 Hz, 1H, H_{23}), 3.19 (dd, J = 15.2, 11.0 Hz, 1H, H_8), 3.04 (dd, J = 15.2, 9.3 Hz, 1H, $H_{22'}$), 2.99 (dd, J = 15.8, 9.9 Hz, 1H, $H_{23'}$), 2.93 – 2.82 (m, 2H, H_{12}). ^{13}C NMR (125 MHz, D_2O:DMSO-d_6 (85:15)): δ = 174.6 (CO, C_{13}), 172.1 (CO, C_{10}), 169.6 (CO, C_{14}), 167.2 (CO, C_{19}), 166.8 (CO, C_5), 137.0 (C, C_{15}), 134.0 (C, C3), 133.4 (C, C1), 129.7 (CH, C_4), 129.65 (CH, C_{16}), 129.60 (CH, C_2), 116.3 (CH, C_{17}), 111.5 (CH, C_{18}), 55.8 (CH, C_6), 54.4 (CH, C_{20}), 51.0 (CH, C_{11}), 53.4 (CH, C_{24}), 45.0 (CH_2, C_8), 41.2 (CH_2, C_9), 39.5 (CH_2, C_{22}), 37.9 (CH_2, C_{23}), 35.9 (CH_2, C_{12}).

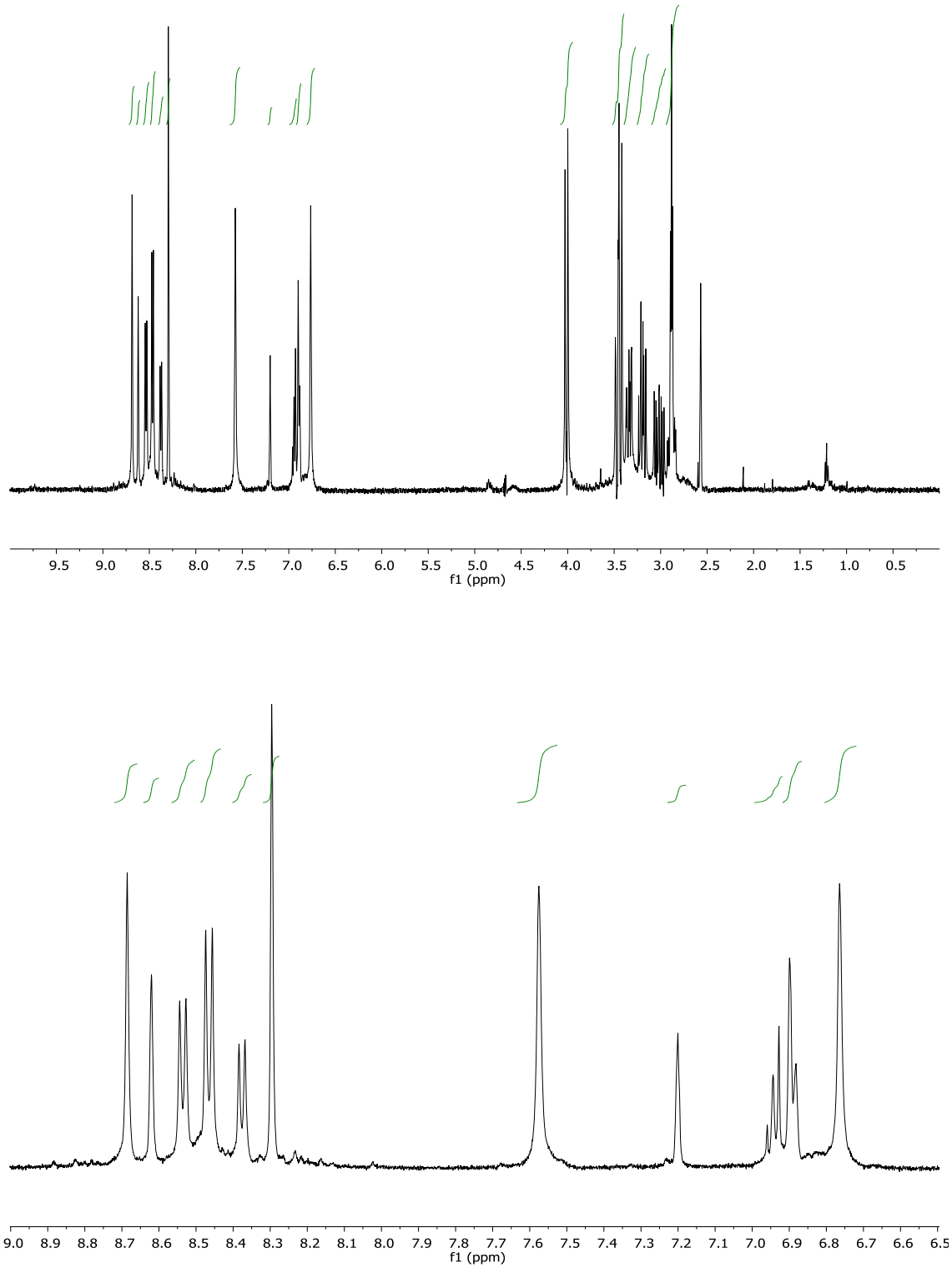


Figure S17: ^{1}H (500 MHz, 298 K in H_2O:DMSO-d_6 (85:15), with water suppression by excitation sculpting scheme) spectrum of **Ia** (8.5 mM phosphate buffer, pH 6.5), and expansion of the amide region (9.0 – 6.5 ppm).

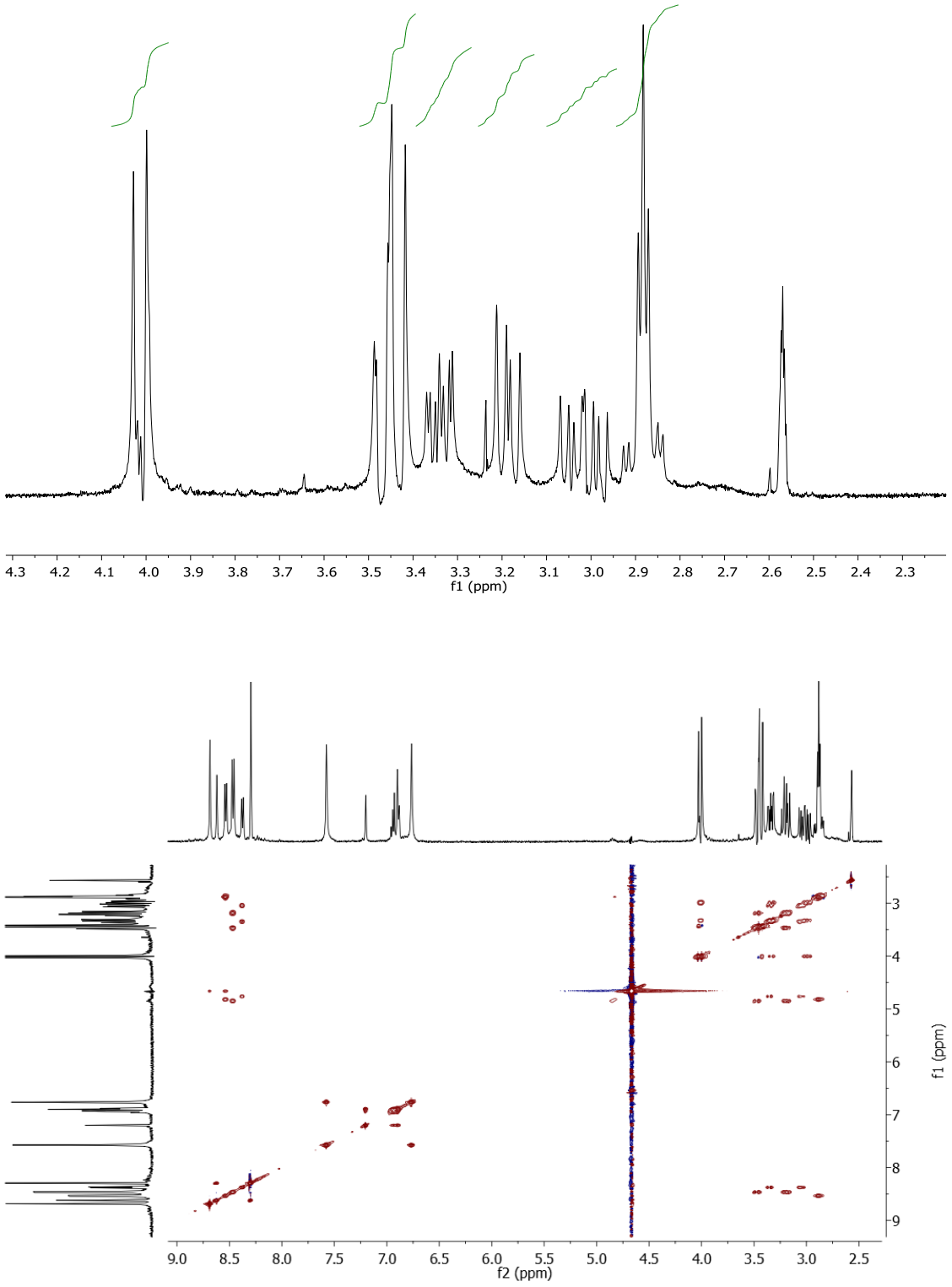


Figure S18: Expansion of the aliphatic region (4.3 – 2.3 ppm) of ^{1}H (500 MHz, 298 K in H_2O:DMSO-d_6 (85:15), with water suppression by excitation sculpting scheme) and ^{1}H 2D TOCSY (500 MHz, 298 K in H_2O:DMSO-d_6 (85:15), with water suppression by excitation sculpting scheme) spectra of **Ia** (8.5 mM phosphate buffer, pH 6.5).

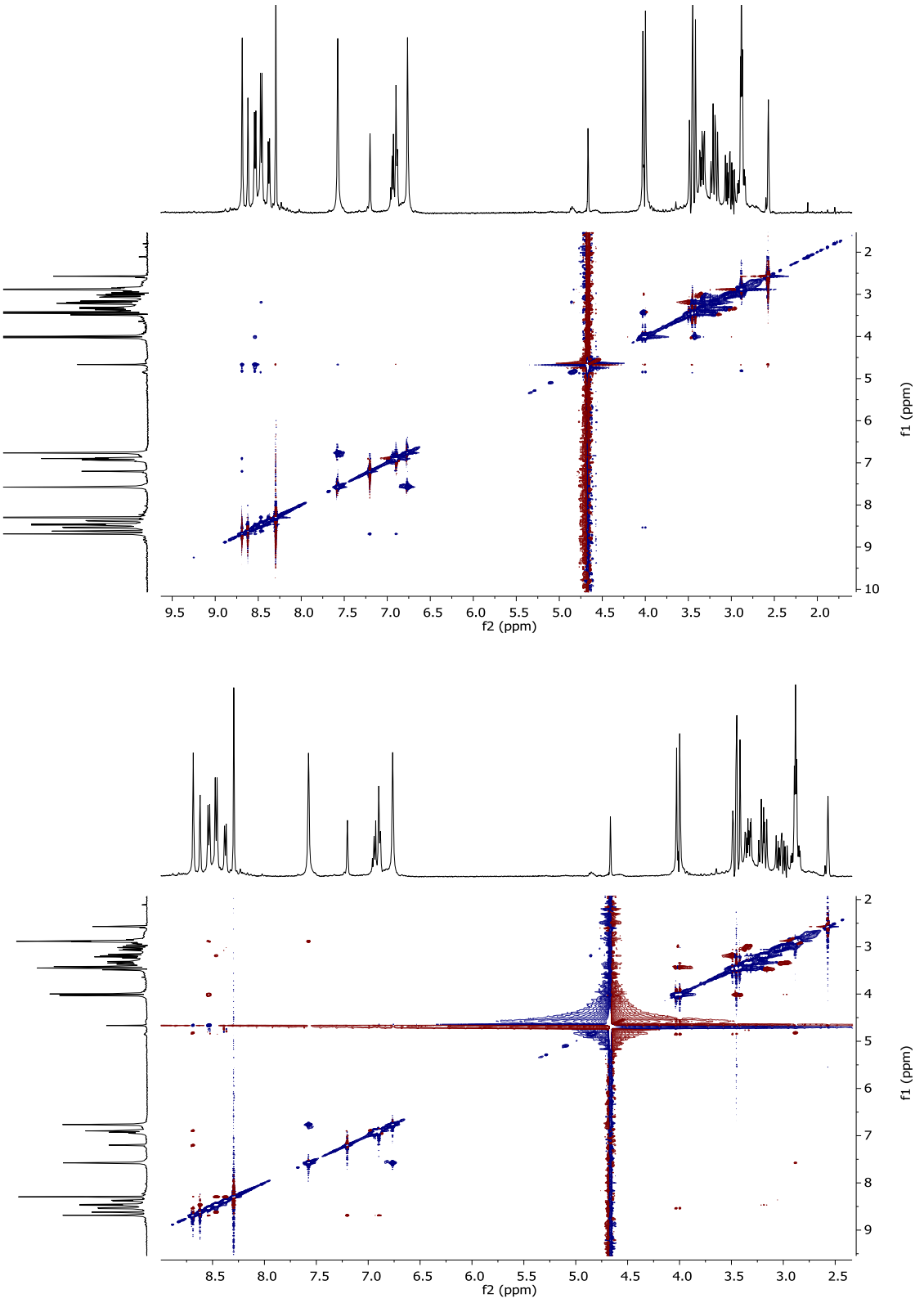


Figure S19: ^{1}H NOESY (500 MHz, 298 K in H_2O:DMSO-d_6 (85:15), with water suppression by excitation sculpting scheme) and ^{1}H ROESY (500 MHz, 298 K in H_2O:DMSO-d_6 (85:15), with water suppression by excitation sculpting scheme) spectra of **Ia** (8.5 mM phosphate buffer, pH 6.5).

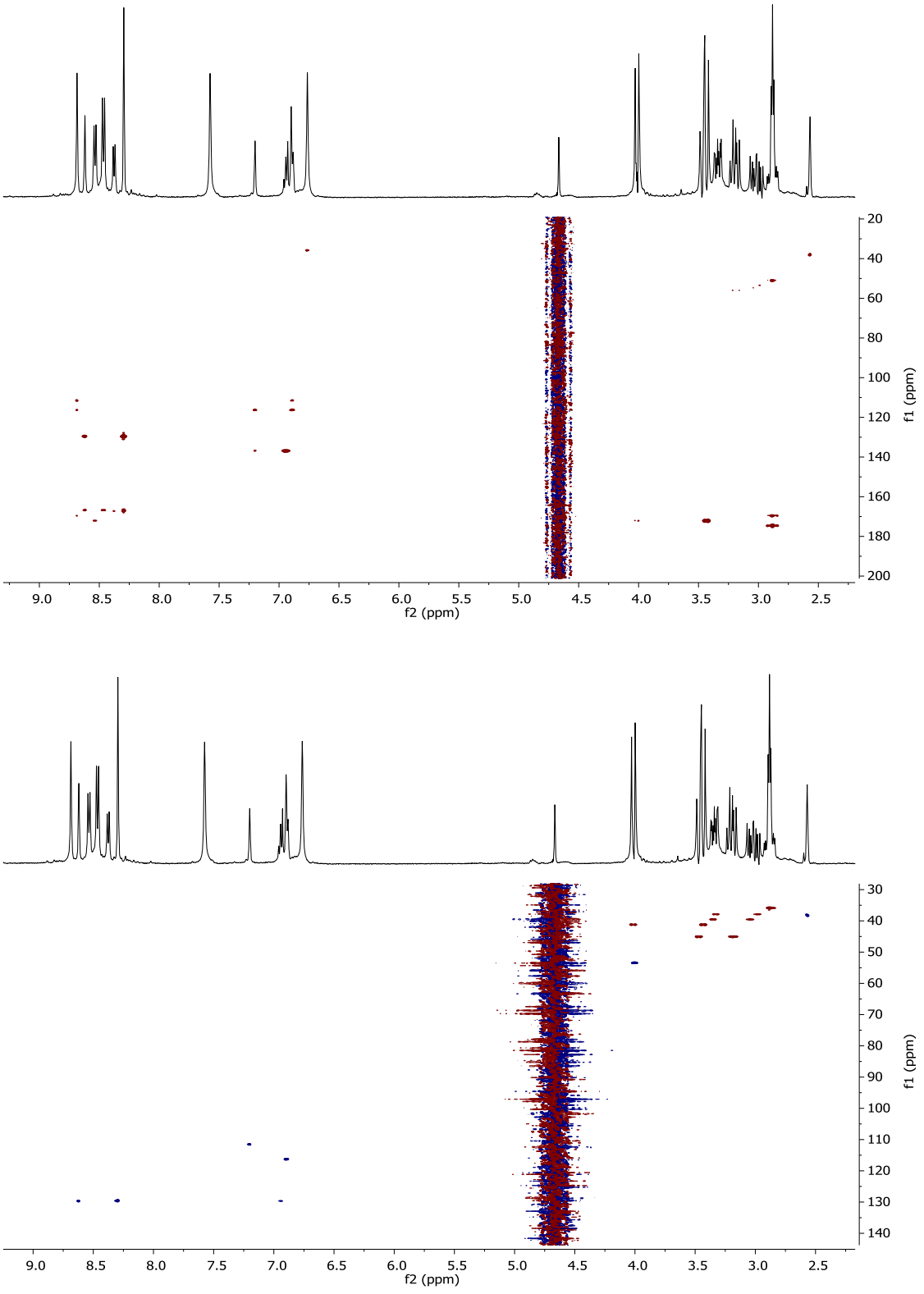


Figure S20: $^{1}H/^{13}C$ gHMBC (500 MHz, 298 K in H_2O:DMSO-d_6 (85:15), with water suppression by excitation sculpting scheme) and $^{1}H/^{13}C$ gHSQC (500 MHz, 298 K in H_2O:DMSO-d_6 (85:15), with water suppression by excitation sculpting scheme) of **Ia** (8.5 mM phosphate buffer, pH 6.5).

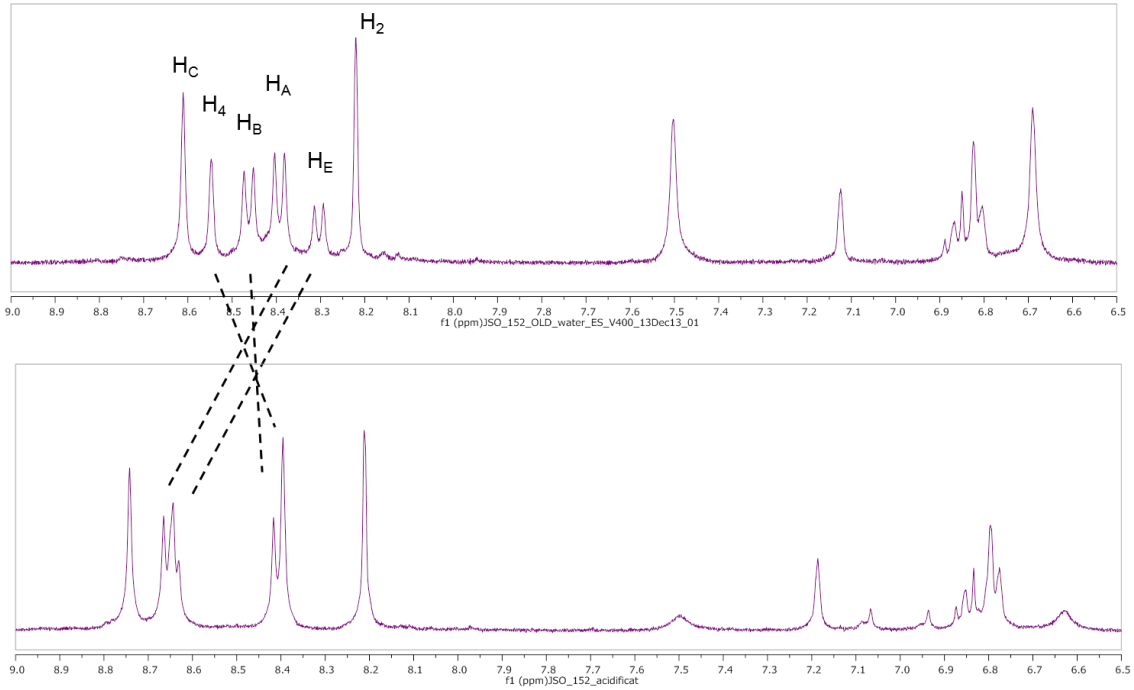


Figure S21: Expansion of the amide region (9.0 – 6.5 ppm) of ^{1}H (400 MHz, 298 K in H_2O:DMSO-d_6 (85:15), with water suppression by excitation sculpting scheme) spectrum of **Ia**. Top: 8.5 mM phosphate buffer, pH 6.5. Bottom: after acidification with TFA, pH ~2. The differences in chemical shift for protons H_A, H_E (moving downfield) and H_4 (moving upfield) are consistent with an unfolding of the proposed conformation for **1a23a**.

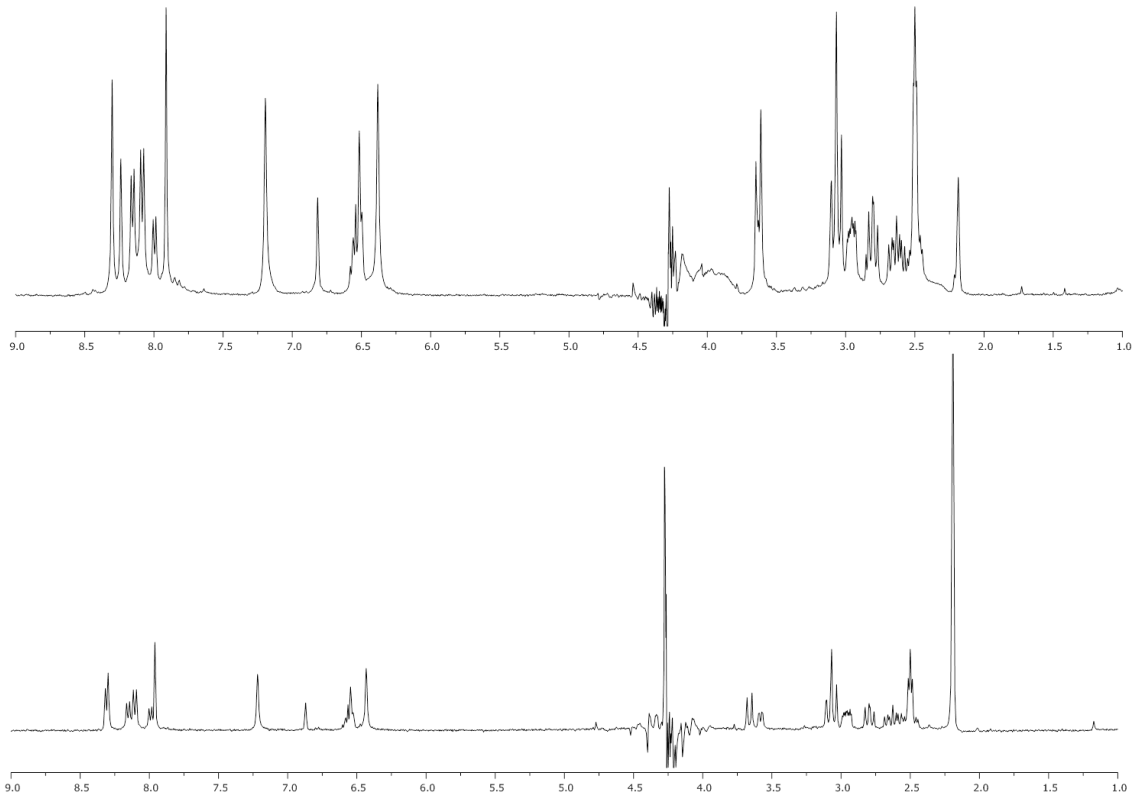


Figure S22: ^{1}H-NMR (400 MHz, 298K) with water suppression by excitation sculpting for **1a23a**. Top: H_2O:DMSO-d_6 (85:15, 8.5 mM phosphate buffer, pH 6.5). Bottom: H_2O:DMSO-d_6 (75:25, 8.5 mM phosphate buffer, pH 6.5). Subtle changes are appreciated, in a more organic solvent the folded structure of **1a23a** is more favored as can be seen by downfield shift for protons H_4 and H_2.

MOLECULAR MODELLING OF **1a23a** (**Ia**)

All the theoretical calculations were performed with Spartan 06 software operating in a Dell workstation. Monte Carlo conformation searches were performed without restrictions by generating 10000-20000 geometries, which were minimized subsequently using the MMFFaq force field. This version of the force field takes into account water solvent as a continuum medium. This force field has proved to be the most suitable for the conformational analysis of pseudopeptide and peptoid molecules.[4] The obtained local minima were ordered following the corresponding MMFFaq energies. The process was repeated several times starting from different initial geometries to ensure mapping all the conformational space. The corresponding conformational searches of the same molecule starting from different geometries rendered identical results. This fact ensures the fidelity and reliability of the results from this conformational analysis. We considered all the carboxylic groups as carboxylate anions and the amine function of the pendant cysteine as the ammonium cation. The global minimum for the **1a23a** (**Ia**) compund is shown in Figure 2 of the manuscript. The superposition of the energetically accessible local minima found for this molecule is shown in figure S23.

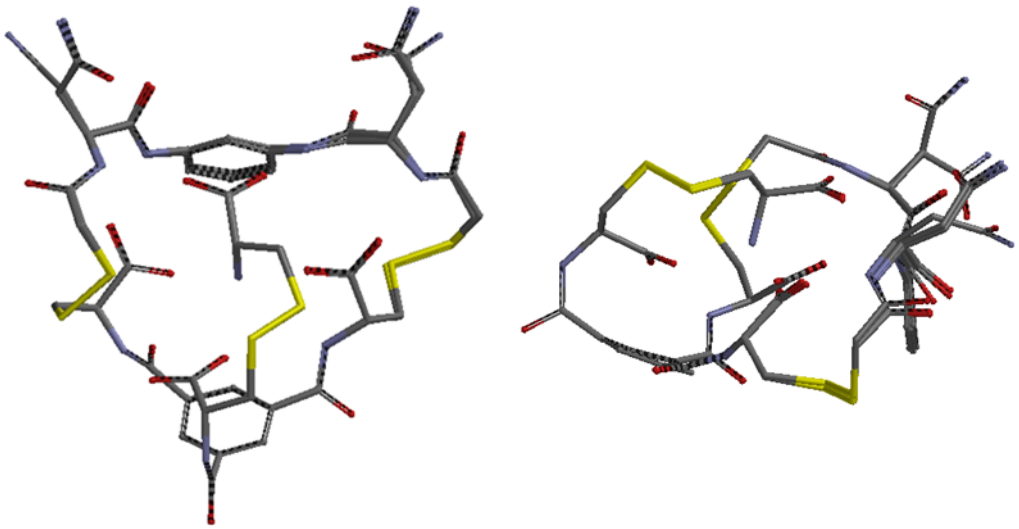


Figure S23: Top and side views of the superposition of the accessible local minima for **1a23a** (**Ia**). Hydrogen atoms have been omitted for clarity.

Related references:

4. (a) W. Brandt, T. Herberg, L. Wessjohann, *Biopolymers (Protein Science)*, **2011**, *96*, 651-667; (b) C. F. Rodriquez, G. Orlova, Y. Guo, X. Li, C.-K. Siu, A. C. Hopkinson, K. W. M.Siu, *J. Phys. Chem. B* **2006,** *110*, 7528-7537; (c) E. F. Strittmatter, E. R. Williams, *J. Phys. Chem. A* **2000,** *104*, 6069-6076; (d) M. D. Beachy, D. Chasman, R. B. Murphy, T. A. Halgren, R. A. Friesner, *J. Am. Chem. Soc.* **1997,** *119*, 5908-5920.