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Pseudopeptidic Compounds for the Generation of Dynamic Combinatorial Libraries of Chemically Diverse Macrocycles in Aqueous Media

Joan Atcher, Jordi Solà and Ignacio Alfonso**

J. Atcher, Jordi Solà and I. Alfonso
Department of Biological Chemistry and Molecular Modeling
Institute of Advanced Chemistry of Catalonia, IQAC-CSIC
Jordi Girona 18-26, 08034, Barcelona, Spain
e-mail: jordi.sola@iqac.csic.es
e-mail: ignacio.alfonso@iqac.csic.es

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General methods

Reagents and solvents were purchased from commercial suppliers (Aldrich, Fluka, Merck or Iris Biotech) and were used without further purification. Chromatographic purifications were performed on Biotage® Isolera Prime™ equipment using Biotage® SNAP KP-Sil and Biotage® SNAP KP-C18-HS cartridges for normal- and reversed-phase purifications respectively. TLCs were performed using 6 x 3 cm SiO_2 pre-coated aluminium plates (ALUGRAM® SIL G/UV_{254}).

RP-HPLC analyses were performed on a Hewlett Packard Series 1100 (UV detector 1315A) modular system using a reversed-phase X-Terra C_{18} (15 x 0.46 cm, 5 µm) column. (MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA) mixtures at 1 mL/min were used as mobile phase and the monitoring wavelengths were set at 220 and 254 nm. The temperature of the column was set at 25 °C. The HPLC samples were prepared by dilution with an acidic solution of 89% H_2O, 10% MeCN and 1% TFA. For the analysis of the DCLs a reversed-phase kromaphase C_{18} (25 x 0.46 cm, 5µm) column was used, (MeCN + 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 126 MHz for ^{13}C), a Varian Mercury 400 instrument (400 MHz for ^1H and 101 MHz for ^{13}C) and a Varian Unity 300 (300 MHz for ^1H and 75 MHz for ^{13}C). The chemical shifts (δ) are reported in ppm relative to trimethylsilane (TMS), and coupling constants (J) are reported in Hertz (Hz). Signal assignment was carried out using the necessary 2D NMR spectra including ^1H-^1H gCOSY, ^1H-^{13}C gHSQC and ^1H-^{13}C gHMBC. For describing signals of ^1H NMR spectra de following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, ABq = AB quartet, quint = quintet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, dq = doublet of quartets, qd = quartet of doublets, ddd = double doublet of doublets, ddt = double doublet of triplets, m = multiplet, and br = broad signal.

HRMS analyses were carried out at the IQAC Mass Spectrometry Facility, using UPLC-ESI-TOF equipment: [Acquity UPLC® BEH C_{18} 1.7 mm, 2.1x100 mm, LCT Premier Xe, Waters]. (MeCN + 20 mM HCOOH and H_2O + 20 mM HCOOH) mixtures at 0.3 mL/min were used as mobile phase. The characterization of the pure products and intermediates was performed in flow injection analysis (FIA) mode.

Synthesis of the building blocks

Synthesis of tritylsulfanyl acetic acid

This compound was prepared as previously described.[1] To a solution of mercaptoacetic acid (4.60 g, 49.9 mmol) and triphenylmethanol (13.0 g, 49.9 mmol) in chloroform (50 mL), trifluoroacetic acid (TFA, 5.0 mL, 65 mmol) was added. After the mixture was stirred at room temperature for 2 hours, volatiles were removed *in vacuum*. The crude product was recrystallized from dichloromethane/hexane to give 13.9 g of tritylsulfanyl acetic acid (83% yield) as a white solid. Rf of the product in AcOEt/Hexane, 3:7, (v/v): 0.39. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.42 (d, J = 7.3 Hz, 6H, CH_{Ar}), 7.30 (t, J = 7.6 Hz, 6H, CH_{Ar}), 7.23 (t, J = 7.3 Hz, 3H, CH_{Ar}), 3.03 (s, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.7 (1 x CO), 144.0 (3 x C_{Ar}), 129.6 (6 x CH_{Ar}), 128.3 (6 x CH_{Ar}), 127.2 (3 x CH_{Ar}), 67.4 (1 x C), 34.5 (1 x CH_2). HRMS (ESI–) calcd. for $C_{21}H_{18}O_2S$ $[2M–H]^−$ (m/z): 667.1982, found: 667.1996.

Synthesis of intermediates 1a-j and 1l

Synthesis of 1a: to a solution of Fmoc-L-Asn(Trt)-OH (4.24 g, 7.11 mmol) in dry DMF (15 mL), HOBt (1.25 g, 9.27 mmol) and DCCD (2.23 g, 10.8 mmol) were added under inert atmosphere of Ar. The resulting mixture was cooled down to 0 °C in an ice-water bath. Then, a solution of *m*-phenylenediamine (334 mg, 3.09 mmol) in dry DMF (10 mL) was added via cannula under inert atmosphere of Ar. The mixture was stirred at room temperature for 60 hours, after which complete conversion of the starting material was observed by TLC (Rf of **1a** in 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 AcOEt/hexane as eluent (from 30% to 50% AcOEt) to give 2.05 g of **1a** (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 (br s, 2H, NHCOC*H), 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, CONHTrt), 6.54 (br s, 2H, NHFmoc), 4.68 (br s, 2H, C*H), 4.51–4.32 (m, 4H, $COOCH_2$), 4.20 (t, J = 7.0 Hz, 2H, CH), 3.16 (d, J = 15.7 Hz, 2H, CH_2C*H), 2.66 (dd, J = 15.7, 6.9 Hz, 2H, CH_2C*H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.8 (2 x CO), 169.0 (2 x CO), 156.4 (2 x

[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.

CO), 144.2 (6 x C_{Ar}), 143.8 (4 x C_{Ar}), 141.4 (4 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 $COOCH_2$), 52.2 (2 x C*H), 47.2 (2 x CH), 38.9 (2 x CH_2C^*H).

Synthesis of 1b: this compound was obtained as described above for **1a**, starting from Fmoc-L-Gln(Trt)-OH. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 25% to 40% AcOEt, Rf of **1b** in AcOEt/hexane, 3:2 (v/v): 0.50) to give 1.12 g of **1b** (47% yield) as a white solid. HRMS (ESI+) calcd. for $C_{84}H_{72}N_6O_8$ $[M+H]^+$ (m/z): 1293.5484, found: 1293.5472. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.84 (s, 2H, $N\underline{H}COC^*H$), 7.89 (s, 1H, CH_{Ar}), 7.75 (d, *J* = 7.1 Hz, 4H, CH_{Ar}), 7.61–7.52 (m, 4H, CH_{Ar}), 7.38 (t, *J* = 7.1 Hz, 4H, CH_{Ar}), 7.31–7.18 (m, 34H, CH_{Ar}), 7.10 (t, *J* = 8.0 Hz, 1H, CH_{Ar}), 7.03 (s, 2H, $N\underline{H}Trt$), 6.98 (d, *J* = 7.4 Hz, 2H, CH_{Ar}), 6.09 (d, *J* = 4.8 Hz, 2H, $N\underline{H}Fmoc$), 4.43–4.30 (m, 4H, $COOCH_2$), 4.20 (t, *J* = 7.1 Hz, 2H, CH), 4.17–4.08 (m, 2H, C*H), 2.67–2.55 (m, 2H, $C^*HCH_2C\underline{H}_2$), 2.50–2.38 (m, 2H, $C^*HCH_2C\underline{H}_2$), 2.19–2.08 (m, 2H, $C^*HC\underline{H}_2$), 2.03–1.89 (m, 2H, $C^*HC\underline{H}_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.5 (2 x CO), 169.5 (2 x CO), 156.5 (2 x CO), 144.5 (6 x C_{Ar}), 143.9 (4 x C_{Ar}), 141.4 (4 x C_{Ar}), 138.2 (2 x C_{Ar}), 128.8 (13 x CH_{Ar}), 128.2 (12 x CH_{Ar}), 127.8 (4 x CH_{Ar}), 127.2 (10 x CH_{Ar}), 125.3 (4 x CH_{Ar}), 120.1 (4 x CH_{Ar}), 115.9 (2 x CH_{Ar}), 111.6 (1 x CH_{Ar}), 71.0 (2 x C), 67.2 (2 x $COO\underline{C}H_2$), 54.4 (2 x C*H), 47.3 (2 x CH), 34.0 (2 x $C^*HCH_2\underline{C}H_2$), 30.4 (2 x $C^*H\underline{C}H_2$).

Synthesis of 1c: this compound was obtained as described above for **1a**, starting from Fmoc-L-Ser(^{t}Bu)-OH. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 25% to 40% AcOEt, Rf of **1c** in AcOEt/hexane, 3:2 (v/v): 0.83) to give 1.05 g of **1c** (45% 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 (br s, 2H, $N\underline{H}COC^*H$), 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 (br s, 2H, $C^*HN\underline{H}CO$), 4.44 (d, *J* = 7.0 Hz, 4H, $COOCH_2$), 4.35 (br s, 2H, C*H), 4.25 (t, *J* = 6.9 Hz, 2H, CH), 3.92 (br s, 2H, $C^*HC\underline{H}_2$), 3.45 (t, *J* = 8.7 Hz, 2H, $C^*HC\underline{H}_2$), 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 $COO\underline{C}H_2$), 61.9 (2 x $C^*H\underline{C}H_2$), 54.8 (2 x C*H), 47.3 (2 x CH), 27.6 (6 x CH_3).

Synthesis of 1d: this compound was obtained as described above for **1a**, starting from Fmoc-L-Thr(^{t}Bu)-OH. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 25% to 40% AcOEt, Rf of **1d** in AcOEt/hexane, 3:7 (v/v): 0.46) to give 1.61 g of **1d** (67% yield) as a white solid. HRMS (ESI–) calcd. for $C_{52}H_{58}N_4O_8$ $[M+HCOO]^-$ (m/z): 911.4237, found: 911.4254. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.24 (s, 2H, N<u>H</u>COC*H), 7.92 (s, 1H, CH_{Ar}), 7.78 (d, *J* = 7.5 Hz, 4H, CH_{Ar}), 7.63 (d, *J* = 7.5 Hz, 4H, CH_{Ar}), 7.41 (t, *J* = 7.5 Hz, 4H, CH_{Ar}), 7.37–7.19 (m, 7H, CH_{Ar}), 6.12 (d, *J* = 4.9 Hz, 2H, C*HN<u>H</u>CO), 4.49–4.21 (m, 10H, 4H x CH_2 + 2H x CH + 2H x C*HNH + 2H x C*<u>H</u>CH_3), 1.38 (s, 18H, C(C<u>H</u>$_3$)$_3$), 1.10 (d, *J* = 6.3 Hz, 6H, C*HC<u>H</u>$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 167.6 (2 x CO), 156.2 (2 x CO), 143.8 (4 x C_{Ar}), 141.4 (4 x C_{Ar}), 138.4 (2 x C_{Ar}), 129.8 (1 x CH_{Ar}), 127.9 (4 x CH_{Ar}), 127.2 (4 x CH_{Ar}), 125.3 (4 x CH_{Ar}), 120.2 (4 x CH_{Ar}), 115.3 (2 x CH_{Ar}), 110.9 (1 x CH_{Ar}), 76.3 (2 x C), 67.2 (2 x CH_2), 67.1 (2 x <u>C</u>*HCH_3), 59.1 (2 x C*HNH), 47.3 (2 x CH), 28.3 (6 x C(<u>C</u>H$_3$)$_3$), 16.9 (2 x C*H<u>C</u>H$_3$).

Synthesis of 1e: this compound was obtained as described above for **1a**, starting from Fmoc-L-Tyr(^{t}Bu)-OH. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 25% to 40% AcOEt, Rf of **1e** in AcOEt/hexane, 2:3 (v/v): 0.46) to give 1.46 g of **1e** (53% yield) as a white solid. HRMS (ESI+) calcd. for $C_{62}H_{62}N_4O_8$ $[M+H]^+$ (m/z): 991.4640, found: 991.4622. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.91 (br s, 2H, N<u>H</u>COC*H), 7.74 (d, *J* = 7.6 Hz, 4H, CH_{Ar}), 7.60–7.48 (m, 5H, CH_{Ar}), 7.37 (t, *J* = 7.5 Hz, 4H, CH_{Ar}), 7.31–7.22 (m, 4H, CH_{Ar}), 7.14–6.99 (m, 7H, CH_{Ar}), 6.86 (d, *J* = 8.4 Hz, 4H, CH_{Ar}), 5.57 (br s, 2H, C*HN<u>H</u>CO), 4.50 (br s, 2H, C*H), 4.44–4.25 (m, 4H, $COOCH_2$), 4.19 (t, *J* = 6.9 Hz, 2H, CH), 3.14–2.93 (m, 4H, C*HC<u>H</u>$_2$), 1.26 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.5 (2 x CO), 156.5 (2 x CO), 154.6 (2 x C_{Ar}), 143.7 (4 x C_{Ar}), 141.4 (4 x C_{Ar}), 137.8 (2 x C_{Ar}), 131.1 (2 x C_{Ar}), 129.9 (4 x CH_{Ar}), 129.5 (1 x CH_{Ar}), 127.9 (4 x CH_{Ar}), 127.3 (4 x CH_{Ar}), 125.2 (4 x CH_{Ar}), 124.6 (4 x CH_{Ar}), 120.1 (4 x CH_{Ar}), 116.1 (2 x CH_{Ar}), 111.7 (1 x CH_{Ar}), 78.7 (2 x C), 67.4 (2 x COO<u>C</u>H$_2$), 57.3 (2 x C*H), 47.2 (2 x CH), 38.0 (2 x C*H<u>C</u>H$_2$), 28.9 (6 x CH_3).

Synthesis of 1f: this compound was obtained as described above for **1a**, starting from Fmoc-L-Trp(Boc)-OH. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 30% to 35% AcOEt, Rf of **1f** in AcOEt/hexane, 2:3 (v/v): 0.59) to give 1.33 g of **1f** (43% yield) as a white solid. HRMS (ESI–) calcd. for $C_{68}H_{64}N_6O_{10}$ $[M+HCOO]^-$ (m/z): 1169.4666, found:1169.5189. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.23 (br s, 2H, N<u>H</u>COC*H), 8.08 (br s, 2H, CH_{Ar}), 7.71 (d, *J* = 7.6 Hz, 4H, CH_{Ar}), 7.62–7.40 (m, 9H, CH_{Ar}), 7.34 (t, *J* = 7.5 Hz, 4H, CH_{Ar}), 7.29–6.98 (m, 11H, CH_{Ar}), 5.72 (br s, 2H, C*HN<u>H</u>CO), 4.67 (br s, 2H, C*H), 4.33 (br s, 4H, $COOCH_2$), 4.18–4.10 (m, 2H, $COOCH_2C\underline{H}$), 3.30–3.06 (m, 4H, $C^*HC\underline{H}_2$), 1.56 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.7 (2 x CO), 156.6 (2 x CO), 149.6 (2 x CO), 143.7 (4 x C_{Ar}), 141.4 (4 x C_{Ar}), 137.8 (2 x C_{Ar}), 135.6 (2 x C_{Ar}), 130.2 (2 x C_{Ar}), 129.4 (1 x CH_{Ar}), 127.8 (4 x CH_{Ar}), 127.2 (4 x CH_{Ar}), 125.2 (4 x CH_{Ar}), 124.8 (2 x CH_{Ar}), 124.6 (2 x CH_{Ar}), 122.9 (2 x CH_{Ar}), 120.1 (4 x CH_{Ar}), 119.1 (2 x CH_{Ar}), 116.3 (2 x CH_{Ar}), 115.5 (2 x CH_{Ar}), 115.3 (2 x C_{Ar}), 112.0 (1 x CH_{Ar}), 83.9 (2 x C), 67.5 (2 x $COO\underline{C}H_2$), 55.8 (2 x C*H), 47.1 (2 x CH), 28.2 (6 x CH_3), 28.1 (2 x $C^*H\underline{C}H_2$).

Synthesis of 1g: this compound was obtained as described above for **1a**, starting from Fmoc-L-Asp(^{t}Bu)-OH. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 25% to 40% AcOEt, Rf of **1g** in AcOEt/hexane, 2:3 (v/v): 0.34) to give 978 mg of **1g** (42% yield) as a white solid. HRMS (ESI+) calcd. for $C_{52}H_{54}N_4O_{10}$ $[M+Na]^+$ (m/z): 917.3732, found: 917.3764. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.57 (s, 2H, N<u>H</u>COC*H), 7.80 (s, 1H, CH_{Ar}), 7.76 (d, *J* = 7.4 Hz, 4H, CH_{Ar}), 7.59 (d, *J* = 6.3 Hz, 4H, CH_{Ar}), 7.39 (t, *J* = 7.3 Hz, 4H, CH_{Ar}), 7.34 – 7.20 (m, 7H, CH_{Ar}), 6.11 (d, *J* = 7.4 Hz, 2H, C*HN<u>H</u>CO), 4.66 (br s, 2H, C*H), 4.45 (d, *J* = 6.4 Hz, 4H, $COOCH_2$), 4.23 (t, *J* = 6.9 Hz, 2H, CH), 2.96 (d, *J* = 16.0 Hz, 2H, $C^*HC\underline{H}_2$), 2.69 (dd, *J* = 17.0, 6.7 Hz, 2H, $C^*HC\underline{H}_2$), 1.45 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.5 (2 x CO), 168.7 (2 x CO), 156.4 (2 x CO), 143.8 (4 x C_{Ar}), 141.5 (4 x C_{Ar}), 138.2 (2 x C_{Ar}), 129.7 (1 x CH_{Ar}), 128.0 (4 x CH_{Ar}), 127.3 (4 x CH_{Ar}), 125.2 (4 x CH_{Ar}), 120.2 (4 x CH_{Ar}), 116.1 (2 x CH_{Ar}), 111.5 (1 x CH_{Ar}), 82.5 (2 x C), 67.5 (2 x $COO\underline{C}H_2$), 51.9 (2 x C*H), 47.3 (2 x CH), 37.5 (2 x $C^*H\underline{C}H_2$), 28.2 (6 x CH_3).

Synthesis of 1h: this compound was obtained as described above for **1a**, starting from Fmoc-L-Glu(^{t}Bu)-OH. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 30% to 40% AcOEt, Rf of **1h** in AcOEt/hexane, 2:3 (v/v): 0.43) to give 1.79 g of **1h** (70% yield) as a white solid. HRMS (ESI+) calcd. for $C_{54}H_{58}N_4O_{10}$ $[M+H]^+$ (m/z): 923.4226, found: 923.4225. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.62 (s, 2H, N<u>H</u>COC*H), 7.84 (s, 1H, CH_{Ar}), 7.75 (d, *J* = 7.5 Hz, 4H, CH_{Ar}), 7.59 (t, *J* = 6.8 Hz, 4H, CH_{Ar}), 7.38 (t, *J* = 7.4 Hz, 4H, CH_{Ar}), 7.33–7.16 (m, 7H, CH_{Ar}), 5.94 (d, *J* = 7.1 Hz, 2H, C*HN<u>H</u>CO), 4.38–4.28 (m, 6H, 4H x $COOCH_2$ + 2H x C*H), 4.21 (t, *J* = 7.0 Hz, 2H, CH), 2.59 – 2.46 (m, 2H, $C^*HCH_2C\underline{H}_2$), 2.43–2.30 (m, 2H, $C^*HCH_2C\underline{H}_2$), 2.22–2.09 (m, 2H, $C^*HC\underline{H}_2$), 2.04–1.93 (m, 2H, $C^*HC\underline{H}_2$), 1.46 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.4 (2 x CO), 169.7 (2 x CO), 156.7 (2 x CO), 143.8 (4 x C_{Ar}), 141.4 (4 x C_{Ar}), 138.2 (2 x C_{Ar}), 129.6 (1 x CH_{Ar}), 127.9 (4 x CH_{Ar}), 127.2

(4 x CH_{Ar}), 125.2 (4 x CH_{Ar}), 120.1 (4 x CH_{Ar}), 115.9 (2 x CH_{Ar}), 111.4 (1 x CH_{Ar}), 81.6 (2 x C), 67.4 (2 x $COO\underline{C}H_2$), 55.2 (2 x C*H), 47.2 (2 x CH), 32.1 (2 x $C^*HCH_2\underline{C}H_2$), 28.3 (2 x $C^*H\underline{C}H_2$), 28.2 (6 x CH_3).

Synthesis of 1i: To a solution of Boc-L-Lys(Cbz)-OH (2.30 g, 6.05 mmol) in dry DMF (15 mL), HBTU (2.55 g, 6.74 mmol) and DIPEA (2.3 mL, 13 mmol) were added. The resulting mixture was cooled down to 0 °C in an ice-water bath. Then, a solution of *m*-phenylenediamine (302 mg, 2.79 mmol) in dry DMF (10 mL) was added via cannula under inert atmosphere of Ar. The mixture was stirred at room temperature for 60 hours, after which complete conversion of the starting material was observed by TLC (Rf of **1i** in AcOEt/hexane, 3:2 (v/v): 0.41). The mixture 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 AcOEt/hexane as eluent (from 40% to 50% AcOEt) to give 1.77 g of **1i** (56% yield) as a white solid. HRMS (ESI+) calcd. for $C_{44}H_{60}N_6O_{10}$ $[M+H]^+$ (m/z): 833.4444, found: 833.4453. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.95 (br s, 2H, $N\underline{H}COC^*H$), 7.73 (br s, 1H, CH_{Ar}), 7.55–7.16 (m, 12H, CH_{Ar}), 7.16–6.97 (m, 1H, CH_{Ar}), 5.72 (br s, 2H, $C^*HN\underline{H}CO$), 5.33–4.84 (m, 6H, 4H x $NHCOOC\underline{H}_2$ + 2H x $N\underline{H}$Cbz), 4.24 (br s, 2H, C*H), 3.16 (br s, 4H, $C\underline{H}_2$NHCbz), 2.00–1.59 (m, 4H, $C^*H\ C\underline{H}_2$), 1.58–1.18 (m, 26H, 4H x $C\underline{H}_2CH_2$NHCbz + 4H x $C^*HCH_2C\underline{H}_2$ + 18H x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.5 (2 x CO), 156.8 (2 x CO), 156.4 (2 x CO), 138.5 (2 x C_{Ar}), 136.7 (2 x C_{Ar}), 129.5 (1 x CH_{Ar}), 128.6 (4 x CH_{Ar}), 128.2 (6 x CH_{Ar}), 115.7 (2 x CH_{Ar}), 111.3 (1 x CH_{Ar}), 80.4 (2 x C), 66.8 (2 x $NHCOO\underline{C}H_2$), 55.4 (2 x C*H), 40.7 (2 x $\underline{C}H_2$NHCbz), 32.2 (2 x $C^*H\underline{C}H_2$), 29.5 (2 x $\underline{C}H_2CH_2$NHCbz), 28.5 (6 x CH_3), 22.9 (2 x $C^*HCH_2\underline{C}H_2$).

Synthesis 1j: This compound was synthesized following the procedure described for **1i** starting from Boc-L-Orn(Alloc)-OH. The crude product was purified by flash chromatography using AcOEt/hexane as eluent (from 45% to 55% AcOEt, Rf of **1j** in AcOEt/hexane, 2:3 (v/v): 0.23) to give 1.43 g of **1j** (85% yield) as a white solid. HRMS (ESI+) calcd. for $C_{34}H_{52}N_6O_{10}$ $[M+H]^+$ (m/z): 705.3818, found: 705.3813. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.92 (br s, 2H, $N\underline{H}COC^*H$), 7.77 (br s, 1H, CH_{Ar}), 7.36–6.97 (m, 3H, CH_{Ar}), 5.89 (ddt, *J* = 17.2, 10.8, 5.6 Hz, 2H, $NHCOOCH_2C\underline{H}CH_2$), 5.66 (br s, 2H, $C^*HN\underline{H}CO$), 5.28 (dq, *J* = 17.2, 1.6 Hz, 2H, $NHCOOCH_2CHC\underline{H}_2$), 5.22–5.07 (m, 4H, 2H x $NHCOOCH_2CHC\underline{H}_2$ + 2H x $N\underline{H}$Alloc), 4.58 (d, *J* = 5.5 Hz, 4H, $NHCOOC\underline{H}_2CHCH_2$), 4.43 (br s, 2H, C*H), 3.40 (br s, 2H, $C\underline{H}_2$NHAlloc), 3.24–3.02 (m, 2H, $C\underline{H}_2$NHAlloc), 1.96–1.77 (m, 2H, $C^*HC\underline{H}_2$), 1.76–1.54 (m, 6H, 2H x $C^*HC\underline{H}_2$ + 4H x $C^*HCH_2C\underline{H}_2$), 1.43 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.3 (2 x CO), 157.2 (2 x CO), 156.4 (2 x CO), 138.5 (2 x C_{Ar}), 133.0 (2 x $NHCOOCH_2\underline{C}HCH_2$), 129.4 (1 x CH_{Ar}), 117.7 (2 x $NHCOOCH_2CH\underline{C}H_2$), 115.7 (2 x CH_{Ar}), 111.5 (1 x CH_{Ar}), 80.3 (2 x C), 65.9 (2 x $NHCOO\underline{C}H_2CHCH_2$), 53.9 (2 x C*H), 38.9 (2 x $\underline{C}H_2$NHAlloc), 30.3 (2 x $C^*H\underline{C}H_2$), 28.5 (6 x CH_3), 26.6 (2 x $C^*HCH_2\underline{C}H_2$).

Synthesis of 1l: this compound was obtained as described above for **1a** starting from Boc-L-Cys(Trt)-OH. In this case dry DCM was used instead of dry DMF as solvent. The crude product was purified by flash chromatography using AcOEt/hexane as eluent (from 25% to 40% AcOEt, Rf of **1l** in AcOEt/hexane, 1:2 (v/v): 0.39) to give 1.49 g of **1l**. (29% yield) as a white solid. HRMS (ESI+) calcd. for $C_{60}H_{62}N_4O_6S_2$ $[M+H]^+$ (m/z): 999.4184, found: 999.4145. ^1H NMR (500 MHz, $CDCl_3$): δ = 8.02 (br s, 2H, $N\underline{H}COC^*H$), 7.64 (s, 1H, CH_{Ar}), 7.44 (d, J = 7.1 Hz, 12H, CH_{Ar}), 7.30 (t, J = 7.6 Hz, 12H, CH_{Ar}), 7.25–7.13 (m, 9H, CH_{Ar}), 4.81 (br s, 2H, $C^*HN\underline{H}CO$), 3.94 (br s, 2H, C*H), 2.85–2.70 (m, 2H, CH_2), 2.62 (dd, J = 13.2, 5.1 Hz, 2H, CH_2), 1.43 (s, 18H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.9 (2 x CO), 156.0 (2 x CO), 144.5 (6 x C_{Ar}), 138.1 (2 x C_{Ar}), 129.7 (12 x CH_{Ar}), 129.5 (1 x CH_{Ar}), 128.2 (12 x CH_{Ar}), 127.1 (6 x CH_{Ar}), 115.7 (2 x CH_{Ar}), 111.0 (1 x CH_{Ar}), 80.9 (2 x C), 67.5 (2 x C), 54.4 (2 x C*H), 33.6 (2 x CH_2), 28.4 (6 x CH_3).

Synthesis of intermediates 2a-j

Synthesis of 2a: compound **1a** (600 mg, 0.47 mmol) was dissolved in 4.0 mL of 20% piperidine in dry DMF. After several minutes stirring at room temperature the product precipitated as a white solid but the mixture was allowed to react 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, obtaining 293 mg of diamine **2a** (75% yield) as a white solid. HRMS (ESI+) calcd. for $C_{52}H_{48}N_6O_4$ $[M+H]^+$ (m/z): 821.3810, found: 821.3832. ^{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).

Synthesis of 2b: 531 mg of **2b** (white solid, 74% yield) were obtained from **1b** as described above for **2a**. HRMS (ESI+) calcd. for $C_{54}H_{52}N_6O_4$ $[M+H]^+$ (m/z): 849.4123, found: 849.4135. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.46 (s, 2H, N$\underline{H}$COC*H), 7.82 (t, *J* = 1.8 Hz, 1H, CH_{Ar}), 7.35–7.19 (m, 33H, CH_{Ar}), 6.93 (s, 2H, N$\underline{H}$Trt), 3.40 (t, *J* = 6.5 Hz, 2H, C*H), 2.53–2.45 (m, 4H, C*H$CH_2C\underline{H}_2$), 2.13–1.94 (m, 4H, C*HC$\underline{H}_2$), 1.68 (br s, 4H, NH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.3 (2 x CO), 171.8 (2 x CO), 144.7 (6 x C_{Ar}), 138.4 (2 x C_{Ar}), 129.7 (1 x CH_{Ar}) , 128.8 (12 x CH_{Ar}), 128.1 (12 x CH_{Ar}), 127.2 (6 x CH_{Ar}), 115.2 (2 x CH_{Ar}), 110.5 (1 x CH_{Ar}), 70.7 (2 x C), 54.8 (2 x C*H), 34.1 (2 x C*H$CH_2\underline{C}H_2$), 31.0 (2 x C*H$\underline{C}H_2$).

Synthesis of 2c: 522 mg of **2c** (white solid, quantitative yield) were obtained from **1c** as described above for **2a**. 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 (br s, 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).

Synthesis of 2d: 445 mg of **2d** (white solid, 64% yield) were obtained from **1d** as described above for **2a**. HRMS (ESI+) calcd. for $C_{22}H_{38}N_4O_4$ $[M+H]^+$ (m/z): 423.2966, found: 423.2956. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.63 (s, 2H, N$\underline{H}$COC*H), 7.82 (t, *J* = 2.1 Hz, 1H, CH_{Ar}), 7.40–7.33 (m, 2H, CH_{Ar}), 7.32–7.23 (m, 1H, CH_{Ar}), 4.23 (qd, *J* = 6.3, 2.5 Hz, 2H, C*$\underline{H}CH_3$), 3.25 (d, *J* = 2.5 Hz, 2H, C*$\underline{H}NH_2$), 1.95 (br s, 4H, NH_2), 1.21 (d, J = 6.3 Hz, 6H, C*HC$\underline{H}_3$), 1.17 (s, 18H, C(C$\underline{H}_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.3 (2 x CO), 138.7 (2 x C_{Ar}), 129.6 (1 x CH_{Ar}), 115.0 (2 x CH_{Ar}), 110.4 (1 x CH_{Ar}), 74.4 (2 x C), 67.8 (2 x $\underline{C}$*HCH_3), 60.4 (2 x C*HNH_2), 28.6 (6 x C($\underline{C}H_3)_3$), 20.5 (2 x C*H$\underline{C}H_3$).

Synthesis of 2e: 812 g of **2e** (white solid, quantitative yield) were obtained from **1e** as described above for **2a**. HRMS (ESI+) calcd. for $C_{32}H_{42}N_4O_4$ $[M+H]^+$ (m/z): 547.3279, found: 547.3280. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.48 (s, 2H, N$\underline{H}$COC*H), 7.92 (t, J = 2.1 Hz, 1H, CH_{Ar}), 7.40 (dd, J = 8.1, 2.0 Hz, 2H, CH_{Ar}), 7.32–7.25 (m, 1H, CH_{Ar}), 7.14 (d, J = 8.4 Hz, 4H, CH_{Ar}), 6.95 (d, J = 8.4 Hz, 4H, CH_{Ar}), 3.70 (dd, J = 9.6, 3.8 Hz, 2H, C*H), 3.32 (dd, J = 14.0, 3.8 Hz, 2H, C*HC$\underline{H}_2$), 2.72 (dd, J = 13.9, 9.7 Hz, 2H, C*HC$\underline{H}_2$), 1.99 (br s, 4H, NH_2), 1.33 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.8 (2 x CO), 154.5 (2 x C_{Ar}), 138.4 (2 x C_{Ar}), 132.5 (2 x C_{Ar}), 129.8 (4 x CH_{Ar}), 129.7 (1 x CH_{Ar}), 124.6 (4 x CH_{Ar}), 115.2 (2 x CH_{Ar}), 110.4 (1 x CH_{Ar}), 78.6 (2 x C), 57.0 (2 x C*H), 40.2 (2 x CH_2), 29.0 (6 x CH_3).

Synthesis of 2f: 781 mg of **2f** (white solid, quantitative yield) were obtained from **1f** as described above for **2a**. HRMS (ESI+) calcd. for $C_{38}H_{44}N_6O_6$ $[M+H]^+$ (m/z): 681.3395, found: 681.3399. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.57 (s, 2H, N$\underline{H}$COC*H), 8.14 (d, J = 8.3 Hz, 2H, CH_{Ar}), 7.94 (t, J = 2.1 Hz, 1H, CH_{Ar}), 7.65 (dd, J = 7.7, 1.0 Hz, 2H, CH_{Ar}), 7.50 (s, 2H, CH_{Ar}), 7.43 (dd, J = 7.8, 2.1 Hz, 2H, CH_{Ar}), 7.38–7.22 (m, 5H, CH_{Ar}), 3.84 (dd, J = 9.8, 3.6 Hz, 2H, C*H), 3.49 (ddd, J = 14.7, 3.7, 1.2 Hz, 2H, C*HC$\underline{H}_2$), 2.88 (dd, J = 14.8, 9.6 Hz, 2H, C*HC$\underline{H}_2$), 1.66 (s, 18H, CH_3), 1.58 (s, 4H, NH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.7 (2 x CO), 149.7 (2 x CO), 138.4 (2 x C_{Ar}), 135.8 (2 x C_{Ar}), 130.3 (2 x C_{Ar}), 129.7 (1 x CH_{Ar}), 124.9 (2 x CH_{Ar}), 124.3 (2 x CH_{Ar}), 122.9 (2 x CH_{Ar}), 119.3 (2 x CH_{Ar}), 116.7 (2 x CH_{Ar}), 115.5 (2 x CH_{Ar}), 115.2 (2 x C_{Ar}), 110.5 (1 x CH_{Ar}), 83.9 (2 x C), 55.4 (2 x C*H), 30.6 (2 x CH_2), 28.3 (6 x CH_3).

Synthesis of 2g: 531 mg of **2g** (white solid, quantitative yield) were obtained from **1g** as described above for **2a**. HRMS (ESI+) calcd. for $C_{22}H_{34}N_4O_6$ $[M+H]^+$ (m/z): 451.2551, found: 451.2560. ^{1}H NMR (400 MHz, MeOD-d_4): δ = 7.92 (t, J = 2.0 Hz, 1H, CH_{Ar}), 7.38–7.34 (m, 2H, CH_{Ar}), 7.29–7.24 (m, 1H, CH_{Ar}), 3.75 (dd, J = 6.7, 6.0 Hz, 2H, C*H), 2.74 (dd, J = 16.2, 6.0 Hz, 2H, CH_2), 2.62 (dd, J = 16.2, 6.7 Hz, 2H, CH_2), 1.43 (s, 18H, CH_3). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 174.5 (2 x CO), 172.1 (2 x CO), 140.0 (2 x C_{Ar}), 130.2(1 x CH_{Ar}), 116.9 (2 x CH_{Ar}), 112.8 (1 x CH_{Ar}), 82.3 (2 x C), 53.6 (2 x C*H), 41.5 (2 x CH_2), 28.3 (6 x CH_3).

Synthesis of 2h: 1.05 g of **2h** (white solid, quantitative yield) were obtained from **1h** as described above for **2a**. HRMS (ESI+) calcd. for $C_{24}H_{38}N_4O_6$ $[M+H]^+$ (m/z): 479.2864, found: 479.2882. ^{1}H NMR (400 MHz, MeOD-d_4): δ = 7.94 (t, J = 2.0 Hz, 1H, CH_{Ar}), 7.37–7.33 (m, 2H, CH_{Ar}), 7.30–7.23 (m, 1H, CH_{Ar}), 3.45 (dd, J = 7.2, 6.1 Hz, 2H, C*H), 2.44–2.30 (m, 4H, $C^*HCH_2C\underline{H}_2$), 2.07–1.96 (m, 2H, $C^*HC\underline{H}_2$), 1.92–1.80 (m, 2H, $C^*HC\underline{H}_2$), 1.43 (s, 18H, CH_3). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 175.5 (2 x CO), 174.2 (2 x CO), 140.0 (2 x C_{Ar}), 130.2 (1 x CH_{Ar}), 117.1(2 x CH_{Ar}), 113.1 (1 x CH_{Ar}), 81.7 (2 x C), 56.1 (2 x C*H), 32.7 (2 x $C^*HCH_2\underline{C}H_2$), 31.5 (2 x $C^*H\underline{C}H_2$), 28.3 (6 x CH_3).

Synthesis of 2i·2TFA: 530 mg of **1i** (0.72 mmol) were dissolved in DCM (10 mL) and TFA (1.5 mL) was added. The mixture was stirred at room temperature for 4 hours and then concentrated under reduced pressure. Diethyl ether was then added over the residue and the precipitate formed was filtered and washed with diethyl ether, obtaining 474 mg of **2i**·2TFA (white solid, 93% yield) HRMS (ESI+) calcd. for $C_{34}H_{44}N_6O_6$ $[M+H]^+$ (m/z): 633.3395, found: 633.3389. ^{1}H NMR (500 MHz, MeOD-d_4): δ = 8.10 (t, J = 2.0 Hz, 1H, CH_{Ar}), 7.41–7.24 (m, 13H, CH_{Ar}), 5.01 (ABq, δ_A = 5.04, δ_B = 4.99, J = 12.5 Hz, 4H, $NHCOOC\underline{H}_2$), 3.95 (t, J = 6.5 Hz, 2H, C*H), 3.13 (t, J = 6.8 Hz, 4H, $C\underline{H}_2NHCbz$), 2.04–1.84 (m, 4H, $C^*HC\underline{H}_2$), 1.56 (quint, J = 7.0 Hz, 4H, $C\underline{H}_2CH_2NHCbz$), 1.51–1.38 (m, 4H, $C^*HCH_2C\underline{H}_2$). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 168.6 (2 x CO), 159.0 (2 x CO), 139.7 (2 x C_{Ar}), 138.3 (2 x C_{Ar}), 130.5 (1 x CH_{Ar}), 129.4 (4 x CH_{Ar}), 128.9 (2 x CH_{Ar}), 128.7 (4 x CH_{Ar}), 117.4 (2 x CH_{Ar}), 113.0 (1 x CH_{Ar}), 67.4 (2 x $NHCOO\underline{C}H_2$), 55.1 (2 x C*H), 41.1 (2 x $\underline{C}H_2NHCbz$), 32.3 (2 x $C^*H\underline{C}H_2$), 30.5 (2 x $\underline{C}H_2CH_2NHCbz$), 23.0 (2 x $C^*HCH_2\underline{C}H_2$).

Synthesis of 2j·2TFA: 1.27 g of **2j**·2TFA (white solid, 94% yield) were obtained from **1j** as described above for **2i**·2TFA. HRMS (ESI+) calcd. for $C_{24}H_{36}N_6O_6$ $[M+H]^+$ (m/z): 505.2769, found: 505.2786. ^{1}H NMR (400 MHz, MeOD-d_4): δ = 8.08 (t, J = 1.8 Hz, 1H, CH_{Ar}), 7.40–7.35 (m, 2H, CH_{Ar}), 7.34–7.29 (m, 1H, CH_{Ar}), 5.90 (ddt, J = 17.3, 10.6, 5.4 Hz, 2H, $NHCOOCH_2C\underline{H}CH_2$), 5.27 (dd, J = 17.3, 1.7 Hz, 2H, $NHCOOCH_2CHC\underline{H}_2$), 5.15 (dd, J = 10.3, 1.0 Hz, 2H, $NHCOOCH_2CHC\underline{H}_2$), 4.52 (dt, J = 5.4, 1.5 Hz, 4H, $NHCOOC\underline{H}_2CHCH_2$), 4.02 (t, J = 6.5 Hz, 2H, C*H), 3.18 (td, J = 6.8, 1.7 Hz, 4H, $C\underline{H}_2NHAlloc$), 2.0–1.85 (m, 4H, $C^*HC\underline{H}_2$), 1.74–1.55 (m, 4H, $C^*HCH_2C\underline{H}_2$). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 168.5 (2 x CO), 159.0 (2 x CO), 139.7 (2 x C_{Ar}), 134.4 (2 x $NHCOOCH_2\underline{C}HCH_2$), 130.5 (1 x CH_{Ar}), 117.5 (2 x $NHCOOCH_2CH\underline{C}H_2$), 117.3 (2 x CH_{Ar}), 112.9 (1 x CH_{Ar}), 66.4 (2 x $NHCOO\underline{C}H_2CHCH_2$), 54.8 (2 x C*H), 40.8 (2 x $\underline{C}H_2NHAlloc$), 30.1 (2 x $C^*H\underline{C}H_2$), 26.6 (2 x $C^*HCH_2C\underline{H}_2$).

Synthesis of intermediates 3a-k

Synthesis of 3a: tritylsulfanyl acetic acid (501 mg, 1.50 mmol) was dissolved in dry DMF (20 mL) and EDC·HCl (312 mg, 1.63 mmol), HOBt (228 mg, 1.69 mmol) and DIPEA (1.6 mL, 4.59 mmol) were added over the solution. The reaction mixture was cooled down to 0 °C in an ice-water bath and **2a** (585 mg, 0.715 mmol) was added over the mixture. The mixture was stirred at room temperature under an inert atmosphere of Ar for 48 hours, and the formation of the product was followed by TLC. The mixture was diluted with DCM, washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, and dried under reduced pressure. The crude product was purified by flash chromatography using AcOEt/hexane as eluent (from 40% to 60% AcOEt, Rf of **3a** in AcOEt/hexane, 1:1 (v/v): 0.46) to give 758 mg of **3a** (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, N<u>H</u>COC*H), 7.60 (t, *J* = 2.0 Hz, 1H, CH_{Ar}), 7.54–7.00 (m, 65H, 2H x C*HN<u>H</u>CO + 63H x CH_{Ar}), 6.91 (s, 2H, N<u>H</u>Trt), 4.49 (td, *J* = 7.5, 3.0 Hz, 2H, C*H), 3.05 (ABq, δ_A = 3.08, δ_B = 3.02, *J* = 15.7 Hz, 4H, C<u>H</u>$_2$STrt), 2.96–2.83 (m, 2H, C<u>H</u>$_2$C*H), 2.39 (dd, *J* = 15.7, 7.8 Hz, 2H, C<u>H</u>$_2$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 <u>C</u>H$_2$C*H), 36.2 (2 x <u>C</u>H$_2$STrt).

Synthesis of 3b: this compound was obtained as described above for **3a**, starting from **2b**. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 30% to 40% AcOEt, Rf of **3b** in AcOEt/hexane, 1:1 (v/v): 0.23) to give 593 mg of **3b** (66% yield) as a white solid. HRMS (ESI+) calcd. for $C_{96}H_{84}N_6O_6S_2$ $[M+H]^+$ (m/z): 1481.5967, found: 1481.5916. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.76 (s, 2H, N<u>H</u>COC*H), 7.73 (t, *J* = 1.9 Hz, 1H, CH_{Ar}), 7.44–7.38 (m, 12H, CH_{Ar}), 7.31–6.96 (m, 55H, 51H x CH_{Ar} + 2H x C*HN<u>H</u>CO + 2H x N<u>H</u>Trt), 4.03 (q, *J* = 6.8 Hz, 2H, C*H), 3.06 (ABq, δ_A = 3.07, δ_B = 3.05, *J* = 15.8Hz, 4H, C<u>H</u>$_2$STrt), 2.59–2.49 (m, 2H, C*HCH$_2$C<u>H</u>$_2$), 2.39–2.29 (m, 2H, C*HCH$_2$C<u>H</u>$_2$), 2.03–1.92 (m, 2H, C*HC<u>H</u>$_2$), 1.81–1.70 (m, 2H, C*HC<u>H</u>$_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.5 (2 x CO), 168.8 (2 x CO), 168.6 (2 x CO), 144.5 (6 x C_{Ar}), 144.1 (6 x C_{Ar}), 138.2 (2 x C_{Ar}), 129.7 (12 x CH_{Ar}), 129.2 (1 x CH_{Ar}), 128.8 (12 x CH_{Ar}), 128.3 (12 x CH_{Ar}), 128.1 (12 x CH_{Ar}), 127.2 (6 x CH_{Ar}), 127.1(6 x CH_{Ar}), 115.9 (2 x CH_{Ar}), 111.4 (1 x CH_{Ar}), 70.9 (2 x C), 68.0 (2 x C), 53.1 (2 x C*H), 36.3 (2 x $\underline{C}H_2STrt$), 34.2 (2 x $C^*HCH_2\underline{C}H_2$), 30.3 (2 x $C^*H\underline{C}H_2$).

Synthesis of 3c: this compound was obtained as described above for **3a** starting from **2c**. The crude product was purified by flash chromatography using AcOEt/hexane as eluent (from 35% to 45% AcOEt, Rf of **3c** in AcOEt/hexane, 2:3 (v/v): 0.27) to give 612 mg of **3c** (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. ^1H NMR (500 MHz, $CDCl_3$): δ = 8.68 (s, 2H, $N\underline{H}COC^*H$), 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, $C^*HN\underline{H}CO$), 4.24–4.18 (dt, *J* = 9.7, 4.6 Hz, 2H, C*H), 3.71 (dd, *J* = 8.6, 4.3 Hz, 2H, $C^*HC\underline{H}_2$), 3.20–3.08 (m, 6H, 2H x $C^*HC\underline{H}_2$ + 4H x $C\underline{H}_2STrt$), 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 $C^*H\underline{C}H_2$), 53.5 (2 x C*H), 36.2 (2 x $\underline{C}H_2STrt$), 27.6 (6 x CH_3).

Synthesis of 3d: this compound was obtained as described above for **3a** starting from **2d**. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 20% to 35% AcOEt, Rf of **3d** in AcOEt/hexane, 3:7 (v/v): 0.28) to give 608 mg of **3d** (57% yield) as a white solid. HRMS (ESI–) calcd. for $C_{64}H_{70}N_4O_6S_2$ $[M+HCOO]^-$ (m/z): 1099.4719, found: 1099.4722. ^1H NMR (500 MHz, $CDCl_3$): δ = 9.16 (s, 2H, $N\underline{H}COC^*H$), 7.70 (s, 1H, CH_{Ar}), 7.43 (d, *J* = 7.5 Hz, 12H, CH_{Ar}), 7.33–7.17 (m, 23H, 21H x CH_{Ar} + 2H x $C^*HN\underline{H}CO$), 4.24–4.13 (m, 4H, 2H x $C^*\underline{H}NH$ + 2H x $C^*\underline{H}CH_3$), 3.07 (ABq, δ_A = 3.10, δ_B = 3.03, *J* = 15.4 Hz, 4H, CH_2), 1.33 (s, 18H, $C(C\underline{H}_3)_3$), 0.94 (d, *J* = 6.4 Hz, 6H, $C^*HC\underline{H}_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.5 (2 x CO), 167.4 (2 x CO), 144.1 (6 x C_{Ar}), 138.4 (2 x C_{Ar}), 129.9 (1 x CH_{Ar}), 129.7 (12 x CH_{Ar}), 128.2 (12 x CH_{Ar}), 127.1 (6 x CH_{Ar}), 115.3 (2 x CH_{Ar}), 110.7 (1 x CH_{Ar}), 76.3 (2 x C), 67.9 (2 x C), 66.2 (2 x $\underline{C}^*HCH_3$), 58.3 (2 x C*HNH), 36.6 (2 x CH_2), 28.3 (6 x $C(\underline{C}H_3)_3$), 17.0 (2 x $C^*H\underline{C}H_3$).

Synthesis of 3e: this compound was obtained as described above for **3a**, starting from **2e**. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 30% to 40% AcOEt, Rf of **3e** in AcOEt/hexane, 2:3 (v/v): 0.39) to give 1.07 g of **3e** (79% yield) as a white solid. HRMS (ESI–) calcd. for $C_{74}H_{74}N_4O_6S_2$ $[M+HCOO]^-$ (m/z): 1223.5032, found: 1223.4956. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.73 (s, 2H, $N\underline{H}COC^*H$), 7.51 (t, *J* = 2.0 Hz, 1H, CH_{Ar}), 7.36 (d, *J* = 7.2 Hz, 12H, CH_{Ar}), 7.29–7.22 (m, 14H, CH_{Ar}), 7.18 (t, *J* = 7.3 Hz, 6H,

CH_{Ar}), 7.14–7.09 (m, 1H, CH_{Ar}), 7.06 (d, *J* = 8.4 Hz, 4H, CH_{Ar}), 6.86 (d, *J* = 8.4 Hz, 4H, CH_{Ar}), 6.61 (d, *J* = 7.3 Hz, 2H, C*HN<u>H</u>CO), 4.37 (q, *J* = 7.1 Hz, 2H, C*H), 3.12 (ABq, δ_A = 3.15, δ_B = 3.09, *J* = 16.3 Hz, 4H, C<u>H</u>$_2$STrt), 2.91 (d, *J* = 7.1 Hz, 4H, C*HC<u>H</u>$_2$), 1.28 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.0 (2 x CO), 168.4 (2 x CO), 154.6 (2 x C_{Ar}), 144.0 (6 x C_{Ar}), 138.0 (2 x C_{Ar}), 131.1 (2 x C_{Ar}), 130.0 (4 x CH_{Ar}), 129.6 (12 x CH_{Ar}), 129.4 (1 x CH_{Ar}), 128.4 (12 x CH_{Ar}), 127.3 (6 x CH_{Ar}), 124.6 (4 x CH_{Ar}), 115.9 (2 x CH_{Ar}), 111.3 (1 x CH_{Ar}), 78.6 (2 x C), 68.2 (2 x C), 55.9 (2 x C*H), 36.9 (2 x C*H<u>C</u>H$_2$), 36.0 (2 x <u>C</u>H$_2$STrt), 29.0 (6 x CH_3).

Synthesis of 3f: this compound was obtained as described above for **3a** starting from **2f**. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 30% to 40% AcOEt, Rf of **3f** in AcOEt/hexane, 2:3 (v/v): 0.54) to give 977 mg of **3f** (88% yield) as a white solid. HRMS (ESI+) calcd. for $C_{80}H_{76}N_6O_8S_2$ $[M+Na]^+$ (m/z): 1335.5058, found: 1335.5060. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (d, *J* = 8.4 Hz, 2H, CH_{Ar}), 7.94 (s, 2H, N<u>H</u>COC*H), 7.55 (d, *J* = 7.8 Hz, 2H, CH_{Ar}), 7.46 (s, 1H, CH_{Ar}), 7.40 (s, 2H, CH_{Ar}), 7.34 (d, *J* = 7.4 Hz, 12H, CH_{Ar}), 7.29–7.02 (m, 25H, CH_{Ar}), 6.61 (d, *J* = 7.2 Hz, 2H, C*HN<u>H</u>CO), 4.52 (q, *J* = 7.0 Hz, 2H, C*H), 3.21–2.98 (m, 8H, 4H x C<u>H</u>$_2$STrt + 4H x C*HC<u>H</u>$_2$), 1.59 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.2 (2 x CO), 168.5 (2 x CO), 149.6 (2 x CO), 144.0 (6 x C_{Ar}), 138.0 (2 x C_{Ar}), 135.6 (2 x C_{Ar}), 130.2 (2 x C_{Ar}), 129.6 (12 x CH_{Ar}), 129.4 (1 x CH_{Ar}), 128.3 (12 x CH_{Ar}), 127.2 (6 x CH_{Ar}), 124.9 (2 x CH_{Ar}), 124.5 (2 x CH_{Ar}), 123.0 (2 x CH_{Ar}), 119.2 (2 x CH_{Ar}), 116.1 (2 x CH_{Ar}), 115.4 (2 x CH_{Ar}), 115.3 (2 x C_{Ar}), 111.7 (1 x CH_{Ar}), 83.8 (2 x C), 68.2 (2 x C), 54.5 (2 x C*H), 36.0 (2 x CO<u>C</u>H$_2$), 28.3 (6 x CH_3), 27.0 (2 x C*H<u>C</u>H$_2$).

Synthesis of 3g: this compound was obtained as described above for **3a** starting from **2g**. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 25% to 45% AcOEt, Rf of **3g** in AcOEt/hexane, 2:3 (v/v): 0.30) to give 644 mg of **3g** (69% yield) as a white solid. HRMS (ESI+) calcd. for $C_{64}H_{66}N_4O_8S_2$ $[M+Na]^+$ (m/z): 1105.4214, found: 1105.4236. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.54 (s, 2H, N<u>H</u>COC*H), 7.63 (s, 1H, CH_{Ar}), 7.40 (d, *J* = 7.9 Hz, 12H, CH_{Ar}), 7.31–7.16 (m, 23H, 21H x CH_{Ar} + 2H x C*HN<u>H</u>CO), 4.54 (td, *J* = 7.6, 4.0 Hz, 2H, C*H), 3.16 (ABq, δ_A = 3.18, δ_B = 3.14, *J* = 16.3 Hz, 4H, C<u>H</u>$_2$STrt), 2.69 (dd, *J* = 17.2, 3.9 Hz, 2H, C*HC<u>H</u>$_2$), 2.44 (dd, *J* = 17.1, 7.8 Hz, 2H, C*HC<u>H</u>$_2$), 1.43 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.3 (2 x CO), 169.0 (2 x CO), 168.0 (2 x CO), 144.0 (6 x C_{Ar}), 138.3 (2 x C_{Ar}), 129.6 (13 x CH_{Ar}), 128.3 (12 x CH_{Ar}), 127.26 (6 x CH_{Ar}), 116.0 (2 x CH_{Ar}), 111.3 (1 x

CH_{Ar}), 82.3 (2 x C), 68.2 (2 x C), 50.4 (2 x C*H), 36.6 (2 x CH_2), 36.0 (2 x CH_2), 28.2 (6 x CH_3).

Synthesis of 3h: this compound was obtained as described above for **3a**, starting from **2h**. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 30% to 40% AcOEt, Rf of **3h** in AcOEt/hexane, 2:3 (v/v): 0.20) to give 285 mg of **3h** (74% yield) as a white solid. HRMS (ESI+) calcd. for $C_{66}H_{70}N_4O_8S_2$ $[M+H]^+$ (m/z): 1111.4708, found: 1111.4696. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.72 (s, 2H, $N\underline{H}COC^*H$), 7.78 (t, J = 1.8 Hz, 1H, CH_{Ar}), 7.44–7.37 (m, 12H, CH_{Ar}), 7.30–7.14 (m, 21H, CH_{Ar}), 6.80 (d, J = 7.2 Hz, 2H, $C^*HN\underline{H}CO$), 4.26 (q, J = 6.8 Hz, 2H, C*H), 3.14 (ABq, δ_A = 3.16, δ_B = 3.12, J = 16.1Hz, 4H, $C\underline{H}_2STrt$), 2.45–2.34 (m, 2H, $C^*HCH_2C\underline{H}_2$), 2.28–2.17 (m, 2H, $C^*HCH_2C\underline{H}_2$), 2.08–1.96 (m, 2H, $C^*HC\underline{H}_2$), 1.83–1.71 (m, 2H, $C^*HC\underline{H}_2$), 1.44 (s, 18H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.1 (2 x CO), 169.0 (2 x CO), 168.8 (2 x CO), 144.0 (6 x C_{Ar}), 138.4 (2 x C_{Ar}), 129.6 (12 x CH_{Ar}), 129.5 (1 x CH_{Ar}), 128.3 (12 x CH_{Ar}), 127.2 (6 x CH_{Ar}), 115.7 (2 x CH_{Ar}), 111.0 (1 x CH_{Ar}), 81.3 (2 x C), 68.1 (2 x C), 53.5 (2 x C*H), 36.1 (2 x $\underline{C}H_2STrt$), 32.0 (2 x $C^*HCH_2\underline{C}H_2$), 28.2 (6 x CH_3), 27.8 (2 x $C^*H\underline{C}H_2$).

Synthesis of 3i: this compound was obtained as described above for **3a** starting from **2i**. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 50% to 65% AcOEt, Rf of **3i** in AcOEt/hexane, 3:2 (v/v): 0.47) to give 1.70 g of **3i** (83% yield) as a white solid. HRMS (ESI+) calcd. for $C_{76}H_{76}N_6O_8S_2$ $[M+H]^+$ (m/z): 1265.5239, found: 1265.5187. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.64 (br s, 2H, $N\underline{H}COC^*H$), 7.62 (br s, 1H, CH_{Ar}), 7.47–7.03 (m, 43H, CH_{Ar}), 6.60 (d, J = 7.4 Hz, 2H, $C^*HN\underline{H}CO$), 5.03 (s, 4H, $NHCOOC\underline{H}_2$), 4.97 (br s, 2H, $N\underline{H}Cbz$), 4.23 (br s, 2H, C*H), 3.22–2.99 (m, 8H, 4H x $C\underline{H}_2NHCbz$ + 4H x $C\underline{H}_2STrt$), 1.92–1.64 (m, 4H, $C^*HC\underline{H}_2$), 1.57–1.34 (m, 4H, $C\underline{H}_2CH_2NHCbz$), 1.33–1.09 (m, 4H, $C^*HCH_2C\underline{H}_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.7 (2 x CO), 169.2 (2 x CO), 156.6 (2 x CO), 144.1 (6 x C_{Ar}), 138.4 (2 x C_{Ar}), 136.8 (2 x C_{Ar}), 129.6 (13 x CH_{Ar}), 128.6 (4 x CH_{Ar}), 128.3 (12 x CH_{Ar}), 128.2 (6 x CH_{Ar}), 127.2 (6 x CH_{Ar}), 115.9 (2 x CH_{Ar}), 111.4 (1 x CH_{Ar}), 68.1 (2 x C), 66.7 (2 x $NHCOO\underline{C}H_2$), 54.3 (2 x C*H), 40.6 (2 x $\underline{C}H_2NHCbz$), 36.2 (2 x $\underline{C}H_2STrt$), 31.6 (2 x $C^*H\underline{C}H_2$), 29.5 (2 x $\underline{C}H_2CH_2NHCbz$), 22.7 (2 x $C^*HCH_2\underline{C}H_2$).

Synthesis of 3j: this compound was obtained as described above for **3a**, starting from **2j**. The residue was purified by flash chromatography using AcOEt/hexane as eluent (from 50% to 75% AcOEt, Rf of **3j** in AcOEt/hexane, 4:1 (v/v): 0.51) to give 281 mg of **3j** (15% yield) as a white solid. HRMS (ESI+) calcd. for

$C_{66}H_{68}N_6O_8S_2$ $[M+Na]^+$ (m/z): 1159.4432, found: 1159.4454. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.75 (br s, 2H, N$\underline{H}$COC*H), 7.74 (br s, 1H, CH_{Ar}), 7.42–7.35 (m, 12H, CH_{Ar}), 7.33–7.23 (m, 15H, CH_{Ar}), 7.22–7.14 (m, 6H, CH_{Ar}), 6.79 (d, *J* = 6.1 Hz, 2H, C*HN$\underline{H}$CO), 5.86 (ddt, *J* = 16.3, 10.7, 5.5 Hz, 2H, $NHCOOCH_2C\underline{H}CH_2$), 5.25 (dd, *J* = 17.2, 1.6 Hz, 2H, $NHCOOCH_2CHC\underline{H}_2$), 5.14 (dd, *J* = 10.6, 1.4 Hz, 2H, $NHCOOCH_2CHC\underline{H}_2$), 5.02 (br s, 2H, N$\underline{H}$Alloc), 4.56 (d, *J* = 5.2 Hz, 4H, $NHCOOC\underline{H}_2CHCH_2$), 4.52 (s, 2H, C*H), 3.41 (br s, 2H, $C\underline{H}_2$NHAlloc), 3.21–2.96 (m, 6H, 2H x $C\underline{H}_2$NHAlloc + 4H x $C\underline{H}_2$STrt), 1.89–1.66 (m, 2H, C*H$C\underline{H}_2$), 1.58–1.35 (m, 6H, 2H x C*H$C\underline{H}_2$ + 4H x C*H$CH_2C\underline{H}_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.9 (2 x CO), 169.1 (2 x CO), 157.1 (2 x CO), 144.1 (6 x C_{Ar}), 138.5 (2 x C_{Ar}), 133.0 (2 x $NHCOOCH_2\underline{C}HCH_2$), 129.7 (12 x CH_{Ar}), 129.4 (1 x CH_{Ar}), 128.3 (12 x CH_{Ar}), 127.2 (6 x CH_{Ar}), 117.7 (2 x $NHCOOCH_2CH\underline{C}H_2$), 115.6 (2 x CH_{Ar}), 111.0 (1 x CH_{Ar}), 68.0 (2 x C), 65.8 (2 x $NHCOO\underline{C}H_2CHCH_2$), 52.9 (2 x C*H), 39.7 (2 x $\underline{C}H_2$NHAlloc), 36.3 (2 x $\underline{C}H_2$STrt), 30.0 (2 x C*H$\underline{C}H_2$), 26.4 (2 x C*H$CH_2\underline{C}H_2$).

Synthesis of 3k: To a solution of **3j** (132 mg, 0.116 mmol) in dry DCM (3.0 mL), $PhSiH_3$ (343 μL, 2.79 mmol) was added under inert atmosphere of Ar. Then a solution of $Pd(PPh_3)_4$ (18 mg, 15 μmol) in dry DCM (2.0 mL) was added. The mixture was stirred at room temperature for 1 hour, after which complete conversion of the starting material was observed by TLC. The crude mixture was filtered through a bed of Celite® and the filtrate was concentrated to dryness under reduced pressure, obtaining diamine **5j** as a brownish solid that was no further purified. HRMS (ESI+) calcd. for $C_{58}H_{60}N_6O_4S_2$ $[M+H]^+$ (m/z): 969.4190, found: 969.4178. The residue was re-dissolved in DCM (5.0 mL), and triethylamine (54 μL, 0.39 mmol) and *N,N′*-di-Boc-*N″*-triflylguanidine (139 mg, 0.355 mmol) were added. The mixture was stirred for 2 hours under inert atmosphere of Ar, until the reaction was completed as evidenced by TLC (Rf of **3k** in AcOEt/Hexane, 2:3 (v/v): 0.30). The mixture was diluted with DCM, washed with 2M aqueous $NaHSO_4$, 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 AcOEt/hexane as eluent (from 40% to 45% AcOEt) to give 106.7 mg of **3k** (63% yield over the last two steps) as a white solid. HRMS (ESI–) calcd. for $C_{80}H_{96}N_{10}O_{12}S_2$ $[M-H]^-$ (m/z): 1451.6578, found: 1451.6572. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.46 (s, 2H, NH), 8.51–8.33 (m, 4H, 2H x N$\underline{H}$COC*H + 2H x NH), 7.67–7.63 (m, 1H, CH_{Ar}), 7.41 (d, *J* = 7.2 Hz, 12H, CH_{Ar}), 7.31–7.16 (m, 21H, CH_{Ar}), 7.08 (d, *J* = 7.7 Hz, 2H, C*HN$\underline{H}$CO), 4.34 (q, *J* = 6.8 Hz, 2H, C*H), 3.43 (q, *J* = 5.8 Hz, 4H, $C\underline{H}_2$NH), 3.14 (s, 4H, $C\underline{H}_2$STrt), 1.90–1.75 (m, 2H, C*H$C\underline{H}_2$), 1.72–1.13 (m, 42H, 2H x C*HC$\underline{H}_2$ + 4H x C*HCH$_2$C$\underline{H}_2$ + 36H x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.3 (2 x CO), 168.9 (2 x CO), 167.5 (2 x C-guanidine), 152.5 (2 x CO), 147.0 (2 x CO), 144.1 (6 x C_{Ar}), 138.6 (2 x C_{Ar}), 129.7 (12 x CH_{Ar}), 129.3 (1 x CH_{Ar}), 128.3 (12 x CH_{Ar}), 127.2 (6 x CH_{Ar}), 116.2 (2 x CH_{Ar}), 111.8 (1 x CH_{Ar}), 90.1 (2 x C), 81.0 (2 x C), 68.0 (2 x C), 53.1 (2 x C*H), 41.9 (2 x CH_2NH), 36.3 (2 x $\underline{C}H_2$STrt), 29.9 (2 x C*H$\underline{C}H_2$), 28.1 (12 x CH_3), 25.5 (2 x C*HCH$_2\underline{C}H_2$).

Synthesis of building blocks 4a-l

Synthesis of 4a: to a solution of **3a** (230 mg, 0.16 mmol) in DCM (1.0 mL), TFA (8.5 mL), TIS (332 µL, 1.28 mmol) and EDT (160 µL, 1.91 mmol) were added rapidly and under stirring. The reaction mixture was stirred at room temperature for 2 hours, 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 by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 5% to 30% MeCN in H_2O). After lyophilisation 37.8 mg of **4a** (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: 485.1279. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.97 (s, 2H, N$\underline{H}$COC*H), 8.34 (d, J = 7.7 Hz, 2H, C*HN$\underline{H}$CO), 7.93 (t, J = 2.0 Hz, 1H, H^1), 7.36 (s, 2H, NH_2), 7.29 (dd, J = 7.6, 2.0 Hz, 2H, H^3), 7.24–7.15 (m, 1H, H^4), 6.91 (s, 2H, NH_2), 4.67 (q, J = 7.1 Hz, 2H, C*H), 3.17 (d, J = 7.9 Hz, 4H, C$\underline{H}_2$SH), 2.73 (t, J = 7.9 Hz, 2H, SH), 2.62–2.41 (m, 4H, C$\underline{H}_2$C*H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.1 (2 x $CONH_2$), 169.5 (2 x $\underline{C}$OC*H), 169.4 (2 x $\underline{C}OCH_2$), 139.1 (2 x C^2), 128.6 (1 x C^4), 114.6 (2 x C^3), 110.9 (1 x C^1), 50.9 (2 x C*H), 37.1 (2 x $\underline{C}H_2$C*H), 27.0 (2 x CH_2SH).

Synthesis of 4b: this compound was obtained as described above for **4a**, starting from **3b**. The product was purified by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 5% to 30% MeCN in H_2O) and 31.3 mg of **4b** (48% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{20}H_{28}N_6O_6S_2$ $[M+H]^+$ (m/z): 513.1590, found: 513.1592. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.09 (s, 2H, N$\underline{H}$COC*H), 8.30 (d, J = 7.7 Hz, 2H, C*HN$\underline{H}$CO), 7.96 (t, J = 2.0 Hz, 1H, H^1), 7.35–7.27 (m, 4H, 2H x H^3 + 2H x NH_2), 7.26–7.20 (m, 1H, H^4), 6.77 (s, 2H, NH_2), 4.39 (td, J = 8.0, 5.7 Hz, 2H, C*H), 3.25–3.12 (m, 4H, C$\underline{H}_2$SH), 2.75 (t, J = 8.0 Hz, 2H, SH), 2.22–2.04 (m, 4H, C*HCH$_2$C$\underline{H}_2$), 1.99–1.88 (m, 2H, C*HC$\underline{H}_2$), 1.88–1.76 (m, 2H, C*HC$\underline{H}_2$). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 173.3 (2 x $CONH_2$), 170.0 (2 x $\underline{C}$OC*H), 169.6 (2 x $\underline{C}OCH_2$), 139.0 (2 x C^2), 128.6 (1 x C^4), 114.3 (2 x C^3), 110.4 (1 x C^1), 53.1 (2 x C*H), 31.1 (2 x C*HCH$_2\underline{C}H_2$), 27.8 (2 x C*H$\underline{C}H_2$), 26.7 (2 x $\underline{C}H_2$SH).

Synthesis of 4c: this compound was obtained as described above for **4a** starting from **3c**. The product was purified by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 5% to 30% MeCN in H_2O) and 42.4 mg of **4c** (51% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{16}H_{22}N_4O_6S_2$ $[M+H]^+$ (m/z): 431.1059, found: 431.1054. ^{1}H NMR (400 MHz, MeOD-d_4): δ = 7.92 (t, *J* = 2.0 Hz, 1H, H^1), 7.37–7.30 (m, 2H, H^3), 7.29–7.23 (m, 1H, H^4), 4.56 (t, *J* = 5.3 Hz, 2H, C*H), 3.93–3.82 (m, 4H, $C\underline{H}_2OH$), 3.28 (s, 4H, $C\underline{H}_2SH$). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 173.1 (2 x $\underline{C}OCH_2$), 170.3 (2 x $\underline{C}$OC*H), 139.7 (2 x C^2), 129.9 (1 x C^4), 117.3 (2 x C^3), 113.4 (1 x C^1), 62.8 (2 x $\underline{C}H_2OH$), 57.2 (2 x C*H), 27.9 (2 x $\underline{C}H_2SH$).

Synthesis of 4d: this compound was obtained as described above for **4a** starting from **3d**; 79.6 mg of **4d** (92% yield) were obtained as a white solid which required no further purification. HRMS (ESI+) calcd. for $C_{18}H_{26}N_4O_6S_2$ $[M+H]^+$ (m/z): 459.1372, found: 459.1371. ^{1}H NMR (400 MHz, MeOD-d_4): δ = 9.80 (s, 2H, N$\underline{H}$COC*H), 8.18 (d, *J* = 8.2 Hz, 2H, C*HN$\underline{H}$CO), 7.95–7.89 (m, 1H, H^1), 7.36–7.30 (m, 2H, H^3), 7.29–7.23 (m, 1H, H^4), 4.48–4.42 (m, 2H, C*$\underline{H}$NH), 4.24 (qd, *J* = 6.4, 3.9 Hz, 2H, C*$\underline{H}CH_3$), 3.35–3.29 (m, 4H, $C\underline{H}_2$), 1.24 (d, *J* = 6.3 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 173.3 (2 x $\underline{C}OCH_2$), 170.4 (2 x $\underline{C}$OC*H), 139.5 (2 x C^2), 129.8 (1 x C^4), 117.2 (2 x C^3), 113.3 (1 x C^1), 68.4 (2 x $\underline{C}$*HCH_3), 60.6 (2 x C*HNH), 28.0 (2 x CH_2), 20.0 (2 x CH_3).

Synthesis of 4e: this compound was obtained as described above for **4a** starting from **3e**; 63.7 mg of **4e** (91% yield) were obtained as a white solid which required no further purification. HRMS (ESI+) calcd. for $C_{28}H_{30}N_4O_6S_2$ $[M+H]^+$ (m/z): 583.1685, found: 583.1696. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.11 (s, 2H, N$\underline{H}$COC*H), 9.17 (br s, 2H, OH), 8.34 (d, *J* = 8.1 Hz, 2H, C*HN$\underline{H}$CO), 7.88 (t, *J* = 2.1 Hz, 1H, H^1), 7.32–7.25 (m, 2H, H^3), 7.25–7.18 (m, 1H, H^4), 7.06 (d, *J* = 8.5 Hz, 4H, H^6), 6.64 (d, *J* = 8.5 Hz, 4H, H^7), 4.58 (td, *J* = 8.4, 5.5 Hz, 2H, C*H), 3.12 (d, *J* = 7.9 Hz, 4H, $C\underline{H}_2SH$), 2.92 (dd, *J* = 13.8, 5.4 Hz, 2H, C*$HC\underline{H}_2$), 2.75 (dd, *J* = 13.8, 8.9 Hz, 2H, C*$HC\underline{H}_2$), 2.63 (t, *J* = 7.9 Hz, 2H, SH). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.0 (2 x $\underline{C}$OC*H), 169.4 (2 x $\underline{C}OCH_2$), 155.8 (2 x C^8), 139.0 (2 x C^2), 130.1 (4 x C^6), 128.8 (1 x C^4), 127.4 (2 x C^5), 114.9 (4 x C^7), 114.6 (2 x C^3), 110.6 (1 x C^1), 55.3 (2 x C*H), 37.1 (2 x C*$H\underline{C}H_2$), 26.9 (2 x CH_2SH).

Synthesis of 4f: this compound was obtained as described above for **4a** starting from **3f**. The product was purified by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 30% to 65% MeCN in H_2O) and 51.3 mg of **4f** (44% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{32}H_{32}N_6O_4S_2$ $[M+H]^+$ (m/z): 629.2004, found: 629.2003. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.82 (d, *J* = 2.4 Hz, 2H, H^5), 10.17 (s, 2H, N$\underline{H}$COC*H), 8.37 (d, *J* = 7.9 Hz, 2H, C*HN$\underline{H}$CO), 7.93 (s, 1H, H^1), 7.64 (d, J = 7.8 Hz, 2H, H^{10}), 7.35–7.27 (m, 4H, 2H x H^3 + 2H x H^7), 7.24–7.18 (m, 1H, H^4), 7.16 (d, J = 2.4 Hz, 2H, H^{13}), 7.05 (ddd, J = 8.1, 6.9, 1.2 Hz, 2H, H^8), 6.97 (ddd, J = 8.0, 7.0, 1.1 Hz, 2H, H^9), 4.72 (td, J = 8.0, 5.8 Hz, 2H, C*H), 3.23–3.11 (m, 6H, 4H x $C\underline{H}_2SH$ + 2H x $C^*HC\underline{H}_2$), 3.02 (dd, J = 14.6, 8.2 Hz, 2H, $C^*HC\underline{H}_2$), 2.65 (t, J = 8.0 Hz, 2H, SH). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.3 (2 x $\underline{C}$OC*H), 169.4 (2 x $\underline{C}OCH_2$), 139.0 (2 x C^2), 136.0 (2 x C^6), 128.8 (1 x C^4), 127.3 (2 x C^{11}), 123.6 (2 x C^{13}), 120.9 (2 x C^8), 118.5 (2 x C^{10}), 118.2 (2 x C^9), 114.7 (2 x C^3), 111.3 (2 x C^7), 110.8 (1 x C^1), 109.6 (2 x C^{12}), 54.4 (2 x C*H), 28.0 (2 x $C^*H\underline{C}H_2$), 27.0 (2 x CH_2SH).

Synthesis of 4g: this compound was obtained as described above for **4a** starting from **3g**. The product was purified by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 5% to 30% MeCN in H_2O) and 49.1 mg of **4g** (53% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{18}H_{22}N_4O_8S_2$ $[M+H]^+$ (m/z): 487.0957, found: 487.0956. ^1H NMR (400 MHz, MeOD-d_4): δ = 7.85 (t, J = 2.1 Hz, 1H, H^1), 7.35–7.29 (m, 2H, H^3), 7.28–7.22 (m, 1H, H^4), 4.90–4.81 (m, 2H, C*H), 3.24 (s, 4H, $C\underline{H}_2SH$), 2.91 (dd, J = 16.6, 6.4 Hz, 2H, $C^*HC\underline{H}_2$), 2.78 (dd, J = 16.6, 7.0 Hz, 2H, $C^*HC\underline{H}_2$). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 173.6 (2 x COOH), 173.4 (2 x $\underline{C}OCH_2$), 170.9 (2 x $\underline{C}$OC*H), 139.8 (2 x C^2), 130.1 (1 x C^4), 117.6 (2 x C^3), 113.8 (1 x C^1), 52.3 (2 x C*H), 36.8 (2 x $C^*H\underline{C}H_2$), 28.1 (2 x CH_2SH).

Synthesis of 4h: this compound was obtained as described above for **4a** starting from **3h**. The product was purified by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 5% to 30% MeCN in H_2O) and 49.6 mg of **4h** (58% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{20}H_{26}N_4O_8S_2$ $[M+H]^+$ (m/z): 515.1270, found: 515.1271. ^1H NMR (400 MHz, MeOD-d_4): δ = 7.90 (t, J = 2.0 Hz, 1H, H^1), 7.35–7.30 (m, 2H, H^3), 7.29–7.23 (m, 1H, H^4), 4.53 (dd, J = 8.7, 5.3 Hz, 2H, C*H), 3.24 (s, 4H, $C\underline{H}_2SH$), 2.45 (t, J = 7.6 Hz, 4H, $C^*HCH_2C\underline{H}_2$), 2.25–2.12 (m, 2H, $C^*HC\underline{H}_2$), 2.08–1.96 (m, 2H, $C^*HC\underline{H}_2$). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 176.0 (2 x COOH), 173.1 (2 x $\underline{C}OCH_2$), 171.4 (2 x $\underline{C}$OC*H), 139.5 (2 x C^2), 129.8 (1 x C^4), 117.0 (2 x C^3), 113.1 (1 x C^1), 54.5 (2 x C*H), 30.7 (2 x $C^*HCH_2\underline{C}H_2$), 28.3 (2 x $C^*H\underline{C}H_2$), 27.7 (2 x CH_2SH).

Synthesis of 4i·2TFA: a solution of **3i** (64.3 mg, 0.051 mmol) in dry DCM (3.3 mL) was cooled down to 0 °C in an ice-water bath. Then triisobutylsilane (TIS, 55 µL, 0.21 mmol) and 800 µL of a solution of HBr in CH_3COOH (33 wt. %) were added under stirring. After 40 minutes stirring at 0 °C, diethyl ether was added over the reaction mixture and the product was filtered off and washed with diethyl ether. The product was purified by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 2% to 12% MeCN in H_2O). During the purification the Br^- anions were exchanged by TFA^- and 31.8 mg of **4i**·2TFA (84% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{22}H_{36}N_6O_4S_2$ $[M+H]^+$ (m/z): 513.2318, found: 513.2319. ^1H NMR (400 MHz, MeOD-d_4): δ = 8.01–7.96 (m, 1H, H^1), 7.35–7.19 (m, 3H, 2H x H^3 + 1H x H^4), 4.49 (dd, *J* = 8.5, 5.5 Hz, 2H, C*H), 3.24 (s, 4H, $C\underline{H}_2SH$), 2.93 (t, *J* = 7.6 Hz, 4H, $C\underline{H}_2NH_3^+$), 2.01–1.87 (m, 2H, $C^*HC\underline{H}_2$), 1.86–1.63 (m, 6H, 2H x $C^*HC\underline{H}_2$ + 4H x $C\underline{H}_2CH_2NH_3^+$), 1.62–1.39 (m, 4H, $C\underline{H}_2CH_2C^*H$). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 173.5 (2 x $\underline{C}OCH_2$), 172.1 (2 x $\underline{C}OC^*H$), 139.9 (2 x C^2), 130.2 (1 x C^4), 117.5 (2 x C^3), 113.6 (1 x C^1), 55.3 (2 x C*H), 40.5 (2 x $CH_2NH_3^+$), 32.9 (2 x $C^*H\underline{C}H_2$), 28.2 (2 x $\underline{C}H_2CH_2NH_3^+$), 28.1 (2 x CH_2SH), 23.8 (2 x $C^*HCH_2\underline{C}H_2$).

Synthesis of 4j·2TFA: to a solution of **3j** (133 mg, 0.117 mmol) in dry DCM (3.0 mL), $PhSiH_3$ (345 μL, 2.80 mmol) was added under inert atmosphere of Ar. Then a solution of $Pd(PPh_3)_4$ (18 mg, 15 μmol) in dry DCM (2.0 mL) was added. The mixture was stirred at room temperature for 1 hour, after which complete conversion of the starting material was observed by TLC. The crude mixture was filtered through a bed of Celite® and the filtrate was concentrated to dryness under reduced pressure. The resulting residue was re-dissolved in DCM (1.0 mL), and TFA (4.5 mL), TIS (242 µL, 0.933 mmol) and EDT (117 µL, 1.40 mmol) were added rapidly and under stirring. The reaction mixture was stirred at room temperature for 1 hour, 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 and washed with diethyl ether. The product was purified by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 2% to 10% MeCN in H_2O) and 46.9 mg of **4j**·2TFA were obtained as a white solid (56% yield). HRMS (ESI+) calcd. for $C_{20}H_{32}N_6O_4S_2$ $[M+H]^+$ (m/z): 485.2005, found: 485.2007. ^1H NMR (400 MHz, MeOD-d_4): δ = 8.02–7.97 (m, 1H, H^1), 7.34–7.18 (m, 3H, 2H x H^3 + 1H x H^4), 4.54 (dd, *J* = 8.0, 5.5 Hz, 2H, C*H), 3.25 (s, 4H, $C\underline{H}_2SH$), 3.07–2.90 (m, 4H, $C\underline{H}_2NH_3^+$), 2.05–1.89 (m, 2H, C*H $C\underline{H}_2$), 1.88–1.68 (m, 6H, 2H x $C^*HC\underline{H}_2$ + 4H, C*H $CH_2C\underline{H}_2$). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 173.5 (2 x $\underline{C}OCH_2$), 171.6 (2 x $\underline{C}OC^*H$), 139.9 (2 x C^2), 130.2 (1 x C^4), 117.5 (2 x C^3), 113.5 (1 x C^1), 54.8 (2 x C*H), 40.3 (2 x $CH_2NH_3^+$), 30.4 (2 x $C^*H\underline{C}H_2$), 28.1 (2 x CH_2SH), 25.0 (2 x $C^*HCH_2\underline{C}H_2$).

Synthesis of 4k·2TFA: this compound was obtained as described above for **4a**, starting from **3k**. The product was purified by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 5% to 10% MeCN in H_2O) and 14.6 mg of **4k**·2TFA (27% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{22}H_{36}N_{10}O_4S_2$ $[M+H]^+$ (m/z): 569.2441, found: 569.2435. ^1H NMR (400 MHz, MeOD-d_4): δ = 7.99 (s, 1H, H^1), 7.33–7.20 (m, 3H, 2 x H^3 + 1 x H^4), 4.52 (dd, *J* = 8.3, 5.6 Hz, 2H, C*H), 3.29–3.11 (m, 8H, 4H x $C\underline{H}_2SH$ + 4H x $C\underline{H}_2NH$), 2.00–1.87 (m, 2H, $C^*HC\underline{H}_2$), 1.86–1.57 (m, 6H, 2H x $C^*HC\underline{H}_2$ + 4H x $C^*HCH_2C\underline{H}_2$). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 172.1 (2 x $\underline{C}OCH_2$), 170.5 (2 x $\underline{C}OC^*H$), 157.2 (2 x C^5), 138.4 (2 x C^2), 128.8 (1 x C^4), 116.1 (2 x C^3), 112.2 (1 x C^1), 53.6 (2 x C*H), 40.6 (2 x CH_2NH), 29.2 (2 x $C^*H\underline{C}H_2$), 26.7 (2 x CH_2SH), 24.9 (2 x $C^*HCH_2\underline{C}H_2$).

Synthesis of 4l·2TFA: this compound was obtained as described above for **4a**, starting from **2l**. The product was purified by reversed-phase flash chromatography using a mixture of MeCN + 0.07% (v/v) TFA and H_2O + 0.1% (v/v) TFA as mobile phase (gradient: from 0% to 10% MeCN in H_2O) and 43.2 mg of **4l**·2TFA (40% yield) were obtained as a white solid. HRMS (ESI+) calcd. for $C_{12}H_{18}N_4O_2S_2$ $[M+H]^+$ (m/z): 315.0949, found: 315.0950. ^1H NMR (400 MHz, MeOD-d_4): δ = 8.07 (t, *J* = 2.0 Hz, 1H, H^1), 7.41–7.36 (m, 2H, H^3), 7.36–7.30 (m, 1H, H^4), 4.14 (dd, *J* = 7.2, 5.1 Hz, 2H, C*H), 3.16 (dd, *J* = 14.7, 5.1 Hz, 2H, CH_2), 3.04 (dd, *J* = 14.7, 7.2 Hz, 2H, CH_2). ^{13}C NMR (101 MHz, MeOD-d_4): δ = 166.7 (2 x CO), 139.6 (2 x C^2), 130.5 (1 x C^4), 117.5 (2 x C^3), 113.0 (1 x C^1), 56.9 (2 x C*H), 26.3 (2 x CH_2).

Characterization of building blocks 4a-l

Building block 4a

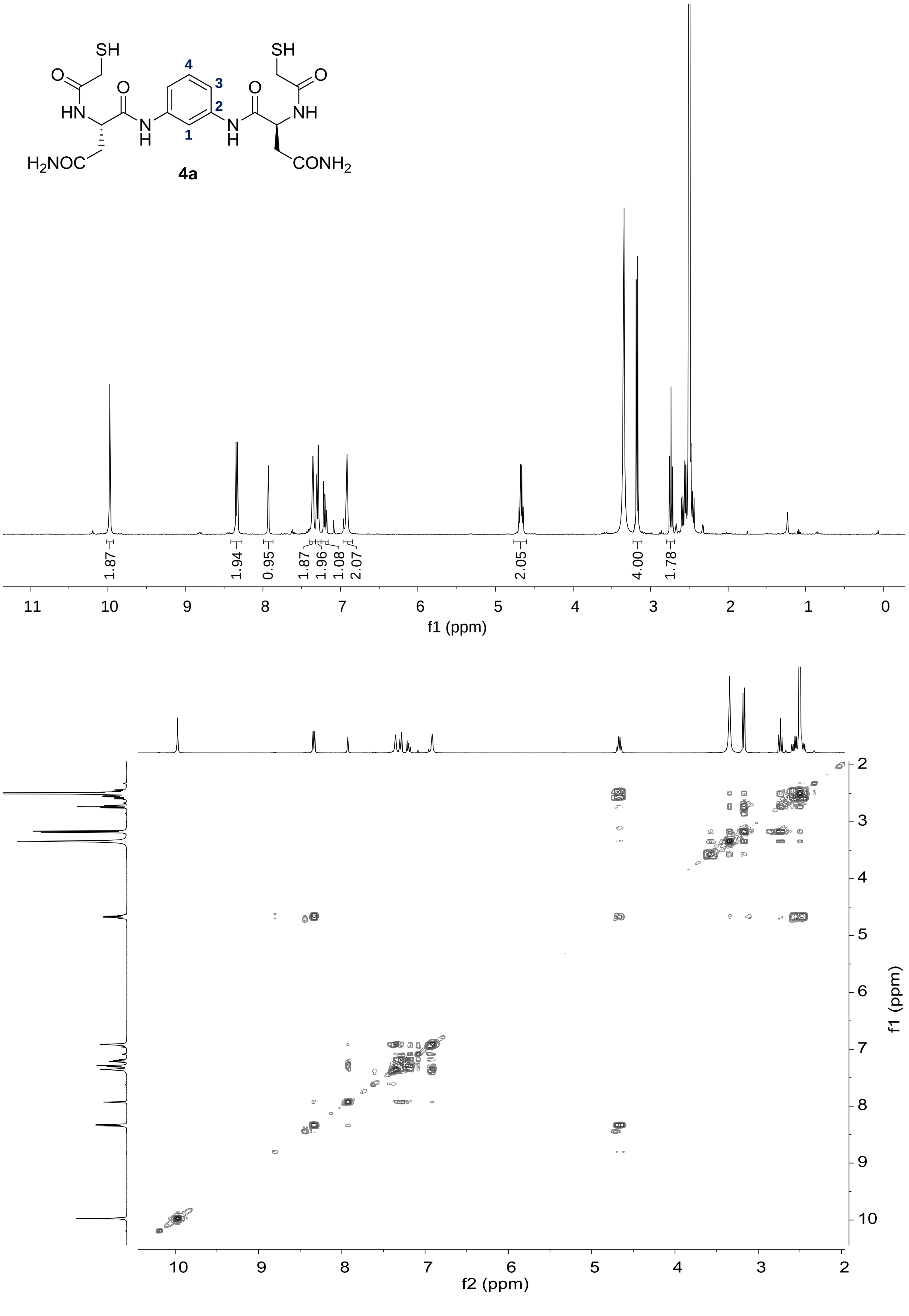


Figure S1. ^{1}H (400 MHz, 298 K in DMSO-d_6) and ^{1}H-^{1}H gCOSY (400 MHz, 298 K in DMSO-d_6) spectra of **4a**.

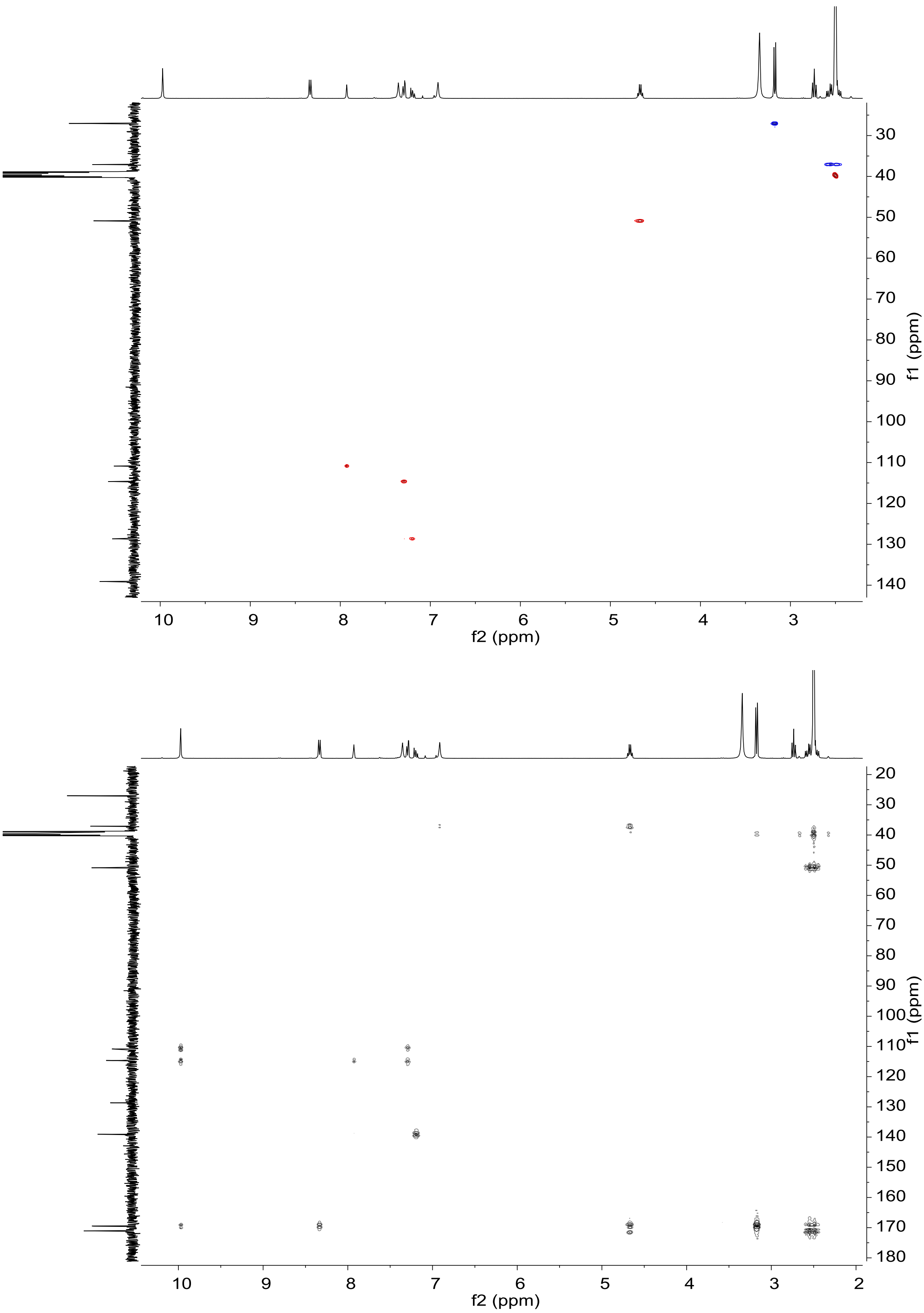


Figure S2. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in DMSO-d_6) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in DMSO-d_6) spectra of **4a**.

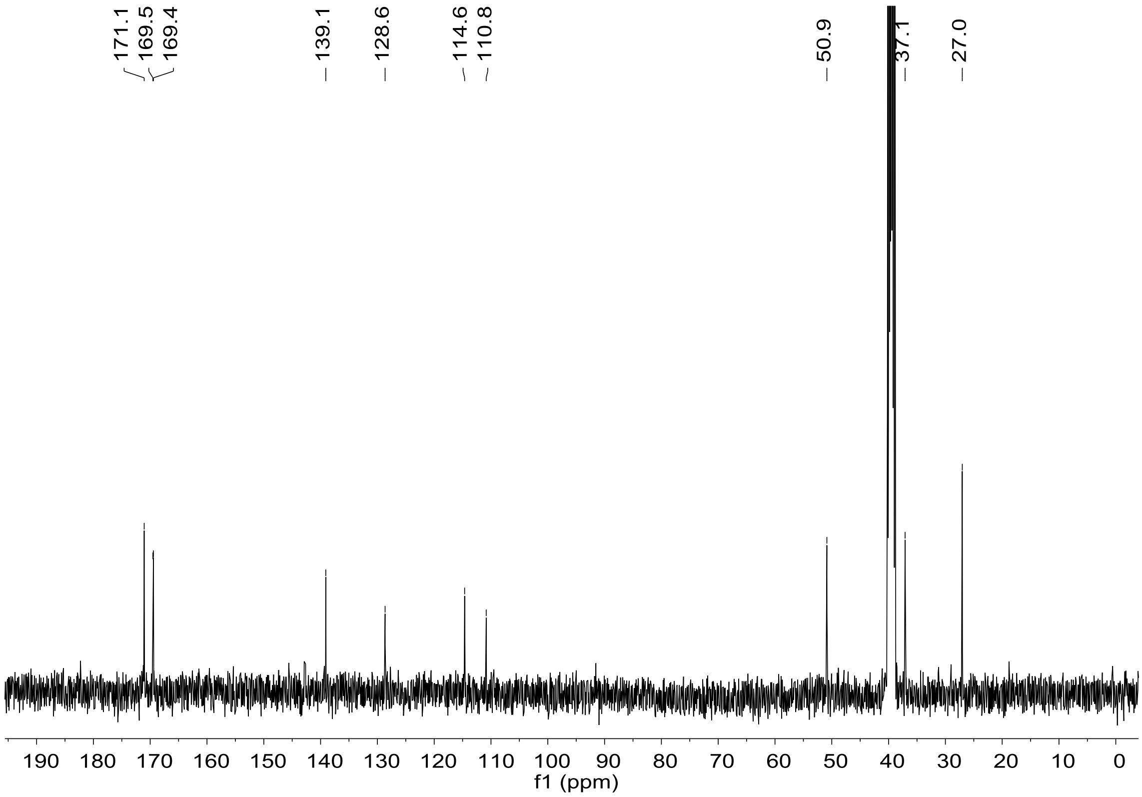


Figure S3. ^{13}C (101 MHz, 298 K in DMSO-d_6) spectrum of **4a**.

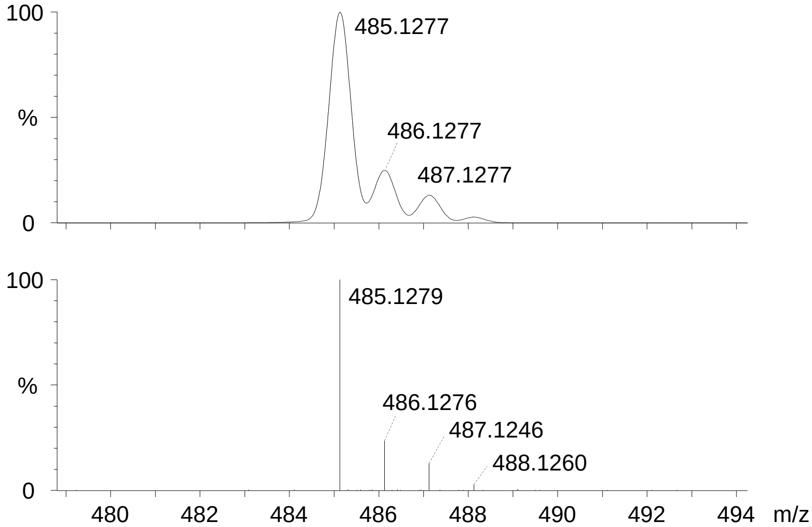
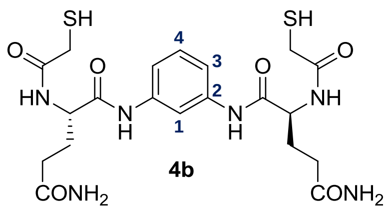
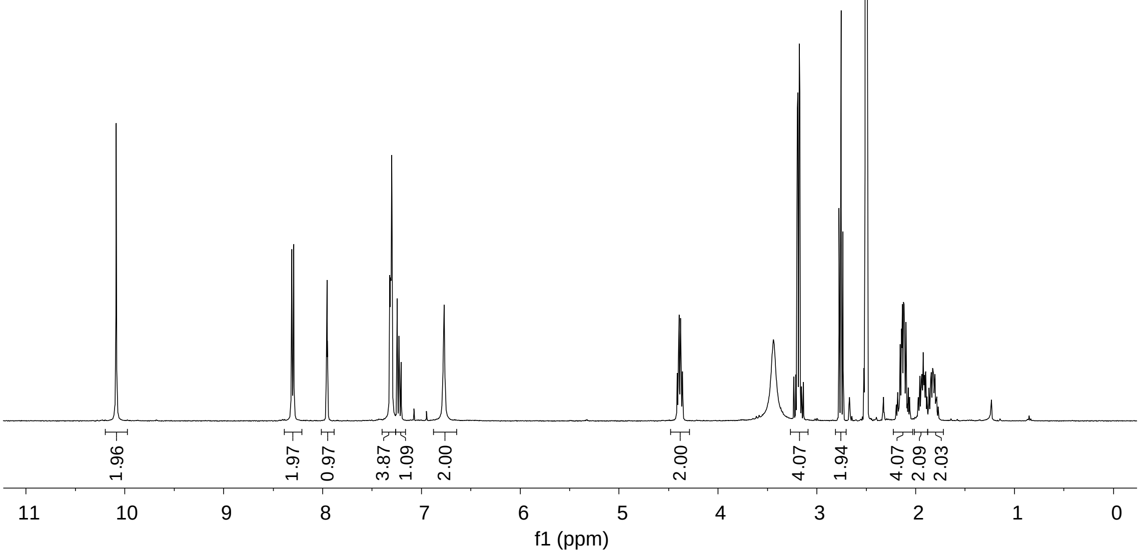


Figure S4. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4a**.

Building block 4b





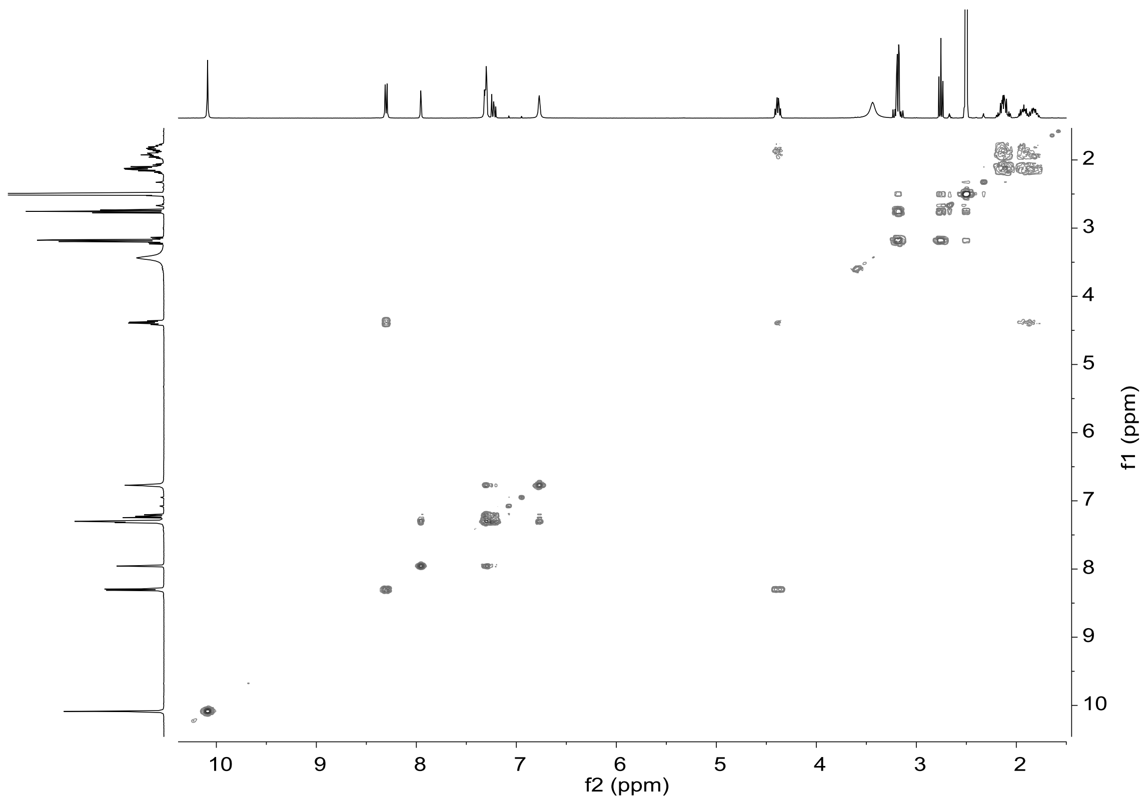
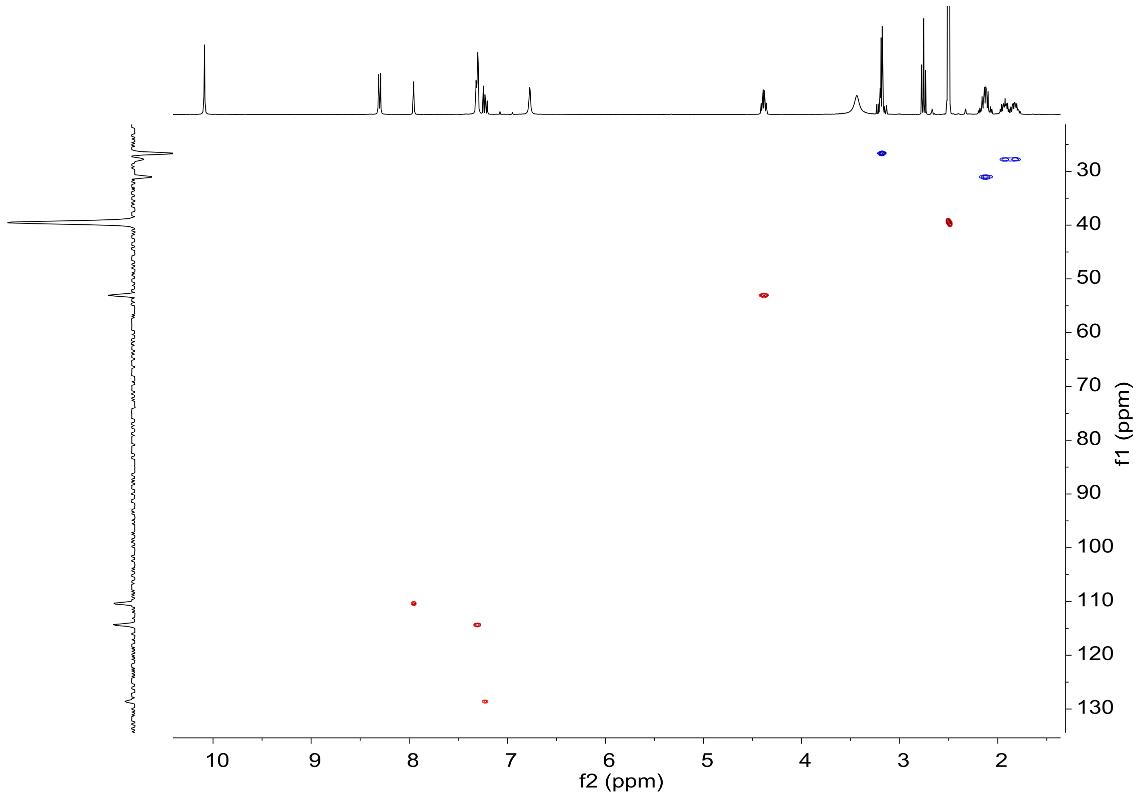


Figure S5. [1]H (400 MHz, 298 K in DMSO-d_6) and [1]H-[1]H gCOSY (400 MHz, 298 K in DMSO-d_6) spectra of **4b**.



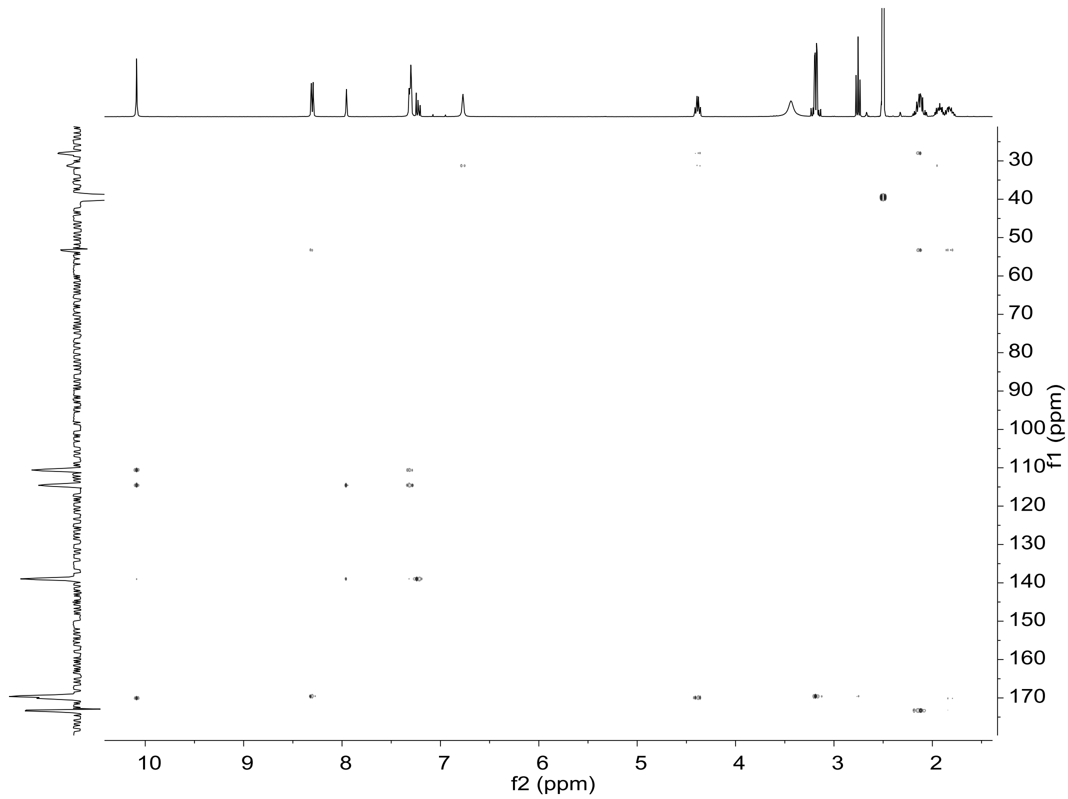


Figure S6. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in DMSO-d_6) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in DMSO-d_6) spectra of **4b**.

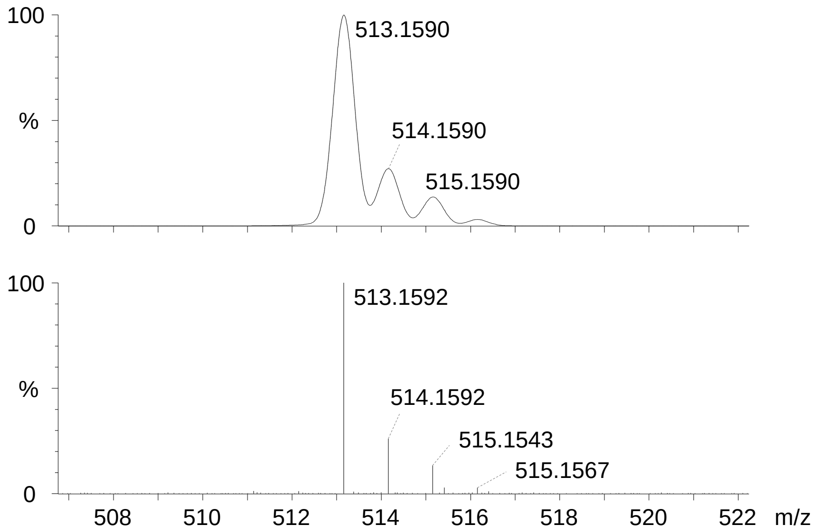


Figure S7. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4b**.

Building block 4c

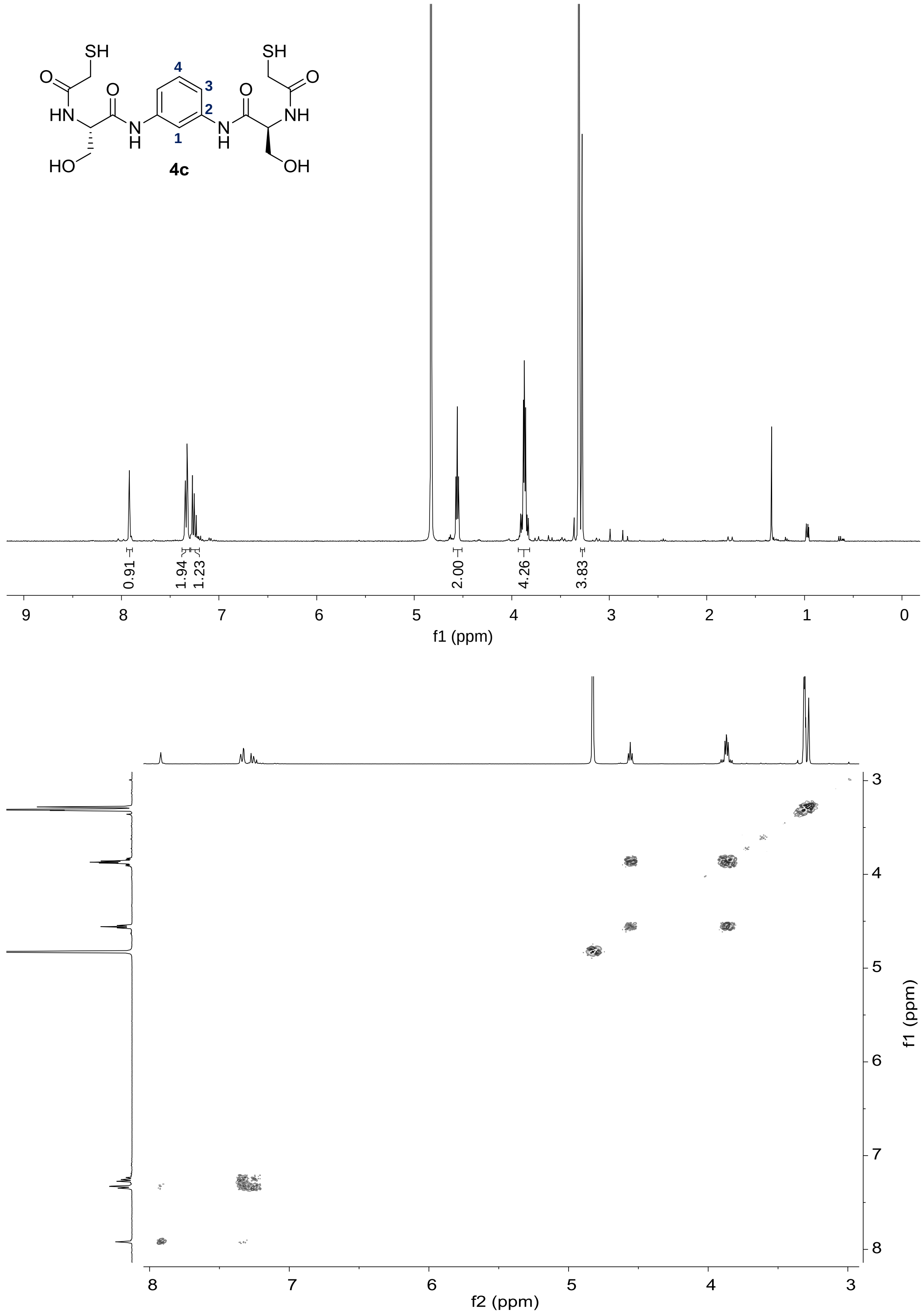


Figure S8. [1]H (400 MHz, 298 K in MeOD-d_4) and [1]H-[1]H gCOSY (400 MHz, 298 K in MeOD-d_4) spectra of **4c**.

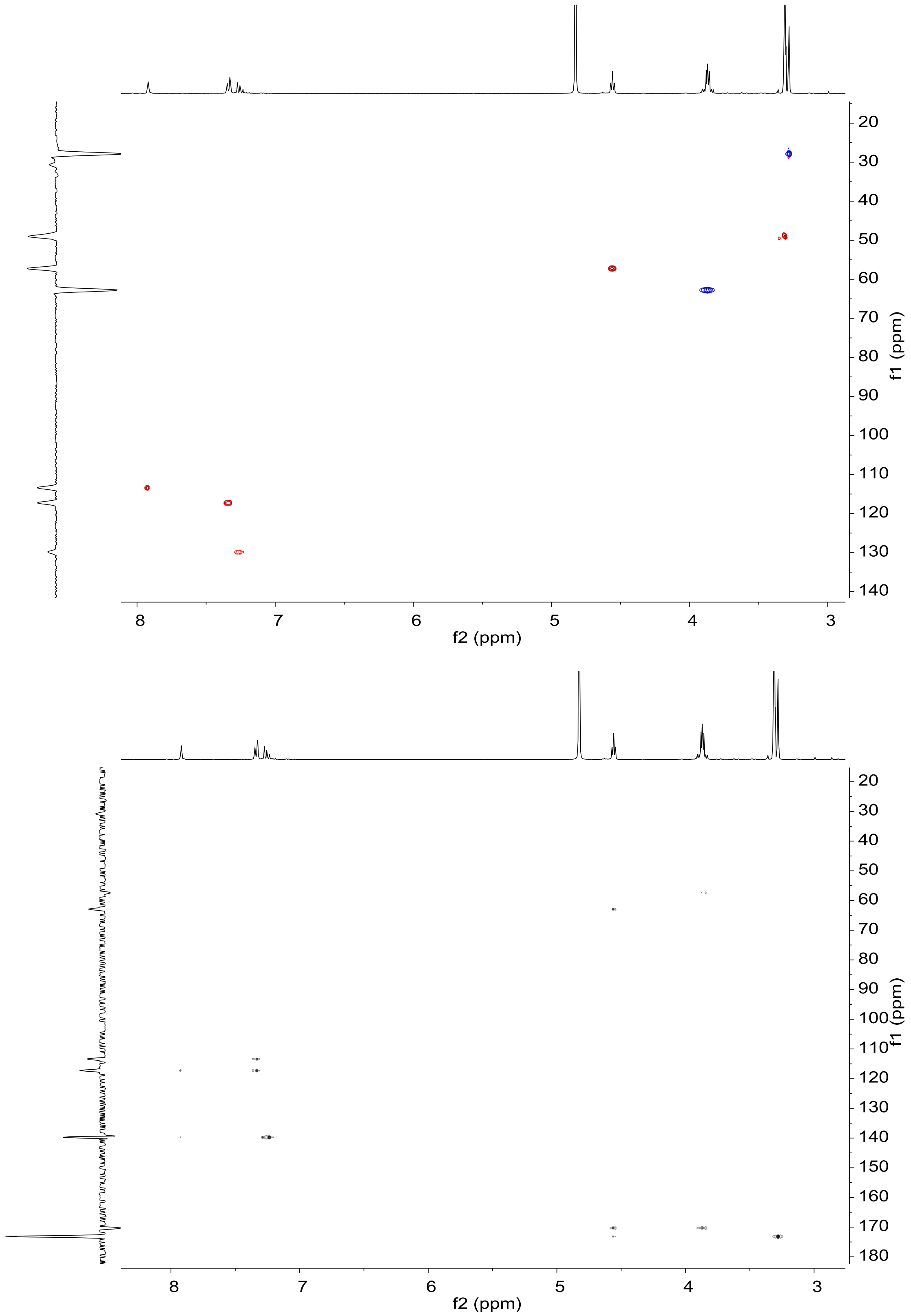


Figure S9. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in MeOD-d_4) spectra of **4c**.

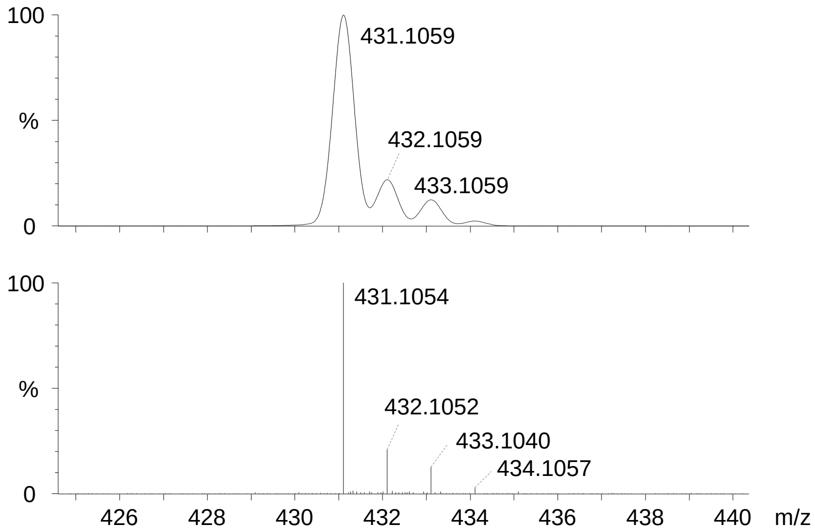
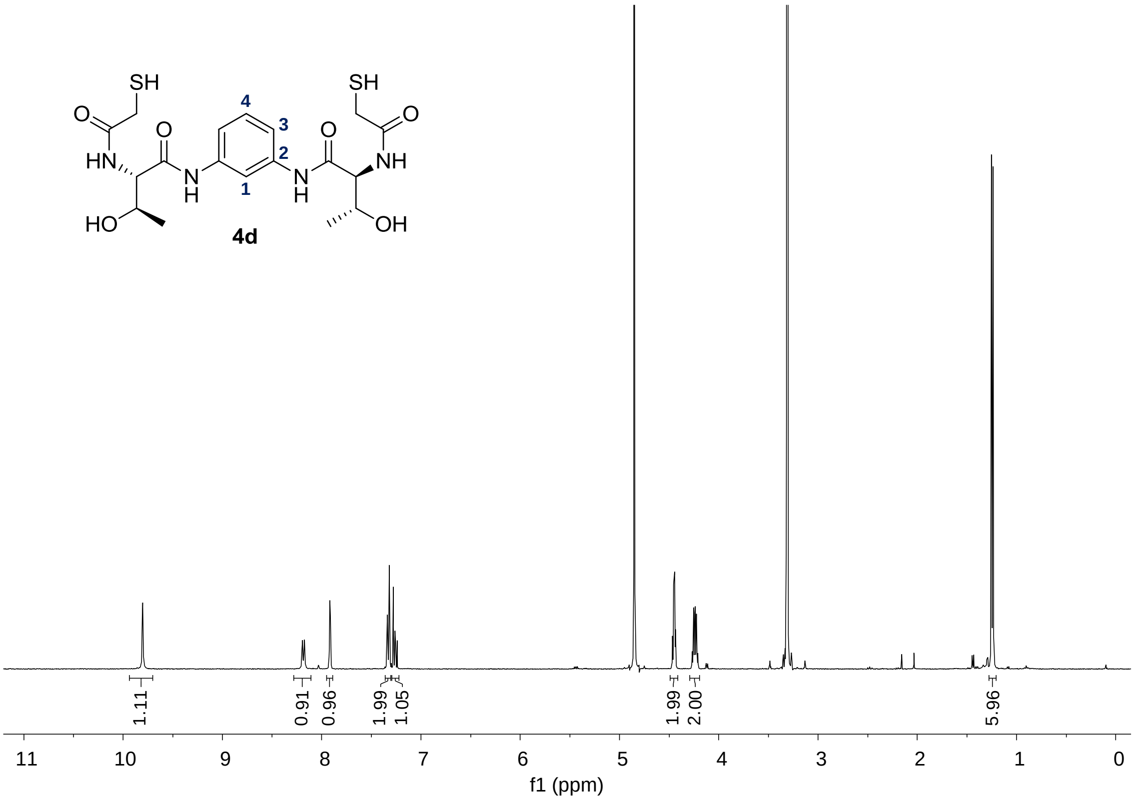


Figure S10. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4c**.

Building block 4d



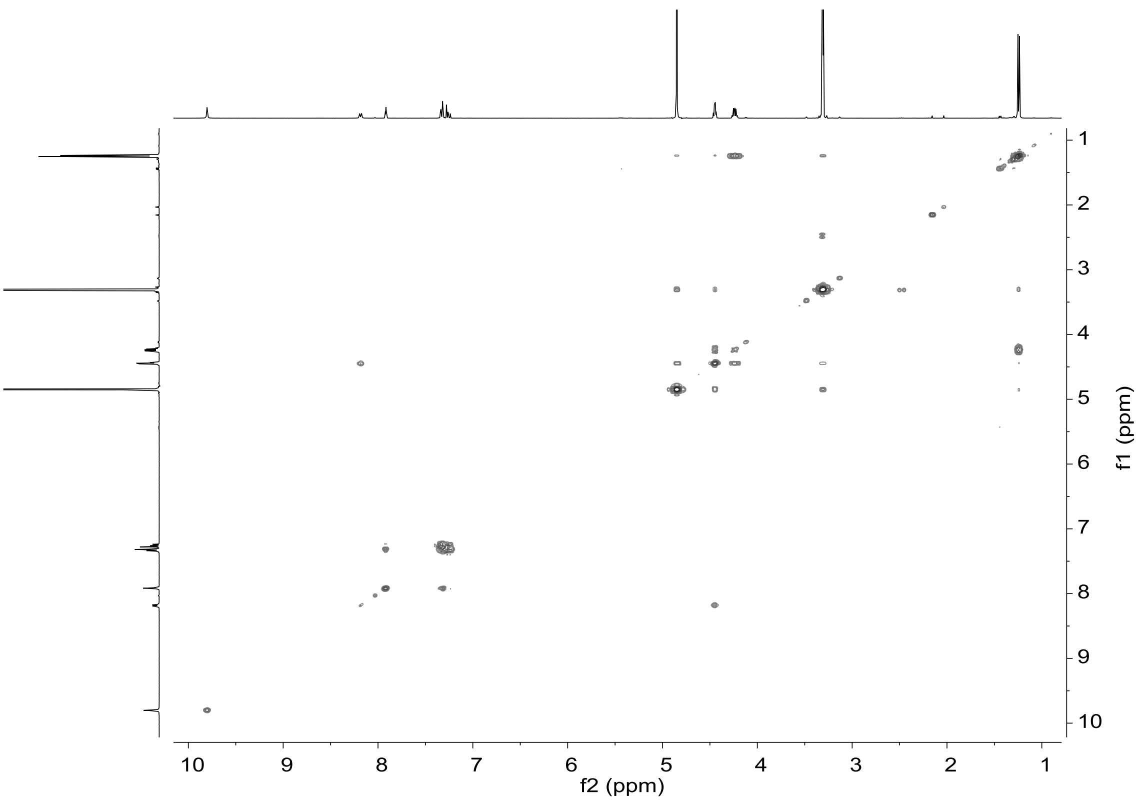
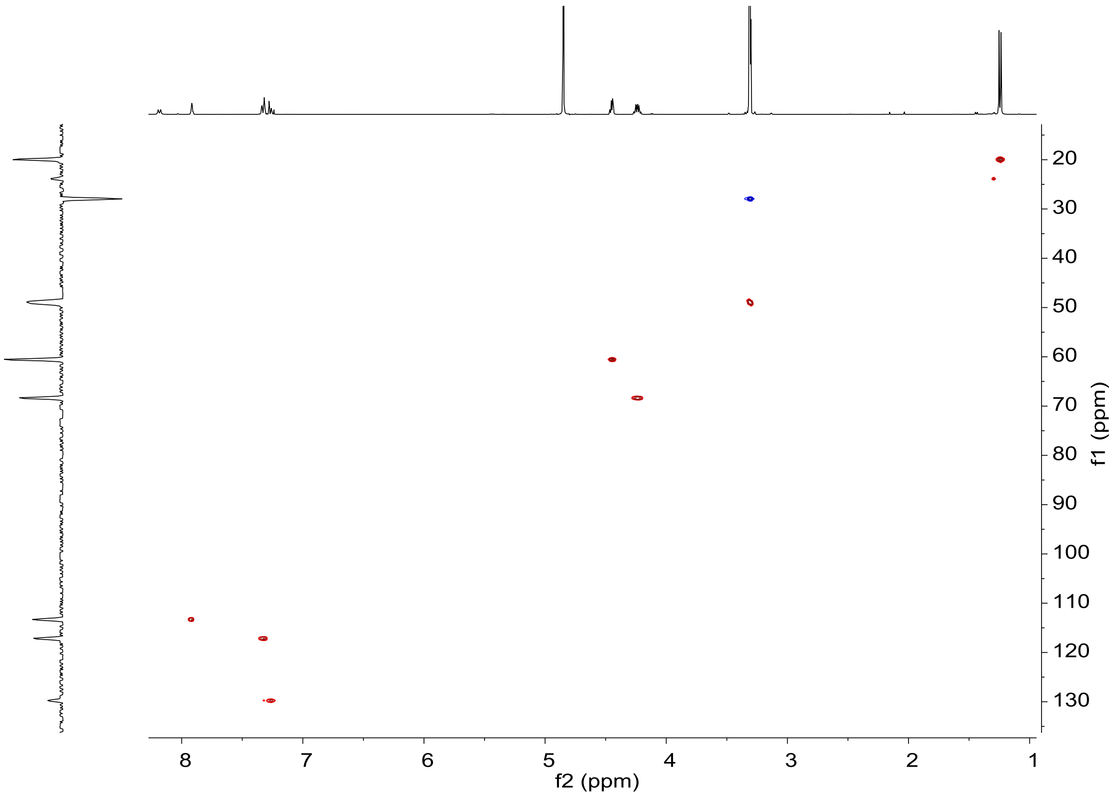


Figure S11. ^{1}H (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{1}H gCOSY (400 MHz, 298 K in MeOD-d_4) spectra of **4d**.



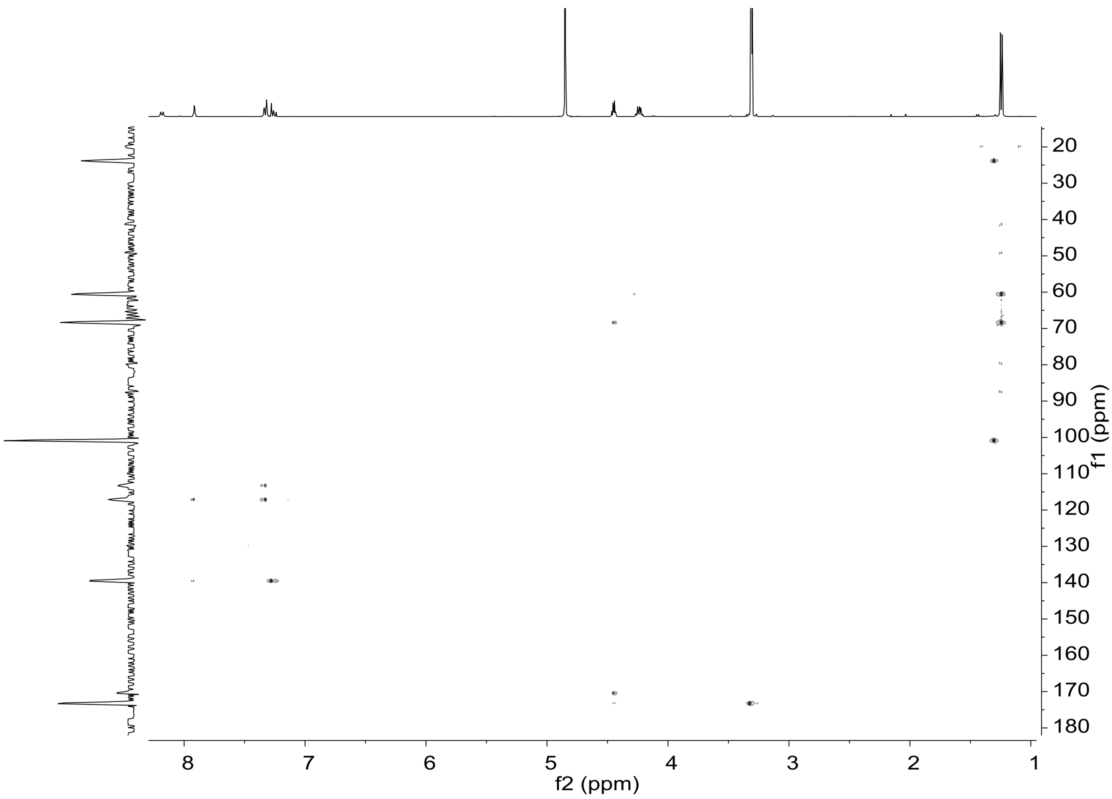


Figure S12. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in MeOD-d_4) spectra of **4d**.

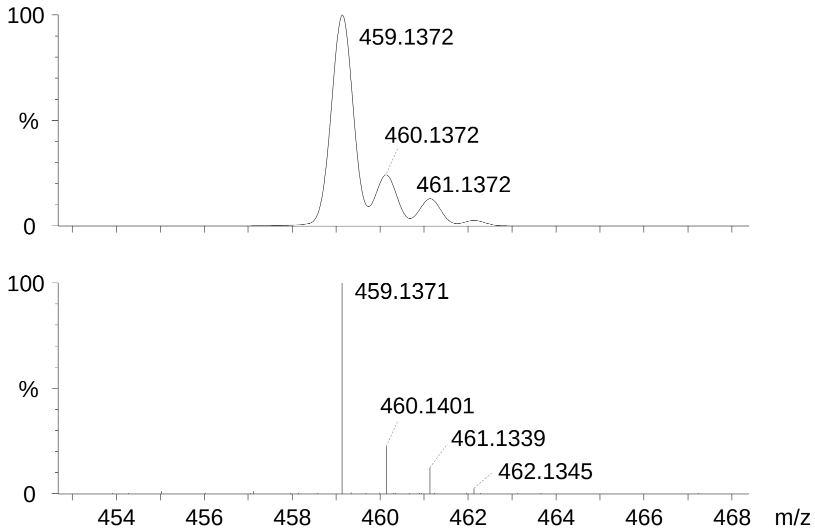
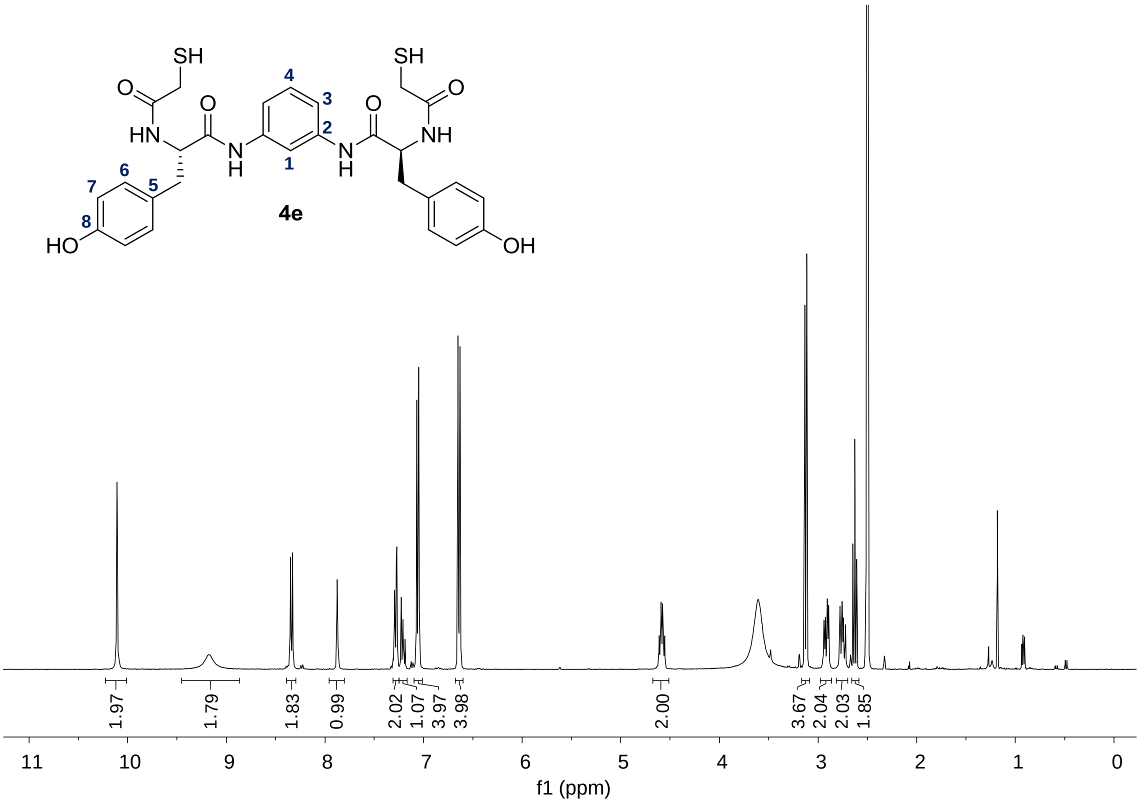


Figure S13. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4d**.

Building block 4e



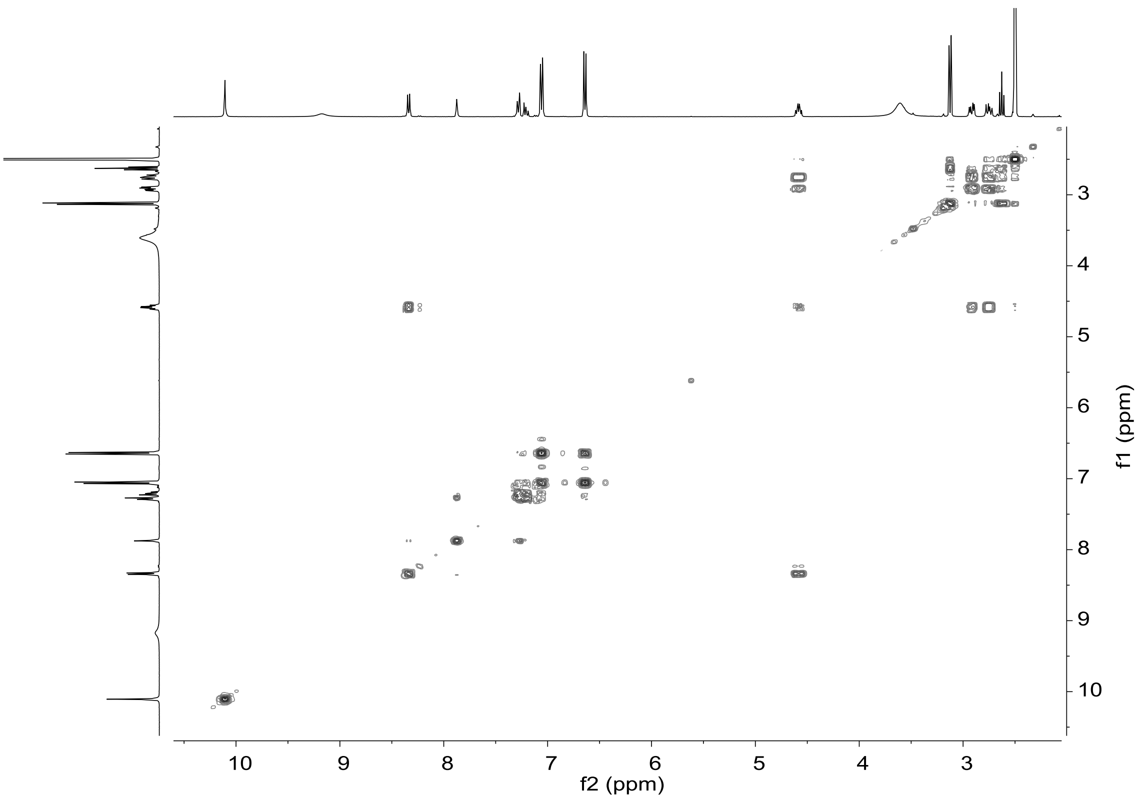
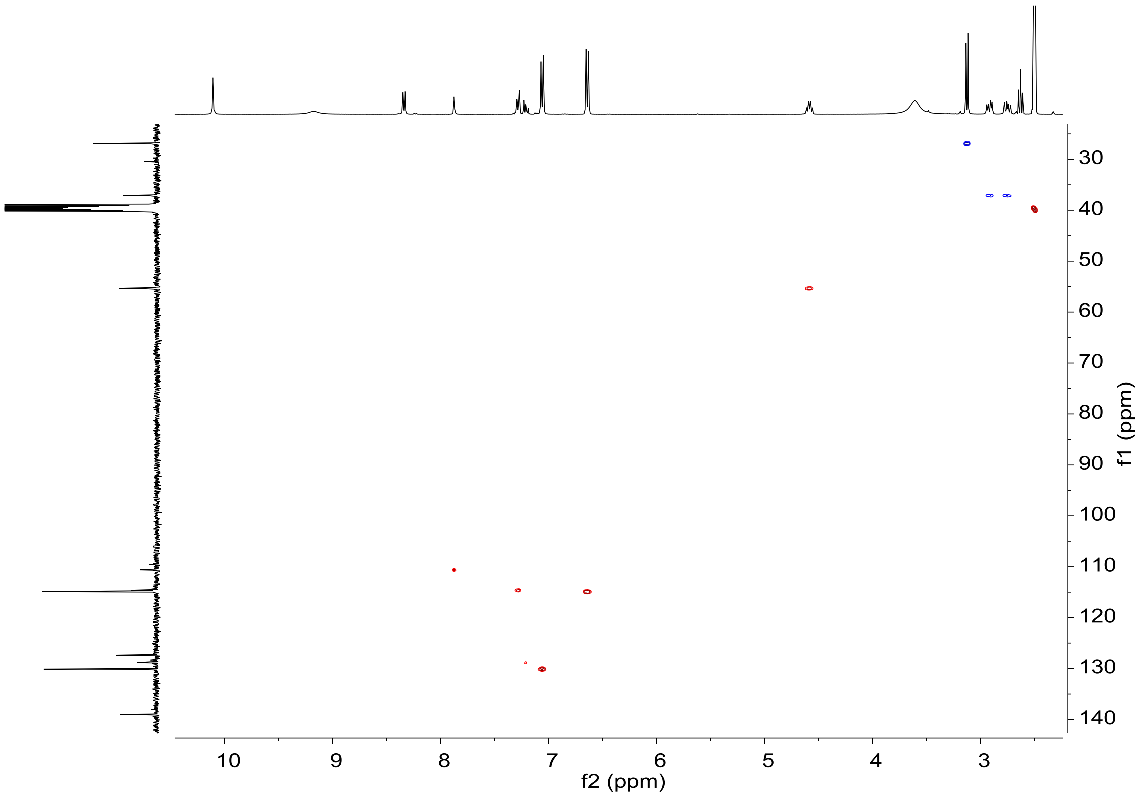


Figure S14. ^{1}H (400 MHz, 298 K in DMSO-d_6) and ^{1}H-^{1}H gCOSY (400 MHz, 298 K in DMSO-d_6) spectra of **4e**.



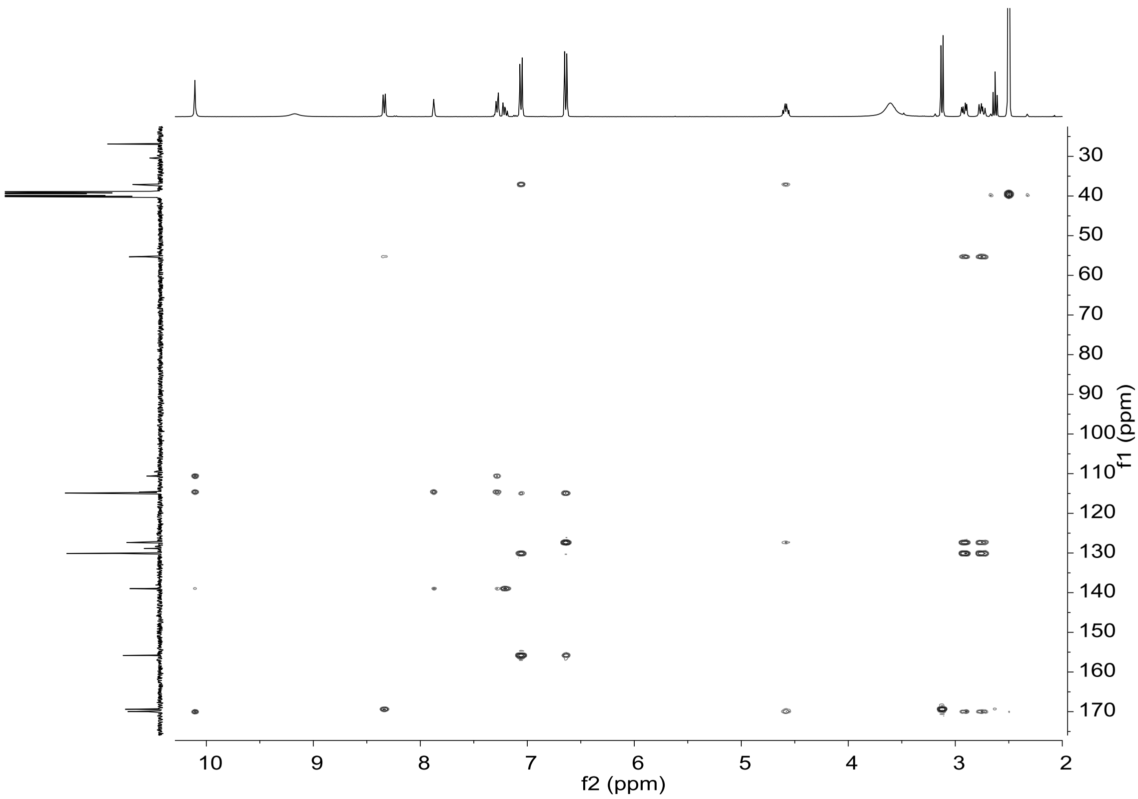


Figure S15. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in DMSO-d_6) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in DMSO-d_6) spectra of **4e**.

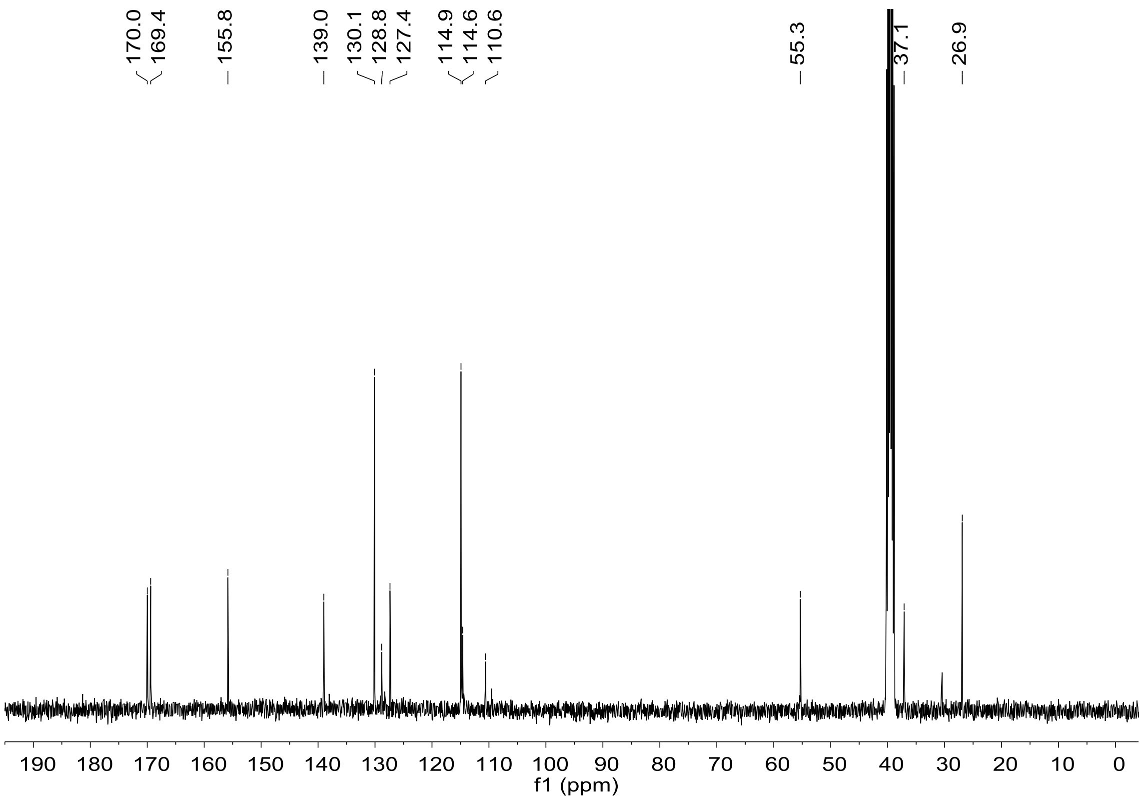


Figure S16. ^{13}C (101 MHz, 298 K in DMSO-d_6) spectrum of **4e**.

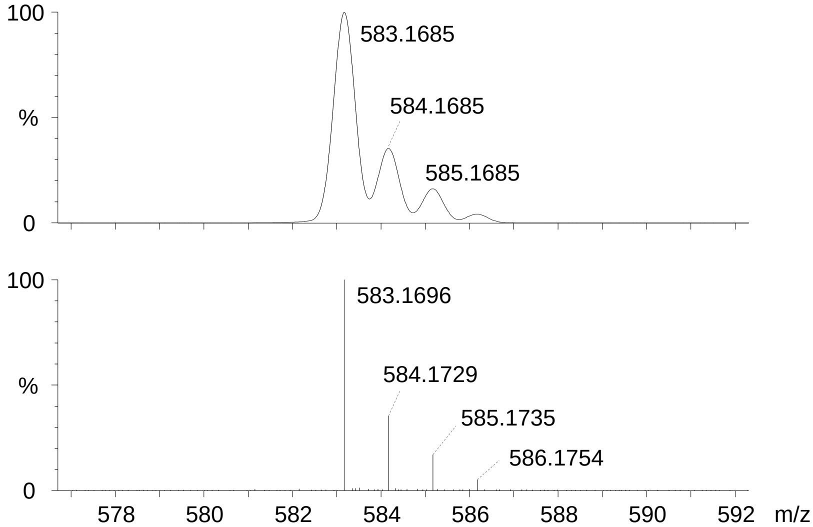


Figure S17. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4e**.

Building block 4f

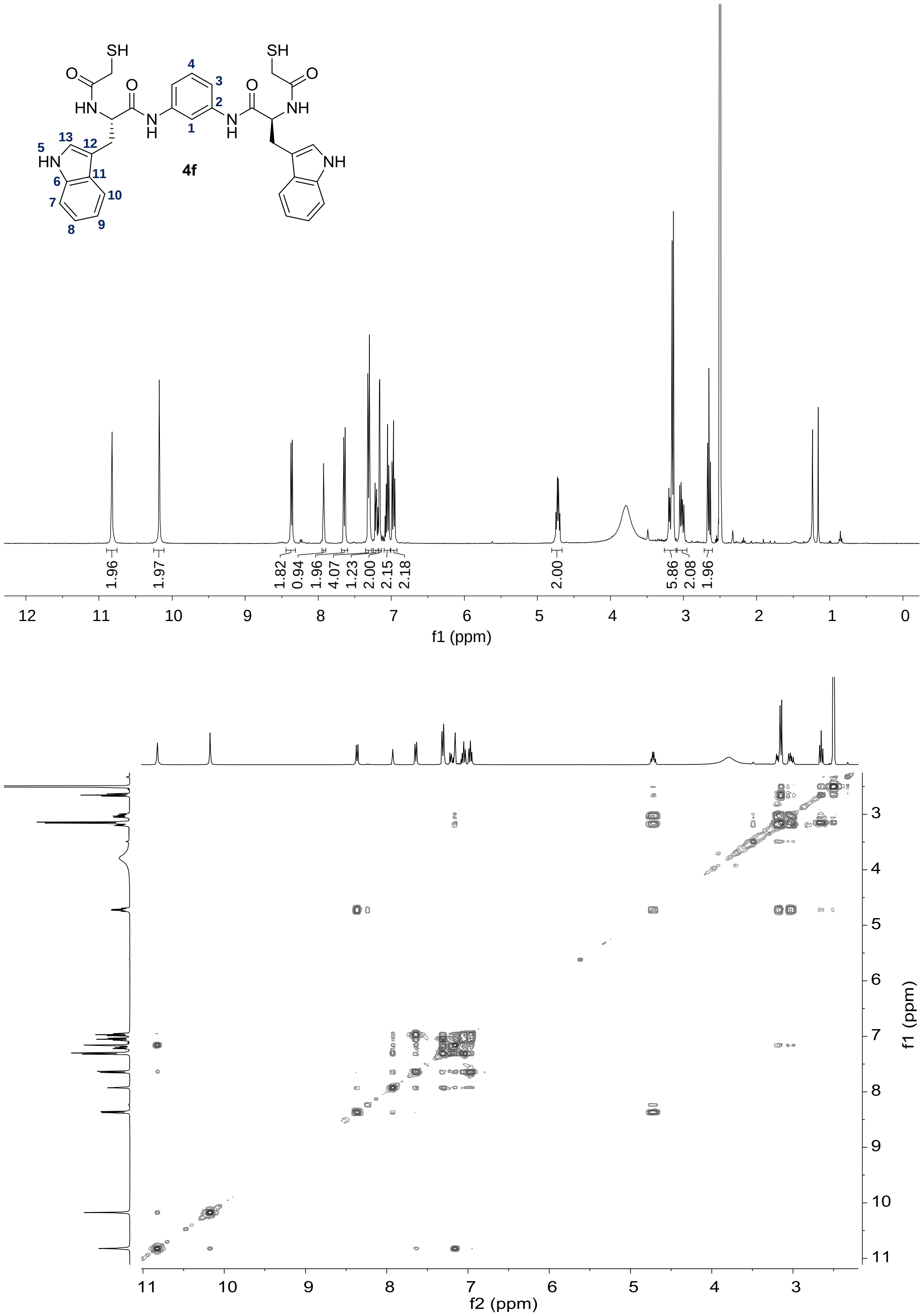
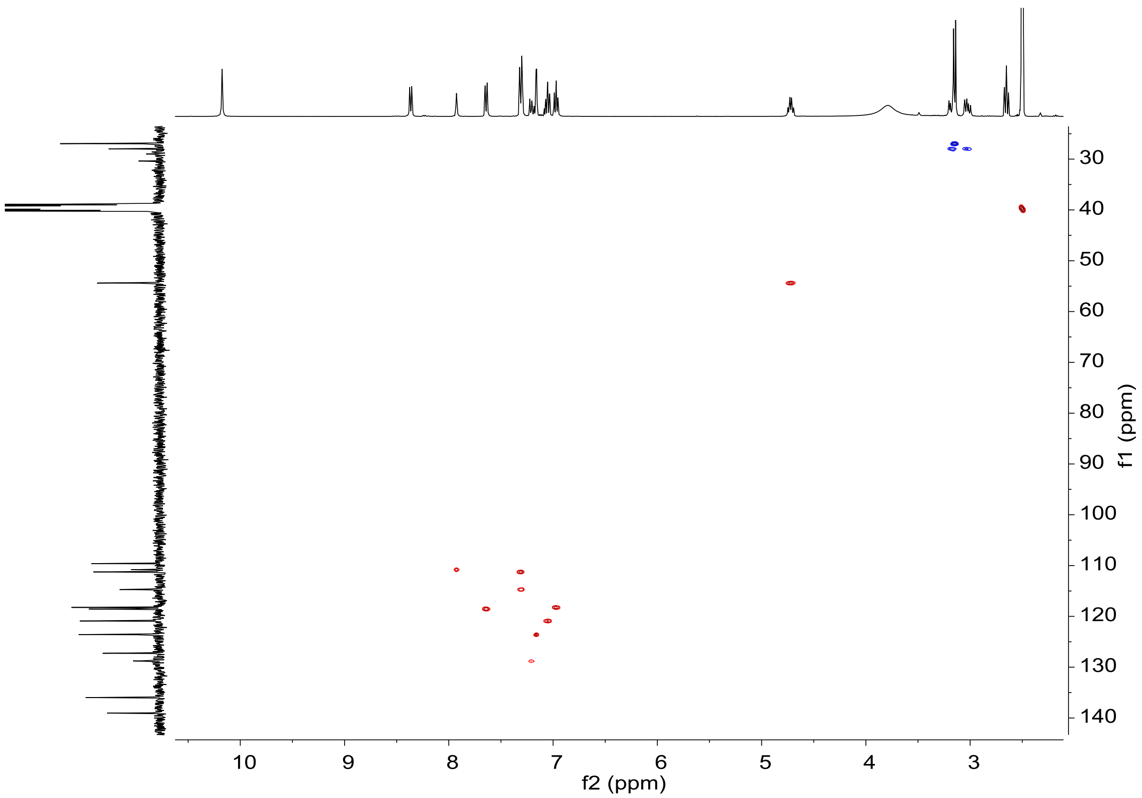


Figure S18. ^{1}H (400 MHz, 298 K in DMSO-d_6) and ^{1}H-^{1}H gCOSY (400 MHz, 298 K in DMSO-d_6) spectra of **4f**.



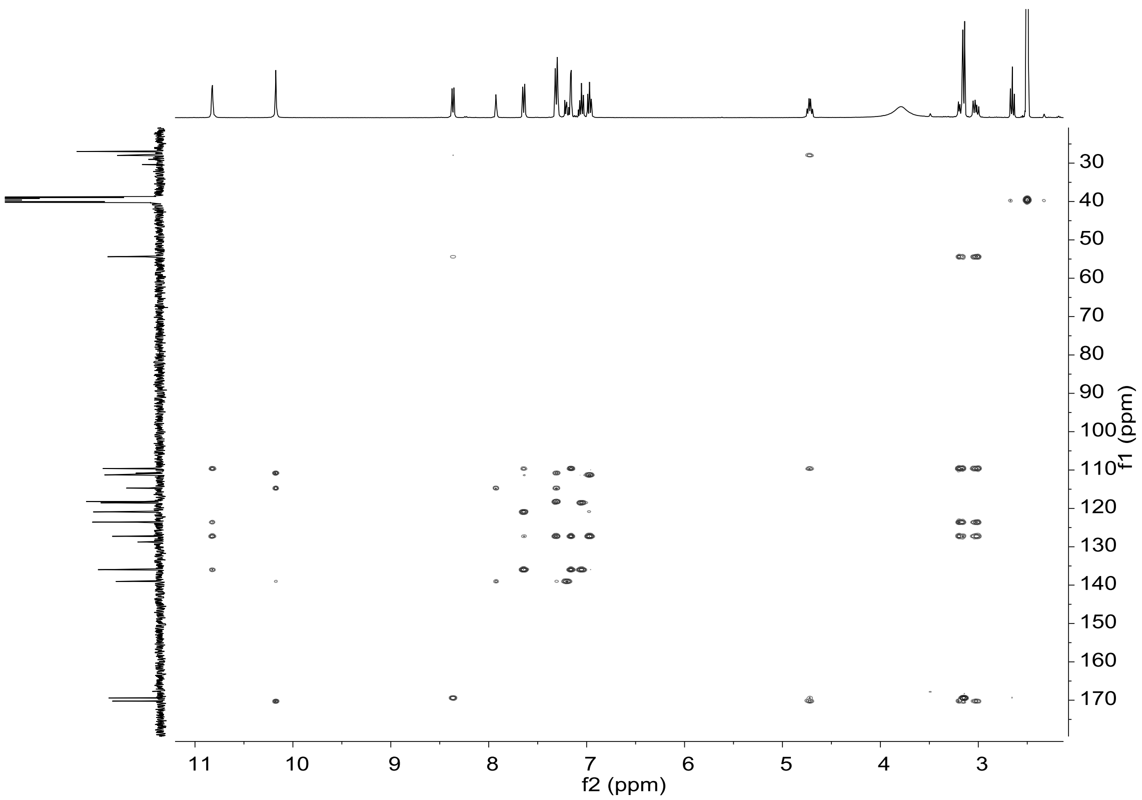


Figure S19. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in DMSO-d_6) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in DMSO-d_6) spectra of **4f**.

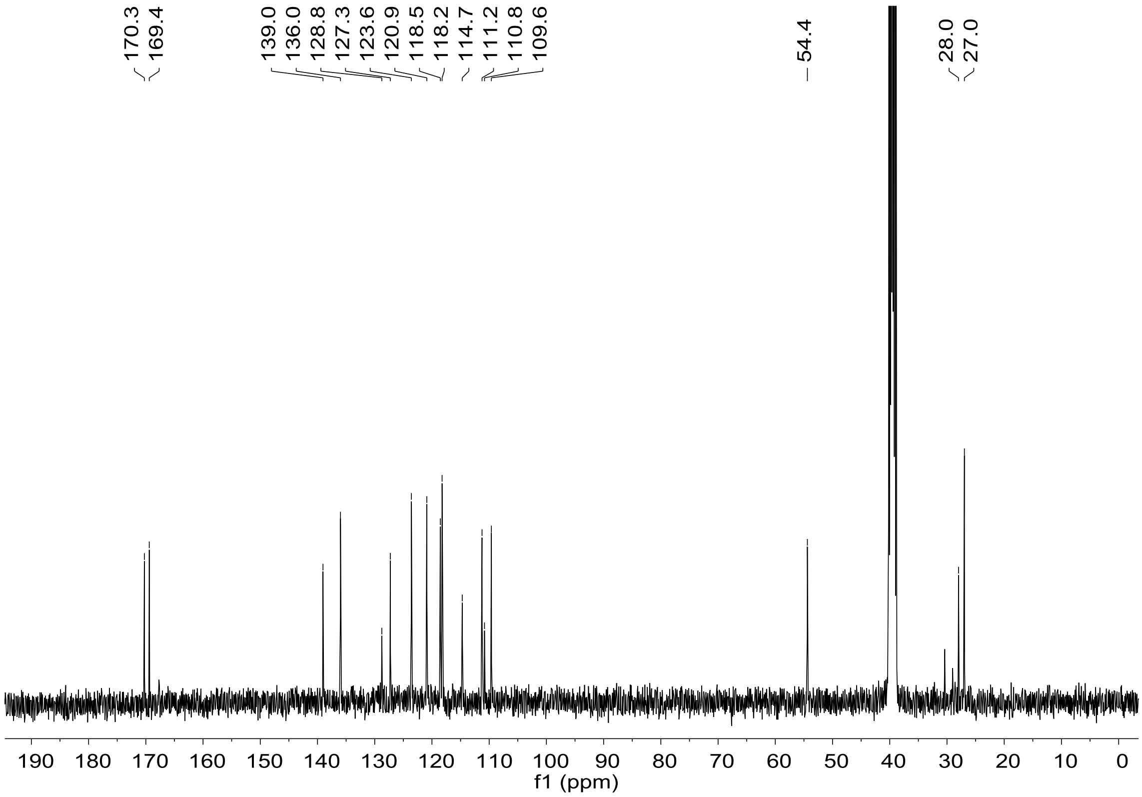


Figure S20. ^{13}C (101 MHz, 298 K in DMSO-d_6) spectrum of **4f**.

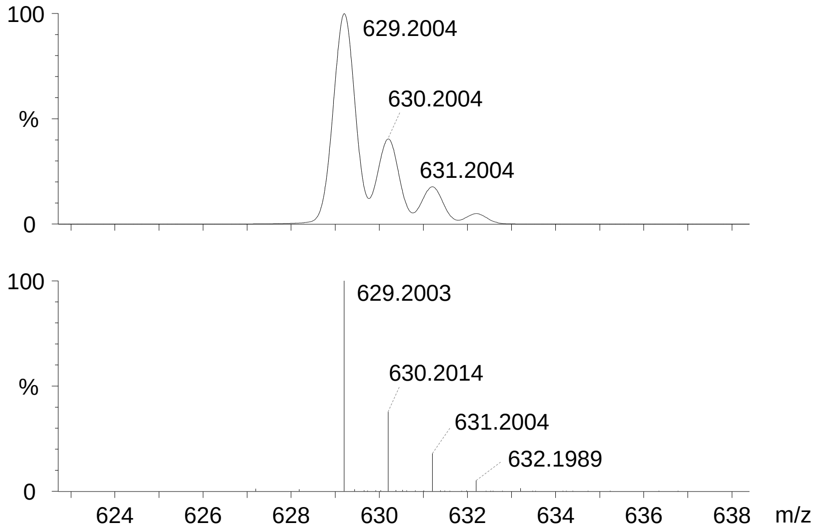


Figure S21. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4f**.

Building block 4g

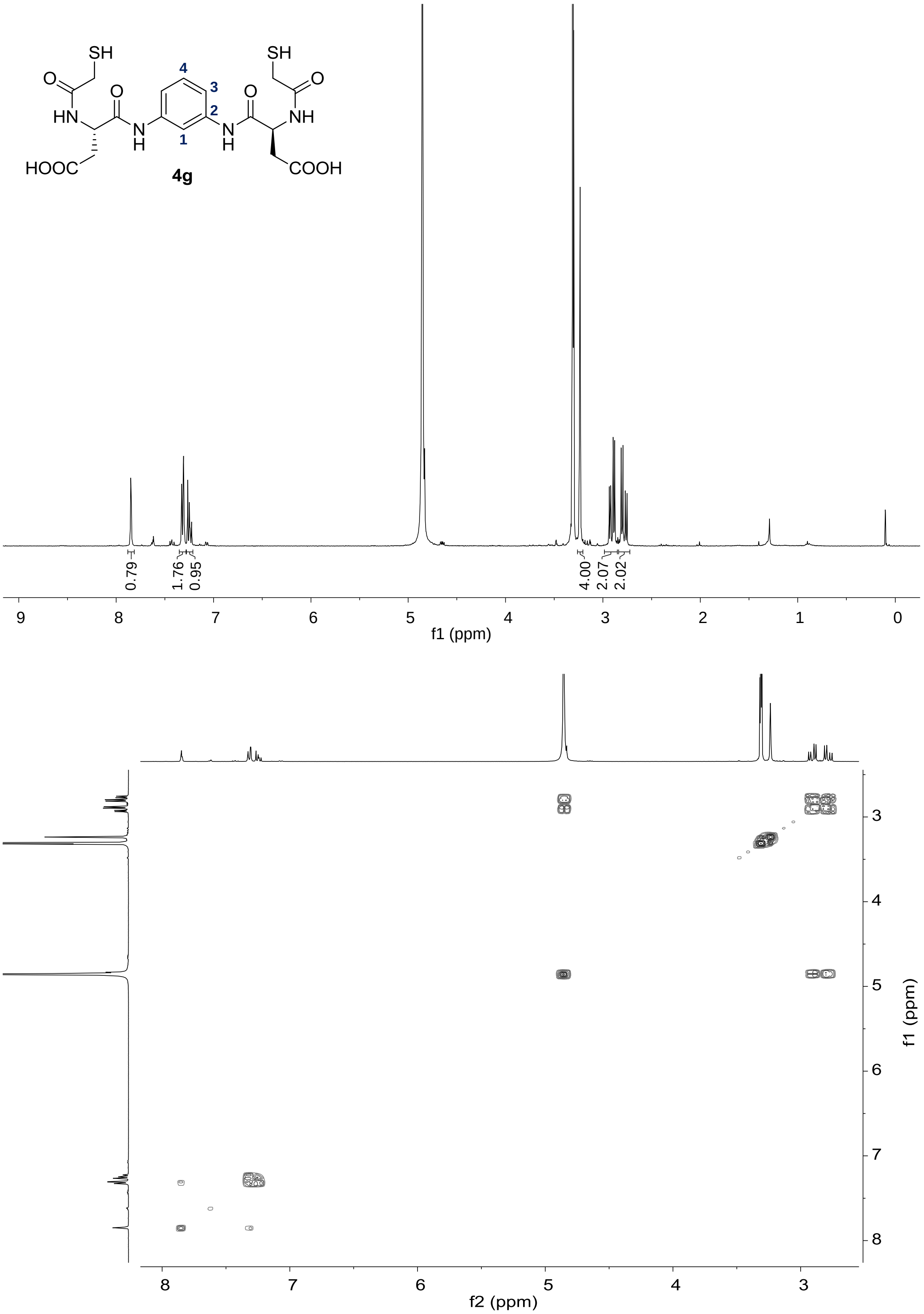
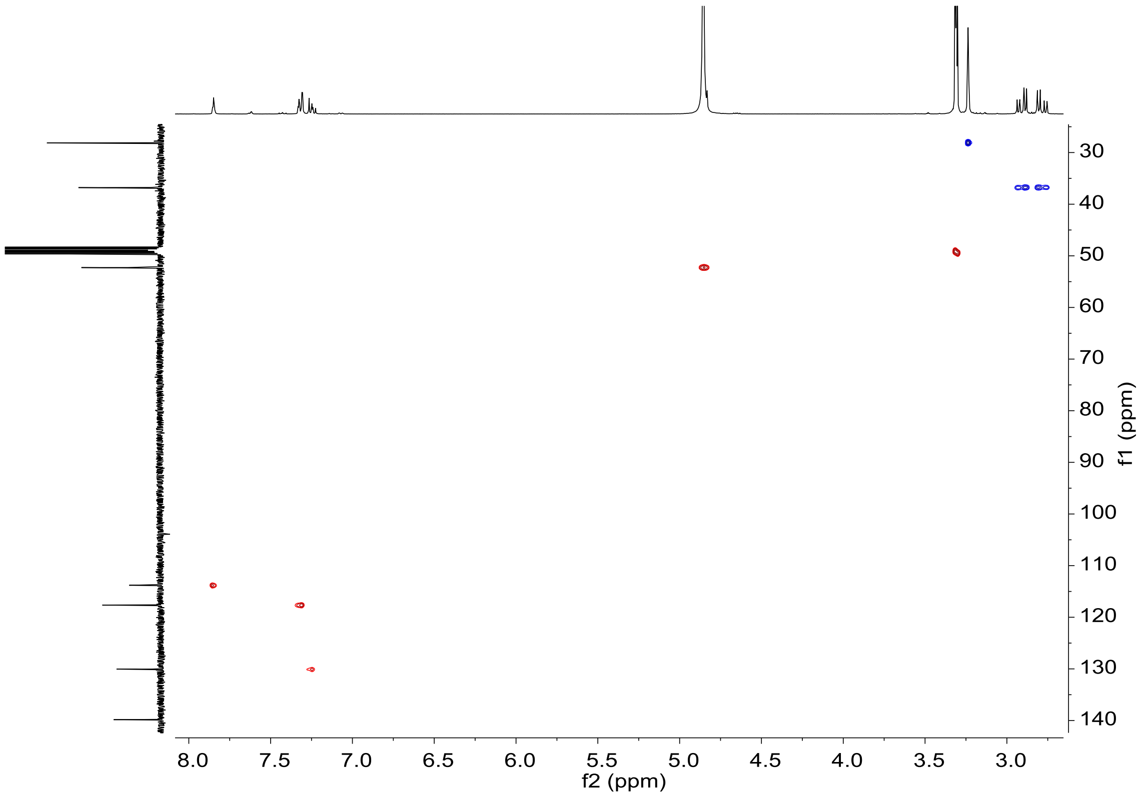


Figure S22. ^{1}H (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{1}H gCOSY (400 MHz, 298 K in MeOD-d_4) spectra of **4g**.



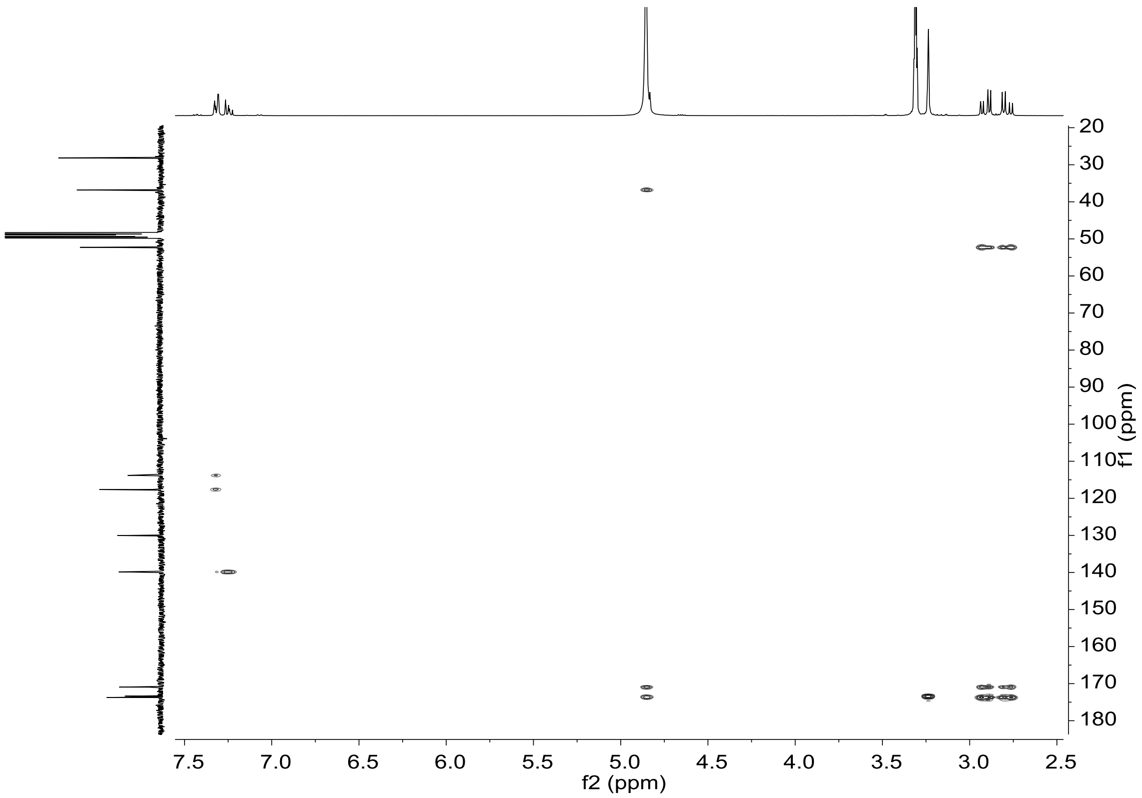


Figure S23. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in MeOD-d_4) spectra of **4g**.

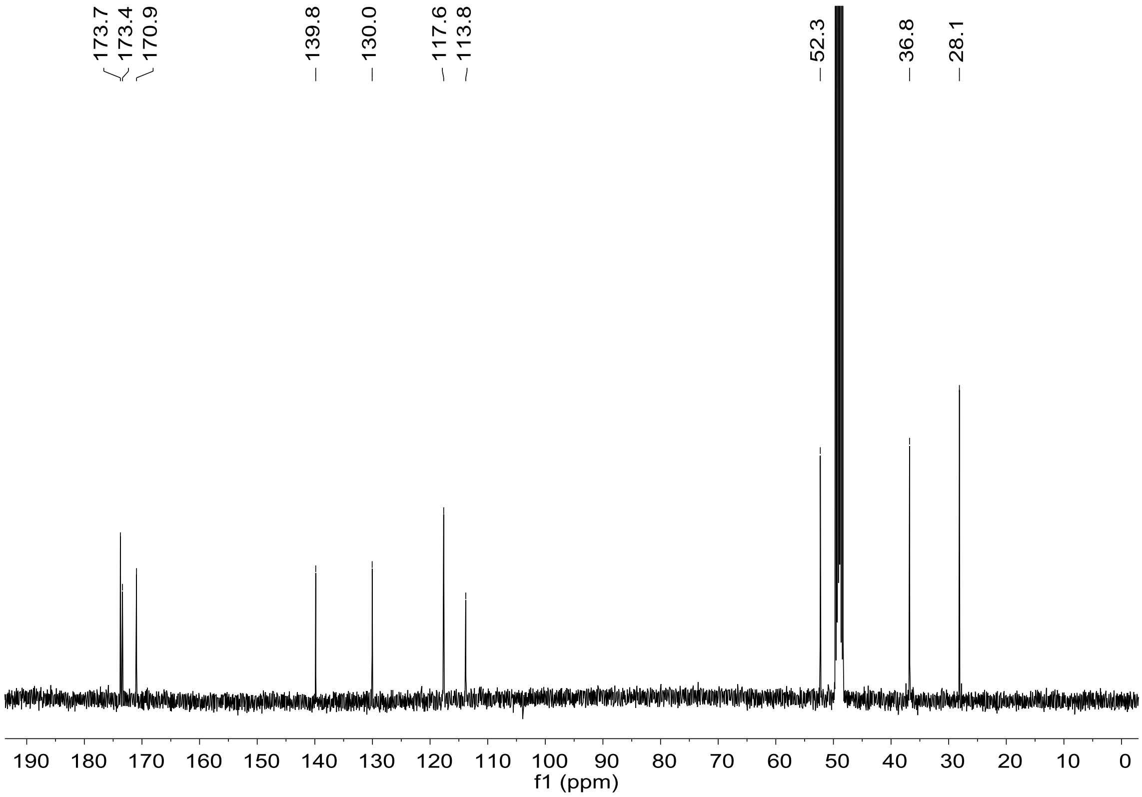


Figure S24. ^{13}C (101 MHz, 298 K in MeOD-d_4) spectrum of **4g**.

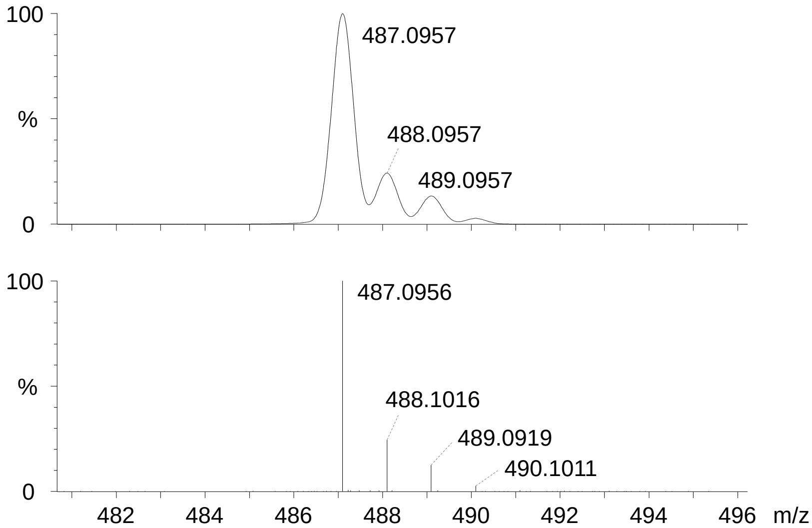


Figure S25. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4g**.

Building block 4h

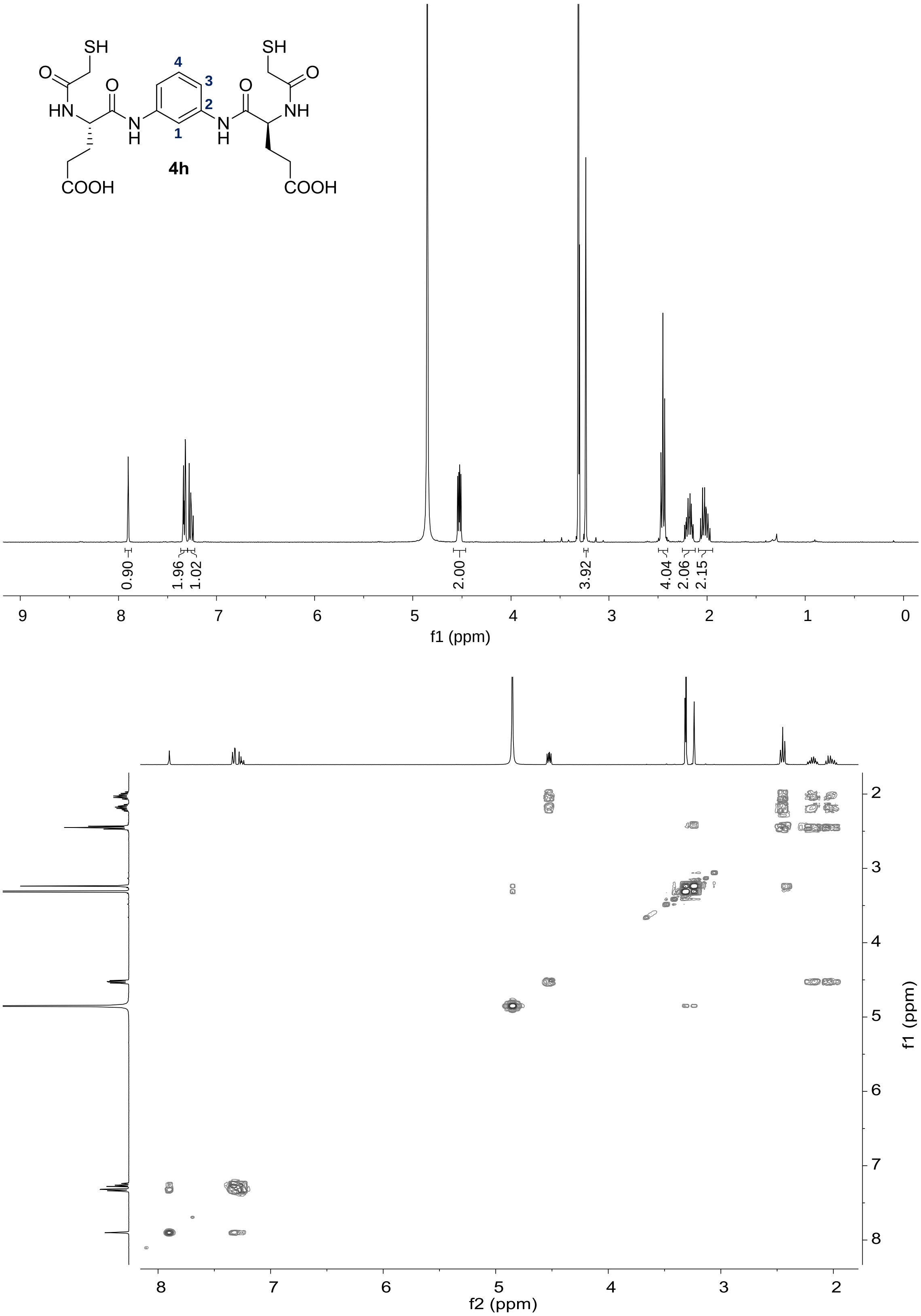


Figure S26. [1]H (400 MHz, 298 K in MeOD-d_4) and [1]H-[1]H gCOSY (400 MHz, 298 K in MeOD-d_4) spectra of **4h**.

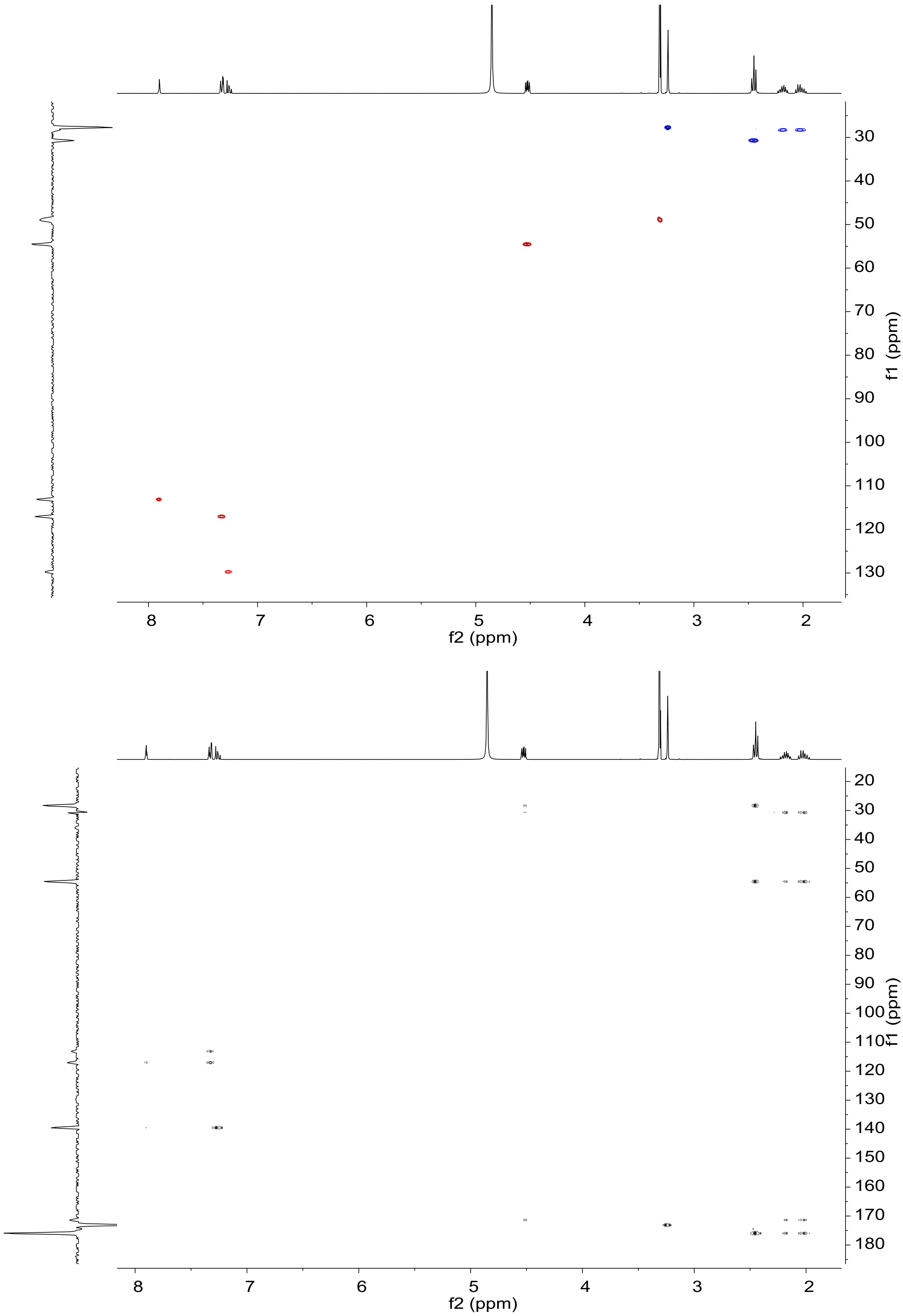


Figure S27. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in MeOD-d_4) spectra of **4h**.

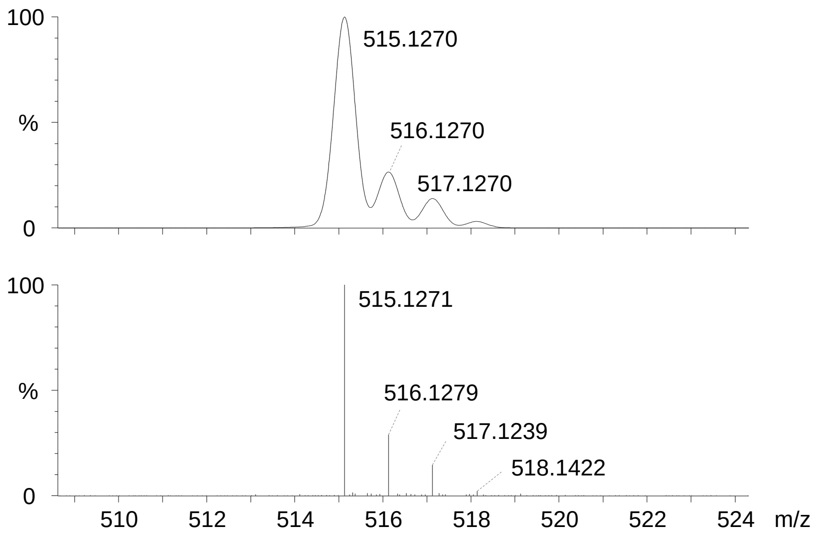


Figure S28. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4h**.

Building block 4i

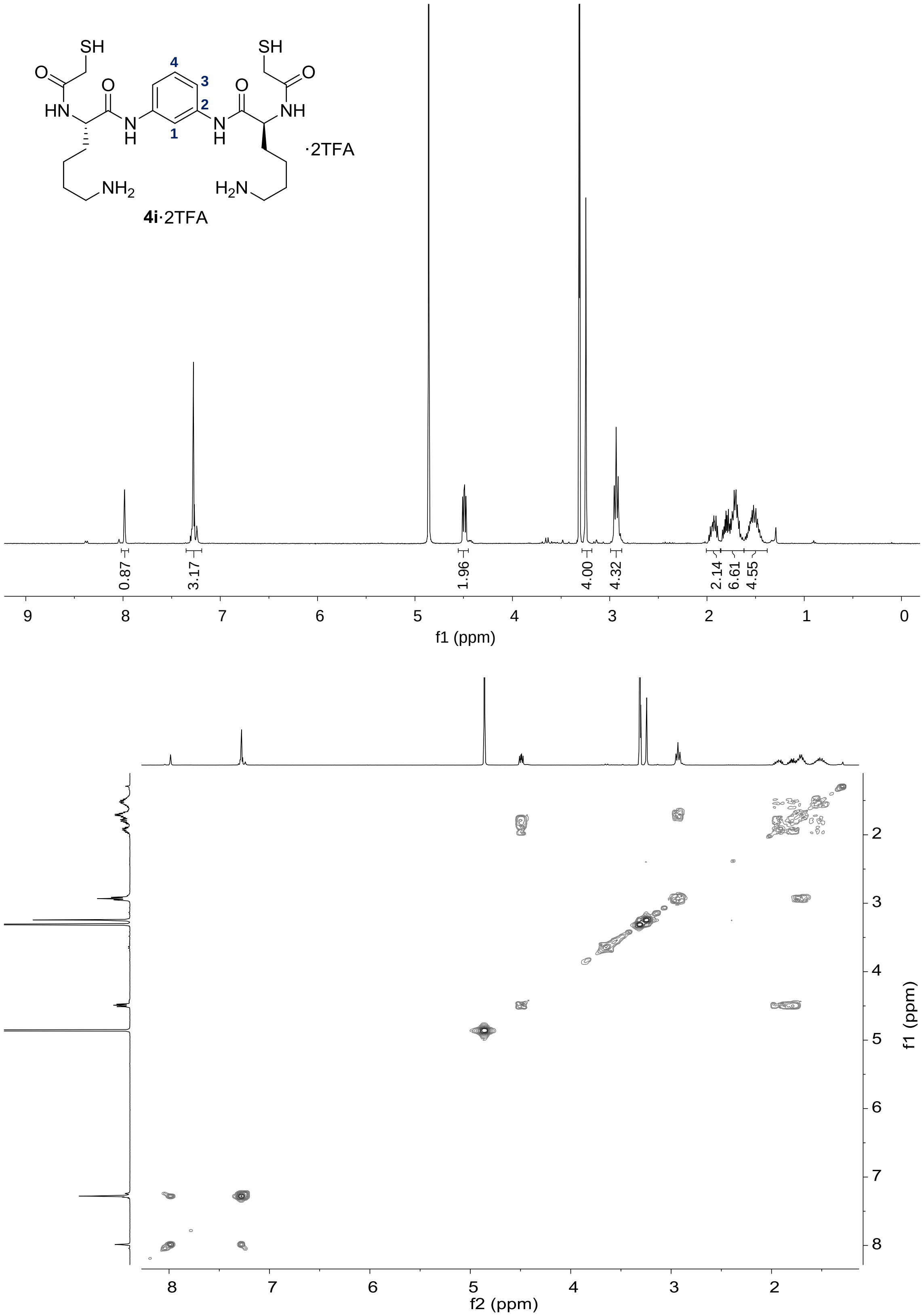


Figure S29. ^{1}H (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{1}H gCOSY (400 MHz, 298 K in MeOD-d_4) spectra of **4i**·2TFA.

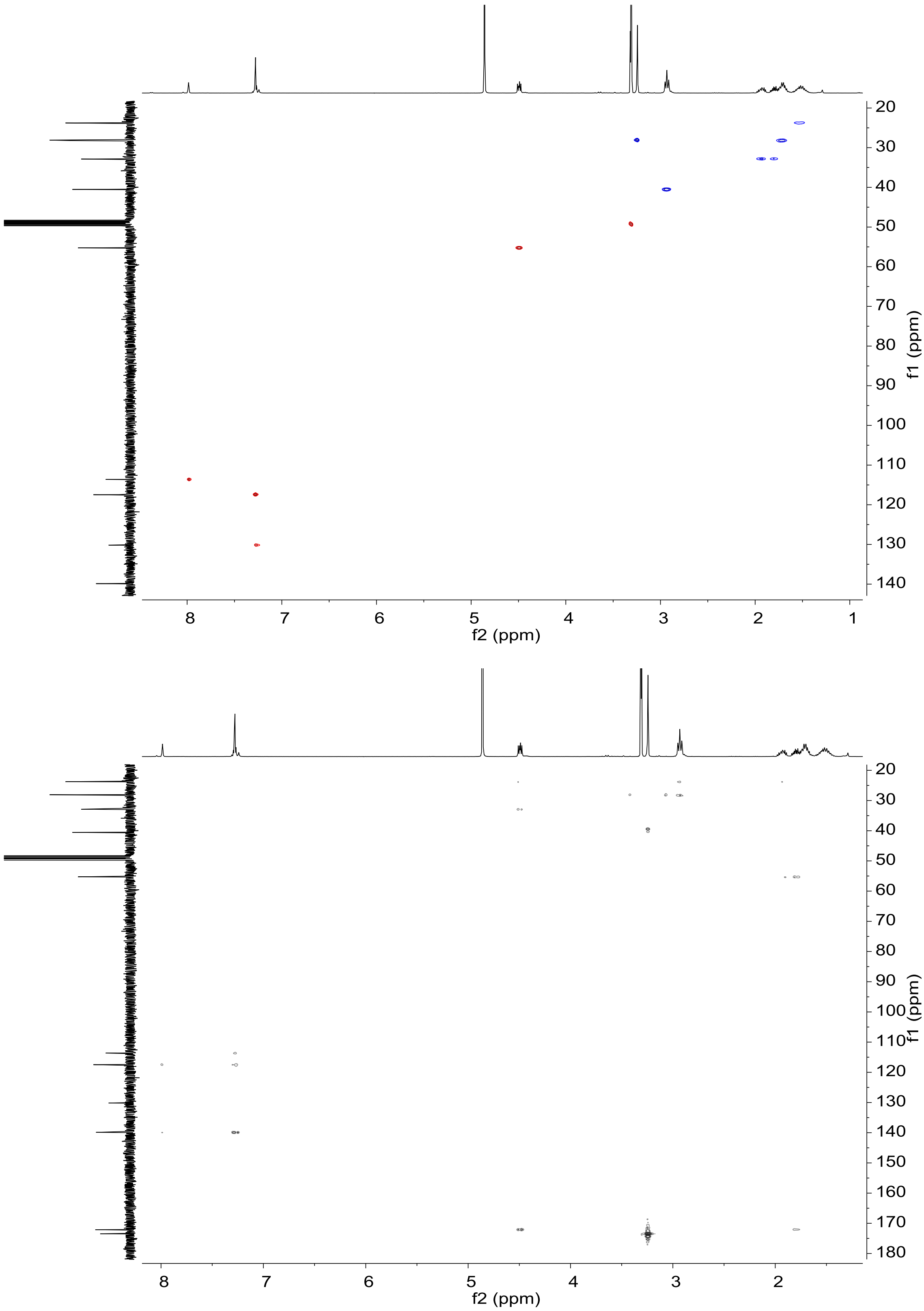


Figure S30. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in MeOD-d_4) spectra of **4i**·2TFA.

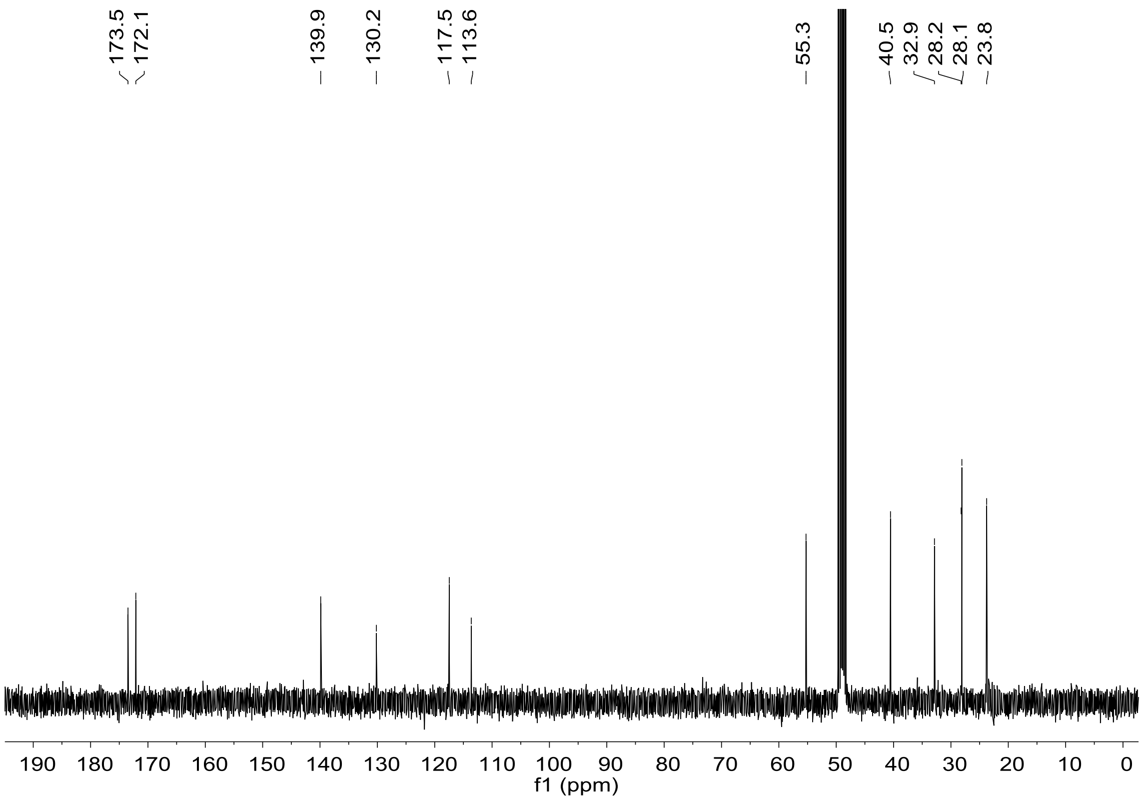


Figure S31. ^{13}C (101 MHz, 298 K in MeOD-d_4) spectrum of **4i**·2TFA.

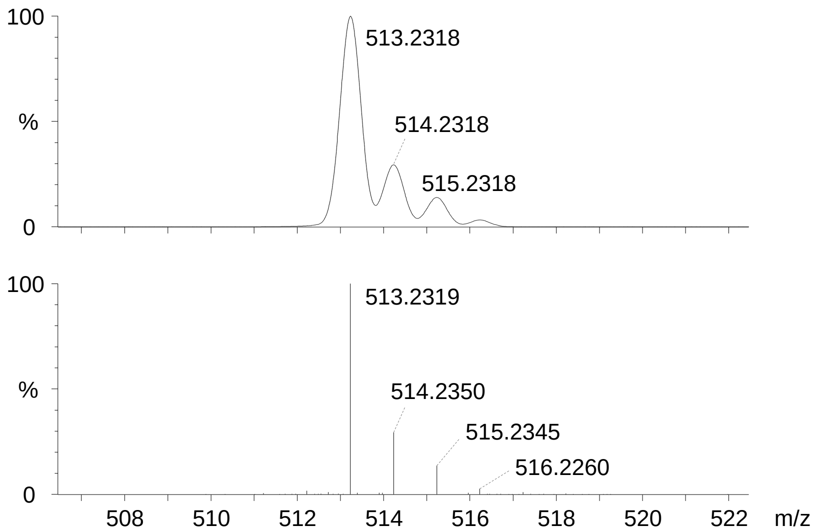


Figure S32. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4i**.

Building block 4j

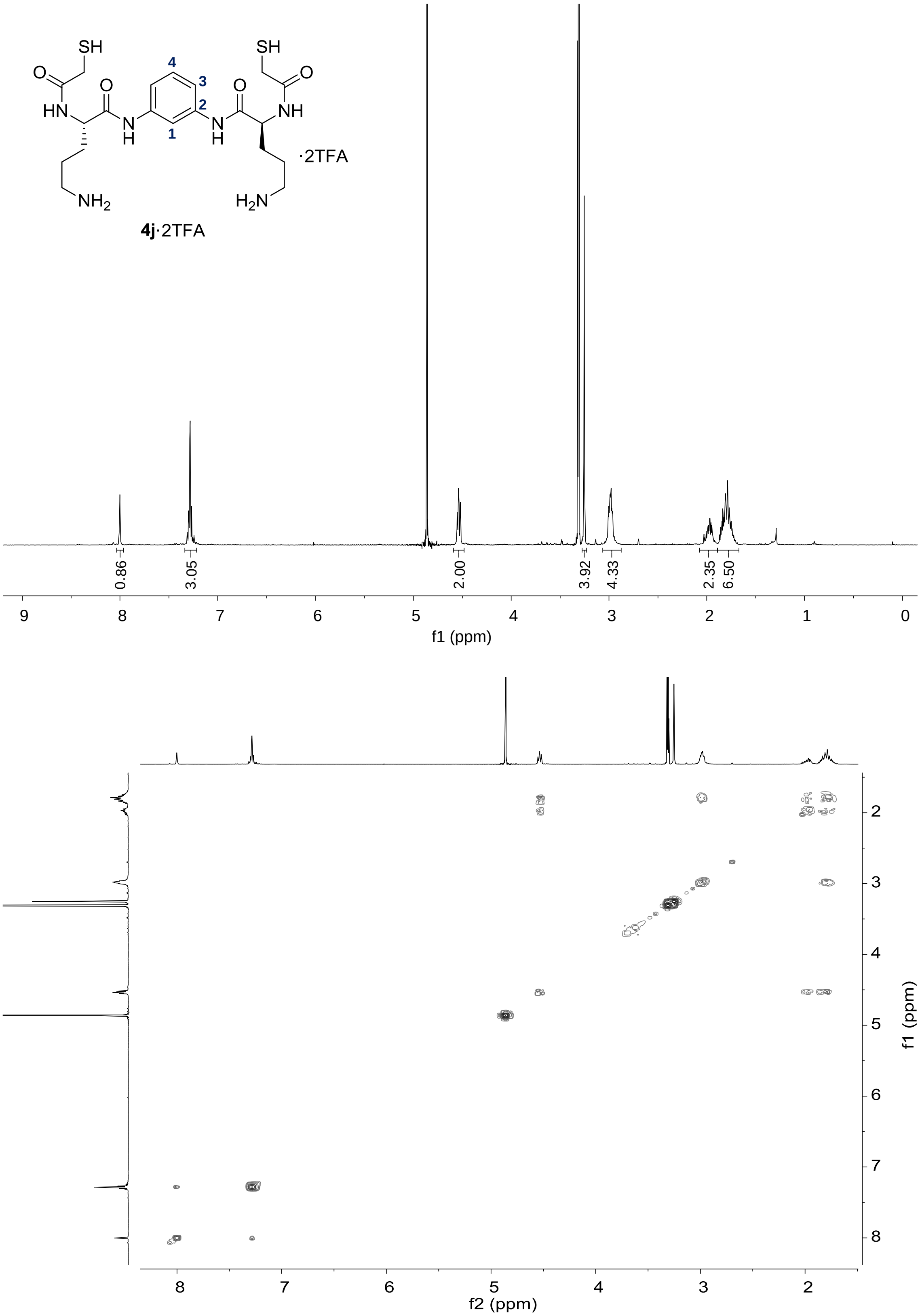
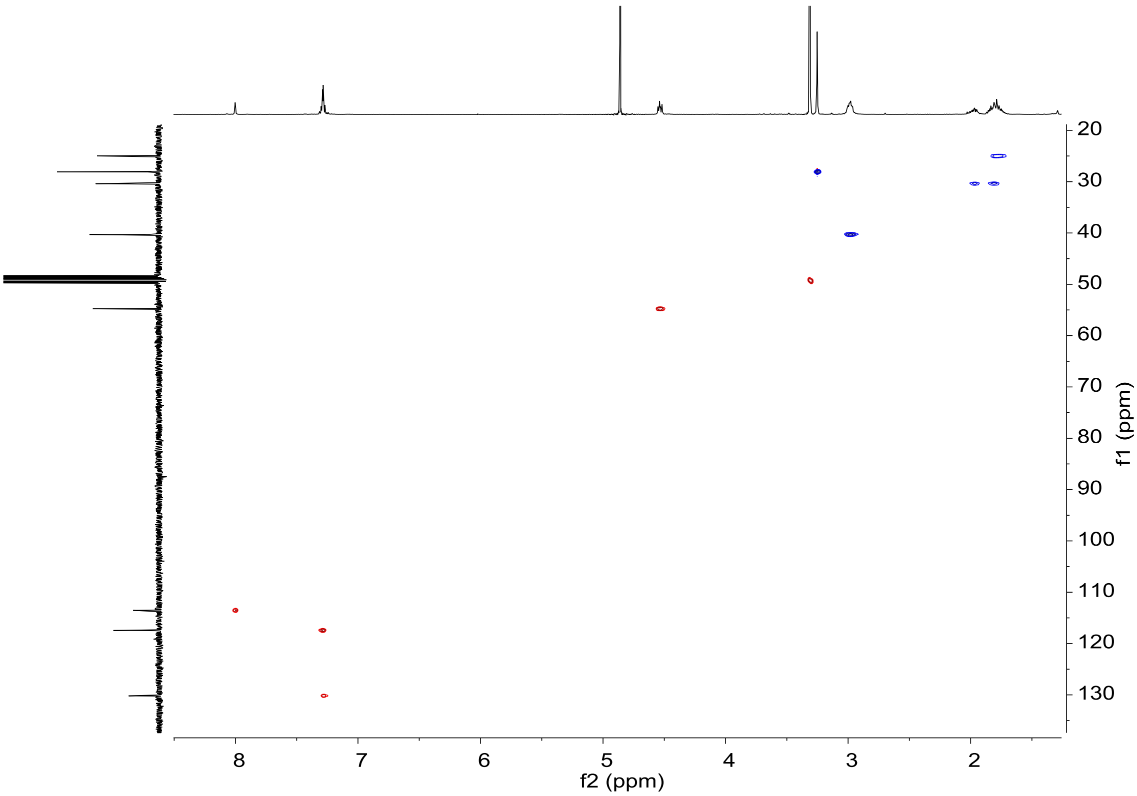


Figure S33. ^{1}H (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{1}H gCOSY (400 MHz, 298 K in MeOD-d_4) spectra of **4j**·2TFA.



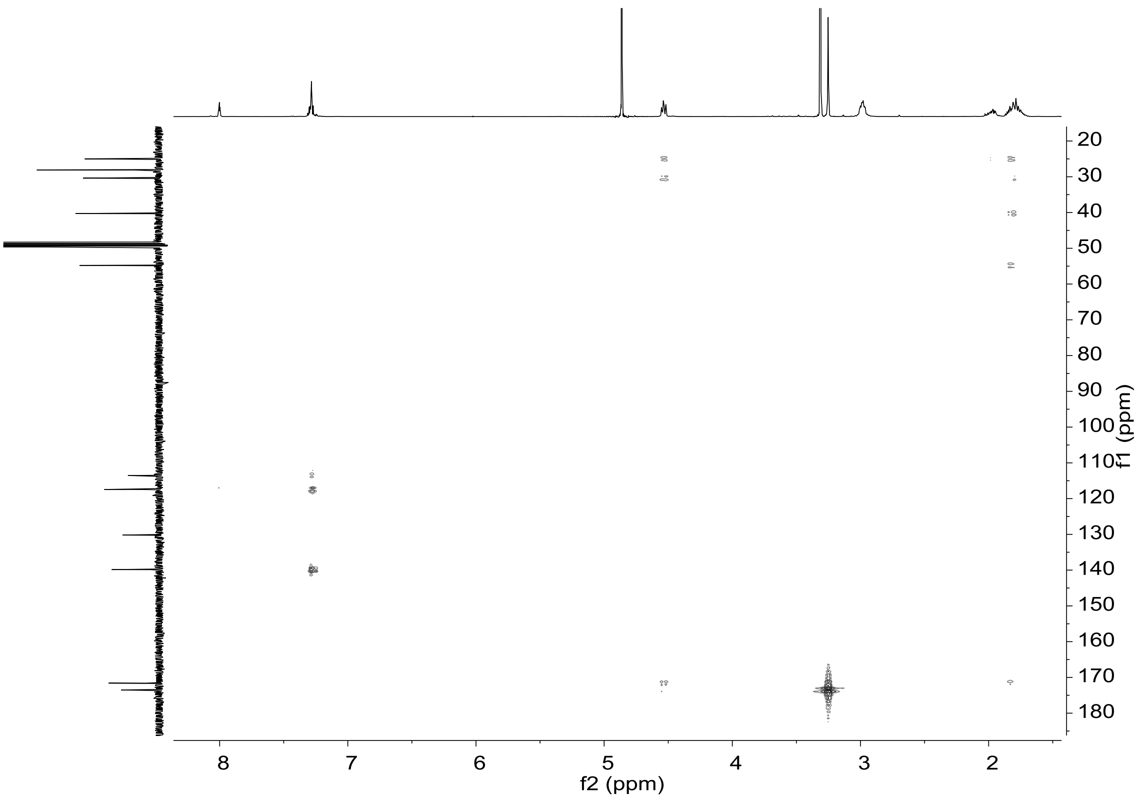


Figure S34. [1]H-[13]C gHSQC (400 MHz, 298 K in MeOD-d_4) and [1]H-[13]C gHMBC (400 MHz, 298 K in MeOD-d_4) spectra of **4j**·2TFA.

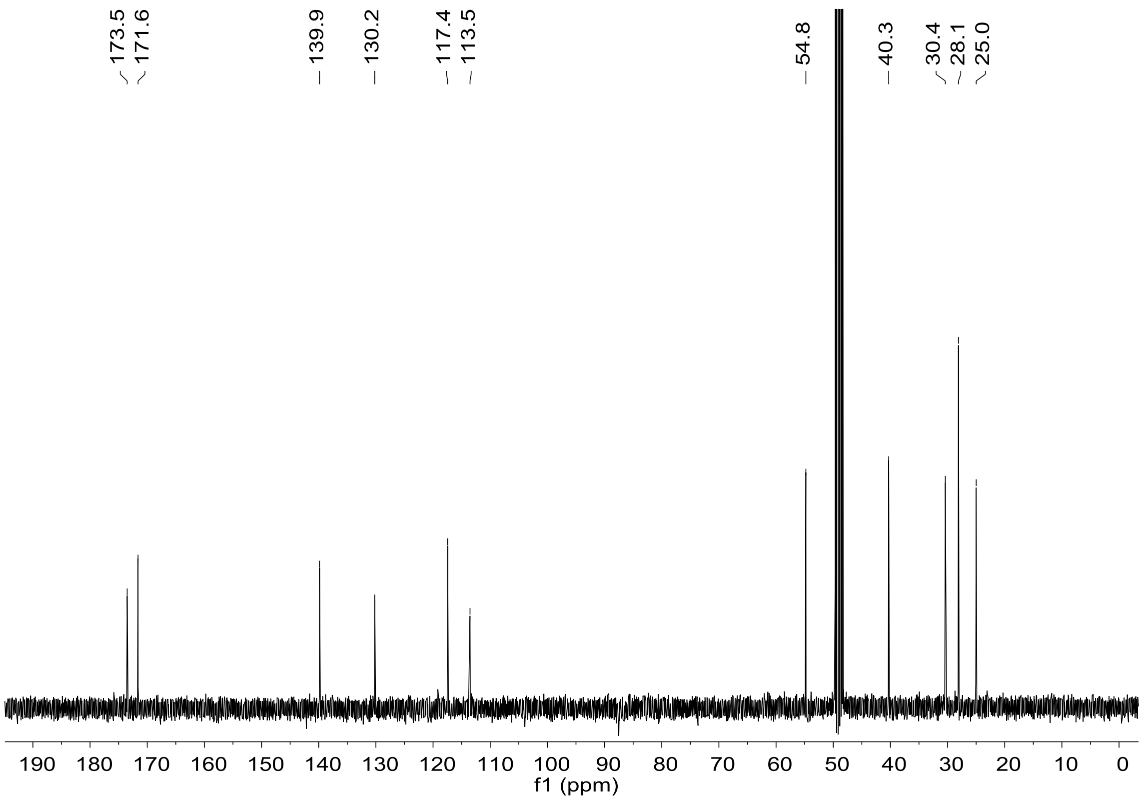


Figure S35. ^{13}C (101 MHz, 298 K in MeOD-d_4) spectrum of **4j**·2TFA.

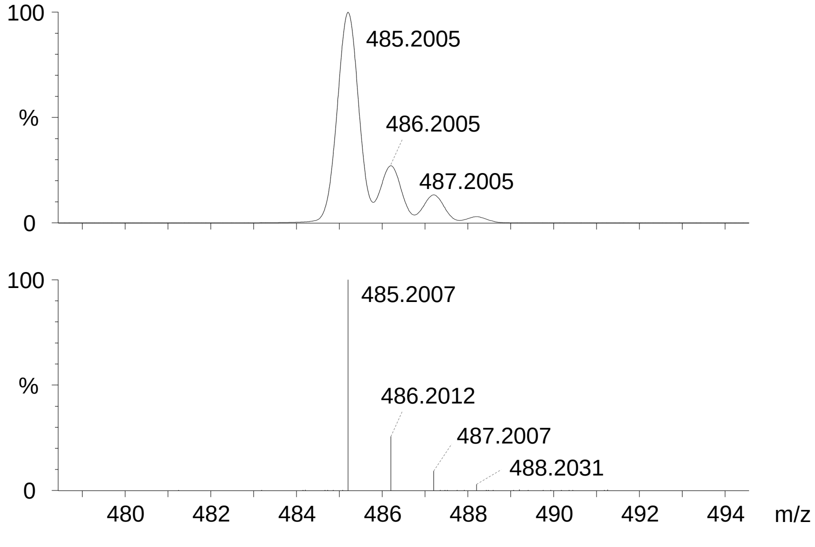
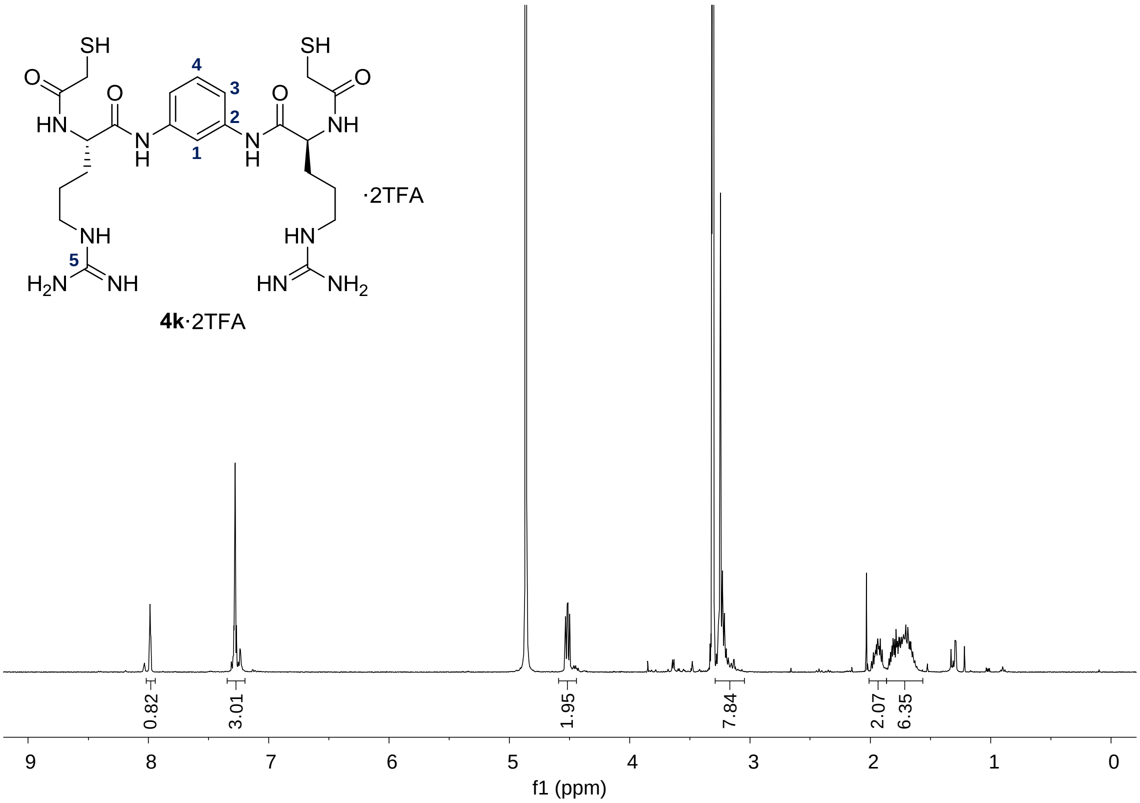


Figure S36. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4j**.

Building block 4k



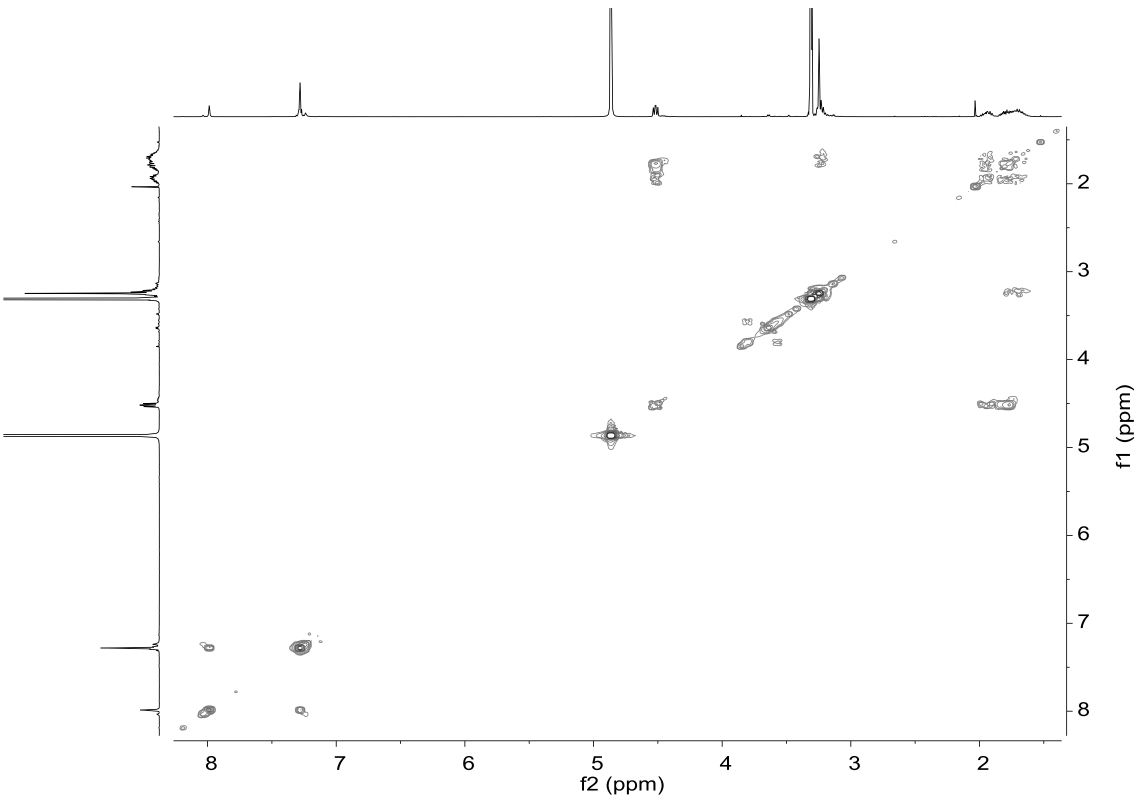
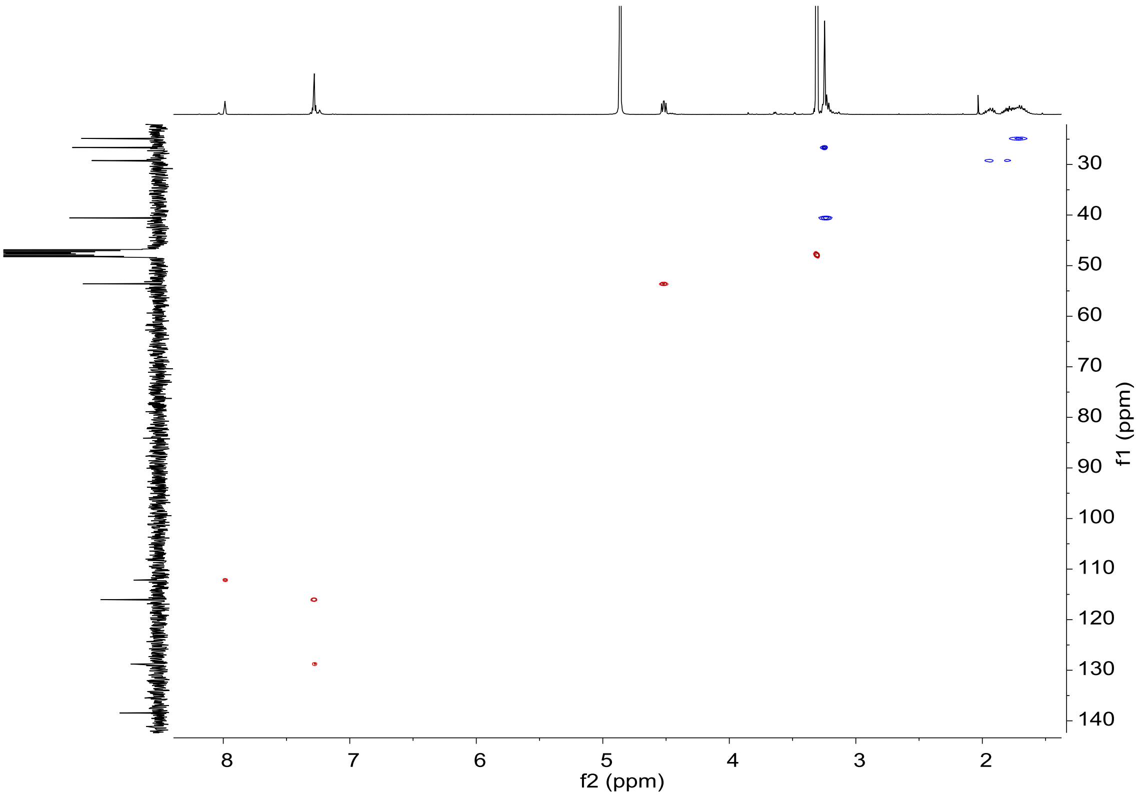


Figure S37. ^{1}H (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{1}H gCOSY (400 MHz, 298 K in MeOD-d_4) spectra of **4k**·2TFA.



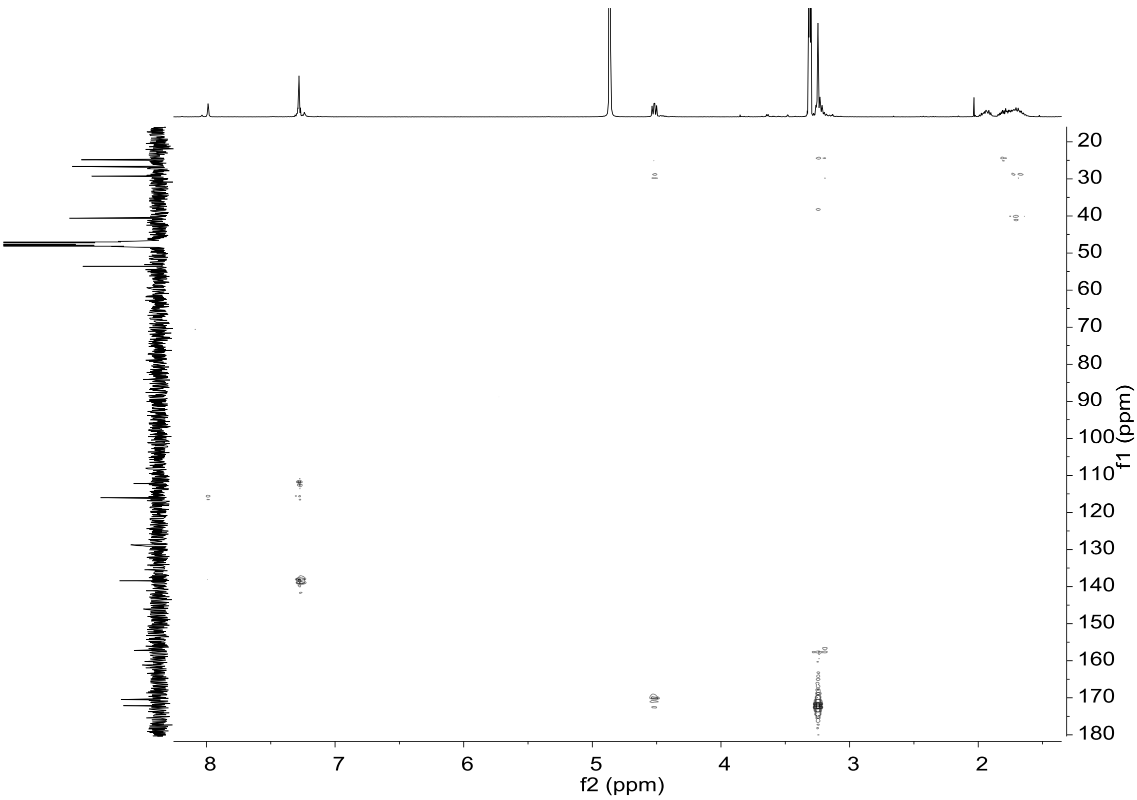


Figure S38. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in MeOD-d_4) spectra of **4k**·2TFA.

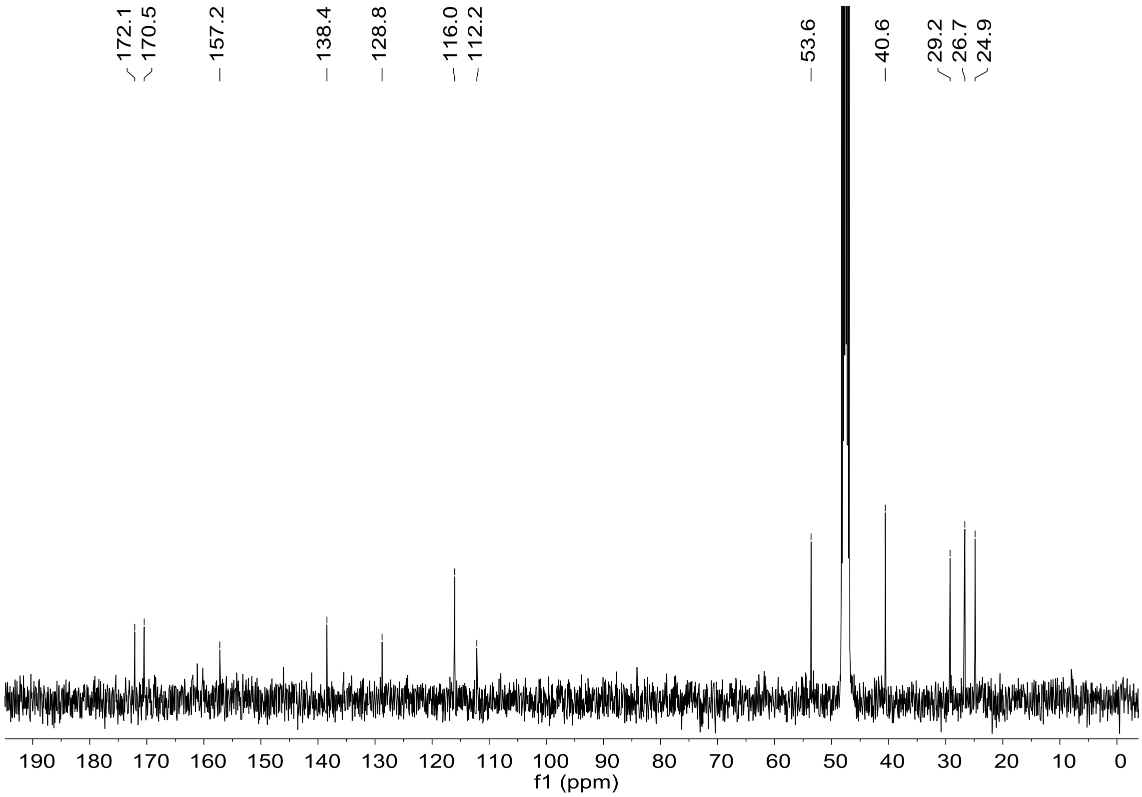


Figure S39. ^{13}C (101 MHz, 298 K in MeOD-d_4) spectrum of **4k**·2TFA.

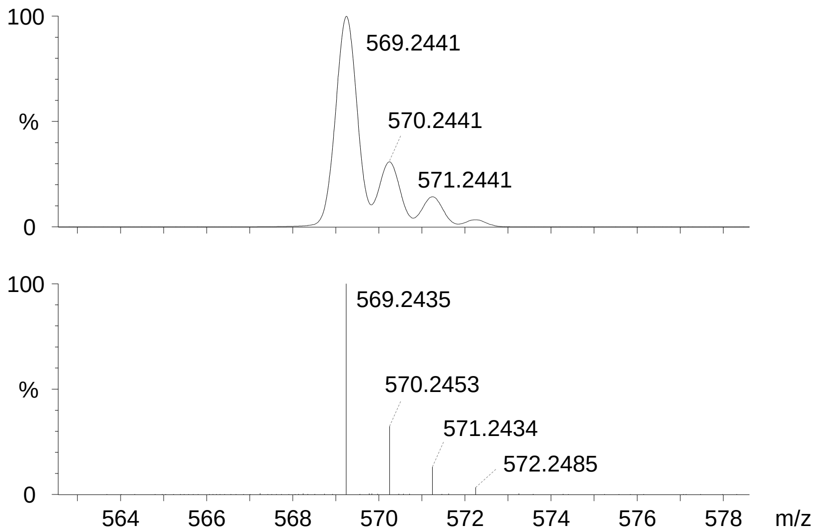
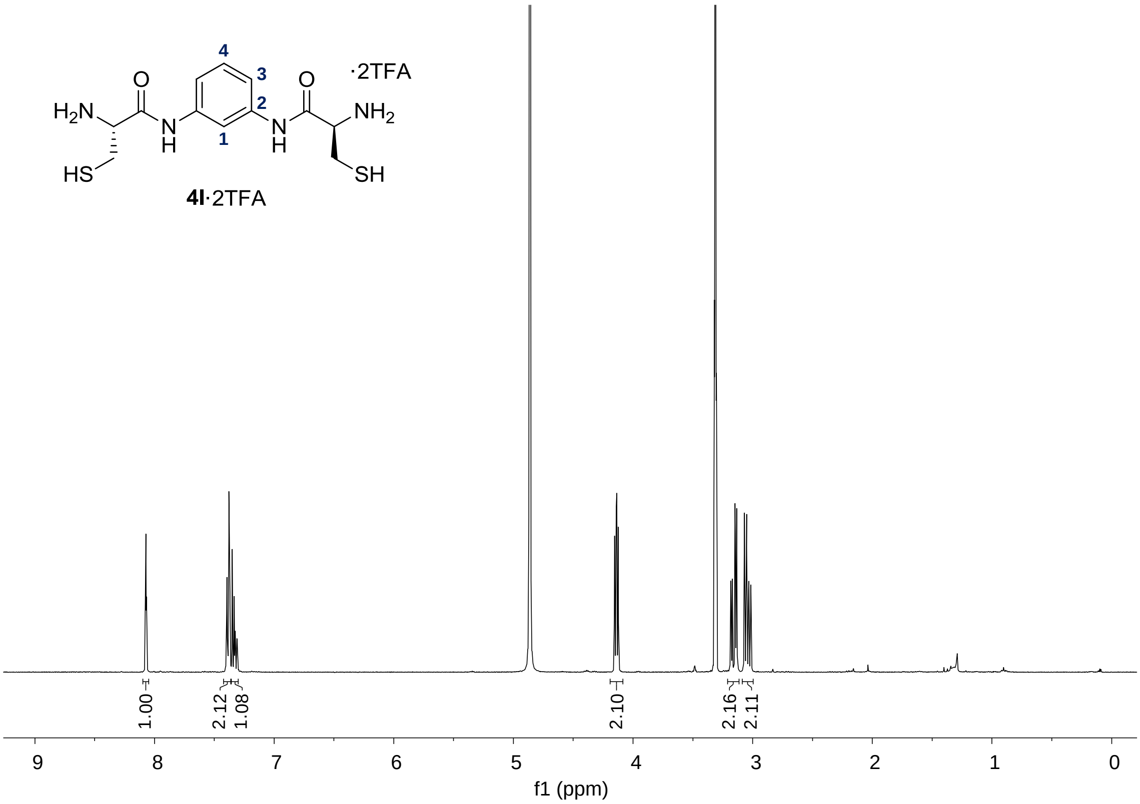


Figure S40. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4k**.

Building block 4l



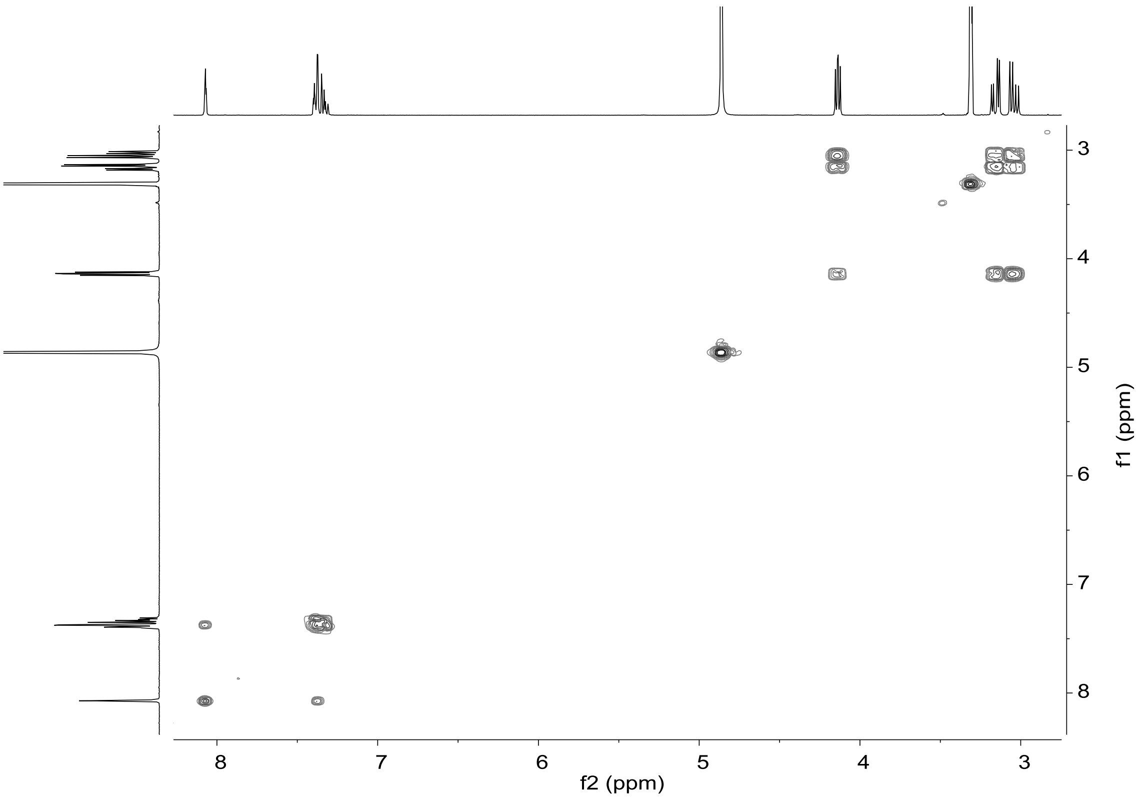
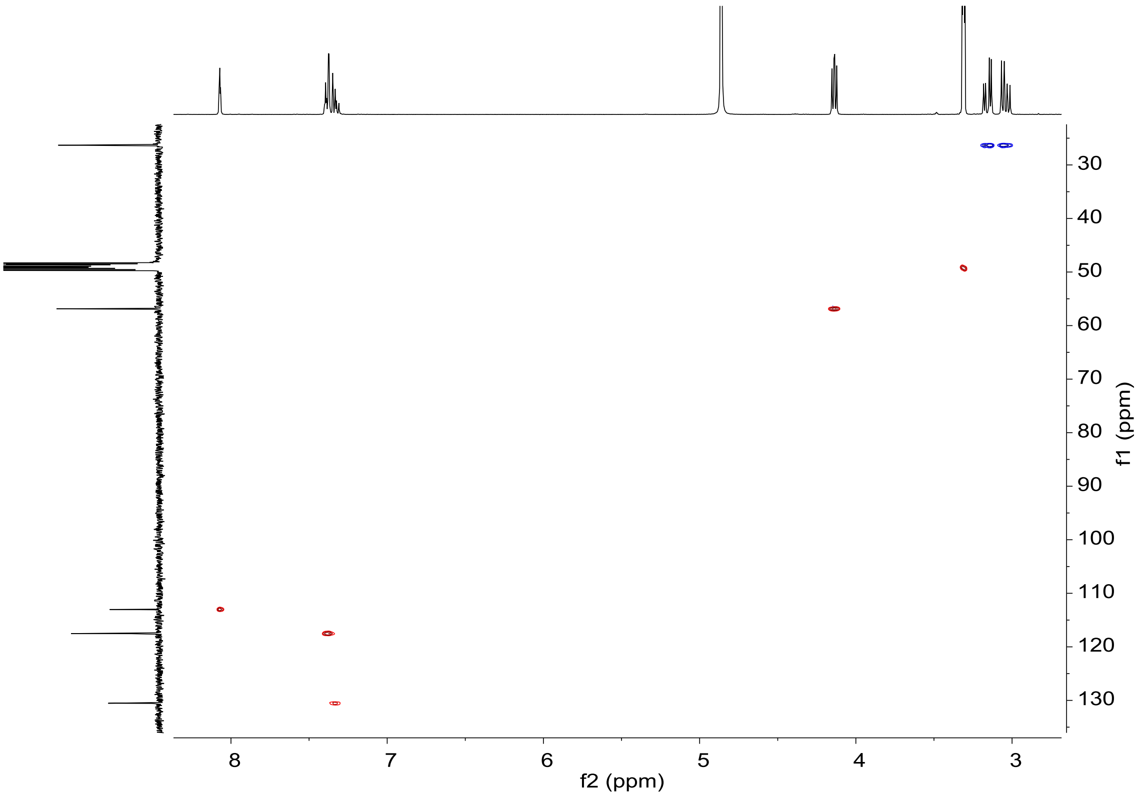


Figure S41. ^{1}H (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{1}H gCOSY (400 MHz, 298 K in MeOD-d_4) spectra of **4l**·2TFA.



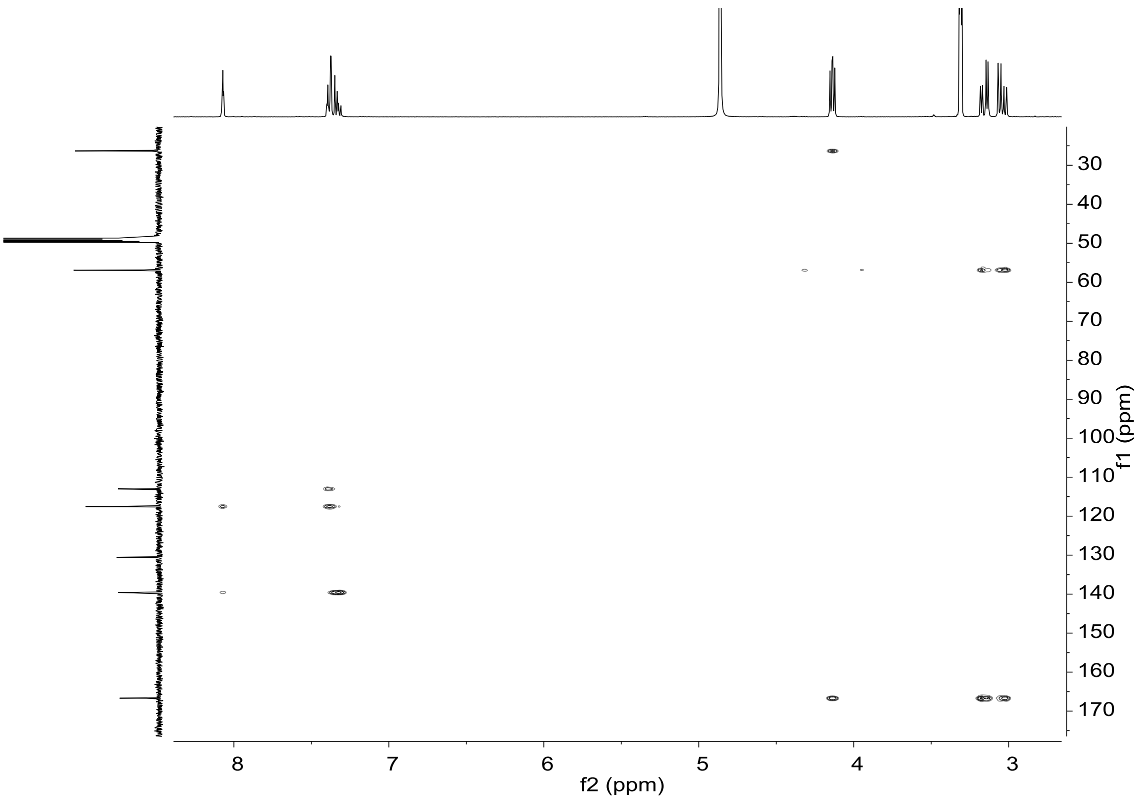


Figure S42. ^{1}H-^{13}C gHSQC (400 MHz, 298 K in MeOD-d_4) and ^{1}H-^{13}C gHMBC (400 MHz, 298 K in MeOD-d_4) spectra of **4l**·2TFA.

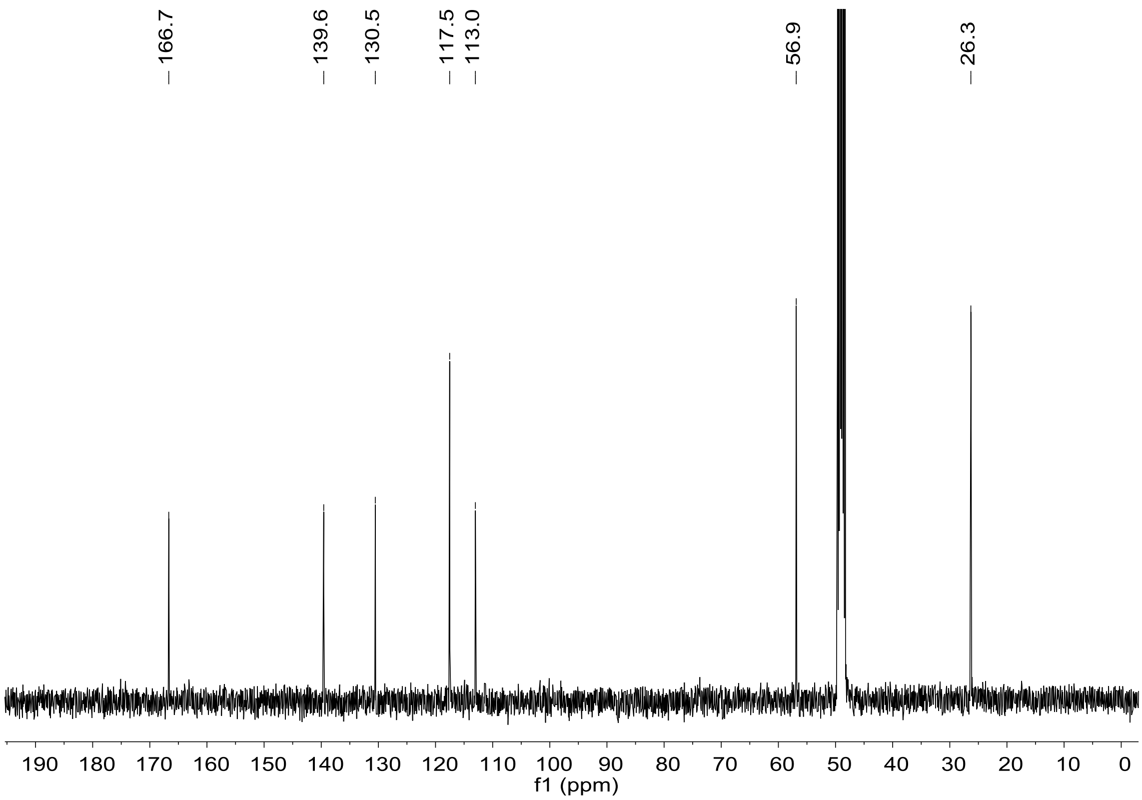


Figure S43. ^{13}C (101 MHz, 298 K in MeOD-d_4) spectrum of **4l**·2TFA.

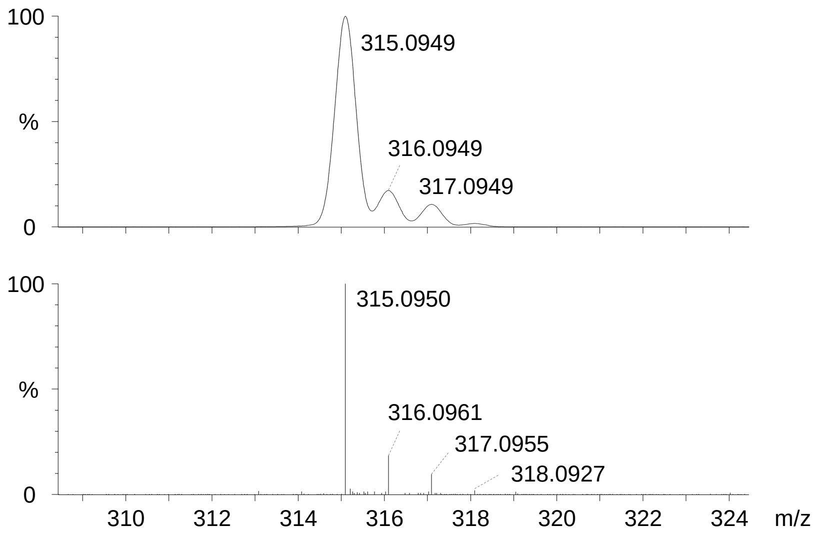


Figure S44. Experimental (lower trace) and simulated (upper trace) ESI-TOF mass spectra for $[M+H]^+$ of **4l**.

MS analysis of the oligomeric disulfides

Homodimers

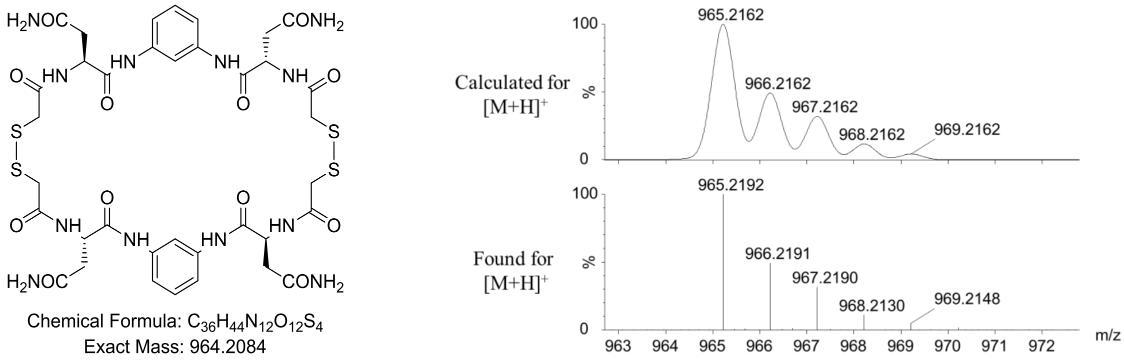


Figure S45. Structure and isotopic pattern of [**4a**]$_2$.

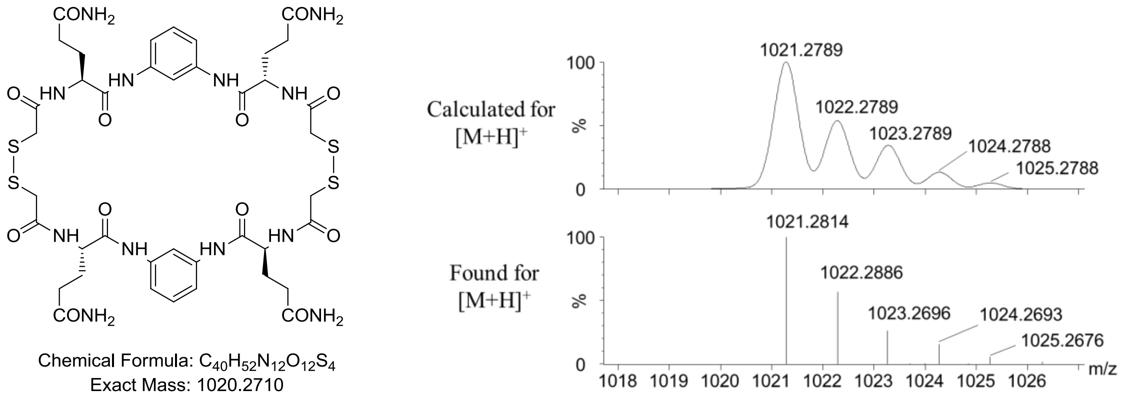


Figure S46. Structure and isotopic pattern of [**4b**]$_2$.

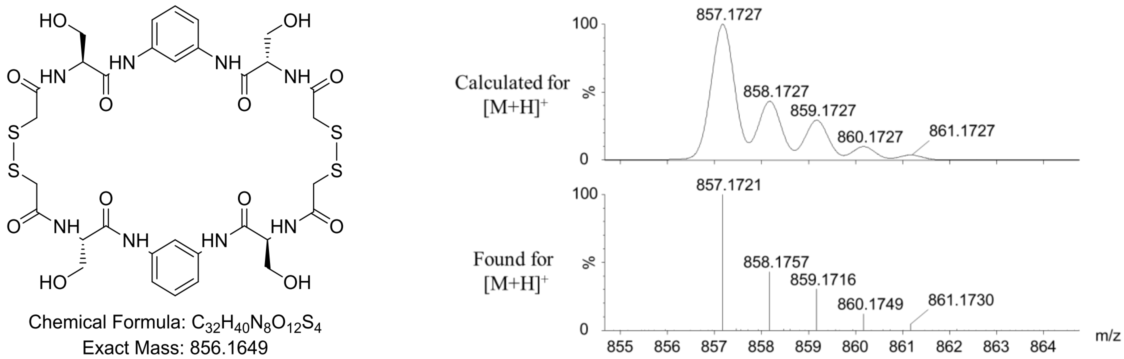


Figure S47. Structure and isotopic pattern of [**4c**]$_2$.

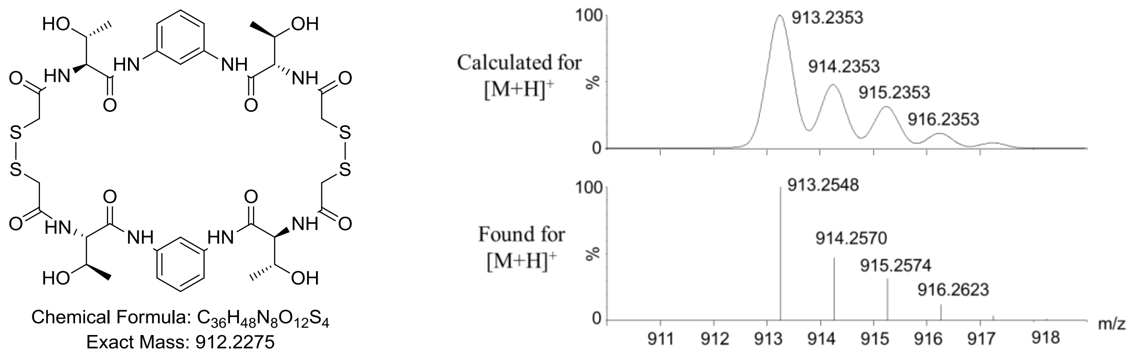


Figure S48. Structure and isotopic pattern of [**4d**]$_2$.

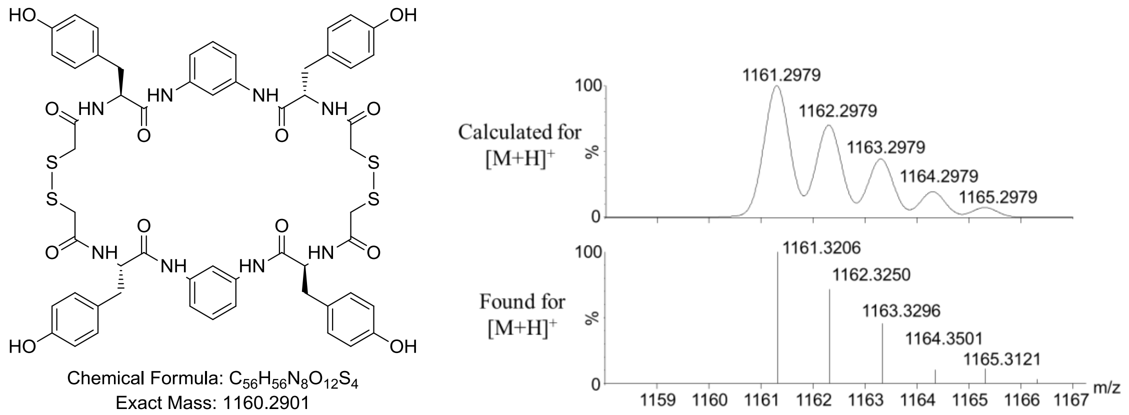


Figure S49. Structure and isotopic pattern of [**4e**]$_2$.

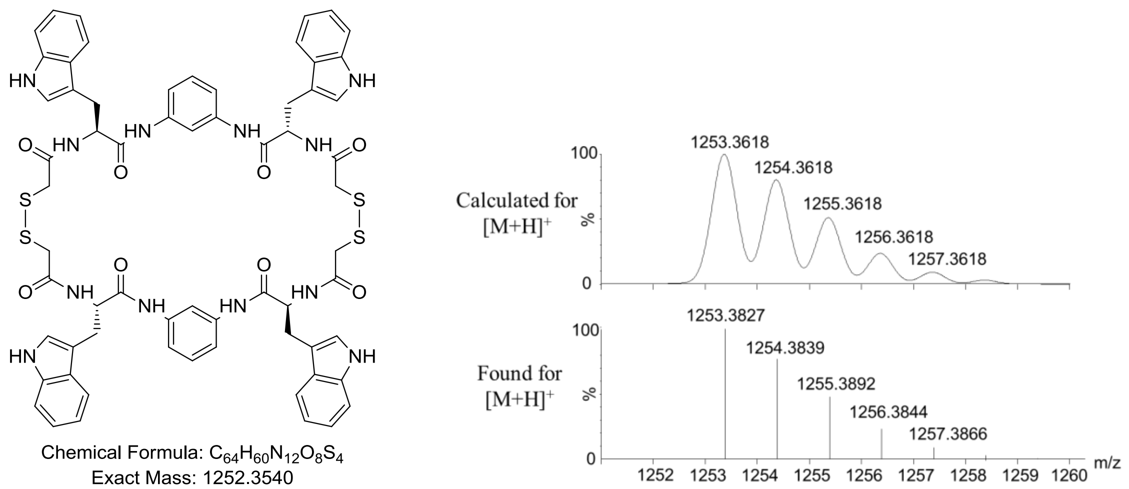


Figure S50. Structure and isotopic pattern of [**4f**]$_2$.

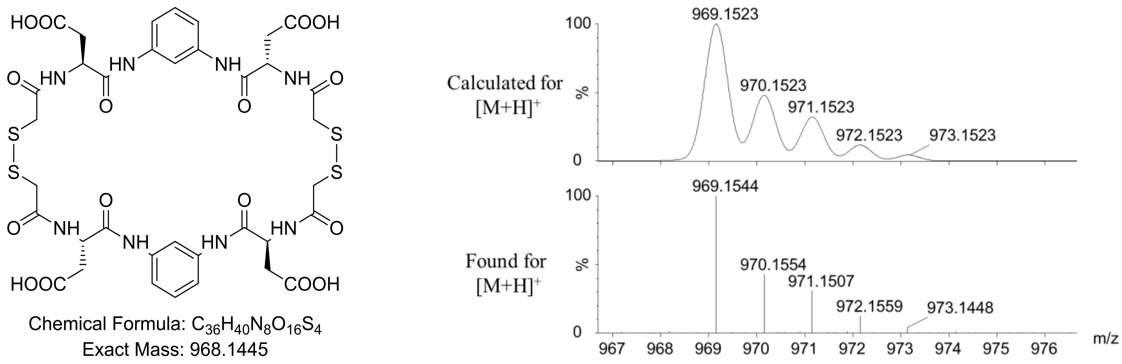


Figure S51. Structure and isotopic pattern of [**4g**]$_2$.

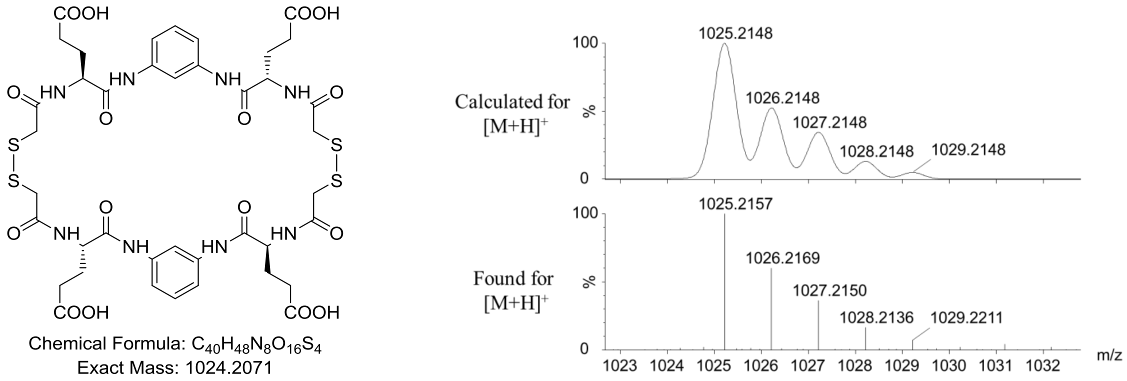


Figure S52. Structure and isotopic pattern of [**4h**]$_2$.

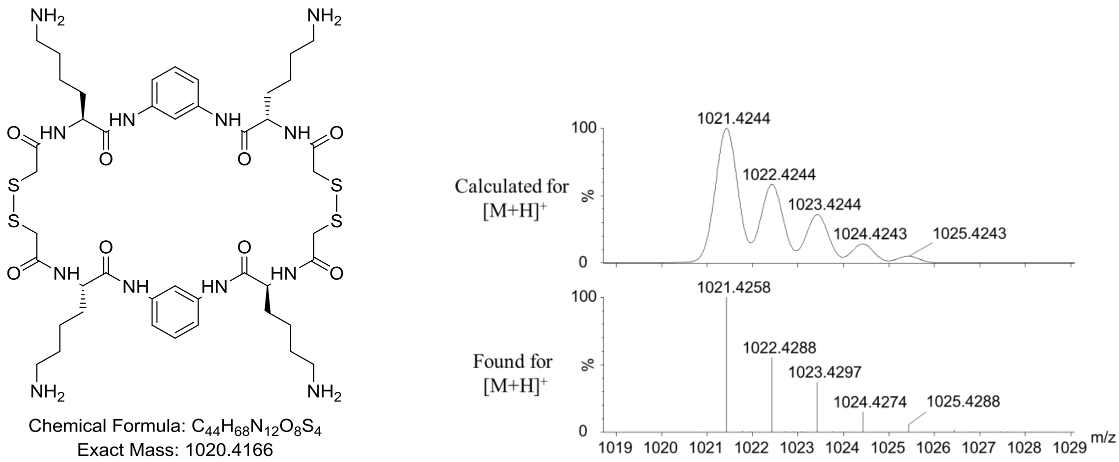


Figure S53. Structure and isotopic pattern of [**4i**]$_2$.

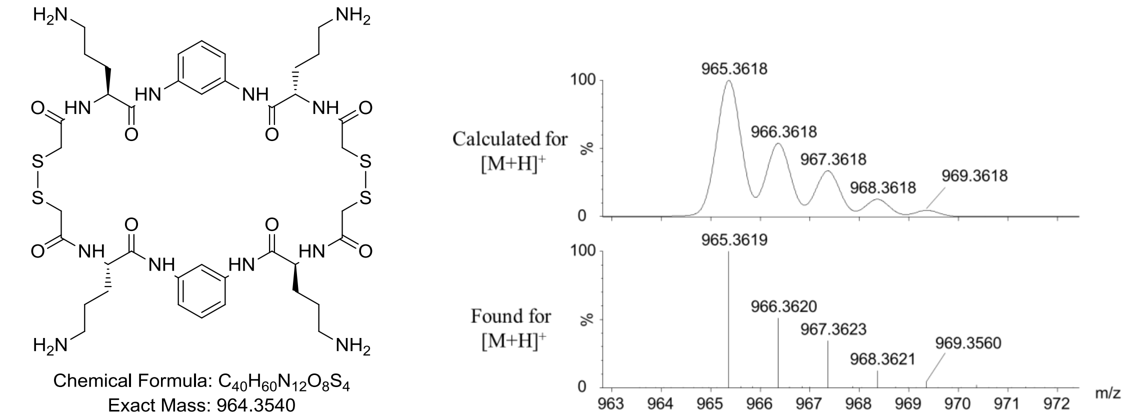


Figure S54. Structure and isotopic pattern of [**4j**]$_2$.

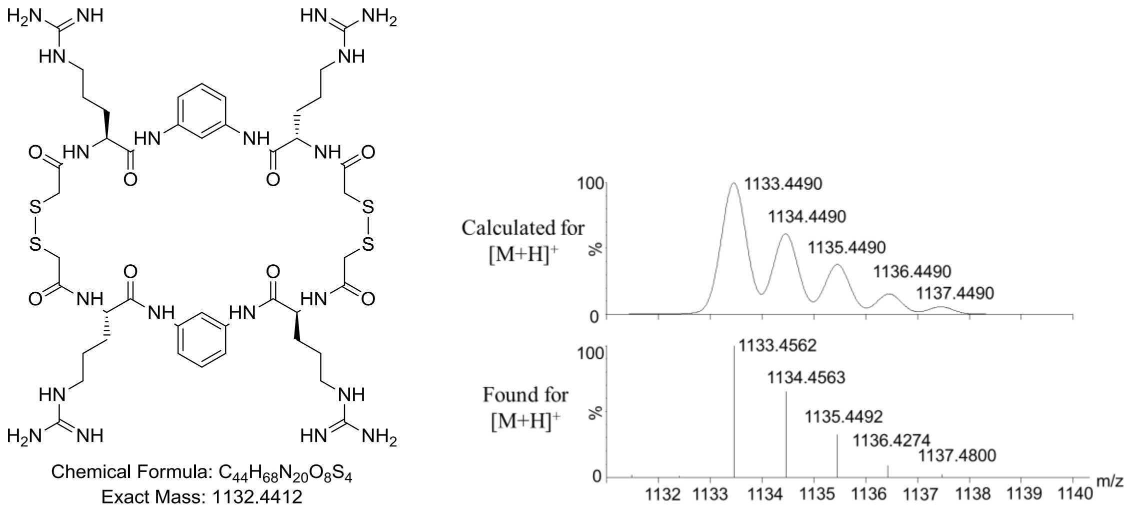


Figure S55. Structure and isotopic pattern of [**4k**]$_2$.

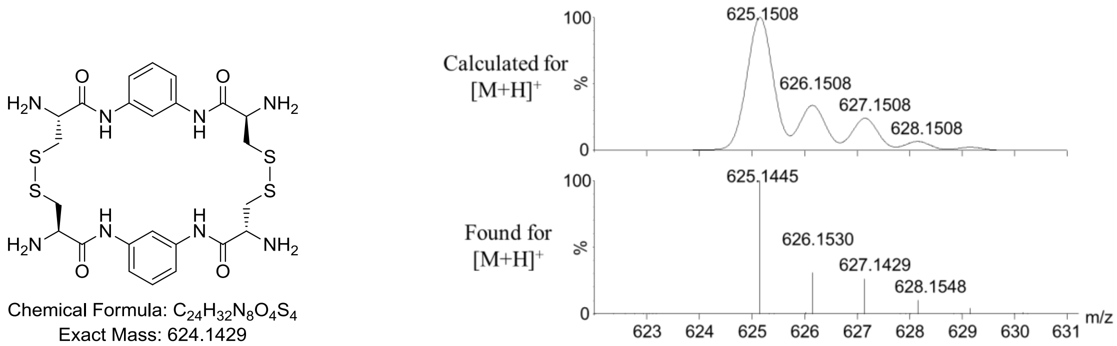


Figure S56. Structure and isotopic pattern of [**4l**]$_2$.

Heterodimers

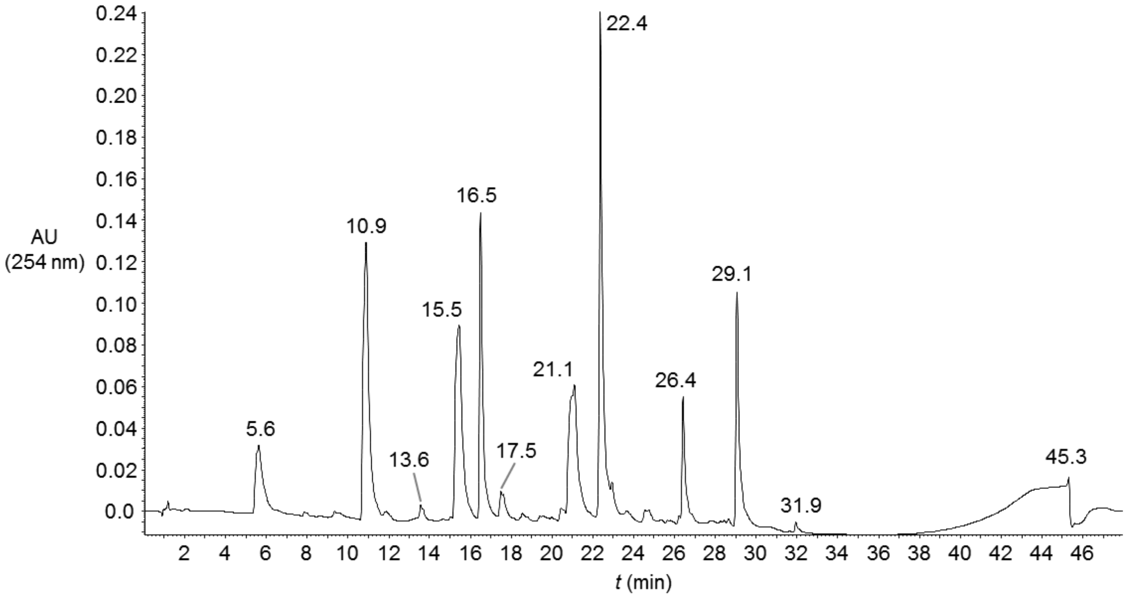


Figure S57. UPLC-UV (254 nm) trace of the mixture of **4d**+**4e**+**4f**+**4j** corresponding to the RP-HPLC trace shown in Figure 2 of the manuscript.

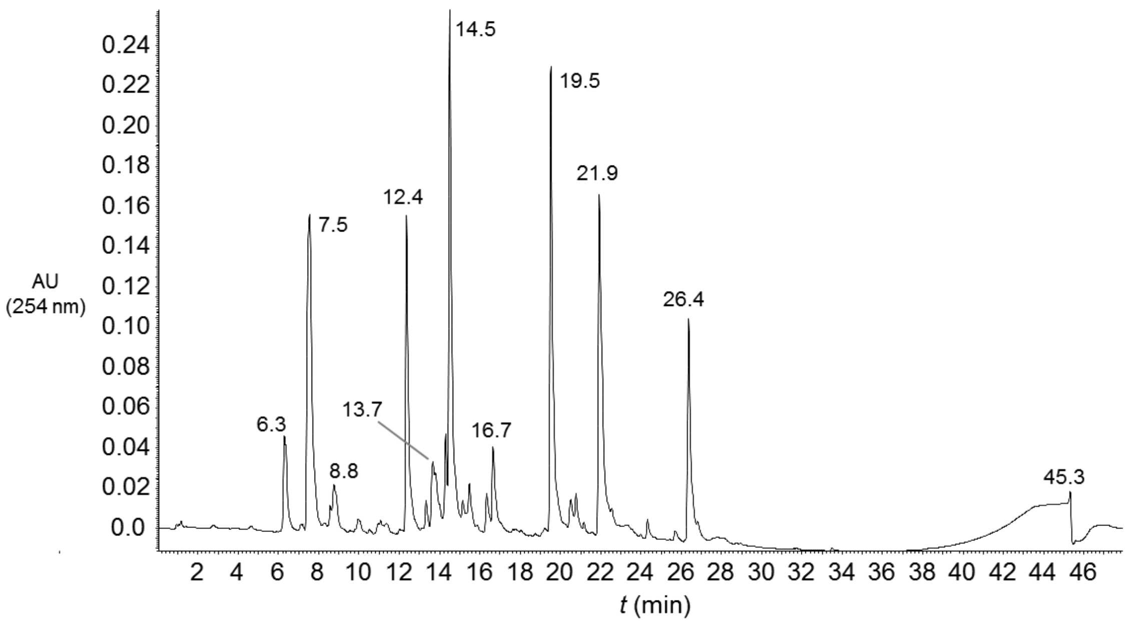


Figure S58. UPLC-UV (254 nm) traces of the mixture of **4b**+**4e**+**4g**+**4l** corresponding to the RP-HPLC trace shown in Figure 3 of the manuscript.

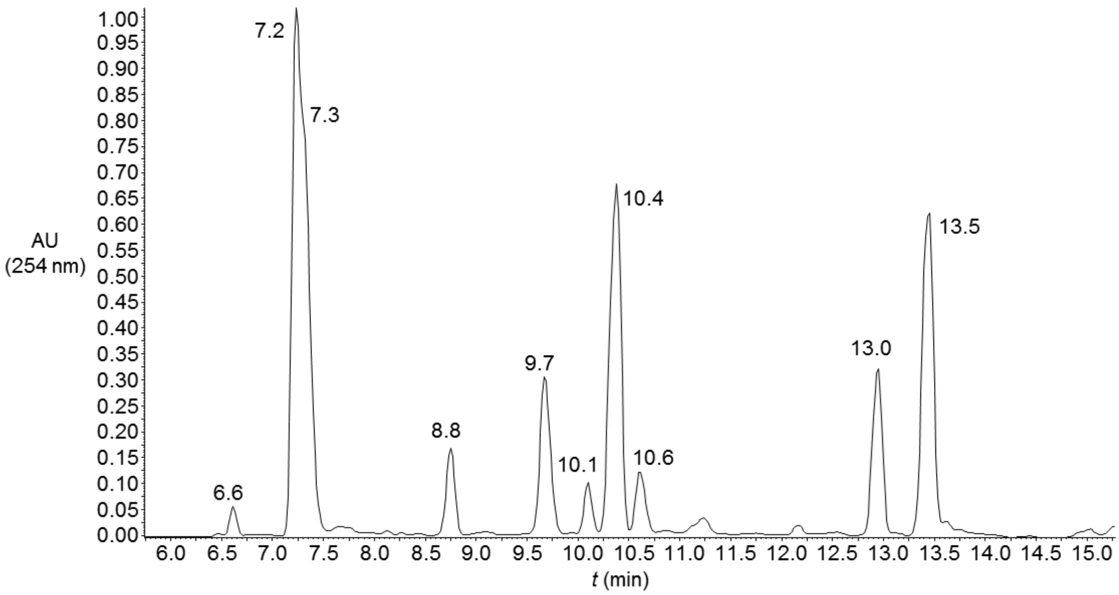


Figure S59. UPLC-UV (254 nm) traces of the mixture of **4d+4e+4h+4i** corresponding to the RP-HPLC trace shown in Figure 4 of the manuscript.

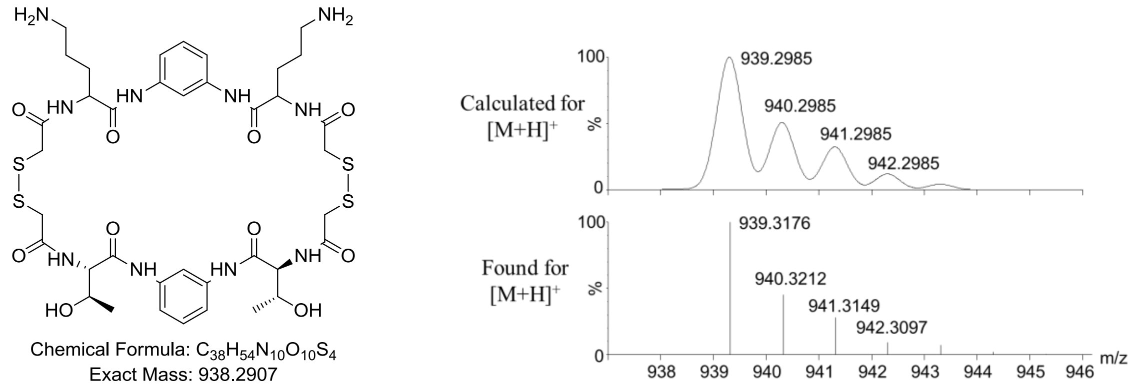


Figure S60. Structure and isotopic pattern of [**4j-4d**] (t_R = 10.9 min in Figure S57).

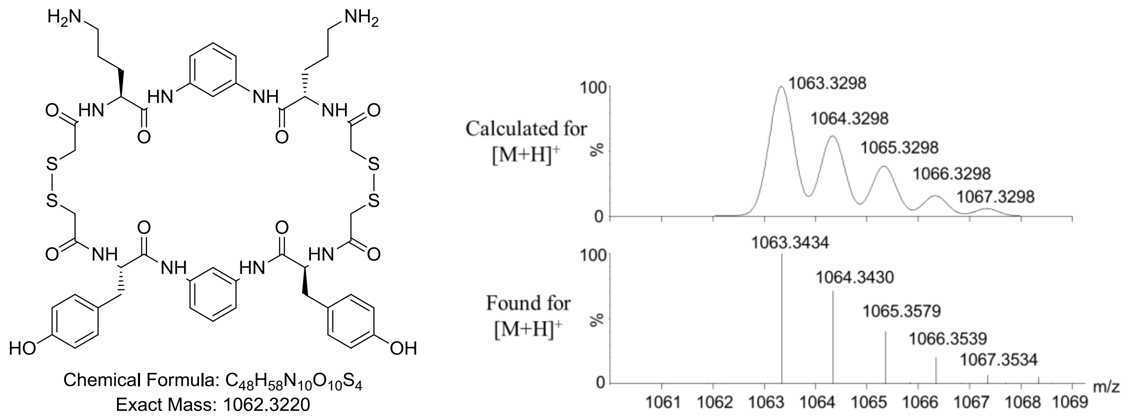


Figure S61. Structure and isotopic pattern of [**4j-4e**] (t_R = 15.5 min in Figure S57).

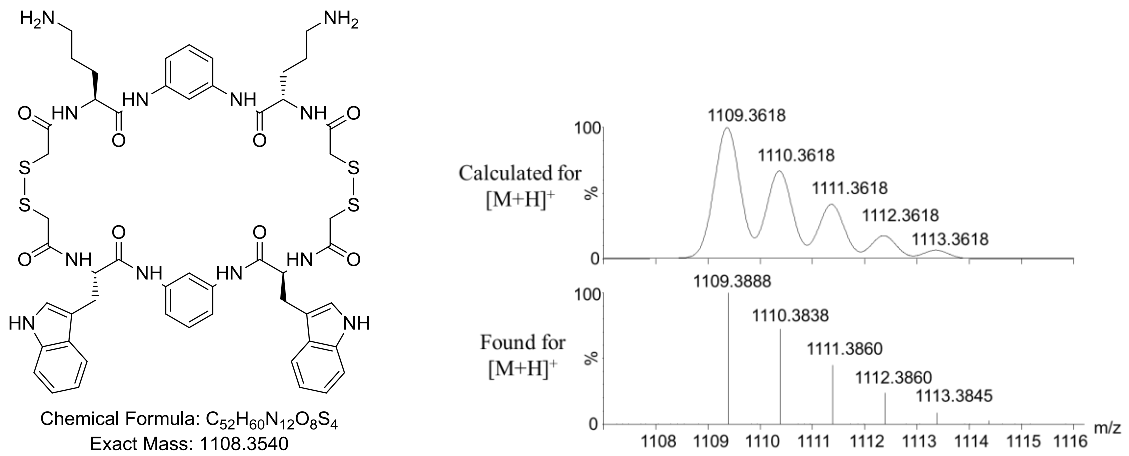


Figure S62. Structure and isotopic pattern of [**4j-4f**] (t_R = 21.1 min in Figure S57).

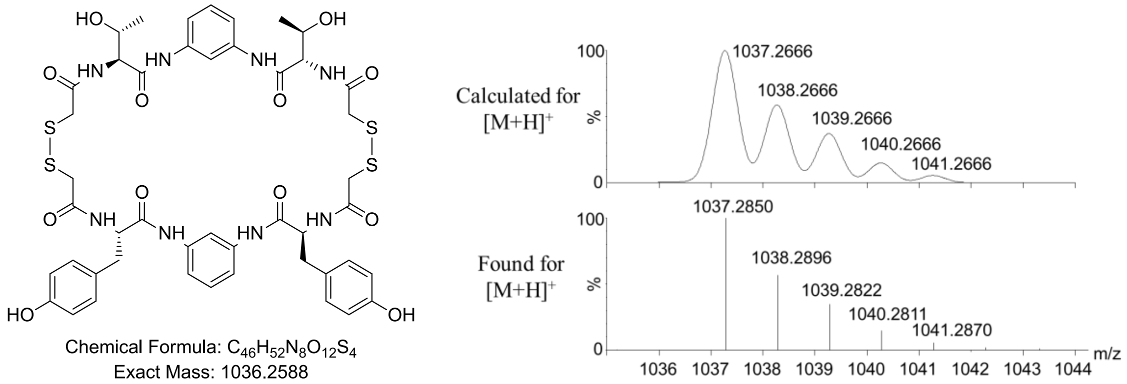


Figure S63. Structure and isotopic pattern of [**4d-4e**] (t_R = 22.4 min in Figure S57 and 13.5 min in Figure S59).

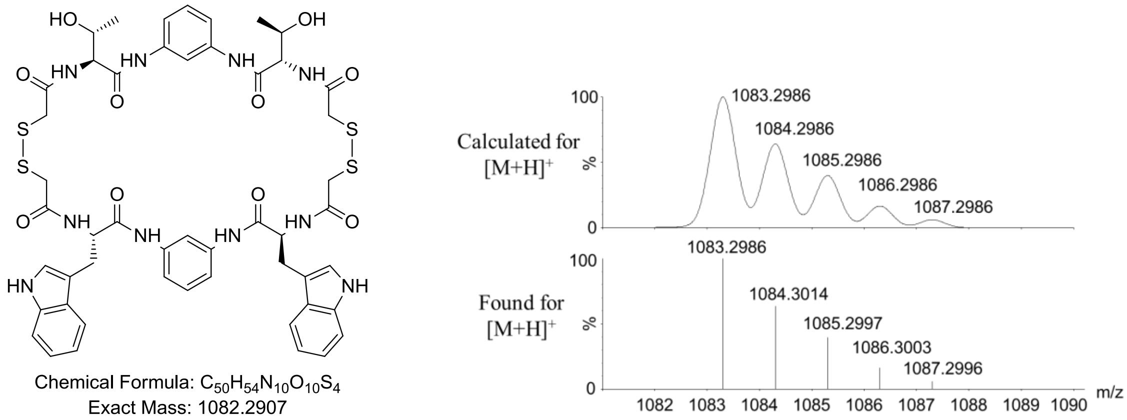


Figure S64. Structure and isotopic pattern of [**4d-4f**] (t_R = 29.1 min in Figure S57).

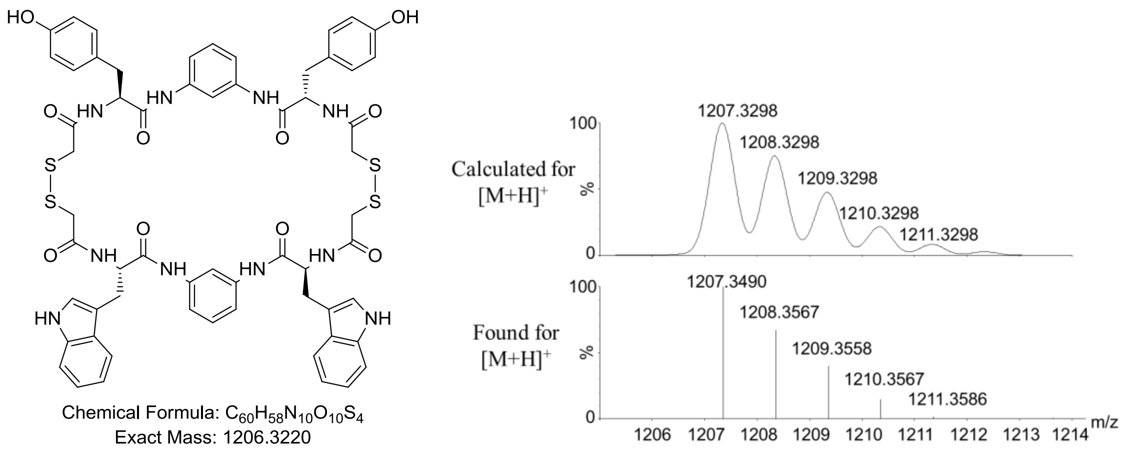


Figure S65. Structure and isotopic pattern of [**4e-4f**] (t_R = 31.9 min in Figure S57).

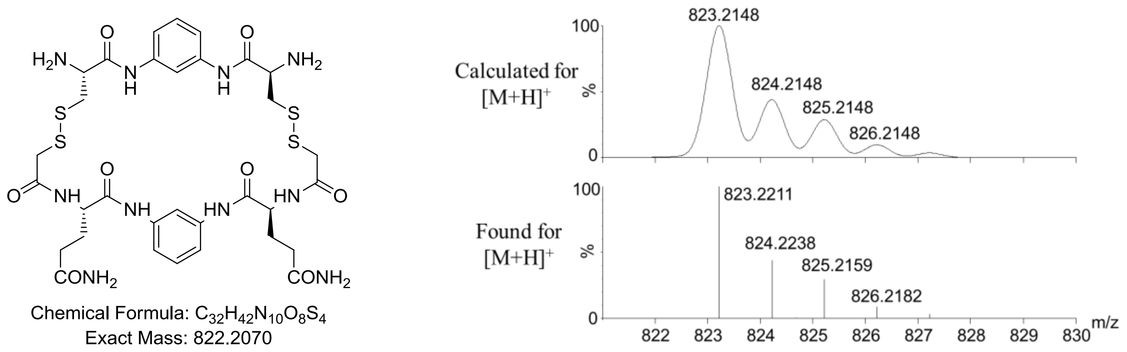


Figure S66. Structure and isotopic pattern of [**4l-4b**] (t_R = 6.3 min in Figure S58).

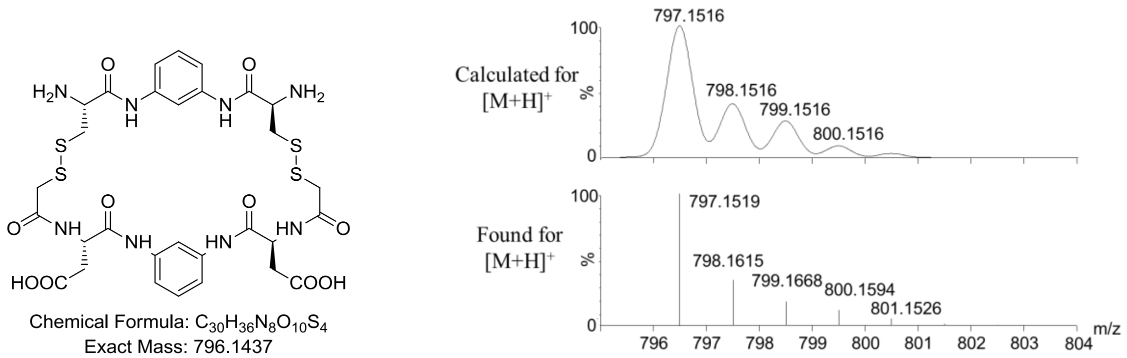


Figure S67. Structure and isotopic pattern of [**4l-4g**] (t_R = 7.5 min in Figure S58).

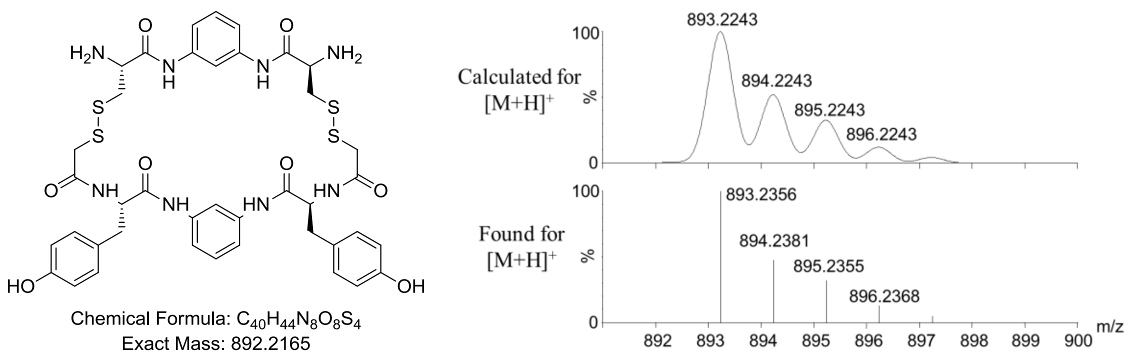


Figure S68. Structure and isotopic pattern of [**4l-4e**] (t_R = 13.7 min in Figure S58).

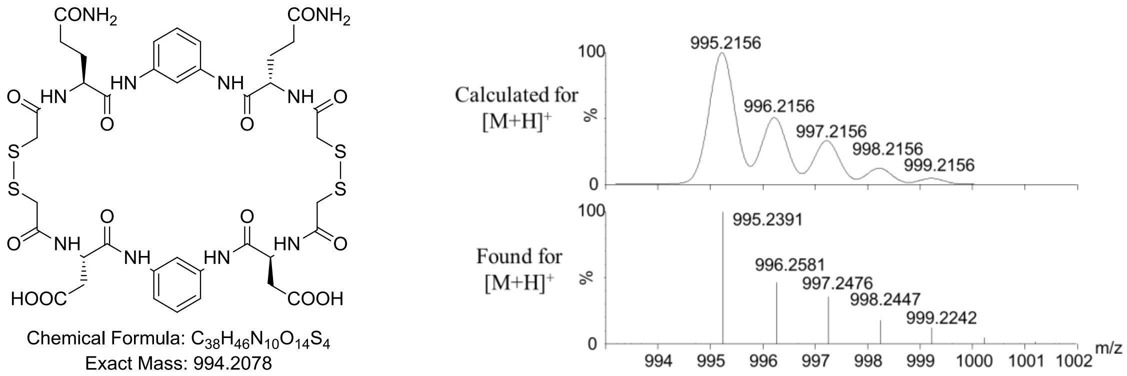


Figure S69. Structure and isotopic pattern of [**4b-4g**] (t_R = 14.5 min in Figure S58).

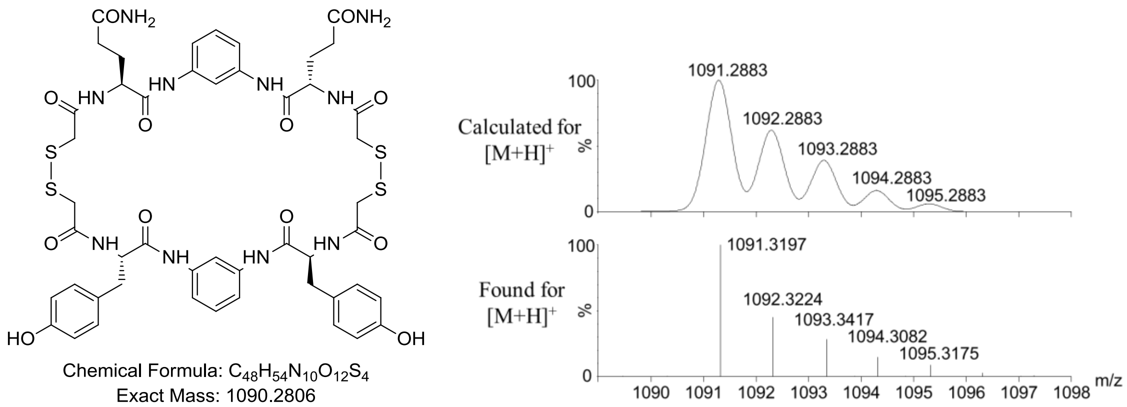


Figure S70. Structure and isotopic pattern of [**4b-4e**] (t_R = 19.5 min in Figure S58).

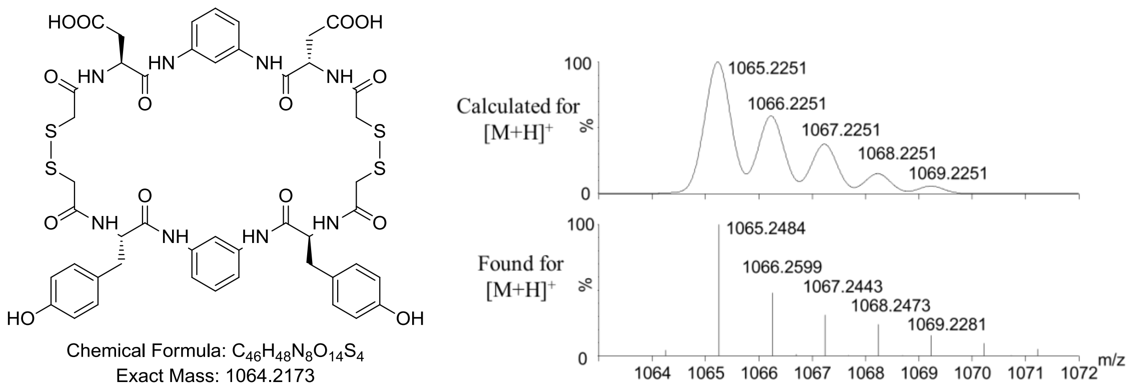


Figure S71. Structure and isotopic pattern of [**4g-4e**] (t_R = 21.9 min in Figure S58).

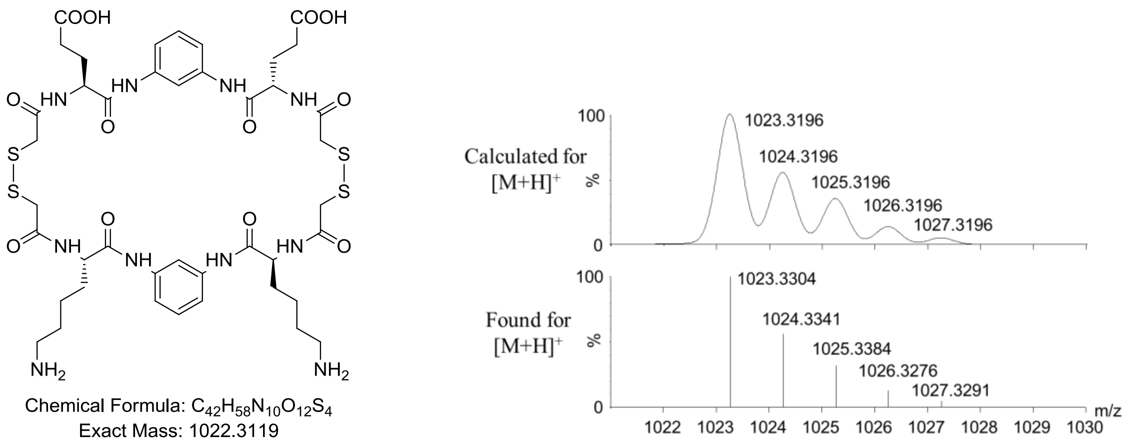


Figure S72. Structure and isotopic pattern of [**4h-4i**] (t_R = 7.2 min in Figure S59).

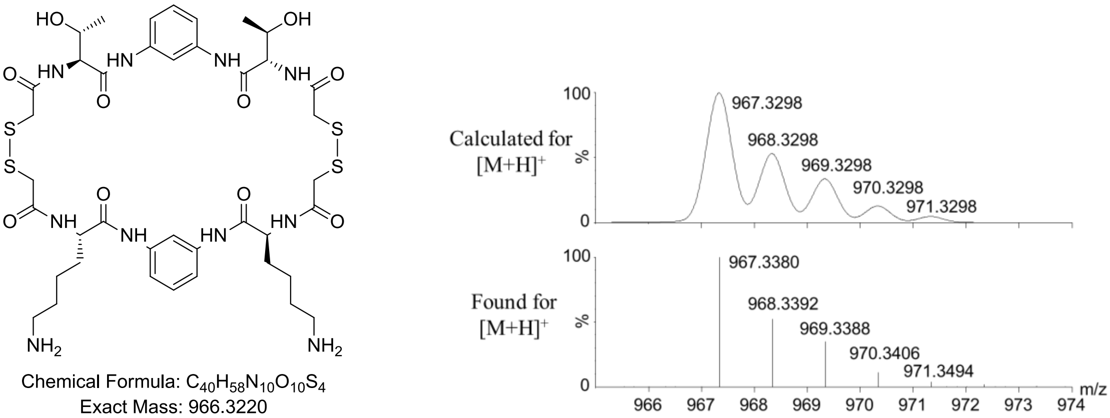


Figure S73. Structure and isotopic pattern of [**4d-4i**] (t_R = 7.3 min in Figure S59).

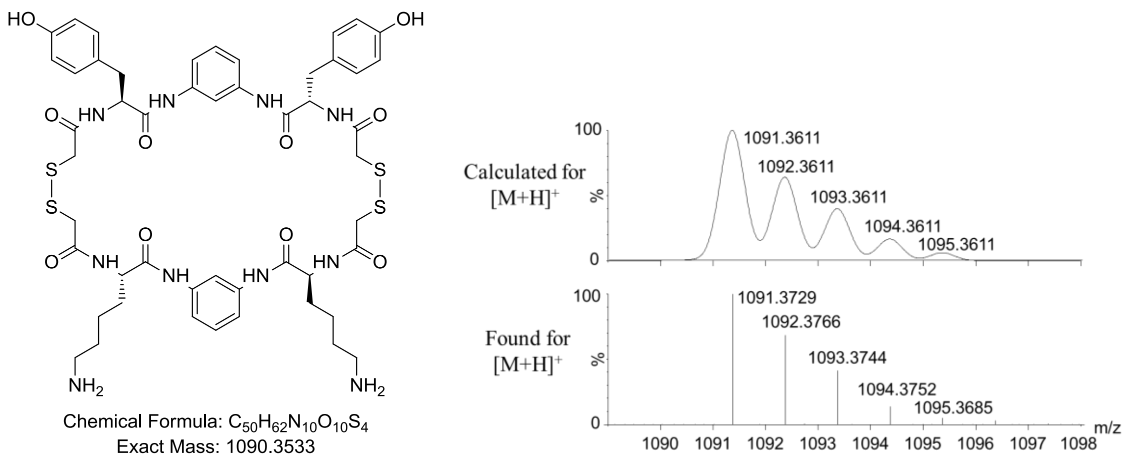


Figure S74. Structure and isotopic pattern of [**4e-4i**] (t_R = 9.7 min in Figure S59).

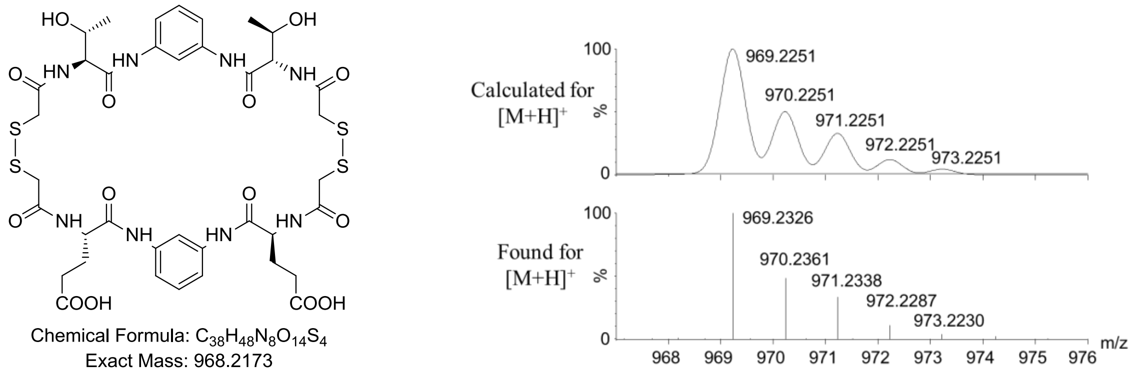


Figure S75. Structure and isotopic pattern of [**4d-4h**] (t_R = 10.4 min in Figure S59).

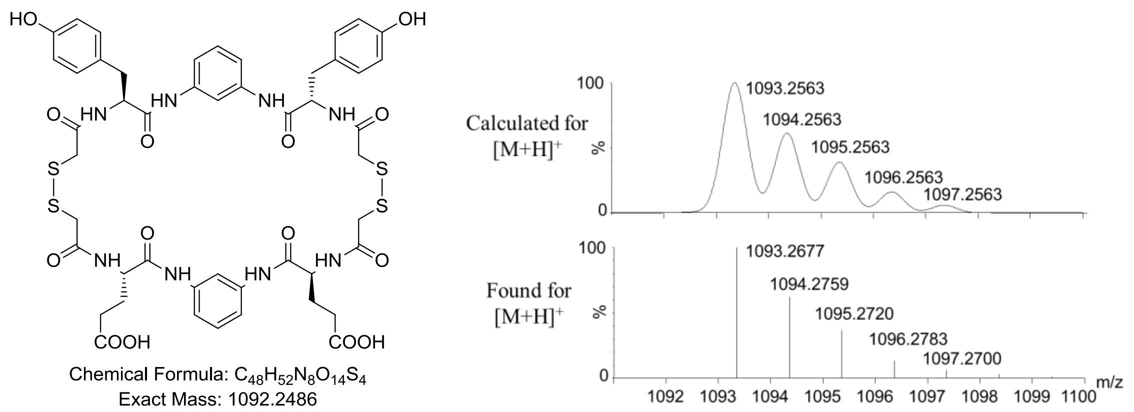


Figure S76. Structure and isotopic pattern of [**4e-4h**] (t_R = 13.0 min in Figure S59).

Trimers

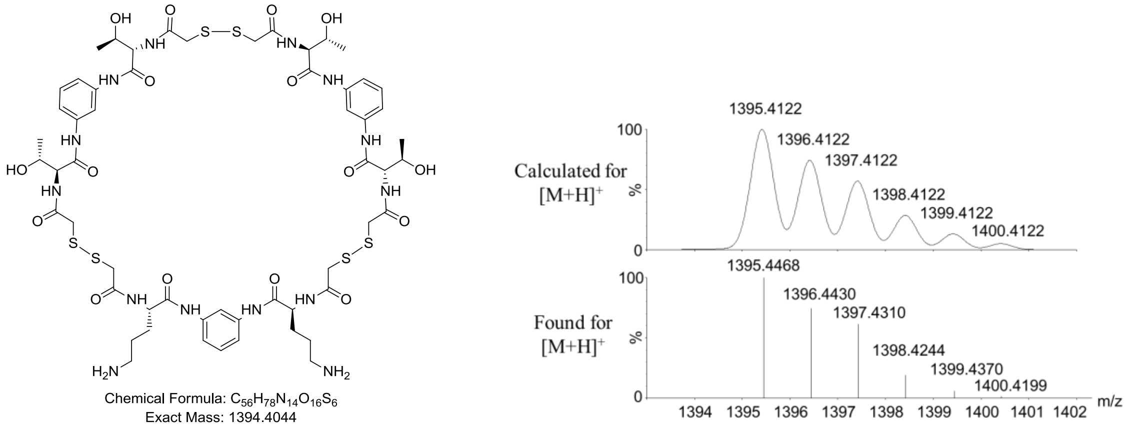


Figure S77. Structure and isotopic pattern of [(**4d**)$_2$-**4j**] (t_R = 13.6 min in Figure S57).

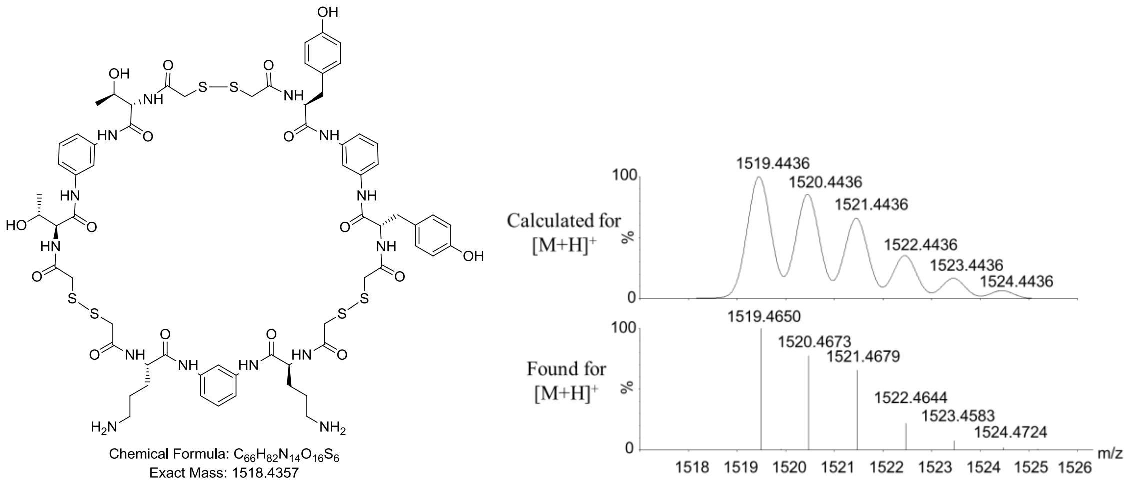


Figure S78. Structure and isotopic pattern of [**4d-4e-4j**] (t_R = 17.5 min in Figure S57).

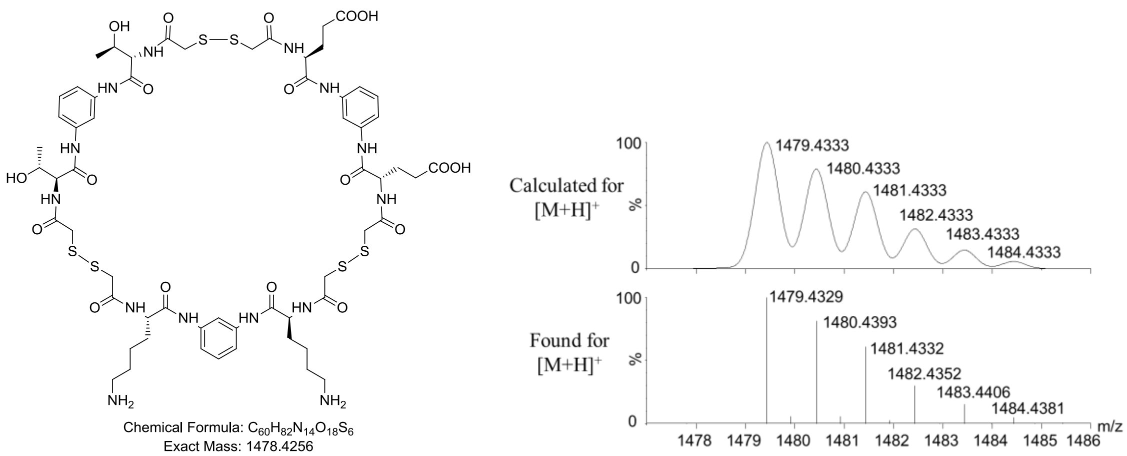


Figure S79. Structure and isotopic pattern of [**4d-4h-4i**] (t_R = 8.8 min in Figure S59).

Stimuli responsiveness of a representative DCL

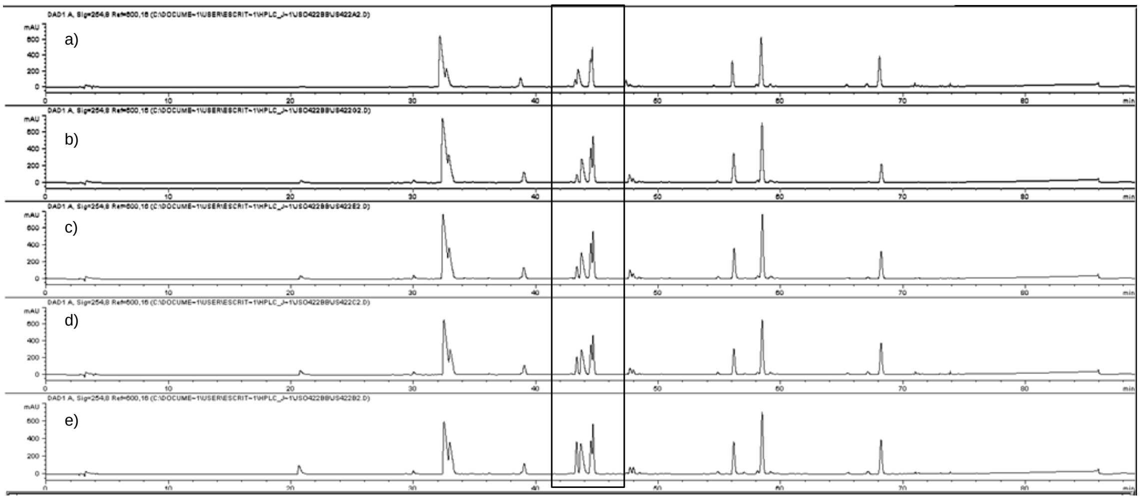


Figure S80. HPLC trace of a DCL formed by the mixture of **4d**, **4e**, **4h** and **4i** at 0.5 mM concentration of each BB, a) in the absence of stimulus b) in the presence of 0.125 mM spermine c) 0.5 mM spermine, d) 2 mM spermine, e) 10 mM spermine.

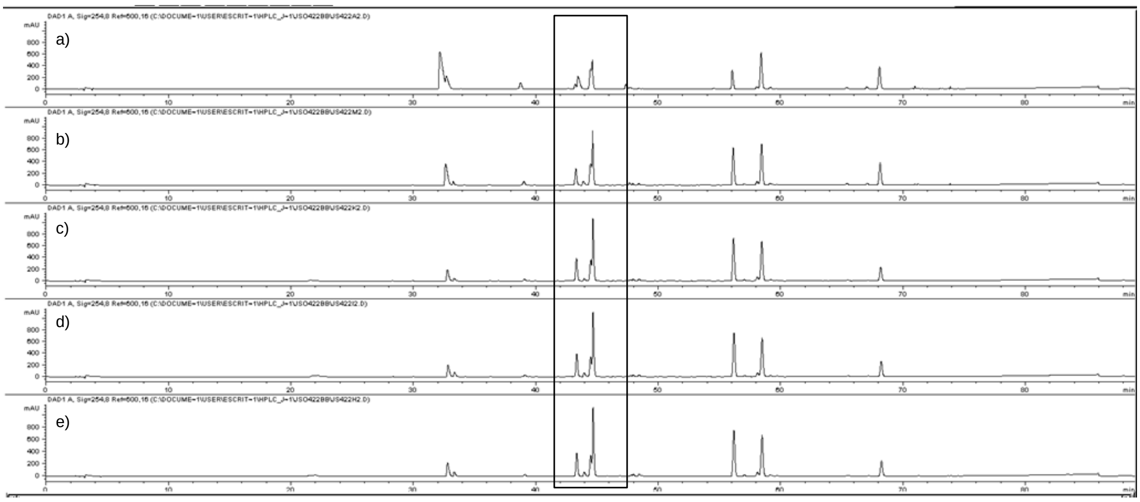


Figure S81. HPLC trace of a DCL formed by the mixture of **4d**, **4e**, **4h** and **4i** at 0.5 mM concentration of each BB, a) in the absence of stimulus, b) in the presence of 0.125 mM phytic acid c) 0.5 mM phytic acid, d) 2 mM phytic acid, e) 10 mM phytic acid.

NMR titration experiments

In order to confirm the host-guest interactions we decided to perform NMR-titrations of the amplified dimers with their corresponding guests, that is phytic acid for [**4d**]$_2$ and spermine for [**4h**]$_2$. To that end we prepared solutions containing the corresponding BBs that give rise to the amplified compounds under conditions similar to those of the libraries. Each homodimer [**4d**]$_2$ and [**4h**]$_2$ was prepared individually by oxidation of its component in a mixture of buffered H_2O:MeCN-d_3 and DMSO-d_6 at 0.5 mM concentration of BBs. The aqueous buffer was adjusted to pH 7.0 using tris(hydroxymethyl)aminomethane-d_{11} (Tris-d_{11}) and the ^{1}H NMR spectra were acquired after 8 days to ensure complete oxidation (also checked by HPLC). The ^{1}H NMR data were consistent with the formation of the respective homodimers [**4d**]$_2$ (Thr) or [**4h**]$_2$ (Glu), although a minor formation of the trimer [**4h**]$_3$ (about 5% mol) could be observed in the latter. Titration of the [**4h**]$_2$ with spermine showed the shift of several signals (see Figure 5 in the main text and Figures S82-S83).

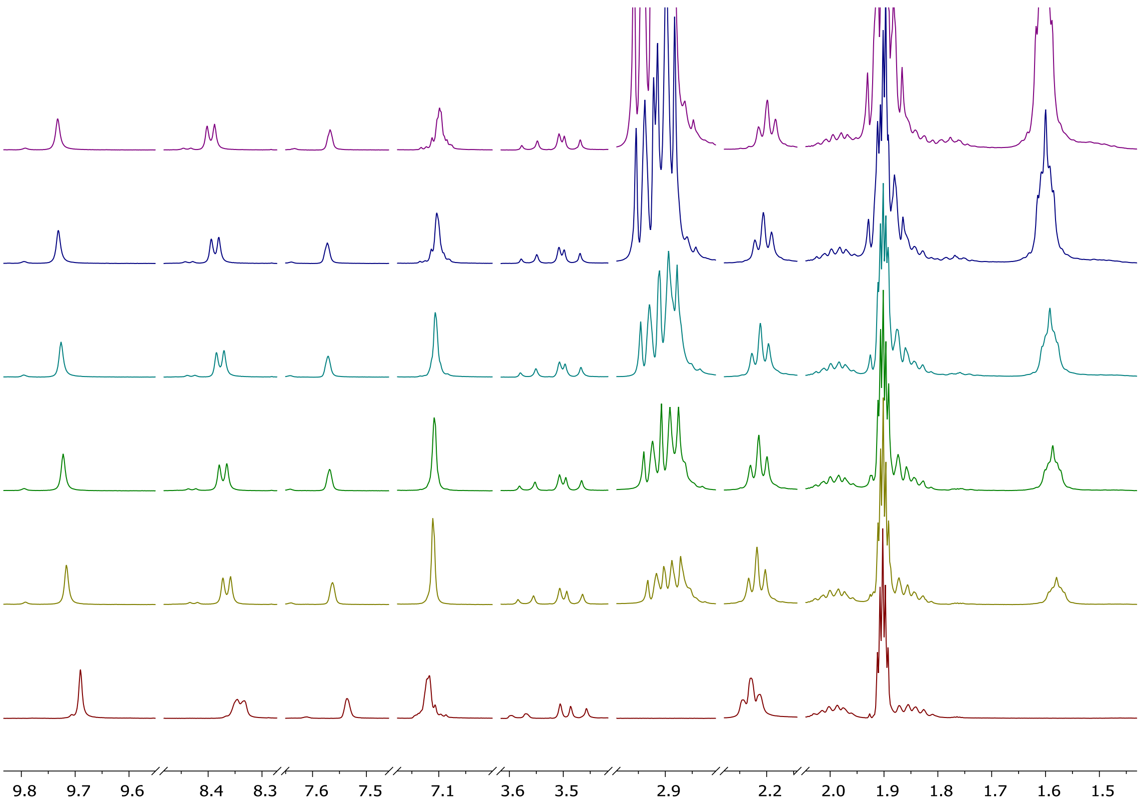


Figure S82. NMR titration experiment (500 MHz, 4:4:2 H_2O:CD_3CN:DMSO-d_6, Tris-d_{11} buffer pH 7.0, 298 K) of [**4h**]$_2$ (125 μM) upon de addition of increasing amounts of spermine (from bottom up: 0, 0.125, 0.25, 0.5, 1.23, 2.41 mM).

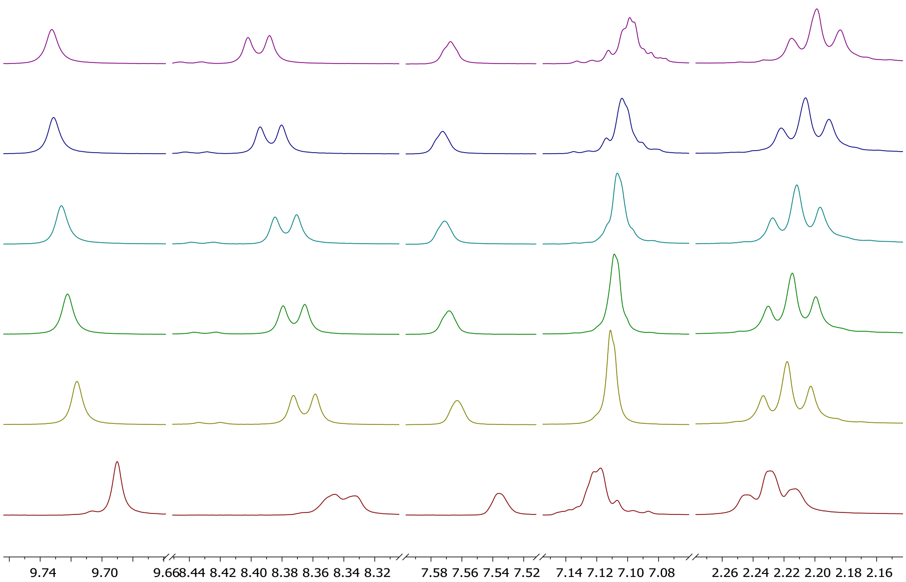


Figure S83. Zoomed region of the spectra shown in Figure S82.

Unfortunately the NMR monitoring of the addition of phytate (up to 10 eq.) to [**4d**]$_2$ was hampered by the close proximity of the key CHOH proton signals (both in host and guest) to the water suppression region. Moreover, the appearance of a slight turbidity during the titration experiment additionally precluded to confirm the host-guest interaction