Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Peptide Macrocyclisation *via* Late-Stage Reductive Amination

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General Methods

^{1}H NMR spectra were recorded at 400 MHz using a Bruker AVANCE 400 or Varian MR-400 spectrometer and 700 MHz using a Bruker Ascend 700 spectrometer. Residual proton-solvent peaks were used as an internal reference for ^{1}H NMR spectra ($CDCl_3$ δ 7.26 ppm, MeOD δ 3.31 ppm and CD_3CN δ 1.94 ppm). Coupling constants (J) are quoted to the nearest 0.1 Hz. The assignment of proton signals was assisted by COSY, ROESY, TOCSY, HSQC and HMBC experiments. ^{13}C NMR spectra were recorded at 101 MHz using a Bruker AVANCE 400 spectrometer and 176 MHz using a Bruker Ascend 700 spectrometer. Solvent peaks were used as an internal reference for ^{13}C NMR spectra ($CDCl_3$ δ 77.16 ppm, MeOD δ 49.00 ppm and CD_3CN δ 1.32 ppm). Assignment of carbon signals was assisted by HSQC and HMBC experiments. The following abbreviations (or combinations thereof) are used to denote ^{1}H NMR multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet. Infrared spectra were recorded on a Perkin-Elmer UATR Two spectrometer as a thin film or solid. UV-Vis absorbance was recorded using a Shimadzu UV-2450 spectrometer. Low-resolution ESI mass spectra were recorded on a Waters LCT Premier TOF (capillary voltage of 2.5 kV and cone voltage of 50 V). High-resolution ESI mass spectra were recorded on a Thermo Fisher Scientific Orbitrap Elite (capillary voltage of 4 kV). Anhydrous solvents were obtained from commercial sources or an MB SPS-800. Commercially available chemicals were used as purchased. Analytical thin-layer chromatography was conducted with aluminium-backed silica gel 60 F254 (0.2 mm) plates supplied by Merck and visualized using UV fluorescence (λ_{max} = 254 nm). Flash chromatography employed Merck Kiesegel 60 silica gel (230–400 mesh). Solvent compositions are given in (*v/v*). PS 40–60 °C refers to petroleum spirits, boiling point fraction 40–60 °C. Microwave irradiation was performed in a CEM Discover microwave reactor. Preparative HPLC was performed on a Waters Alliance Separation Module 2690, with a Waters 996 photodiode array detector. The system was operated using Empower 3 software. All separations employed linear gradients (unless otherwise specified) of water containing 0.1% trifluoroacetic acid and MeCN containing 0.1% trifluoroacetic acid at a constant flow rate of 10 mL/min (preparative HPLC, Alltima, C18, 5 μm; 22 × 250 mm) or 3 mL/min (semi-preparative HPLC, Luna, C18, 5 μm; 10 × 250 mm). All gradients are given in percentage of MeCN (Solvent B). UPLC-MS was performed on a Waters Acquity system outfitted with a Waters UV Detector. Separations employed linear gradients (unless otherwise specified) of water containing 0.1% formic acid (Solvent A) and MeCN containing 0.1% formic acid (Solvent B) at a constant flow rate (0.25 mL/min, BEH,

C18, 1.7 μm). Automated Fmoc-SPPS was carried out on a Biotage Initiator+ Alstra microwave peptide synthesiser, with procedures as described below.

Literature Compounds:

Compounds **5**, **6a**, **7a** and **2** were all synthesised according to literature procedures reported by Vincent and co-workers.[1]

General Procedure A: Automated Peptide Assembly

Automated Assembly:

Peptides were elongated using automated iterative Fmoc-solid-phase peptide synthesis (Fmoc-SPPS) according to the following general protocols:

Deprotection: The resin was treated with 20% piperidine/DMF (1 × 3 min then 1 × 10 min) and washed with DMF (4 × 4.5 mL).

General amino acid coupling: The resin was treated with Fmoc-protected amino acid (4.0 equiv., 0.4 M in DMF), ethyl cyano(hydroxyimino)acetate (Oxyma Pure®) (4.0 equiv., 0.5 M in DMF), and *N,N'*-diisopropylcarbodiimide (DIC) (4.0 equiv., 0.5 M in DMF). The fritted syringe was sealed and agitated for 20 minutes under microwave irradiation (50 °C, 200 W). The resin was then washed with DMF (4 × 4.5 mL).

Capping: Ac_2O/DIEA (4 equiv., 0.3 M in NMP) was added to the resin. The reaction was agitated for 10 minutes at room temperature. The resin was then washed with DMF (4 × 4.5 mL).

Cleavage: The resin was dissolved in DCM and transferred to a 6 mL or 12 mL fritted syringe then washed with DMF (5 × 3 mL), DCM (5 × 3 mL). A mixture of TFA/TIPS/H_2O (3 mL, 90:5:5 *v/v/v*) was added to the resin. After 2 h, the resin was washed with TFA (2 × 3 mL) and DCM (2 × 3 mL).

Work-Up*:* The combined cleavage solutions were concentrated under a gentle stream of nitrogen, and the oily residue obtained was then treated with ice-cold Et_2O to precipitate the peptide. Peptides were further purified by preparative reverse-phase HPLC using the conditions indicated in each synthetic protocol described below.

General Procedure B: Peptide Macrocyclisation *via* Reductive Amination

Linear peptide diamine (1.0 equiv.) was diluted in sodium borate reduction buffer (40 mM sodium borate, 100 mM $NaBH_3CN$, pH 9) to a concentration of 1 mM. To this was added dialdehyde **1, 2** or **3** (3.0 equiv., 60 mM in MeCN). The reaction was stirred for 16 – 24 h and monitored *via* UPLC-MS analysis. If incomplete, additional aldehyde was added (1.0 – 3.0 equiv.) and the reaction stirred for a further 16 – 24 h. The crude mixture was concentrated to a volume of approximately 1 mL under a stream of nitrogen and diluted with MeCN containing 0.1% TFA. The mixture was then purified *via* preparative reverse phase HPLC (direct injection of crude reaction mixture; eluent as noted) to afford the pure macrocyclic peptide after lyophilisation. For the purpose of yield calculations, the protonation states of the peptide starting materials and peptide products were assumed to be consistent.

Optimisation of Reaction Solvent and Additives (see Manuscript Table 1)

Linear peptide diamine **9** (5.8 – 13 mmol, 1.0 equiv.) was dissolved in the appropriate solvent to a concentration of 1 mM. To this was added the additive (specified in table), dialdehyde **1** (1.5 equiv., 60 mM in MeCN) and $NaBH_3CN$ (10 equiv., 80 mM in MeCN). The reaction was stirred at room temperature for 24 h and subsequently concentrated under a stream of nitrogen, diluted in water/MeCN (50/50, v/v) and purified *via* preparative reverse phase HPLC (5% B for 3 min, then 5% – 40% B, over 30 minutes) to afford the pure macrocyclic peptide after lyophilisation.

Crude HPLC Trace of Optimised Reaction Conditions (from Entry 7, Table 1):

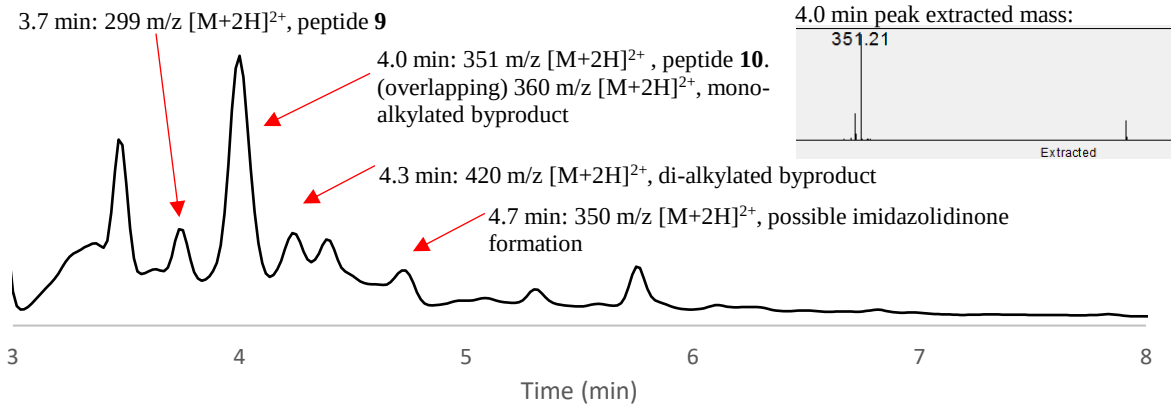
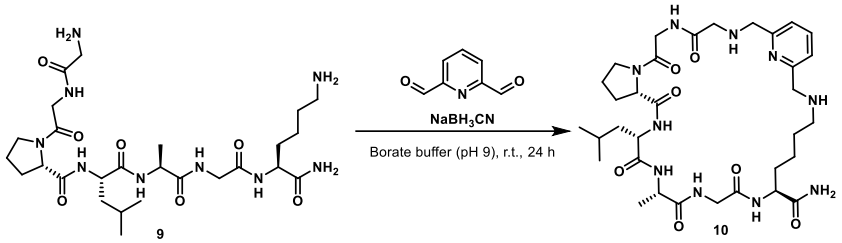


*Figure S1. Model reaction for the cyclisation of peptide **9** with dialdehyde using optimised reaction conditions (see Entry 7, Table 1 in the manuscript). Gradient:* 5% B for 1 min, then 5% to 40% B over 5 min, λ = 210 nm). Inset: extracted mass of product peak at t = 4.0 min.

Optimisation of Equivalents of Reductant in 40 mM Borate Buffer



Optimisation was carried out using linear peptide (**9**) on a 0.5 mg scale, at 1 mM concentration with respect to the peptide. Linear peptide (**9**) was dissolved in 40 mM sodium borate buffer and to this was added aldehyde **1** (1.5 equiv., 60 mM in MeCN) and 10, 20 or 100 equivalents of $NaBH_3CN$ (as a solution in 40 mM borate buffer). The reaction was capped and stirred at room temperate. At t = 24 h, an aliquot of each reaction was analysed *via* UPLC-MS, with the relative conversion quantified by peak area in the UV chromatogram (λ = 210). Employing 100 equivalents of $NaBH_3CN$ led to complete consumption of peptide starting material (**9**), with no observed increase in the production of over alkylated by-products. Accordingly, 100 equivalents of $NaBH_3CN$ were used for all subsequent cyclisations.

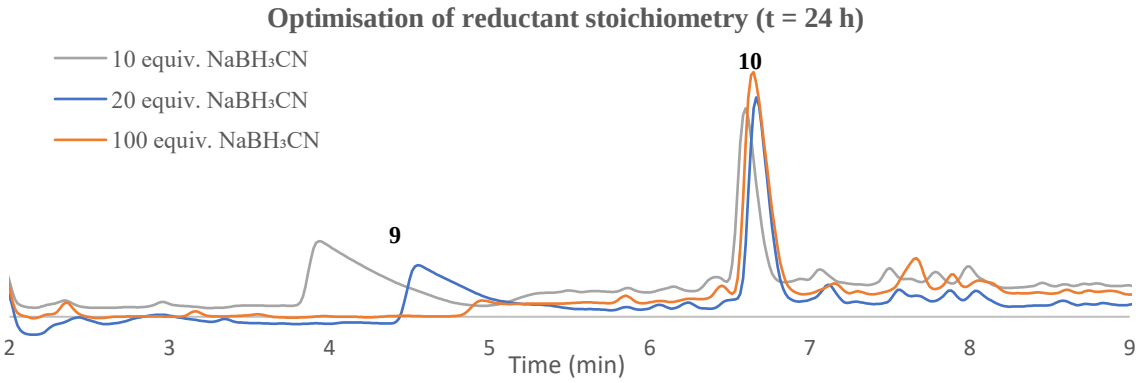
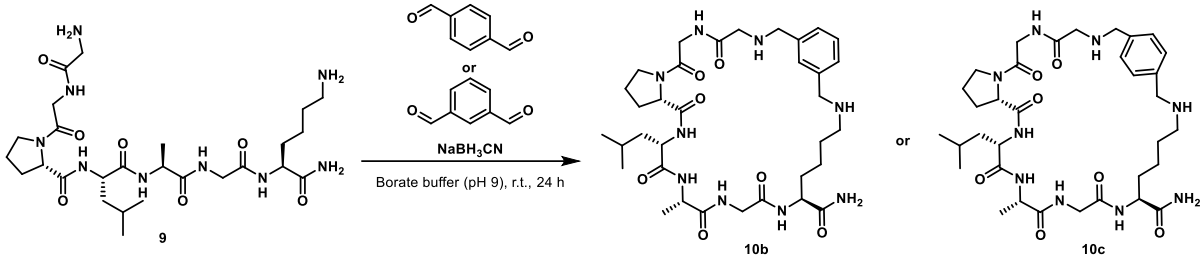


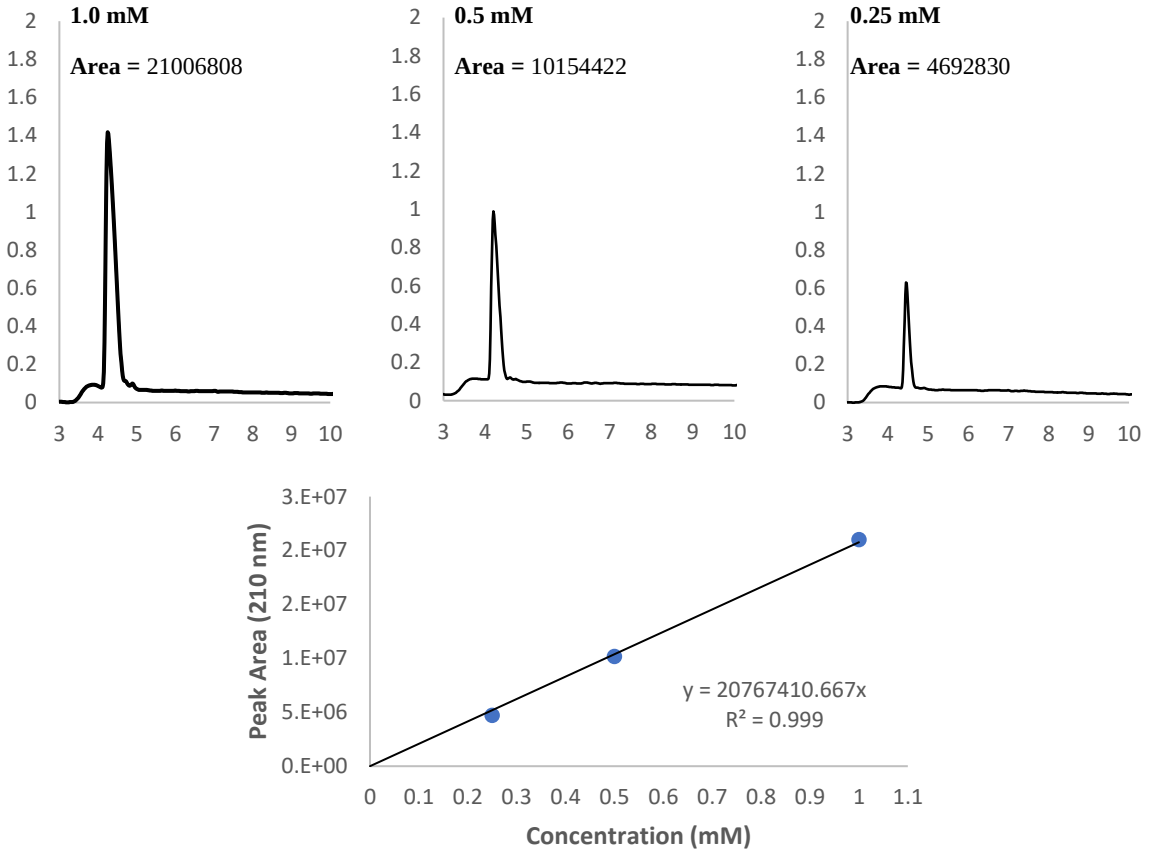
Figure S2. Optimisation of reductant stoichiometry (t = 24 h). Gradient: 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm.

Cyclisation of Peptide 9 with Isophthalaldehyde and Terephthalaldehyde

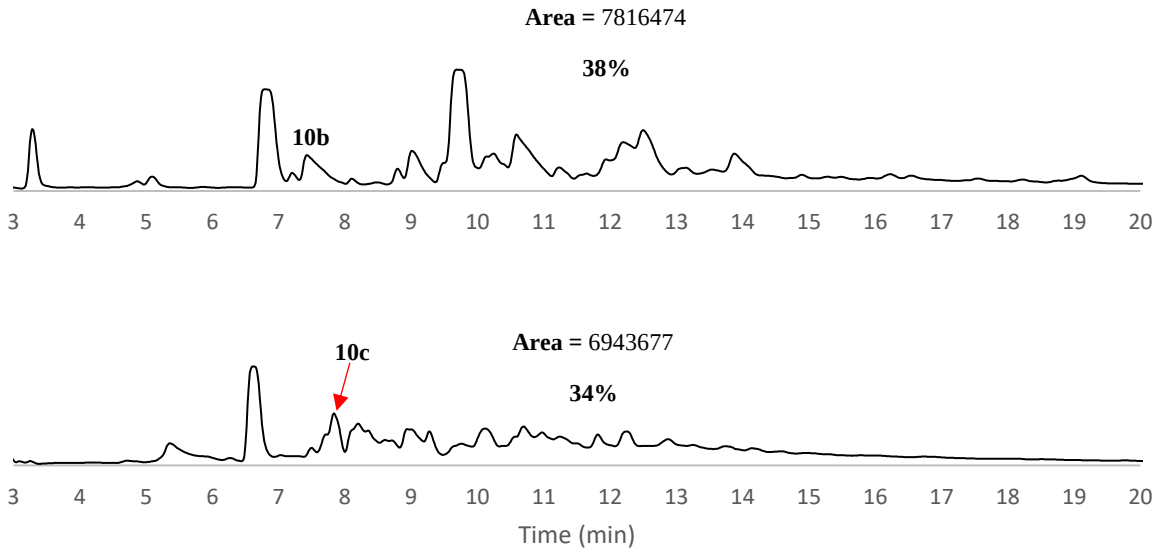


Linear peptide diamine (1.0 equiv.) was diluted in sodium borate reduction buffer (40 mM sodium borate, 100 mM $NaBH_3CN$, pH 9) to a concentration of 1 mM. To this was added isophthalaldehyde or terephthalaldehyde (3.0 equiv., 60 mM in MeCN). The reaction was stirred at rt for 24 h and evaluated by analytical reverse-phase UPLC (5 to 40% B over 30 min, λ = 210 nm). To estimate reaction yield, the area of the product was integrated and compared to a standard curve derived from the integration (λ = 210 nm) of stock samples of peptide **9** prepared at concentrations of 1.0 mM, 0.5 mM, and 0.25 mM. Yields are estimated assuming the extinction coefficient for **9** and **10b**/**10c** are comparable at λ = 210 nm.

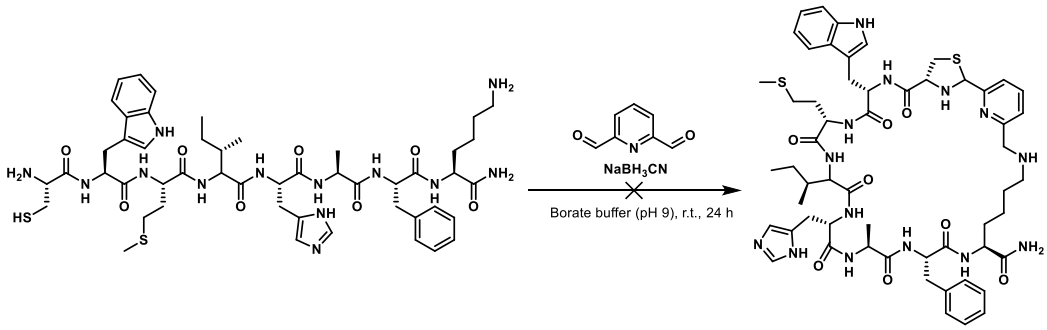
Standard curve preparation (Gradient: 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm.)



Crude reaction traces: 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm.



Attempted Cyclisation of an N-terminal Cysteine Peptide



Macrocyclisation of a peptide bearing an N-terminal cysteine residue was attempted *via* general procedure B. After 3 h, the mixture was analysed *via* UPLC-MS, which revealed two major peaks corresponding to $[M+2H]^{2+}$ of a putative N-terminal thiazolidine[2, 3] peptide macrocycle (**Figure S3**). However, leaving the reaction to stir overnight led to degradation of the product before isolation (**Figure S4**), suggesting that unprotected cysteine residues may not be tolerated under the reaction conditions.

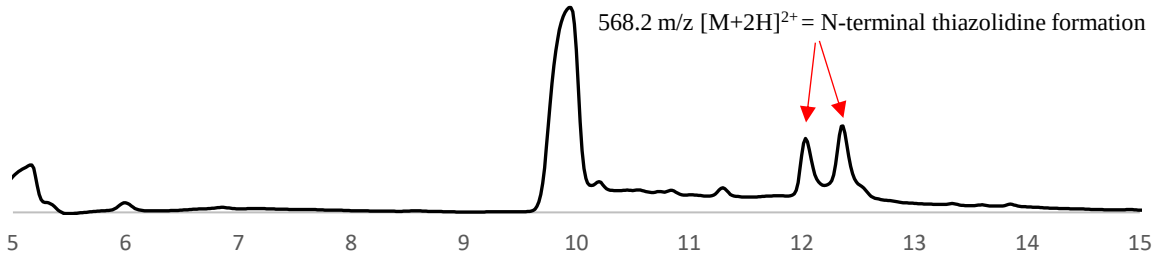


Figure S3. Attempted macrocyclisation of an N-terminal cysteine peptide at t = 3 h. Gradient: 20% B for 1 min, then 20% to 80% B over 10 min, λ = 210 nm).

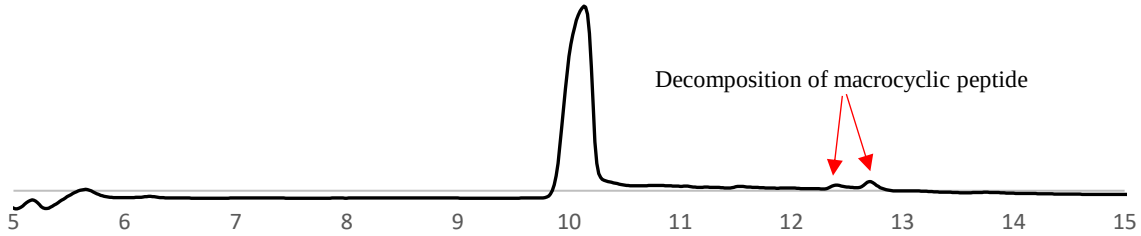
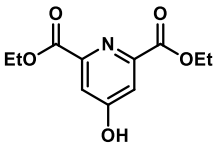


Figure S4. Attempted macrocyclisation of an N-terminal cysteine peptide at t = 24 h. Gradient: 20% B for 1 min, then 20% to 80% B over 10 min, λ = 210 nm).

Synthesis of Dialdehyde linkers

Compounds **5**, **6a**, **7a** and **2** were all synthesised according to literature procedures reported by Vincent and co-workers.[1]

Diethyl 4-hydroxypyridine-2,6-dicarboxylate (5)

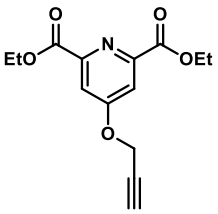


5

A solution of chelidamic acid **4** (2.0 g, 10.9 mmol, 1.0 equiv.) in dry EtOH (50 mL) under a nitrogen atmosphere was cooled to 0 °C. To this solution was added thionyl chloride (7.9 mL, 109 mmol, 10.0 equiv.) dropwise. The solution was allowed to warm to room temperature and magnetically stirred for 9 h. The solution was concentrated under reduced pressure and purified *via* flash chromatography on silica gel (MeOH/DCM: 2/98) to give diethyl 4-hydroxypyridine-

2,6-dicarboxylate **5** (1.89 g, 72%) as a yellow solid. Spectra matched literature values.[1] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.37 (s, 2H), 4.45 (q, *J* = 7.1 Hz, 4H), 1.41 (t, *J* = 7.1 Hz, 6H).

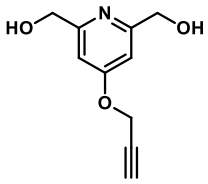
Diethyl 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarboxylate (6a)



6a

To a solution of diethyl 4-hydroxypyridine-2,6-dicarboxylate **5** (450 mg, 1.9 mmol, 1.0 equiv.) in acetone (30 mL) was added K_2CO_3 (520 mg, 3.8 mmol, 2.0 equiv.) and propargyl bromide (1 M solution in PhMe, 7.52 mL, 7.5 mmol, 4.0 equiv.) under a nitrogen atmosphere. The solution was heated to reflux and magnetically stirred for 2 h. The solution was subsequently cooled to room temperature, diluted with DCM (30 mL) and washed with water (3 x 20 mL). The organic layer was dried over $NaSO_4$, filtered, and concentrated under reduced pressure to give diethyl 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarboxylate **6a** (437 mg, 84%) as a yellow oil. Spectra matched literature values.[1] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.87 (s, 2H), 4.86 (d, *J* = 2.4 Hz, 2H), 4.48 (q, *J* = 7.2 Hz, 4H), 2.62 (t, *J* = 2.4 Hz, 1H), 1.46 (t, *J* = 7.2 Hz, 6H).

(4-(prop-2-yn-1-yloxy)pyridine-2,6-diyl)dimethanol (7a)

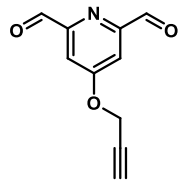


7a

A solution of diethyl 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarboxylate **6a** (437 mg, 1.6 mmol, 1.0 equiv.) in dry EtOH (5 mL) under a nitrogen atmosphere was cooled to 0 °C. To this was added $NaBH_4$ (298 mg, 7.9 mmol, 5.0 equiv.) portion-wise. The solution was allowed to warm to room temperature and magnetically stirred for 8 h. The solution was cooled to 0 °C and water was added dropwise until effervescence ceased. The mixture was extracted with EtOAc (2 x 5 mL), and the organic layer dried over $NaSO_4$, filtered, and concentrated under reduced pressure to give (4-(prop-2-yn-1-yloxy)pyridine-2,6-diyl)dimethanol **7a** (251 mg, 82%) as a

light-yellow solid. Spectra matched literature values.[1] **^{1}H NMR** (400 MHz, MeOD) δ 7.07 (s, 2H), 4.88 (d, *J* = 2.4 Hz, 2H), 4.64 (s, 4H), 3.06 (t, *J* = 2.4 Hz, 1H).

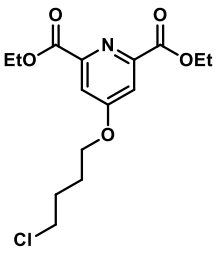
4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarbaldehyde (2)



2

A solution of oxalyl chloride (215 μL, 2.5 mmol, 2.2 equiv.) in DCM (20 mL) under a nitrogen atmosphere was cooled to -78 °C. To this was added DMSO (356 μL, 5.0 mmol, 4.0 equiv.) dropwise and the solution was magnetically stirred for 5 minutes. (4-(prop-2-yn-1-yloxy)pyridine-2,6-diyl)dimethanol **7a** (220 mg, 1.1 mmol, 1.0 equiv.) was added dropwise, followed by Et_3N (1.59 mL, 11.0 mmol, 10.0 equiv.). The solution was allowed to warm to room temperature and was diluted with water (50 mL). The mixture was extracted with DCM (3 x 25 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified *via* flash chromatography on silica gel (MeOH/DCM: 1/99) to give 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarbaldehyde **2** (150 mg, 70%) as a yellow solid. Spectra matched literature values.[1] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.12 (s, 2H), 7.73 (s, 2H), 4.88 (d, *J* = 2.4 Hz, 2H), 2.62 (t, *J* = 2.4 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 192.3, 165.8, 155.0, 112.0, 77.9, 76.2, 56.6.

Diethyl 4-(4-chlorobutoxy)pyridine-2,6-dicarboxlate (6b)

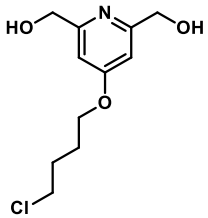


6b

To a solution of diethyl 4-hydroxypyridine-2,6-dicarboxylate **5** (478 mg, 2.1 mmol, 1.0 equiv.) in DMF (25 mL) under a nitrogen atmosphere was added 1-bromo-4-chlorobutane (1.4 mL, 12.5 mmol, 6.0 equiv.) and K_2CO_3 (1.15 g, 8.4 mmol, 4.0 equiv.). The solution was stirred at 50 °C for 3 h. The solution was filtered and concentrated under reduced pressure and the crude residue was purified *via* flash chromatography on silica gel (EtOAc/Petroleum ether: 1/1) to

give **6b** (509 mg, 77%) as a clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.77 (s, 2H), 4.47 (q, *J* = 7.1 Hz, 4H), 4.18 (t, *J* = 5.7 Hz , 2H), 3.63 (t, *J* = 6.1 Hz, 2H), 2.05 – 1.97 (m, 4H), 1.46 (t, *J* = 7.1 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.9, 164.9, 150.4, 114.4, 68.2, 62.6, 44.5, 29.1, 26.4, 14.4. **IR:** ν_{max} **=** 2980, 2961, 2929, 2879, 1741, 1722, 1594, 1446, 1337, 1237, 1226, 1041, 1020, 781 cm^{-1}. **HRMS (ESI-TOF):** calc'd $C_{15}H_{21}NO_5{}^{35}Cl$ $[M+H]^+$ 330.1108; found 330.1104.

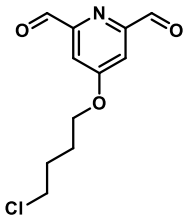
(4-(4-chlorobutoxy)pyridine-2,6-diyl)dimethanol (7b)



7b

To an oven-dried round-bottom flask under a nitrogen atmosphere was added diethyl ester **6b** (55 mg, 0.17 mmol, 1.0 equiv.) followed by EtOH (10 mL). The resulting solution was magnetically stirred and cooled to 0 °C. To this solution was added $NaBH_4$ (32 mg, 0.83 mmol, 5.0 equiv.) and the solution was allowed to warm to room temperature and stirred for a further 16 h. 1 M HCl (aq) was added to the solution until effervescence ceased. The solution was then diluted with water (50 mL) and the organic layer was extracted with EtOAc (5 x 15 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the product diol **7b** (37.2 mg, 91%) as a white solid. **^{1}H NMR** (400 MHz, MeOD) δ 6.97 (s, 2H), 4.61 (s, 4H), 4.20 – 4.06 (m, 2H), 3.72 – 3.57 (m, 2H), 2.04 – 1.89 (m, 4H). **^{13}C NMR** (101 MHz, MeOD) δ 168.7, 163.7, 106.4, 68.5, 65.3, 45.4, 30.4, 27.5. **IR:** ν_{max} **=** 3367 (br), 2954, 2900, 2655, 1601, 1572, 1438, 1352, 1309, 1303, 1150, 1089, 1050, 1032, 866, 671 cm^{-1}. **HRMS (ESI-TOF):** calc'd $C_{11}H_{17}NO_3{}^{35}Cl$ $[M+H]^+$ 246.0897; found 246.0798. **M.P** = 109-111 °C.

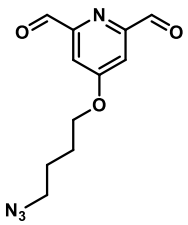
4-(4-chlorobutoxy)pyridine-2,6-dicarbaldehyde (8)



8

To an oven-dried round-bottom flask under a nitrogen atmosphere was added oxalyl chloride (289 μL, 3.4 mmol, 2.5 equiv.) and dry dichloromethane (40 mL). This solution was magnetically stirred and cooled to -78 °C. Dimethyl sulfoxide (421 μL, 5.9 mmol, 4.4 equiv.) was added dropwise, and the solution was stirred at -78 °C for a further 15 minutes. Diol **7b** (331 mg, 1.4 mmol, 1.0 equiv.) was added dropwise, followed by triethylamine (1.88 mL, 14 mmol, 10 equiv.) dropwise. The solution was allowed to warm to room temperature before being diluted with H_2O (50 mL). The aqueous layer was extracted with DCM (3 × 25 mL). The combined organic fractions were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified *via* flash chromatography on silica gel (EtOAc/Petroleum ether 3:7) to give dialdehyde **8** (260 mg, 80%) as a yellow oil which solidified upon freezing. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.11 (s, 2H), 7.63 (s, 2H), 4.20 (t, *J* = 5.8 Hz, 2H), 3.63 (t, *J* = 6.0 Hz, 2H), 2.06 – 1.97 (m, 4H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 192.5, 167.0, 154.9, 111.5, 68.5, 44.5, 29.1, 26.3. **IR:** ν_{max} = 2922, 2847, 1710, 1592, 1557, 1448, 1361, 1313, 716, 652 cm^{-1}. **HRMS (ESI-TOF):** calc'd $C_{11}H_{12}NO_3{}^{35}ClNa$ $[M+Na]^+$ 264.0403; found 264.0408. **M.P** = 41-42 °C.

4-(4-azidobutoxy)pyridine-2,6-dicarbaldehyde (3)



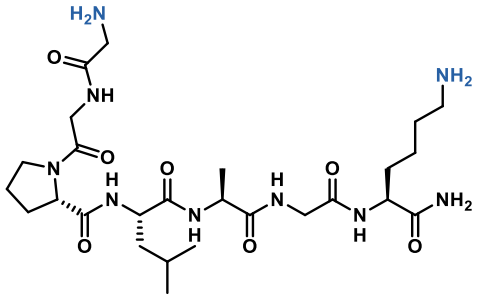
3

To a solution of dialdehyde **8** (100 mg, 4.1 mmol, 1.0 equiv.) in dry DMF (5 mL) under a nitrogen atmosphere was added NaN_3 (135 mg, 21 mmol, 5.0 equiv.). The solution was magnetically stirred at room temperature for 3 h. The reaction solution was then concentrated under a stream of nitrogen, redissolved in DCM (20 mL) and washed with H_2O (2 × 20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under

reduced pressure. The crude mixture was purified *via* flash chromatography on silica gel (100% EtOAc) to give azido-dialdehyde **3** (78 mg, 76%) as a dark yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.11 (s, 2H), 7.63 (s, 2H), 4.18 (t, *J* = 6.1 Hz, 2H), 3.39 (t, J = 6.6 Hz, 2H), 2.02 – 1.90 (m, 2H), 1.85 – 1.76 (m, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 192.5, 167.0, 154.9, 111.5, 68.7, 51.1, 26.2, 25.6. **IR:** ν_{max} = 3084, 2953, 2847, 2095, 1709, 1592, 1361, 1312, 715, 700 cm^{-1}. **HRMS (ESI-TOF):** calc'd $C_{11}H_{12}N_4O_3Na$ $[M+Na]^+$ 271.0807; found 271.0811.

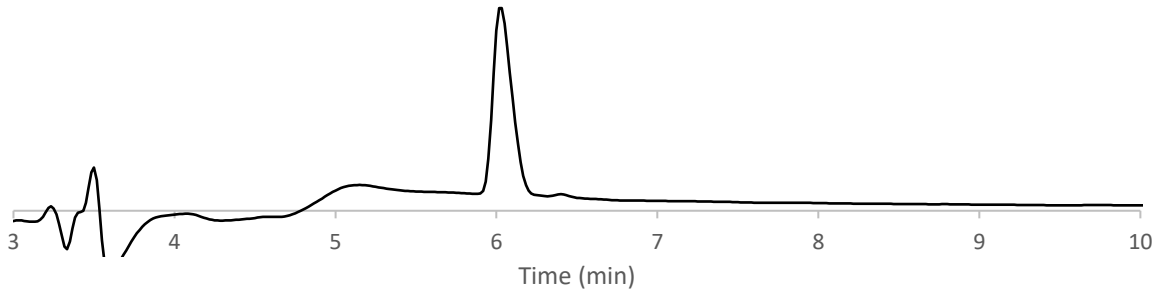
Experimental procedures and characterization data for peptide starting materials

Peptide 9



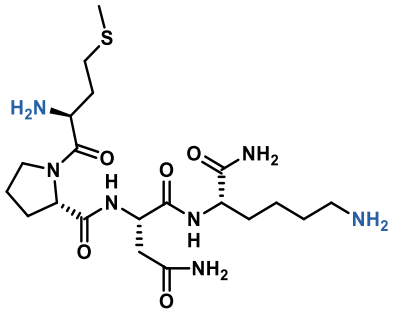
9

Peptide **9** was prepared according to general procedure A on a 583 µmol scale. TFA cleavage afforded the crude peptide after workup. The crude peptide was purified *via* reverse-phase preparative HPLC (15% B for 3 min, then 15% – 60% B, over 20 minutes) to afford peptide **9** as a white solid following lyophilisation. **^{1}H NMR** (400 MHz, MeOD) δ 4.50 – 4.29 (m, 3H), 4.28 – 4.17 (m, 1H), 4.14 (s, 2H), 3.99 – 3.91 (m, 1H), 3.84 – 3.77 (m, 1H), 3.75 (s, 2H), 3.73 – 3.66 (m, 1H), 3.65 – 3.56 (m, 1H), 2.94 (t, *J* = 7.4 Hz, 2H), 2.43 – 2.10 (m, 1H), 2.09 – 1.84 (m, 4H), 1.83 – 1.56 (m, 6H), 1.55 – 1.33 (m, 2H), 1.40 (d, *J* = 7.2 Hz, 3H), 0.96 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 6.2 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{26}H_{48}N_9O_7$ $[M+H]^+$ 598.3677; found 598.3673. **UPLC trace:**



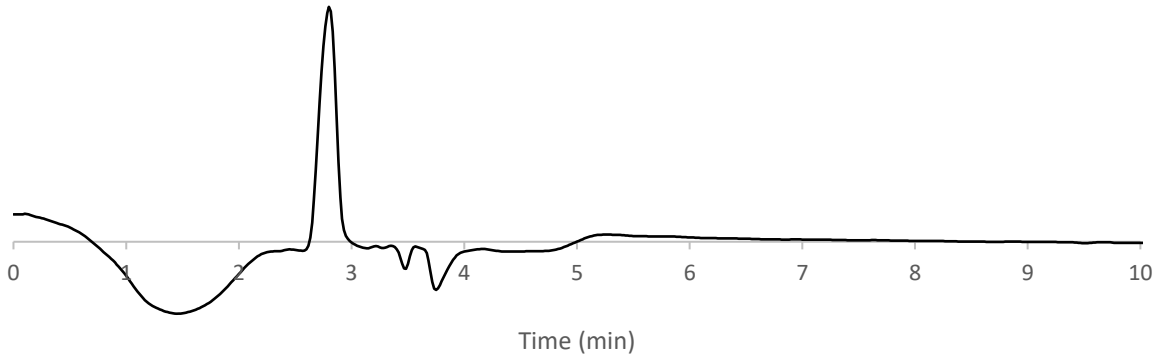
Purified peptide **9** (Rt = 6.1 min, 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm).

Peptide SI-1



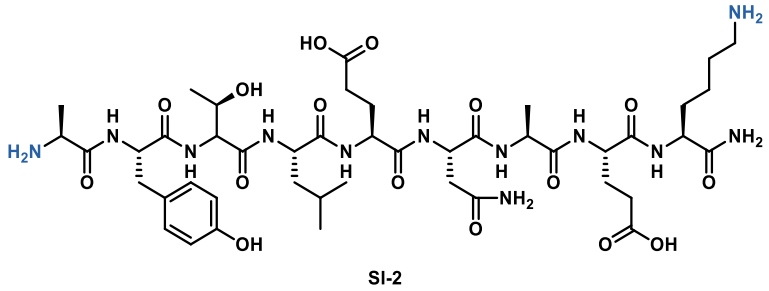
SI-1

Peptide **SI-1** was prepared according to general procedure A on a 120 µmol scale. TFA cleavage afforded the crude peptide after workup. The crude peptide was purified *via* reverse phase preparative HPLC (0% B for 3 min, then 0% – 60% B, over 20 minutes) to afford peptide **SI-1** as a white fluffy solid following lyophilisation. **^{1}H NMR** (700 MHz, MeOD) δ 4.62 (t, *J* = 6.3 Hz, 1H), 4.51 (dd, *J* = 8.5, 5.3 Hz, 1H), 4.41 – 4.37 (m, 1H), 4.34 – 4.30 (m, 1H), 3.78 – 3.72 (m, 1H), 3.72 – 3.66 (m, 1H), 2.93 (m, 2H), 2.83 – 2.66 (m, 4H), 2.31 – 2.19 (m, 2H), 2.16 (s, 3H), 2.14 – 2.06 (m, 2H), 2.05 – 1.97 (m, 2H), 1.96 – 1.88 (m, 1H), 1.74 – 1.64 (m, 3H), 1.51 – 1.43 (m, 2H). **HRMS (ESI-TOF):** calc'd $C_{20}H_{38}N_7O_5S$ $[M+H]^+$ 488.2655; found 488.2656. **UPLC trace:**



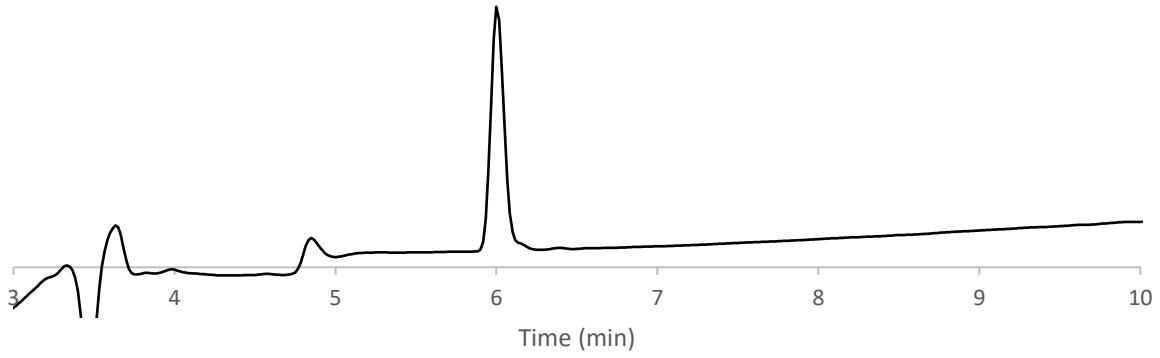
Purified peptide **SI-1** (Rt = 2.8 min, 10% B for 1 min, then 10% to 60% B over 10 min, λ = 210 nm).

Peptide SI-2



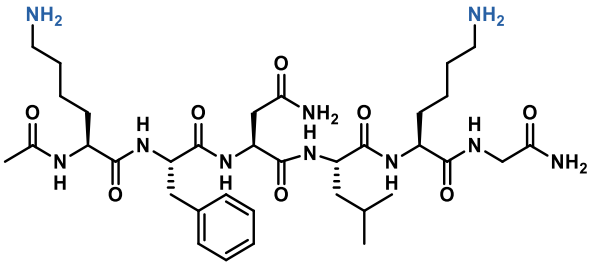
SI-2

Peptide **SI-2** was prepared according to general procedure A on a 120 µmol scale. TFA cleavage afforded the crude peptide after workup. The crude peptide was purified *via* reverse-phase preparative HPLC (20% B for 3 min, then 20% – 60% B, over 20 minutes) to afford peptide **SI-2** as a white fluffy solid following lyophilisation. **^{1}H NMR** (700 MHz, MeOD) δ 7.08 (d, *J* = 8.5 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 4.76 – 4.70 (m, 1H), 4.61 – 4.54 (m, 1H), 4.47 – 4.42 (m, 1H), 4.38 – 4.32 (m, 1H), 4.32 – 4.27 (m, 1H), 4.26 – 4.20 (m, 2H), 4.21 – 4.14 (m, 2H), 3.94 – 3.86 (m, 1H), 3.12 (dd, *J* = 14.2, 5.6 Hz, 1H), 2.99 – 2.93 (m, 2H), 2.90 – 2.84 (m, 1H), 2.82 (dd, *J* = 15.6, 7.9 Hz, 1H), 2.75 (dd, *J* = 15.5, 5.6 Hz, 1H), 2.57 – 2.49 (m, 1H), 2.47 – 2.39 (m, 4H), 2.21 – 2.13 (m, 1H), 2.14 – 2.06 (m, 1H), 2.07 – 2.00 (m, 2H), 1.97 – 1.90 (m, 1H), 1.87 – 1.79 (m, 1H), 1.77 – 1.67 (m, 2H), 1.68 – 1.63 (m, 3H), 1.60 – 1.52 (m, 1H), 1.48 (d, *J* = 7.1 Hz, 3H), 1.47 (d, *J* = 7.3 Hz, 3H), 1.22 (d, *J* = 6.4 Hz, 3H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.5 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{45}H_{73}N_{12}O_{16}$ $[M+H]^+$ 1037.5267; found 1037.5273. **UPLC trace:**



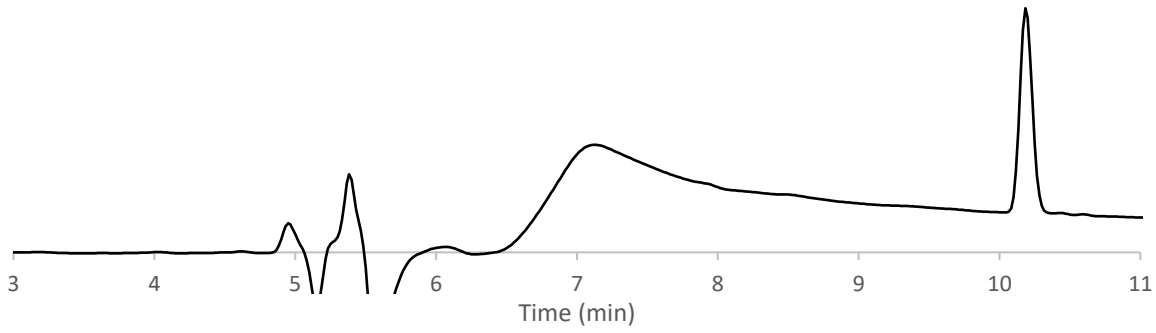
Purified peptide **SI-2** (Rt = 6.0 min, 10% B for 1 min, then 10% to 60% B over 10 min, λ = 210 nm).

Peptide SI-3



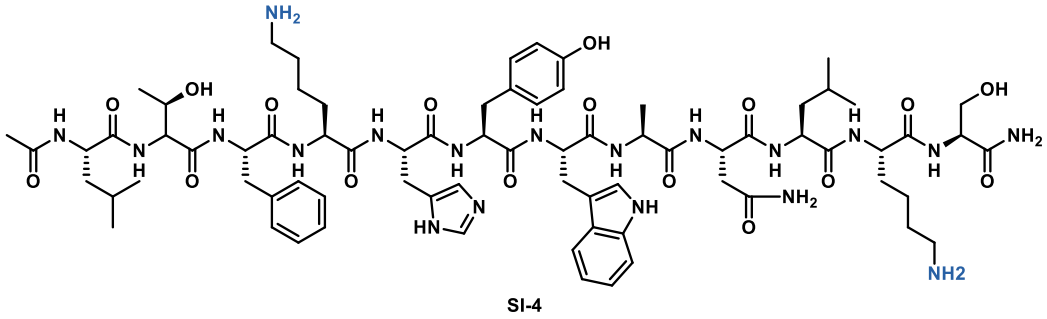
SI-3

Peptide **SI-3** was prepared according to general procedure A on a 120 µmol scale. TFA cleavage afforded the crude peptide after workup. The crude peptide was purified *via* reverse-phase preparative HPLC (20% B for 3 min, then 20% – 60% B, over 20 minutes) to afford peptide **SI-3** as a white fluffy solid following lyophilisation. **^{1}H NMR** (400 MHz, MeOD) δ 7.41 – 7.14 (m, 5H), 4.66 (t, *J* = 6.4 Hz, 1H), 4.63 – 4.55 (m, 1H), 4.37 – 4.22 (m, 2H), 4.21 – 4.12 (m, 1H), 3.85 (d, *J* = 6.0 Hz, 2H), 3.19 (dd, *J* = 14.0, 5.1 Hz, 1H), 3.04 – 2.77 (m, 6H), 2.73 (dd, *J* = 15.6, 6.1 Hz, 1H), 1.96 (s, 3H), 1.90 – 1.53 (m, 10H), 1.52 – 1.40 (m, 2H), 1.38 – 1.20 (m, 3H), 0.97 (d, *J* = 6.3 Hz, 3H), 0.92 (d, *J* = 6.3 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{35}H_{59}N_{10}O_8$ $[M+H]^+$ 747.4517; 747.4500. **UPLC trace:**

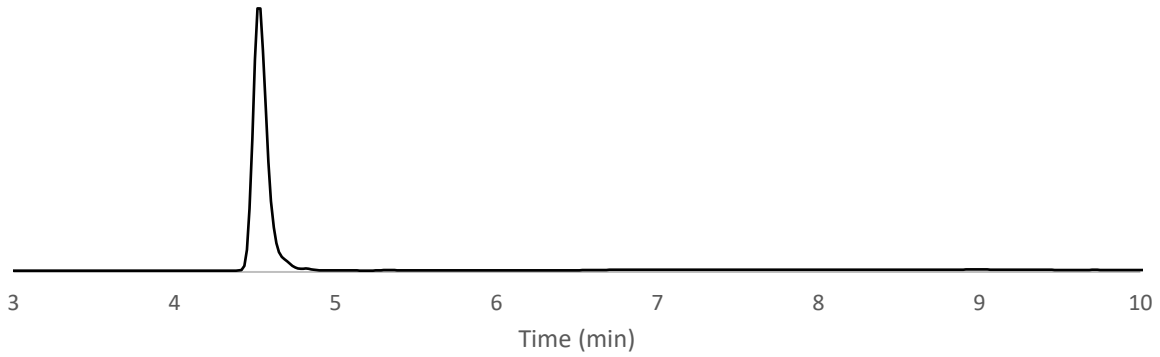


Purified peptide **SI-3** (Rt = 10.2 min, 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm).

Peptide SI-4

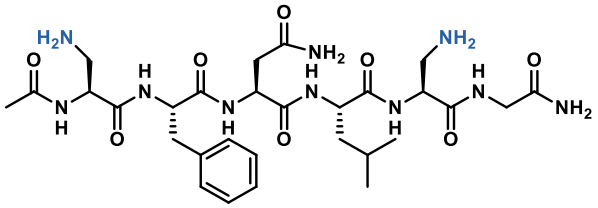


Peptide **SI-4** was prepared according to general procedure A on a 120 µmol scale. TFA cleavage afforded the crude peptide after workup. The crude peptide purified *via* reverse-phase preparative HPLC (30% B for 3 min, then 30% – 70% B, over 20 minutes) to afford peptide **SI-4** as a white fluffy solid following lyophilisation. **^{1}H NMR** (700 MHz, MeOD) δ 8.72 (s, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.29 – 7.24 (m, 5H), 7.19 (t, *J* = 7.1 Hz, 1H), 7.14 – 7.10 (m, 2H), 7.03 (t, *J* = 7.4 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 2H), 6.51 (d, *J* = 8.0 Hz, 2H), 4.51 – 4.46 (m, 1H), 4.46 – 4.38 (m, 2H), 4.37 – 4.33 (m, 1H), 4.33 – 4.29 (m, 1H), 4.25 (t, *J* = 8.1 Hz, 1H), 4.21 – 4.16 (m, 2H), 4.13 – 4.11 (m, 3H), 4.08 – 4.01 (m, 2H), 3.96 – 3.91 (m, 1H), 3.87 (dd, *J* = 11.6, 4.0 Hz, 1H), 3.29 – 3.23 (m, 2H), 3.19 – 3.11 (m, 3H), 3.09 – 3.04 (m, 1H), 3.02 – 2.97 (m, 1H), 2.96-2.90 (m, 2H), 2.88 – 2.83 (m, 2H), 2.83 – 2.79 (m, 1H), 2.69 (dd, *J* = 16.2, 4.6 Hz, 1H), 2.11 (s, 3H), 1.92 – 1.82 (m, 3H), 1.81 – 1.75 (m, 1H), 1.73 – 1.64 (m, 5H), 1.61 – 1.56 (m, 5H), 1.51 – 1.43 (m, 6H), 1.40 – 1.27 (m, 2H), 1.16 (d, *J* = 5.5 Hz, 3H), 0.98 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.77 (d, *J* = 6.5 Hz, 3H), 0.73 (d, *J* = 6.5 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{75}H_{111}N_{19}O_{17}$ $[M+2H]^{2+}$ 774.9203; found 774.9199. **UPLC trace:**



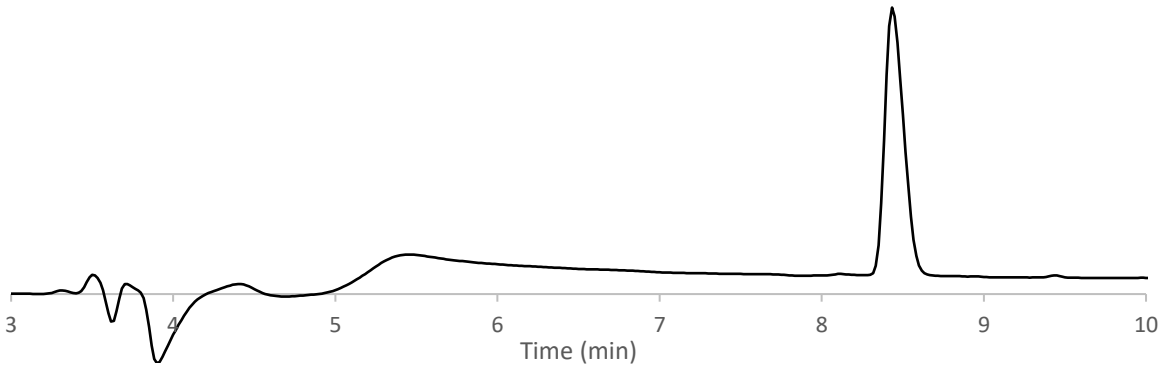
Purified peptide **SI-4** (Rt = 4.5 min, 20% B for 1 min, then 20% to 80% B over 10 min, λ = 210 nm).

Peptide SI-5



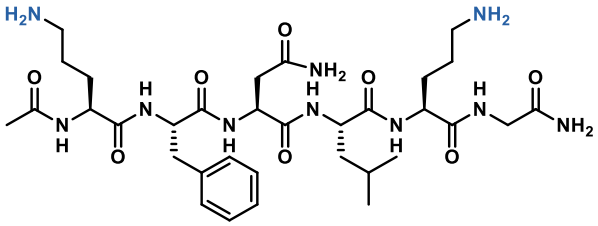
SI-5

Peptide **SI-5** was prepared according to general procedure A on a 50 µmol scale. TFA cleavage afforded the crude peptide after workup. The crude peptide was purified *via* reverse phase preparative HPLC (20% B for 3 min, then 20% – 60% B, over 20 minutes) to afford peptide **SI-5** as a white fluffy solid following lyophilisation. **^{1}H NMR** (700 MHz, MeOD) δ 7.31 – 7.26 (m, 2H), 7.25 – 7.20 (m, 3H), 4.71 – 4.68 (m, 1H), 4.67 – 4.62 (m, 2H), 4.57 (dd, *J* = 9.3, 5.3 Hz, 1H), 4.32 – 4.28 (m, 1H), 3.95 (d, *J* = 16.9 Hz, 1H), 3.84 (d, *J* = 16.9 Hz, 1H), 3.44 (dd, *J* = 13.2, 5.4 Hz, 1H), 3.31 (dd, *J* = 28.0, 6.3 Hz, 2H, *overlapping with MeOD peak*), 3.16 (dd, *J* = 14.1, 5.3 Hz, 1H), 3.11 (dd, *J* = 13.1, 7.7 Hz, 1H), 2.94 (dd, *J* = 14.1, 9.4 Hz, 1H), 2.88 (dd, *J* = 15.5, 8.0 Hz, 1H), 2.76 (dd, *J* = 15.5, 5.0 Hz, 1H), 1.95 (s, 3H), 1.83 – 1.63 (m, 3H), 0.98 (d, *J* = 6.3 Hz, 3H), 0.92 (d, *J* = 6.3 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{29}H_{47}N_{10}O_8$ $[M+H]^+$ 663.3578; 663.3579. **UPLC trace:**



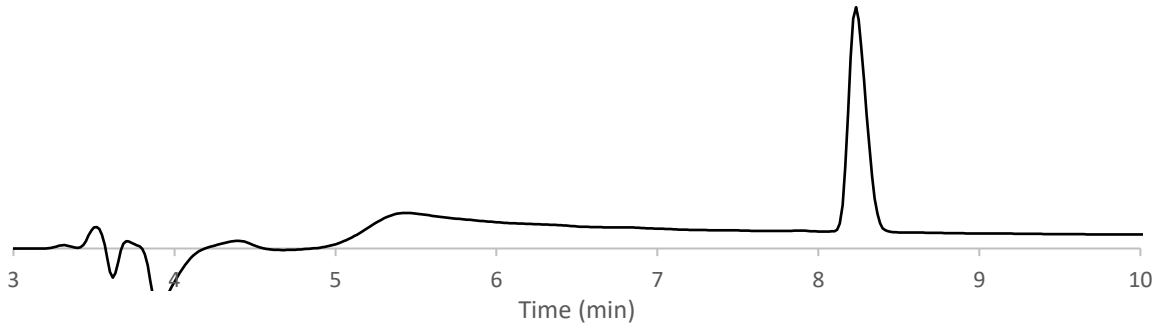
Purified peptide **SI-5** (Rt = 8.5 min, 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm).

Peptide SI-6



SI-6

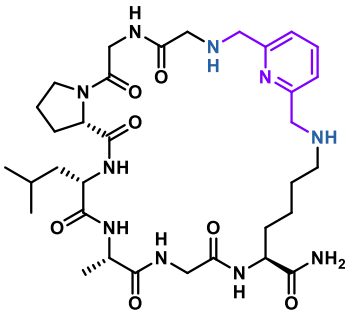
Peptide **SI-6** was prepared according to general procedure A on a 120 µmol scale. TFA cleavage afforded the crude peptide after workup. The crude peptide was purified *via* reverse phase preparative HPLC (20% B for 3 min, then 20% – 60% B, over 20 minutes) to afford peptide **SI-6** as a white fluffy solid following lyophilisation. **^{1}H NMR** (700 MHz, MeOD) δ 7.30 – 7.26 (m, 2H), 7.25 – 7.19 (m, 3H), 4.67 – 4.65 (m, 1H), 4.58 (dd, *J* = 9.1, 5.4 Hz, 1H), 4.33 (dd, *J* = 9.2, 5.2 Hz, 1H), 4.31 – 4.27 (m, 2H), 3.88 – 3.84 (m, 2H), 3.16 (dd, *J* = 14.1, 5.4 Hz, 1H), 2.96 – 2.93 (m, 3H), 2.92 – 2.89 (m, 2H), 2.84 (dd, *J* = 15.7, 6.9 Hz, 1H), 2.75 (dd, *J* = 15.7, 5.8 Hz, 1H), 2.00 – 1.96 (m, 1H), 1.95 (s, 3H), 1.85 – 1.80 (m, 1H), 1.78 – 1.71 (m, 4H), 1.68 – 1.62 (m, 5H), 0.97 (d, *J* = 6.5 Hz, 3H), 0.92 (d, *J* = 6.5 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{33}H_{55}N_{10}O_8$ $[M+H]^+$ 719.4204; 719.4204. **UPLC trace:**



Purified peptide **SI-6** (Rt = 8.2 min, 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm).

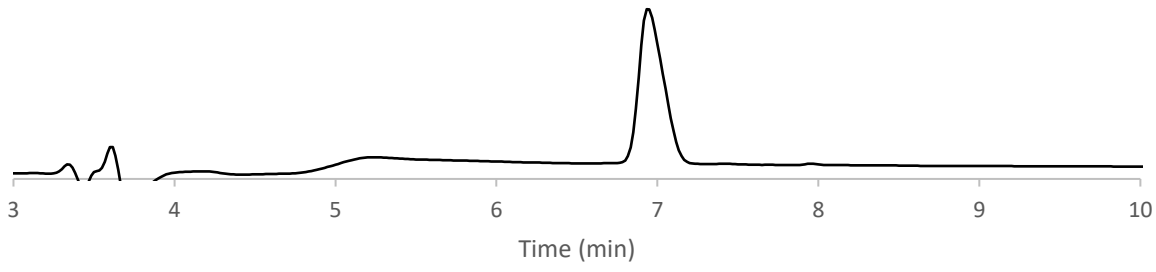
Experimental procedures and characterization data for pyridinyl macrocyclic products

Peptide 10



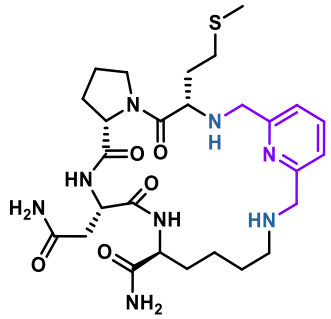
10

Macrocyclic peptide **10** was prepared according to general procedure B from linear peptide **9** (6.23 mg, 7.5 μmol). At time = 24 h, an additional 1.5 equiv. of dialdehyde **1** was added, and the reaction continued to stir. At time = 29 h the reaction was purified *via* reverse phase preparative HPLC (0% B for 5 minutes, then 15% B for 3 minutes, then 15% – 60% B, over 20 minutes) to afford peptide macrocycle **10** (4.38 mg, 63%) as a white solid after lyophilisation. **^{1}H NMR** (700 MHz, MeOD) δ 7.98 (t, *J* = 7.8 Hz, 1H), 7.52 (dd, *J* = 7.8, 2.8 Hz, 2H), 4.56 (s, 2H), 4.51 – 4.47 (m, 2H), 4.46 – 4.41 (m, 2H), 4.31 – 4.23 (m, 2H), 4.17 – 4.08 (m, 1H), 4.05 – 3.98 (m, 2H), 3.96 – 3.83 (m, 3H), 3.72 – 3.68 (m, 1H), 3.62 – 3.57 (m, 1H), 3.18 – 3.13 (m, 2H), 2.28 – 2.22 (m, 1H), 2.06 – 2.04 (m, 2H), 1.94 (app s, 2H), 1.89 – 1.85 (m, 1H), 1.83 – 1.78 (m, 2H), 1.69 – 1.62 (m, 3H), 1.58 – 1.53 (m, 1H), 1.48 – 1.43 (m, 1H), 1.35 (d, *J* = 7.1 Hz, 3H), 0.98 (d, *J* = 6.4 Hz, 3H), 0.93 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR** (176 MHz, MeOD) δ 176.6, 175.6, 175.1, 175.1, 172.0, 170.0, 167.3, 152.6, 152.1, 140.6, 124.2, 124.1, 62.3, 54.4, 53.7, 51.6, 51.4, 51.2, 49.5, 49.4, 47.9, 44.0, 42.8, 41.0, 32.2, 30.6, 26.3, 26.0, 25.9, 23.6, 23.2, 22.3, 16.8. **HRMS (ESI-TOF):** calc'd $C_{33}H_{53}N_{10}O_7$ $[M+H]^+$ 701.4099; found 701.4083. **UPLC trace:**



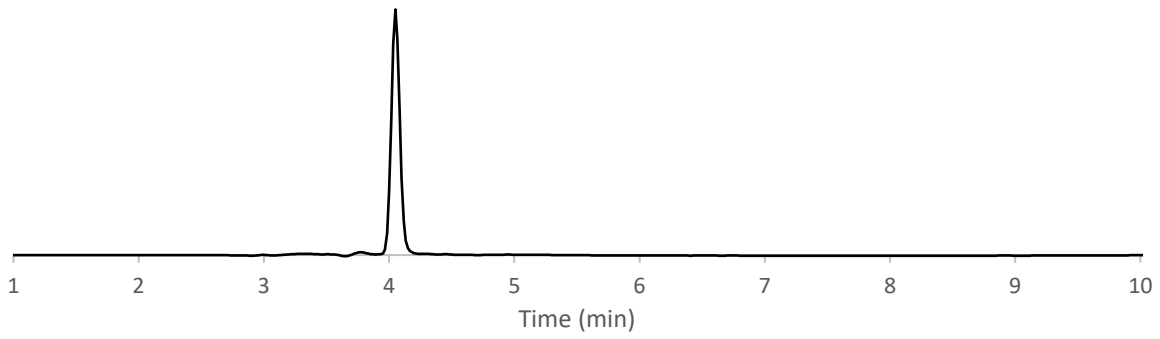
Purified peptide **10** (Rt = 7.0 min, 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm).

Peptide 11



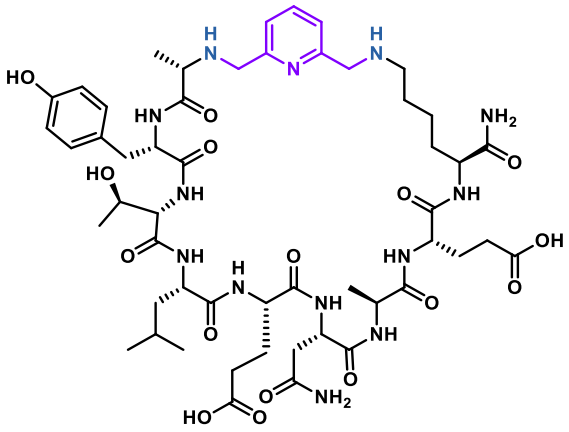
11

Macrocyclic peptide **11** was prepared according to general procedure B from linear peptide **SI-1** (3.22 mg, 4.5 μmol). At time = 24 h, an additional 2.0 equiv. of dialdehyde **1** was added, and the reaction continued to stir. At time = 30 h the reaction was purified *via* reverse phase preparative HPLC (10% B for 3 minutes, then 10% – 60% B, over 20 minutes) to afford peptide macrocycle **11** (1.95 mg, 53%) as a white solid after lyophilisation. **^{1}H NMR** (400 MHz, MeOD) δ 7.97 (t, *J* = 7.8 Hz, 1H), 7.53 – 7.46 (m, 2H), 4.78 – 4.70 (m, 1H), 4.68 – 4.61 (m, 2H), 4.59 – 4.53 (m, 1H), 4.50 – 4.45 (m, 1H), 4.44 – 4.36 (m, 1H), 4.32 – 4.28 (m, 2H), 3.86 – 3.71 (m, 2H), 3.24 – 3.15 (m, 1H), 2.87 – 2.63 (m, 5H), 2.42 – 2.27 (m, 2H), 2.20 (s, 3H), 2.17 – 2.13 (m, 2H), 2.10 – 2.03 (m, 2H), 1.98 – 1.86 (m, 2H), 1.81 – 1.67 (m, 2H), 1.59 – 1.45 (m, 2H). **HRMS (ESI-TOF):** calc'd $C_{27}H_{43}N_8O_5S$ $[M+H]^+$ 591.3077; found 591.3079. **UPLC trace:**



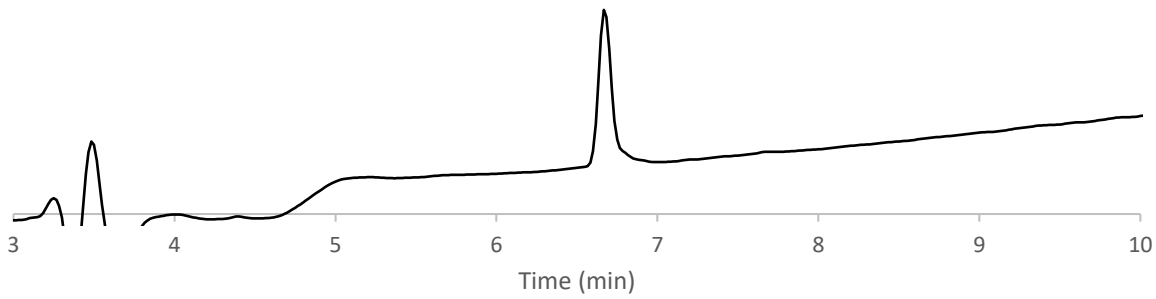
Purified peptide **11** (Rt = 4.1 min, 10% B for 1 min, then 10% to 60% B over 10 min, λ = 262 nm).

Peptide 12



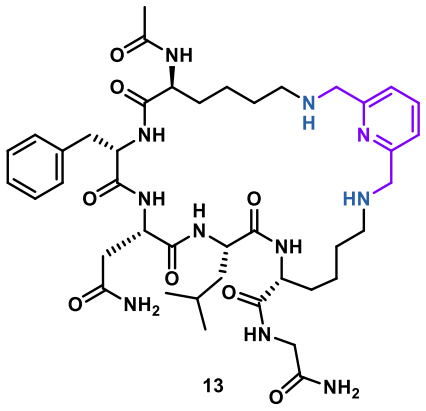
12

Macrocyclic peptide **12** was prepared according to general procedure B from linear peptide **SI-2** (6.35 mg, 5.0 μmol). At time = 24 h, an additional 2.0 equiv. of dialdehyde **1** was added, and the reaction continued to stir. At time = 32 h the reaction was purified *via* reverse phase preparative HPLC (0% B for 5 minutes, then 20% B for 3 minutes, then 20% – 60% B, over 20 minutes) to afford peptide macrocycle **12** (1.57 mg, 23%) as a white solid after lyophilisation. **^{1}H NMR** (700 MHz, MeOD) δ 7.95 (t, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.12 – 7.04 (m, 2H), 6.71 – 6.63 (m, 2H), 4.76 (dd, *J* = 10.3, 5.4 Hz, 1H), 4.57 (d, *J* = 3.6 Hz, 1H), 4.52 – 4.47 (m, 2H), 4.47 – 4.41 (m, 2H), 4.30 – 4.25 (m, 2H), 4.24 – 4.21 (m, 1H), 4.19 – 4.14 (m, 2H), 4.12 – 4.08 (m, 1H), 4.04 (q, *J* = 7.0 Hz, 1H), 3.91 (d, *J* = 14.4 Hz, 1H), 3.21 – 3.17 (m, 3H), 2.89 – 2.83 (m, 3H), 2.52 – 2.41 (m, 5H), 2.27 – 2.20 (m, 1H), 2.14 – 2.07 (m, 2H), 2.02 – 1.95 (m, 2H), 1.89 – 1.77 (m, 4H), 1.71 – 1.64 (m, 2H), 1.62 (d, *J* = 7.1 Hz, 3H), 1.57 – 1.55 (m, 1H), 1.49 (d, *J* = 7.4 Hz, 3H), 1.22 (d, *J* = 6.3 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.95 (d, *J* = 6.6 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{52}H_{78}N_{13}O_{16}$ $[M+H]^+$ 1140.5689; found 1140.5686. **UPLC trace:**



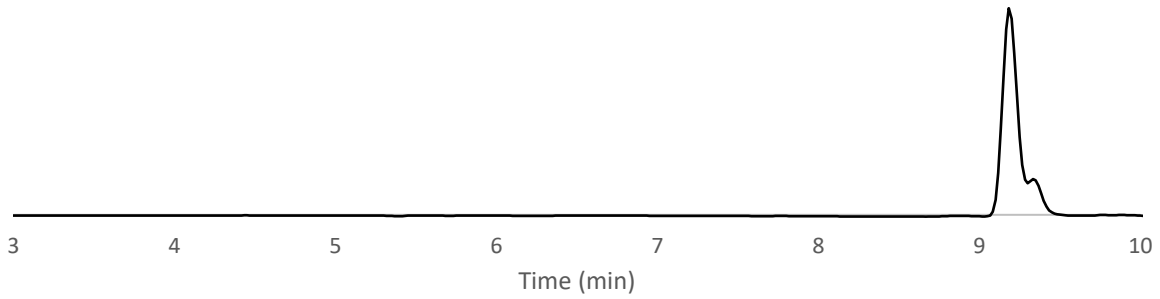
Purified peptide **12** (Rt = 6.7 min, 10% B for 1 min, then 10% to 60% B over 10 min, λ = 230 nm).

Peptide 13



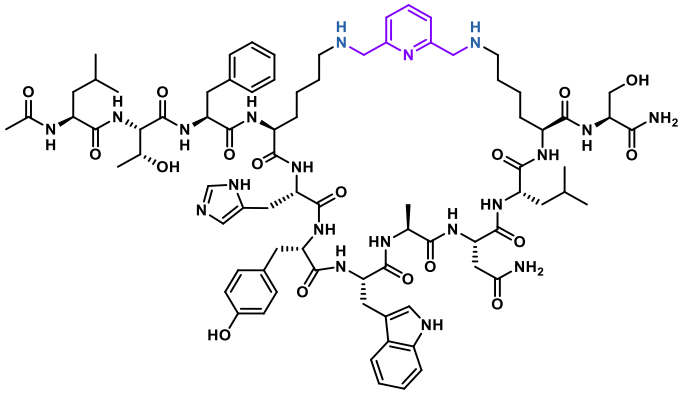
13

Macrocyclic peptide **13** was prepared according to general procedure B from linear peptide **SI-3** (3.46 mg, 3.5 μmol). At time = 24 h, an additional 1.5 equiv. of dialdehyde **1** was added, and the reaction continued to stir. At time = 48 h the reaction was purified *via* reverse phase preparative HPLC (20% B for 3 minutes, then 20% – 60% B, over 20 minutes) to afford peptide macrocycle **13** (2.47 mg, 65%) as a fluffy white solid. **^{1}H NMR** (700 MHz, MeOD) δ 7.97 (t, *J* = 7.8 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.30 – 7.25 (m, 2H), 7.24 – 7.17 (m, 3H), 4.55 – 4.51 (m, 1H), 4.50 – 4.46 (m, 3H), 4.45 (s, 2H), 4.40 – 4.35 (m, 1H), 4.30 – 4.26 (m, 1H), 4.20 – 4.16 (m, 1H), 3.85 (d, *J* = 1.5 Hz, 2H), 3.19 – 3.13 (m, 4H), 3.13 – 3.08 (m, 1H), 2.98 (dd, *J* = 14.0, 9.4 Hz, 1H), 2.76 – 2.69 (m, 2H), 1.96 (s, 3H), 1.95 – 1.92 (m, 1H), 1.85 – 1.76 (m, 5H), 1.73 – 1.68 (m, 4H), 1.65 – 1.60 (m, 1H), 1.59 – 1.54 (m, 1H), 1.51 – 1.44 (m, 1H), 1.41 – 1.34 (m, 2H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.91 (d, *J* = 6.3 Hz, 3H). **^{13}C NMR** (176 MHz, MeOD) δ 175.2, 174.8, 174.6, 174.3, 174.0, 174.0, 173.9, 173.6, 152.7, 152.6, 140.4, 138.4, 130.3, 129.6, 127.9, 124.1, 124.1, 56.9, 55.3, 54.4, 54.3, 52.4, 51.6, 51.6, 51.3, 51.2, 49.5, 49.4, 43.1, 40.8, 37.5, 37.2, 32.0, 31.8, 26.5, 26.3, 25.9, 23.5, 23.5, 23.3, 22.5, 21.7. **HRMS (ESI-TOF):** calc'd $C_{42}H_{64}N_{11}O_8$ $[M+H]^+$ 850.4939; found 850.4942. **UPLC trace:**



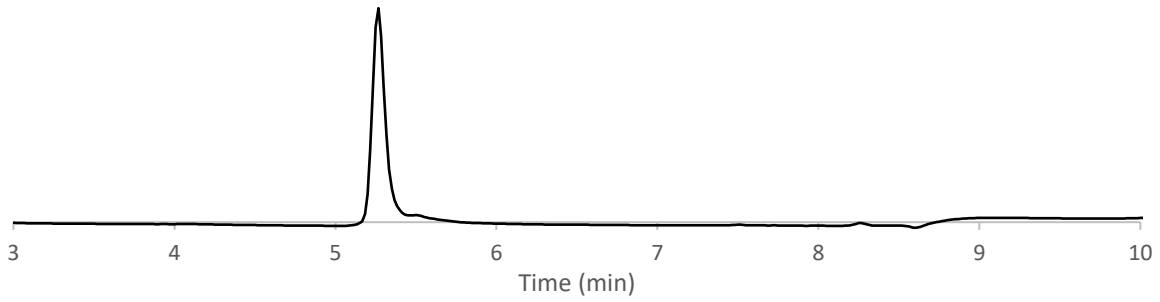
Purified peptide **13** (Rt 9.2 min, 5% B for 1 min, then 5% to 60% B over 10 min, λ = 210 nm).

Peptide 14



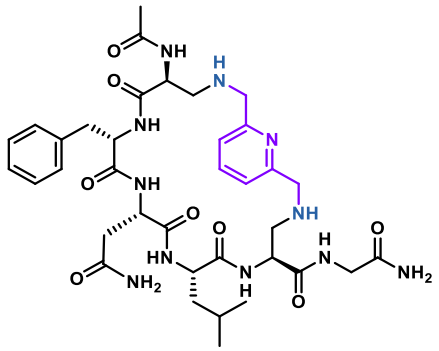
14

Macrocyclic peptide **14** was prepared according to general procedure B from linear peptide **SI-4** (5.06 mg, 4.5 μmol). At time = 22 h the reaction was purified *via* reverse phase preparative HPLC (0% B for 5 minutes, then 20% B for 3 minutes, then 20% – 80% B, over 20 minutes) to afford peptide macrocycle **14** (1.92 mg, 36%) as a white fluffy solid after lyophilisation. **^{1}H NMR** (700 MHz, MeOD) δ 8.76 (s, 1H), 7.83 (t, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.39 (dd, *J* = 17.2, 8.0 Hz, 2H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.29 – 7.24 (m, 5H), 7.19 – 7.16 (m, 1H), 7.14 (s, 1H), 7.09 (t, *J* = 7.3 Hz, 1H), 6.97 (t, *J* = 7.6 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 2H), 6.45 (d, *J* = 8.0 Hz, 2H), 4.48 – 4.39 (m, 4H), 4.38 – 4.32 (m, 3H), 4.29 (dd, *J* = 16.9, 8.9 Hz, 2H), 4.26 – 4.22 (m, 2H), 4.16 – 4.10 (m, 3H), 4.09 – 4.00 (m, 2H), 3.96 (dd, *J* = 11.7, 6.7 Hz, 1H), 3.87 (dd, *J* = 11.7, 4.1 Hz, 1H), 3.29 (d, *J* = 4.9 Hz, 1H), 3.26 – 3.11 (m, 6H), 3.09 – 3.01 (m, 2H), 2.97 (dd, *J* = 13.9, 7.8 Hz, 2H), 2.88 (dd, *J* = 16.5, 9.4 Hz, 1H), 2.71 – 2.65 (m, 1H), 2.12 (s, 3H), 2.08 – 1.85 (m, 7H), 1.85 – 1.75 (m, 5H), 1.74 – 1.66 (m, 3H), 1.63 – 1.55 (m, 6H), 1.53 – 1.48 (m, 2H), 1.46 (d, *J* = 7.3 Hz, 3H), 1.18 (d, *J* = 6.1 Hz, 3H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.77 (d, *J* = 6.5 Hz, 3H), 0.68 (d, *J* = 6.5 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{82}H_{115}N_{20}O_{17}$ $[M+H]^+$ 1651.8749; found 1651.8778. **UPLC Trace:**



Purified peptide **14** (Rt = 5.3 min, 20% B for 1 min, then 20% to 80% B over 10 min, λ = 210 nm).

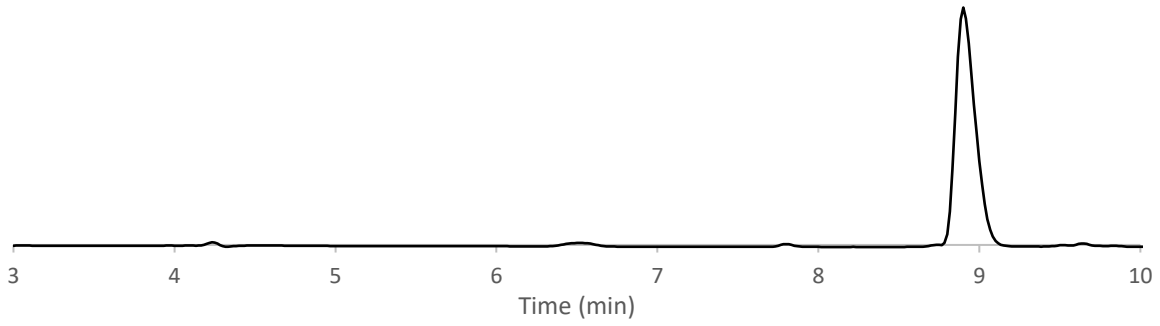
Peptide 15



15

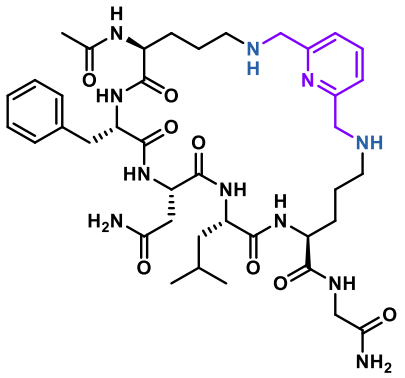
Macrocyclic peptide **15** was prepared according to general procedure B from linear peptide **SI-5** (6.20 mg, 6.96 μmol). At time = 24 h, an additional 1.5 equiv. of dialdehyde **1** was added, and the reaction continued to stir. At time = 46 h the reaction was purified *via* reverse phase preparative HPLC (0% B for 5 minutes, then 20% B for 3 minutes, then 20% – 60% B, over 20 minutes) to afford peptide macrocycle **15** (2.69 mg, 39%) as a fluffy white solid. **^{1}H NMR** (700 MHz, MeOD) δ 7.99 (t, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.33 – 7.22 (m, 5H), 5.16 (d, *J* = 9.4 Hz, 1H), 4.81 – 4.79 (m, 1H), 4.73 (d, *J* = 15.6 Hz, 1H), 4.52 – 4.46 (m, 3H), 4.44 (t, J = 5.6 Hz, 1H), 4.35 – 4.30 (m, 1H), 4.10 – 4.06 (m, 1H), 3.91 (s, 2H), 3.73 (dd, *J* = 13.4, 4.4 Hz, 1H), 3.64 – 3.60 (m, 1H), 3.48 – 3.42 (m, 1H), 3.22 – 3.19 (m, 1H), 3.02 – 2.98 (m, 1H), 2.74 – 2.70 (m, 2H), 1.89 (s, 3H), 1.70 – 1.55 (m, 4H), 0.91 – 0.89 (m, 6H). **HRMS (ESI-TOF):** calc'd $C_{36}H_{52}N_{11}O_8$ $[M+H]^+$ 766.4004; found 766.4004.

UPLC trace:



Purified peptide **15** (Rt = 8.9 min, 5% B for 1 min, then 5% to 40% B over 10 min, λ = 262 nm).

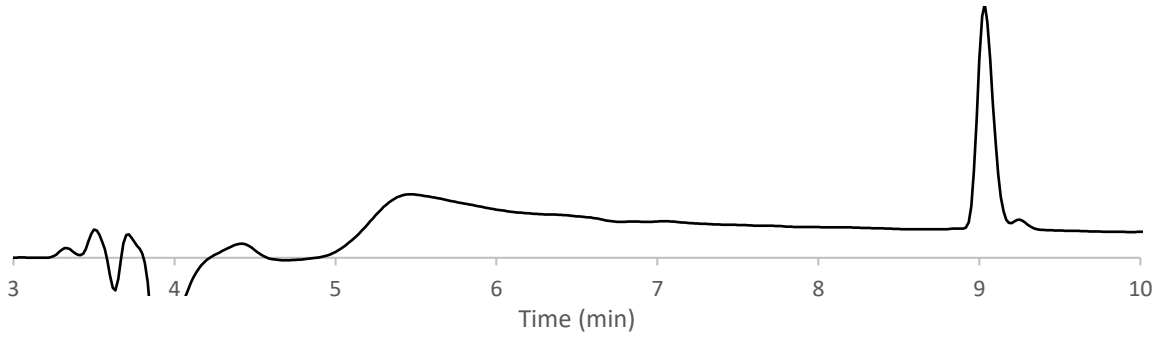
Peptide 16



16

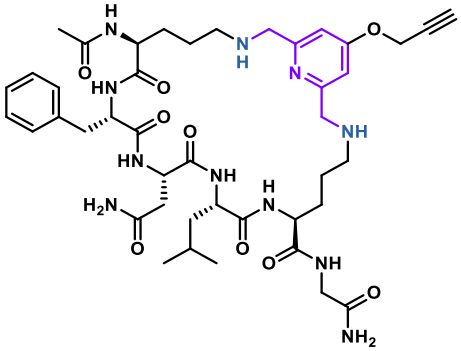
Macrocyclic peptide **16** was prepared according to general procedure B from linear peptide **SI-6** (4.98 mg, 5.3 μmol). At time = 24 h, an additional 1.0 equiv. of dialdehyde **1** was added, and the reaction continued to stir. At time = 30 h the reaction was purified *via* reverse phase preparative HPLC (0% B for 5 minutes, then 20% B for 3 minutes, then 20% – 60% B, over 20 minutes) to afford peptide macrocycle **16** (2.30 mg, 42%) as a fluffy white solid. **^{1}H NMR** (700 MHz, MeOD) δ 7.96 (t, *J* = 7.8 Hz, 1H), 7.55 – 7.42 (m, 2H), 7.33 – 7.27 (m, 2H), 7.26 – 7.18 (m, 3H), 4.64 – 4.59 (m, 1H), 4.50 – 4.47 (m, 2H), 4.46 – 4.43 (m, 4H), 4.29 (dd, *J* = 10.6, 4.5 Hz, 1H), 4.17 – 4.13 (m, 1H), 3.85 (d, *J* = 0.9 Hz, 2H), 3.20 – 3.14 (m, 4H), 3.10 – 3.06 (m, 1H), 3.01 – 2.98 (m, 1H), 2.79 – 2.72 (m, 2H), 2.02 – 1.97 (m, 1H), 1.95 (s, 3H), 1.93 – 1.85 (m, 2H), 1.83 – 1.76 (m, 5H), 1.72 – 1.63 (m, 3H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{40}H_{60}N_{11}O_8$ $[M+H]^+$ 822.4626; found 822.4626.

UPLC trace:



Purified peptide **16** (Rt = 9.0 min, 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm).

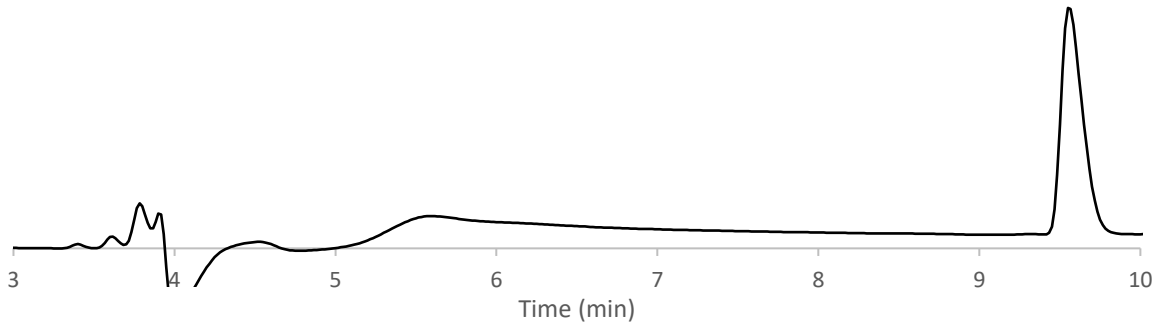
Peptide 17



17

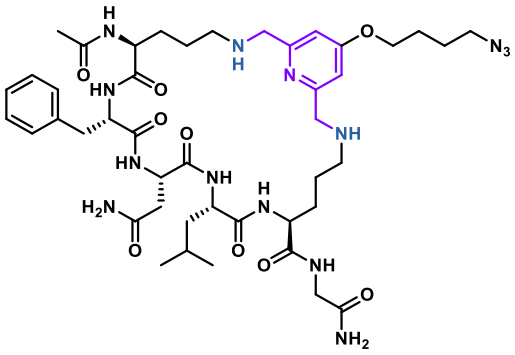
Macrocyclic alkynyl peptide **17** was prepared according to general procedure B from linear peptide **SI-6** (7.00 mg, 7.4 μmol). At time = 20 h the reaction was purified *via* reverse phase preparative HPLC (0% B for 5 minutes, then 10% B for 3 minutes, then 10% – 60% B, over 20 minutes) to afford peptide macrocycle **17** (2.57 mg, 31%) as a white solid. **^{1}H NMR** (700 MHz, MeOD) δ 7.32 – 7.27 (m, 2H), 7.26 – 7.21 (m, 3H), 7.16 – 7.13 (m, 1H), 7.13 – 7.11 (m, 1H), 4.92 (d, *J* = 2.3 Hz, 2H), 4.64 – 4.60 (m, 1H), 4.52 – 4.47 (m, 1H), 4.44 (dd, *J* = 9.0, 3.9 Hz, 1H), 4.42 – 4.39 (m, 2H), 4.37 (s, 2H), 4.30 – 4.26 (m, 1H), 4.17 – 4.12 (m, 1H), 3.85 (s, 2H), 3.22 – 3.12 (m, 5H), 3.08 – 3.04 (m, 1H), 3.01 – 2.97 (m, 1H), 2.78 – 2.72 (m, 2H), 2.00 – 1.96 (m, 1H), 1.95 (s, 3H), 1.92 – 1.85 (m, 2H), 1.83 – 1.74 (m, 5H), 1.71 – 1.64 (m, 3H), 0.96 (d, *J* = 6.5 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{43}H_{62}N_{11}O_9$ $[M+H]^+$ 876.4733; found 876.4732.

UPLC trace:



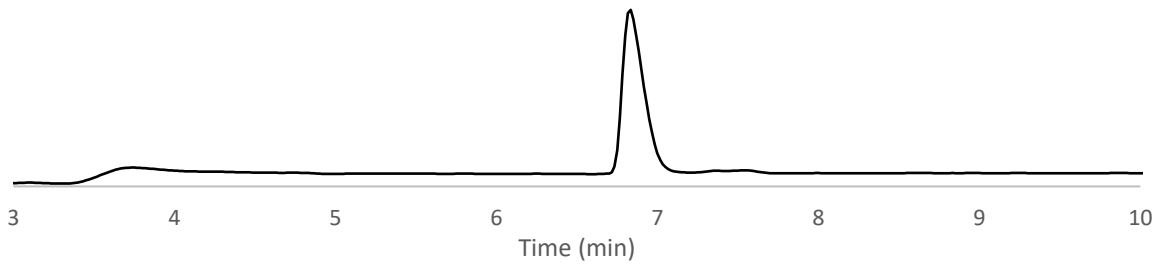
Purified peptide **17** (Rt = 9.6 min, 5% B for 1 min, then 5% to 40% B over 10 min, λ = 210 nm).

Peptide 18



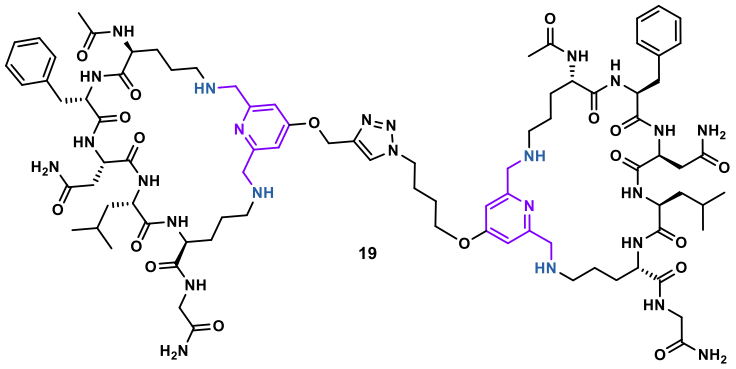
18

Macrocyclic azido peptide **18** was prepared according to general procedure B using 50/50 borate buffer/CH_3CN (v/v) from linear peptide **SI-6** (10.00 mg, 10.6 μmol). At time = 22 h the reaction was purified *via* reverse phase preparative HPLC (20% B for 3 minutes, then 20% – 80% B over 20 minutes) to afford peptide macrocycle **18** (6.25 mg, 51%) as a white solid. 2.22 mg (22%) of starting material **SI-6** was also recovered. **^{1}H NMR** (700 MHz, MeOD) δ 7.32 – 7.27 (m, 2H), 7.25 – 7.20 (m, 3H), 7.07 – 7.03 (m, 2H), 4.59 (t, *J* = 6.5 Hz, 1H), 4.47 (dd, *J* = 9.1, 5.8 Hz, 1H), 4.43 (dd, *J* = 9.5, 4.3 Hz, 1H), 4.40 – 4.37 (m, 1H), 4.36 – 4.34 (m, 3H), 4.31 – 4.27 (m, 1H), 4.17 (t, *J* = 6.2 Hz, 2H), 4.15 – 4.11 (m, 1H), 3.85 (d, *J* = 3.4 Hz, 2H), 3.38 (t, *J* = 6.7 Hz, 2H), 3.20 – 3.11 (m, 4H), 3.09 – 3.04 (m, 1H), 3.00 (dd, *J* = 14.1, 9.1 Hz, 1H), 2.76 – 2.74 (m, 2H), 2.00 – 1.96 (m, 1H), 1.95 (s, 3H), 1.93 – 1.87 (m, 4H), 1.82 – 1.76 (m, 7H), 1.73 – 1.68 (m, 2H), 1.66 – 1.62 (m, 1H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H). **HRMS (ESI-TOF):** calc'd $C_{44}H_{67}N_{14}O_9$ $[M+H]^+$ 935.5215; found 935.5221. **UPLC trace:**

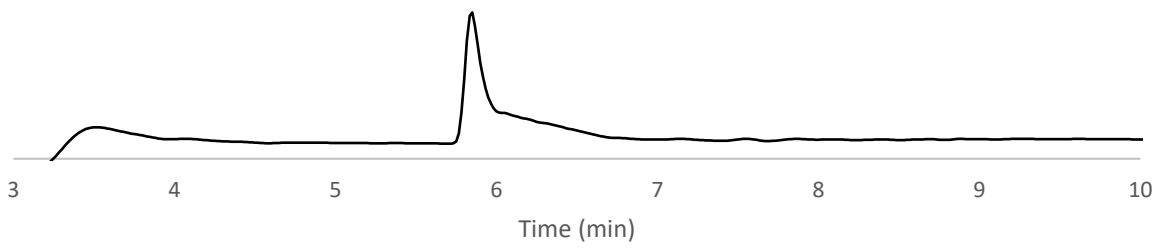


Purified peptide **18** (Rt = 6.8 min, 5% B for 1 min, then 5% to 60% B over 10 min, λ = 210 nm).

Peptide 19



Bis-macrocyclic triazole peptide **19** was prepared *via* procedures adapted from Finn and co-workers.[4] Peptide **17** (2.0 mg, 1.8 μmol, 1.0 equiv.) and peptide **18** (3.0 mg, 2.6 μmol, 1.5 equiv.) were dissolved in phosphate buffer (100 mM, pH 7). To this was added sodium ascorbate (10 equiv., 100 mM in water), aminoguanidine (10 equiv., 100 mM in water), $CuSO_4$ (0.5 equiv., 20 mM in water) and THPTA (0.5 equiv., 50 mM in water) to afford a solution with a final concentration of 2.0 mM with respect to the alkynyl peptide. The solution was capped and magnetically stirred for 24 h and subsequently purified *via* reverse phase preparative HPLC (5% B for 3 minutes, then 5% – 60% B over 40 minutes) to afford the bis-macrocyclic triazole peptide **19** (3.04 mg, 74%) as a fluffy light yellow solid. Cyclic azido peptide starting material **18** was recovered (0.56 mg, 18%). **^{1}H NMR** (700 MHz, MeOD) δ 8.18 (s, 1H), 7.32 – 7.26 (m, 4H), 7.26 – 7.21 (m, 6H), 7.20 – 7.16 (m, 2H), 7.07 – 7.03 (m, 2H), 5.33 (s, 2H), 4.64 – 4.59 (m, 2H), 4.52 (t, *J* = 7.0 Hz, 2H), 4.50 – 4.47 (m, 2H), 4.44 – 4.41 (m, 2H), 4.39 – 4.34 (m, 8H), 4.30 – 4.27 (m, 2H), 4.18 (t, *J* = 6.3 Hz, 2H), 4.16 – 4.13 (m, 2H), 3.85 (d, *J* = 2.0 Hz, 4H), 3.21 – 3.18 (m, 2H), 3.16 – 3.12 (m, 6H), 3.08 – 3.04 (m, 2H), 3.01 – 2.97 (m, 2H), 2.78 – 2.72 (m, 4H), 2.12 – 2.09 (m, 2H), 1.99 – 1.97 (m, 2H), 1.95 (s, 6H), 1.91 – 1.87 (m, 2H), 1.85 – 1.82 (m, 4H), 1.80 – 1.77 (m, 6H), 1.76 – 1.74 (m, 2H), 1.70 – 1.68 (m, 3H), 1.67 – 1.63 (m, 3H), 1.30 – 1.28 (m, 2H), 0.95 (dd, *J* = 6.5, 1.5 Hz, 6H), 0.92 (dd, *J* = 6.4, 1.5 Hz, 6H). **HRMS (ESI-TOF):** calc'd $C_{87}H_{129}N_{25}O_{18}$ $[M+2H]^{2+}$ 905.9974; found 905.9964 **UPLC trace:**

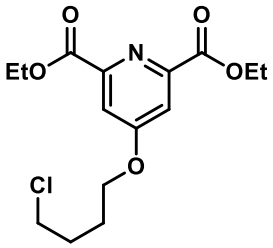


Purified peptide **19** (Rt = 5.8 min, 5% B for 1 min, then 5% to 60% B over 10 min, λ = 210 nm).

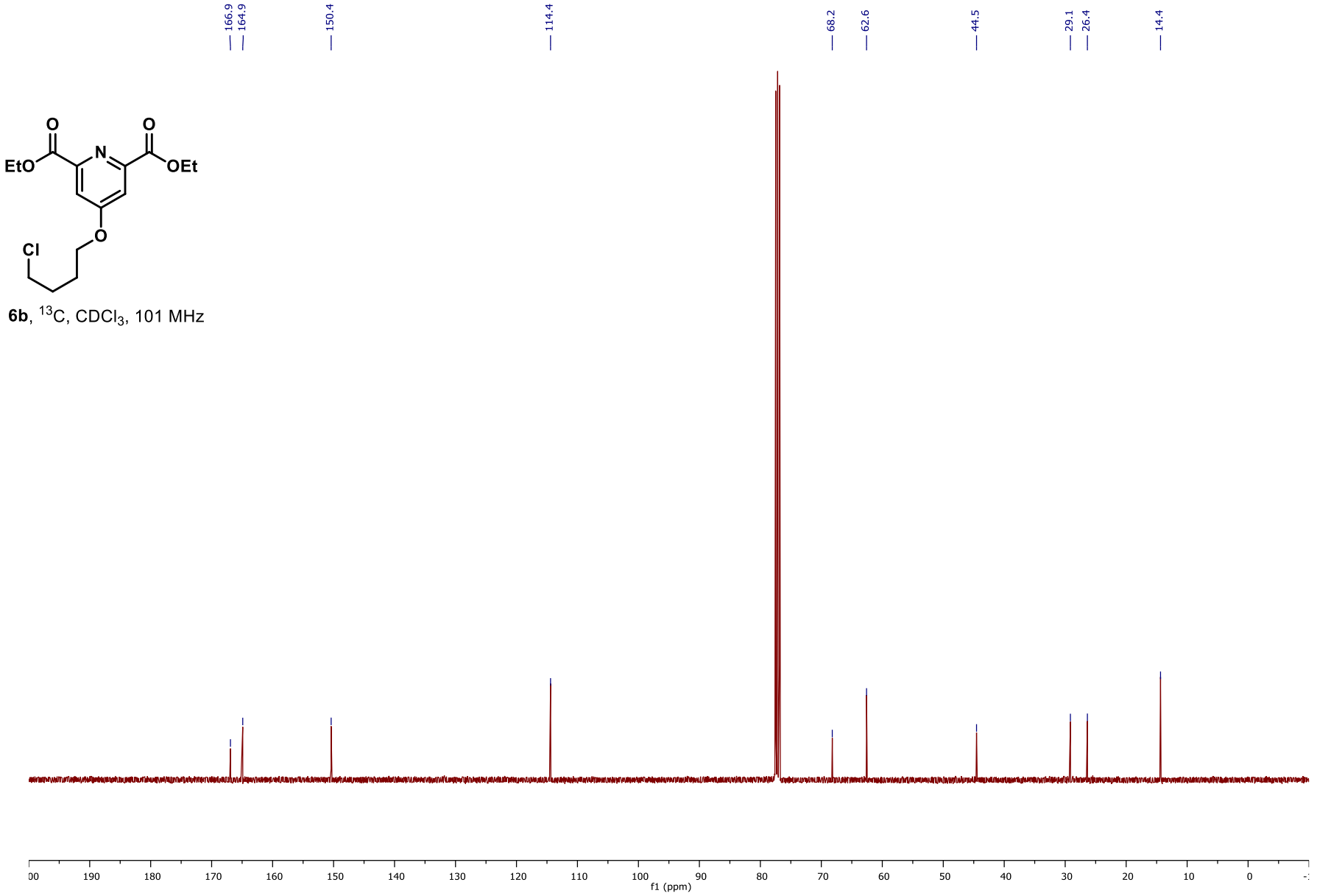
References

1. B. J. Timmer, M. A. Flos, L. M. Jorgensen, D. Proverbio, S. Altun, O. Ramstrom, T. Aastrup and S. P. Vincent, *Chem. Commun.*, 2016, **52**, 12326-12329.
2. L. R. Malins, J. N. deGruyter, K. J. Robbins, P. M. Scola, M. D. Eastgate, M. R. Ghadiri and P. S. Baran, *J. Am. Chem. Soc.*, 2017, **139**, 5233-5241.
3. P. Botti, T. D. Pallin and J. P. Tam, *J. Am. Chem. Soc.*, 1996, **118**, 10018-10024.
4. V. Hong, S. I. Presolski, C. Ma and M. G. Finn, *Angew. Chem. Int. Ed.*, 2009, **48**, 9879-9883.

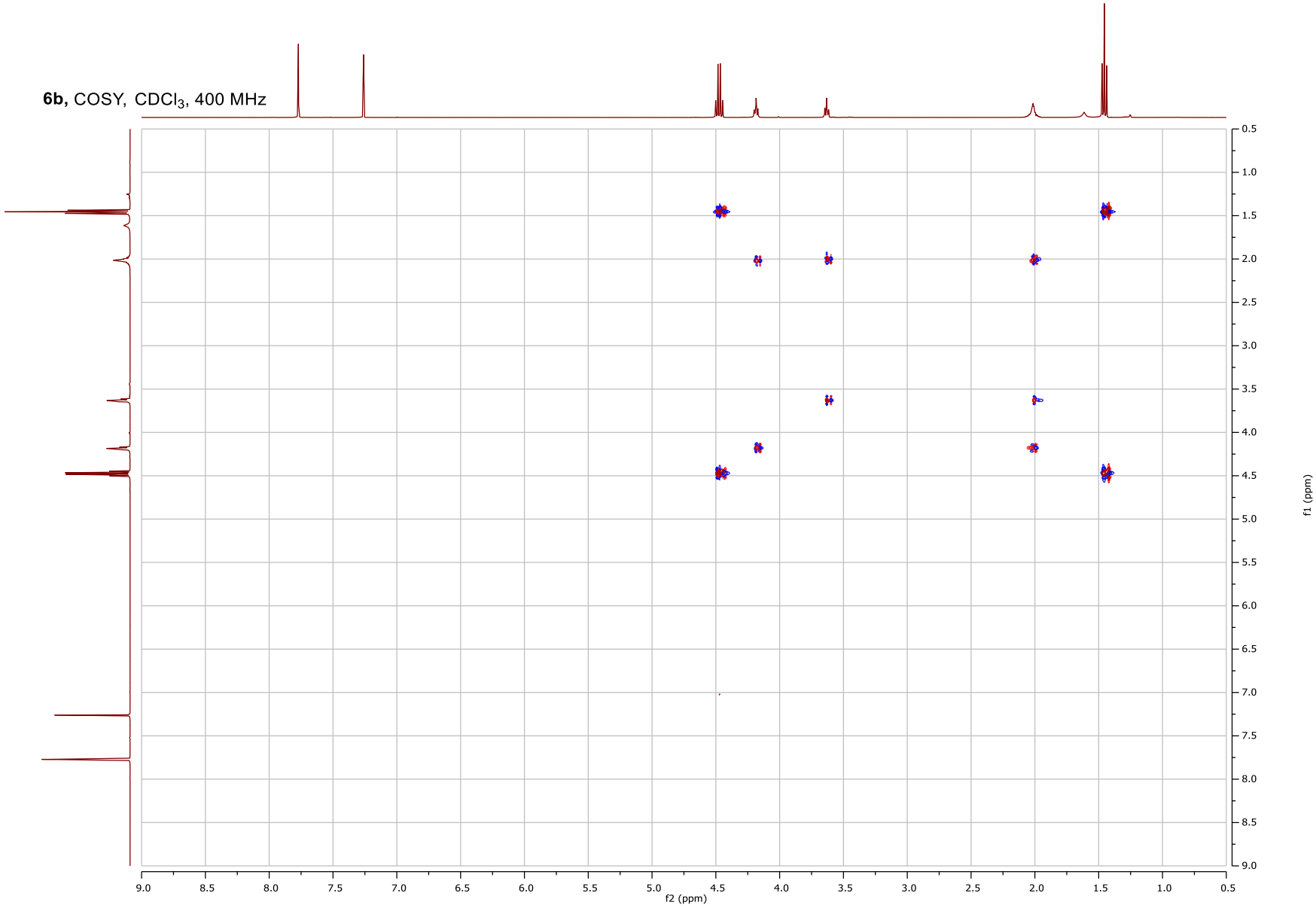
NMR Spectral Data



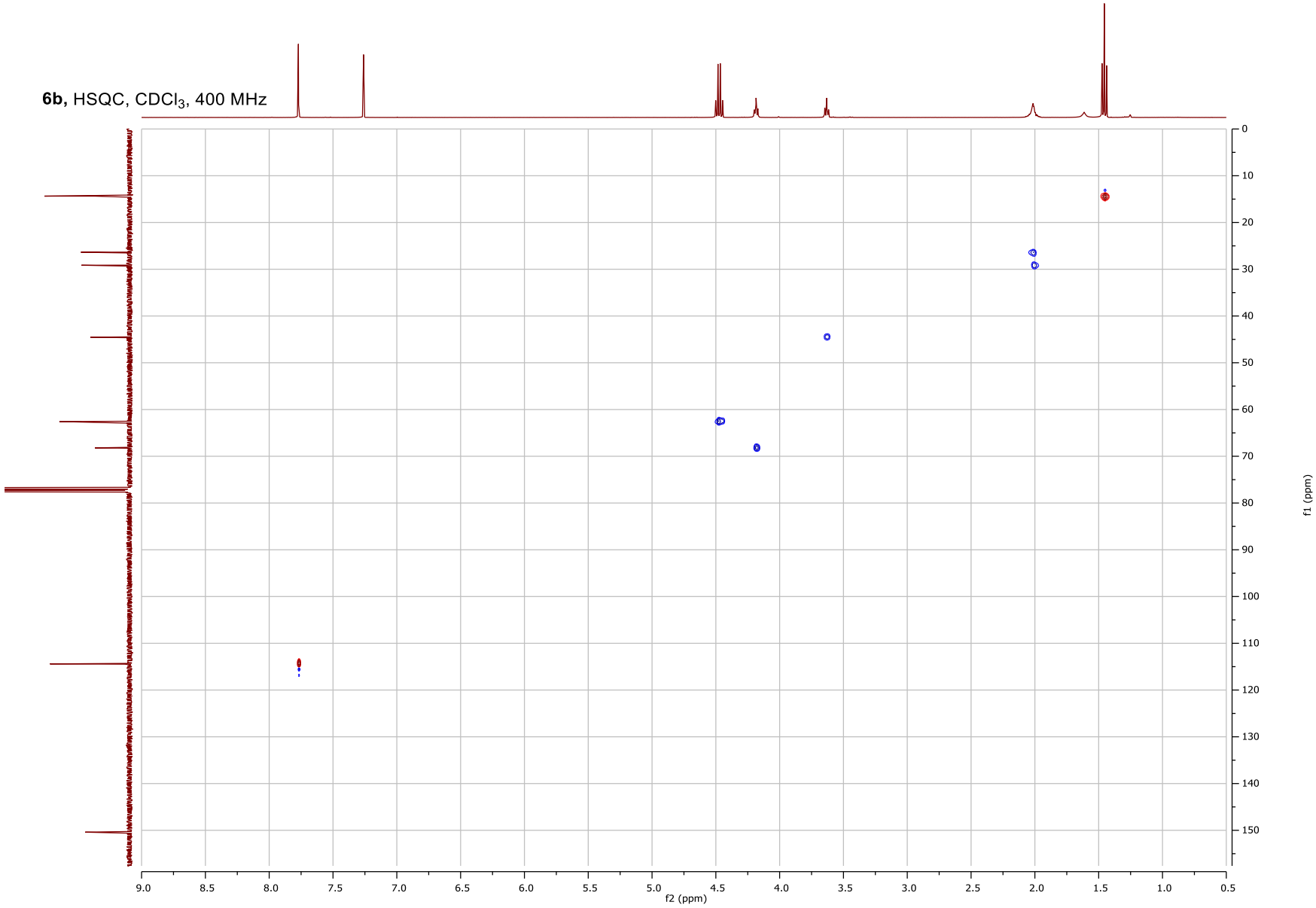
6b, ^{1}H, $CDCl_3$, 400 MHz



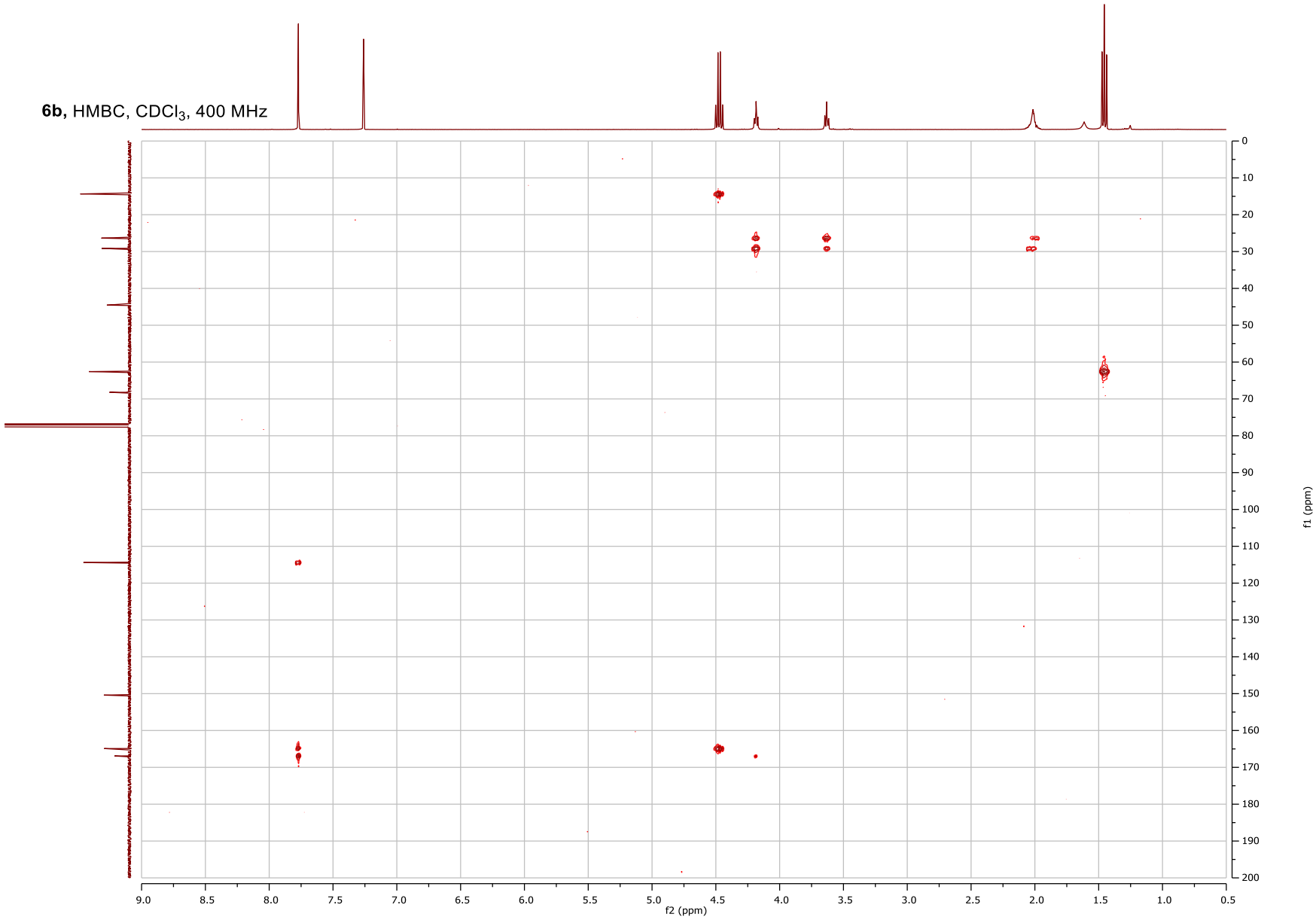
6b, ^{13}C, $CDCl_3$, 101 MHz

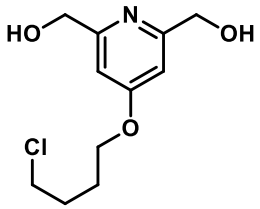

6b, COSY, CDCl3, 400 MHz
f2 (ppm)
f1 (ppm)

6b, HSQC, $CDCl_3$, 400 MHz

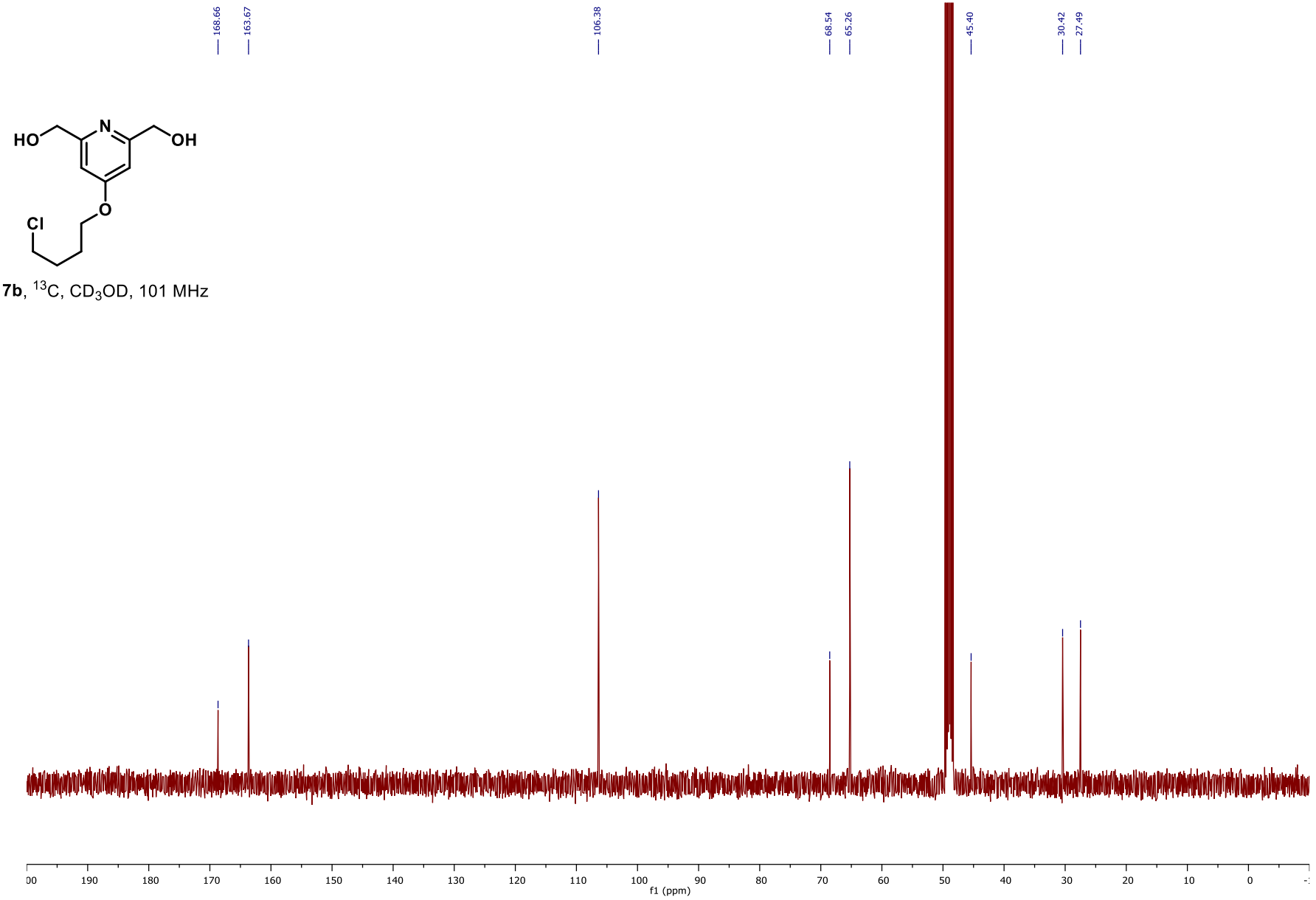


6b, HMBC, $CDCl_3$, 400 MHz



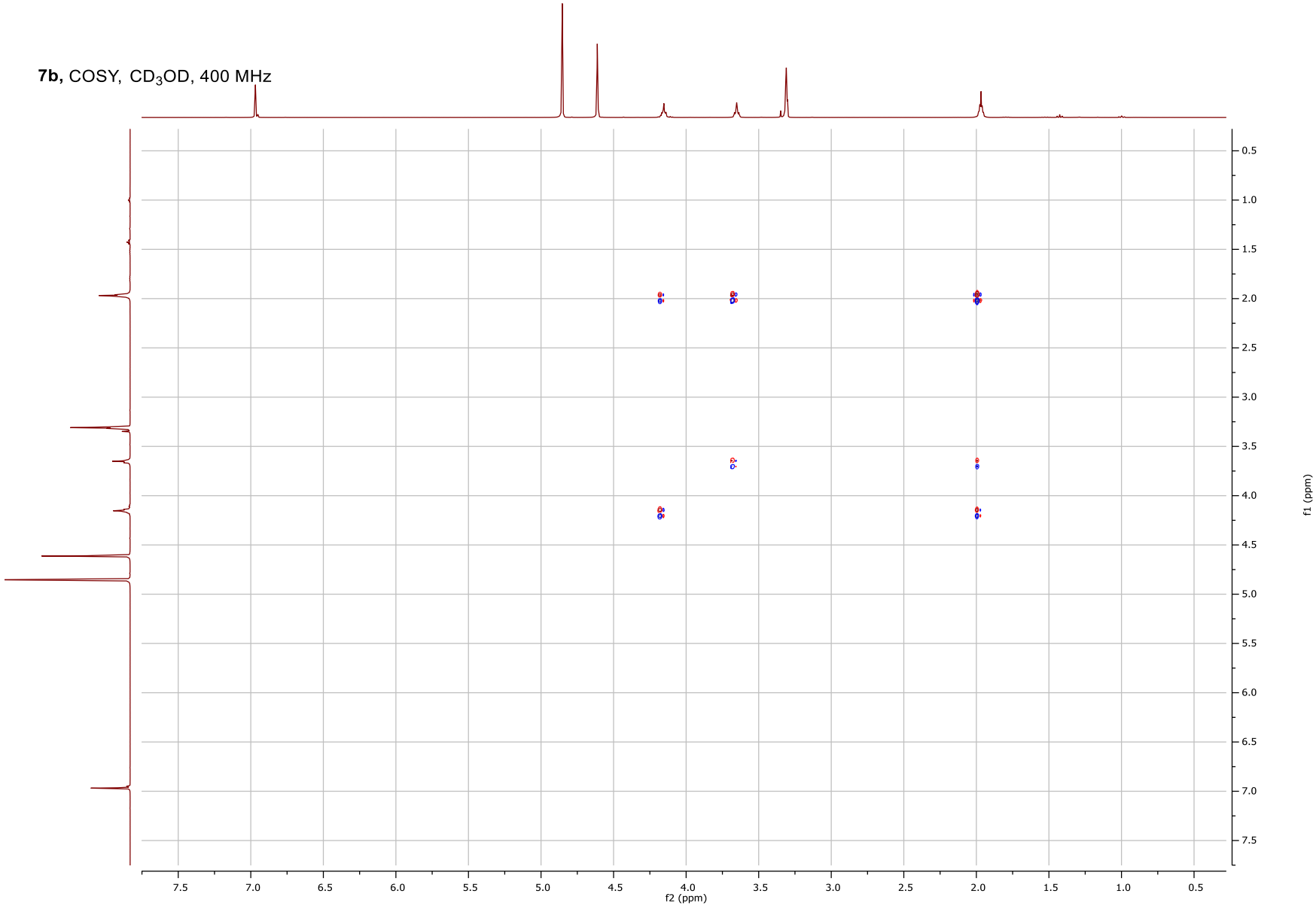


7b, ^{1}H, CD_3OD, 400 MHz

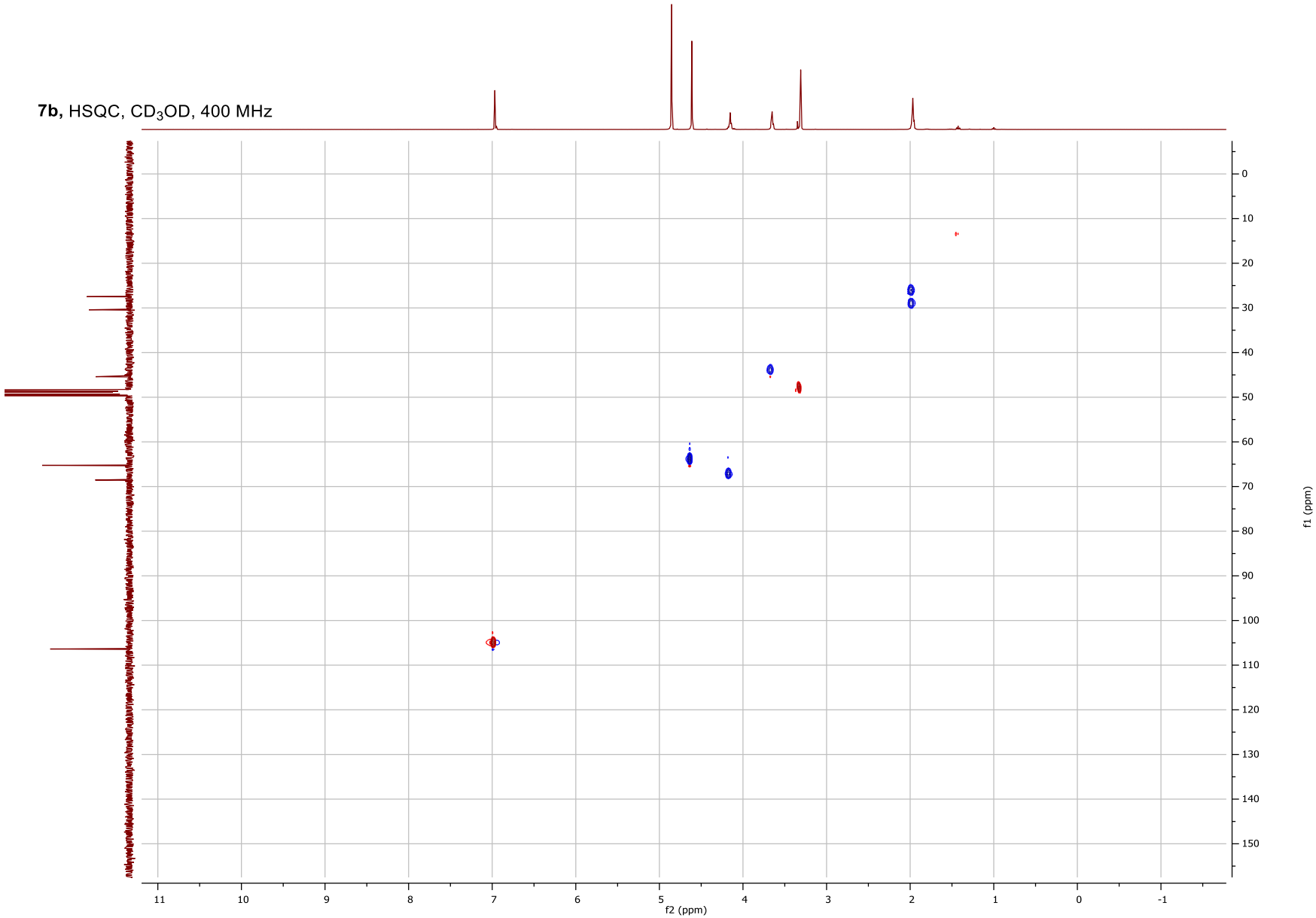


7b, ^{13}C, CD_3OD, 101 MHz

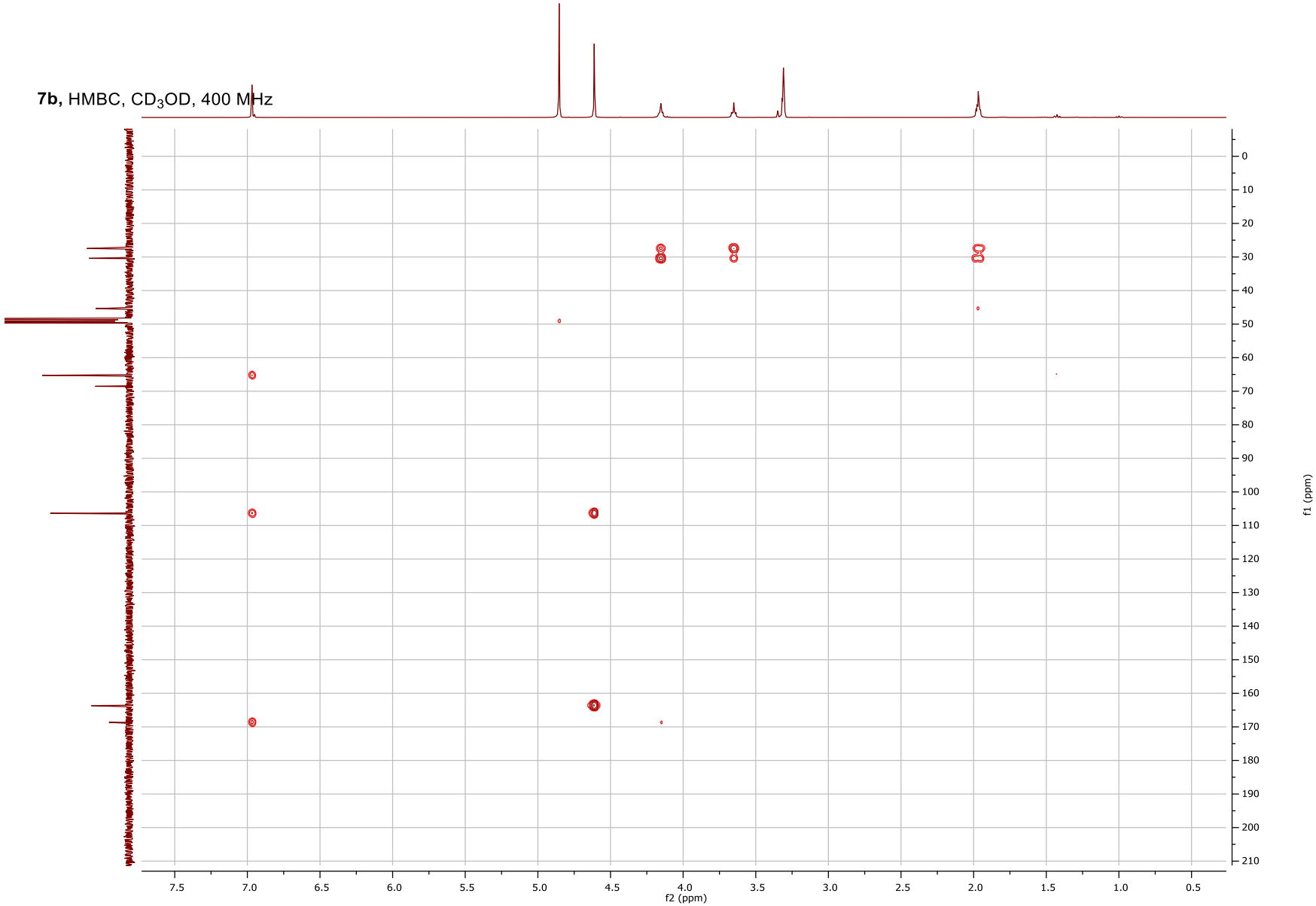
7b, COSY, CD_3OD, 400 MHz

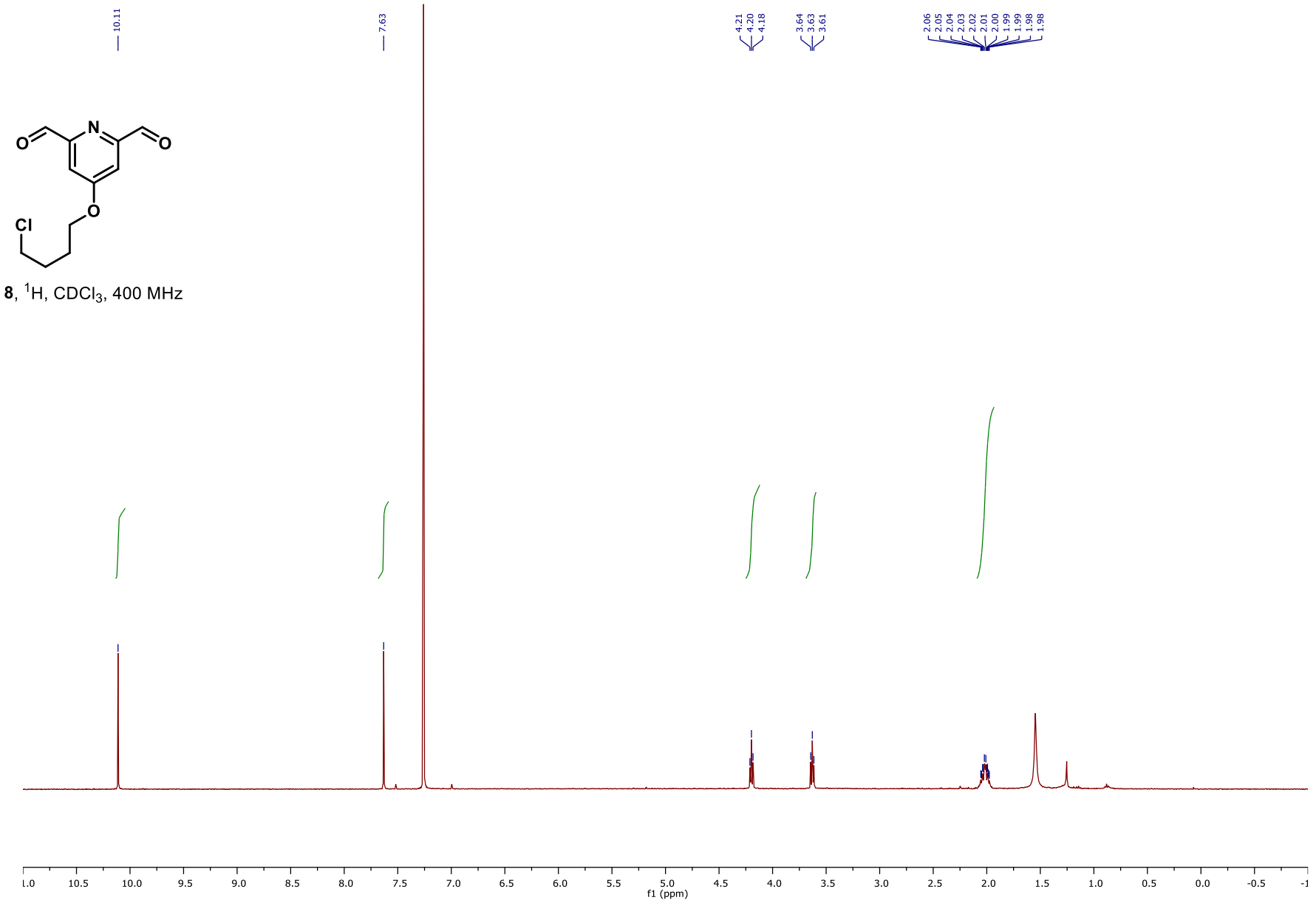


7b, HSQC, CD_3OD, 400 MHz

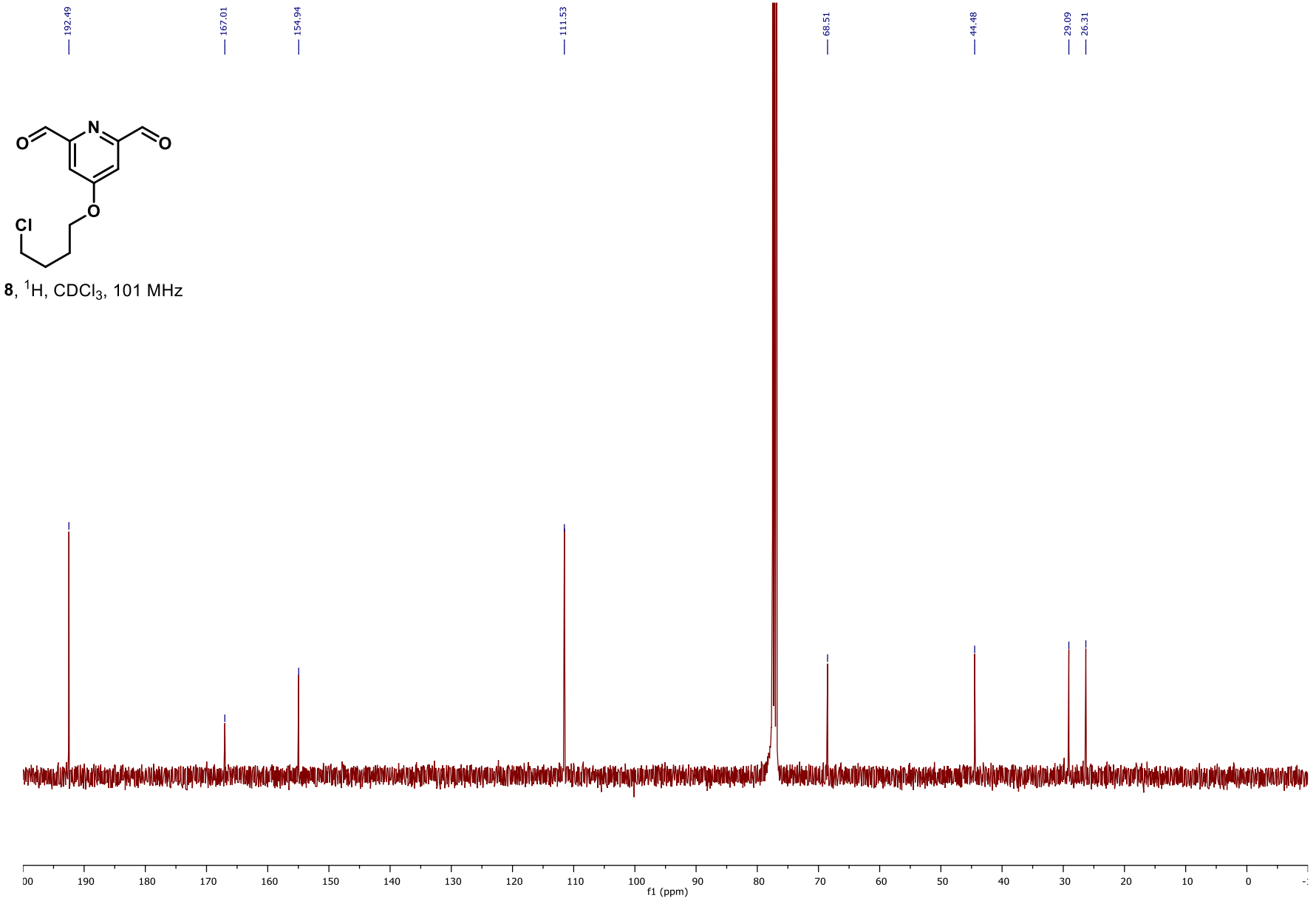


7b, HMBC, CD_3OD, 400 MHz



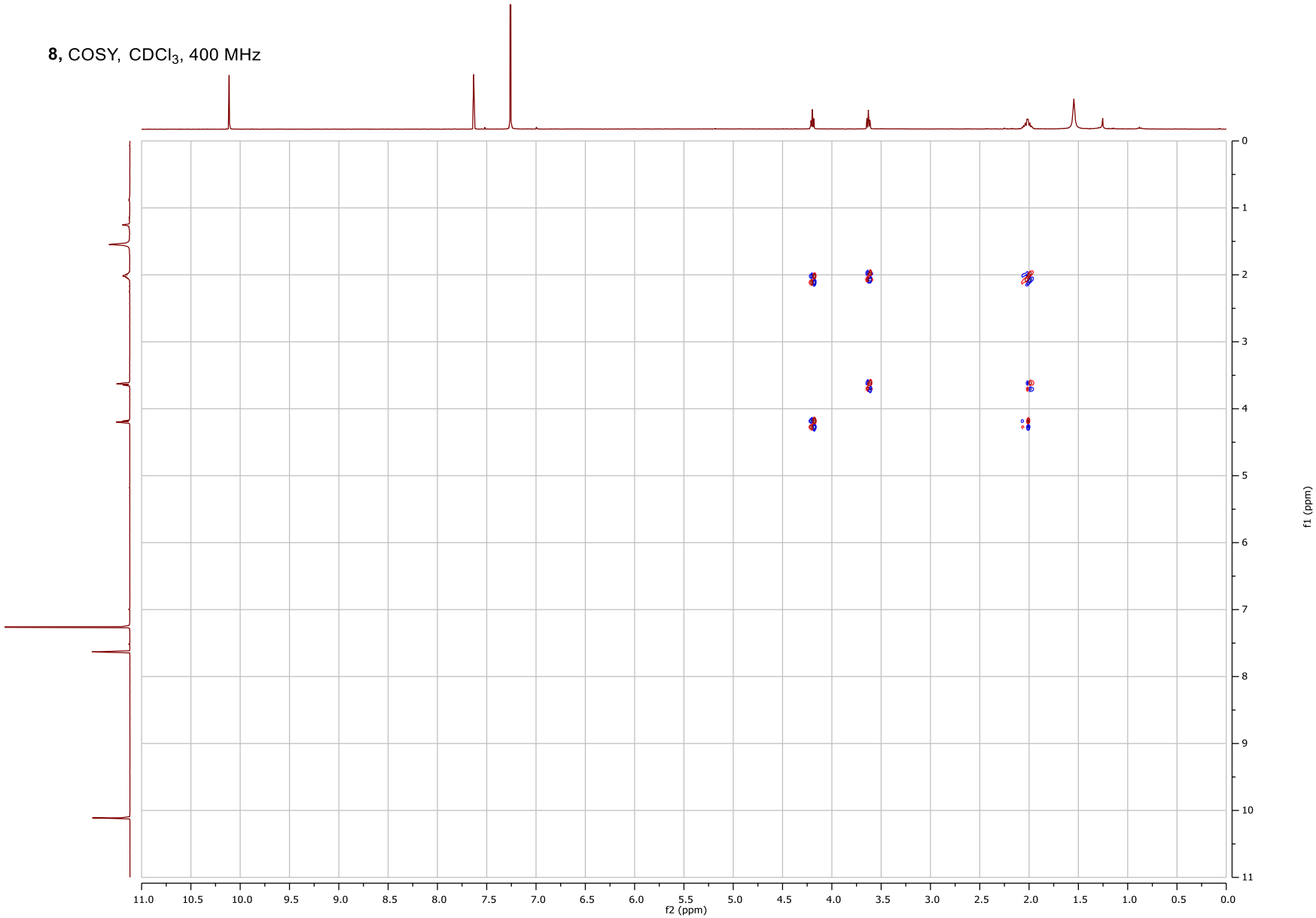


8, ^{1}H, $CDCl_3$, 400 MHz

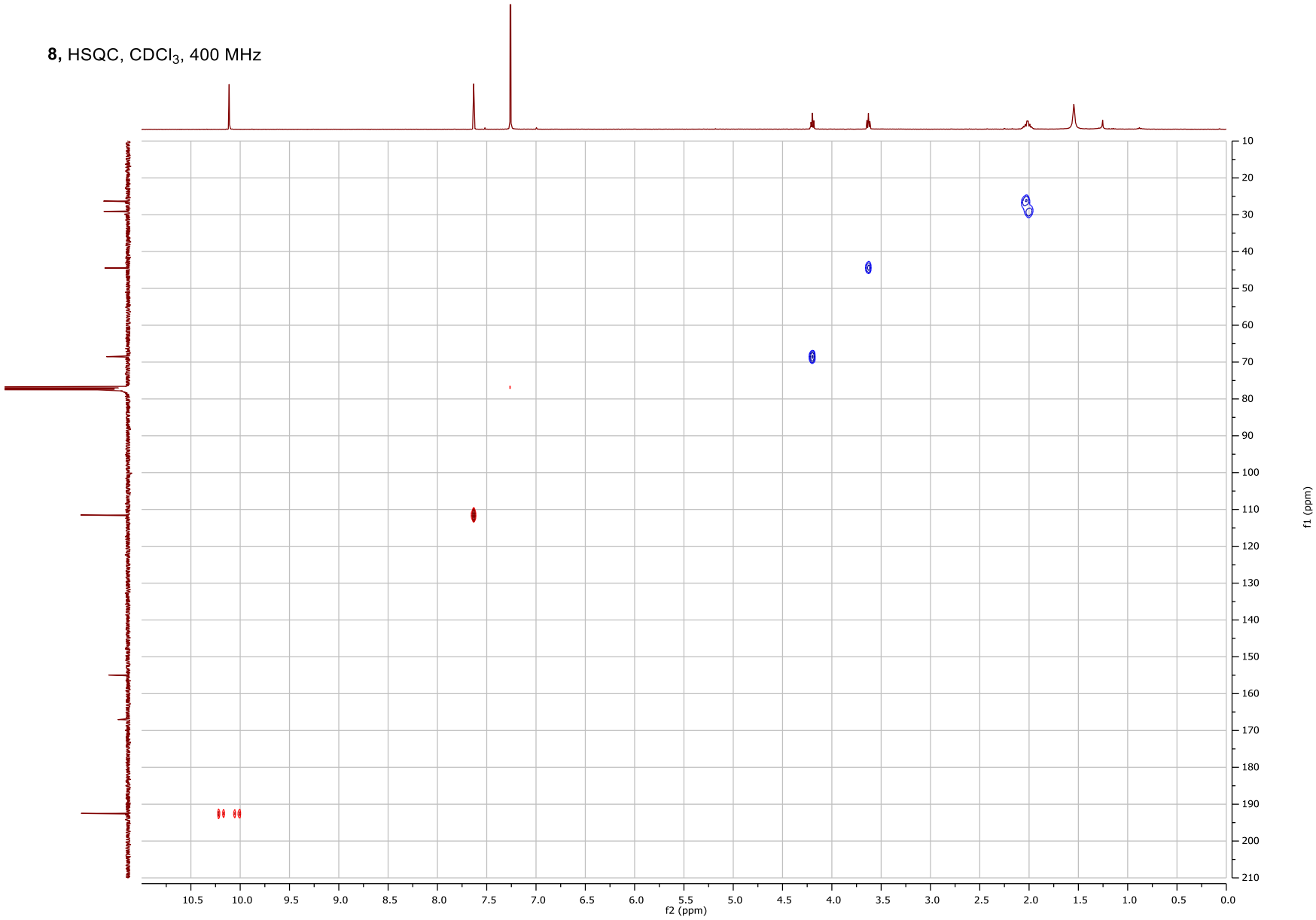


8, ^{1}H, $CDCl_3$, 101 MHz

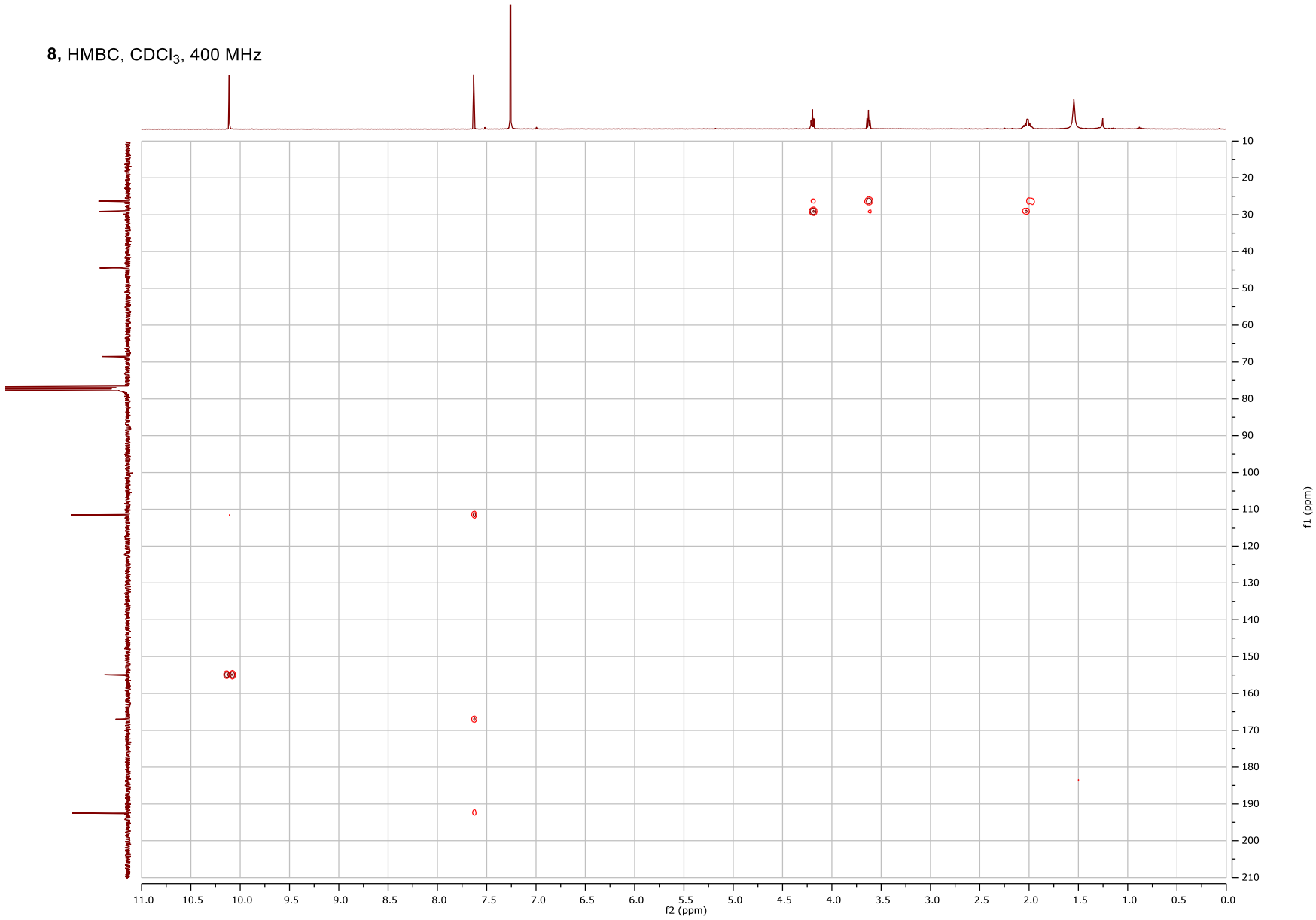
8, COSY, $CDCl_3$, 400 MHz

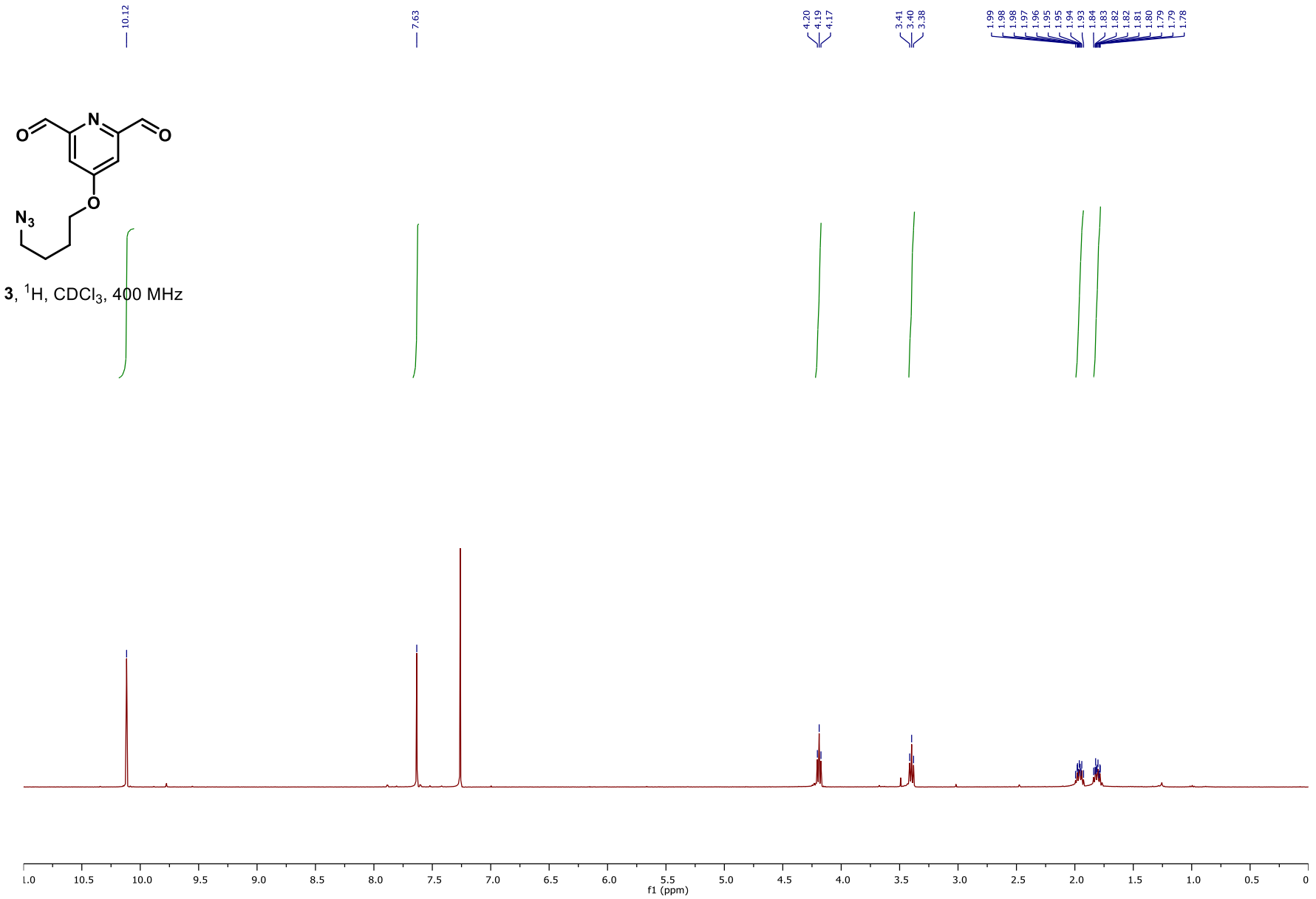


8, HSQC, $CDCl_3$, 400 MHz

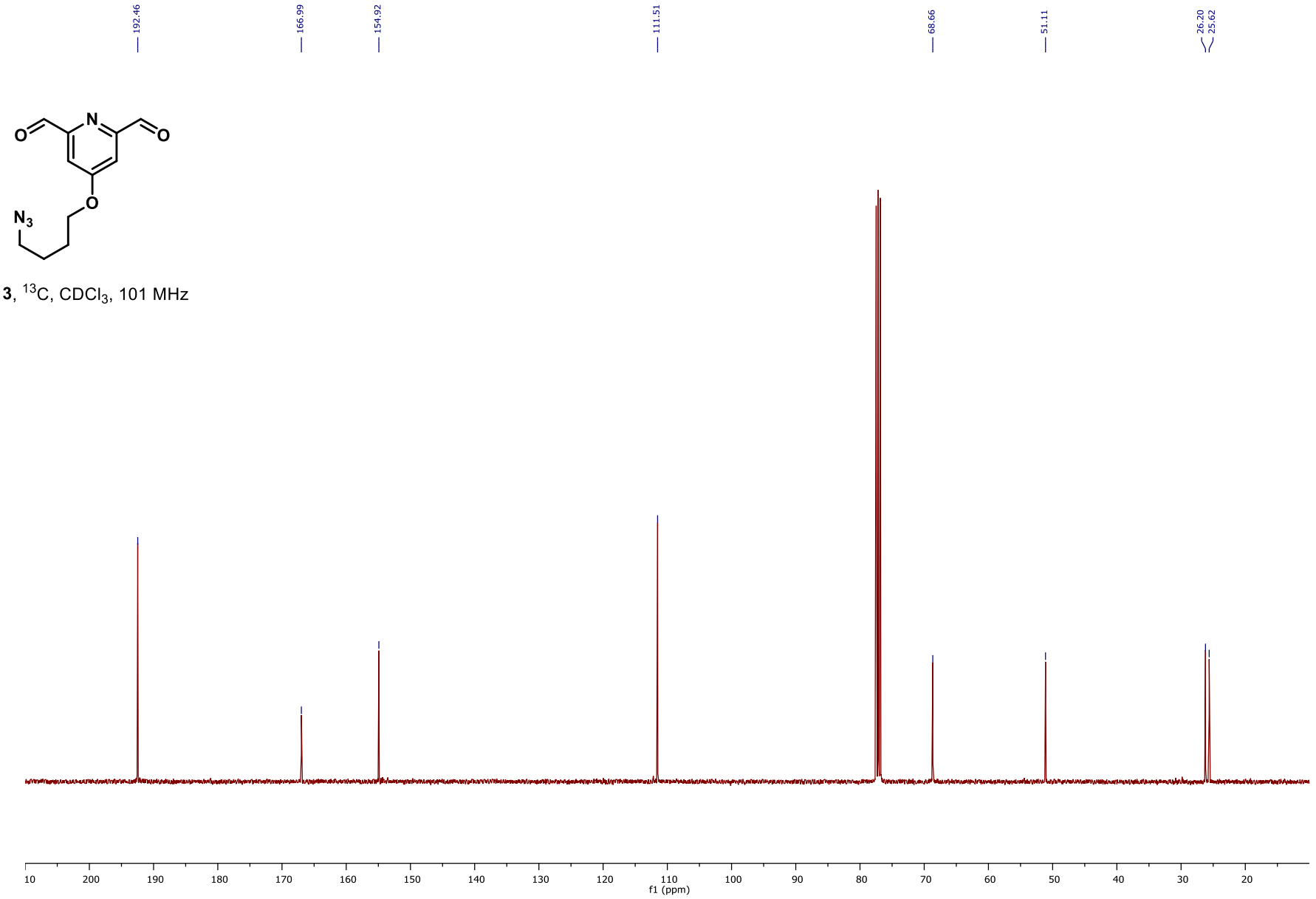


8, HMBC, $CDCl_3$, 400 MHz



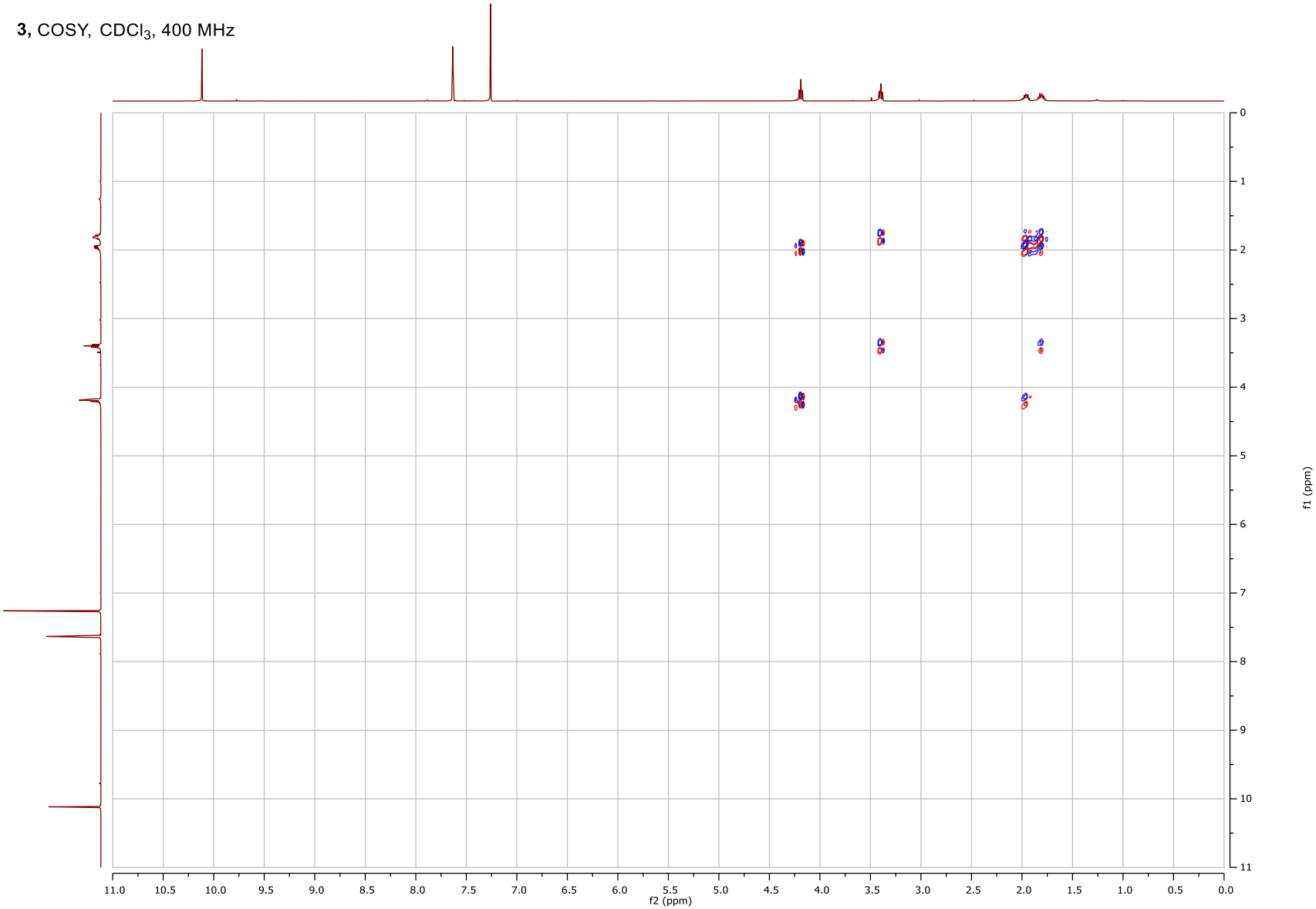


3, ^{1}H, $CDCl_3$, 400 MHz

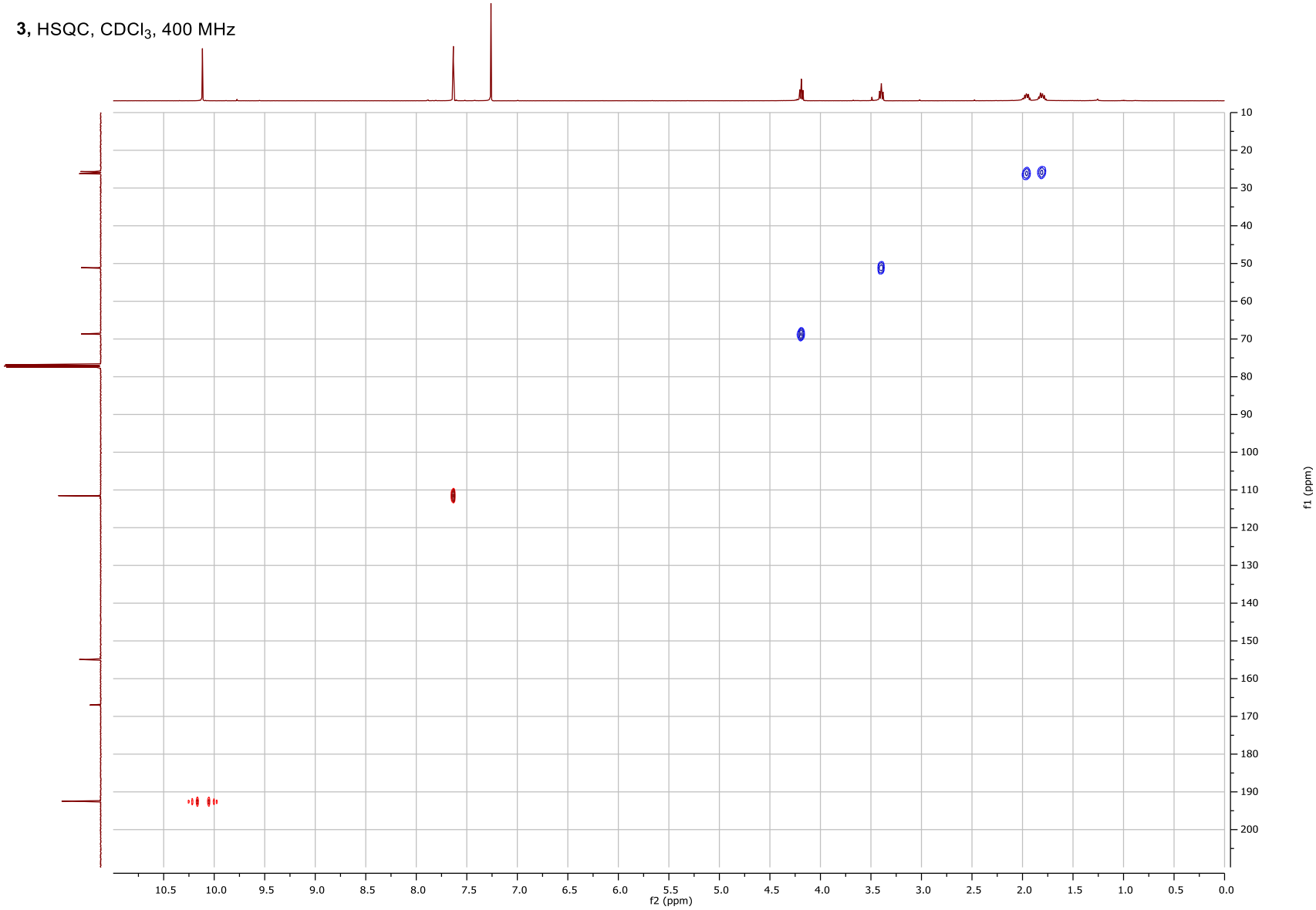


3, ^{13}C, $CDCl_3$, 101 MHz

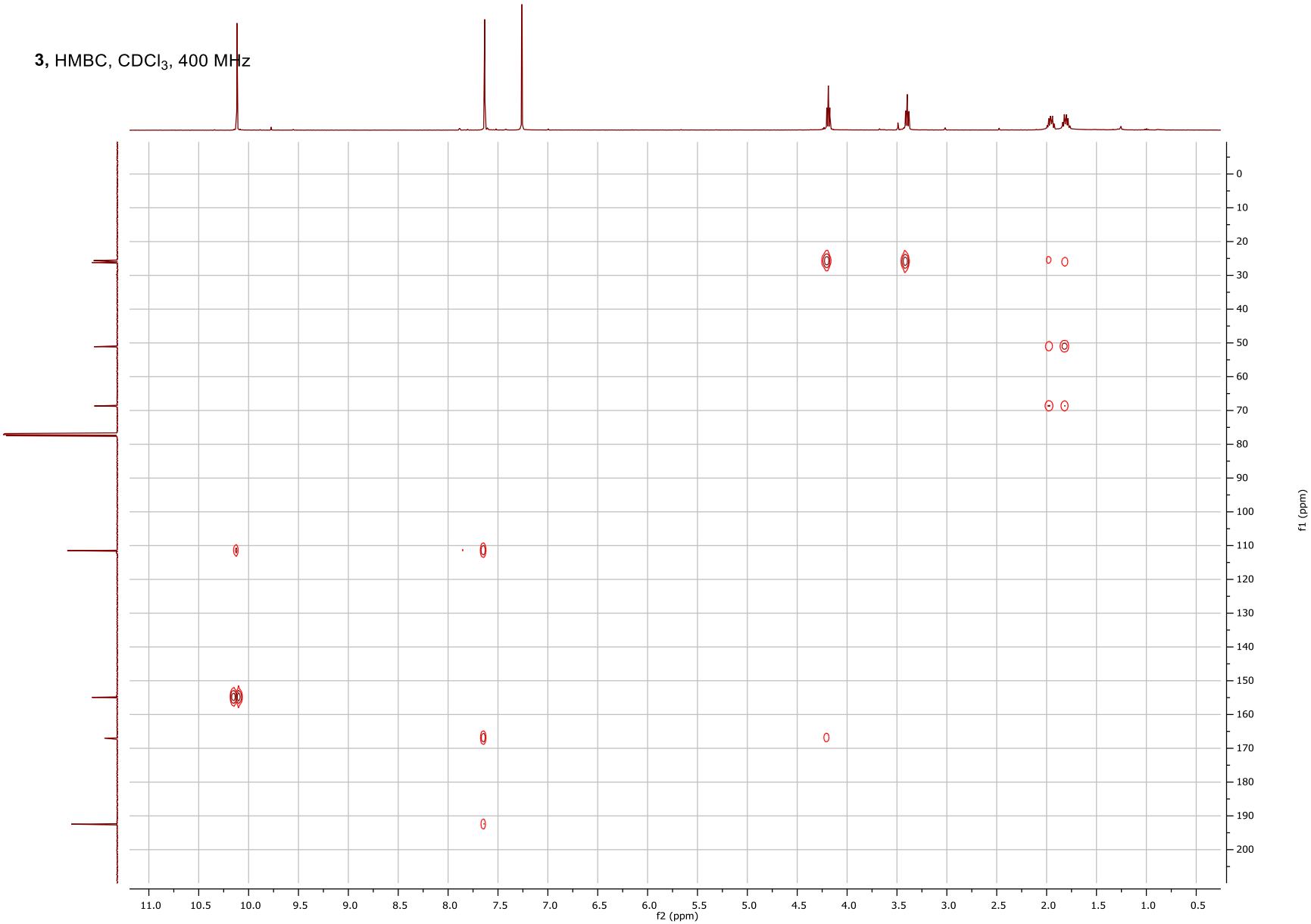
3, COSY, $CDCl_3$, 400 MHz

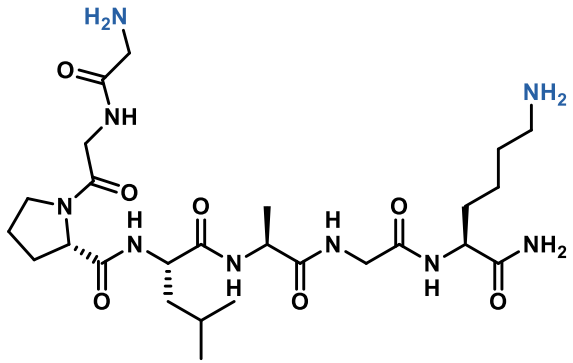


3, HSQC, $CDCl_3$, 400 MHz

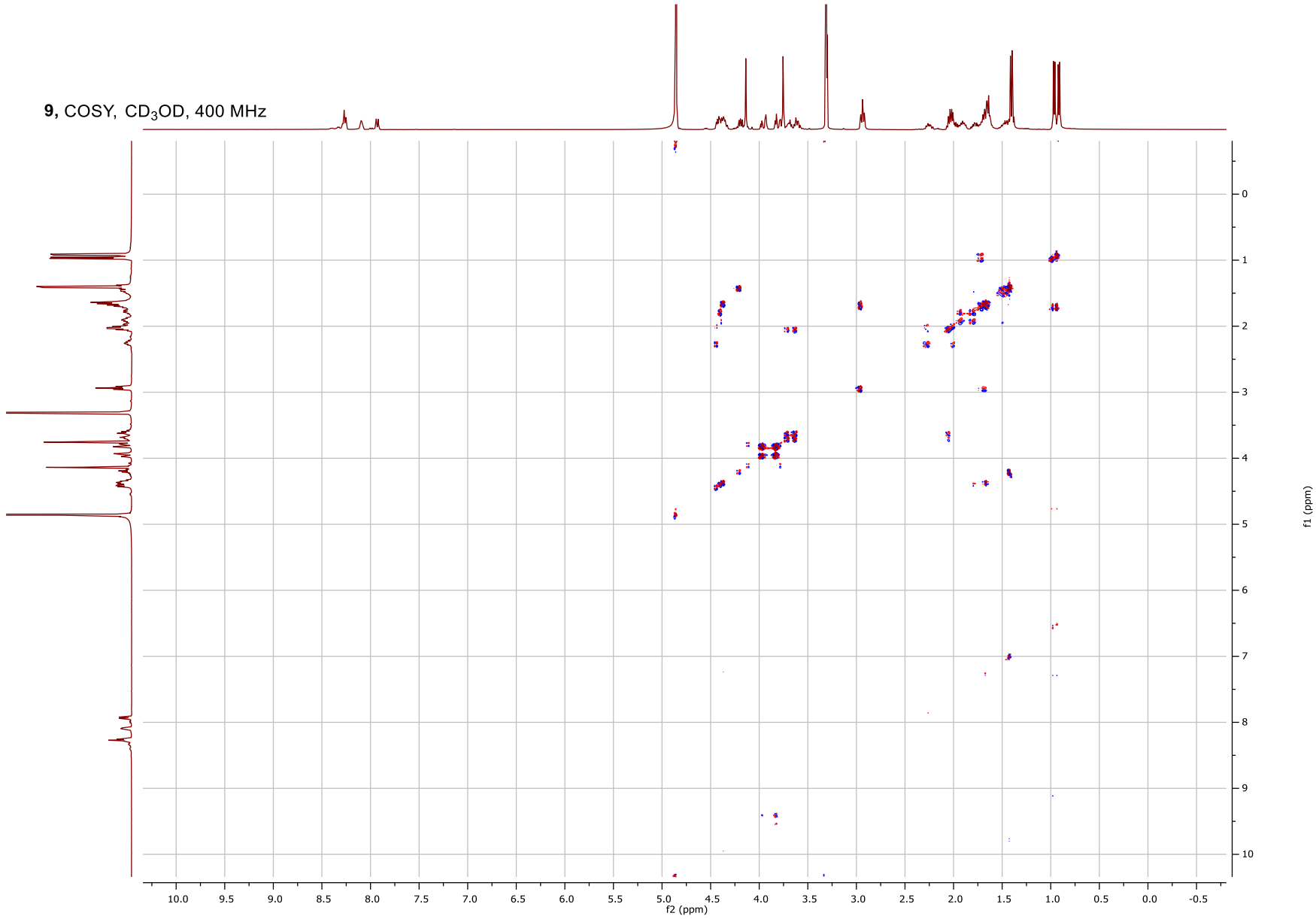


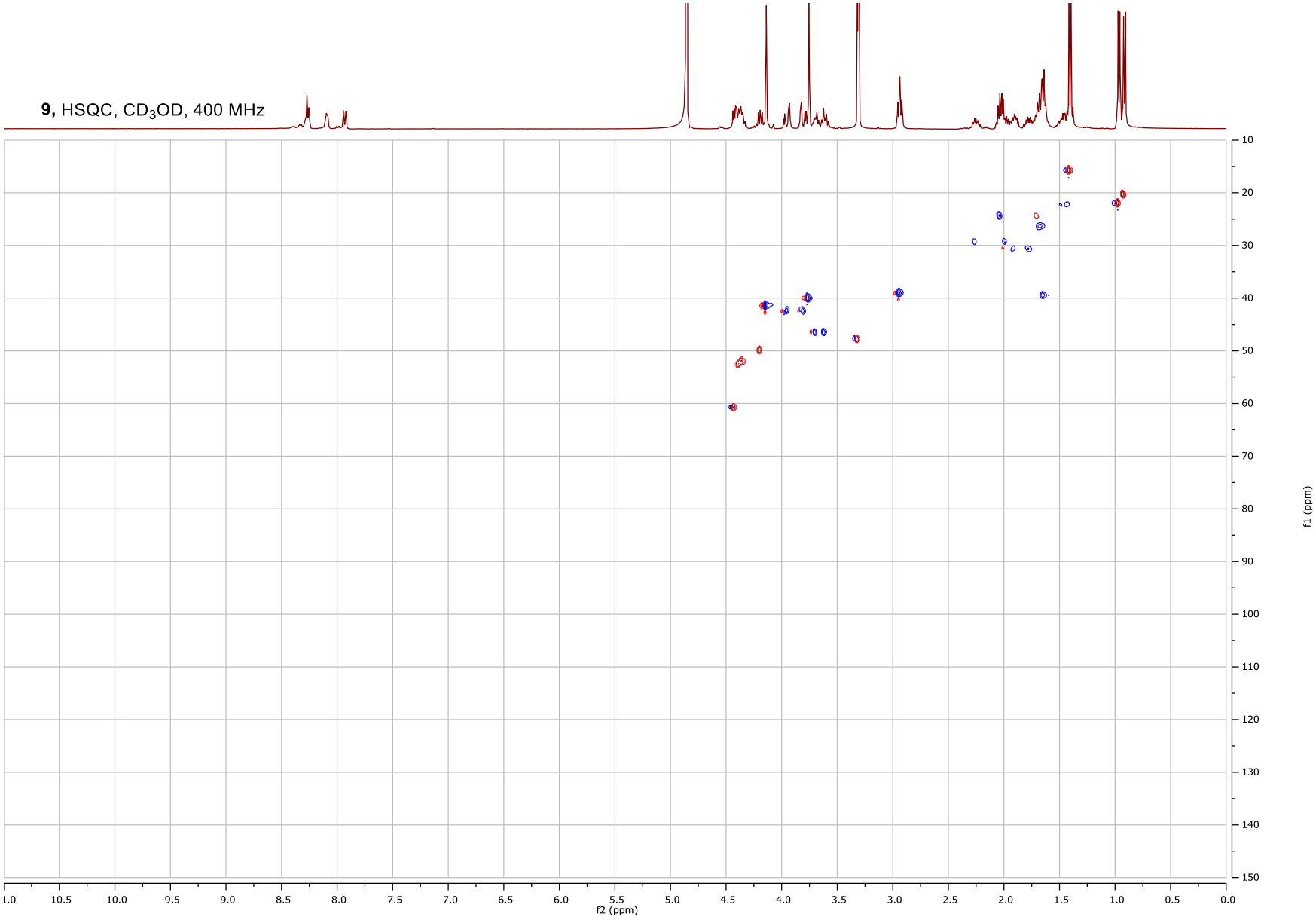
3, HMBC, $CDCl_3$, 400 MHz

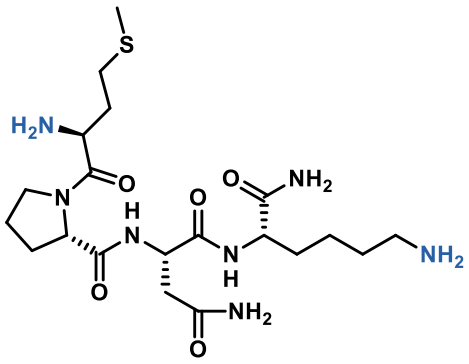




9, ^{1}H, CD_3OD, 400 MHz

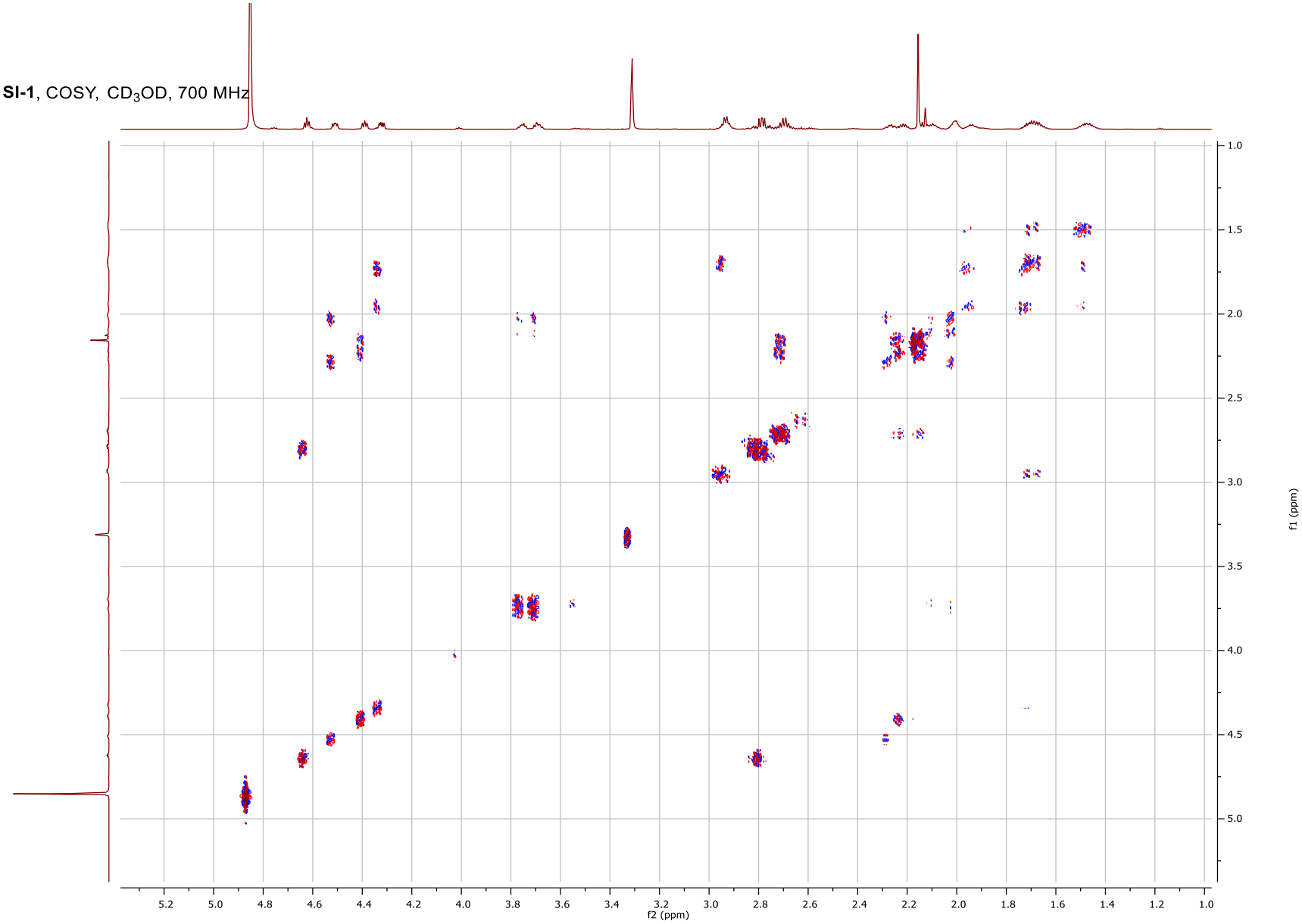

9, COSY, CD3OD, 400 MHz
f2 (ppm)
f1 (ppm)


9, HSQC, CD3OD, 400 MHz
f2 (ppm)
f1 (ppm)

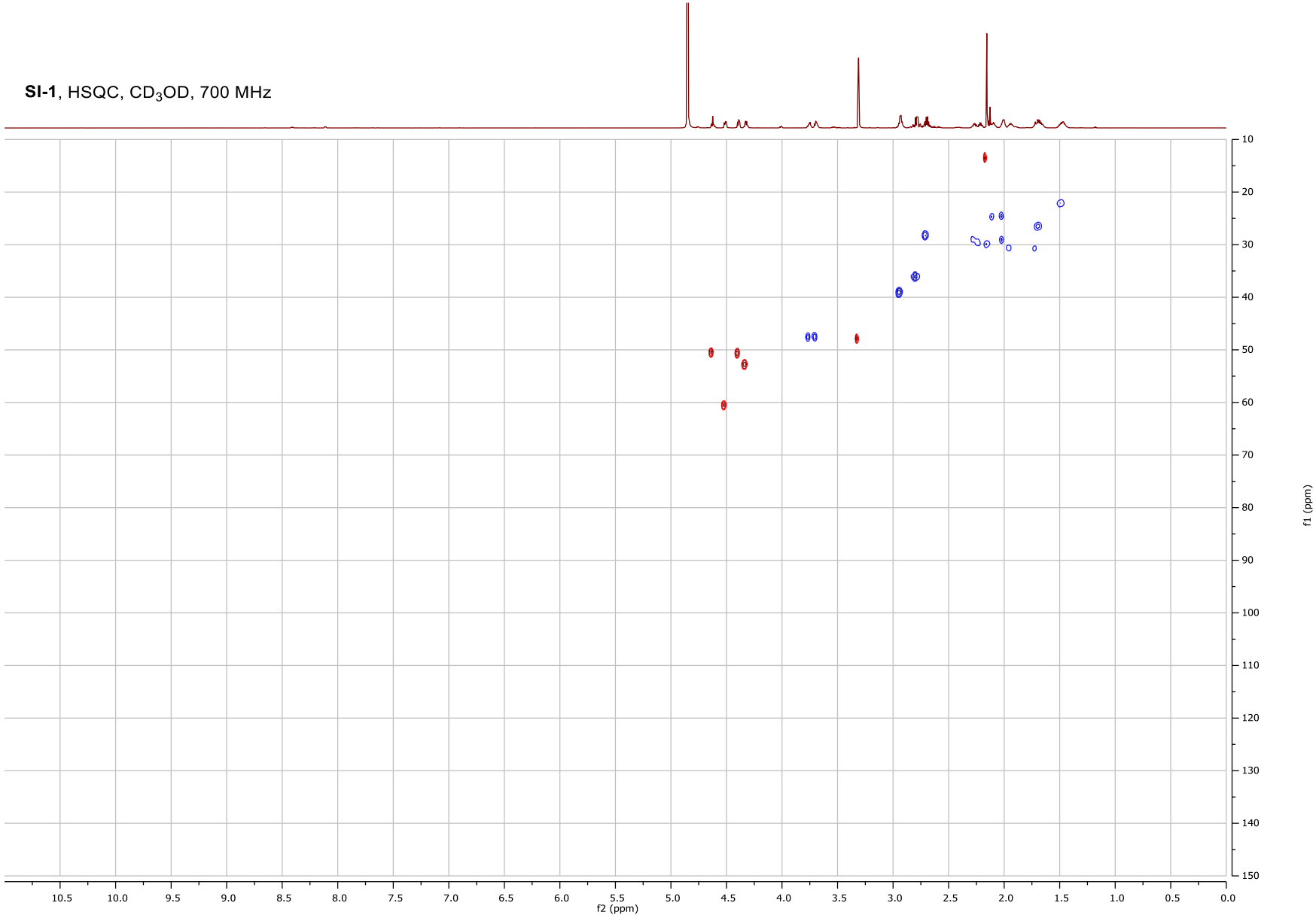


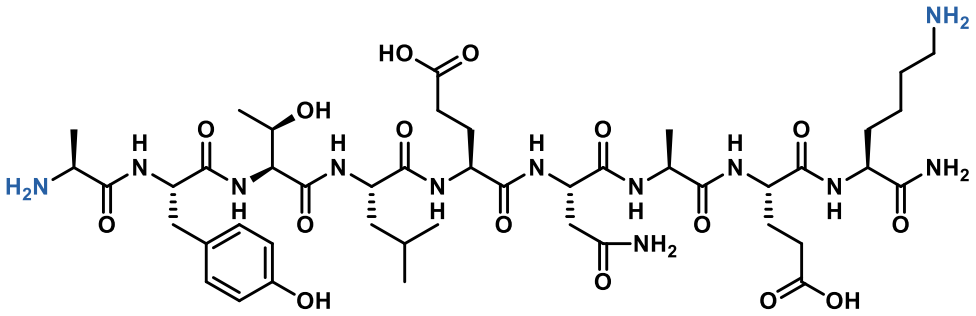
SI-1, ^{1}H, CD_3OD, 700 MHz

SI-1, COSY, CD_3OD, 700 MHz

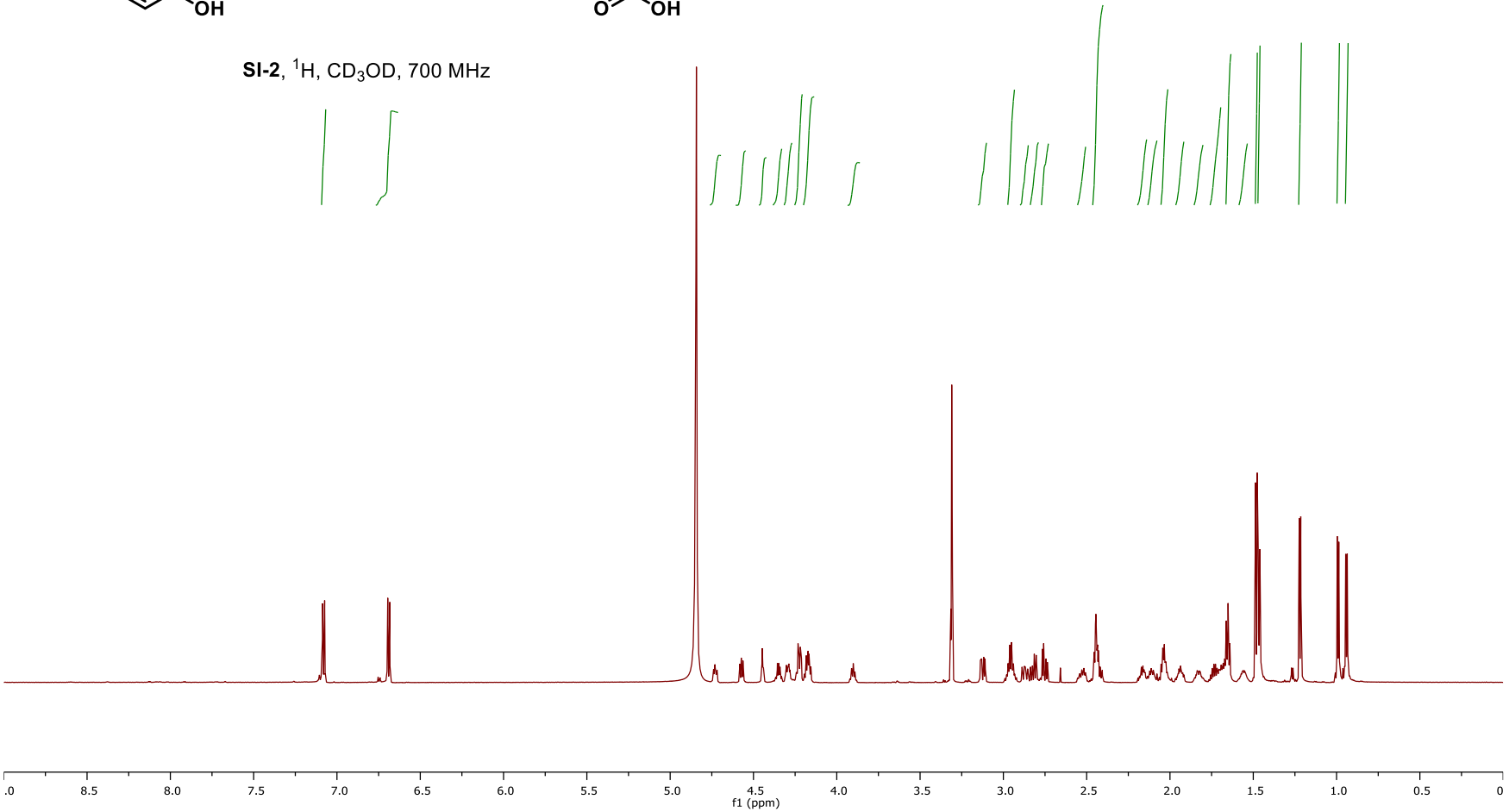


SI-1, HSQC, CD_3OD, 700 MHz

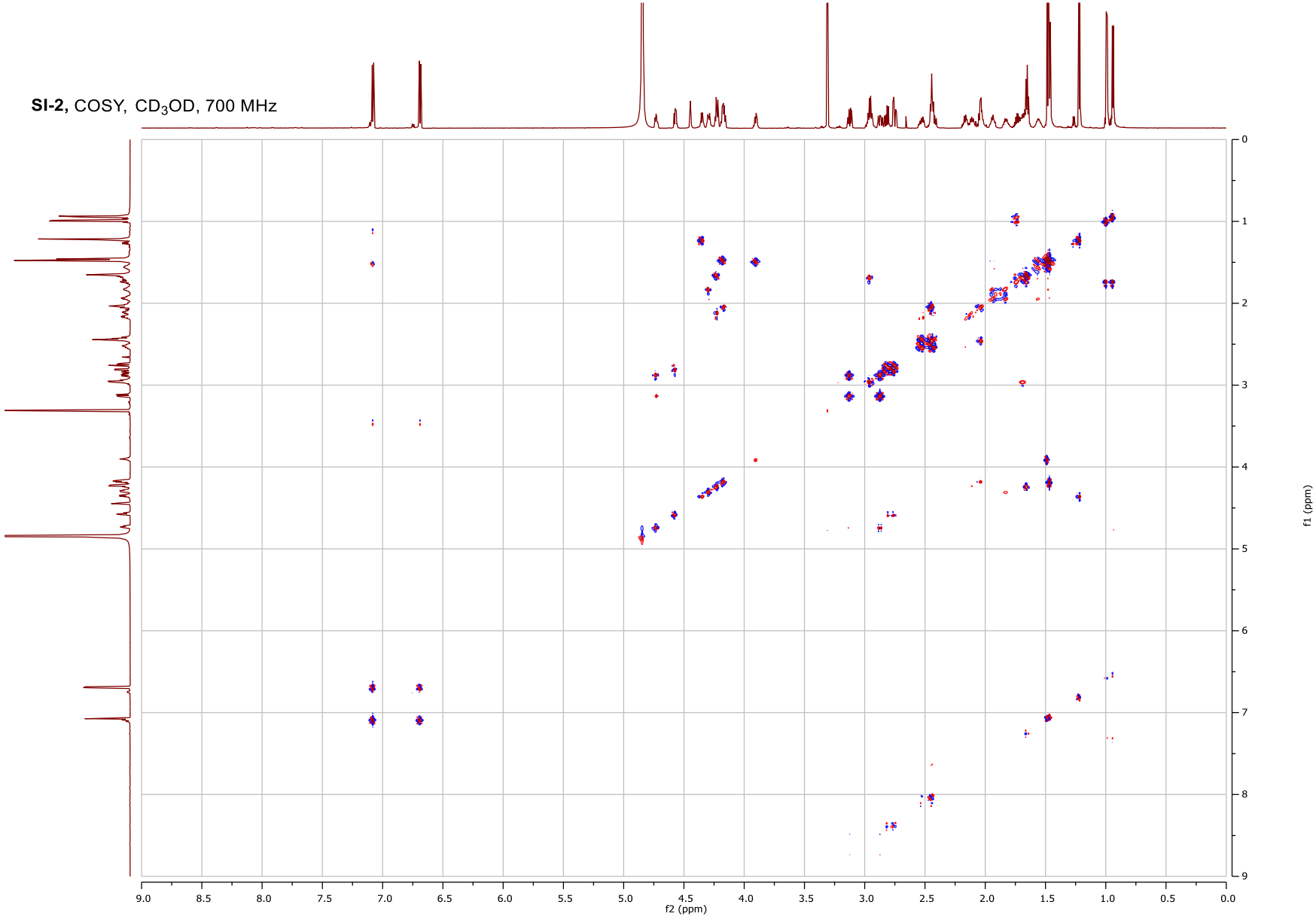




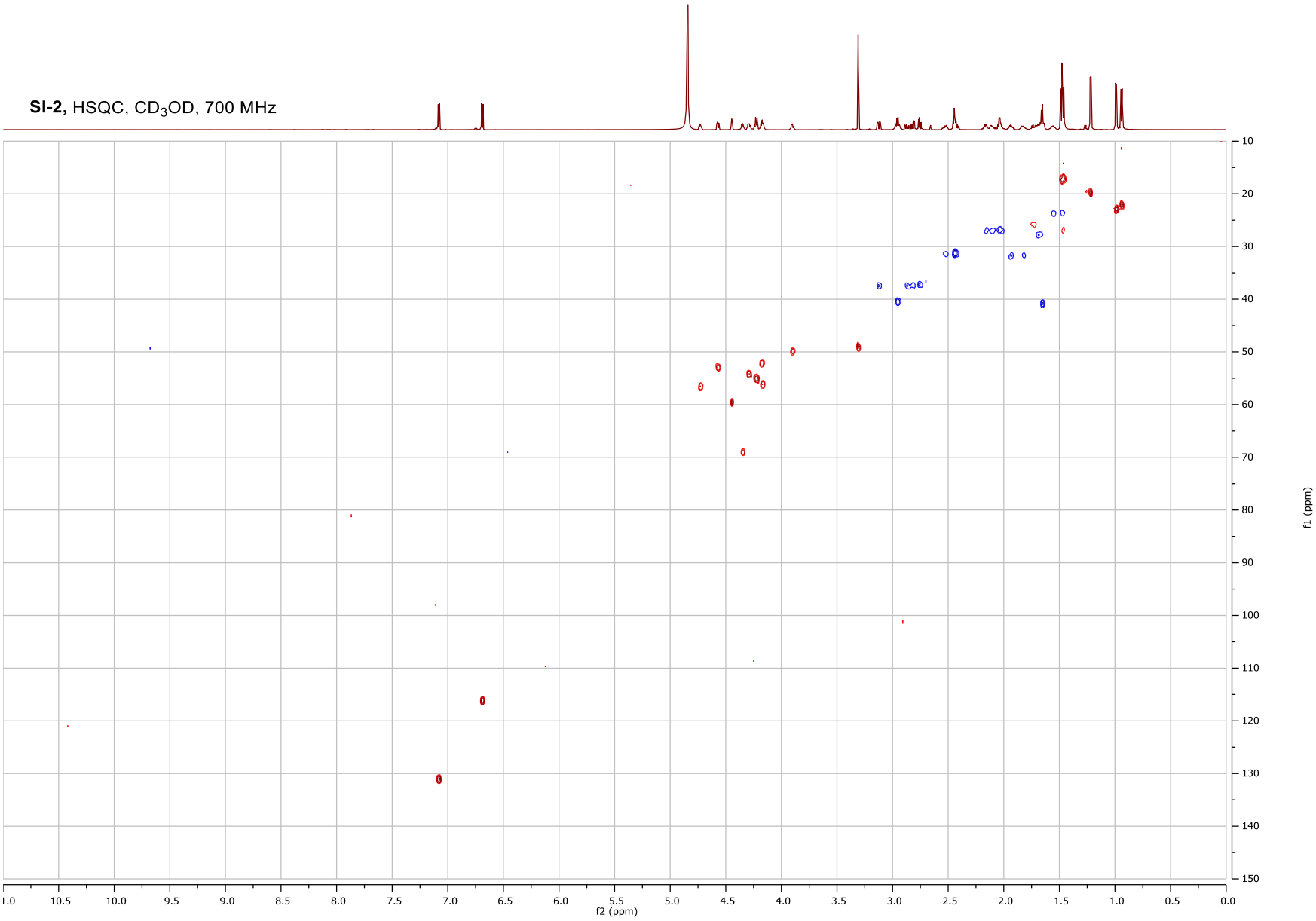
SI-2, ^{1}H, CD_3OD, 700 MHz

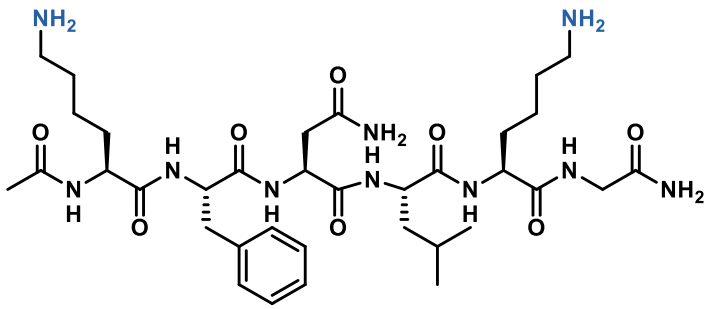


SI-2, COSY, CD_3OD, 700 MHz



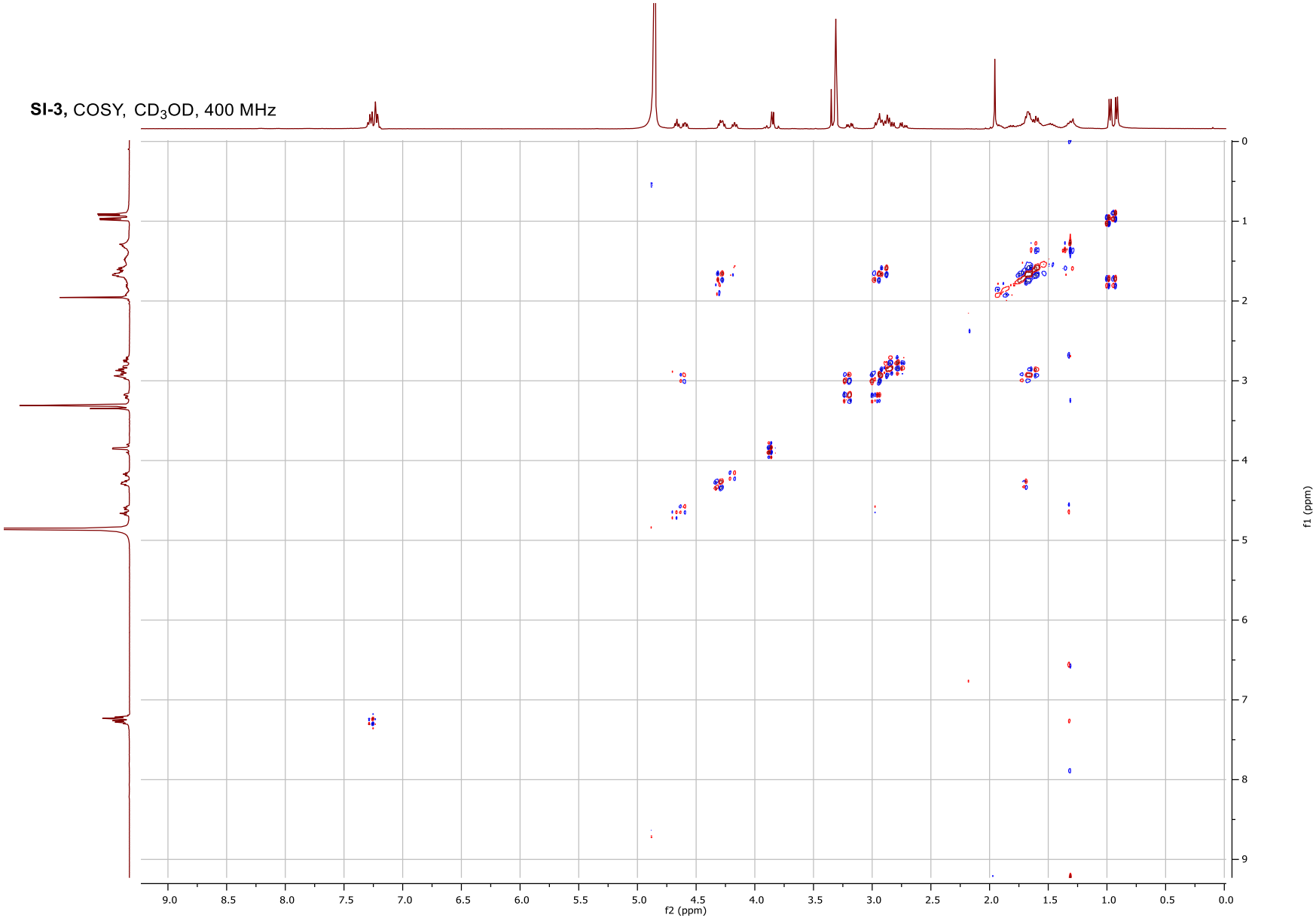
SI-2, HSQC, CD_3OD, 700 MHz



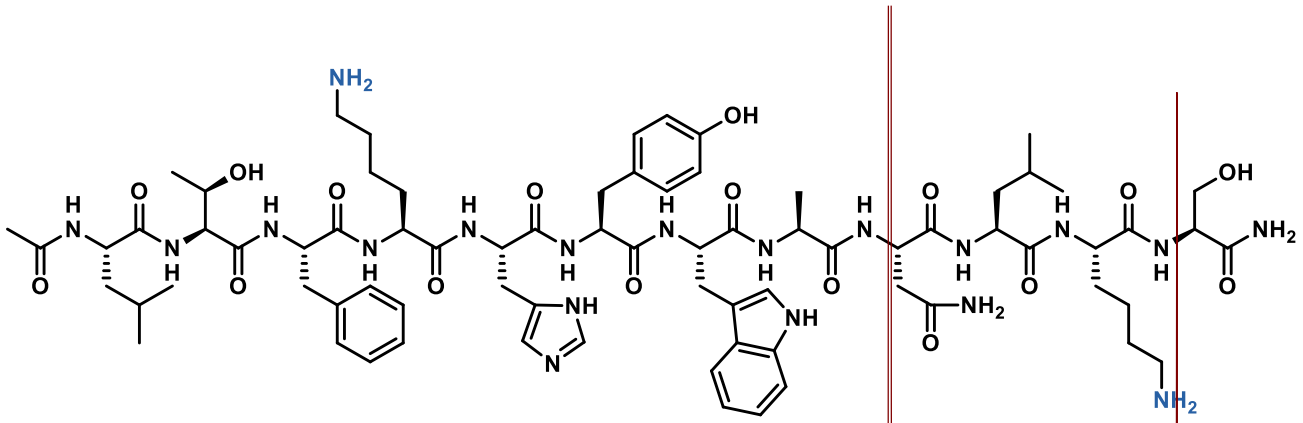


SI-3, ^{1}H, CD_3OD, 400 MHz

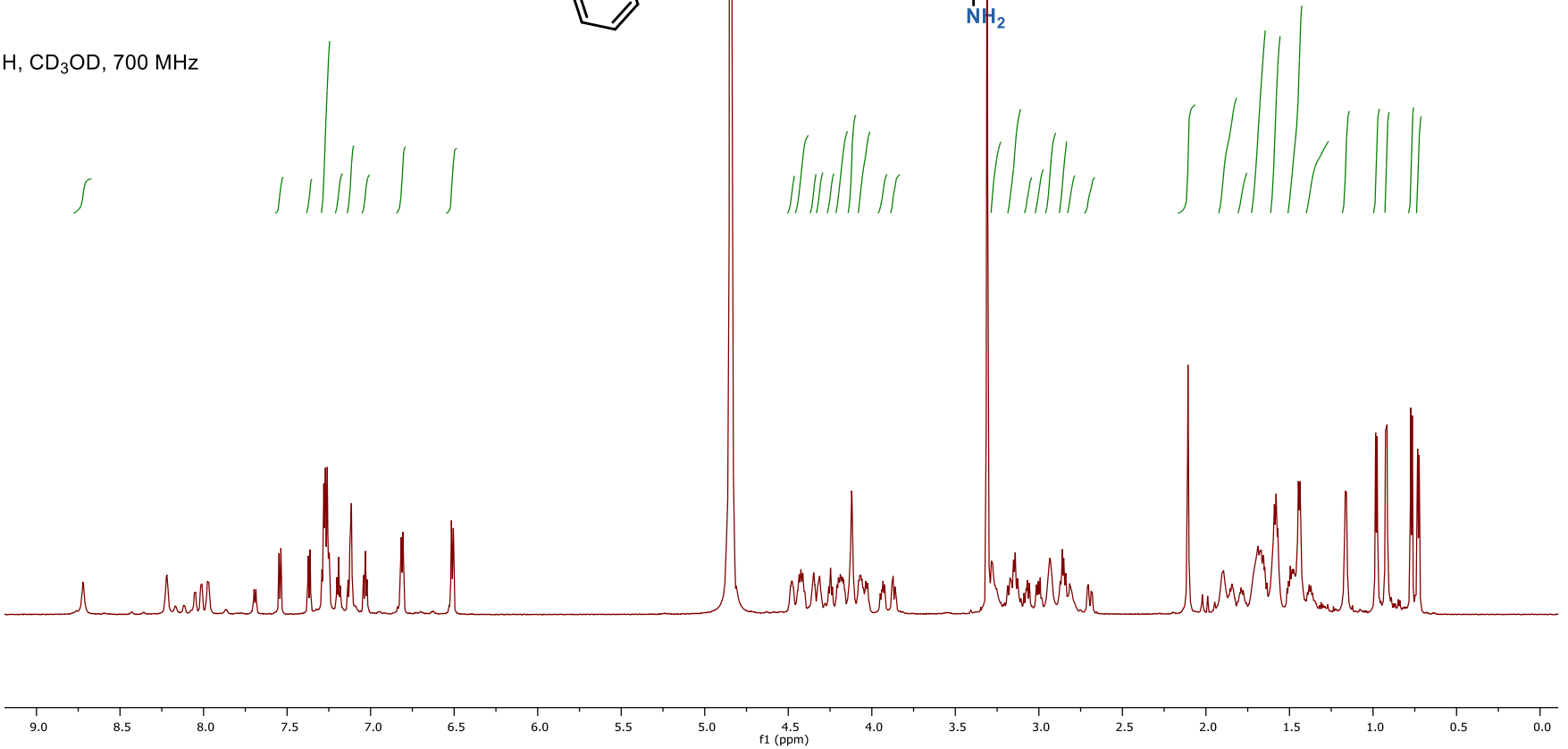
SI-3, COSY, CD_3OD, 400 MHz

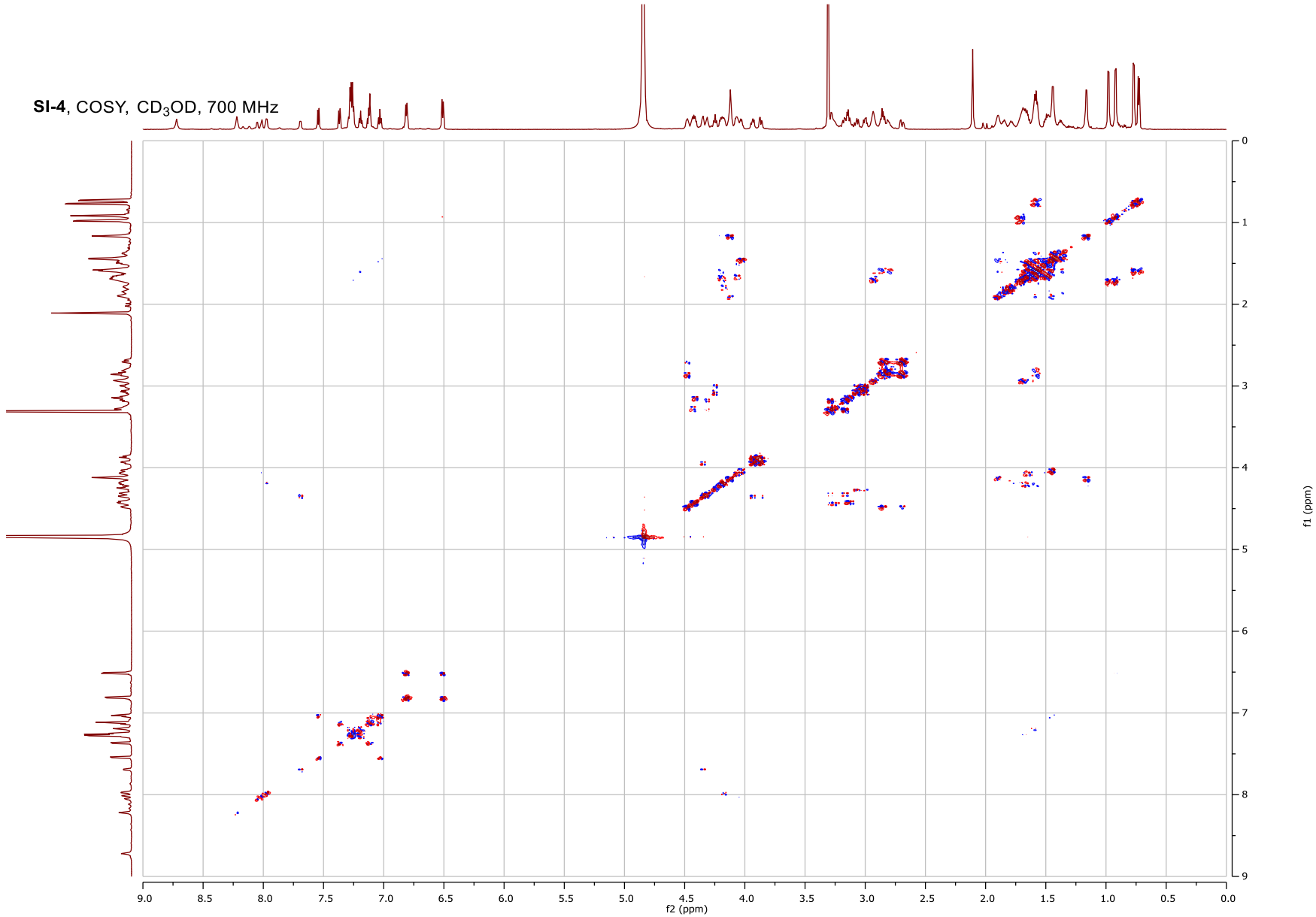


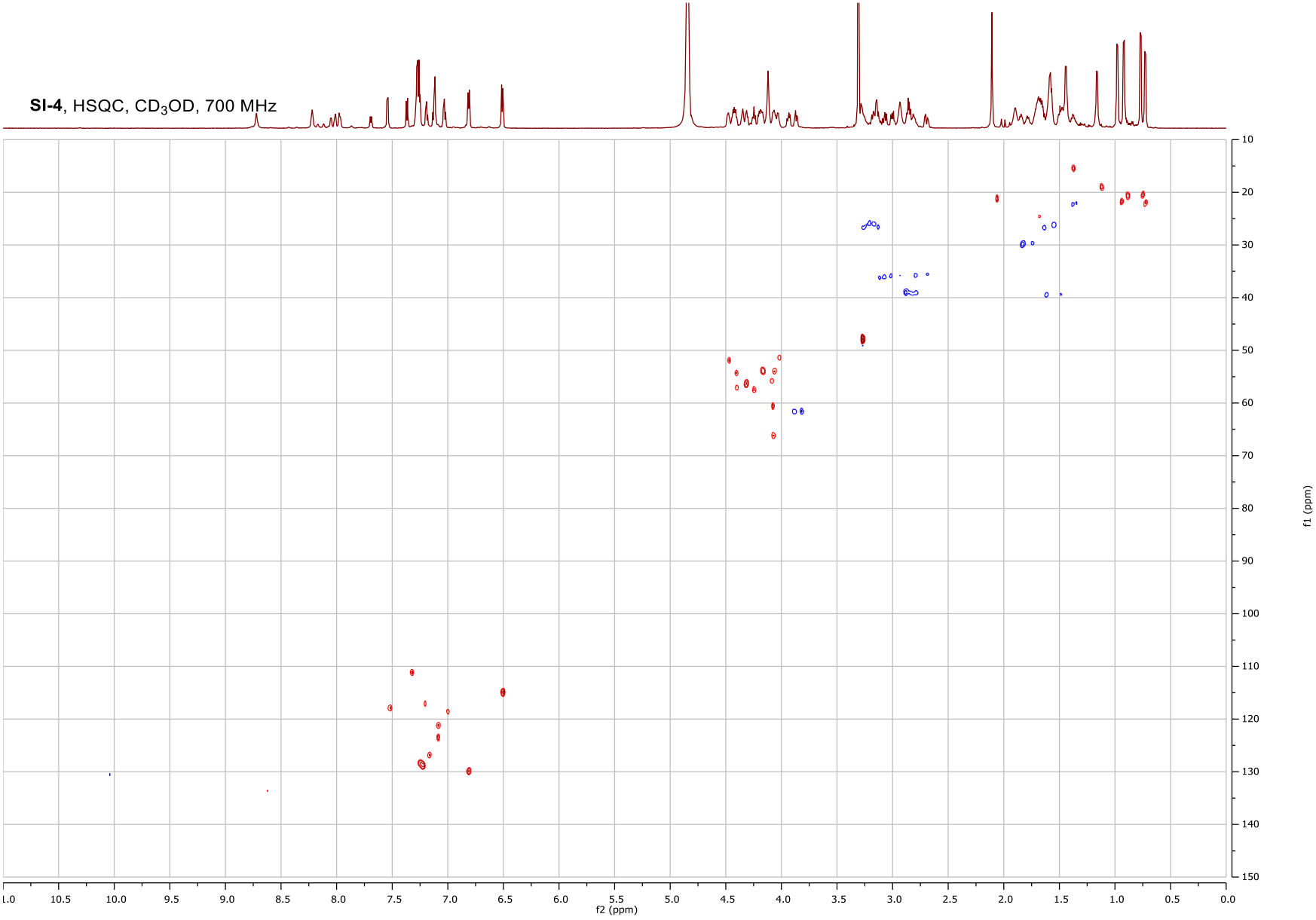
SI-3, HSQC, CD_3OD, 400 MHz

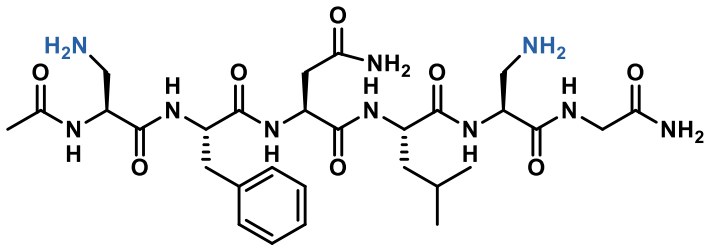


SI-4, ^{1}H, CD_3OD, 700 MHz



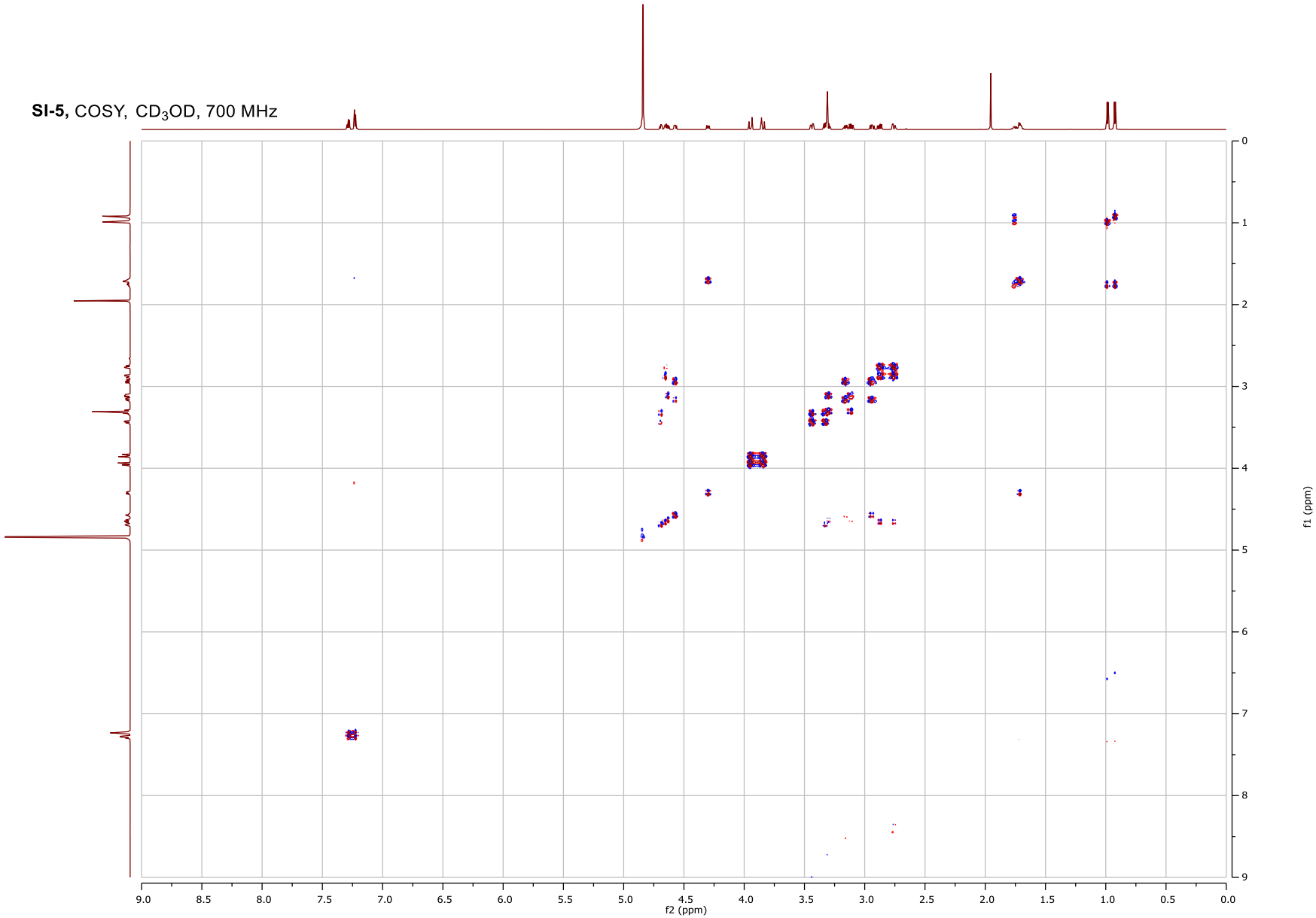

SI-4, COSY, CD3OD, 700 MHz
f2 (ppm)
f1 (ppm)


SI-4, HSQC, CD3OD, 700 MHz
f2 (ppm)
f1 (ppm)

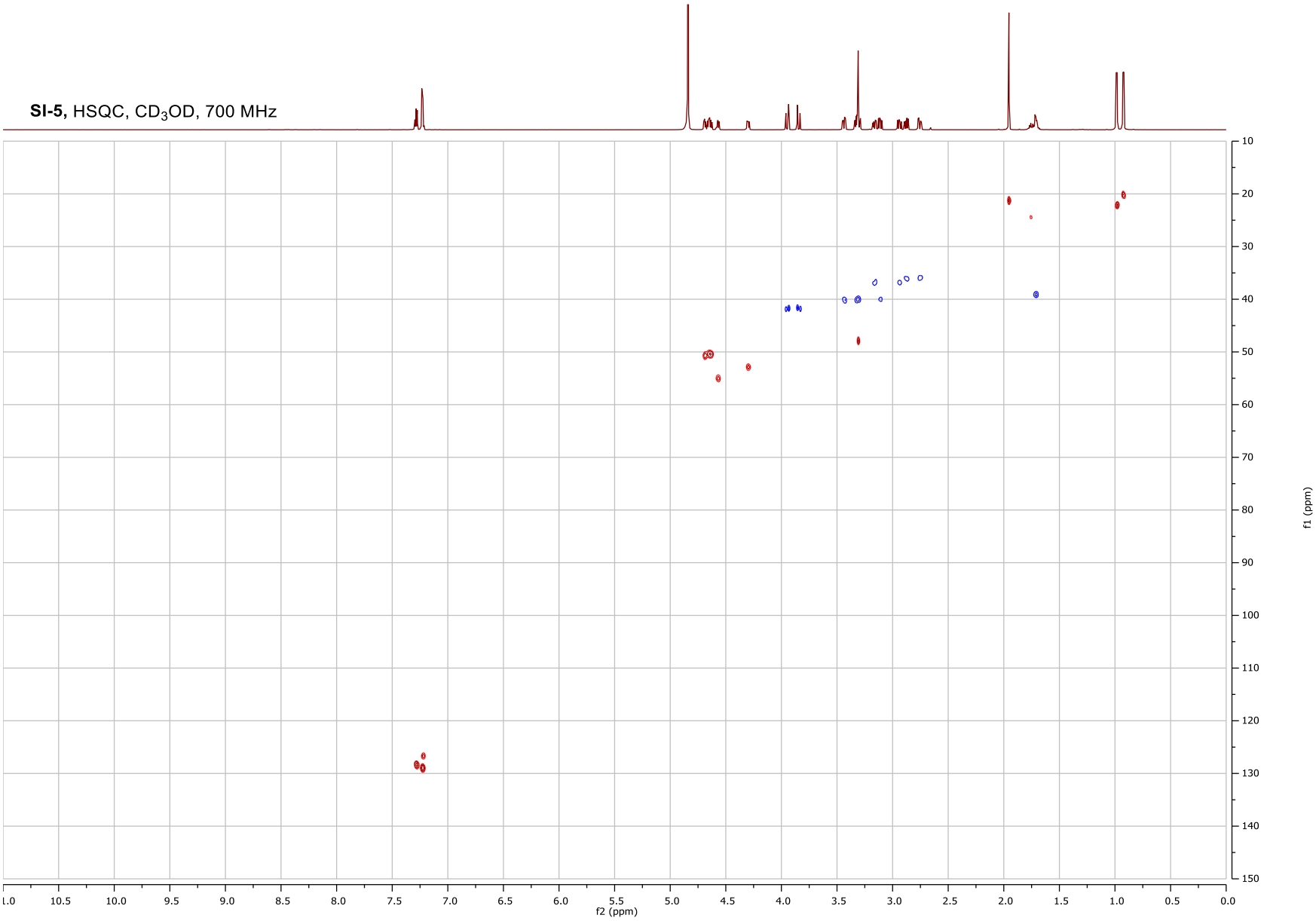


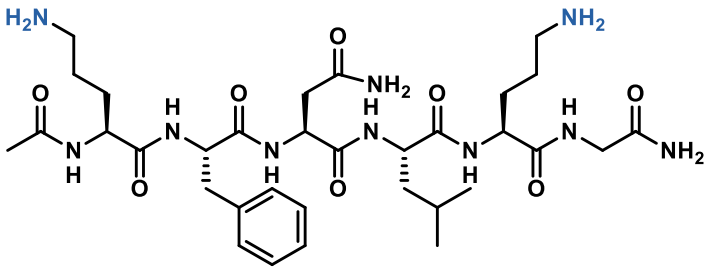
SI-5, ^{1}H, CD_3OD, 700 MHz

SI-5, COSY, CD_3OD, 700 MHz



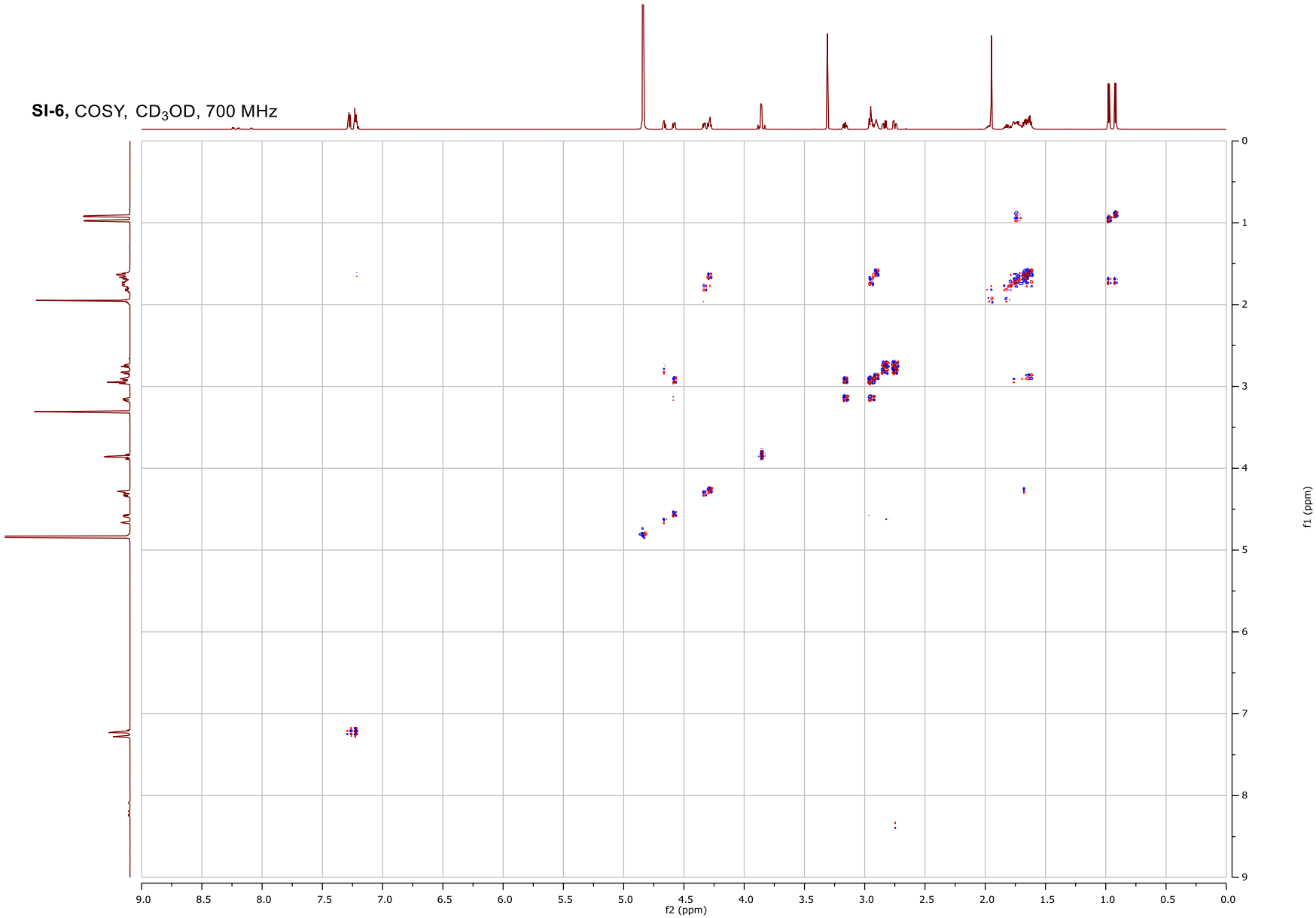
SI-5, HSQC, CD_3OD, 700 MHz



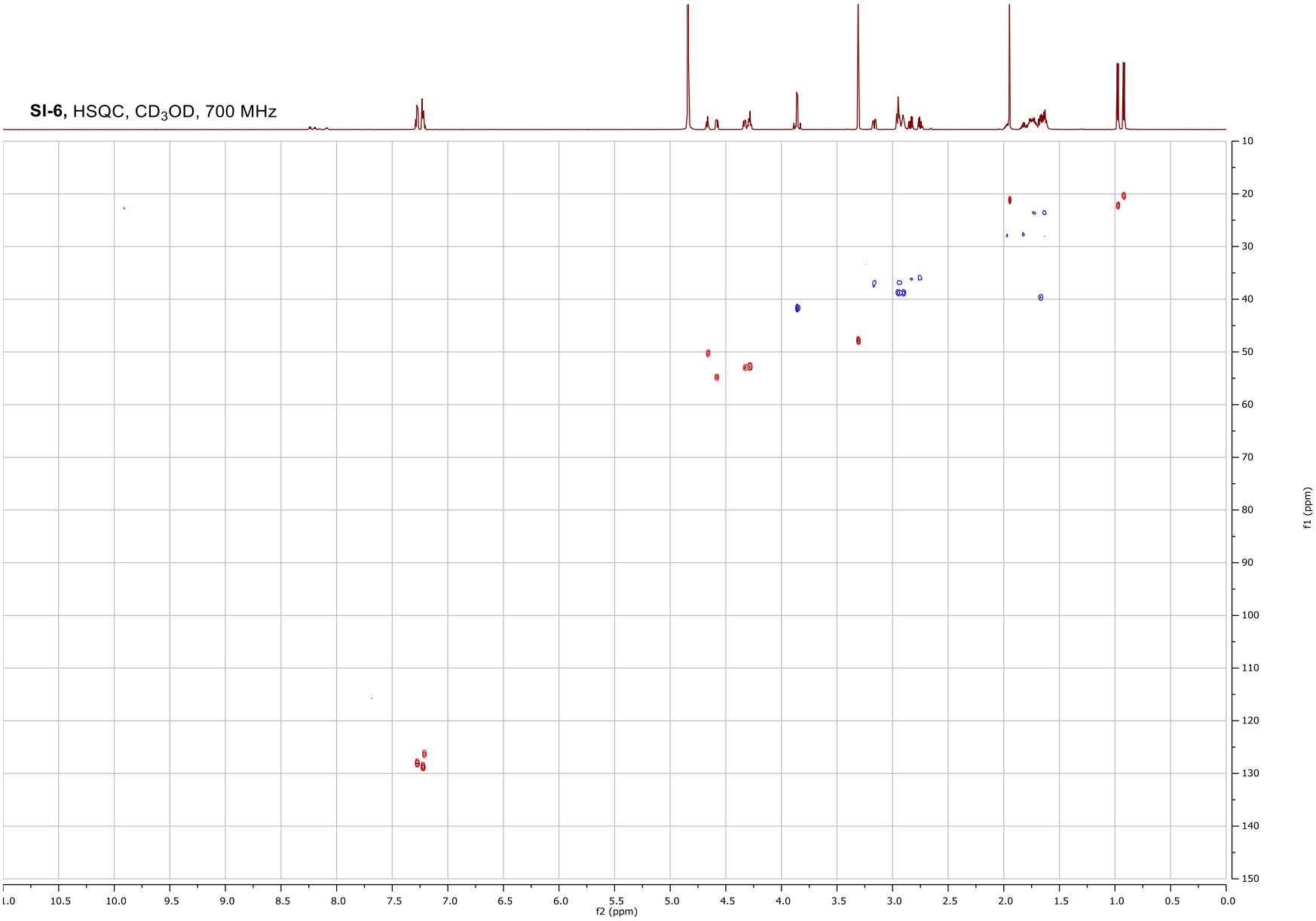


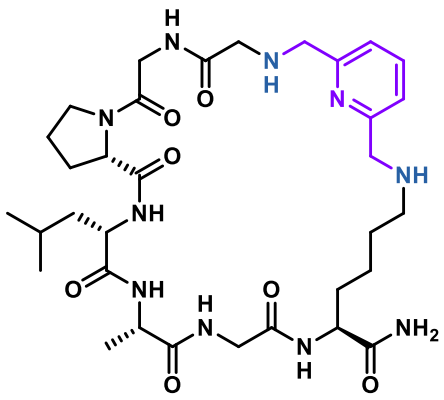
SI-6, ^{1}H, CD_3OD, 700 MHz

SI-6, COSY, CD_3OD, 700 MHz

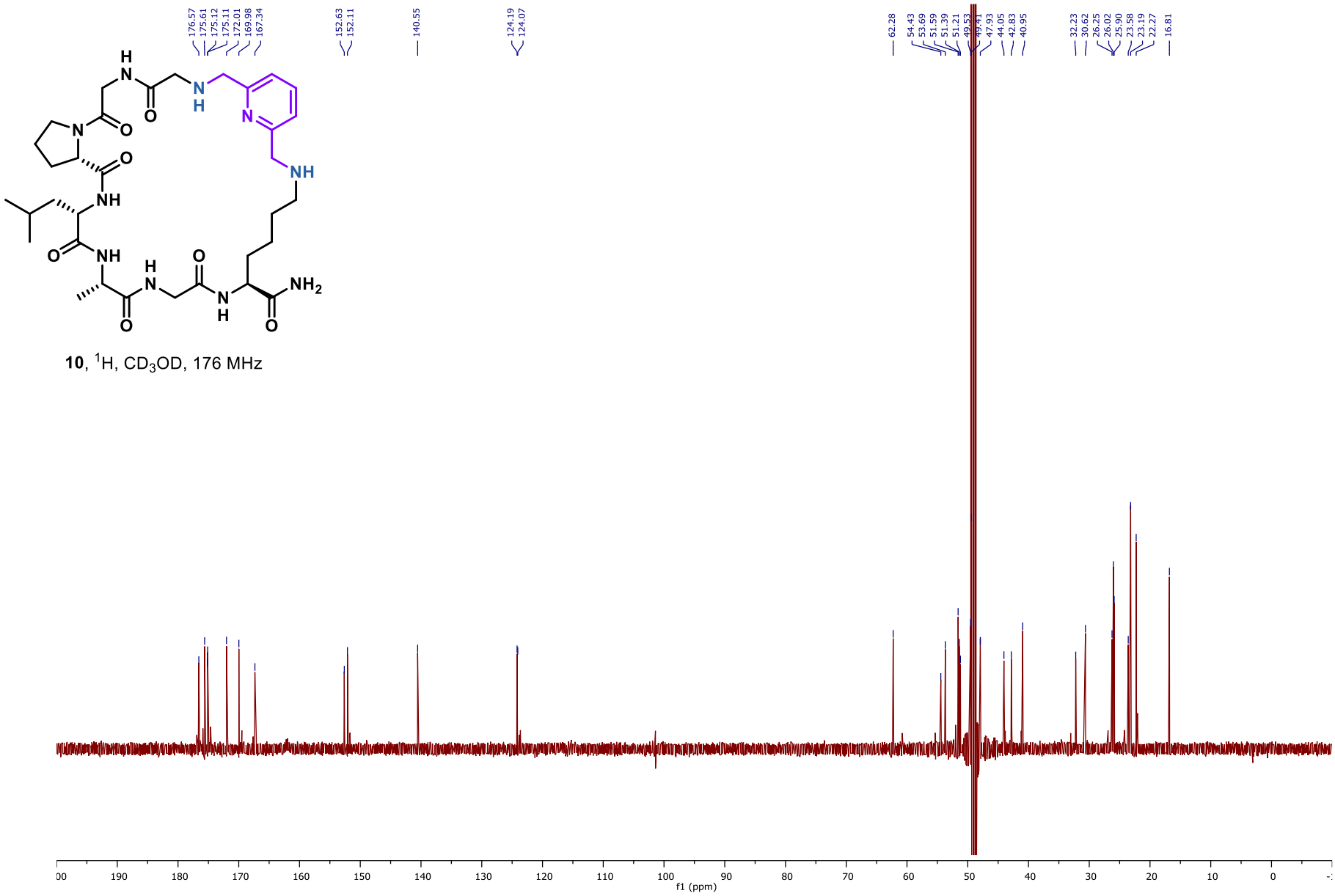


SI-6, HSQC, CD_3OD, 700 MHz





10, ^{1}H, CD_3OD, 700 MHz

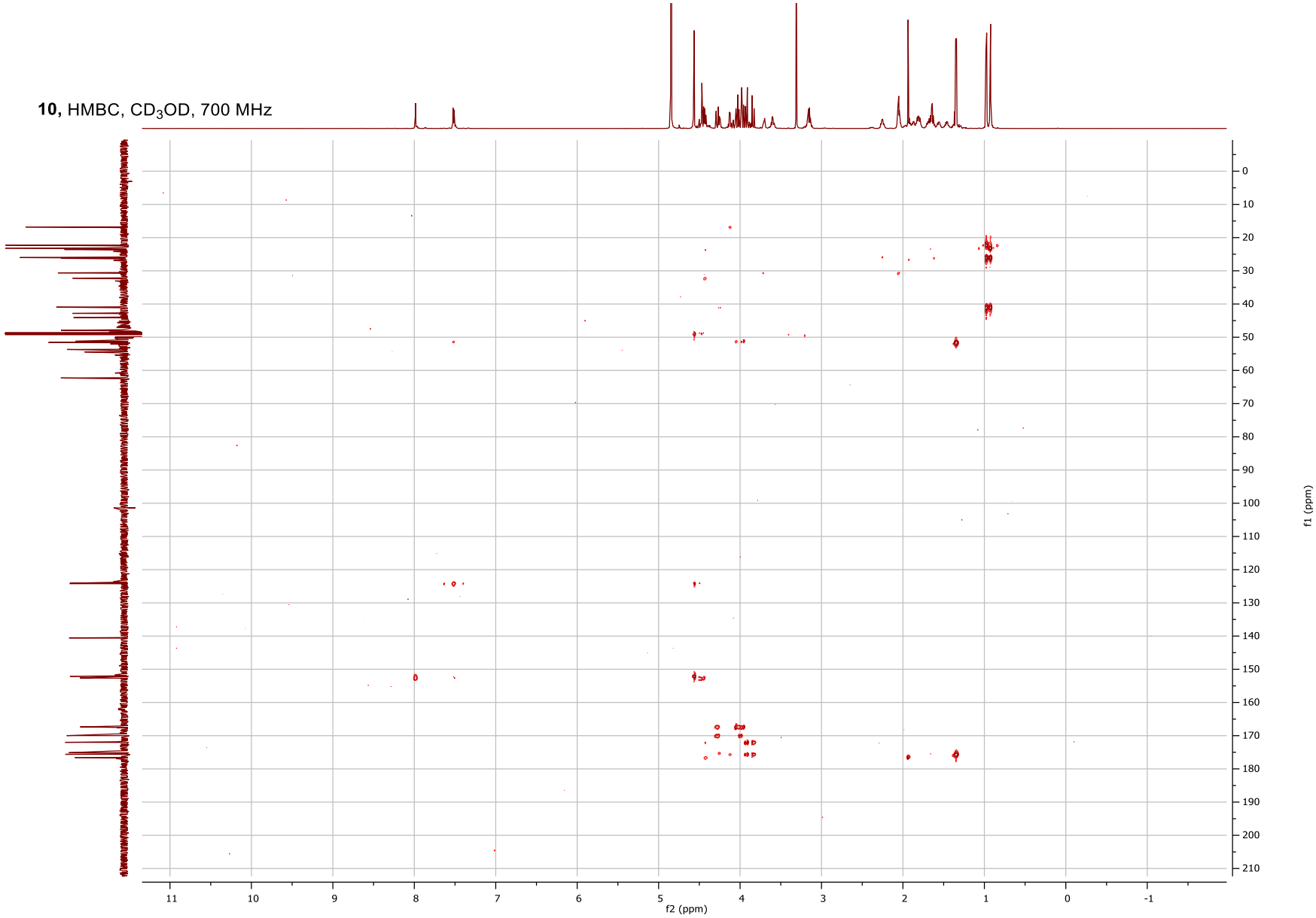


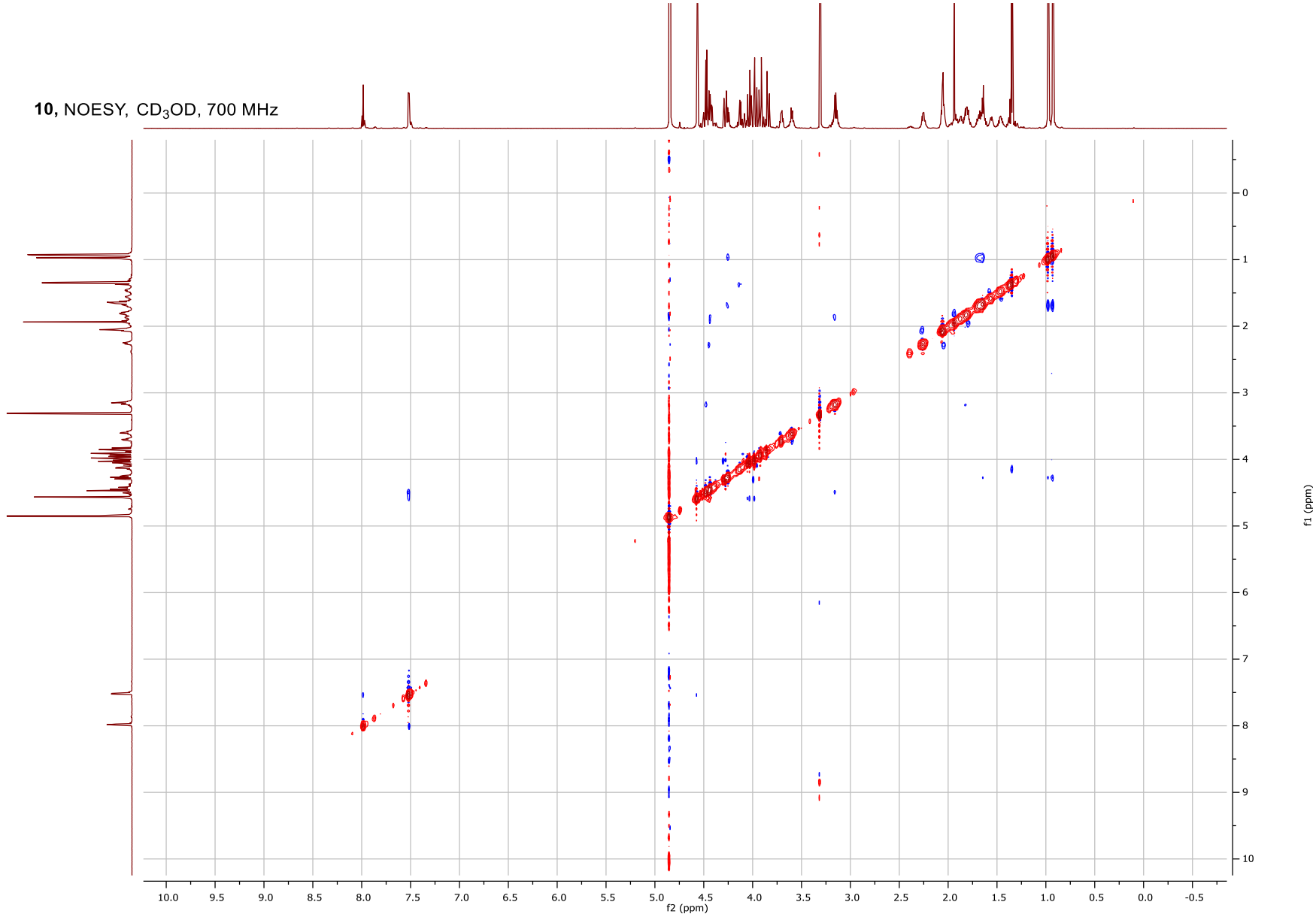
10, ^{1}H, CD_3OD, 176 MHz


10, COSY, CD3OD, 700 MHz
f2 (ppm)
f1 (ppm)

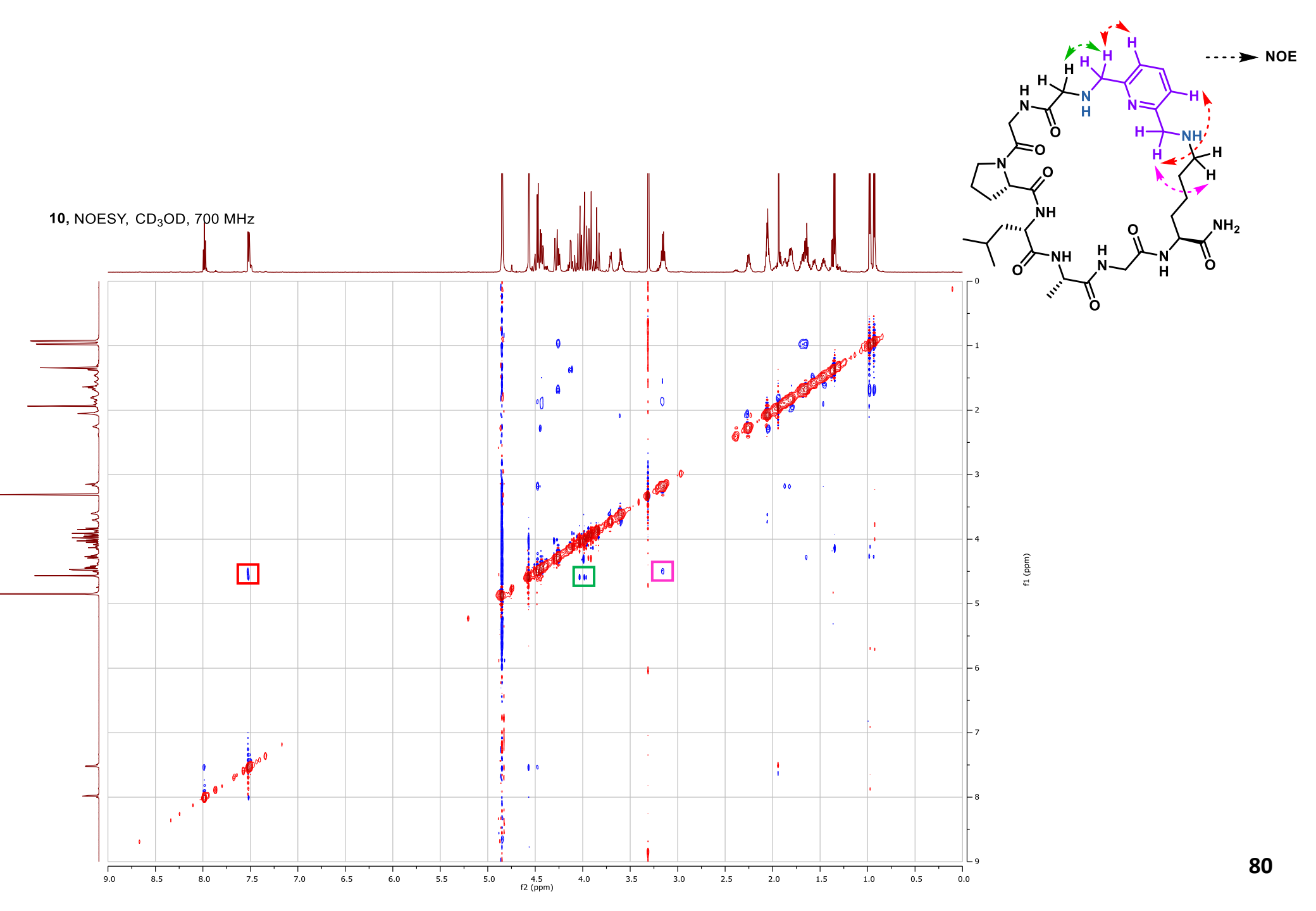
10, HSQC, CD_3OD, 700 MHz

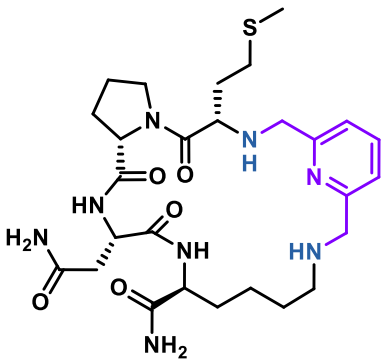
10, HMBC, CD_3OD, 700 MHz




10, NOESY, CD3OD, 700 MHz
f2 (ppm)
f1 (ppm)

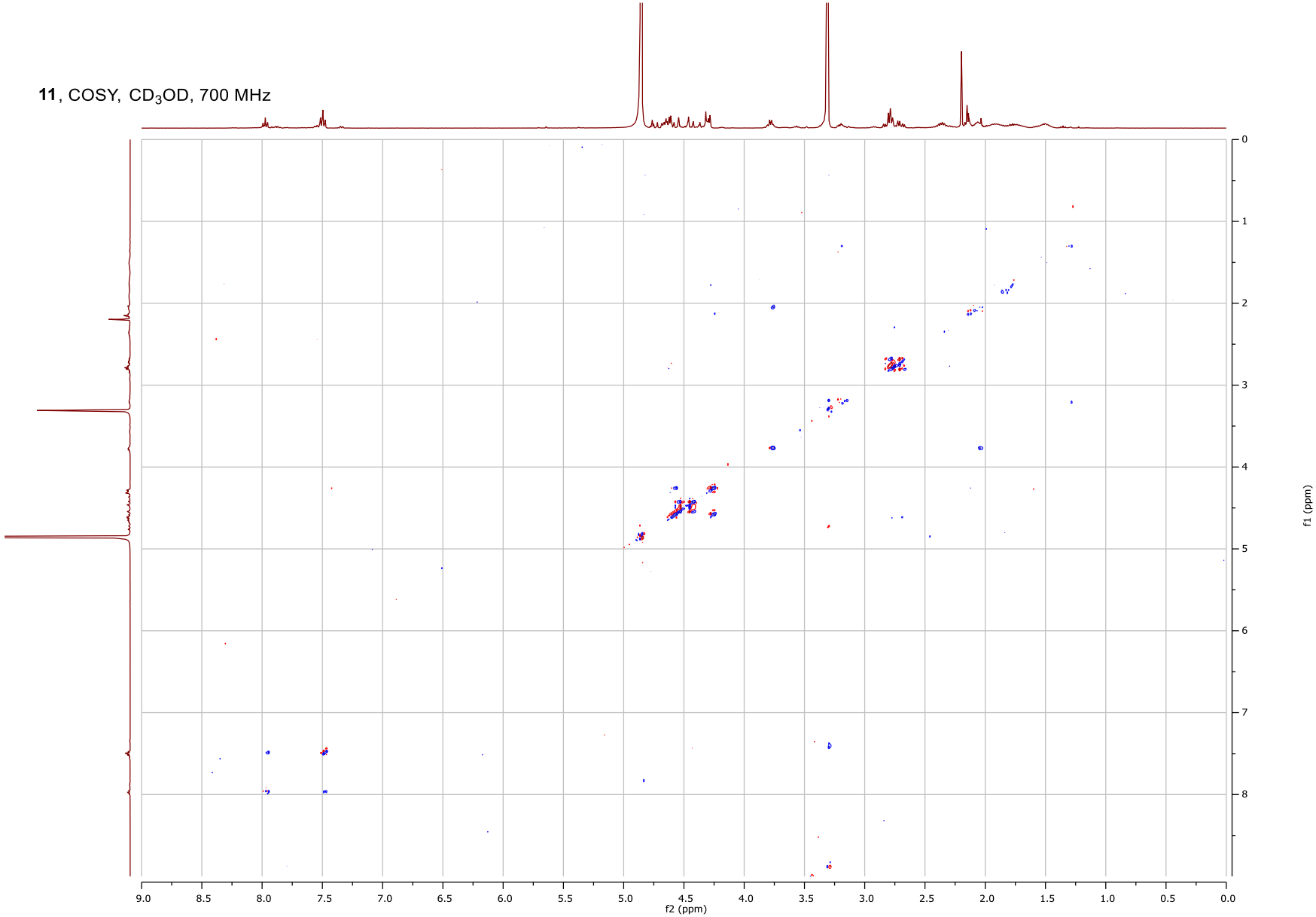
10, NOESY, CD_3OD, 700 MHz

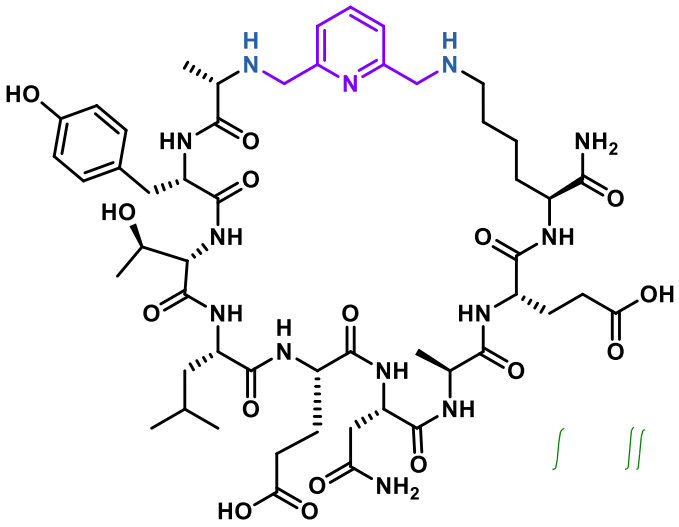




11, ^{1}H, CD_3OD, 400 MHz

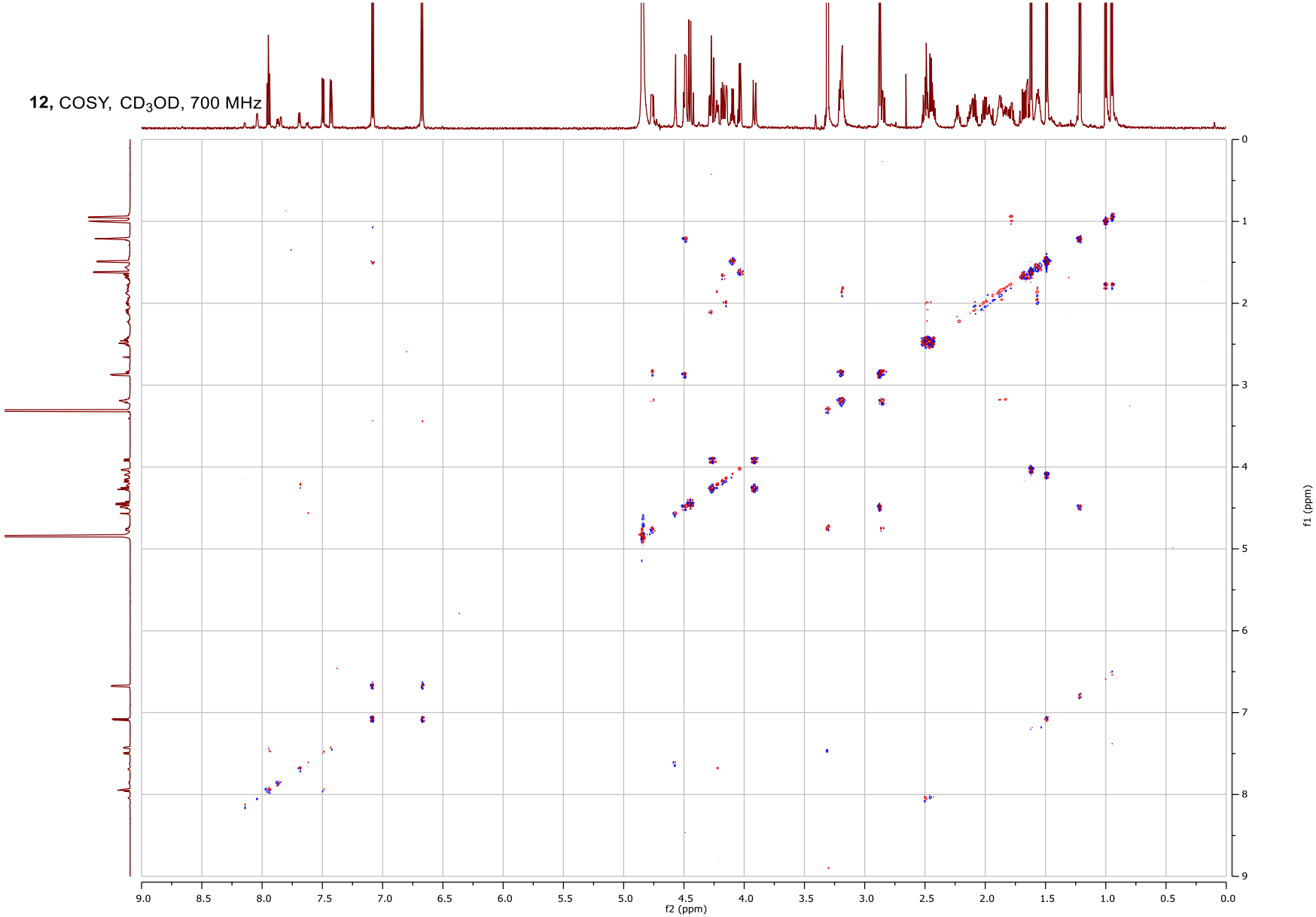
11, COSY, CD_3OD, 700 MHz

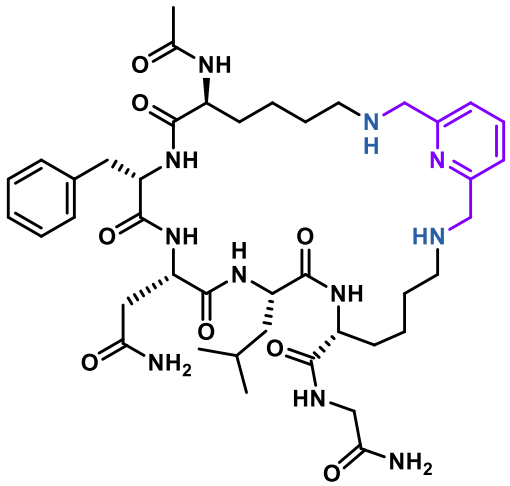




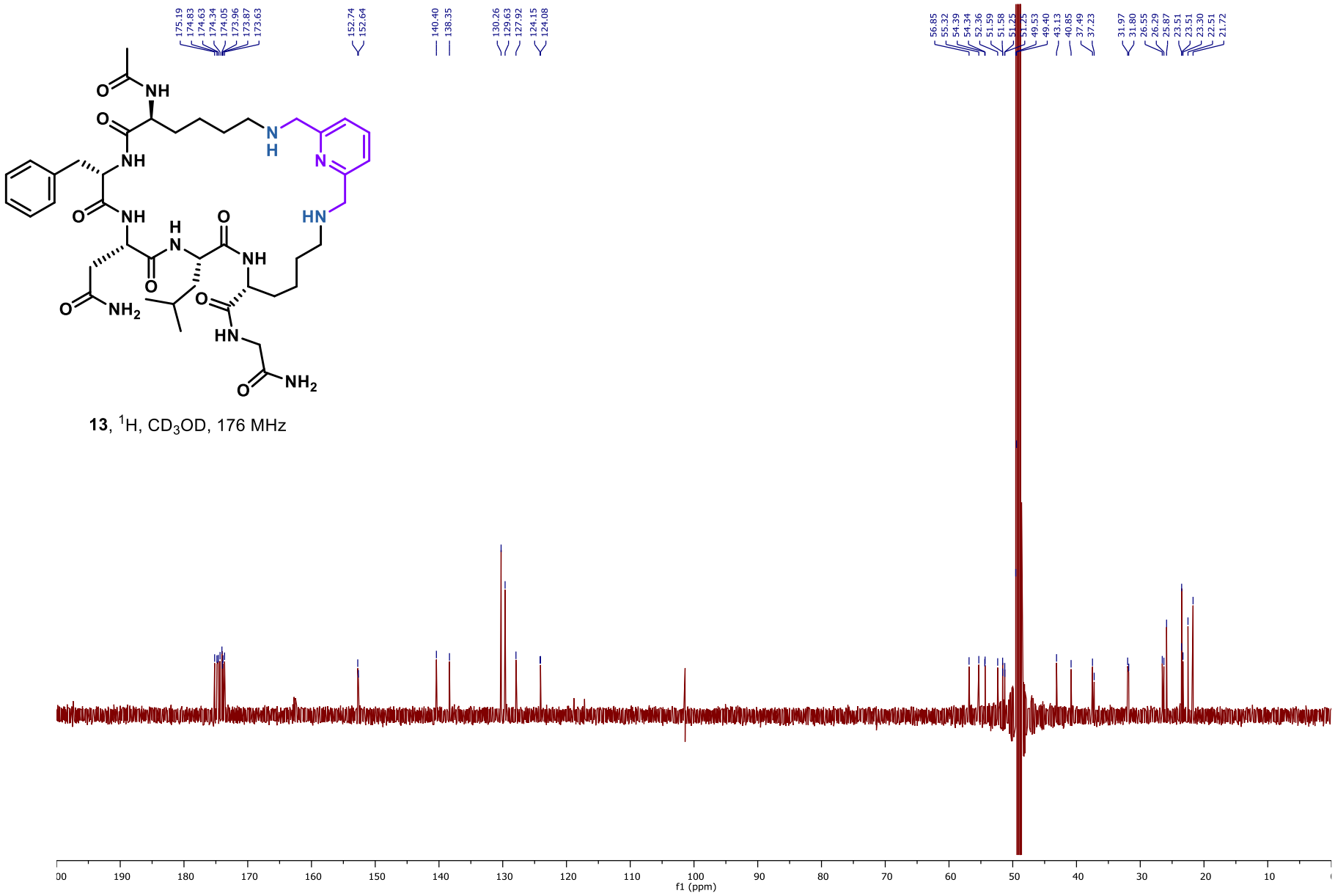
12, ^{1}H, CD_3OD, 700 MHz

12, COSY, CD_3OD, 700 MHz



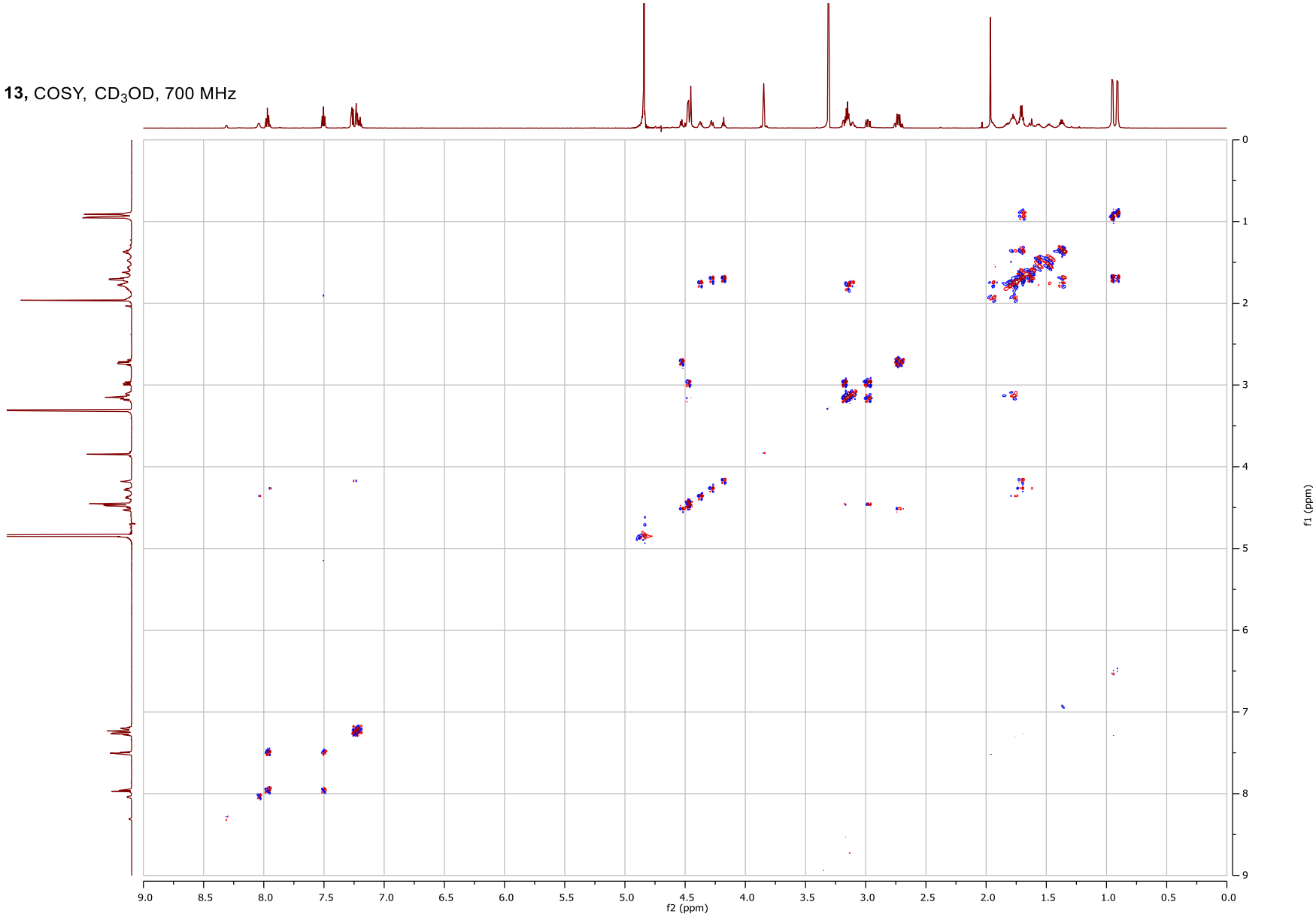


13, ^{1}H, CD_3OD, 700 MHz

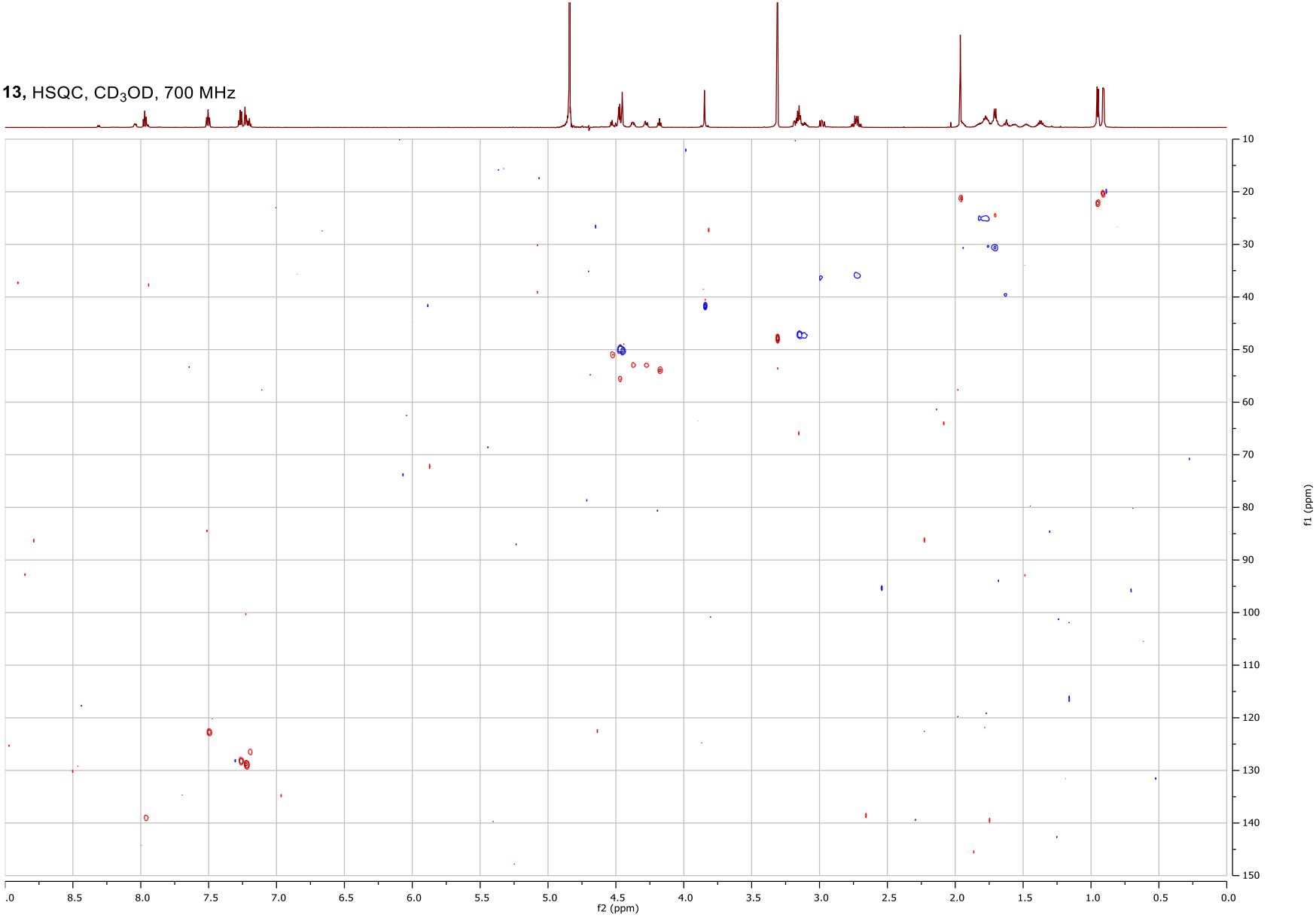


13, ^{1}H, CD_3OD, 176 MHz

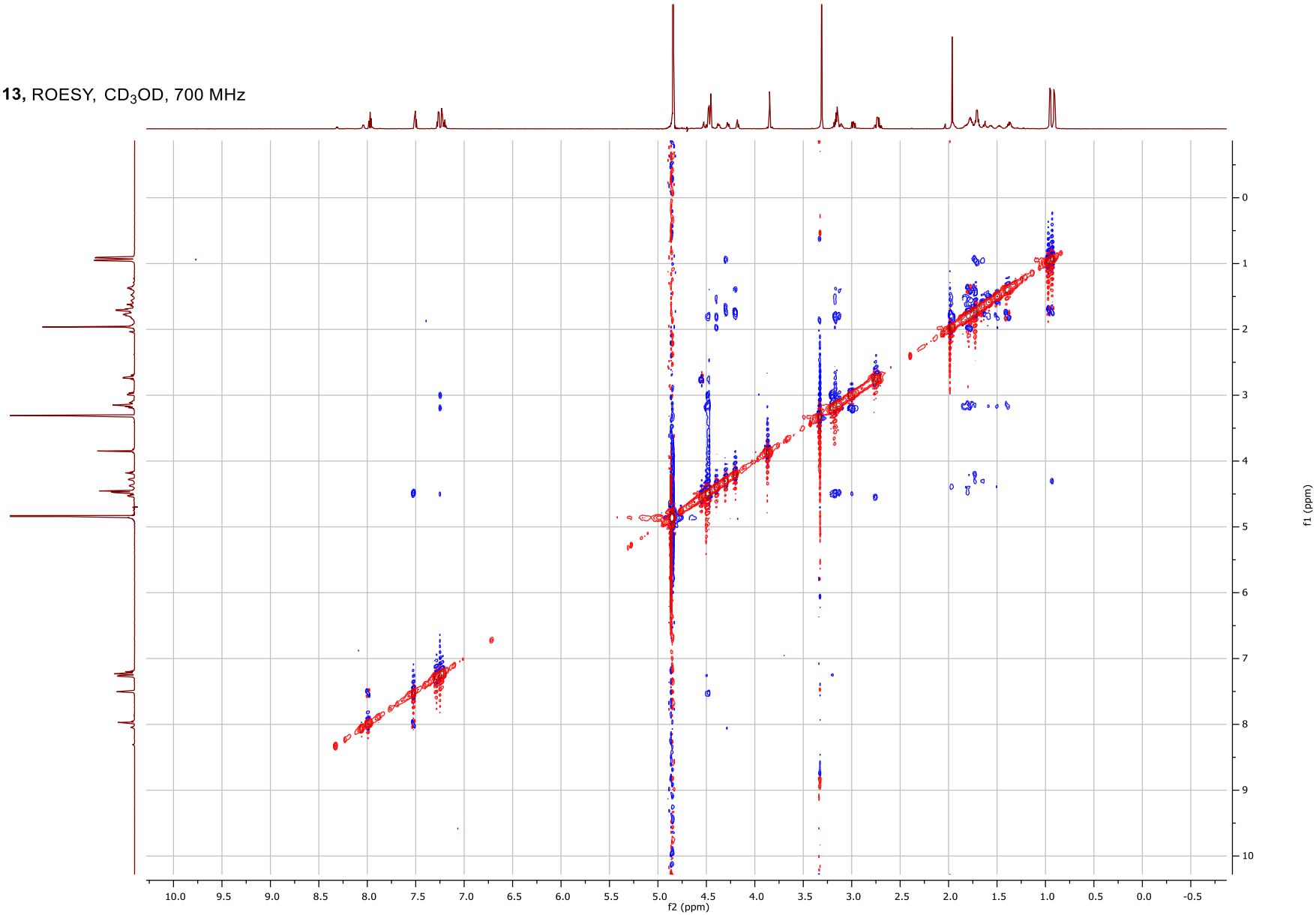
13, COSY, CD_3OD, 700 MHz



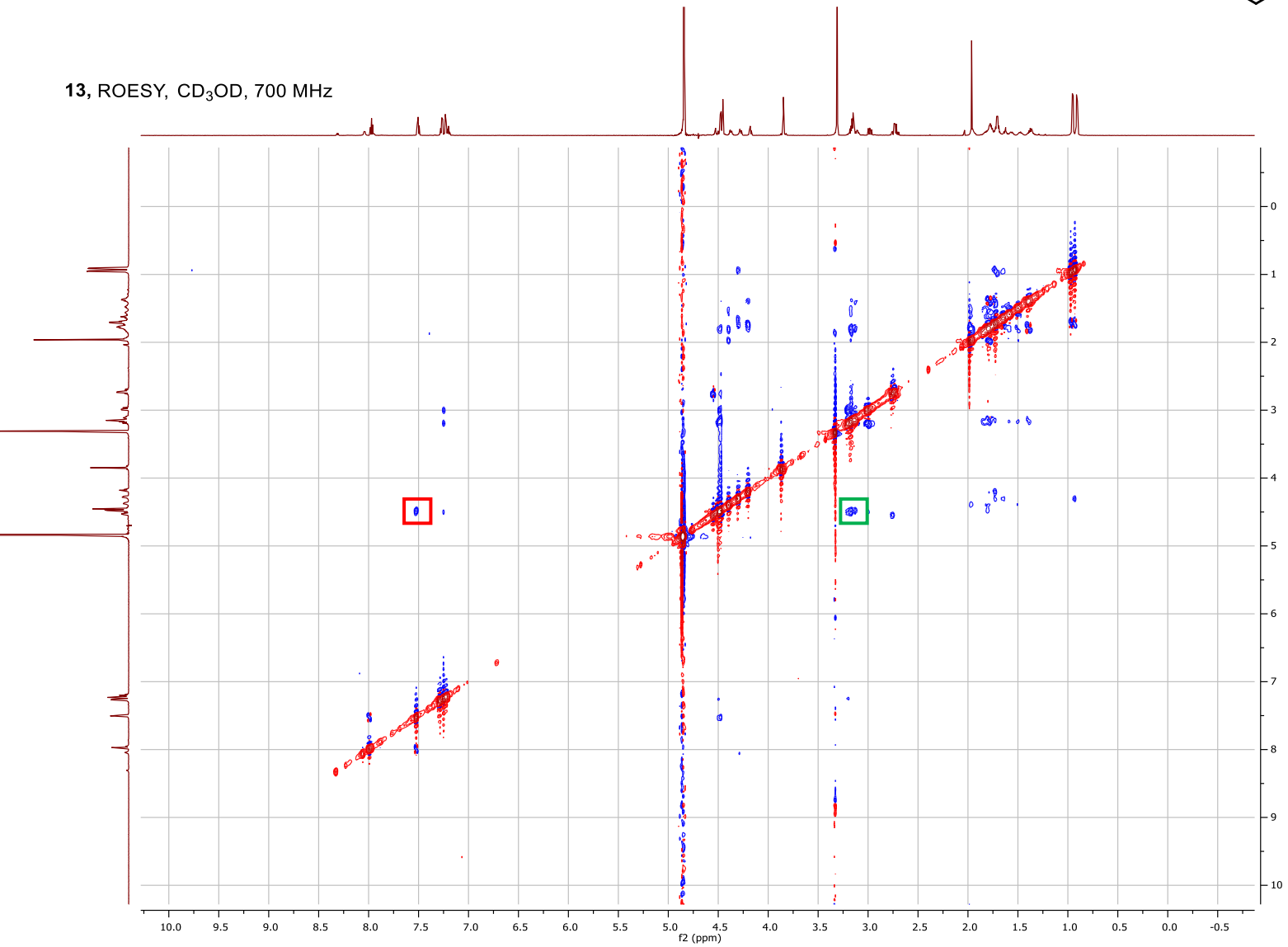
13, HSQC, CD_3OD, 700 MHz

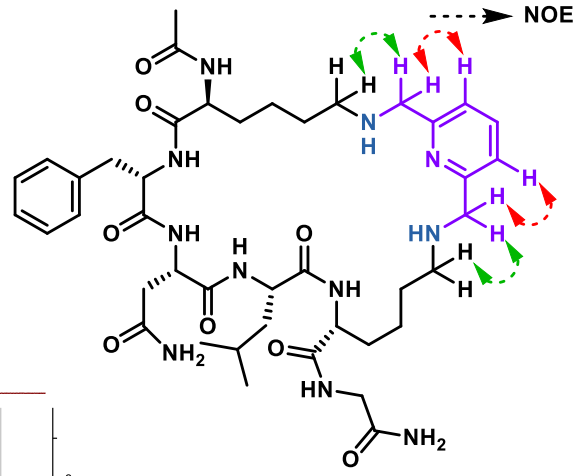


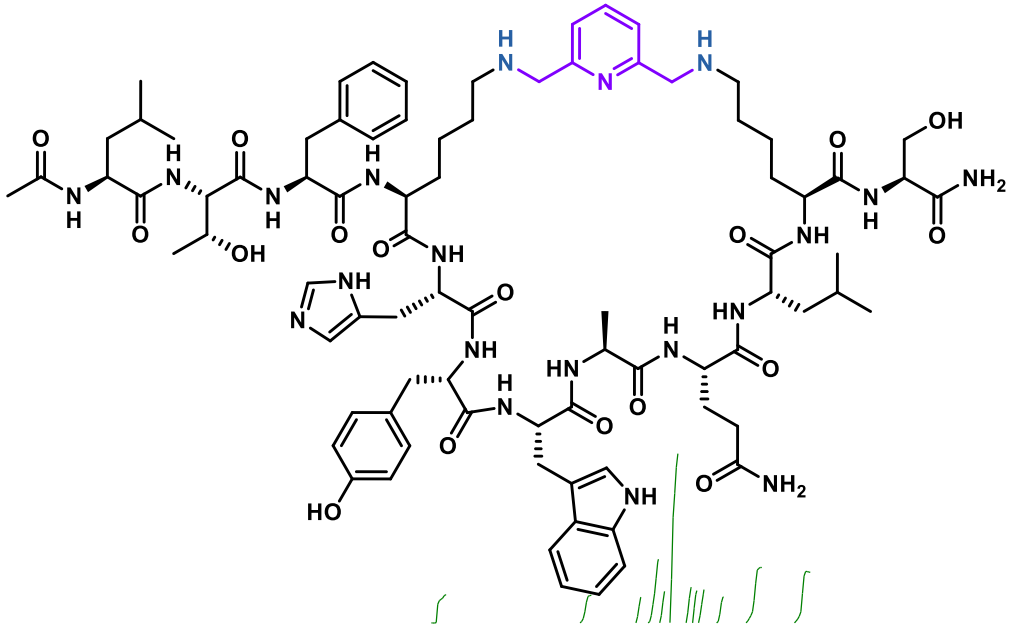
13, ROESY, CD_3OD, 700 MHz



13, ROESY, CD_3OD, 700 MHz

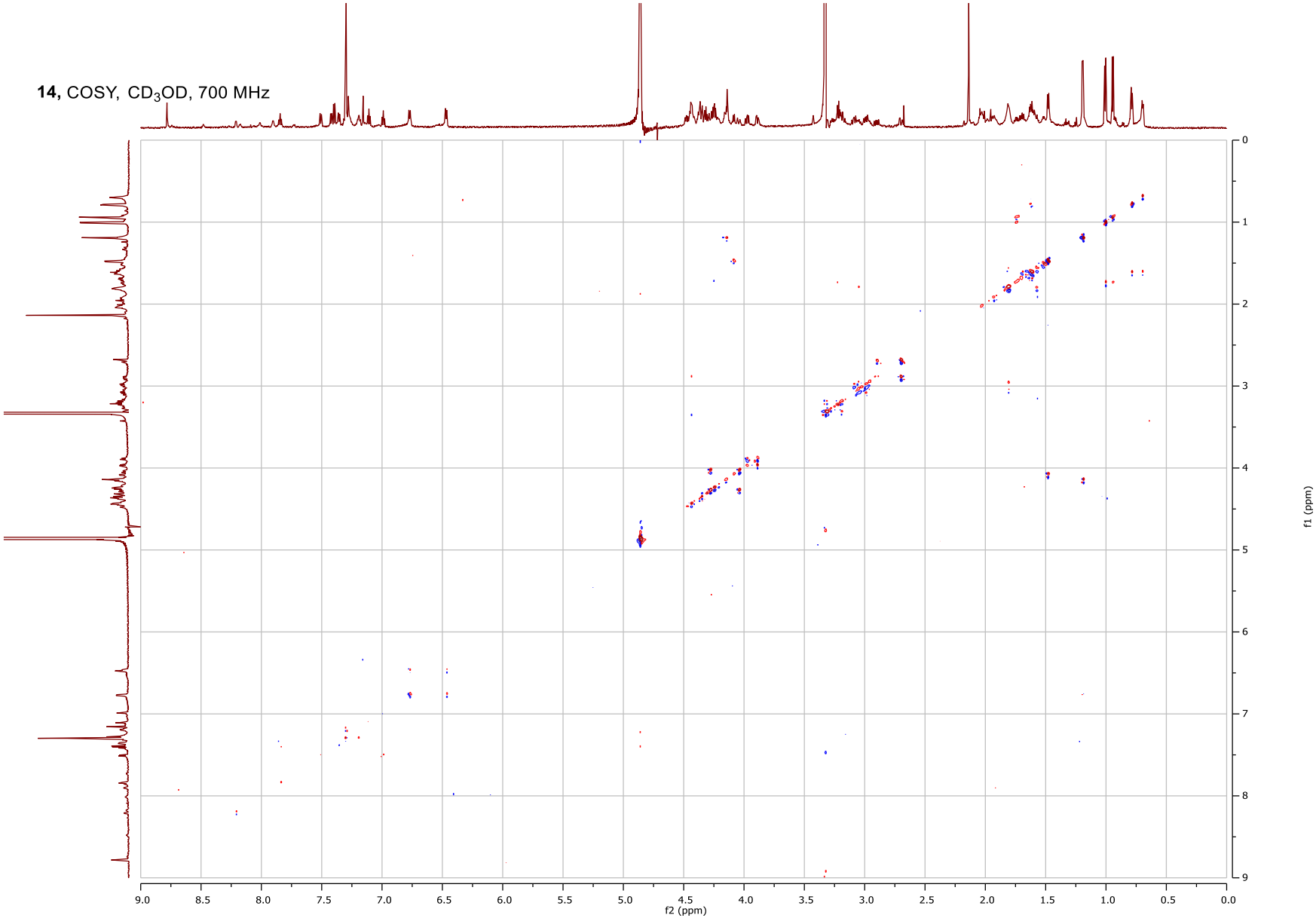


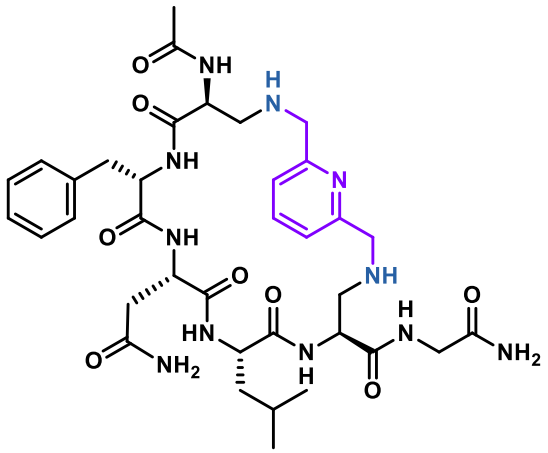




14, ^{1}H, CD_3OD, 700 MHz

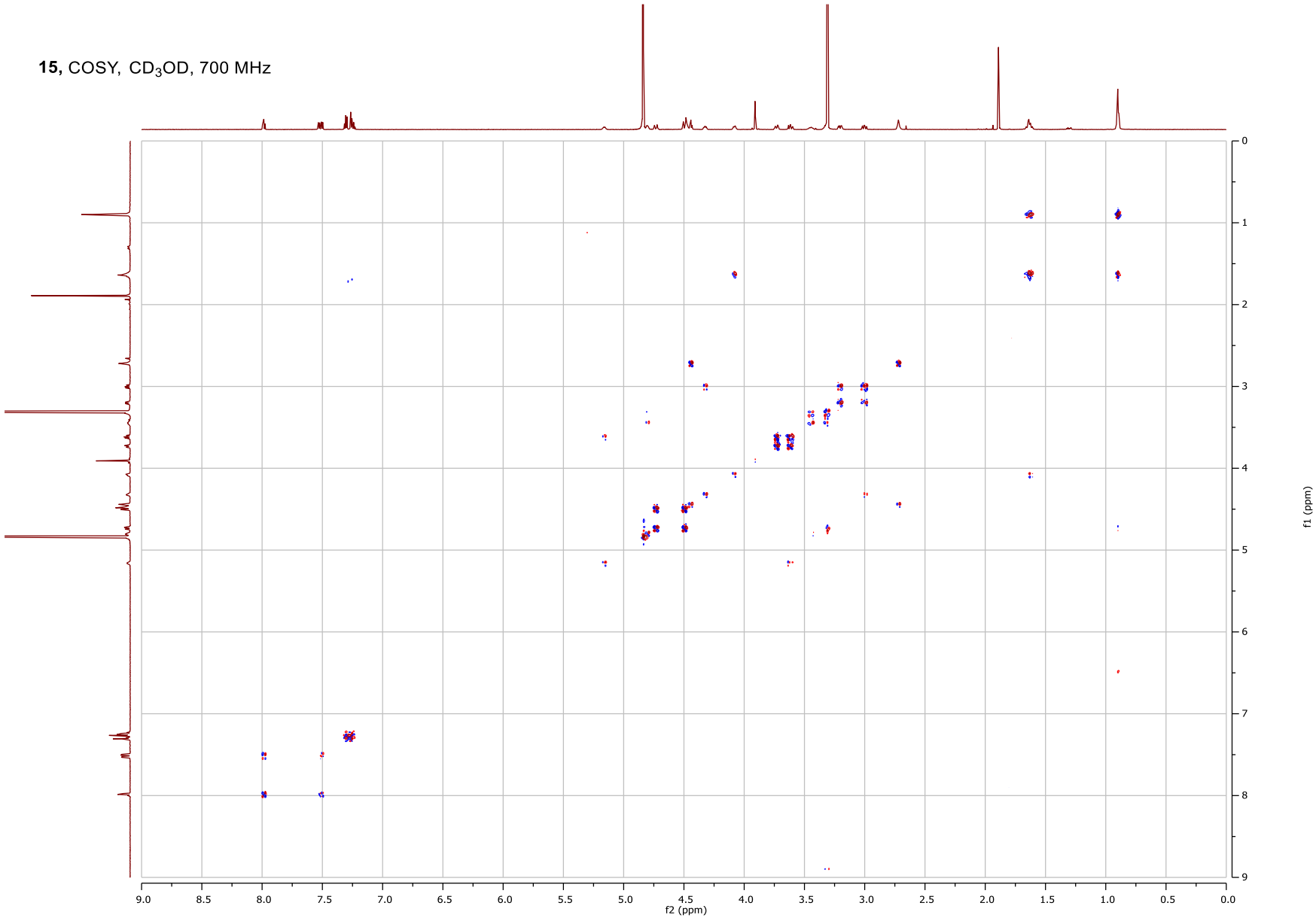
14, COSY, CD_3OD, 700 MHz

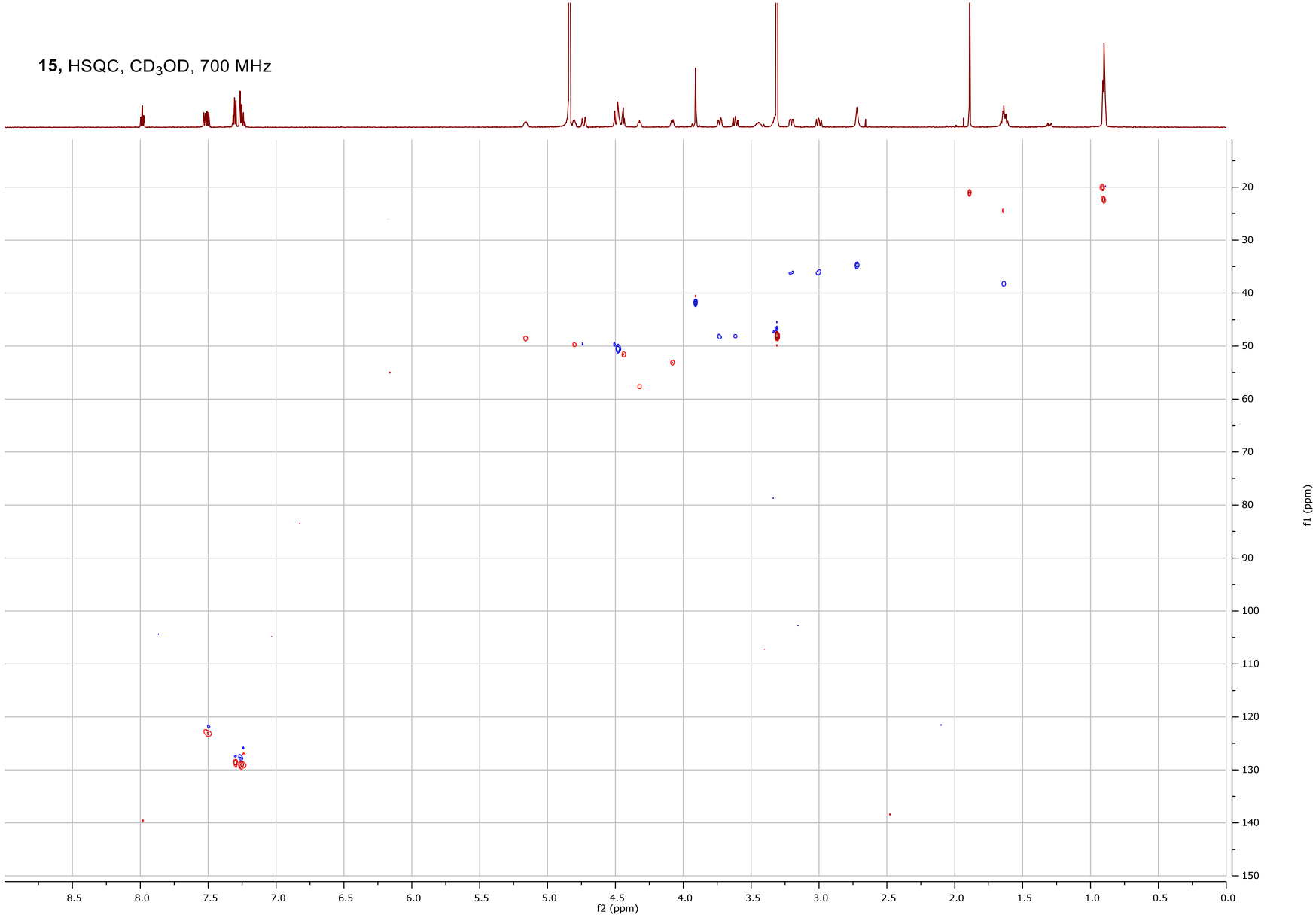


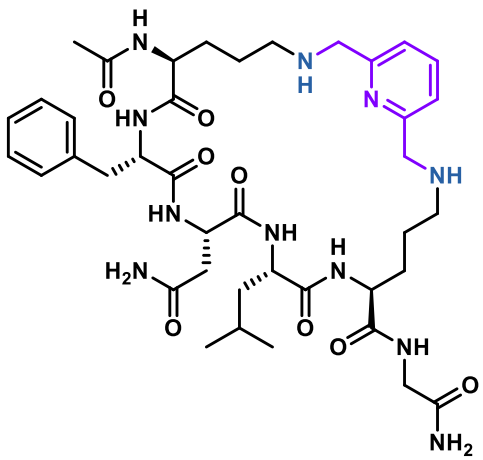


15, ^{1}H, CD_3OD, 700 MHz

15, COSY, CD_3OD, 700 MHz

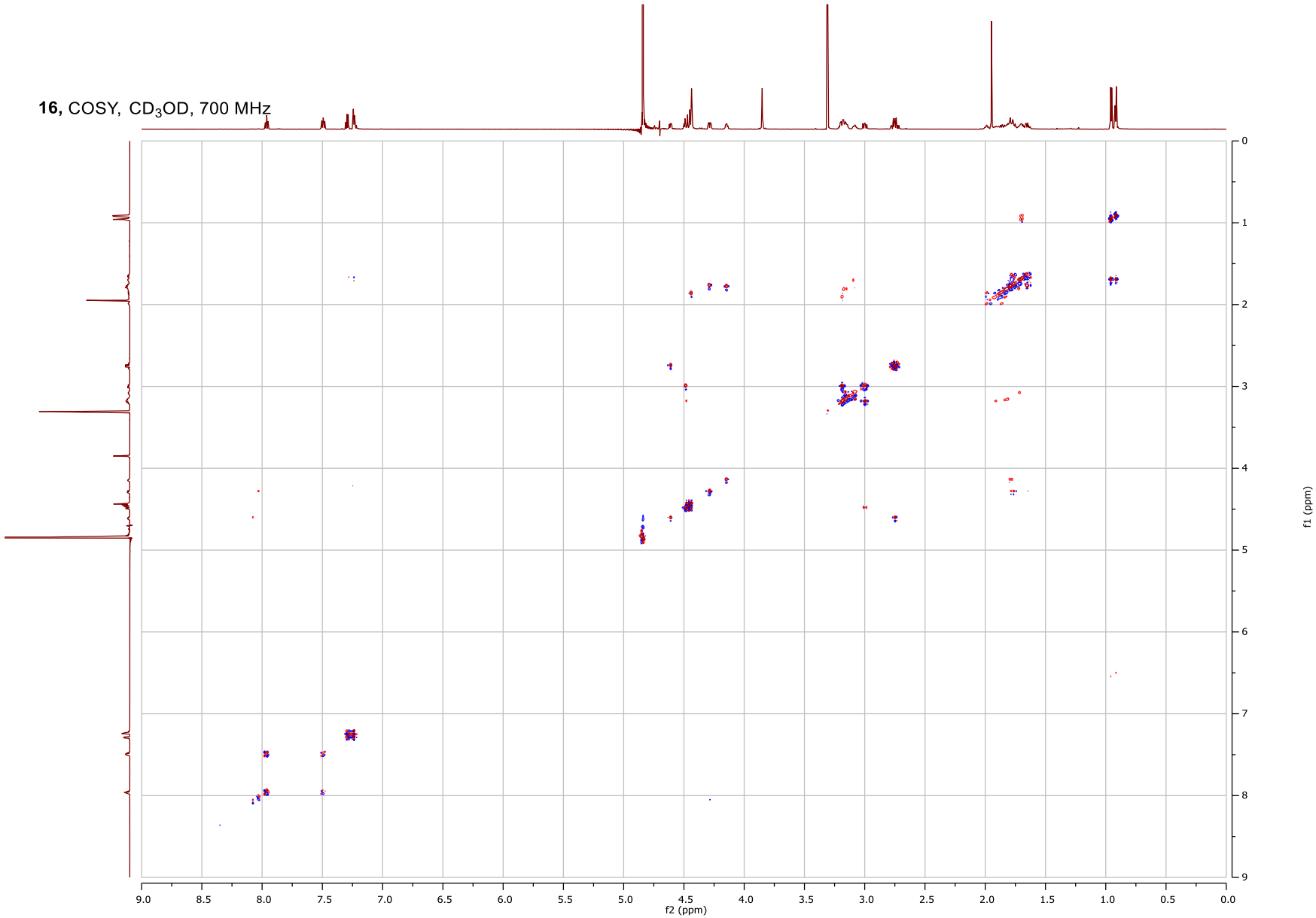


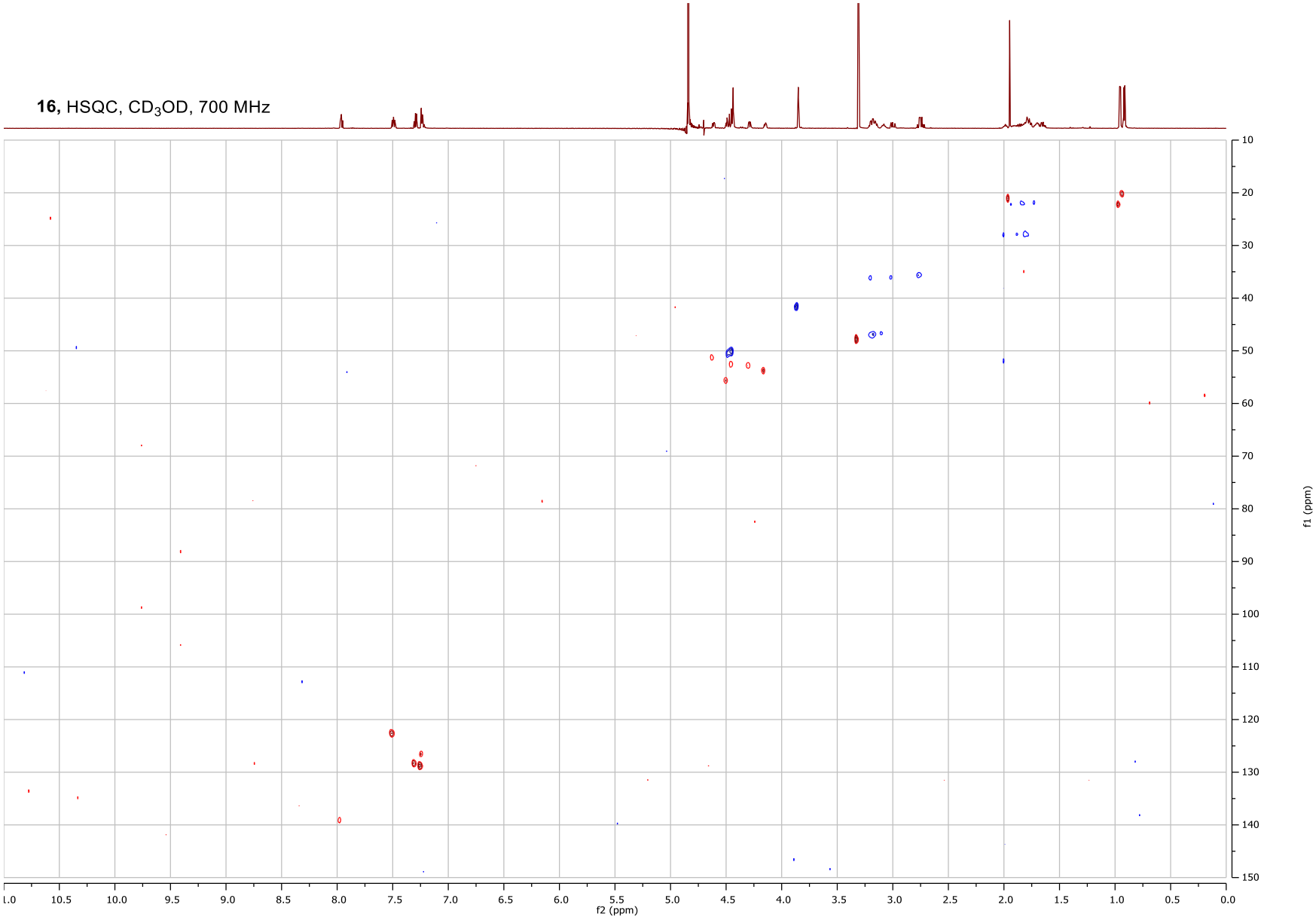

15, HSQC, CD3OD, 700 MHz
f2 (ppm)
f1 (ppm)

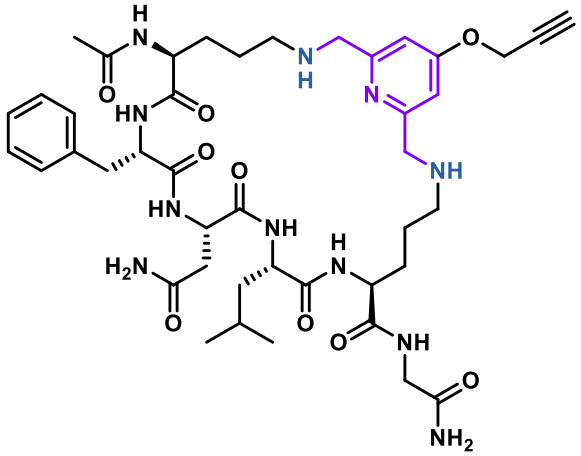


16, ^{1}H, CD_3OD, 700 MHz

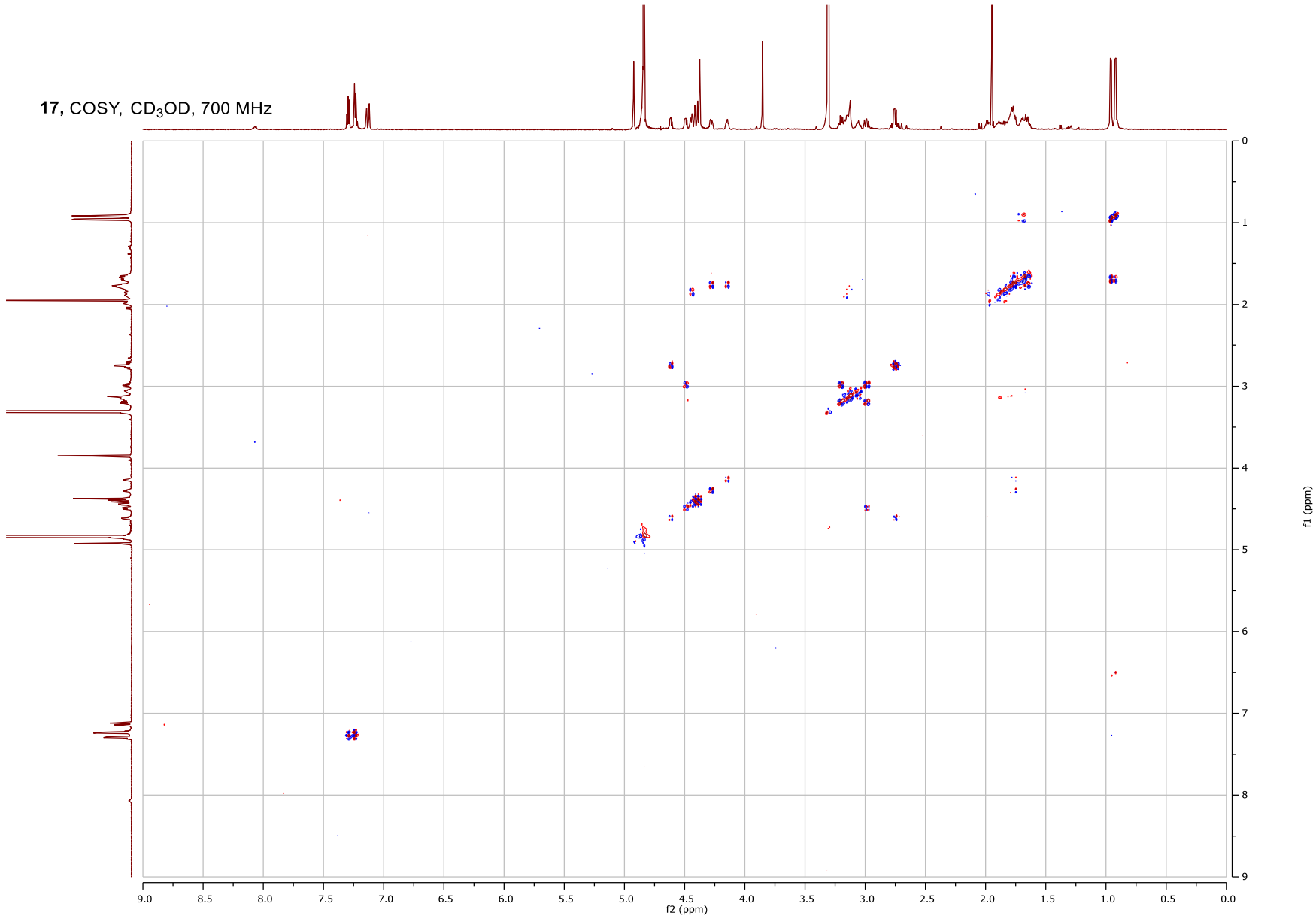
16, COSY, CD_3OD, 700 MHz

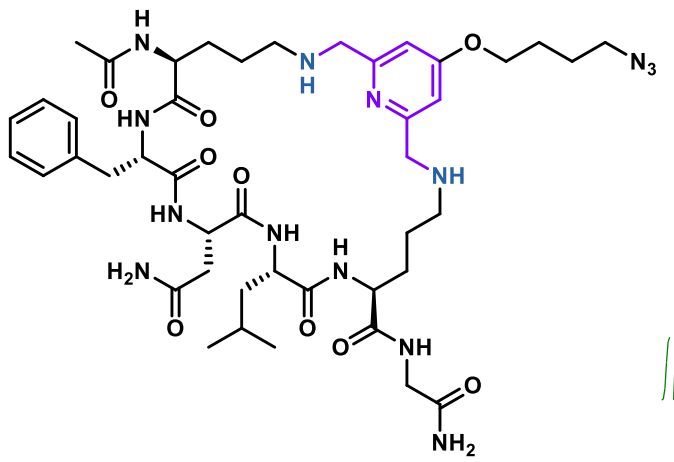



16, HSQC, CD3OD, 700 MHz
f2 (ppm)
f1 (ppm)



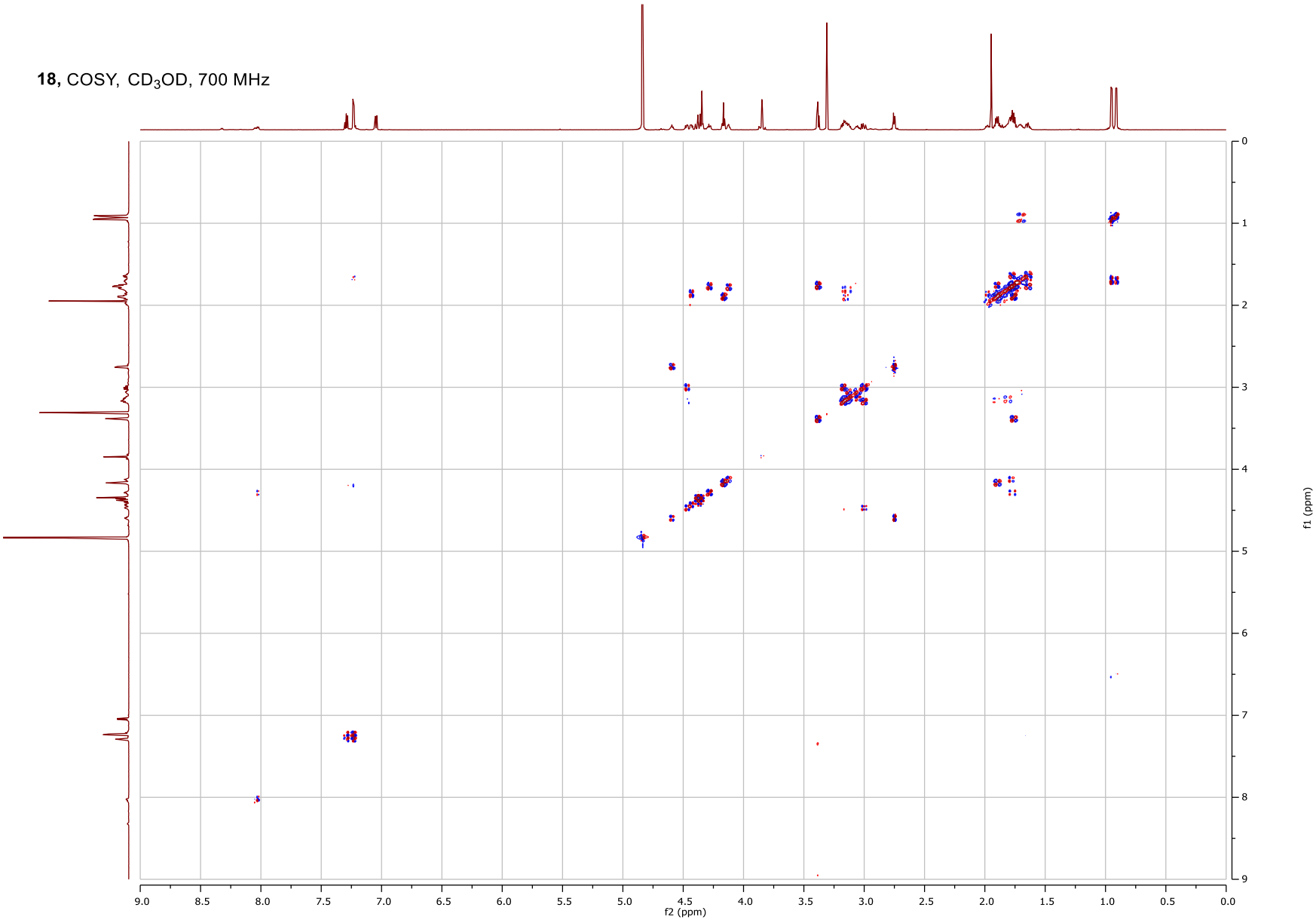
17, ^{1}H, CD_3OD, 700 MHz


17, COSY, CD3OD, 700 MHz
f2 (ppm)
f1 (ppm)

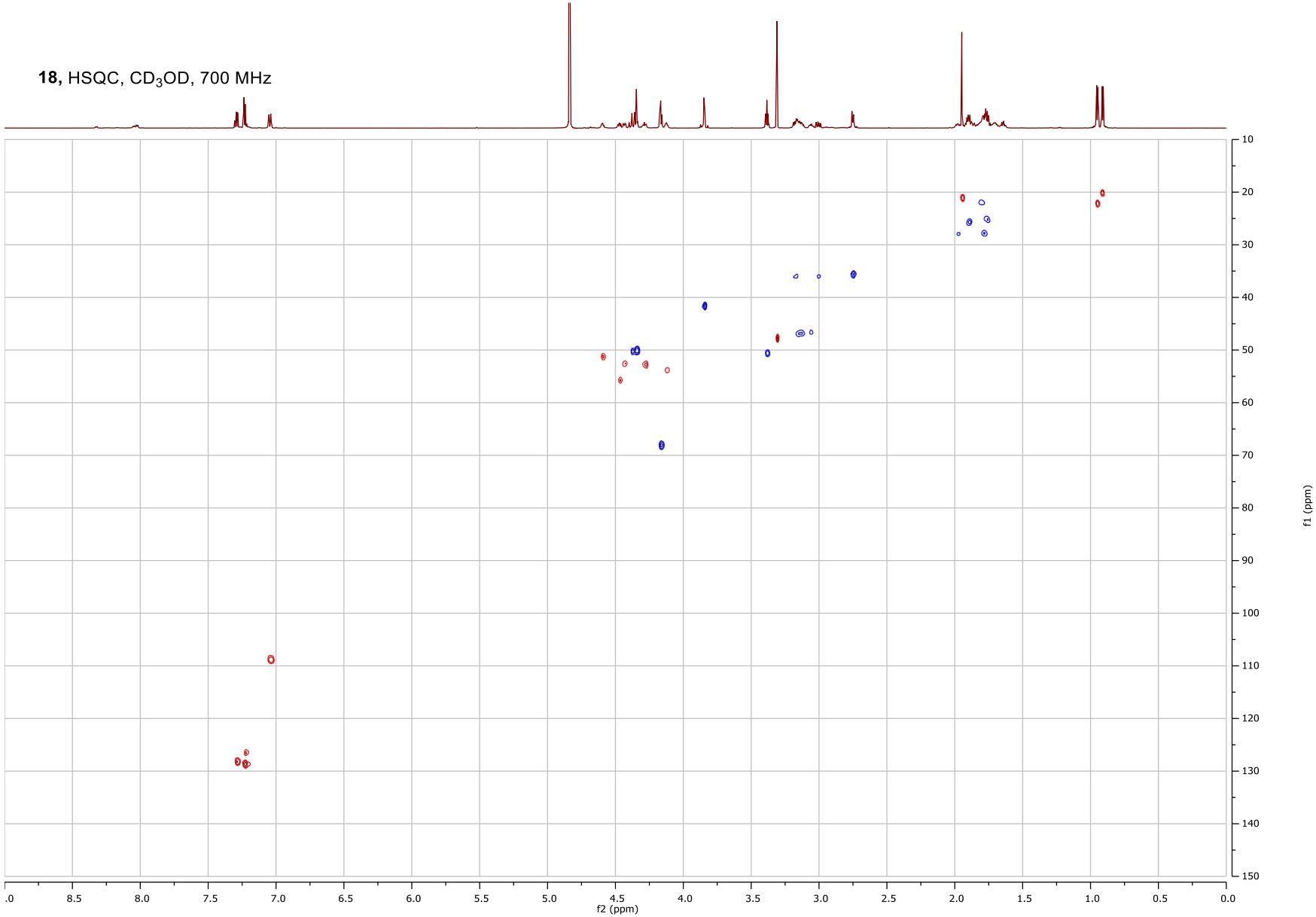


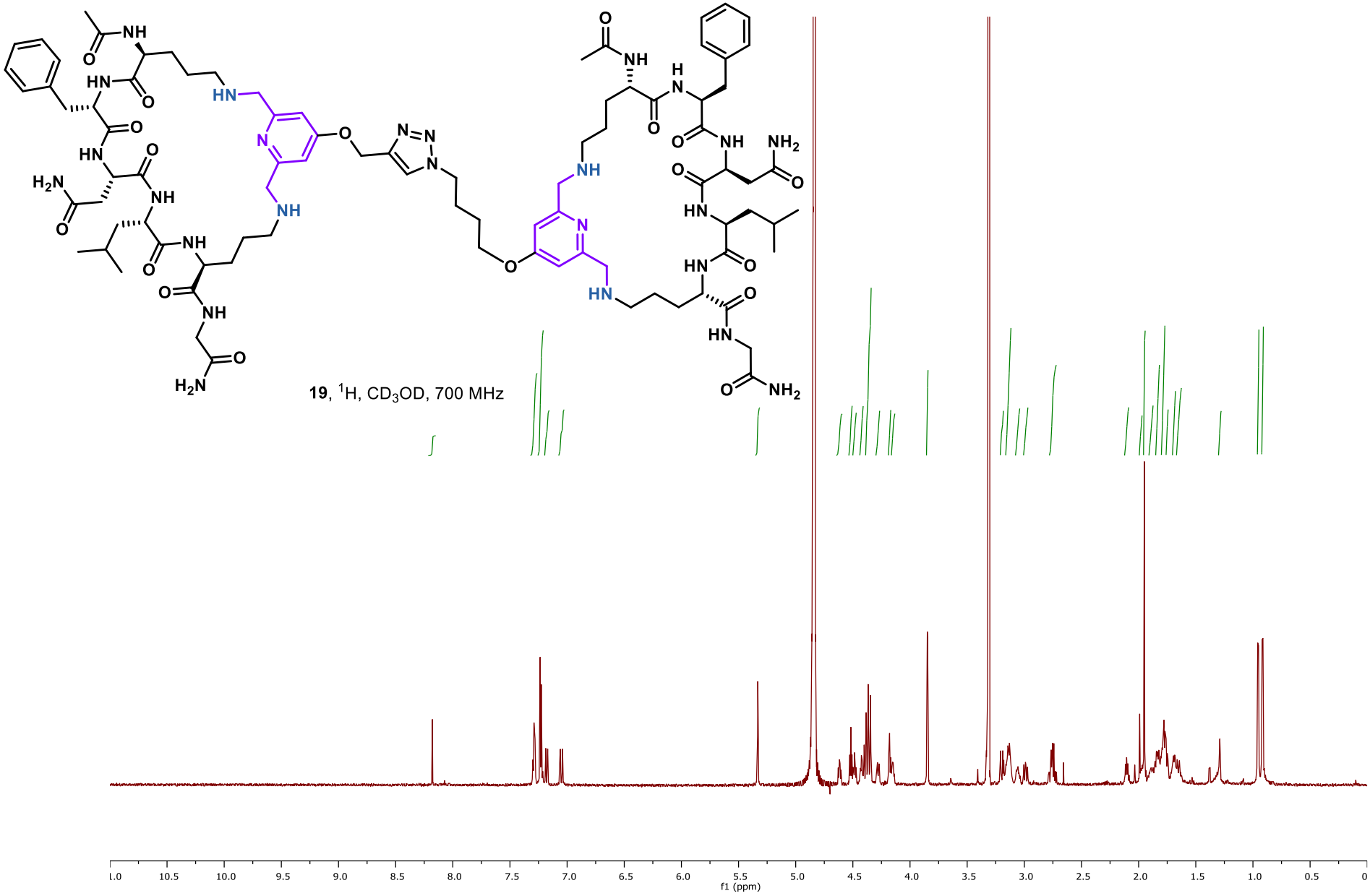
18, ^{1}H, CD_3OD, 700 MHz

18, COSY, CD_3OD, 700 MHz



18, HSQC, CD_3OD, 700 MHz

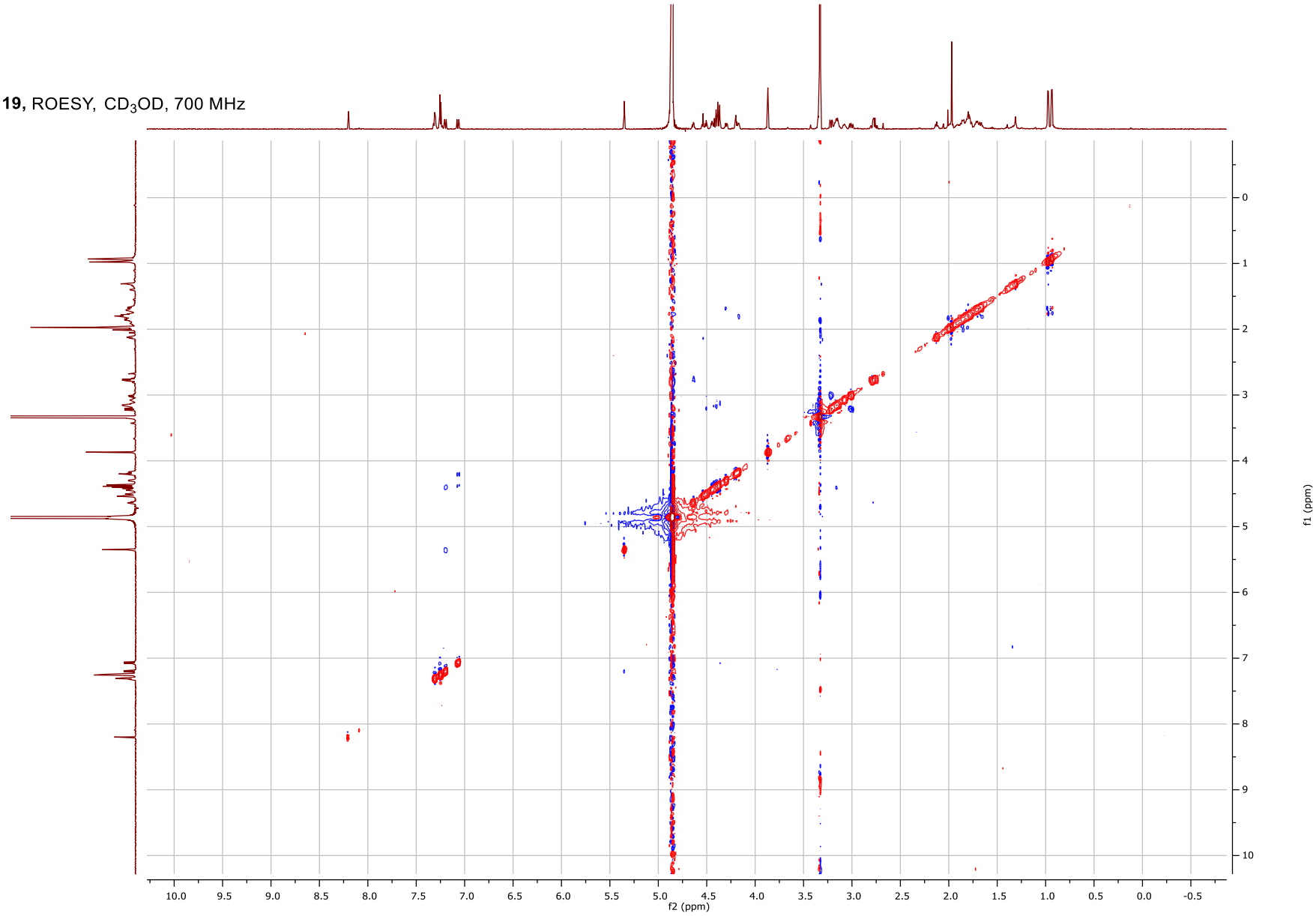


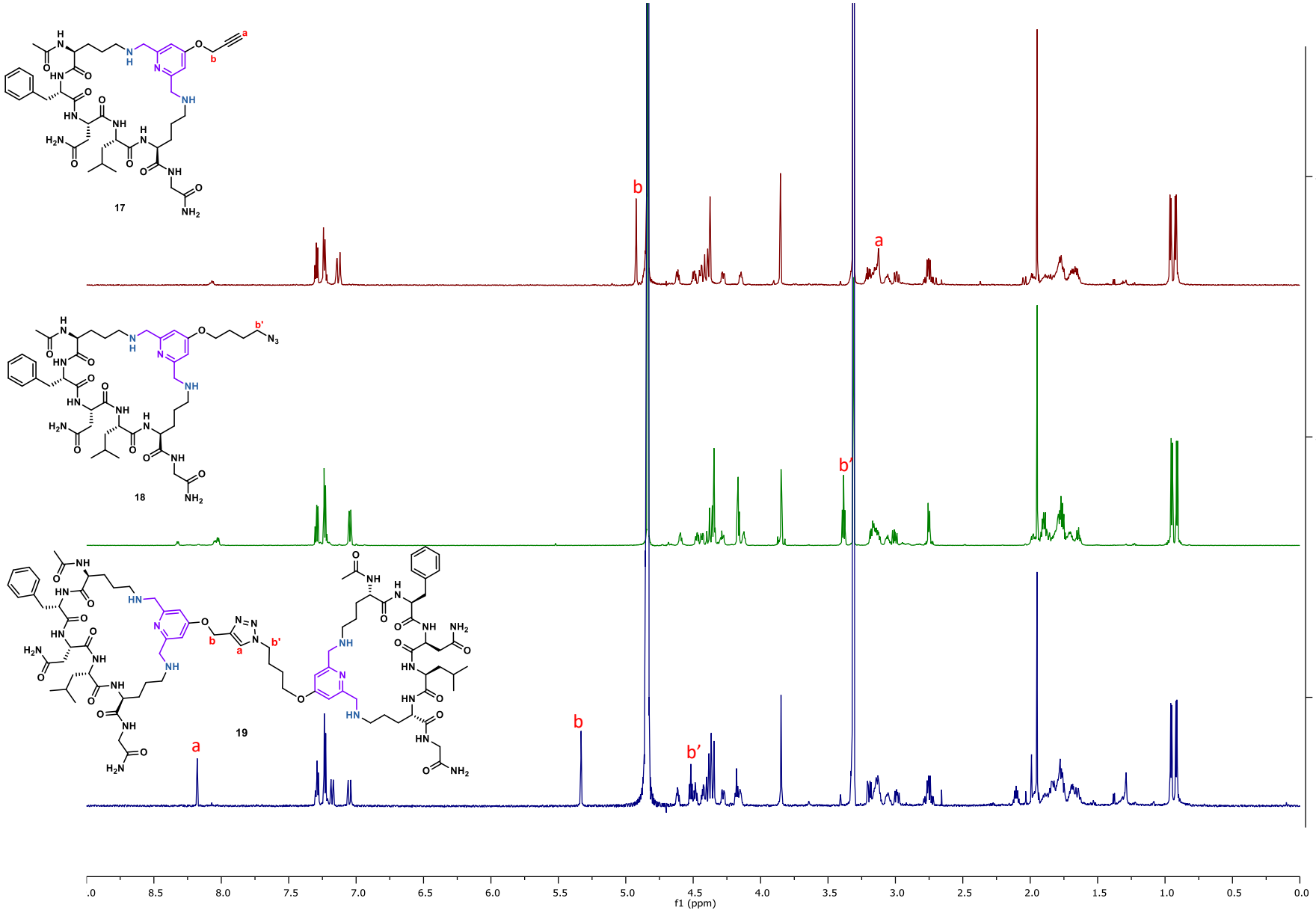

19, 1H, CD3OD, 700 MHz
f1 (ppm)

19, COSY, CD_3OD, 700 MHz

19, TOCSY, CD_3OD, 700 MHz

19, ROESY, CD_3OD, 700 MHz




17
18
19
a
b
b'
f1 (ppm)