SUPPORTING INFORMATION

Modeling linear and cyclic PKS intermediates through atom replacement

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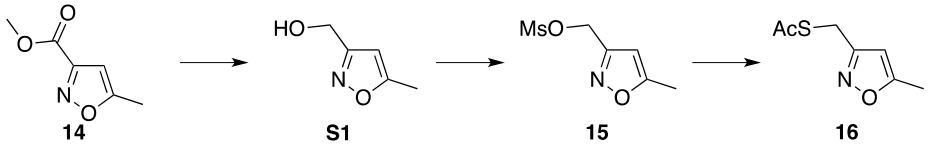
A. General Materials and Methods.

Unless otherwise noted, all reagents and chemical compounds were purchased from Alfa Aesar, Strem Chemicals, Sigma–Aldrich or TCI and used without further purification. Amine **18** was prepared according to [Meier, J. L.; Mercer, A. C.; Rivera, H. Jr.; Burkart, M. D. *J. Am. Chem. Soc.* **2006**, 128, 12174]. Flash chromatography was carried out on 40–63 mesh Geduran Silica Gel 60 (EMD Millipore). Thin layer chromatography (TLC) was conducted on 250 μm Silica Gel 60 F254 glass plates (EMD Millipore). NMR spectra were recorded on a Mercury Plus 400 MHz (Varian), a ECA 500 MHz (Jeol), a DMX 400 MHz (Bruker), a DMX 500 MHz (Bruker) or a VX 500 MHz equipped with XSens cold probe (Varian) spectrometer. FID files were processed using MestRenova version 8.1 (MestreLab Research). NMR spectra were referenced to residual solvent peaks according to S. Budavari, M.J. O'Neil, A. Smith, P.E. Heckelman, The Merck Index, an Encyclopedia of Chemicals, Drugs, and Biologicals, Eleventh Edition, Merck Co., Inc. Rahway, NJ, 1989. Mass spectrometric analyses were conducted on the following instruments: a LCQ Deca (ThermoFinnigan), MAT900XL (ThermoFinnigan), LTQ Orbitrap XL (ThermoScientific), or a LCT Premier (Waters) mass spectrometer. UV spectra were measured with a 1 cm cell on a DU800 (Bruker) spectrophotometer. IR spectra were obtained with a IR100 FT-IR (ThemoNicolet). Reversed-phase HPLC separation was performed using a semi-preparative C18 Luna column (250 x 10 mm) at a flow rate of 2.5 mL/min using 600E pump (Waters) and Lambda-Max model 480 UV detector (Waters). Unless stated otherwise, anhydrous solvents were used for all chemical reactions. Reactions were conducted under Ar atmosphere in a round bottom flask or vial capped with a rubber septa and were stirred using a Teflon coated stir bar. All mixtures are provided as v:v ratios. All protein NMR experiments were conducted on a 500 MHz VS500 spectrometer equipped with an Xsens cryoprobe (Varian) or a 600 MHz Avance system equipped with a cryoprobe (Bruker) at the UC San Diego Biomolecular NMR Facility. Proteins were purified by FPLC gel filtration over a HiPrep 26/60 Sephacryl S-100 HR column (GE Healthcare Life Sciences). Isotopes for NMR experiments were sourced from Cambridge Isotope Laboratories.

B. Chemical Synthesis

B.1. Synthesis of thioacetate 16. A three-step procedure was used to prepare thioester **16** from commercially available **14**.



***S*-((5-methylisoxazol-3-yl)methyl) ethanethioate (16).** The synthesis of thioester **16** was most effectively conducted in a single day through three back-to-back steps from commercially available methyl 5-methylisoxazolecarboxylate (**14**). Ester **14** (25.00 g, 0.177 mol) was dissolved in absolute EtOH (250 mL). After cooling to 0 °C, $NaBH_4$ (18.09 g, 0.478 mol) was added in ~0.5 g portions so that the temperature of the reaction did not exceed 5 °C. After the addition, the mixture was kept at 0 °C for 1 h and slowly warmed over 1.5 h to rt. After 1 h at rt, the flask was cooled to 0 °C with an ice bath and satd. NH_4Cl (200 mL) was added in a drop wise fashion. Once quenched, the mixture was warmed to rt, extracted with EtOAc (3×500 mL), washed with brine, dried with Na_2SO_4, and filtered through a plug of Celite (100 g) washing with EtOAc (1 L) to deliver 19.28 g (96%) of carbinol **S1**.

Carbinol **S1**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.02 (s, 1H), 4.68 (s, 2H), 2.92 (bs, 1H), 2.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.0, 163.9, 100.6, 57.0, 12.4; HRMS *m/z* calcd. for $C_5H_8NO_2$ $[M+H]^+$ 114.0555 found, 114.0551.

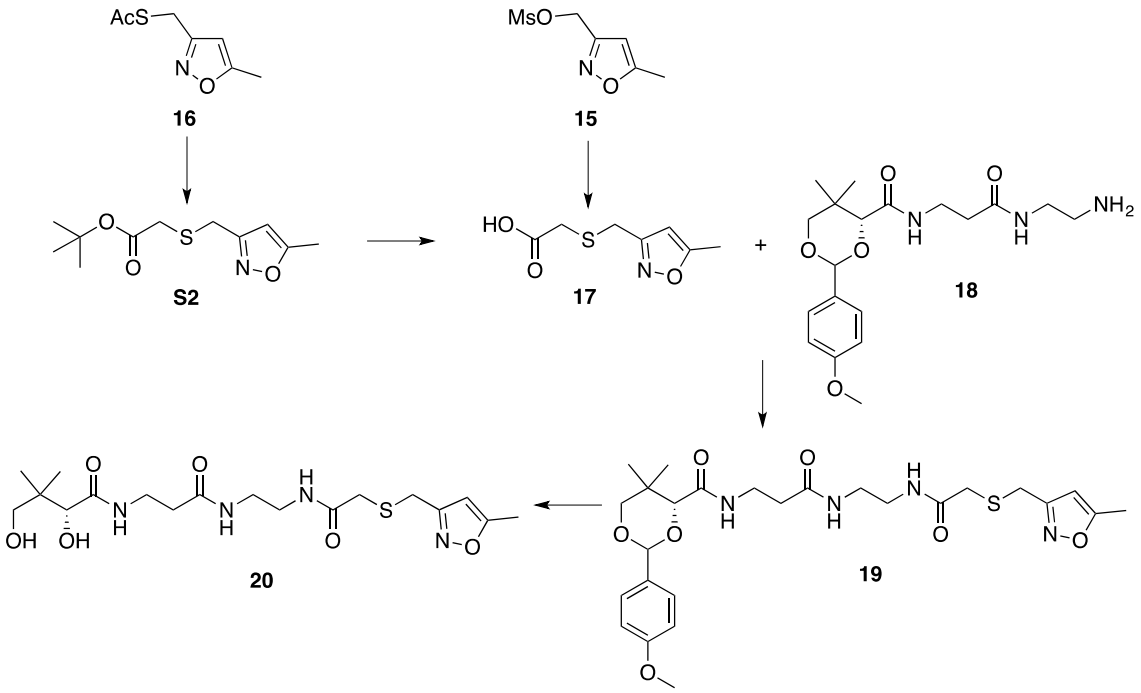
Carbinol **S1** (19.28 g, 0.170 mol) was dried by rotary evaporation of toluene (3×100 mL) and dissolved in dry CH_2Cl_2 (1 L). Freshly distilled Et_3N (31.50 mL, 0.226 mol) was added and the flask was cooled to 0 °C. MsCl (14.00 mL, 0.181 mol) was added drop wise over 20 min. The mixture was kept at 0 °C for 30 min then warmed over 1 h to rt. After 30 min at rt, brine (400 mL) was added. The resulting mixture was extracted with CH_2Cl_2 (3×500 mL), washed with brine, dried with Na_2SO_4, and filtered through a plug of SiO_2 (300 g) washing with a 1:1 CH_2Cl_2:EtOAc (800 mL) to deliver the crude mesylate **15.**

Mesylate **15**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.12 (s, 1H), 5.20 (s, 2H), 3.02 (s, 3H), 2.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.0, 158.2, 101.3, 62.1, 38.1, 12.3; HRMS *m/z* calcd. for $C_6H_{10}NO_4S$ $[M+H]^+$ 192.0331 found, 192.0327.

Immediately after, the entire batch of crude mesylate **15** was dissolved in dry DMF (150 mL). KSAc (20.60 g, 0.180 mol) was added over 5 min as a solid. Within 5 min of the addition, the reaction mixture fused as a solid and became warm (~65 °C). This mixture was allowed to sit for 1 h at which point additional dry DMF (50 mL) was added. After 1.5 h at rt, satd. $NaHCO_3$ (500 mL) was added and the mixture was extracted CH_2Cl_2 (3×500 mL), washed with brine, dried with Na_2SO_4, and filtered through a plug of SiO_2 (300 g) washing with 1:1 CH_2Cl_2:EtOAc (1 L) to deliver the 24.20 g of thioester **16**, which could be used without further purification as the only impurity detectable was ~1% of mesylate **15.** If desired, this material could be purified via flash chromatography to afford 23.10 g of **15** (76% from **14**).

Thioester **16**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.92 (s, 1H), 4.08 (s, 2H), 2.38 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.6, 170.2, 161.2, 101.7, 30.4, 23.9, 12.4; HRMS *m/z* calcd. for $C_7H_9NO_2SNa$ $[M+Na]^+$ 194.0245 found, 194.0246.

B.2. Synthesis of mimetic 11. The synthesis of mimetic **11** was accomplished either from mesylate **15** or thioacetate **16**. The following section provides an overview of the methods and procedures used.



2-(((5-methylisoxazol-3-yl)methyl)thio)acetic acid (17). Thioester **16** (381.2 mg, 2.23 mol) was dissolved in absolute EtOH (22 mL). The solution was degassed by repeated pump and fill with Ar. A degassed solution of NaOH (1.11 mL, 5 N in H_2O, 5.56 mmol) under Ar was added via syringe to this solution at rt. Within 10 min of addition, *tert*-butylbromoacetate (0.35 mL, 2.37 mmol) was added and the mixture was kept at rt for 1 h. The pH was adjusted to 7 by the addition of acetic acid, the mixture was diluted with EtOAc (50 mL) and dried via rotary evaporation. Flash chromatography (hexanes to 2:1 hexanes:EtOAc) afforded 362.0 mg (87%) of acid **17**, as a colorless oil. An additional 32.5 mg (6%) of ester **S2** was also obtained.

Alternatively, acid **17** was prepared from mesylate **15**. Mercaptoacetic acid (1.95 mL, 27.94 mmol) was added to mesylate **15** (4.88 g, 25.52 mmol) in DMF (60 mL). This was followed by sequential addition of NaI (3.83 g, 25.55 mmol) and Et_3N (7.85 mL, 56.27 mmol). The reaction mixture was warmed to 60 °C. After 12 h at 60 °C, the solvent was removed by rotary evaporation. The residue was dissolved in a mixture of EtOAc (100 mL) and H_2O (100 mL). The layers were separated and the water layer was further extracted with EtOAc (3×100 mL). Organic layers were combined and dried over Na_2SO_4. The solvent was removed via rotary evaporation to afford pale yellow solid. Flash chromatography (EtOAc to 9:1 EtOAc:MeOH) afforded 4.23 g (89%) of acid **17**.

Acid **17**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.03 (s, 1H), 3.83 (s, 2H), 3.23 (s, 2H), 2.41 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.8, 170.4, 161.0, 101.6, 32.6, 26.6, 12.5; HR–ESI–TOFMS *m/z* calcd. for $C_7H_9NO_3SNa$ $[M+Na]^+$: 210.0201, found 210.0196.

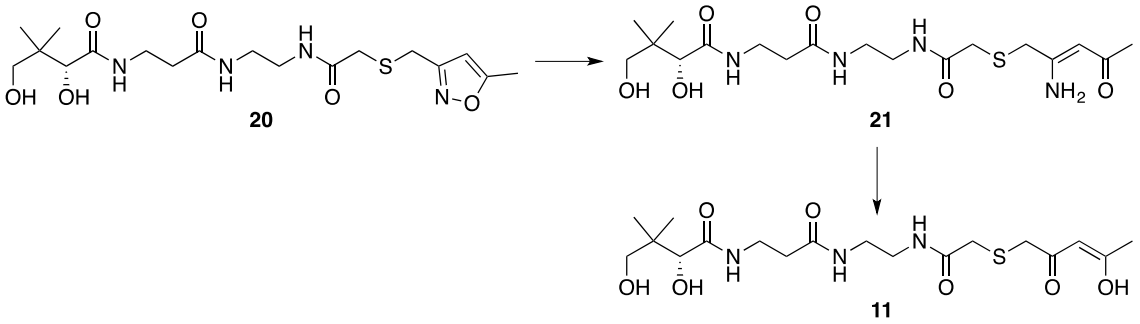
(4*R*)-2-(4-methoxyphenyl)-5,5-dimethyl-*N*-(3-((2-(2-(((5-methylisoxazol-3-yl)methyl)thio)-acetamido)ethyl)amino)-3-oxopropyl)-1,3-dioxane-4-carboxamide (19): EDAC•HCl (768.00 mg, 4.01 mmol) was added to a mixture of acid **17** (300.00 mg, 1.60 mmol) and amine **18** (608.00 mg, 1.60 mmol) dissolved in dry DMF (10 mL). Within 5 min, Et*i*Pr$_2$ (1.40 mL, 8.04 mmol) was added and the mixture was stirred at rt. After 12 h at rt, the solvent was removed by

rotary evaporation. Flash chromatography (EtOAc to 3:1 EtOAc:MeOH) afforded 567.0 mg (64%) of amide **19**, as colorless oil.

Amide **19**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42 (d, *J* = 8.7 Hz, 2H), 7.06 (t, *J* = 6.1 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 5.99 (d, *J* = 1.1 Hz, 1H), 5.45 (s, 1H), 4.07 (s, 1H), 3.81 (s, 3H), 3.70 (d, *J* = 11.4 Hz, 1H), 3.66 (s, 2H), 3.63 (d, *J* = 11.3 Hz, 1H), 3.55 (p, *J* = 6.2 Hz, 2H), 3.34 (m, 4H), 3.07 (s, 2H), 2.44 (t, *J* = 6.2 Hz, 2H), 2.40 (s, 3H), 1.10 (s, 3H), 1.07 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.8, 170.6, 169.6, 169.1, 161.0, 160.3, 130.3, 127.7, 113.8, 101.6, 101.5, 84.0, 78.6, 55.5, 39.9, 39.8, 36.2, 35.1, 35.1, 33.2, 26.4, 22.0, 19.3, 12.5; HR–ESI–TOFMS *m/z* calcd. for $C_{26}H_{36}N_4O_7SNa$ $[M+Na]^+$: 571.2202, found 571.2202.

(*R*)-2,4-dihydroxy-3,3-dimethyl-N-(3-((2-(2-(((5-methylisoxazol-3-yl)methyl)thio)-acetamido)ethyl)amino)-3-oxopropyl)butanamide (20): Amide **19** (567.00 mg, 1.03 mmol) was dissolved in 80% aq. AcOH (10 mL). After 16 h at rt, the solvent was removed via rotary evaporation assisted by azeotropic removal of toluene. Flash chromatography (EtOAc to 4:1 EtOAc:MeOH) afforded 390.0 mg (88%) of mimetic **20**, as a colorless oil.

Mimetic **20**: ^{1}H NMR (CD_3OD, 500 MHz) δ 6.17 (d, *J* = 1.2 Hz, 1H), 3.89 (s, 1H), 3.76 (s, 2H), 3.49 (m, 2H), 3.46 (d, *J* = 11.1 Hz, 1H), 3.38 (d, *J* = 11.0 Hz, 1H), 3.35 (s, 2H), 3.30 (m, 4H), 3.16 (s, 2H), 2.43 (t, *J* = 6.7 Hz, 2H), 2.41 (d, *J* = 1.1 Hz, 3H), 0.91 (s, 6H); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.0, 174.2, 171.9, 171.7, 162.9, 102.7, 77.3, 70.3, 49.8, 40.4, 39.9, 36.6, 36.4, 35.7, 27.3, 21.4, 20.9, 12.0; HRMS *m/z* calcd. for $C_{18}H_{30}N_4O_6SNa$ $[M+Na]^+$: 453.1784, found 453.1780.



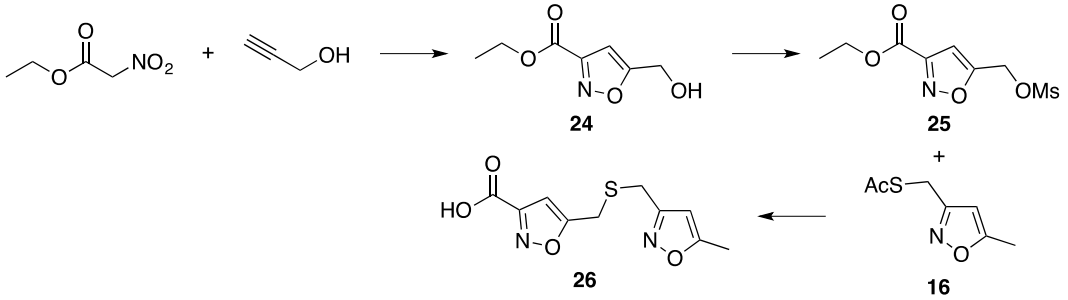
(*R,Z*)-*N*-(3-((2-(2-((2-amino-4-oxopent-2-en-1-yl)thio)acetamido)ethyl)amino)-3-oxopropyl)-2,4-dihydroxy-3,3-dimethylbutanamide (21): Fresh $Mo(CO)_6$ (84.1 mg, 0.32 mmol) was added to **20** (137.0 mg, 0.318 mmol) dissolved in a 25% aq. MeCN (20 mL) at rt. The reaction mixture was warmed to reflux. After 3 h, the reaction was cooled and the solvent removed via rotary evaporation. Flash chromatography (EtOAc to 3:1 EtOAc:MeOH) afforded 64.3 mg (47%) of **21**, as a pale-yellow oil.

Intermediate **21**: ^{1}H NMR (D_2O, 500 MHz) δ 3.96 (s, 1H), 3.52 (m, 2H), 3.49 (d, *J* = 11.5 Hz, 1H), 3.37 (d, *J* = 11.3 Hz, 1H), 3.33 (s, 2H), 3.30 (s, 2H), 3.29 (s, 2H), 3.28 (m, 4H), 2.47 (t, *J* = 6.6 Hz, 2H), 2.03 (s, 3H), 0.90 (s, 3H), 0.87 (s, 3H); ^{13}C NMR (D_2O, 125 MHz) δ 201.8, 177.8, 176.9, 174.9, 165.7, 78.4, 71.0, 51.5, 41.8, 41.3, 41.2, 38.6, 38.1, 38.0, 37.8, 30.9, 23.2, 21.8; HR–ESI–MS *m/z* calcd. for $C_{18}H_{32}N_4O_6SNa$ $[M+Na]^+$: 455.1935, found 455.1936.

(R,Z)-2,4-dihydroxy-N-(3-((2-(2-((4-hydroxy-2-oxopent-3-en-1-yl)thio)acetamido)ethyl)-amino)-3-oxopropyl)-3,3-dimethylbutanamide (11): Intermediate **21** (10.7 mg, 0.025 mmol) was dissolved in 2:2:1 AcOH:H_2O:CH_3CN (1 mL) and stirred at rt. After 3.5 h, the solvent was removed via rotary evaporation. Flash chromatography (1:5 MeOH:EtOAc to 3:5 MeOH:EtOAc) afforded 6.0 mg (56%) of mimetic **11**, as pale-yellow oil.

Mimetic **21**: ^{1}H NMR (CD_3OD, 500 MHz) δ 4.04 (s, 1H), 3.59 (m, 2H), 3.57 (d, *J* = 11.3 Hz, 1H), 3.45 (d, *J* = 11.3 Hz, 1H), 3.40 (m, 4H), 3.37 (s, 2H), 3.36 (s, 2H), 3.31 (s, 2H, minor conformer), 3.30 (s, 2H, minor conformer), 2.55 (m, 2H), 2.35 (s, 1H), 2.11 (s, 3H, minor conformer), 2.08 (s, 3H, minor conformer), 2.04 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.5, 176.1, 174.2, 172.7, 77.3, 70.3, 40.3, 40.0, 38.9, 37.3, 36.6, 36.4, 21.4, 21.0; HR–ESI-TOF–MS *m/z* calcd. for $C_{18}H_{31}N_3O_7SNa$ $[M+Na]^+$: 456.1780 found 456.1775.

B.3. Synthesis of *bis*-isoxazole acid 26. A three-step procedure was developed to access gram quantities of compound **26**. This method was particularly viable for scale due to the fact that acid **26** could be obtained in pure form through acid-base extraction and did not require chromatographic purification.



Ethyl 5-(hydroxymethyl)isoxazole-3-carboxylate (24). Ethylnitroacetate (15.90 mL, 143.23 mmol) and propargyl alcohol (4.35 mL, 75.42 mmol) were dissolved in absolute EtOH (200 mL). DABCO (0.846 g, 7.54 mmol) was added and the mixture was warmed to 80 °C over 30 min. After 72 h at 80 °C, the mixture was dried via rotary evaporation. Flash chromatography (1:1 hexanes:CH_2Cl_2 to CH_2Cl_2 to 10:1 CH_2Cl_2:MeOH) afforded 12.5 g (97%) of carbinol **24**.

Carbinol **24**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.60 (s, 1H), 4.75 (s, 2H), 4.37 (q, *J* = 7.1 Hz, 2H), 4.00 (bs, 1H), 1.35 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.9, 159.9, 156.2, 102.5, 62.4, 56.1, 14.1; HRMS *m/z* calcd. for $C_7H_{10}NO_4$ $[M+H]^+$: 172.0610, found. 171.0608.

Ethyl 5-(((methylsulfonyl)oxy)methyl)isoxazole-3-carboxylate (25). Carbinol **24** (6.41 g, 37.45 mmol) was dried by rotary evaporation of toluene (3×25 mL) and then dissolved in dry CH_2Cl_2 (320 mL). Freshly distilled Et_3N (7.8 mL, 55.91 mol) was added and the flask was cooled to 0 °C. MsCl (3.48 mL, 44.96 mol) was added drop wise over 20 min. The mixture was kept at at 0 °C for 30 min then warmed over 1 h to rt. After 30 min at rt, brine (50 mL) was added. The resulting mixture was extracted with CH_2Cl_2 (3×150 mL), washed with brine, dried with Na_2SO_4, and filtered through a plug of SiO_2 (300 g) washing with 1:1 CH_2Cl_2:EtOAc (500 mL) to deliver the crude 9.31 g (99%) of mesylate **25**, which was used without further purification**.**

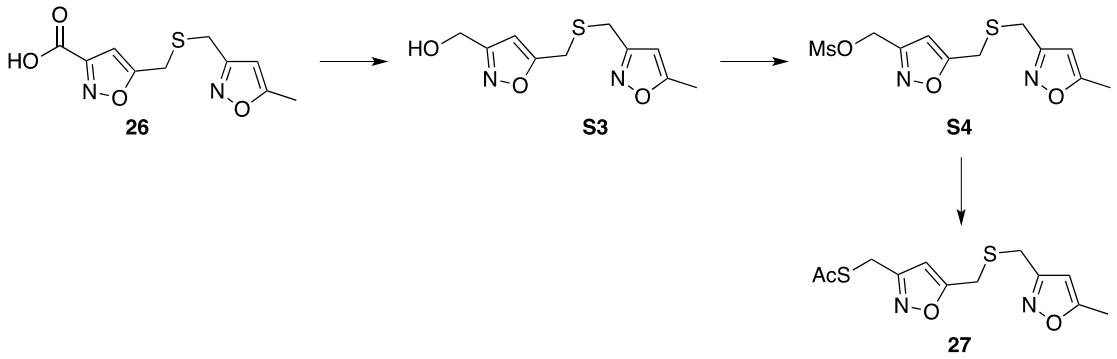
Mesylate **25**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.73 (s, 1H), 5.22 (s, 2H), 4.45 (q, *J* = 7.2 Hz, 2H), 2.13 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.4, 159.3, 156.9, 106.1, 62.7, 59.7, 38.6, 14.2; HRMS *m/z* calcd. for $C_8H_{11}NO_6SNa$ $[M+Na]^+$: 272.0199, found 272.0199.

Sodium 5-((((5-methylisoxazol-3-yl)methyl)thio)methyl)isoxazole-3-carboxylate (26). Thioester **16** (2.30 g, 13.43 mmol) and mesylate **25** (3.04 g, 12.20 mmol) were dissolved in absolute EtOH (140 mL). The solution was degassed by repeated pump and fill with Ar. A degassed solution of NaOH (4.48 mL, 6 N in H_2O, 26.88 mmol) under Ar was added via syringe to this solution at rt over 10 min. The mixture was vigorously stirred for 1.5 h at rt during which time a white precipitate appeared. The resulting mixture was extracted with EtOAc (200 mL). The pH of the aqueous layer was adjusted to 3 by the addition of 1 N HCl and the resulting mixture was extracted with EtOAc (3×100 mL). The combined organic phases were dried with

Na_2SO_4 and concentrated via rotary evaporation to afford 2.63 g (78%) of the *bis*-isoxazole acid salt **26,** as a colorless wax. Samples of **26** contained traces of NaOMs (^{1}H NMR (CD_3OD, 500 MHz) δ 6.47 (s, 1H) and ^{13}C NMR ($CDCl_3$, 125 MHz) δ 39.4) under these conditions, the presence of this material did not interfere with further processing.

Bis-isoxazole acid salt **26**: ^{1}H NMR (CD_3OD, 500 MHz) δ 6.47 (s, 1H), 6.12 (d, *J* = 1.0 Hz, 1H), 3.81 (s, 2H), 3.74 (s, 2H), 2,70 (s, 2H), 2.40 (d, *J* = 0.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.8, 171.2, 163.2, 162.8, 104.1, 102.6, 39.4, 26.9, 26.5, 12.0; HRMS *m/z* calcd. for $C_{10}H_{11}N_2O_4S$ $[M+H]^+$: 255.0177, found 255.0177.

B.4. Synthesis of thioester 27. The preparation of thioacetate **27** was most effectively conducted in through a four-step two-day procedure from acid **26**.



(5-((((5-methylisoxazol-3-yl)methyl)thio)methyl)isoxazol-3-yl)methanol (S3). Acid **26** (2.63 g, 9.53 mmol) was dissolved in toluene (530 mL). After cooling to -80 °C, DIBAL-H (25.86 mL, 31.03 mmol, 1.2 M) was added carefully in a drop wise fashion over 30 min so that the temperature of the reaction did not exceed -70 °C. The mixture was kept at -80 °C for 1 h at which point it was warmed to 0 °C over 1.5 h. After cooling to -20 °C, EtOAc (100 mL) was added carefully, followed satd. solution of Rochelle's salt (200 mL). The mixture was warmed to rt and vigorously stirred for ~4 h when the solution became clear. The resulting mixture was extracted with EtOAc (3×200 mL). The combined organic phases were washed with brine, dried with Na_2SO_4 and concentrated via rotary evaporation to afford a mixture of the corresponding aldehyde and carbinol **S3**. The crude material was dissolved in EtOH (65 mL) and cooled to 0 °C. $NaBH_4$ (784.0 mg, 20.72 mmol) was added in portions over 30 min. The resulting mixture was warmed to rt over 1 h and then extracted with EtOAc (3×200 mL). The combined organic layers were washed with brine, dried with Na_2SO_4 and concentrated via rotary evaporation. Flash chromatography (hexanes to 1:1 hexanes:EtOAc) provided 2.10 g (92%) of **S3**, as a clear wax.

Bis-isoxazole carbinol **S3**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.26 (s, 1H), 5.98 (s, 1H), 4.67 (s, 2H), 3.67 (s, 2H), 3.61 (s, 2H), 3.34 (bs, 1H), 2.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.3, 168.9, 164.0, 161.0, 102.1, 101.4, 56.6, 26.1, 25.5, 12.4; HRMS *m/z* calcd. for $C_{10}H_{12}N_2O_3SNa$ $[M+Na]^+$: 263.0461, found 264.0459.

(5-((((5-methylisoxazol-3-yl)methyl)thio)methyl)isoxazol-3-yl)methyl methane-sulfonate (S4). *Bis*-isoxazole carbinol **S3** (572.3 mg, 2.38 mmol) was dried by rotary evaporation of toluene (3×10 mL) and then dissolved in dry CH_2Cl_2 (25 mL). Freshly distilled Et_3N (0.51 mL, 3.66 mmol) was added and the flask was cooled to 0 °C. MsCl (0.22 mL, 2.84 mmol) was added drop wise over 15 min. The mixture was kept at 0 °C for 30 min then warmed over 1 h to rt. After 30 min at rt, brine (30 mL) was added. The resulting mixture was extracted with CH_2Cl_2 (3×50

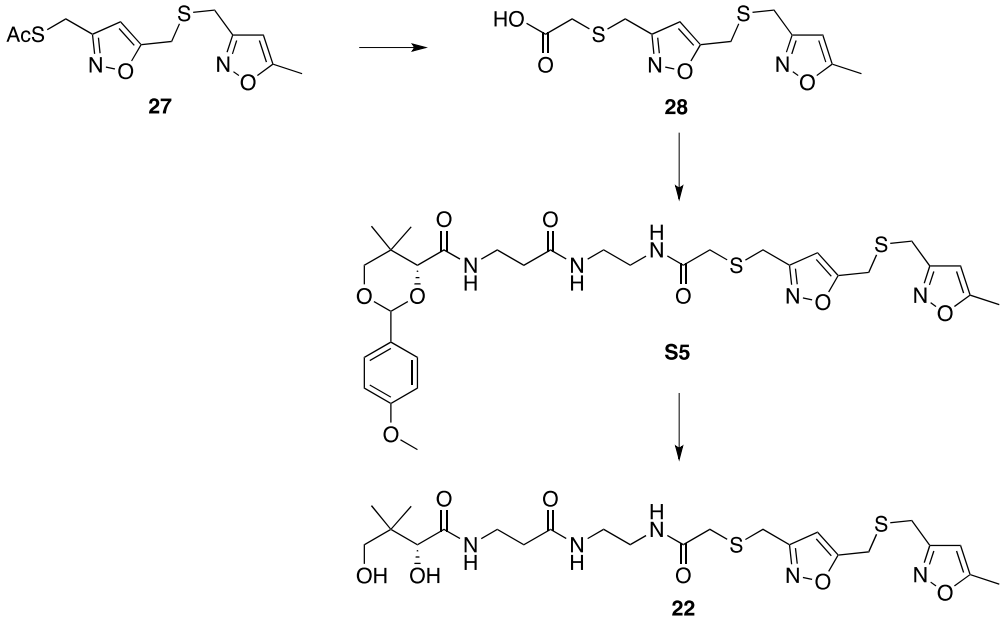
mL), washed with brine, dried with Na_2SO_4, and filtered through a plug of SiO_2 (50 g) washing with 1:1 CH_2Cl_2:EtOAc (200 mL) to deliver the crude *bis*-isoxazole mesylate **S4.**

Bis-isoxazole mesylate **S4**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.36 (s, 1H), 5.98 (dd, *J* = 0.9, 0.7 Hz, 1H), 5.24 (s, 2H), 3.71 (d, *J* = 0.7 Hz, 2H), 3.64 (s, 2H), 3.06 (s, 3H), 2.39 (d, *J* = 0.9, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.3, 170.3, 160.8, 158.2, 102.6, 101.3, 61.9, 38.1, 26.2, 25.4, 12.3; HRMS *m/z* calcd. for $C_{11}H_{14}N_2O_5SNa$ $[M+Na]^+$: 341.0232, found 341.0232.

***S*-((5-((((5-methylisoxazol-3-yl)methyl)thio)methyl)isoxazol-3-yl)methyl) ethane-thioate (27).** The crude *bis*-isoxazole mesylate **S4** was dissolved in dry DMF (5 mL). KSAc (0.38 g, 3.33 mmol) was added over 5 min as a solid. Within 5 min of the addition, the reaction mixture fused as a solid wax and became warm (45-50 °C). This mixture was allowed to sit for 1 h at which point DMF (2.5 mL) was added and the mixture was allowed to sit for 1.5 h until stirring became possible. Satd. $NaHCO_3$ (25 mL) was added and the mixture was extracted with CH_2Cl_2 (3×50 mL), washed with brine, and dried with Na_2SO_4. Flash chromatography (hexanes to 1:1 hexanes:EtOAc) provided 631.8 mg (89%) of **27**, as a clear crystalline solid.

Bis-isoxazole thioacetate **27**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.15 (s, 1H), 5.98 (d, *J* = 1.0 Hz, 1H), 4.11 (s, 2H), 3.68 (s, 2H), 3.66 (s, 2H), 2.42 (d, *J* = 0.9 Hz, 3H), 2.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.4, 170.3, 169.4, 161.2, 161.0, 103.1, 101.4, 30.4, 26.4, 25.7, 23.8, 12.5; HRMS *m/z* calcd. for $C_{12}H_{15}N_2O_3S_2$ $[M+H]^+$: 299.0524, found 299.0523.

B.5 Synthesis of mimetic 22. A three-step procedure was used to prepare mimetic **22** from *bis*-isoxazole thioester **27**.



2-(((5-((((5-methylisoxazol-3-yl)methyl)thio)methyl)isoxazol-3-yl)methyl)thio)acetic acid (28). Thioester **27** (200.5 mg, 0.67 mmol) and *tert*-butylbromoacetate (105.0 µL, 0.71 mmol) were dissolved in absolute EtOH (12 mL). The solution was degassed by repeated pump and fill with Ar. A degassed solution of NaOH (0.34 mL, 6 N in H_2O, 2.04 mmol) under Ar was added via syringe to this solution at rt. The resulting mixture was kept at rt for 1.5 h at which point it was extracted with EtOAc (100 mL). The pH of the aqueous layer was adjusted to 3 by the addition of 1 N HCl and the resulting mixture was extracted with EtOAc (3×60 mL). The

combined organic phases were dried with Na_2SO_4 and concentrated via rotary evaporation to afford 172.4 mg (82%) of *bis*-isoxazole acid **28**, as a colorless oil.

Bis-isoxazole acid **28**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.33 (s, 1H), 6.07 (s, 1H), 3.84 (s, 2H), 3.72 (s, 2H), 3.64 (s, 2H), 3.25 (s, 2H), 2.43 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.5, 170.9, 169.1, 161.7, 161.3, 103.7, 101.9, 34.0, 27.8, 25.7, 25.5, 12.8; HRMS *m/z* calcd. for $C_{12}H_{13}N_2O_4S_2$ $[M-H]^+$: 313.0322, found 313.0325.

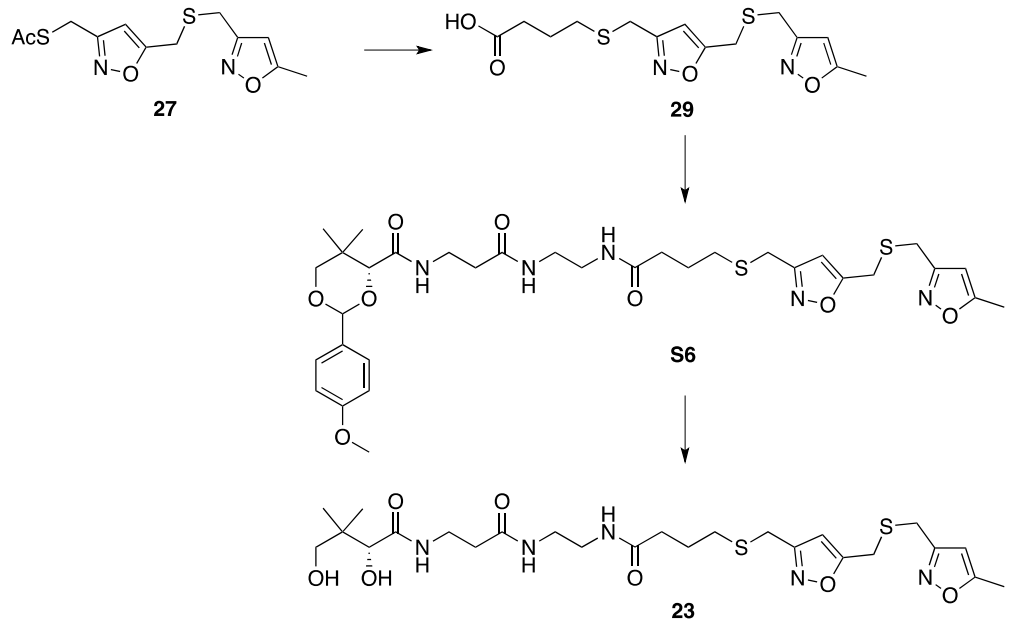
(4*R*)-2-(4-methoxyphenyl)-5,5-dimethyl-*N*-(3-((2-(2-(((5-((((5-methylisoxazol-3-yl)-methyl)thio)methyl)isoxazol-3-yl)methyl)thio)acetamido)ethyl)amino)-3-oxopropyl)-1,3-dioxane-4-carboxamide (S5). EDAC•HCl (324.60 mg, 1.69 mmol) was added to a mixture of acid **28** (213.00 mg, 0.68 mmol) and amine **18** (257.10 mg, 0.68 mmol) dissolved in dry DMF (10 mL). Within 5 min, EtN^iPr_2 (0.60 mL, 3.44 mmol) was added and the reaction mixture was stirred at rt. After 12 h, the solvent was removed by rotary evaporation. Flash chromatography (EtOAc to 4:1 EtOAc:MeOH) afforded 209.0 mg (46%) of amide **S5**, as a colorless oil.

Heptaketide amide **S5**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.98 (s, 1H, NH), 7.65 (t, *J* = 6.0 Hz, 1H, NH), 7.44 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.33 (s, 1H), 6.13 (s, 1H), 5.53 (s, 1H), 4.14 (s, 1H), 3.80 (s, 3H), 3.76 (s, 2H), 3.76 (s, 2H), 3.72 (d, *J* = 11.1 Hz, 1H), 3.71 (s, 2H), 3.67 (d, *J* = 11.3 Hz, 1H), 3.45 (d, *J* = 6.6 Hz, 2H), 3.25 (m, 4H), 3.14 (s, 2H), 2.99 (s, 1H), 2.86 (s, 1H), 2.42 (t, *J* = 6.8 Hz, 2H), 2.40 (s, 3H), 1.10 (s, 3H), 1.03 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) 174.1, 171.8, 171.6, 171.5, 171.4, 162.9, 162.8, 161.7, 131.8, 128.8, 114.5, 103.8, 102.6, 85.1, 79.3, 55.8, 40.4, 39.9, 36.4, 36.4, 36.3, 35.8, 34.0, 27.3, 26.9, 26.4, 22.2, 19.7, 12.1; HR–ESI–TOFMS *m/z* calcd. for $C_{31}H_{41}N_5O_8S_2Na$ $[M+Na]^+$: 698.2294, found 698.2269.

(*R*)-2,4-dihydroxy-3,3-dimethyl-*N*-(3-((2-(2-(((5-((((5-methylisoxazol-3-yl)methyl)-thio)methyl)isoxazol-3-yl)methyl)thio)acetamido)ethyl)amino)-3-oxopropyl)butanamide (22). Amide **S5** (22.8 mg, 0.034 mmol) was dissolved in 80% aq. AcOH (1 mL). After 16 h at rt, the solvent was removed via rotary evaporation assisted by azeotropic removal of toluene. Flash chromatography (EtOAc to 4:1 EtOAc:MeOH) afforded 15.3 mg (81%) of mimetic **22**, as a colorless oil.

Mimetic **22**: ^{1}H NMR (CD_3OD, 500 MHz) δ 6.35 (s, 1H), 6.14 (s, 1H), 3.89 (s, 1H), 3.79 (s, 4H), 3.74 (s, 2H), 3.49 (q, *J* = 6.5 Hz, 2H), 3.46 (d, *J* = 10.8 Hz, 1H), 3.39 (d, *J* = 10.9 Hz, 1H), 3.35 (s, 2H), 3.31 (m, 4H), 3.18 (s, 2H), 2.44 (t, *J* = 6.7 Hz, 2H), 2.41 (d, *J* = 1.0 Hz, 3H), 0.92 (s, 6H); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.0, 174.2, 171.9, 171.8, 171.4, 163.0, 162.9, 103.8, 102.6, 77.3, 70.3, 49.8, 40.4, 39.9, 36.6, 36.4, 35.8, 27.3, 26.9, 26.4, 21.4, 20.9, 12.1; HRMS *m/z* calcd. for $C_{23}H_{35}N_5O_7S_2Na$ $[M+Na]^+$: 580.1876, found 580.1880.

B.6. Synthesis of mimetic 23. A three-step procedure was used to prepare mimetic **23** from *bis*-isoxazole thioester **27**.



4-(((5-((((5-methylisoxazol-3-yl)methyl)thio)methyl)isoxazol-3-yl)methyl)thio)-butanoic acid (29). Thioester **27** (279.0 mg, 0.94 mmol) and *tert*-butyl 4-bromobutanoate (313.0 mg, 1.40 mmol) were dissolved in absolute EtOH (18 mL). The solution was degassed by repeated pump and fill with Ar. A degassed solution of NaOH (0.47 mL, 5 N in H_2O, 2.35 mmol) under Ar was added via syringe to this solution at rt. The resulting mixture was kept at rt for 1.5 h at which point it was extracted with EtOAc (100 mL). The pH of the aqueous layer was adjusted to 3 by the addition of 1 N HCl and the resulting mixture was extracted with EtOAc (3×60 mL). The combined organic phases were dried with Na_2SO_4 and concentrated via rotary evaporation to afford 312.5 mg (97%) of *bis*-isoxazole acid **29**, as a colorless oil.

Bis-isoxazole acid **29**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.26 (s, 1H), 6.02 (d, *J* = 1.1 Hz, 1H), 3.68 (s, 2H), 3.67 (s, 2H), 3.66 (s, 2H), 2.53 (t, *J* = 7.2 Hz, 2H), 2.45 (t, *J* = 7.2 Hz, 2H), 2.42 (d, *J* = 0.9 Hz, 3H), 1.90 (p, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.9, 170.4, 169.1, 162.0, 161.1, 102.9, 101.5, 32.6, 30.7, 26.2, 26.1, 25.7, 23.8, 12.5; HR–ESI–TOFMS *m/z* calcd. for $C_{14}H_{17}N_2O_4S_2$ $[M-H]^+$: 341.0635, found 341.0640.

(4*R*)-2-(4-methoxyphenyl)-5,5-dimethyl-*N*-(3-((2-(4-(((5-((((5-methylisoxazol-3-yl)methyl)thio)methyl)isoxazol-3-yl)methyl)thio)butanamido)ethyl)amino)-3-oxopropyl)-1,3-dioxane-4-carboxamide (S6). EDAC•HCl (28.0 mg, 0.15 mmol) was added to an ice-cold mixture of acid **29** (20.0 mg, 0.06 mmol) and amine **18** (22.1 mg, 0.06 mmol) dissolved in dry CH_2Cl_2 (1 mL). Within 5 min, EtN^iPr_2 (0.05 mL, 0.29 mmol) was added and the reaction mixture was stirred at rt. After 12 h, the solvent was removed by rotary evaporation. Flash chromatography (EtOAc to 4:1 EtOAc:MeOH) afforded 31.8 mg (77%) of **S6**, as a colorless oil.

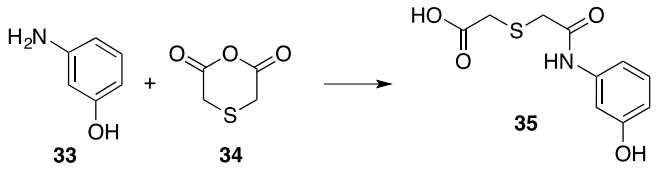
Amide **S6**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.94 (s, 1H, NH), 7.65 (t, *J* = 6.1 Hz, 1H, NH), 7.45 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 6.31 (s, 1H), 6.13 (s, 1H), 5.53 (s, 1H), 5.49 (s, 1H), 4.15 (s, 1H), 3.80 (s, 3H), 3.76 (s, 2H), 3.72 (d, *J* = 11.7 Hz, 1H), 3.71(s, 2H), 3.68 (s, 2H), 3.67 (d, *J* = 11.6 Hz, 1H), 3.48 (m, 3H), 3.35 (s, 3H), 3.23 (m, 3H), 2.47 (t, *J* = 7.2 Hz, 2H), 2.41 (d, *J* = 1.0 Hz, 3H), 2.39(t, *J* = 6.5 Hz, 2H), 2.25 (t, *J* = 7.4 Hz, 2H), 1.84 (p, *J* = 7.4 Hz, 2H), 1.10 (s,

3H), 1.03 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 175.5, 174.1, 171.8, 171.6, 171.2, 163.6, 162.8, 161.7, 131.8, 128.8, 114.5, 103.8, 102.6, 102.5, 85.1, 79.3, 55.7, 49.8, 40.0, 36.4, 36.4, 35.8, 35.8, 34.0, 31.8, 26.9, 26.5, 26.4, 26.1, 22.2, 19.7, 12.1; HR–ESI–TOFMS *m/z* calcd. for $C_{33}H_{45}N_5O_8S_2Na$ $[M+Na]^+$: 726.2607, found 726.2596.

(*R*)-2,4-dihydroxy-3,3-dimethyl-*N*-(3-((2-(4-(((5-(((((5-methylisoxazol-3-yl)methyl)thio)methyl)isoxazol-3-yl)methyl)thio)butanamido)ethyl)amino)-3-oxopropyl)butanamide (23). Amide **S6** (5.8 mg, 8.24 μmol) was dissolved in 80% aq. AcOH (0.2 mL). After 16 h at rt, the solvent was removed via rotary evaporation assisted by azeotropic removal of toluene. Flash chromatography (EtOAc to 3:1 EtOAc:MeOH) afforded 2.8 mg (58%) of mimetic **23**, as a colorless oil.

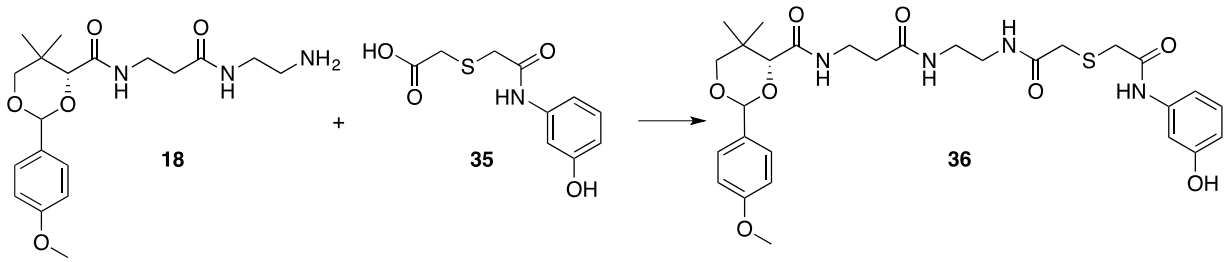
Mimetic **23**: ^{1}H NMR (CD_3OD, 500 MHz) δ 6.33 (s, 1H), 6.14 (s, 1H), 5.49 (s, 1H), 3.89 (s, 1H) 3.78 (s, 2H), 3.73 (s, 2H), 3.70 (s, 2H), 3.48 (q, *J* = 6.7 Hz, 2H), 3.46 (d, *J* = 10.6 Hz, 1H), 3.39 (d, *J* = 11.0 Hz, 1H), 3.35 (s, 2H), 3.27 (m, 3H), 2.50 (t, *J* = 7.2 Hz, 2H), 2.42 (t, *J* = 6.6 Hz, 2H), 2.41 (s, 3H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.87 (p, *J* = 7.5 Hz, 2H), 0.92 (s, 6H); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.1, 175.6, 174.1, 171.8, 171.2, 163.6, 162.9, 103.8, 102.6, 77.3, 70.3, 54.8, 49.8, 40.4, 40.1, 40.0, 36.6, 36.4, 35.8, 31.8, 26.9, 26.5, 26.4, 26.1, 21.4, 20.9, 12.1; HRMS *m/z* calcd. for $C_{25}H_{39}N_5O_7S_2Na$ $[M+Na]^+$: 608.2189, found 608.2200.

B.7. Synthesis of mimetic 30. The first mimetic **30** as shown in Scheme 3 of the manuscript were prepared through a three to four step sequence. The following section provides experimental procedures for these steps.



2-(2-(3-Hydroxyphenylamino)-2-oxoethylthio)acetic acid (35). A mixture of *m*-aminophenol (**33**) (1.14 g, 10.46 mmol) and thiodiglycolic anhydride (**34**) (1.32 g, 10 mmol) was dissolved in dry DMF (5 mL). After 16 h at rt, the solvent was removed via rotary evaporation to afford pale yellow solid residue. Flash chromatography (1:1 hexanes:EtOAc to 9:1 EtOAc:MeOH) afforded 2.37 g (97%) of acid **35**.

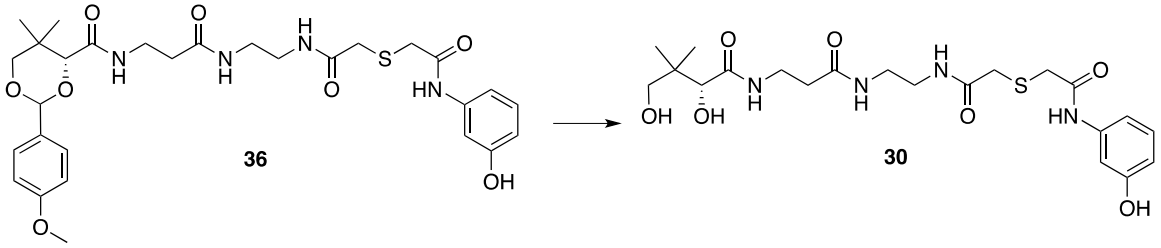
Acid **35**: TLC (2:1 EtOAc/MeOH): R_f = 0.6; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.63 (brs, 1H), 9.95 (s, 1H), 9.37 (s, 1H), 7.16 (t, *J* = 2.1 Hz, 1H), 7.06 (t, *J* = 8.1 Hz, 1H), 6.92 (ddd, *J* = 1.0, 1.9, 8.0 Hz, 1H), 6.45 (ddd, *J* = 1.0, 2.2, 8.1, 1H), 3.42 (s, 2H), 3.39 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.1, 167.3, 157.6, 140.0, 129.4, 110.5, 109.9, 106.3, 35.0, 33.7; HR–ESI–TOFMS *m/z* calcd. for $C_{10}H_{10}NO_4S$ $[M-H]^-$: 240.0331, found 240.0334.



(4R)-N-(3-(2-(2-(2-(3-Hydroxyphenylamino)-2-oxoethylthio)acetamido)ethylamino)-3-oxopropyl)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamide (36). EDAC•HCl

(1.77 g, 9.23 mmol) was added to a mixture of acid **35** (916 mg, 3.8 mmol) and amine **18** (1.44 g, 3.8 mmol) dissolved in dry DMF (10 mL). EtN^iPr_2 (3.22 mL, 18.47 mmol) was added at rt. After 12 h at rt, the solvent was removed by rotary evaporation. Flash chromatography (EtOAc to 9:1 EtOAc:MeOH) provided 720.0 mg (32%) of amide **36**, as colorless oil.

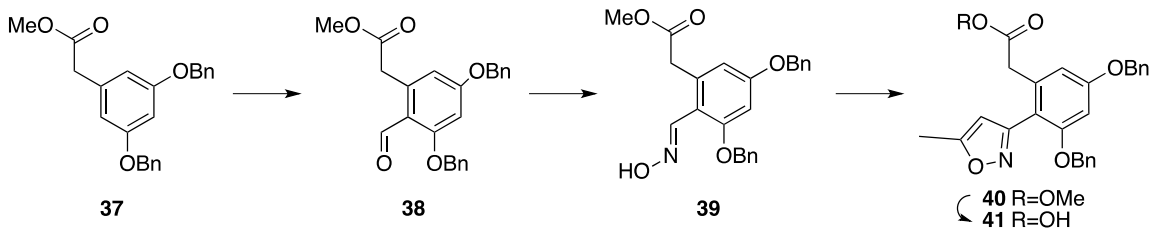
Amide **36**: TLC (9:1 EtOAc/MeOH): R_f = 0.6; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.43 (d, *J* = 8.7 Hz, 2H), 7.17 (t, *J* = 2.2 Hz, 1H), 7.09 (t, *J* = 8.1 Hz, 1H), 6.94 (ddd, *J* = 1.0, 2.0, 8.1 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.53 (ddd, *J* = 1.0, 2.4, 8.1 Hz, 1H), 5.50 (s, 1H), 4.12 (s, 1H), 3.77 (s, 3H), 3.69 (d, *J* = 11.5 Hz, 1H), 3.65 (d, *J* = 11.4 Hz, 1H), 3.46 (t, *J* = 6.6 Hz, 2H), 3.37 (s, 2H), 3.34 (s, 2H), 3.30 (m, 4H), 3.24 (m, 2H), 2.38 (t, *J* = 6.6 Hz, 2H), 1.08 (s, 3H), 1.01 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.1, 172.0, 171.6, 170.0, 161.7, 159.0, 140.6, 131.8, 130.6, 128.8, 114.5, 112.4, 112.3, 108.4, 102.6, 85.1, 79.3, 55.7, 49.6, 40.4, 39.8, 37.6, 36.7, 36.5, 36.3, 34.0, 22.2,19.7; HR–ESI–TOFMS *m/z* calcd. for $C_{29}H_{38}N_4O_8SNa$ $[M+Na]^+$: 625.2308, found 625.2319.



(R)-2,4-Dihydroxy-N-(3-(2-(2-(2-(3-hydroxyphenylamino)-2-oxoethylthio)-acetamido)-ethylamino)-3-oxopropyl)-3,3-dimethylbutanamide (30): Amide **36** (50.0 mg, 0.082 mmol) was dissolved in 80% aq. AcOH (1 mL). After 16 h at rt, the solvent was removed via rotary evaporation assisted by azeotropic removal of toluene. Flash chromatography (EtOAc to 4:1 EtOAc:MeOH) afforded 24.6 mg (62%) mimetic **30**, as a colorless oil.

Mimetic **30**: TLC (1:2 MeOH/EtOAc): R_f = 0.6; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.17 (t, *J* = 2.1 Hz, 1H), 7.10 (t, *J* = 8.1 Hz, 1H), 6.95 (dd, *J* = 2.6, 7.9 Hz, 1H), 6.54 (dd, *J* = 2.4, 8.2 Hz, 1H), 3.89 (s, 1H), 3.48 (m, 2H), 3.45 (d, *J* = 11.4 Hz, 1H), 3.41 (s, 2H), 3.37 (d, *J* = 10.9 Hz, 1H), 3.35 (s, 2H), 3.28 (m, 4H), 2.40 (t, *J* = 6.6 Hz, 2H), 0.91 (s, 6H); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.0, 174.2, 172.1, 170.1, 158.9, 140.6, 130.6, 112.5, 112.3, 108.4, 77.3, 70.3, 40.4, 40.3, 39.9, 37.7, 36.7, 36.6, 36.4, 21.4, 21.0; HR–ESI–TOFMS *m/z* calcd. for $C_{21}H_{32}N_4O_7SNa$ $[M+Na]^+$: 507.1889, found 507.1871.

B.8. Synthesis of mimetic 31. The synthesis of **31** began with the preparation of isoxazole intermediate **41** in 4-steps from ester **37**, as shown in Scheme 4 of the manuscript. A three-step procedure was then used to convert **41** into mimetic **31**. The following section provides experimental procedures for these steps.



Methyl 2-(3,5-bis(benzyloxy)-2-formylphenyl)acetate (38). Ester **37** (3.0 g, 8.23 mmol) was dissolved in DMF (4 mL) and heated to 50 °C. $POCl_3$ (1.16 ml, 12.4 mmol) was added and the

reaction mixture was heated to 100 °C. After 10 min, the reaction was cooled rt. After 16 h at rt, the mixture was cooled to 0˚C and 10% aq. NaOAc (20 mL) was added. After 3 h, the reaction was filtered to give a tan solid. Recrystallization from 95% EtOH afforded 2.25 g (70%) of aldehyde **38**, as a white solid.

Aldehyde **38**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.53 (s, 1H), 7.41 (t, *J* = 4.6 Hz, 4H), 7.37 (m, 6H), 6.59 (d, *J* = 2.3 Hz, 1H), 6.46 (d, *J* = 2.3 Hz, 1H), 5.11 (s, 2H), 5.09, (s, 2H), 3.96 (s, 2H), 3.72 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.1, 171.6, 164.5, 163.9, 139.3, 135.8, 135.8, 128.2, 128.8, 128.5, 128.4, 127.7, 127.4, 117.4, 111.2, 99.2, 70.7, 70.4, 52.0, 40.7; HRMS *m/z* calcd. for $C_{24}H_{22}O_5Na$ $[M+Na]^+$: 413.1350, found 413.1360.

Methyl (E)-2-(3,5-bis(benzyloxy)-2-((hydroxyimino)methyl)phenyl)acetate (39). NaOAc (189.5 mg, 2.31 mmol) and NH_2OH•HCl (97.9 mg, 1.41 mmol) were added sequentially to a suspension of aldehyde **38** (500 mg, 1.28 mmol) in MeOH (9 mL). The resulting reaction mixture was heated to reflux for 1 h. The reaction was allowed to cool to rt and the solvent was removed by rotary evaporation. The resulting solid was dissolved in CH_2Cl_2 (15 mL) and washed with H_2O (2 × 15 mL), brine (15 mL) and the organic phase was dried over Na_2SO_4. The solvent was removed to yield oxime **39**, which was used without further purification. Analytical samples were purified by flash column chromatography (hexanes to 4:1 hexanes:EtOAc).

Oxime **39**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.57 (s, 1H), 7.68 (bs, 1H), 7.39 (m, 6H), 7.35 (m, 4H), 6.56 (d, *J* = 2.3 Hz, 1H), 6.49 (d, *J* = 2.3 Hz, 1H), 5.40 (s, 4H), 3.91 (s, 2H), 3.67 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.1, 171.6, 164.5, 163.9, 139.3, 135.8, 128.8, 128.5, 128.4, 127.7, 127.4, 117.4, 111.2, 99.2, 70.7, 70.4, 52.0, 40.7.

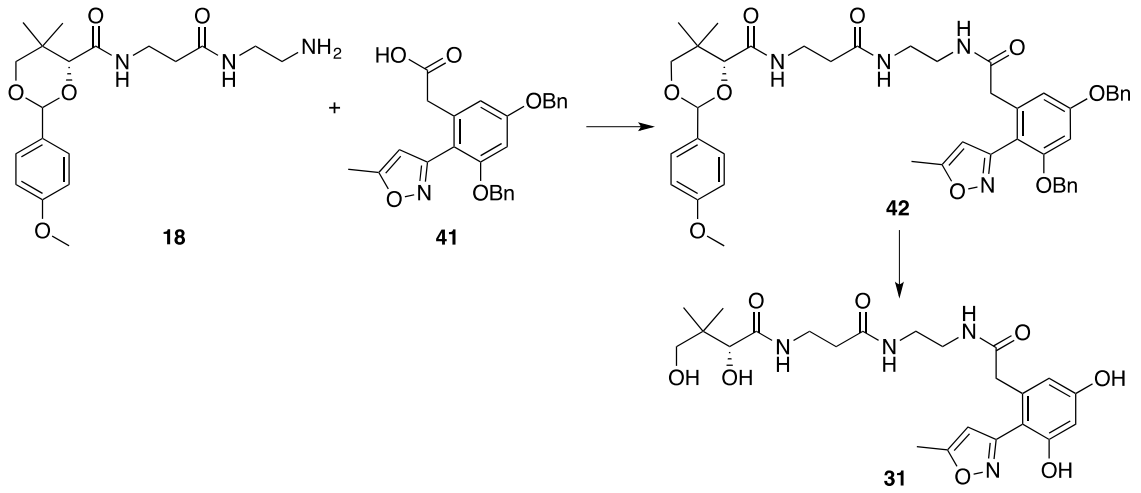
Methyl 2-(3,5-bis(benzyloxy)-2-(5-methylisoxazol-3-yl)phenyl)acetate (40). Et_3N (0.20 mL, 1.41 mmol) was added to a solution of oxime **39** in $CHCl_3$ (6 mL) and the solution was cooled to 0 °C. *N*-Chlorosuccinimide (88.3 mg, 1.41 mmol) was added. After 1.5 h at 0 °C, isopropenyl acetate (1.41 ml, 12.8 mmol) was added and the reaction mixture was heated to 50 °C for 18 h. The reaction mixture was allowed to cool, washed with water (3 × 5 mL), brine (5 mL) and dried over Na_2SO_4. The solvent was removed via rotary evaporation to afford isoxazole **40**, which was used without further purification. Analytical samples were purified by flash column chromatography (20:1 hexanes:EtOAc to 3:1 hexanes:EtOAc).

Isoxazole **40**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 (m, 6H), 7.32 (m, 4H), 6.61 (d, *J* = 2.4 Hz, 1H), 6.59 (d, *J* = 2.4 Hz, 1H), 6.13 (d, *J* = 1.1 Hz, 1H), 5.05 (s, 2H), 5.05 (s, 2H), 3.73 (s, 2H), 3.64 (s, 3H), 2.44 (d, *J* = 1.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.9, 168.3, 160.3, 159.3, 158.2, 136.7, 136.5, 136.1, 128.7, 128.6, 128.2, 127.8, 127.7, 126.9, 112.6, 108.7, 104.6, 100.0, 70.5, 70.2, 52.1, 39.6, 12.4; HRMS *m/z* calcd. for $C_{27}H_{25}NO_5Na$ $[M+Na]^+$: 466.1625, found 466.1626.

2-(3,5-Bis(benzyloxy)-2-(5-methylisoxazol-3-yl)phenyl)acetic acid (41). A 1 M solution of NaOH (7 mL) was added to isoxazole **40** in MeOH (7 mL) and the mixture was heated to 50 °C. After 2 h, the mixture was allowed to cool to rt and the MeOH was removed by rotary evaporation. The resulting aqueous reaction mixture was neutralized with 1 M HCl and extracted with EtOAc (3 × 15 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated via rotary evaporation. The crude mixture was purified by flash column chromotagraphy (100:1 $CHCl_3$:MeOH to 25:1 $CHCl_3$:MeOH) to afford 365.0 mg (65% over 3 steps from **37**) of acid **41**, as a white solid.

Acid **41**: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.16 (bs, 1H), 7.46 (m, 2H), 7.40 (m, 2H), 7.35 (m, 5H), 7.29 (m, 1H), 6.78 (d, *J* = 2.3 Hz, 1H), 6.68 (d, *J* = 2.3 Hz, 1H), 6.24 (d, *J* = 1.0 Hz, 1H), 5.13 (s, 2H), 5.11 (s, 2H), 3.52 (s, 2H), 2.41 (d, *J* = 1.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz)

δ 172.0, 168.1, 159.7, 158.9, 157.4, 136.9, 136.7, 136.7, 128.5, 128.5, 128.0, 128.0, 127.8, 127.2, 111.8, 109.5, 104.5, 99.3, 69.6, 69.5, 11.8; HRMS *m/z* calcd. for $C_{26}H_{23}NO_5Na$ $[M+Na]^+$: 452.1468, found 452.1471.



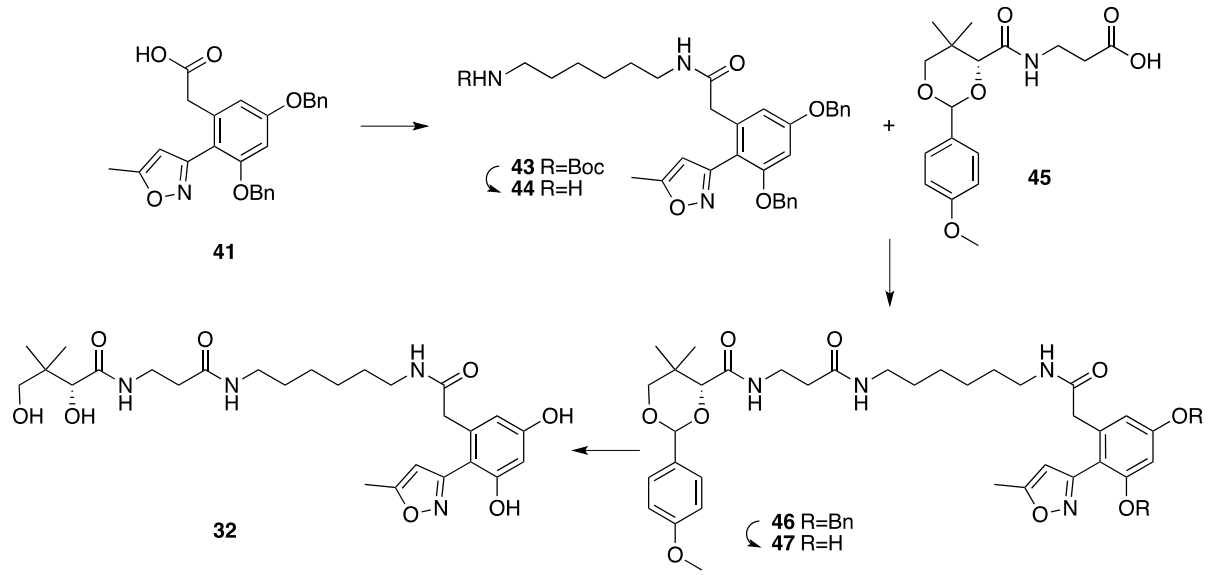
(4*R*)-*N*-(3-((2-(2-(3,5-Bis(benzyloxy)-2-(5-methylisoxazol-3-yl)phenyl)acetamido)-thyl)amino)-3-oxopropyl)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamide (42). EtN^iPr_2 (0.085 mL, 0.49 mmol) was added to a solution of acid **41** (70.0 mg, 0.16 mmol) and HATU (93.2 mg, 0.25 mmol) in DMF (1 mL). After 10 min at rt, amine **18** (74.4 mg, 0.196 mmol) was added to the resulting reaction mixture. After 18 h at rt, the solvent was removed by rotary evaporation. The crude material was purified by flash column chromatography (100:1 CH_2Cl_2:MeOH to 33:1 CH_2Cl_2:MeOH) to afford 73.3 mg (57%) of amide **42**, as a white solid.

Amide **42**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50 (m, 2H), 7.44 (m, 4H), 7.38 (m, 6H), 7.31 (t, J = 1.7 Hz, 1H), 7.29 (m, 1H), 7.22 (m, 1H), 6.91 (t, J = 8.8 Hz, 2H), 6.77 (d, J = 2.4 Hz, 1H), 6.76 (d, J = 2.3 Hz, 1H), 6.29 (d, J = 0.9 Hz, 1H), 5.51 (s, 1H), 5.15 (s, 4H), 4.05 (s, 1H), 3.79 (s, 3H), 3.69 (d, J = 11.0 Hz, 1H), 3.63 (d, J = 11.2 Hz, 1H), 3.42 (s, 2H), 3.41 (m, 2H), 3.21 (m, 4H), 2.45 (d, J = 0.9 Hz, 3H), 2.34 (t, J = 6.5 Hz, 2H), 1.07 (s, 3H), 1.03 (s, 3H); 13C NMR (CDCl3, 125 MHz) δ 171.9, 171.0, 169.4, 169.4, 161.4, 161.0, 160.4, 159.1, 138.7, 138.1, 131.9, 129.5, 129.4, 129.3, 128.9, 128.7, 128.6, 128.6, 128.1, 114.2, 112.8, 109.8, 105.7, 102.0, 100.3, 84.6, 78.9, 71.0, 70.7, 55.6, 42.3, 39.9, 36.4, 35.7, 33.7, 22.2, 19.6, 12.2; HRMS *m/z* calcd. for $C_{45}H_{50}N_4O_9$ $[M+Na]^+$: 813.4270, found 813.3472.

(*R*)-*N*-(3-((2-(2-(3,5-Dihydroxy-2-(5-methylisoxazol-3-yl)phenyl)acetamido)ethyl)amino)-3-oxopropyl)-2,4-dihydroxy-3,3-dimethylbutanamide (31). Amide **42** (38.0 mg, 0.048 mmol) was dissolved in MeOH (2 mL) and 5% Pd on C (10.2 mg, 0.0048 mmol) was added as a powder. The reaction mixture degassed and charged with a H_2 atmosphere. After 4.5 h at rt, the mixture was filtered through a pad of Celite (1 g) and concentrated by rotary evaporation. The resulting tan solid was washed sequentially with hexanes (2 mL) and CH_2Cl_2 (2 mL) providing 13.5 mg (57%) of mimetic **31**, which used without further purification.

Mimetic **30**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.36 (d, *J* = 2.2 Hz, 1H), 6.34 (d, *J* = 2.3 Hz, 1H), 6.25 (d, *J* = 0.9 Hz, 1H), 3.87 (s, 1H), 3.45 (m, 2H), 3.43 (d, *J* = 12.2 Hz, 1H), 3.41 (s, 2H), 3.38 (d, *J* = 11.0 Hz, 1H), 3.24 (m, 4H), 2.45 (d, *J* = 0.9 Hz, 3H), 2.40 (t, *J* = 6.6 Hz, 2H), 0.91 (s, 3H), 0.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.5, 172.9, 172.7, 168.8, 160.1, 159.1, 157.3, 136.3, 109.1, 108.0, 104.4, 101.3, 79.5, 68.9, 48.5, 40.8, 38.9, 38.5, 35.2, 34.9, 19.9, 19.7, 10.7; HRMS *m/z* calcd. for $C_{23}H_{32}N_4O_8$ $[M-H]^-$: 491.2147, found 491.2151.

B.9. Synthesis of mimetic 32. A five-step procedure was used to convert acid intermediate **41** into mimetic **32**. The following section provides experimental procedures for these steps.



***tert*-Butyl (6-(2-(3,5-bis(benzyloxy)-2-(5-methylisoxazol-3-yl)phenyl)acetamido)hexyl)-carbamate (43).** EtN^iPr_2 (0.24 ml, 1.40 mmol) was added to a solution of acid **41** (200.0 mg, 0.47 mmol) and HATU (265.8 mg, 0.70 mmol) in DMF (2 mL). After 10 min at rt, *N*-boc-1,6-hexanediamine (110.8 mg, 0.51 mmol) was added. After 18 h at rt, the solvent was removed by rotary evaporation. The crude material was purified by flash column chromatography (10:1 hexanes:EtOAc to 1:1 hexanes:EtOAc) to afford 220.3 mg (75%) of Boc-amide **43**, as a white solid.

Boc-amide **43**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.61 (m, 1H), 7.42 (m, 2H), 7.38 (m, 2H), 7.34 (m, 4H), 7.29 (m, 2H), 6.82 (d, *J* = 2.3 Hz, 1H), 6.57 (d, *J* =2.3, Hz, 1H), 6.18 (d, *J* = 1.1 Hz, 1H), 5.06 (s, 2H), 5.04 (s, 2H), 3.45 (s, 2H), 3.18 (q, *J* = 6.6 Hz, 2H), 3.06 (q, *J* = 6.7 Hz, 2H), 2.46 (d, *J* = 0.9 Hz, 3H), 1.47 (m, 4H), 1.43 (s, 9H), 1.29 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.0, 168.4, 160.6, 159.6, 158.2, 156.1, 138.3, 136.5, 136.5, 128.7, 128.6, 128.3, 128.0, 127.9, 127.0, 111.2, 107.1, 105.1, 100.0, 79.1, 70.4, 70.3, 42.2, 40.5, 39.5, 30.0, 29.3, 28.5, 26.5, 26.4, 12.4; HRMS *m/z* calcd. for $C_{37}H_{45}N_3O_6Na$ $[M+Na]^+$: 650.3199, found 650.3201

(4*R*)-*N*-(3-((6-(2-(3,5-bis(benzyloxy)-2-(5-methylisoxazol-3-yl)phenyl)acetamido)-hexyl)amino)-3-oxopropyl)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamide (46). Boc-amide **43** (60.0 mg, 0.096 mmol) was dissolved in CH_2Cl_2 (2 mL) and 4 M HCl in dioxanes (100 µL) was added at rt. After 3 h at rt, the solvent was removed to afford amine **44**. EtN^iPr_2 (0.075 ml, 0.430 mmol) was added to a solution of acid **45** (32.4 mg, 0.096 mmol) and HATU (54.4 mg, 0.14 mmol) in DMF (1 mL). After 10 min at rt, amine **44** (54.2 mg, 0.096 mmol) was added as solution in DMF (1 mL). After 18 h at rt, the solvent was removed by rotary evaporation. The crude material was purified by flash column chromatography (100:1 CH_2Cl_2:MeOH to 33:1 CH_2Cl_2:MeOH) to yield 65.4 mg (80%) of amide **46**, as a white foam.

Amide **46**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.49 (m, 2H), 7.45(d, *J* = 8.7 Hz, 2H), 7.37 (m, 7H), 7.30 (m, 1H), 7.26 (t, *J* = 5.9 Hz, 1H), 7.16 (t, *J* = 5.8 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.76 (d, *J* = 2.1 Hz, 1H), 6.75 (d, *J* = 2.4 Hz, 1H), 6.29 (d, *J* = 1.0 Hz, 1H), 5.54 (s, 1H), 5.15 (s, 2H), 5.13 (s, 2H), 4.08 (s, 1H), 3.79 (s, 3H), 3.69 (d, *J* = 11.4 Hz, 1H), 3.64 (d, *J* = 11.3 Hz, 1H), 3.48 (qd, *J* = 6.5, 13.0 Hz, 1H), 3.42 (s, 2H), 3.37 (qd, *J* = 6.3, 12.8 Hz, 1H), 3.11 (qd, *J* = 6.8, 12.8 Hz,

2H), 2.44 (d, *J* = 0.9 Hz, 3H), 2.33 (t, *J* = 6.5 Hz, 2H), 1.41 (m, 4H), 1.27 (m, 4H), 1.07 (s, 3H), 1.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.3, 170.3, 169.2, 169.2, 161.2, 160.9, 160.3, 159.0, 139.1, 137.9, 131.8, 129.3, 129.2, 128.8, 128.5, 128.0, 114.1, 112.6, 109.2, 105.7, 101.8, 100.0, 84.4, 78.8, 70.9, 70.6, 55.5, 42.0, 39.5, 39.4, 36.0, 35.6, 33.6, 26.9, 22.1, 19.5, 12.1; HRMS *m/z* calcd. for $C_{49}H_{58}N_4O_9$ $[M+Na]^+$: 869.4096, found 869.4093.

(4R)-N-(3-((6-(2-(3,5-dihydroxy-2-(5-methylisoxazol-3-yl)phenyl)acetamido)hexyl)amino)-3-oxopropyl)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamide (47). 5% Pd on C (5 mg, 0.002 mmol) was added as a powder to a solution of **46** (20 mg, 0.024 mmol) in EtOAc (1 mL) and MeOH (0.1 mL). The reaction mixture degassed and charged with a H_2 atmosphere. After 5 h at rt, the mixture was filtered through a pad of Celite (1 g) and concentrated by rotary evaporation to afford 10.2 mg (65%) of amide **47**, which was used without further purification.

Amide **47**: ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.95 (s, 1H), 7.45 (m, 2H), 7.34 (t, *J* = 6.0 Hz, 1H), 7.30 (t, *J* = 5.6 Hz, 1H), 6.91 (m, 2H), 6.52 (d, *J* = 2.4 Hz, 1H), 6.43 (d, *J* = 0.9 Hz, 1H), 6.42 (d, *J* =2.4 Hz, 1H), 5.55 (s, 1H), 4.12 (s, 1H), 3.79 (s, 3H), 3.71 (dd, *J* = 0.9, 11.3 Hz, 1H), 3.65 (d, *J* = 11.2 Hz, 1H), 3.50 (m, 1H), 3.45 (m, 1H), 3.42 (s, 2H), 3.13 (m, 4H), 2.46 (d, *J* = 0.9 Hz, 3H), 2.39 (t, *J* = 6.6 Hz, 2H), 1.41 (m, 4H), 1.25 (m, 4H), 1.08 (s, 3H), 1.04 (s, 3H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 171.3, 170.3, 169.2, 169.2, 161.2, 160.9, 160.3, 159.0, 139.1, 137.9, 131.8, 129.3, 128.8, 128.5, 128.0, 114.1, 112.6, 109.2, 105.7, 101.8, 100.0, 84.4, 78.8, 70.9, 70.6, 55.5, 42.0, 39.5, 39.4, 36.0, 35.6, 33.6, 26.9, 22.1, 19.5, 12.1; HRMS *m/z* calcd. for $C_{35}H_{46}N_4O_9$ $[M+Na]^+$: 689.3157, found 689.3159.

***(R)-N-(3-((2-(2-(3,5-dihydroxy-2-(5-methylisoxazol-3-yl)phenyl)acetamido)ethyl)amino)-3-oxopropyl)-2,4-dihydroxy-3,3-dimethylbutanamide* (32).** Amide **47** (4.0 mg, 0.0060 mmol) was dissolved in 80% aq. AcOH (1 mL). After 3.5 h, the reaction was concentrated by rotary evaporation. The resulting light-brown solid was washed with sequentially hexanes (2 mL), CH_2Cl_2 (2 mL) and EtOAc (2 mL) to afford 2.3 mg (63%) of mimetic **32**, as a tan solid.

Mimetic **32**: ^{1}H NMR (CD_3OD, 500 MHz) δ 6.36 (d, *J* = 2.3 Hz, 1H), 6.32 (d, *J* = 2.3 Hz, 1H), 6.26 (d, *J* = 0.9 Hz, 1H), 3.88 (s, 1H), 3.50 (m, 2H), 3.46 (d, *J* = 10.9 Hz, 1H), 3.42 (s, 2H), 3.38 (d, *J* = 11.0 Hz, 1H), 3.13 (m, 4H), 2.46 (d, *J* = 0.9 Hz, 3H), 2.40 (t, *J* = 6.7 Hz, 2H), 1.46 (m, 4H), 1.30 (m, 4H), 0.91 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.1, 173.8, 173.6, 169.9, 161.4, 160.5, 158.7, 138.2, 109.9, 109.3, 105.9, 102.5, 77.2, 70.3, 42.0, 40.4, 36.4, 36.4, 30.8, 30.2, 24.1, 23.8, 21.3, 20.9, 12.0; HRMS *m/z* calcd. for $C_{27}H_{40}N_4O_8$ $[M+Na]^+$: 571.2743, found 571.2739.

C. Protein NMR Studies.

The following methods were used to collect the NMR data presented in Fig. 4 and Fig. 6 of the manuscript.

C.1. Expression, modification, and purfication of *Pseudomonas fluorescens* AcpH. pET29-PfAcpH plasmid was transformed into BL21 (DE3) cells [Kosa, N. M.; Haushalter, R. W.; Smith, A. R.; Burkart, M. D. *Nat. Methods*. **2012**, *9*, 981]. The construct was grown in Luria-Bertani (LB) media containing 50 mg/L kanamycin. Expression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at an OD_{600} of 0.9, and the cells were incubated an additional 16 h at 16 °C. The cells were harvested by centrifugation at 1000 relative centrifugal force (RCF). The pelleted cells were re-suspended in 30 mL of lysis buffer (150 mM NaCl, 50 mM Tris pH 7.5, 10% glycerol) and lysed in a French pressure cell. The lysate was then centrifuged (12000 RCF) for 1 h to remove insoluble debris. The His_6-tagged proteins were purified using Ni-NTA resin (Novagen).

C.2. Preparation of PfAcpH affinity resin. Coupling of *Plasmodium falciparum* PfAcpH to Affi-gel 15 (Bio-Rad) resin was conducted in 0.1 M MOPS pH 7.5 and the resin capped with 0.1 M glycine ethyl ester according to the manufacturer's instructions. By Bradford analysis the resin obtained from this procedure contained 4 mg/mL of PfAcpH.

C.3. Expression, modification, and purification of *apo*-actACP. pET28-actACP (C17S) plasmid was transformed into BL21 (DE3) cells [Haushalter, R. W.; Filipp, F. V.; Ko, K. S.; Yu, R.; Opella, S. J.; Burkart, M. D. ACS Chem. Biol. 2011, 6, 413]. Uniformly labeled ^{15}N-actACP was expressed by culturing cells in M9 minimal media (1 L) supplemented with 1 g ^{15}N-NH_4Cl, 4 g D-glucose, and 50 mg of kanamycin. Uniformly labeled ^{13}C,^{15}N-actACP was prepared by supplementing M9 minimal media (1 L) with 4 g ^{13}C-D-glucose, 1 g ^{15}N-NH_4Cl, and 50 mg kanamycin. Expression was induced with 0.5 mM IPTG at an OD_{600} of 0.9, and the cells were incubated an additional 16 h at 16 °C. The cells were harvested by centrifugation (1000 RCF). The pelleted cells were re-suspended in 30 mL of lysis buffer (150 mM NaCl, 50 mM Tris pH 7.5, 10% glycerol) and lysed in a French pressure cell. The lysate was then centrifuged (12000 RCF) for 1 h to remove insoluble debris. The His_6-tagged proteins were purified using Ni-NTA resin (Novagen) to yield a mixture of *apo*-actACP and *holo*-actACP due to endogenous phosphopantetheinyl transferase activity. It was necessary to "apofy" the actACP mixture by tumbling 10 mL of 300 μM ACP in 150 mM NaCl, 50 mM Tris pH 8.0, 15 mM $MgCl_2$, 1 mM $MnCl_2$, 3 mM tris(2-carboxyethyl)phosphine (TCEP), and 250 μL of Affi-gel 15 (Bio-Rad) containing 4 mg/mL covalently-attached PfAcpH discussed in section C.2 at 37 °C for 12 h [Kosa, N. M.; Haushalter, R. W.; Smith, A. R.; Burkart, M. D. *Nat. Methods*. **2012**, *9*, 981]. The resin was spun down by gentle centrifugation (~200 RCF) and pure *apo*-actACP was isolated from the supernatant.

C.4. Preparation of *crypto*-actACPs 4a, 4b, 5b, 6b, 8a, 8b, and 8c. Loading of the polyketide mimetics **11**, **20**, **22**, **23**, **30**, **31** or **32** was achieved by a previously described one pot chemoenzymatic reaction converting **11**, **20**, **22**, **23**, **30**, **31** or **32** to their corresponding CoA analog *in situ* using ATP and three of the *E. coli* CoA biosynthetic enzymes (CoaA, CoaD, CoaE), followed by loading onto *apo*-actACP by Sfp [Worthington, A. S.; Burkart M. D. *Org. Biomol. Chem*. **2006**, *4*, 44]. In this study, this was achieved by treating 1.5 ml of 400 μM *apo*-actACP with 12.5 mM $MgCl_2$, 8 mM ATP, 2 mM mimetic **11**, **20**, **22**, **23**, **30**, **31** or **32**, 1.5 μM Sfp, 0.5 μM *E. coli* CoaA, 0.7 μM *E. coli* CoaD, 0.6 μM *E. coli* CoaE, 0.02% Triton X, 0.1% NaN_3, and 5 mM TCEP in a 150 mM NaCl and 50 mM Tris pH 7.4 buffer containing 10%

glycerol. After incubation at 37°C for 12 h, the reactions were purified by FPLC into a 50 mM KPi 7.4 buffer. *Crypto*-actACPs eluted between 155-180 mL.

C.5. Preparation of *holo*-actACP. To prepare pure *holo*-actACP, *apo*-actACP from section C.3 was incubated with 50 mM Tris pH 7.5, 150 mM NaCl, 12.5 mM $MgCl_2$, 2 mM coenzyme A, 3 mM TCEP, 0.1% NaN_3 and 1.5 μM Sfp at 37 °C overnight. The solution was purified by FPLC, as described in section C.4.

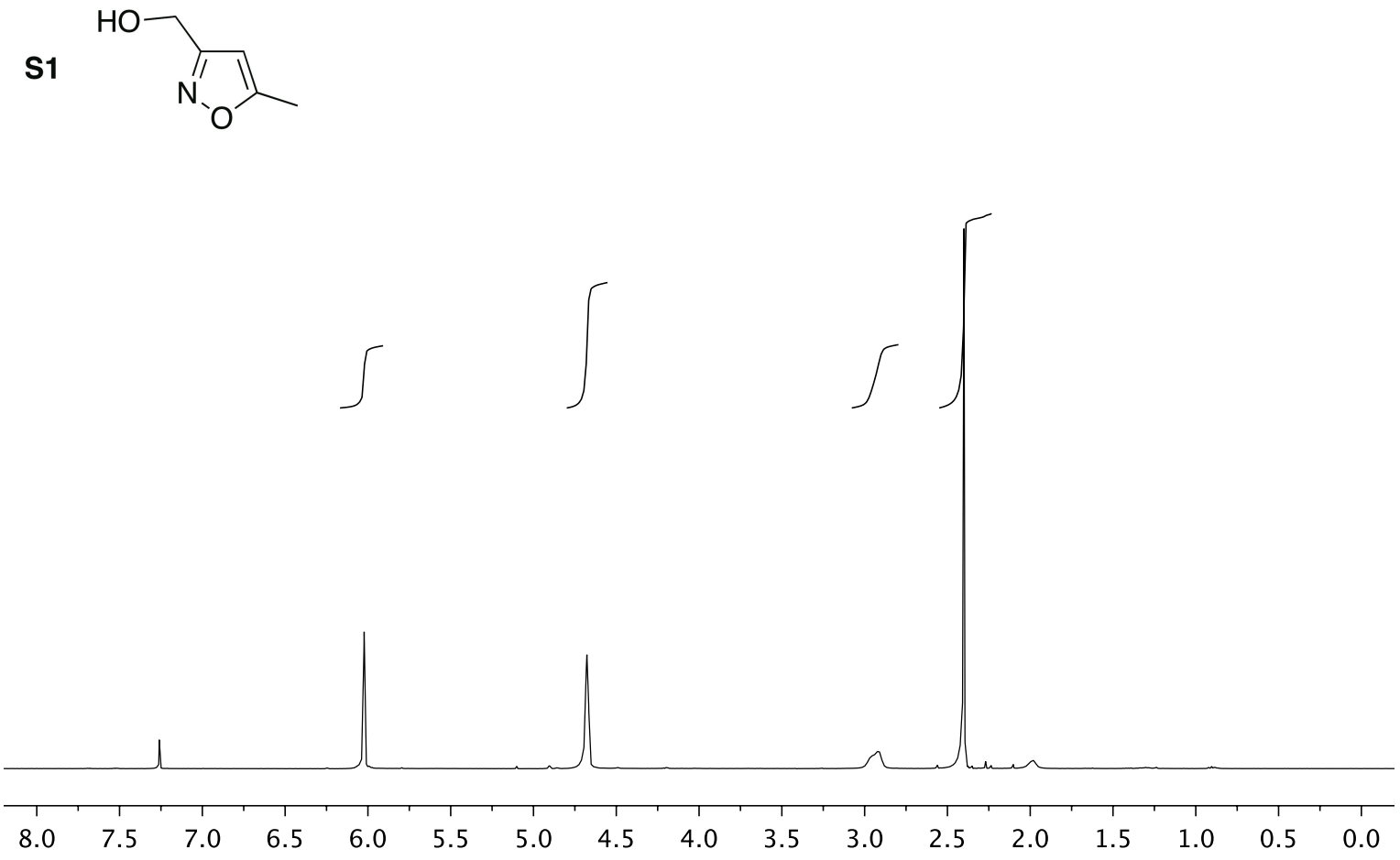
C.6. HPLC analysis of ACP proteins. Loading of polyketide analogs in sections C.4 and C.5 was monitored by HPLC analysis. The *crypto*-actACP proteins were passed over a C18 column (Burdick & Jackson) using 10% solvent B for 5 min, then increasing 10–50% B over 5 min, and finally 50-63.3% of solvent B over 10 min (solvent A = H_2O, 0.05% trifluoroacetic acid; solvent B = CH_3CN 0.05% trifluoroacetic acid). Analyses were conducted on HP 1100 series HPLC (Agilent) equipped with a G1315A DAD detector (Agilent). The elution of the protein was monitored by absorbance at 210 nm.

C.7. Protein NMR experiments. NMR samples were prepared by first concentrating the FPLC pure proteins in 50 mM KPi pH 7.4. A 450 μL aliquot of the appropriate actACP form was then prepared for NMR by adding 50 μL of D_2O, 5 μL of 10% (w/v) NaN_3 and 5 μL of 0.5 M TCEP adjusted to pH 7.3. The final concentration of the crypto-labeled actACP evaluated was as: 1 mM for **4a**, **4b**, **5b**, **6b**, **8a**, **8b**, and **8c**. After adding the sample, each NMR tube was flushed with argon, capped, and sealed with Teflon tape. All NMR spectra were acquired at 37 °C with a 1.2 s recycle delay. HSQC spectra were collected for each sample prepared in the same buffer for CSP analysis, collecting 2048 points (R+I) in the ^1H direct dimension and 256 points (R+I) in the ^{15}N indirect dimension. Chemical shifts observed for the *holo*-actACP were in good agreement with the literature [Haushalter, R. W.; Filipp, F. V.; Ko, K. S.; Yu, R.; Opella, S. J.; Burkart, M. D. *ACS Chem. Biol*. **2011**, *6*, 413]. Chemical shifts were measured by peak maxima, and chemical shift perturbation was calculated using the formula $CSP = [\{(0.2\delta N)^2 + (\delta H)^2\}/2]^{0.5}$.

Because significant but similar peak perturbations were observed for **5b** and **6b**, a $^{13}C,^{15}N$-labeled sample of **5b** was prepared and subjected to an HNCACB experiment with 2048 points (R+I) in the direct ^1H dimension and 96 points (R+I) in both the ^{13}C and ^{15}N indirect dimensions. Standard backbone-assignment techniques correlating the backbone amides to their CA and CB, and those for the neighboring residue were employed to confirm our peak assignments. NMR spectra were processed using nmrPipe (NIH), and analyzed using the Sparky (UC San Francisco) and CARA software suites.

C.8. In Silico Docking experiments. The docking program GOLD was used for docking between the ActACP and the phosphopantetheine-tethered atom replacement probes. Both protein and ligands were prepared for docking by removing waters, adding hydrogens, charges, and converting the pdb files to Mol2 files using the program Chimera 2. The Act ACP ligand-binding pocket was defined as residues within 10 Å of the hydroxyl group of the active site residue Ser 42. A 1.8 Å restraint was placed between the phosphate group from the pantetheine moiety and the active site Ser 42. Docking was performed using the default settings with 100 docking trials. The docking solutions were ranked using the ChemPLP scoring functions.

Figure S1. ^{1}H NMR (400 Mhz) and ^{13}C NMR (100 MHz) spectra of **S1** in $CDCl_3$



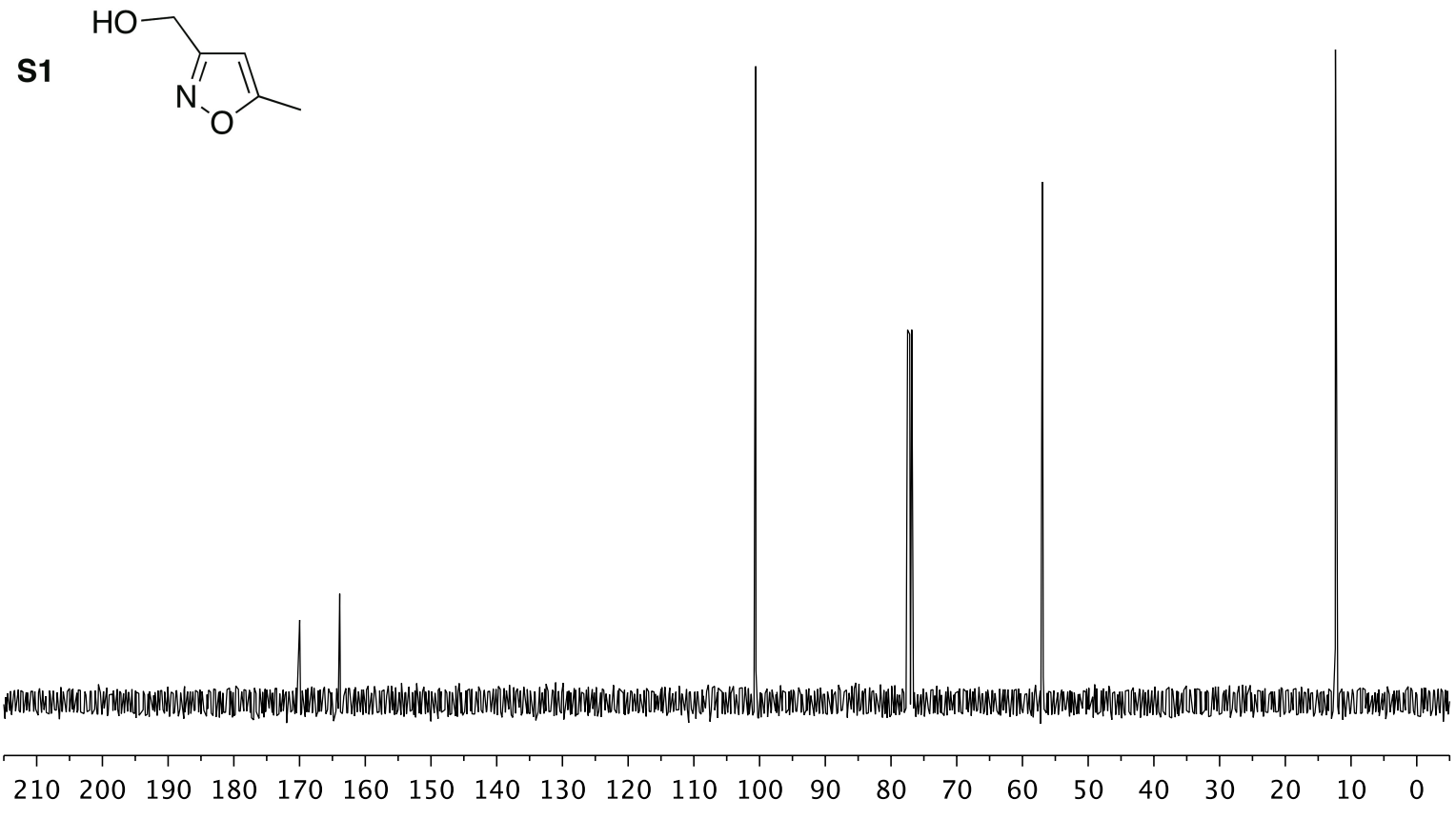
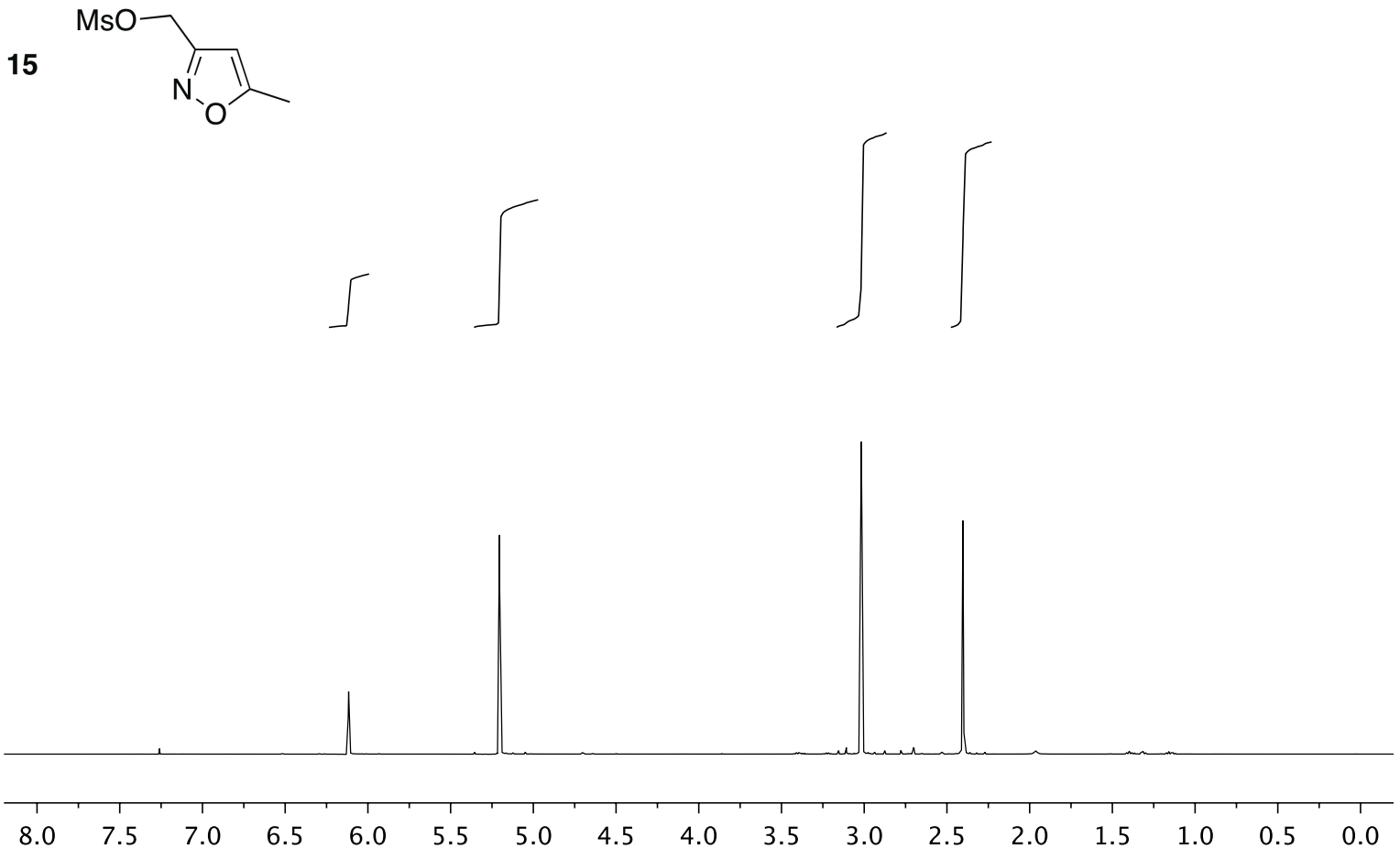


Figure S2. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **15** in $CDCl_3$



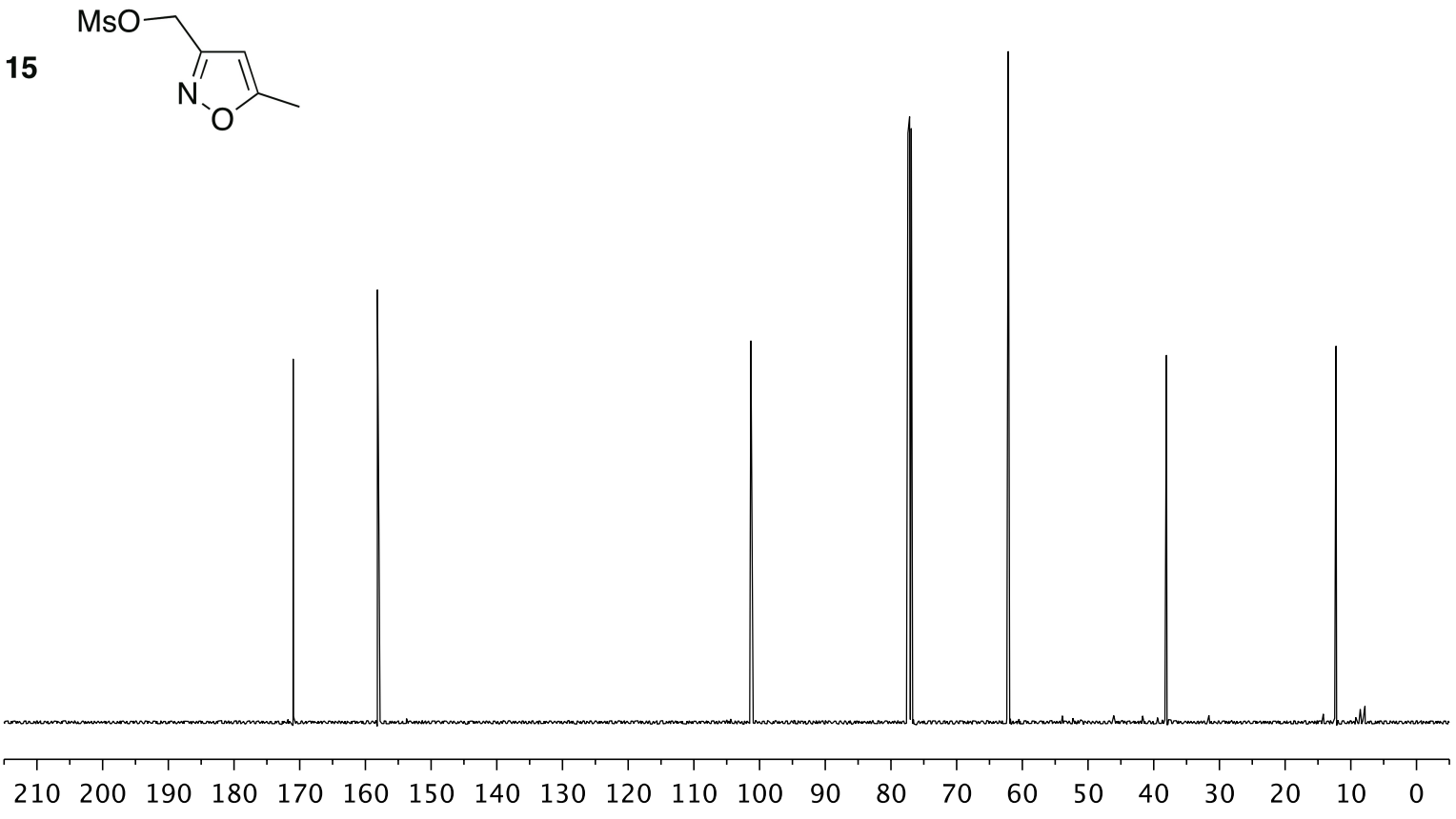
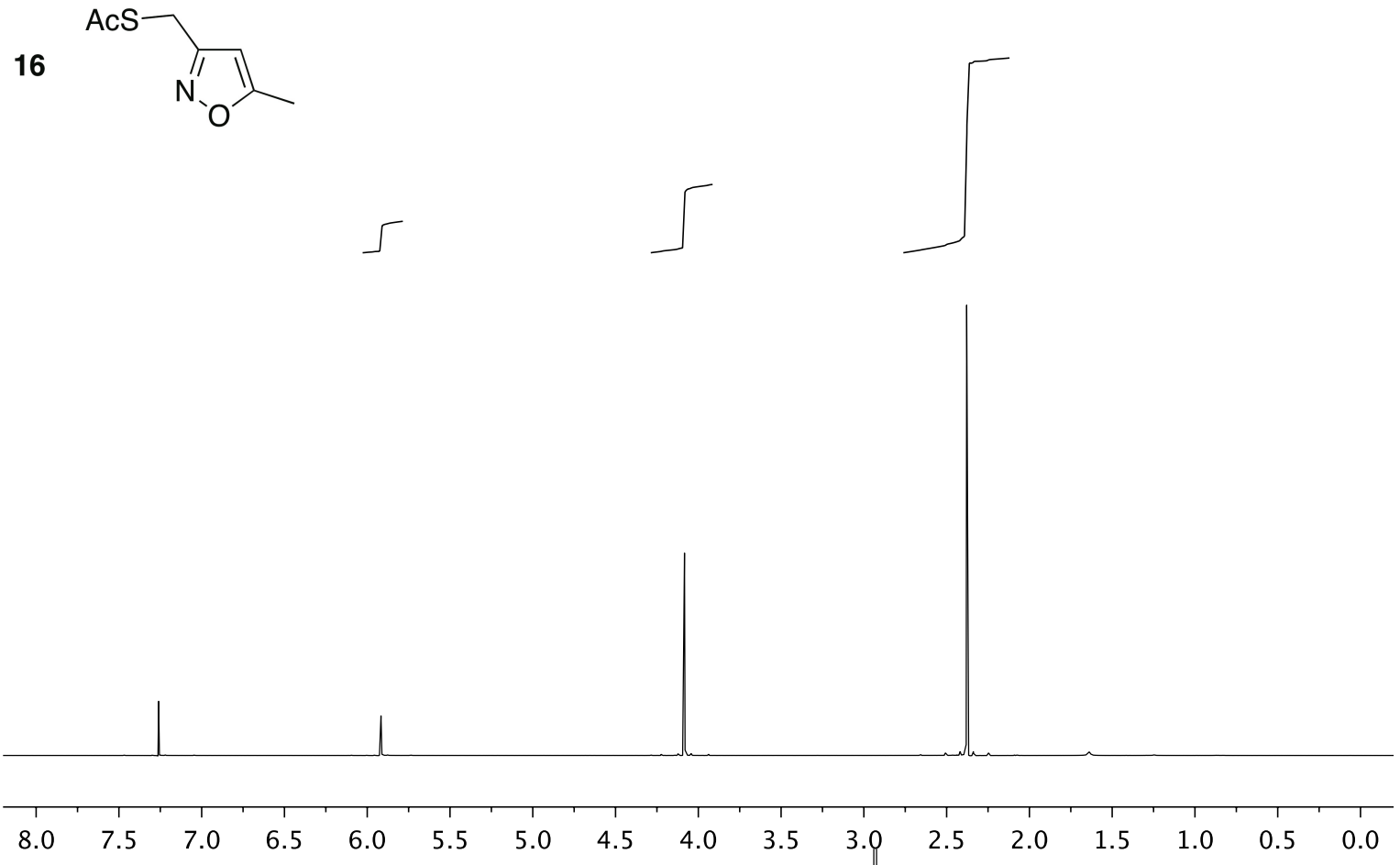


Figure S3. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **16** in $CDCl_3$



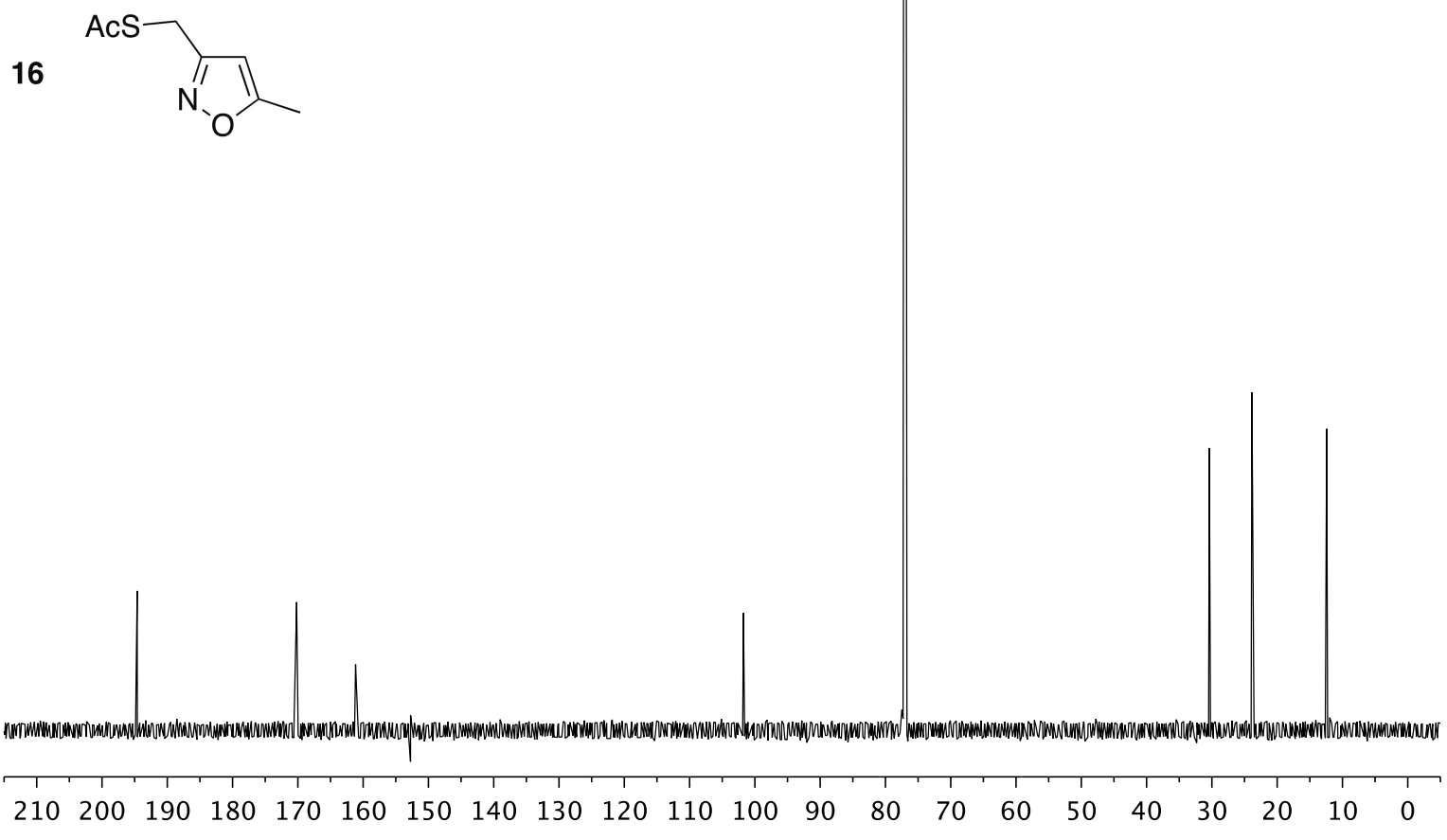
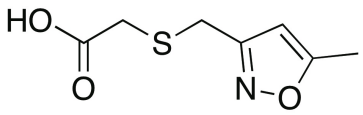
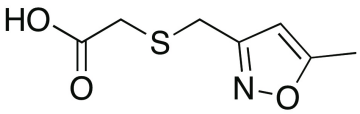


Figure S4. ^{1}H NMR (400 Mhz) and ^{13}C NMR (100 MHz) spectra of **17** in $CDCl_3$

17



17



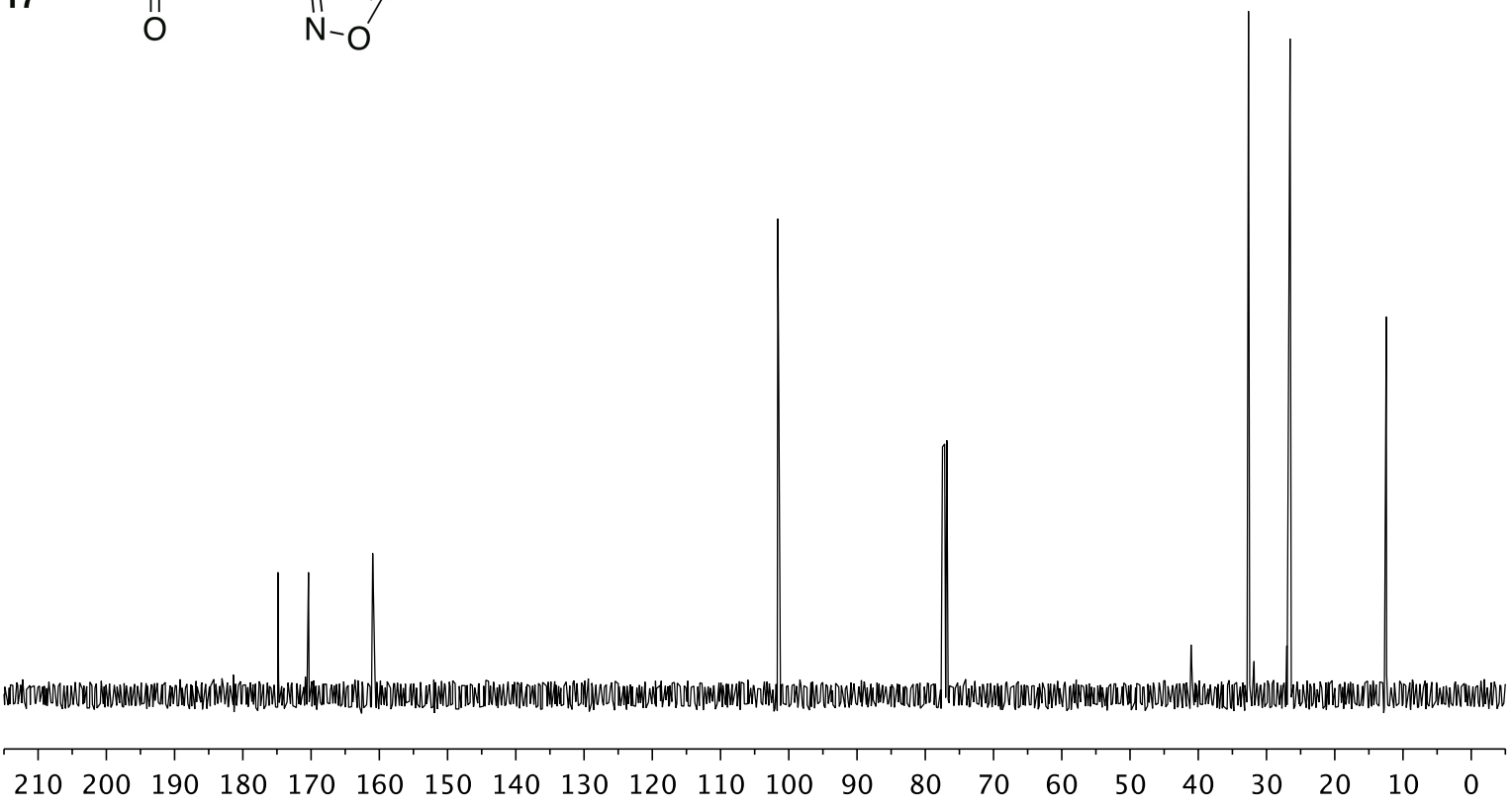
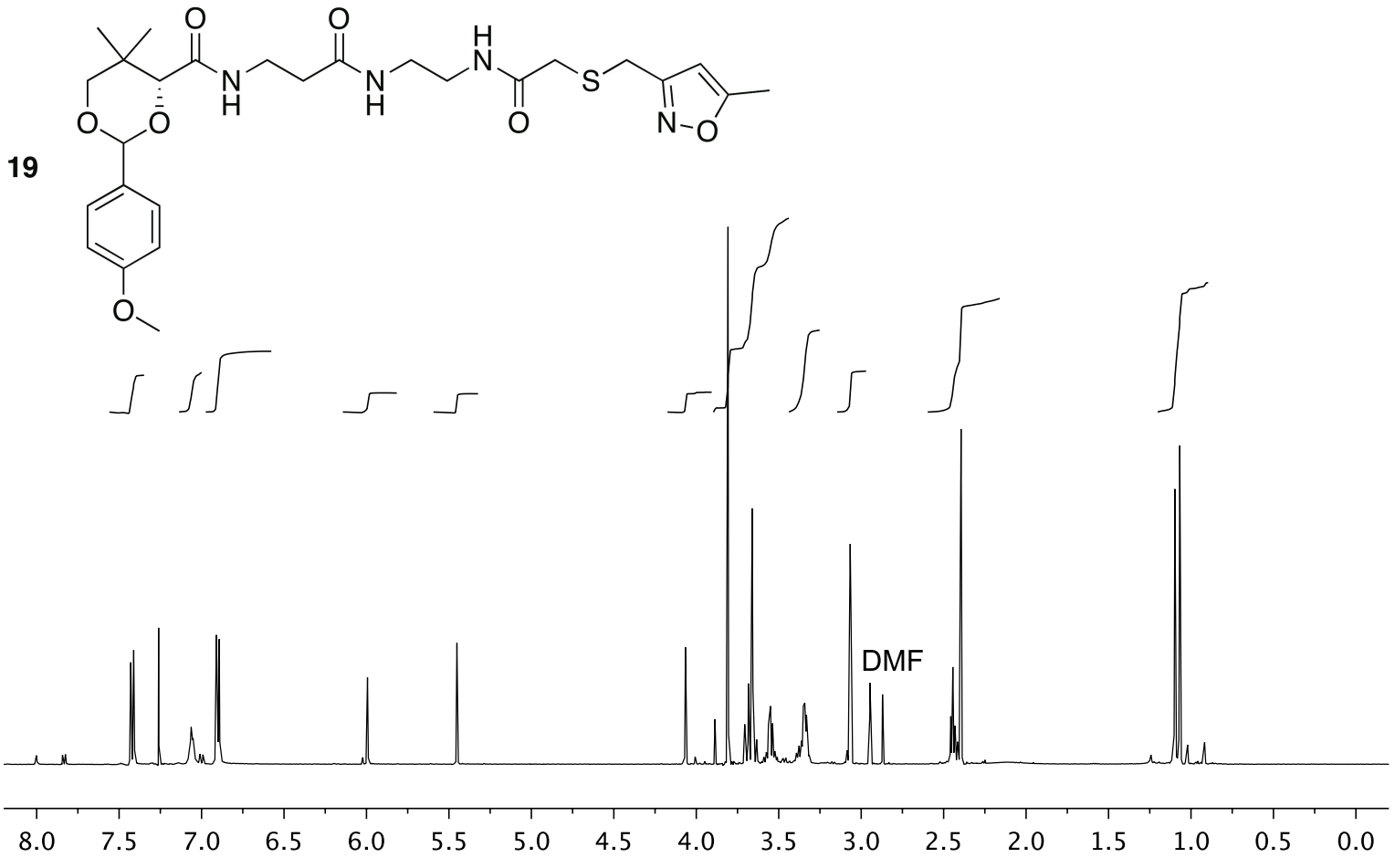


Figure S5. ^{1}H NMR (500 Mhz) and ^{13}C NMR (100 MHz) spectra of **19** in $CDCl_3$



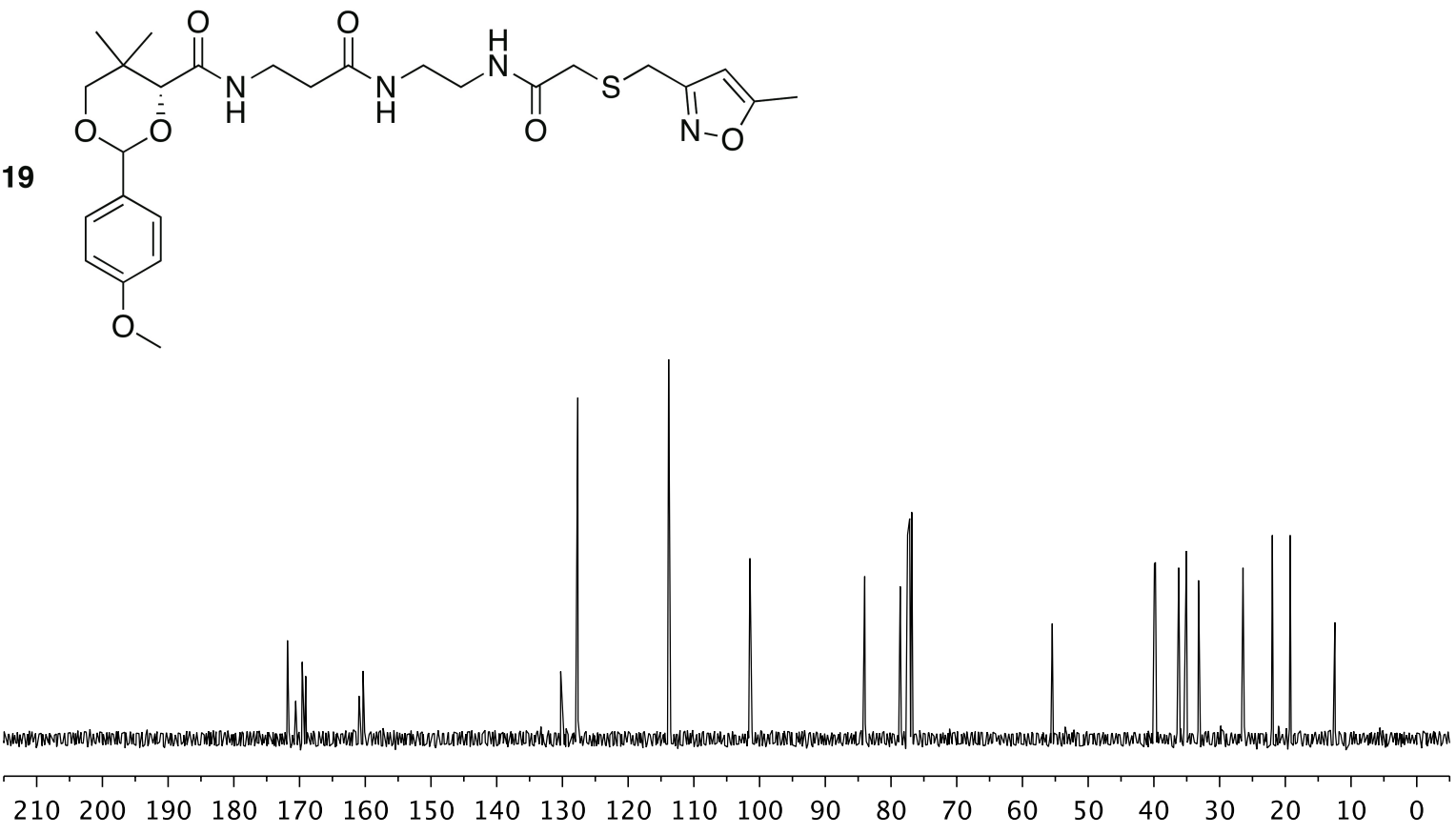
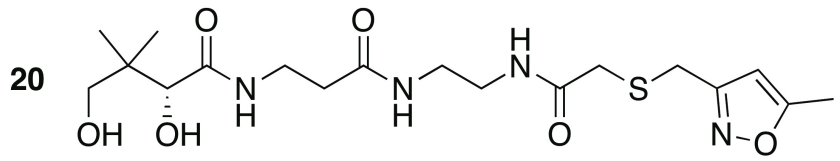


Figure S6. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **20** in CD_3OD



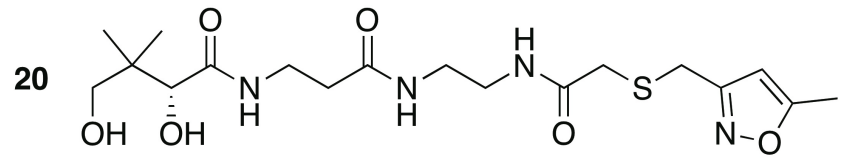
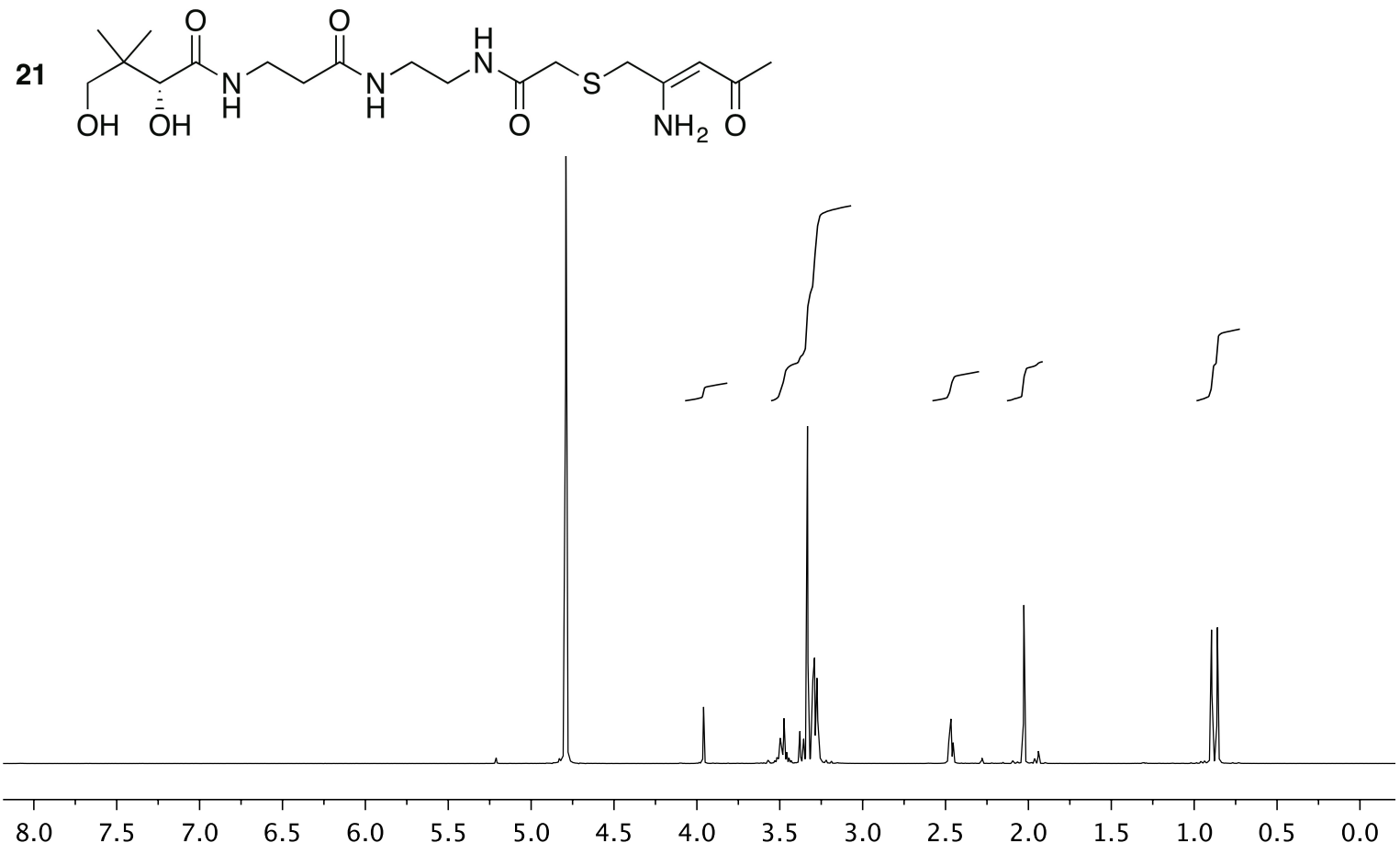


Figure S7. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **21** in D_2O



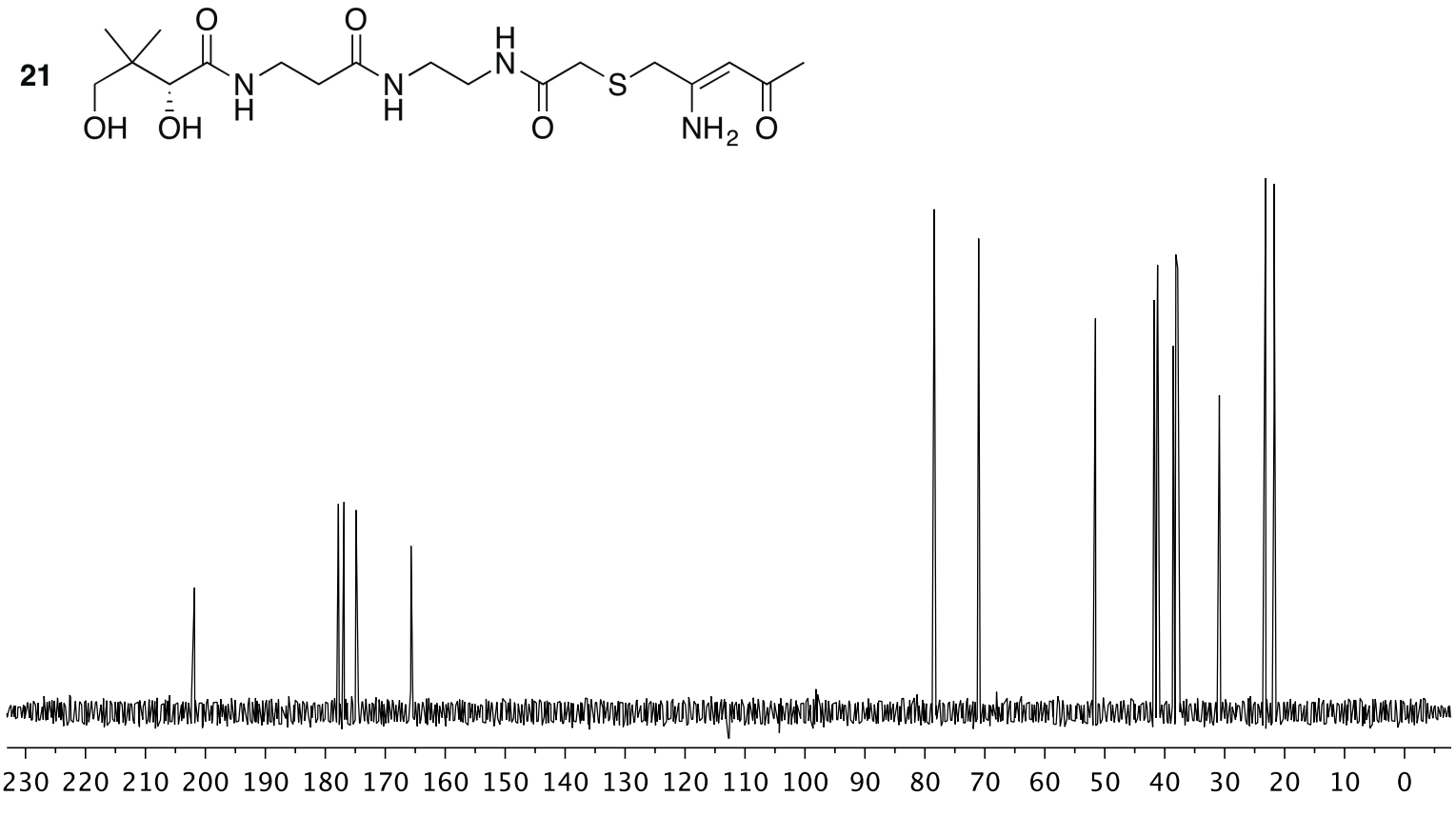
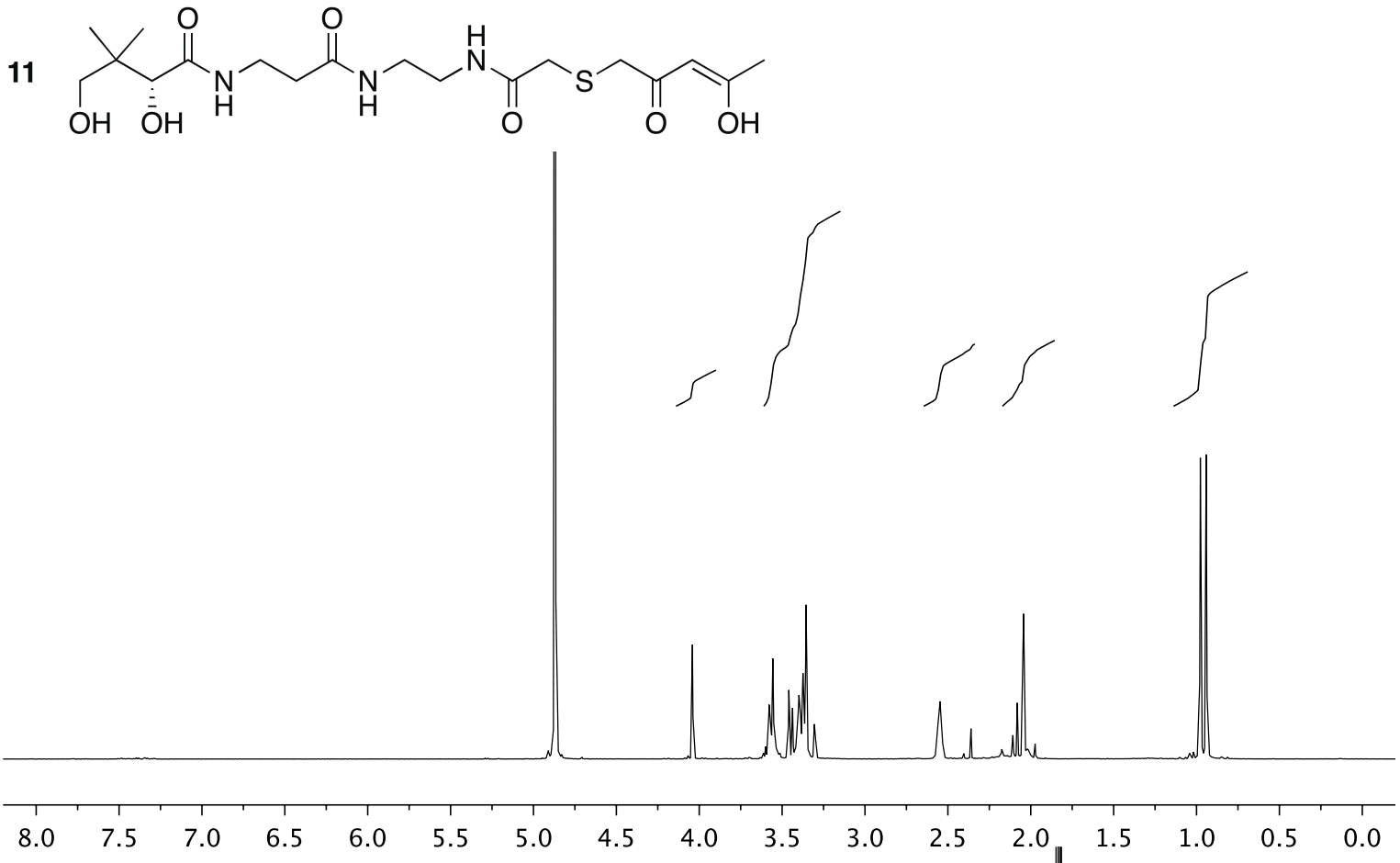


Figure S8. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **11** in CD_3OD



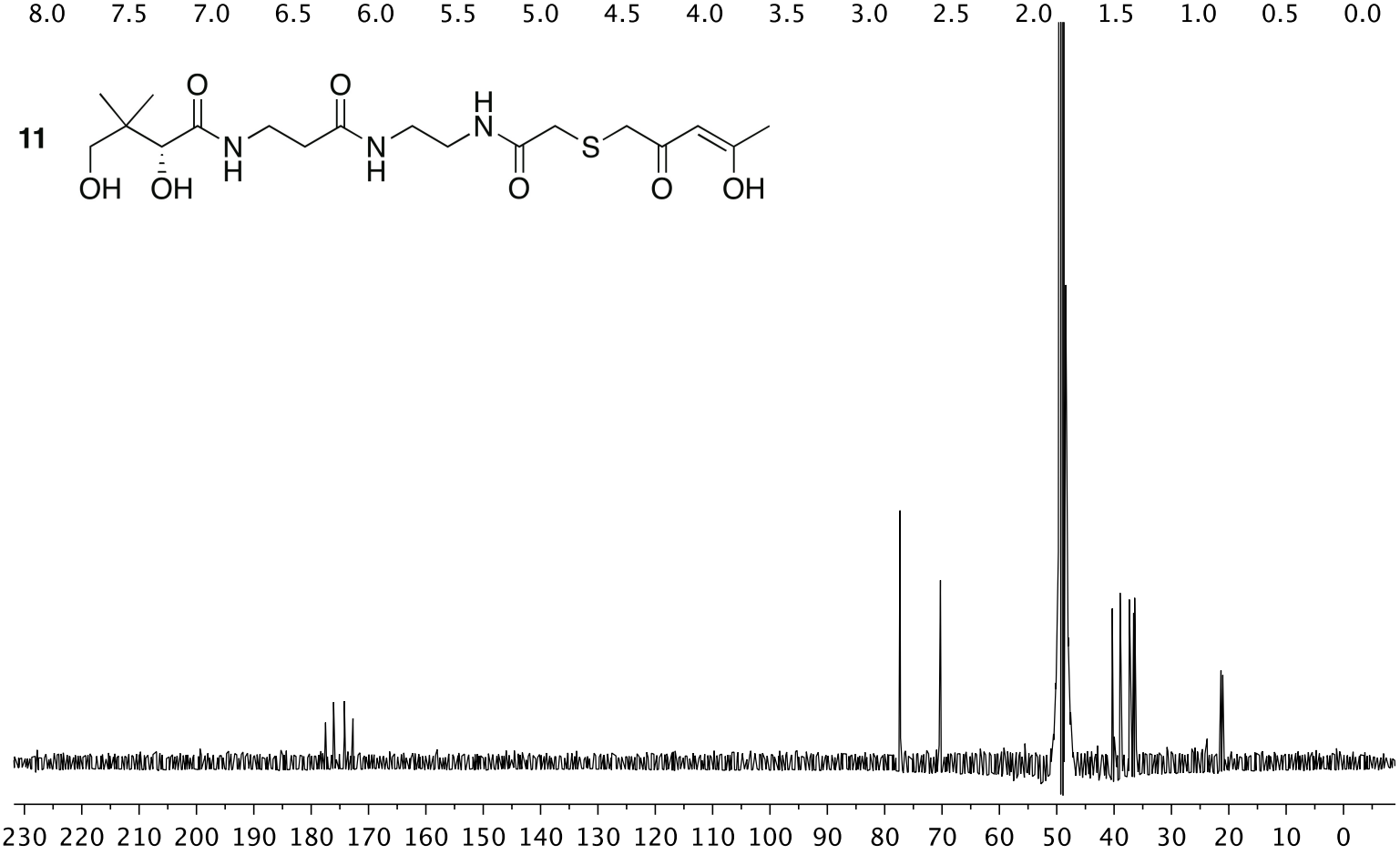
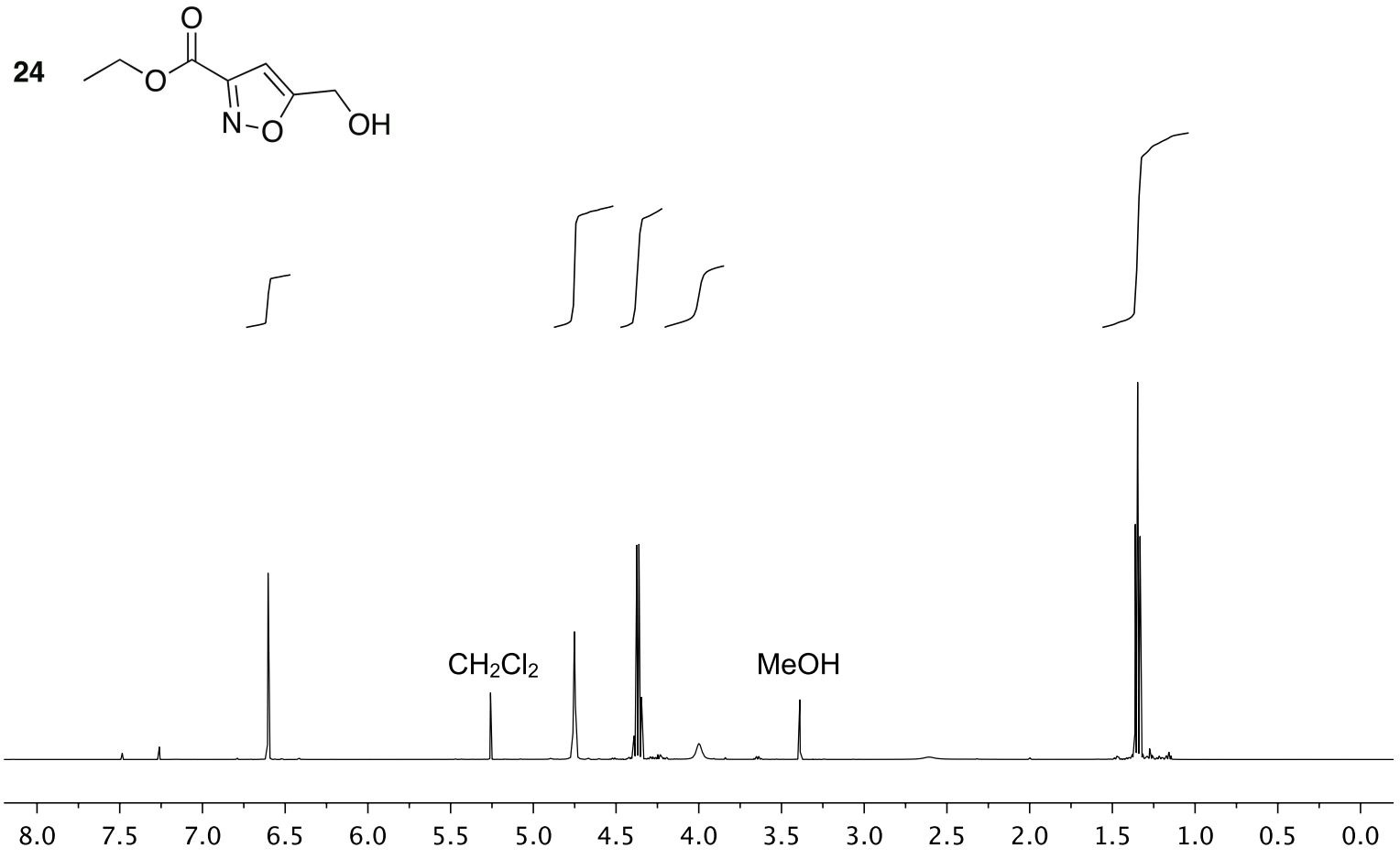


Figure S9. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **24** in $CDCl_3$



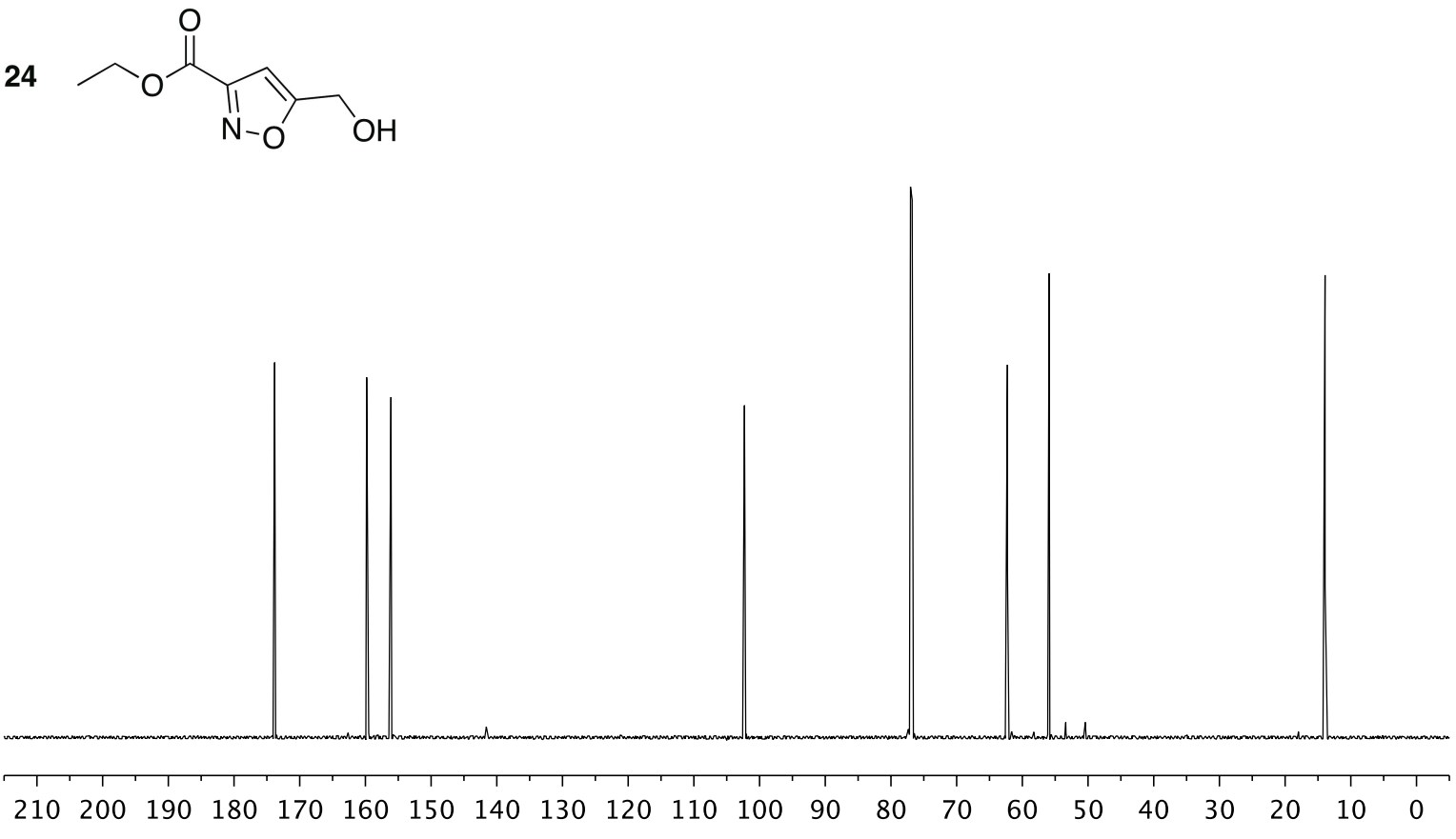
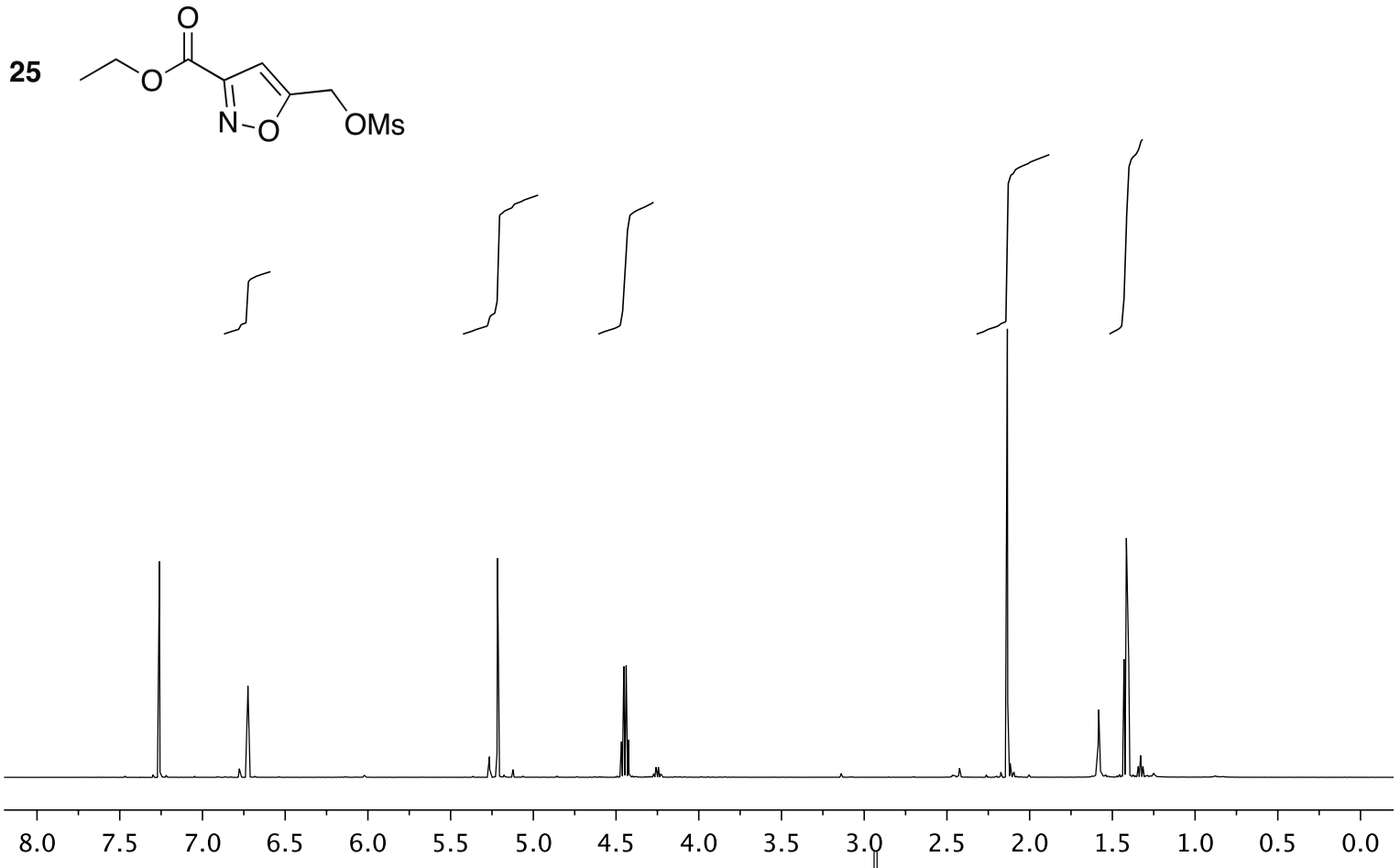


Figure S10. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **25** in $CDCl_3$



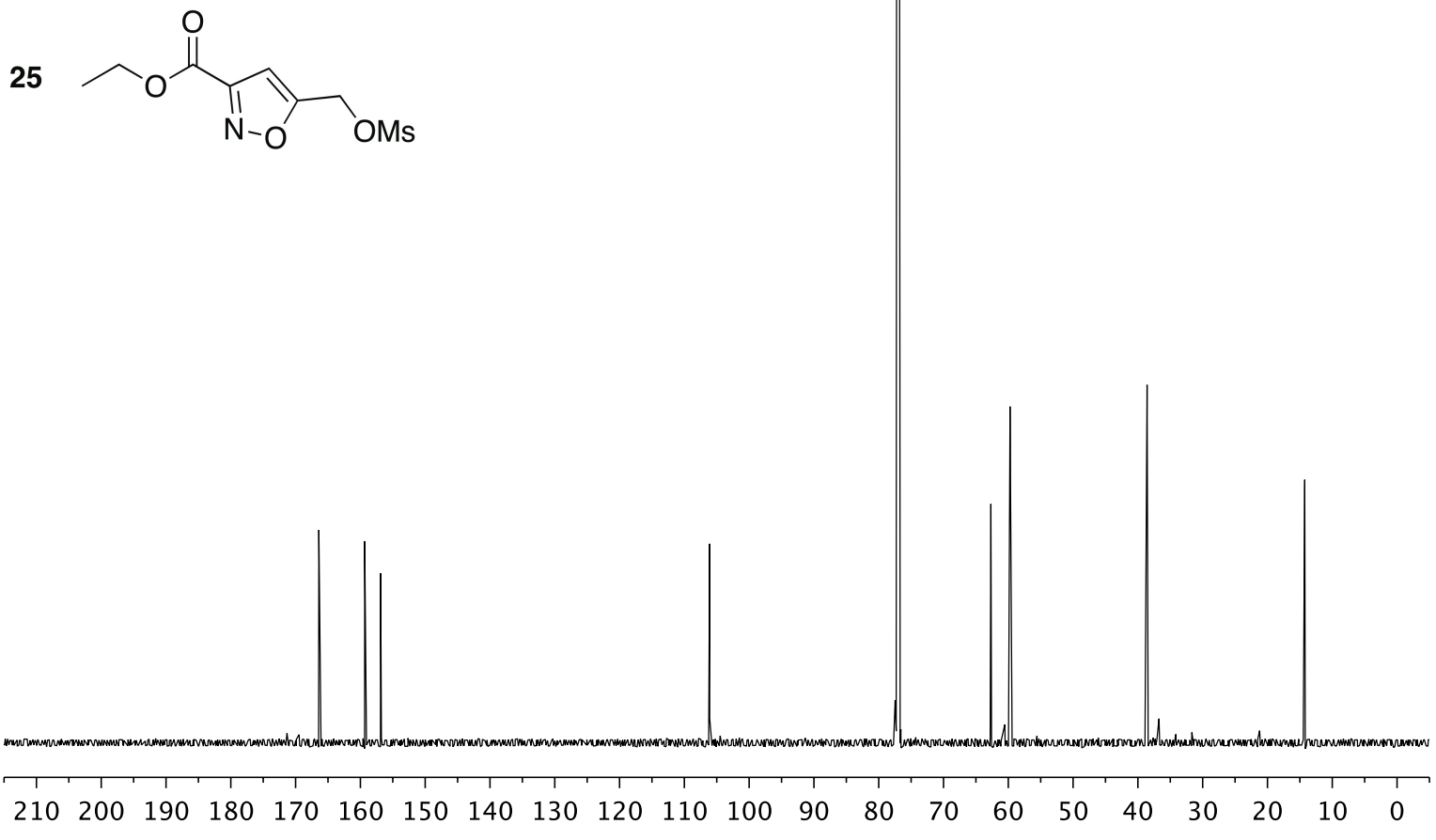


Figure S11. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **26** in CD_3OD

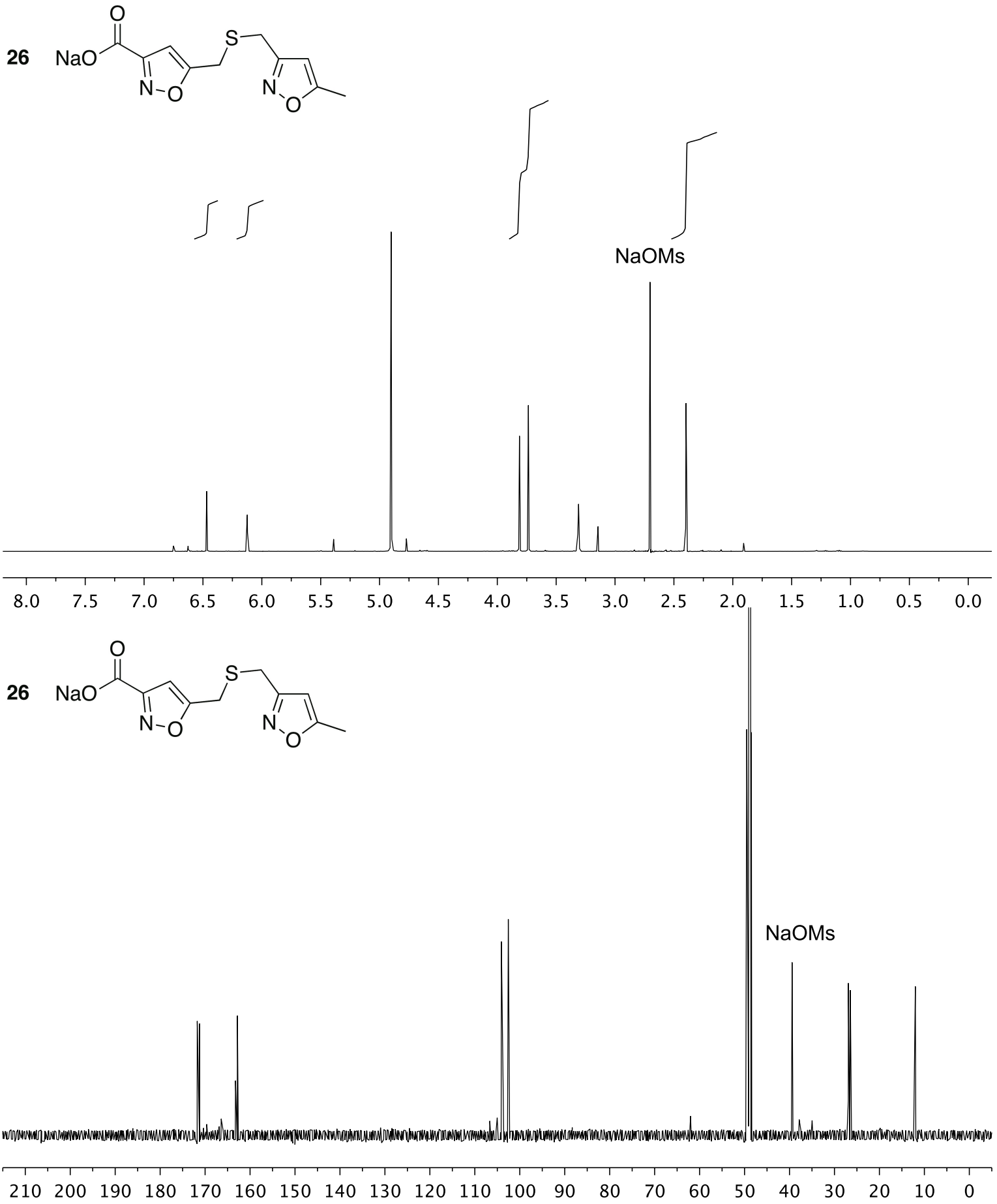
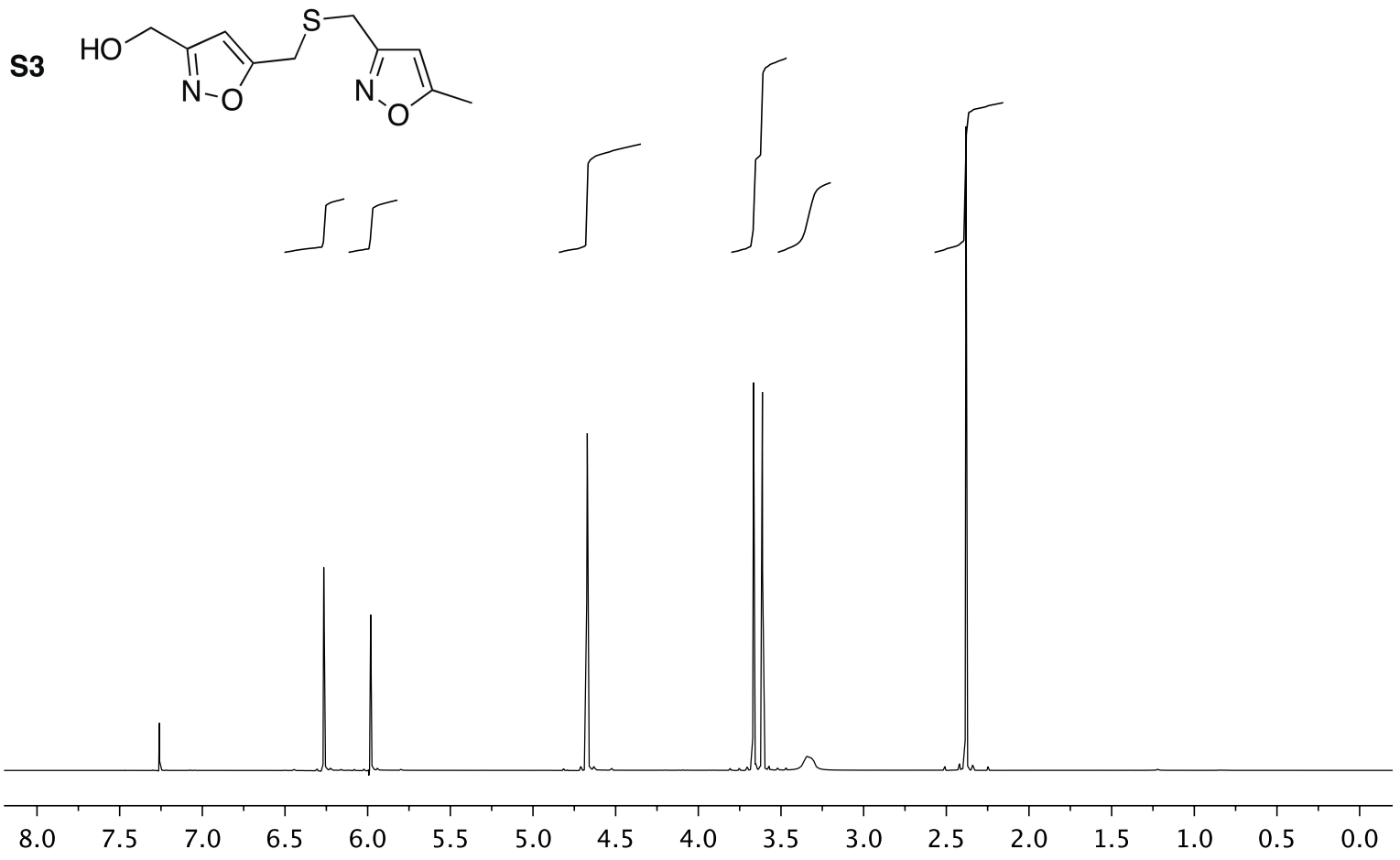


Figure S12. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **S3** in $CDCl_3$



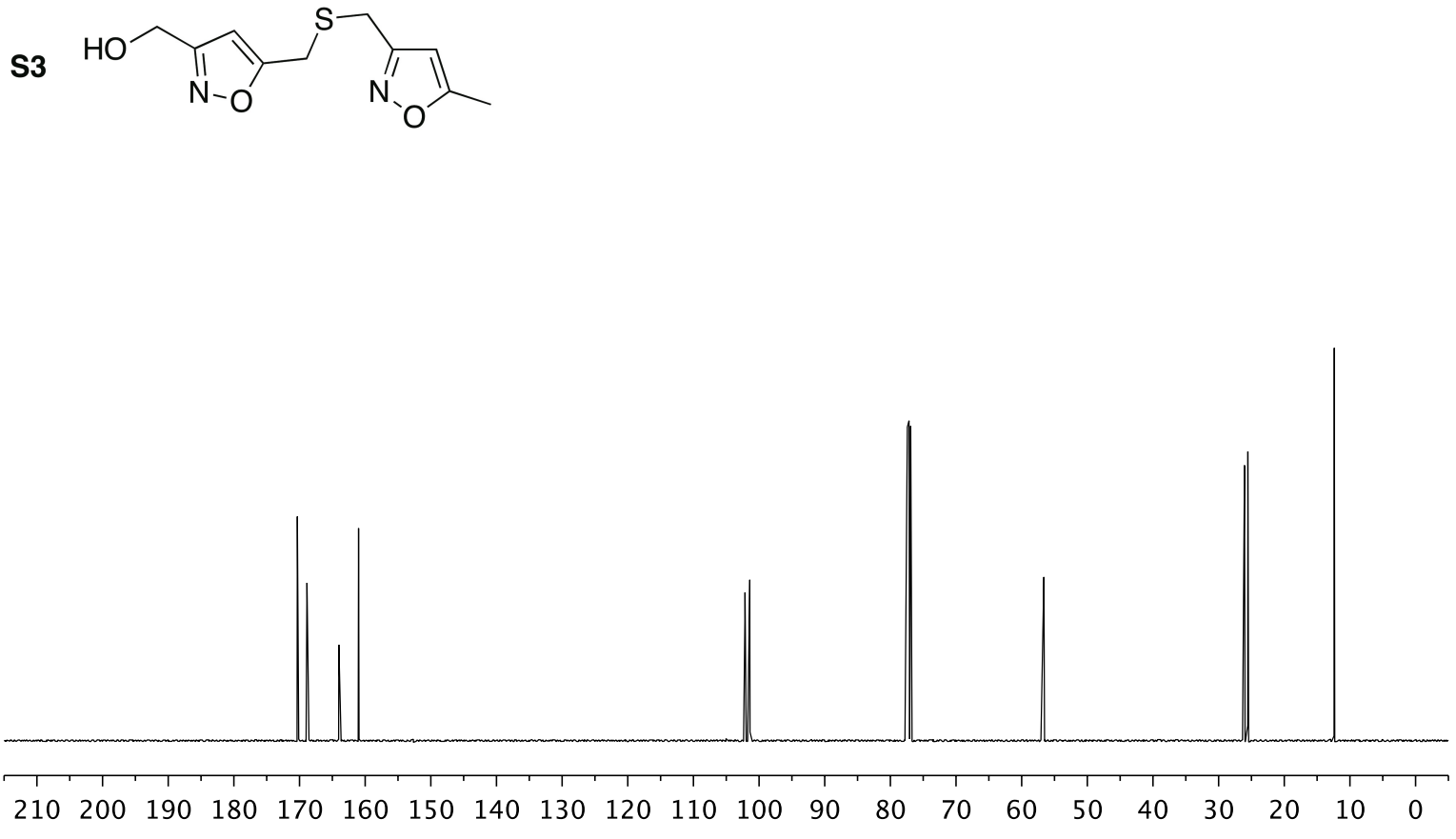
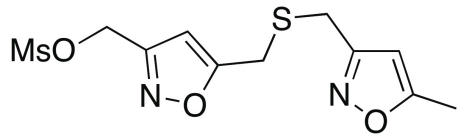
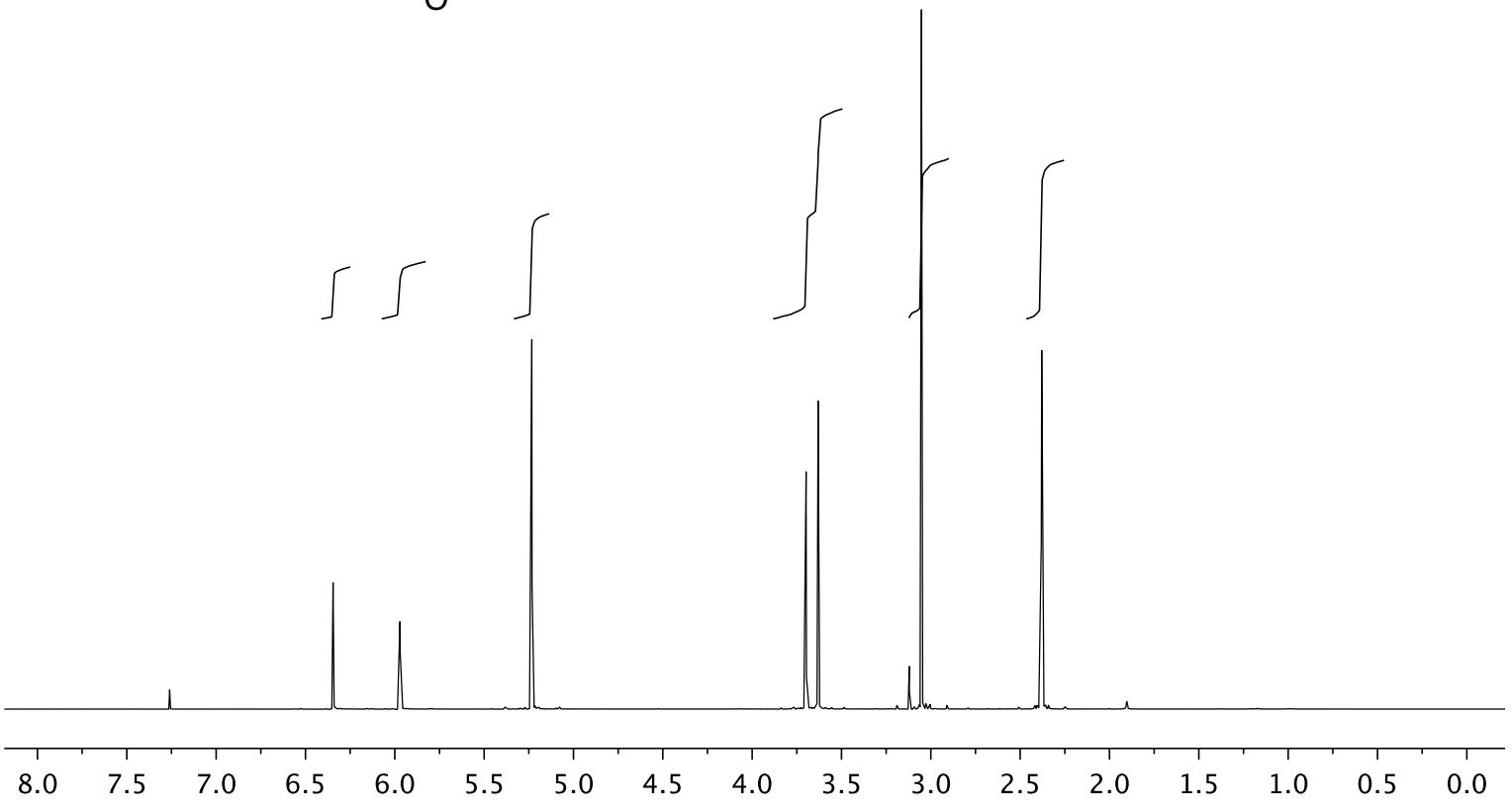


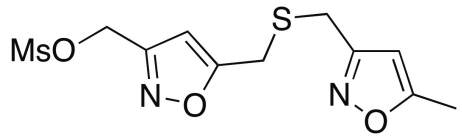
Figure S13. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **S4** in $CDCl_3$

S4





S4



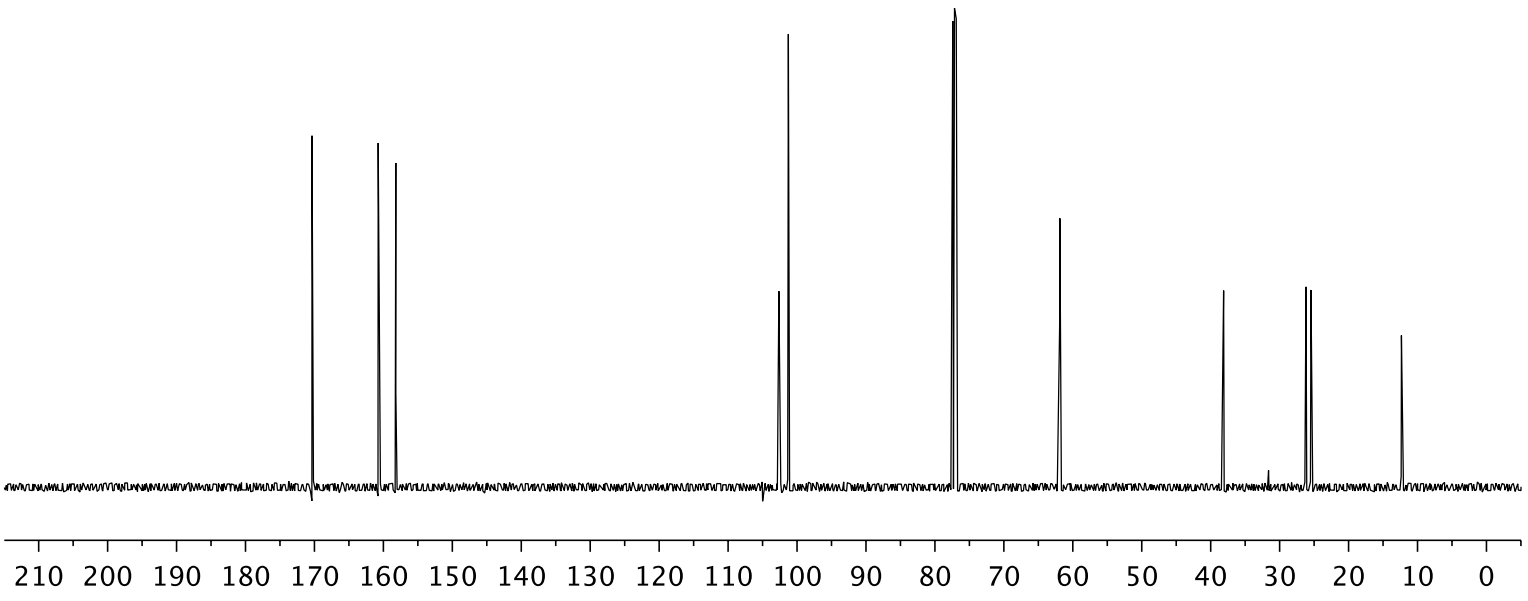
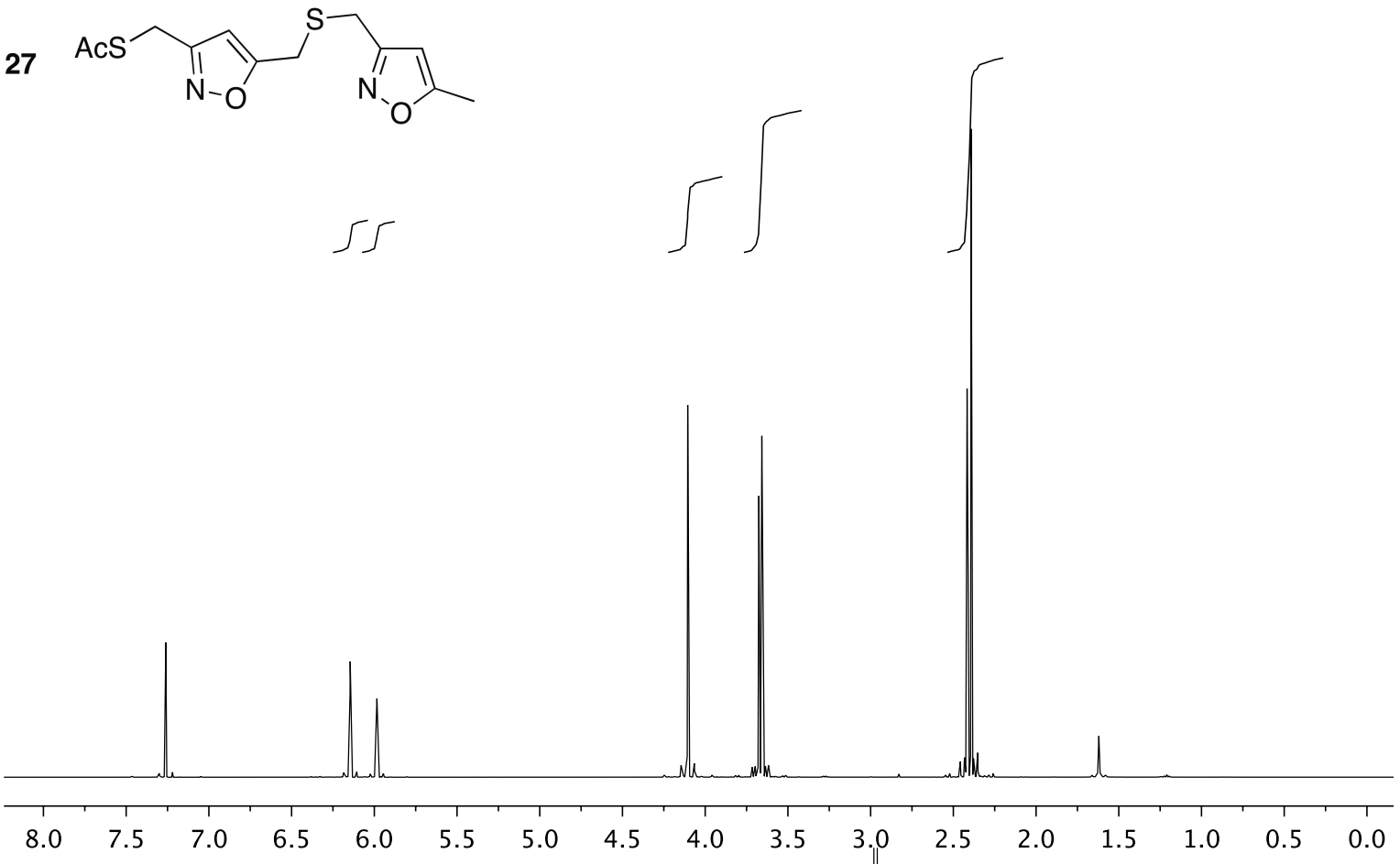


Figure S14. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **27** in $CDCl_3$



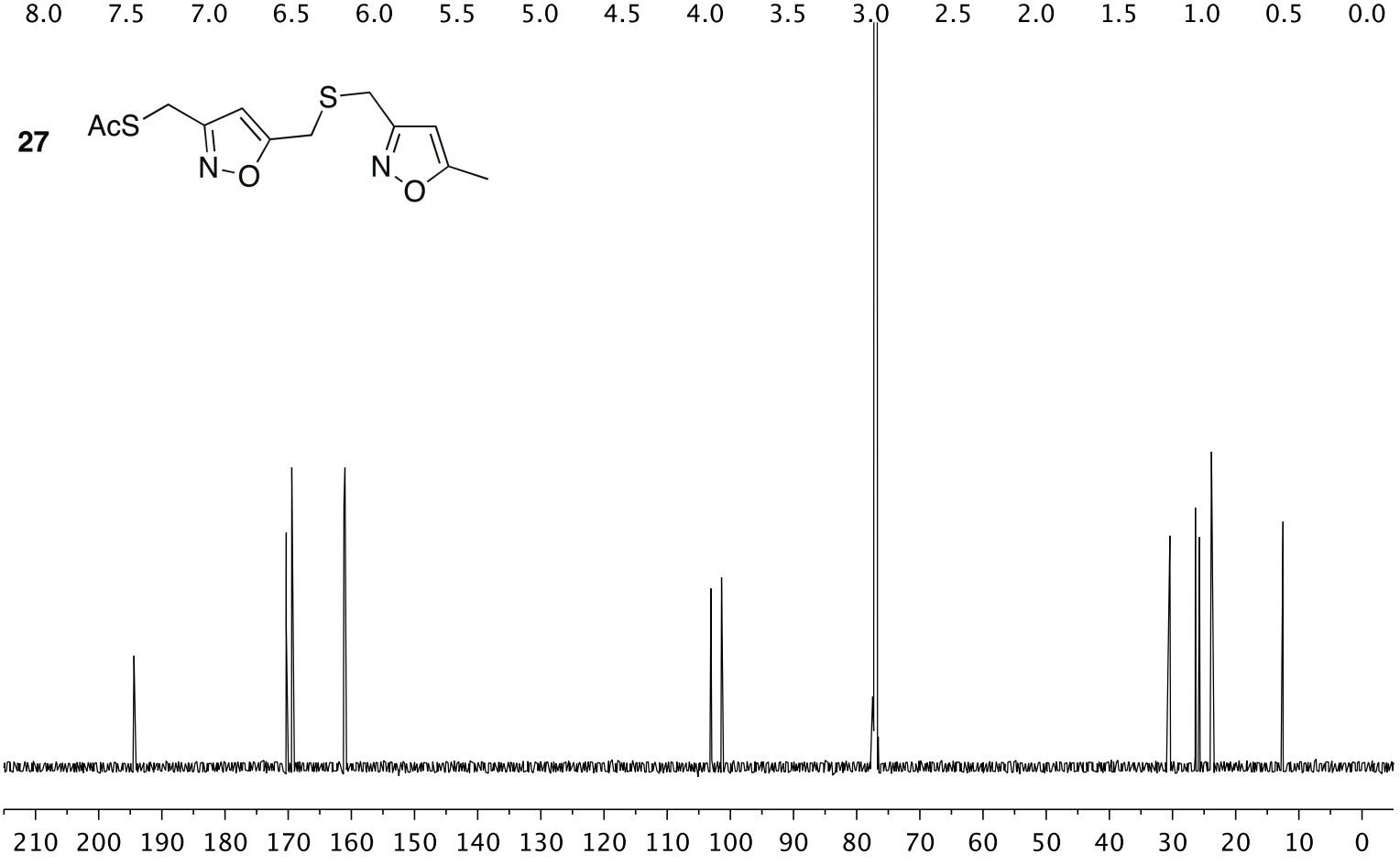
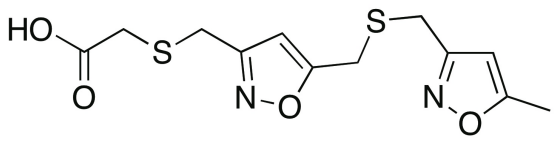
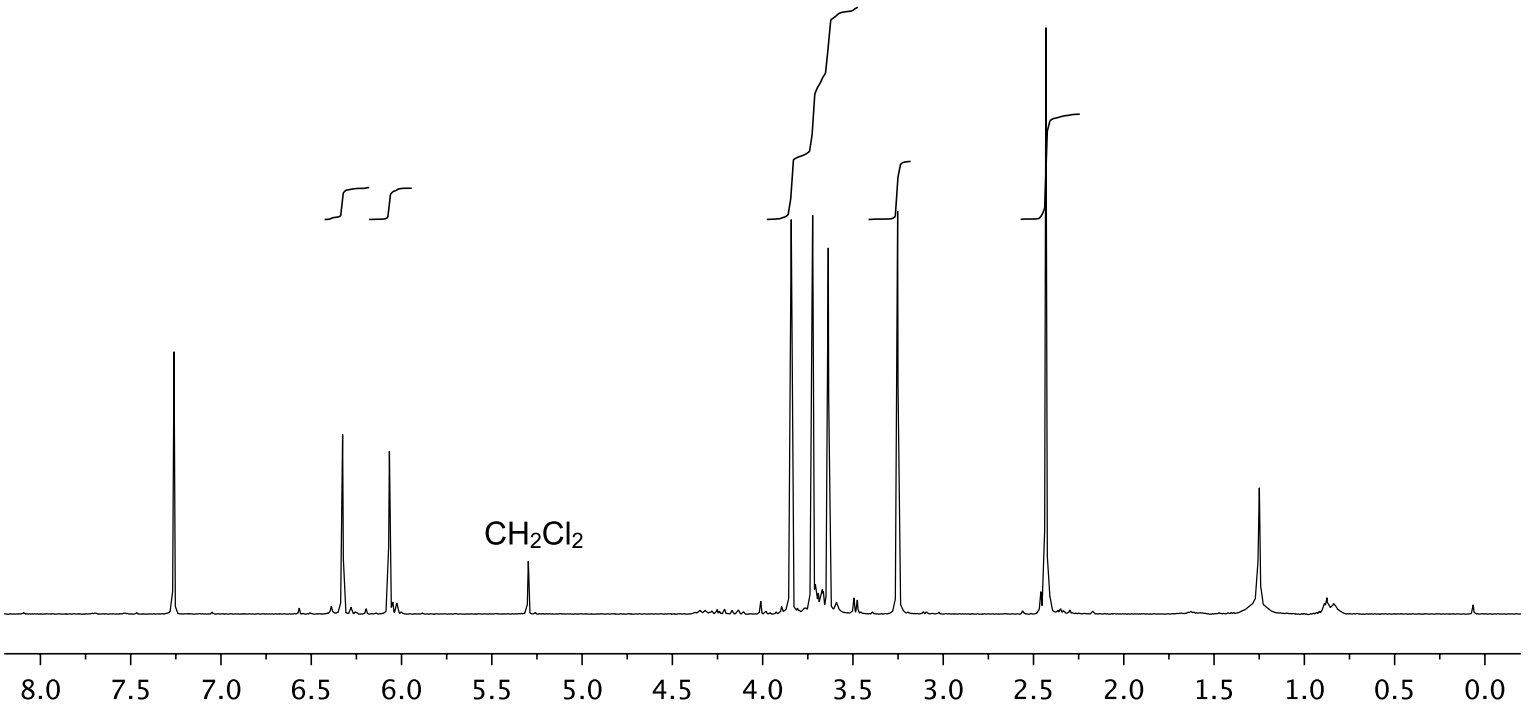


Figure S15. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **28** in $CDCl_3$

28





28

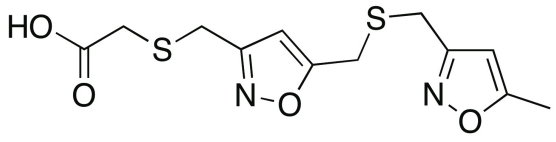
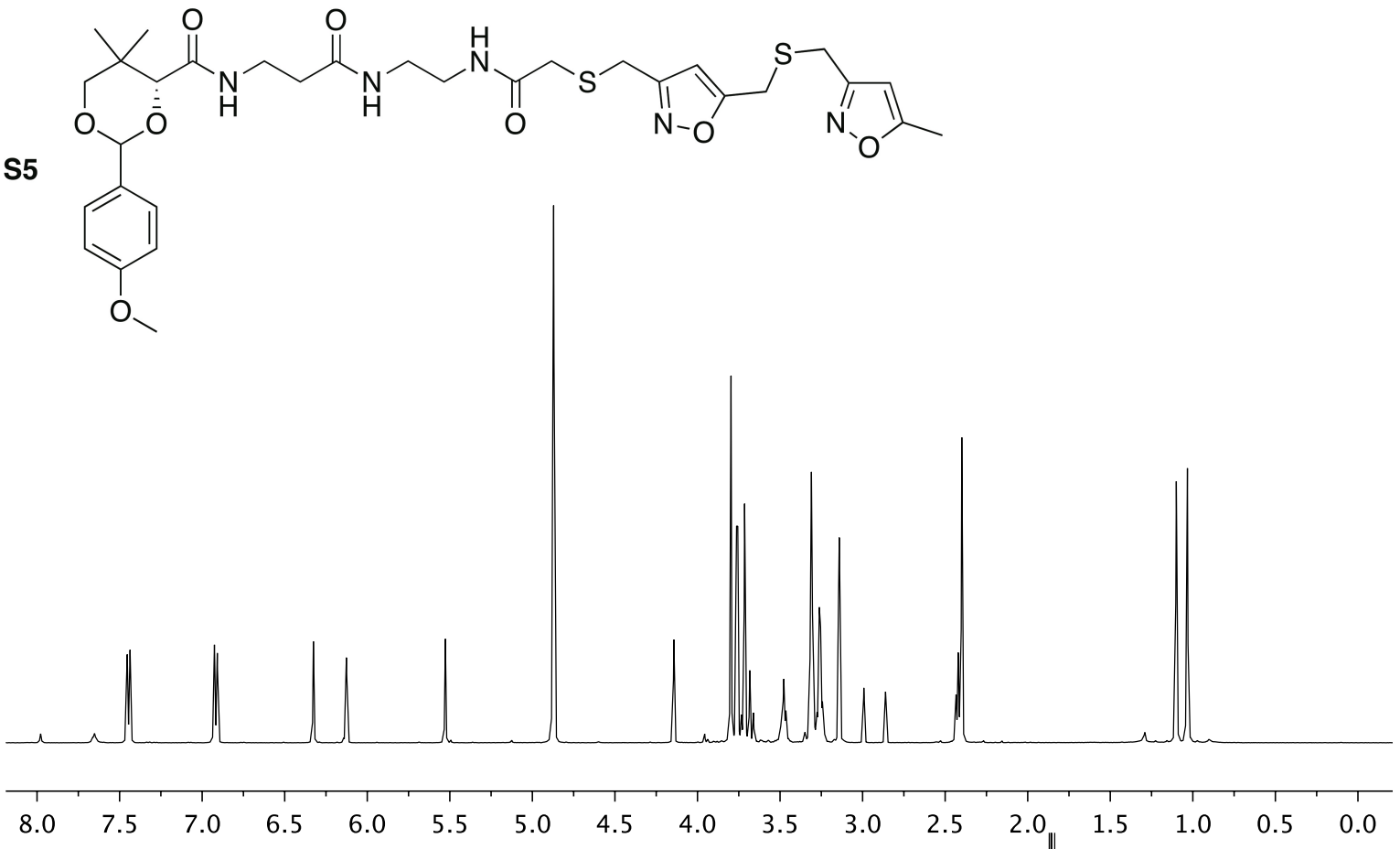


Figure S16. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **S5** in CD_3OD



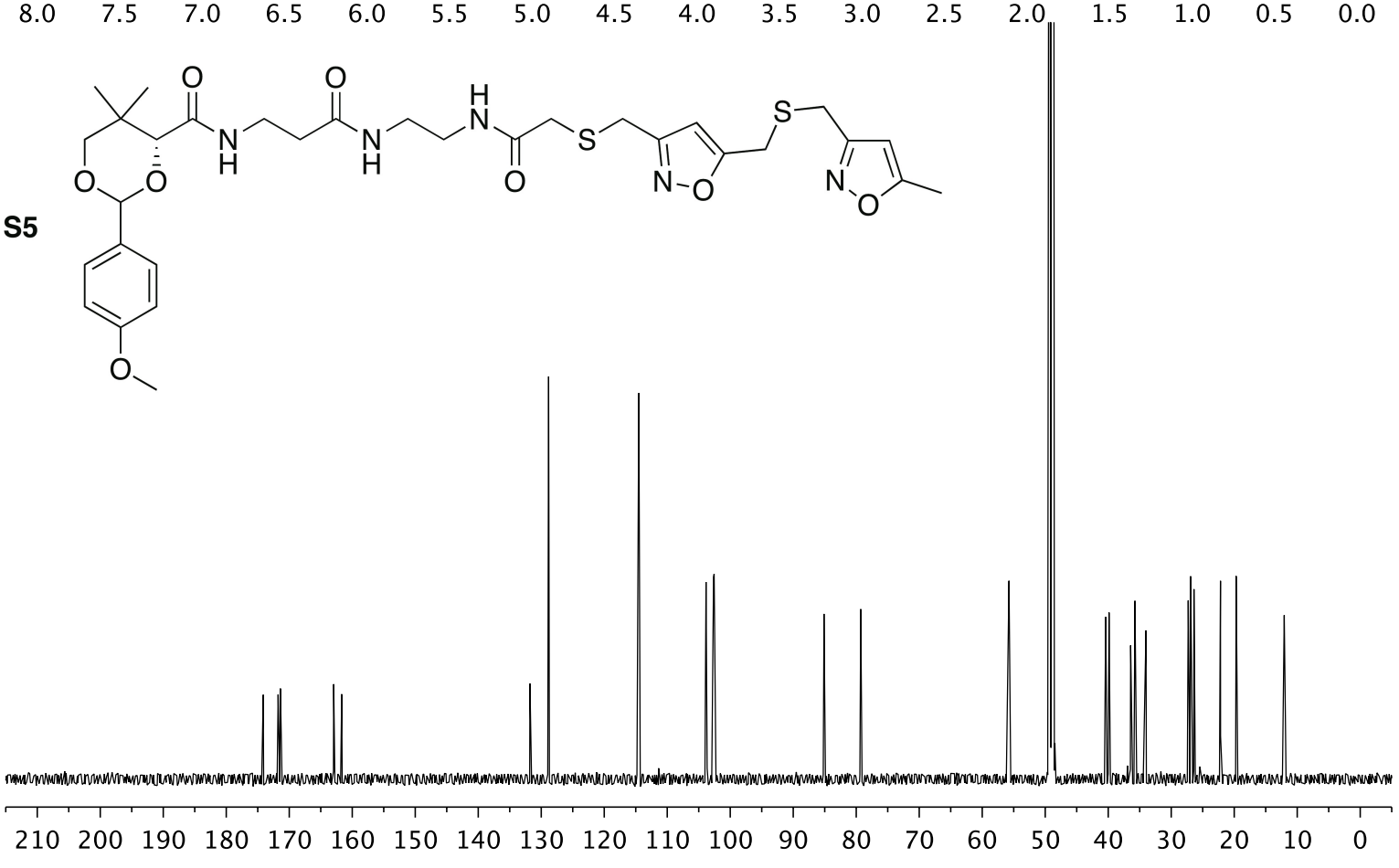
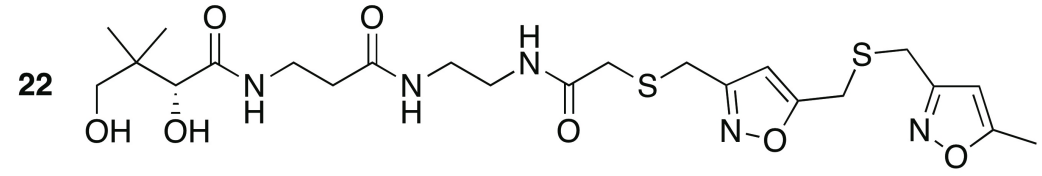
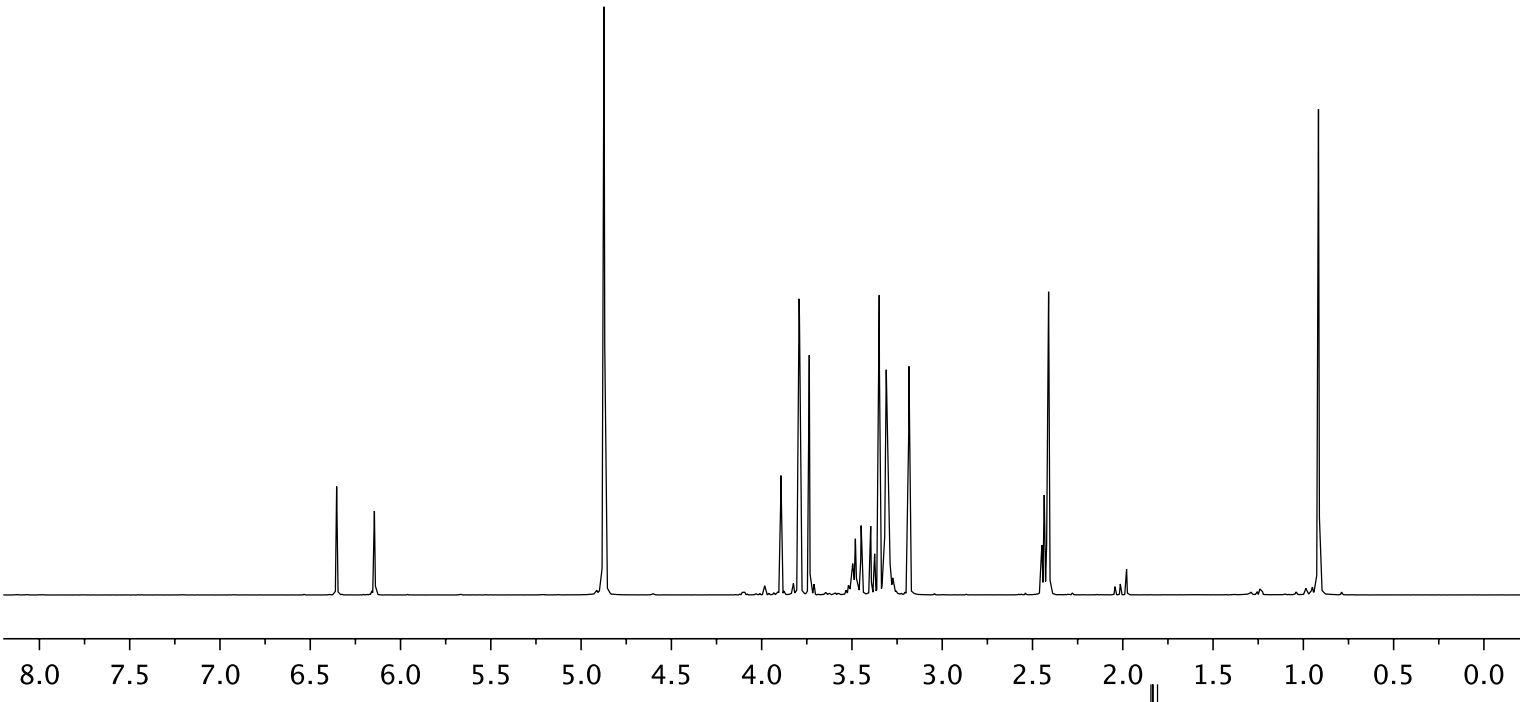


Figure S17. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **22** in CD_3OD





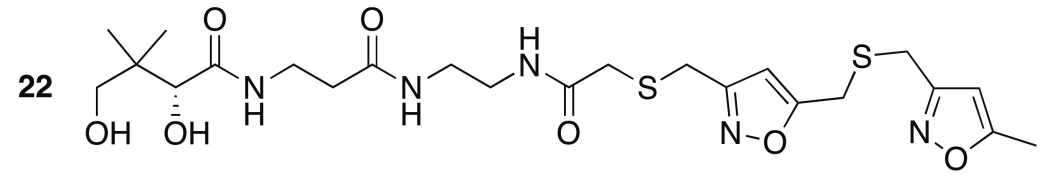
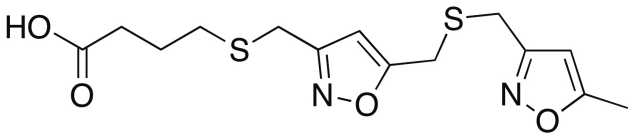
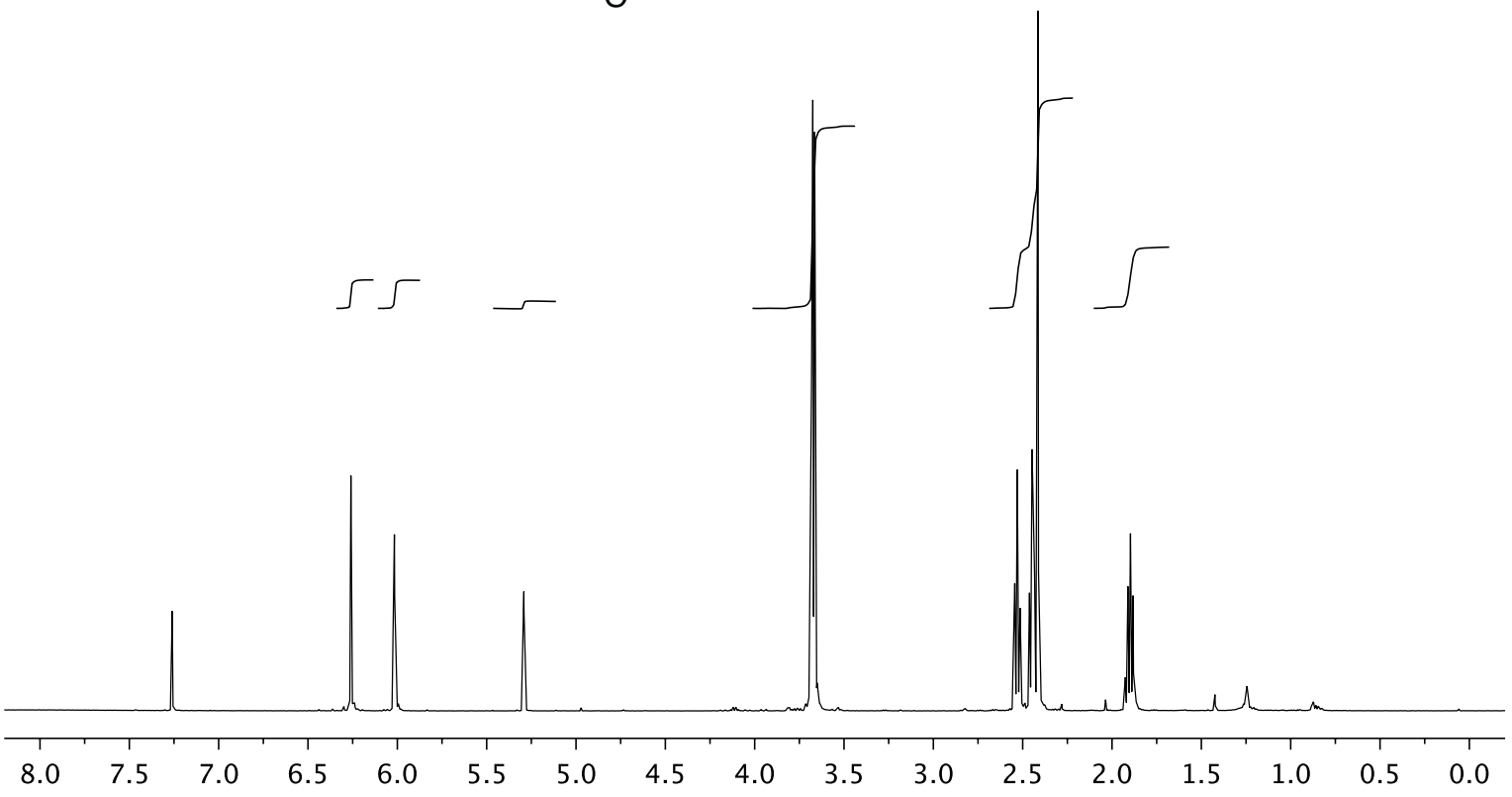


Figure S18. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **29** in $CDCl_3$

29





29

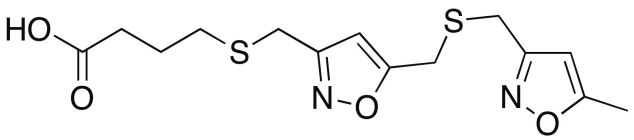


Figure S19. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **S6** in CD_3OD

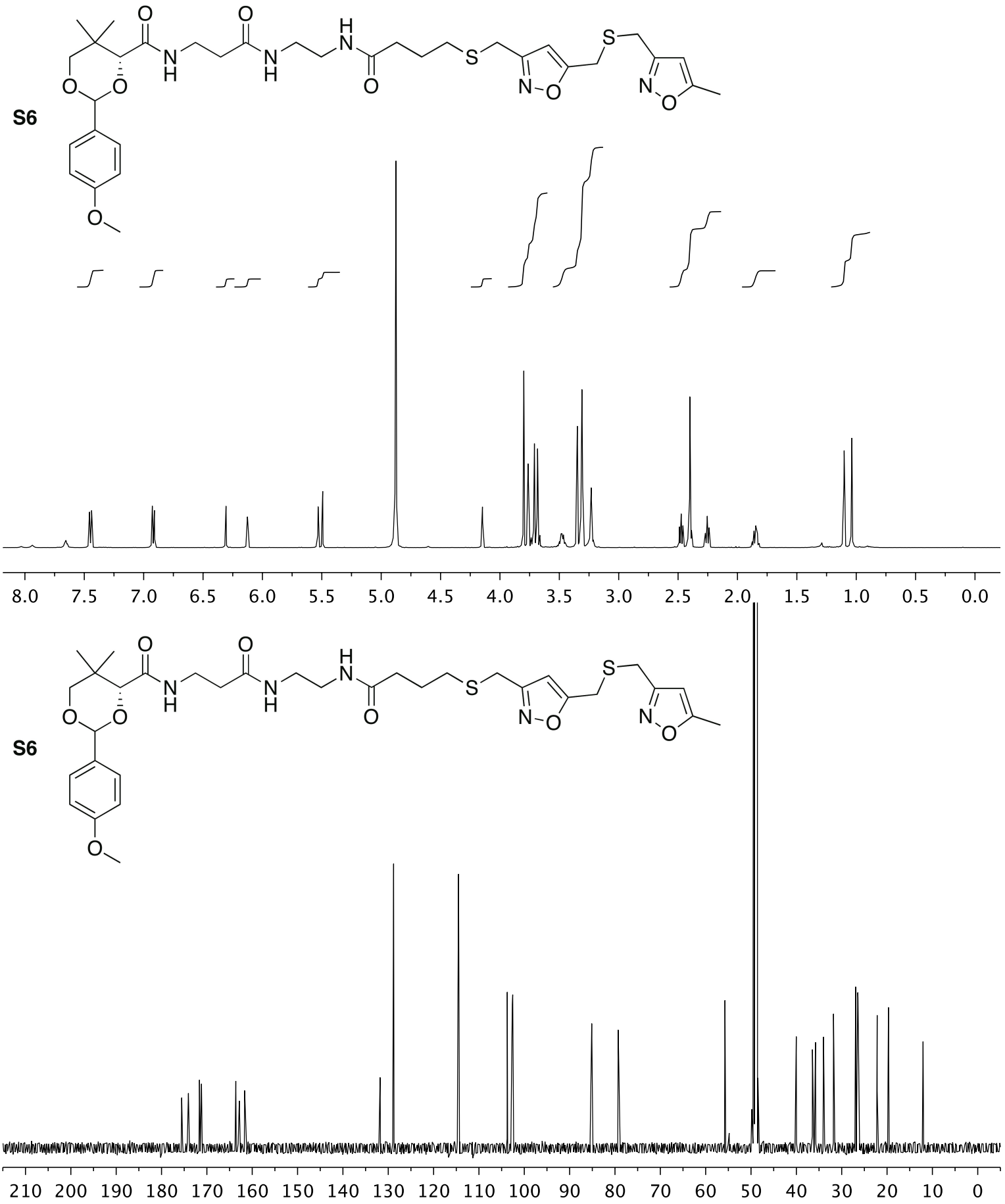
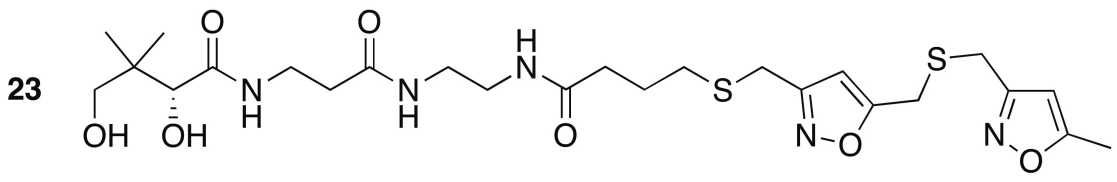


Figure S20. ^{1}H NMR (500 Mhz) and ^{13}C NMR (125 MHz) spectra of **23** in CD_3OD



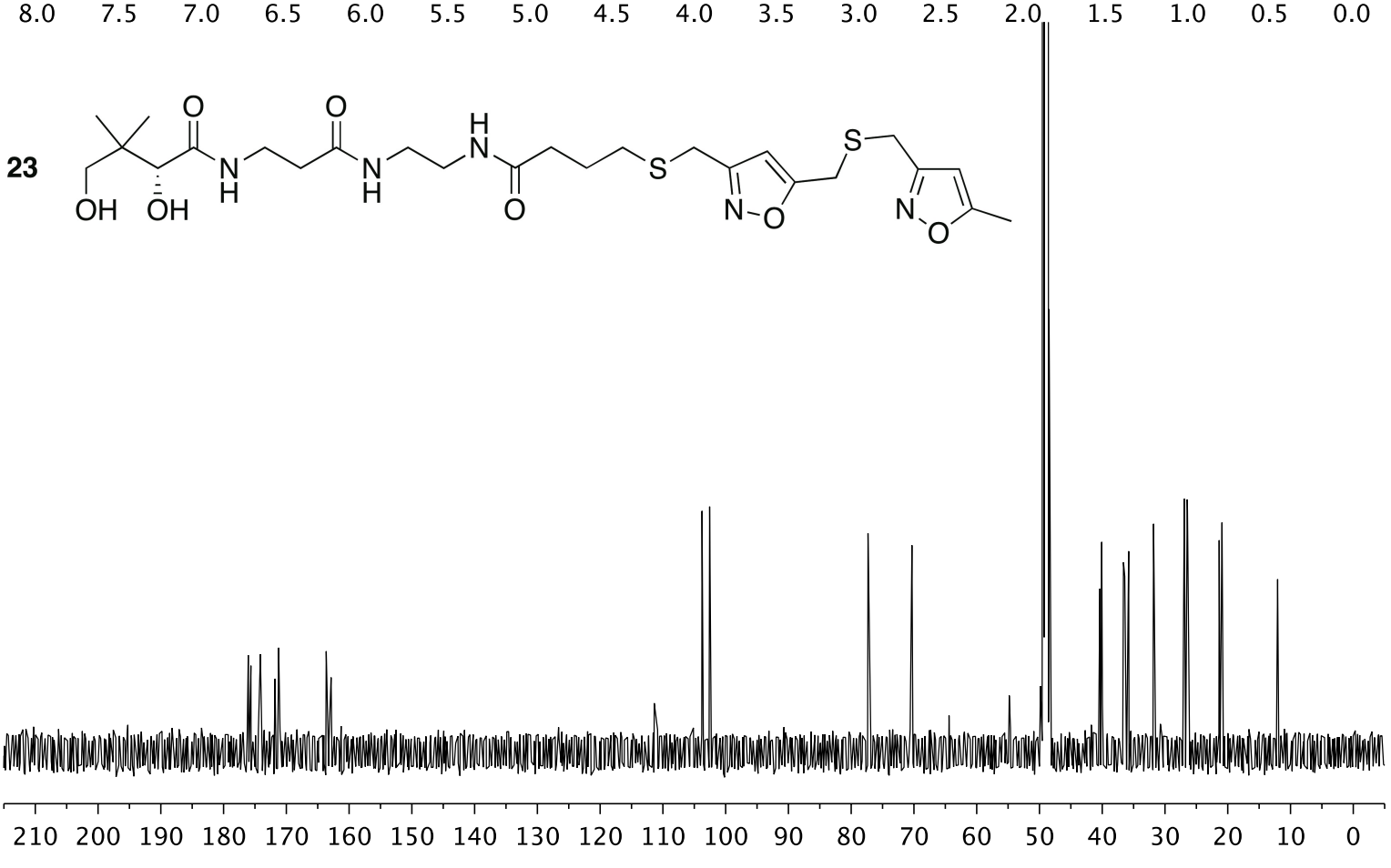
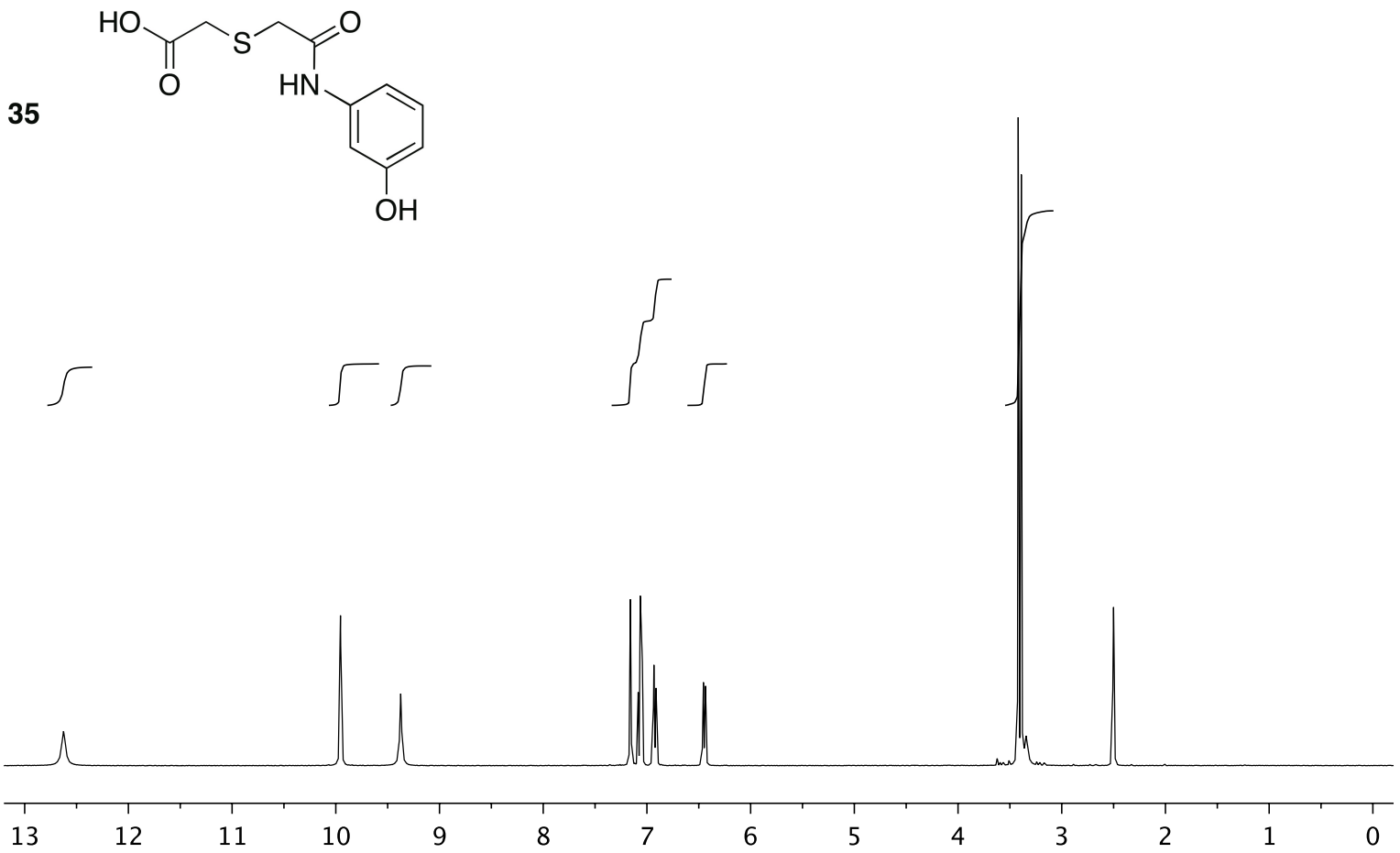


Figure S21. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra of **35** in DMSO-d_6



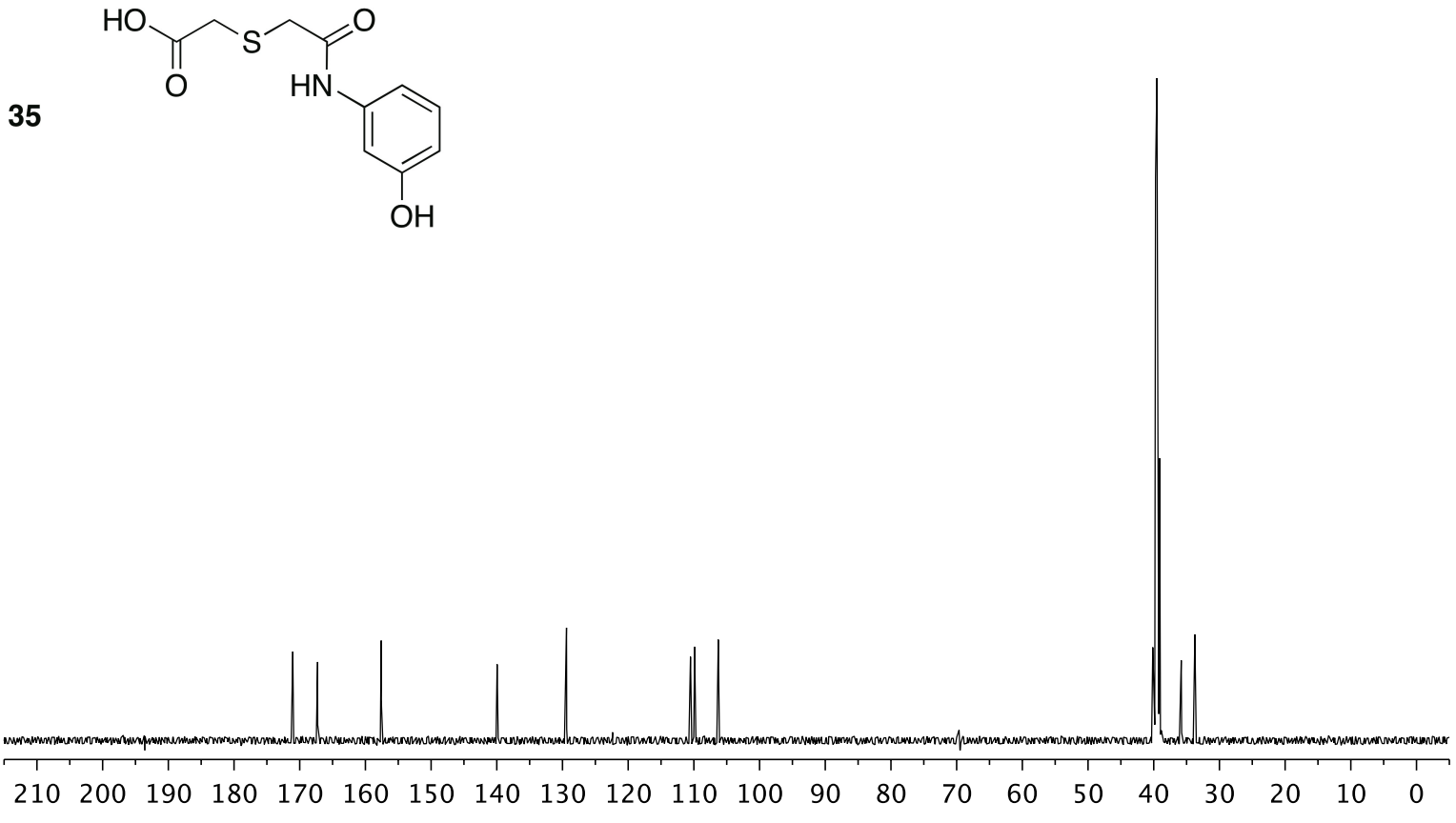
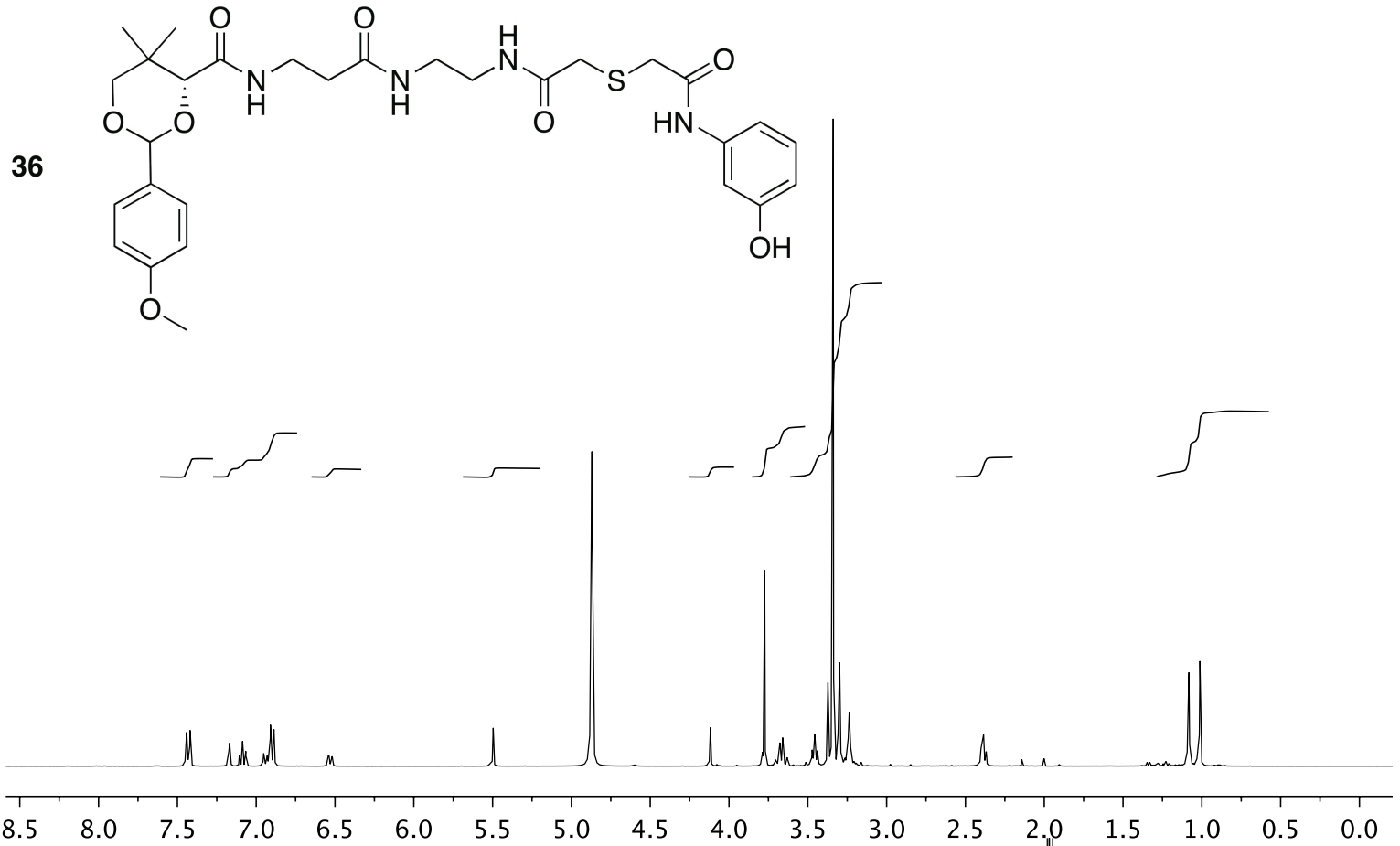


Figure S22. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra of **36** in CD_3OD



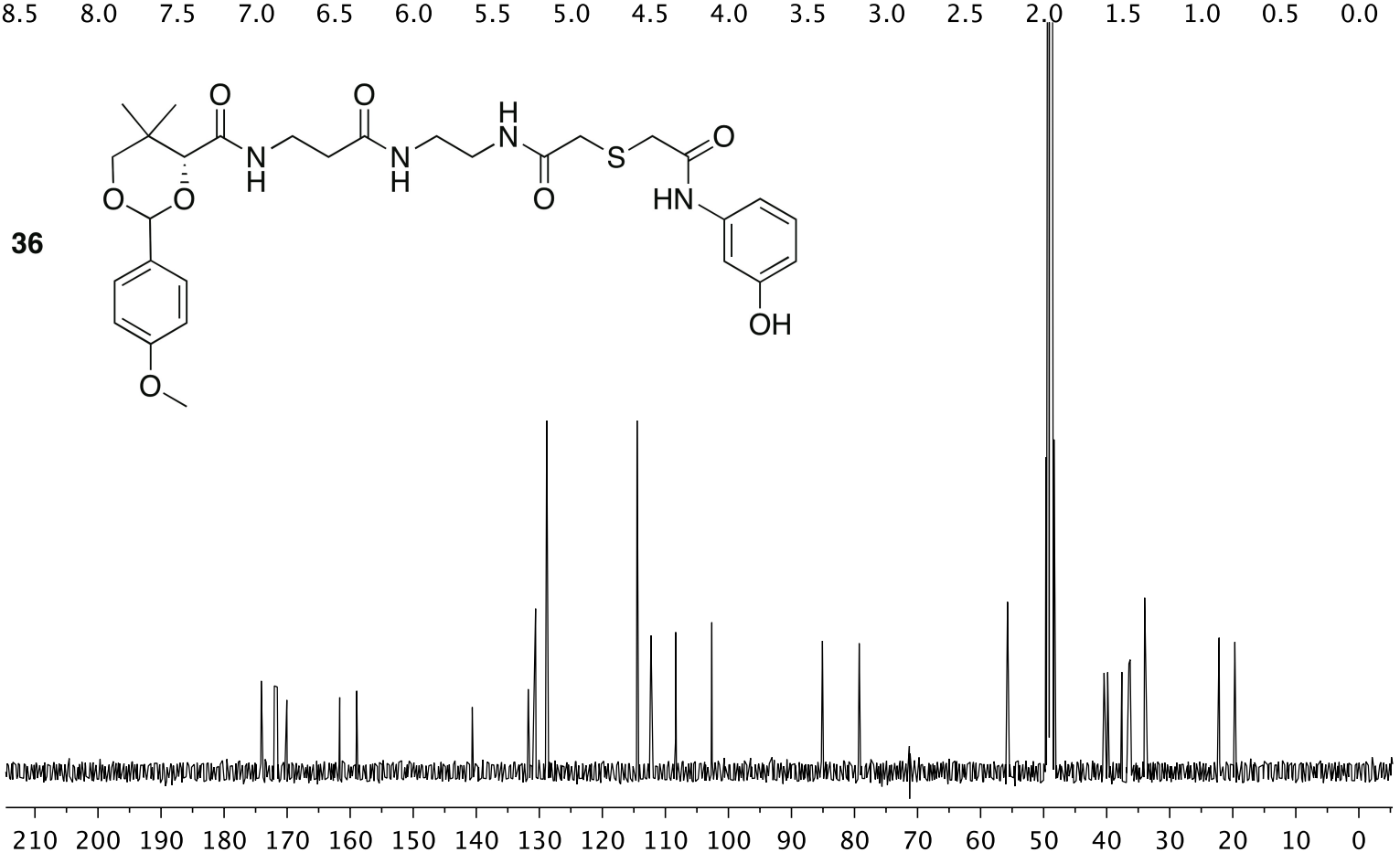
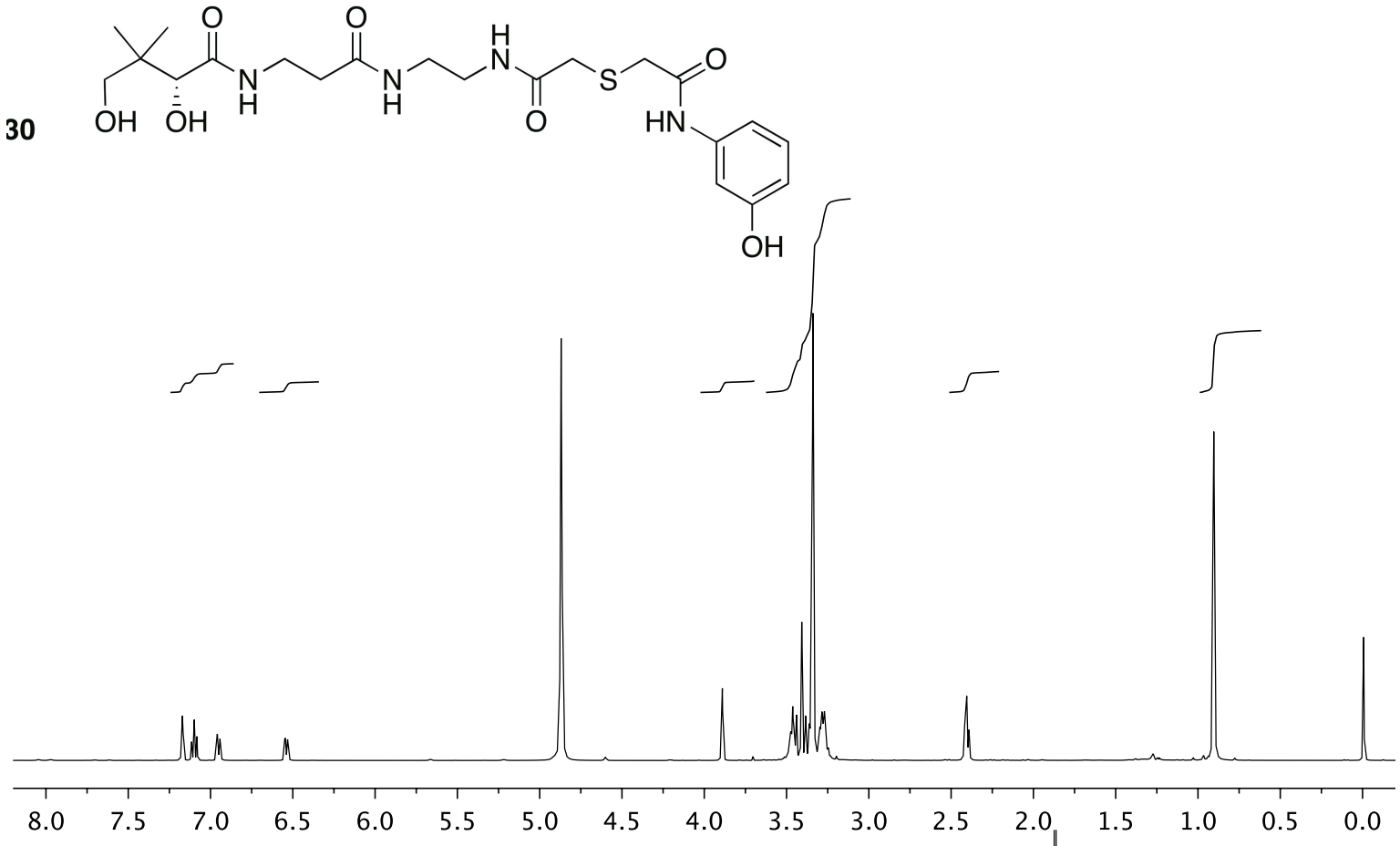


Figure S23. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of 3**0** in CD_3OD



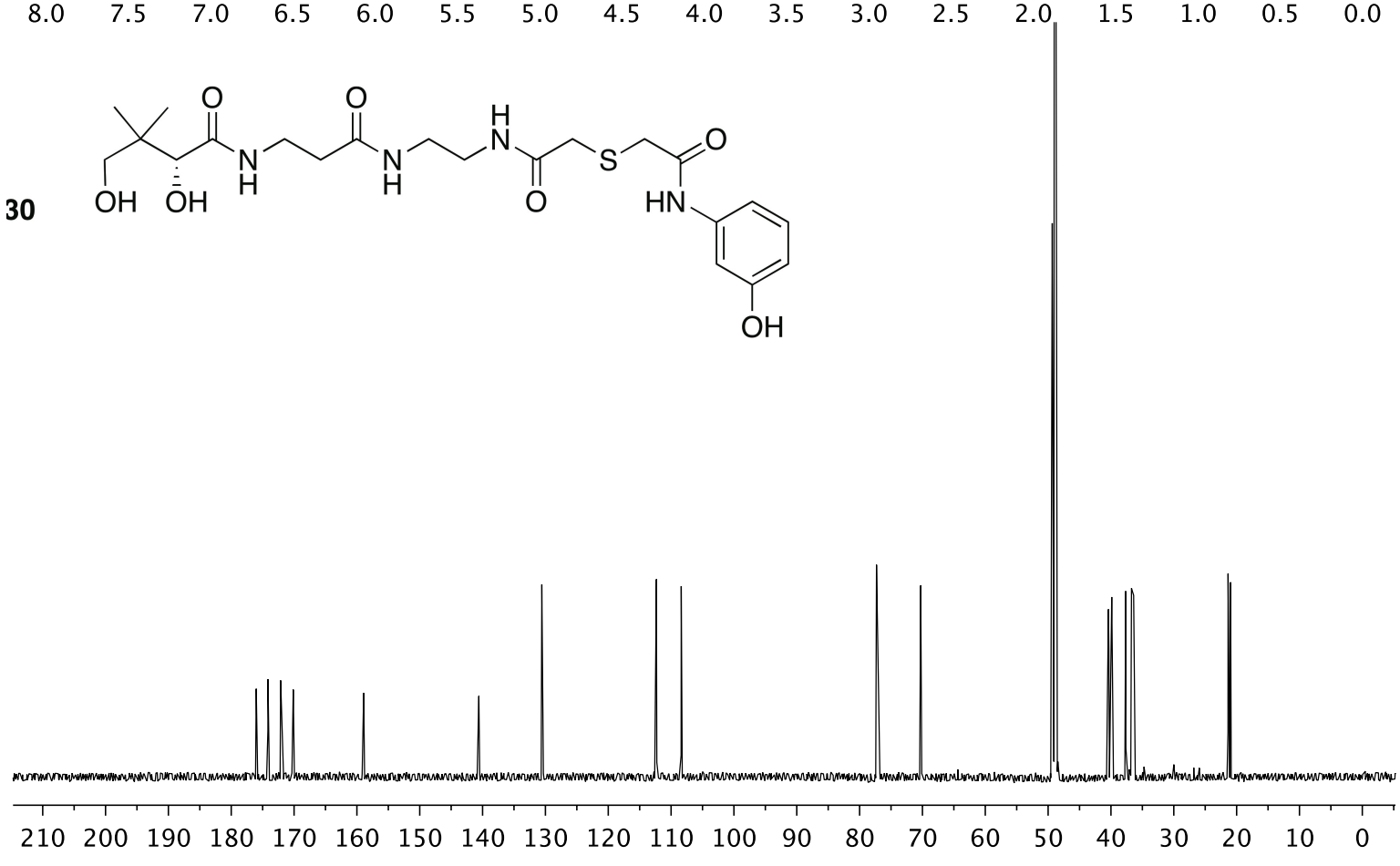
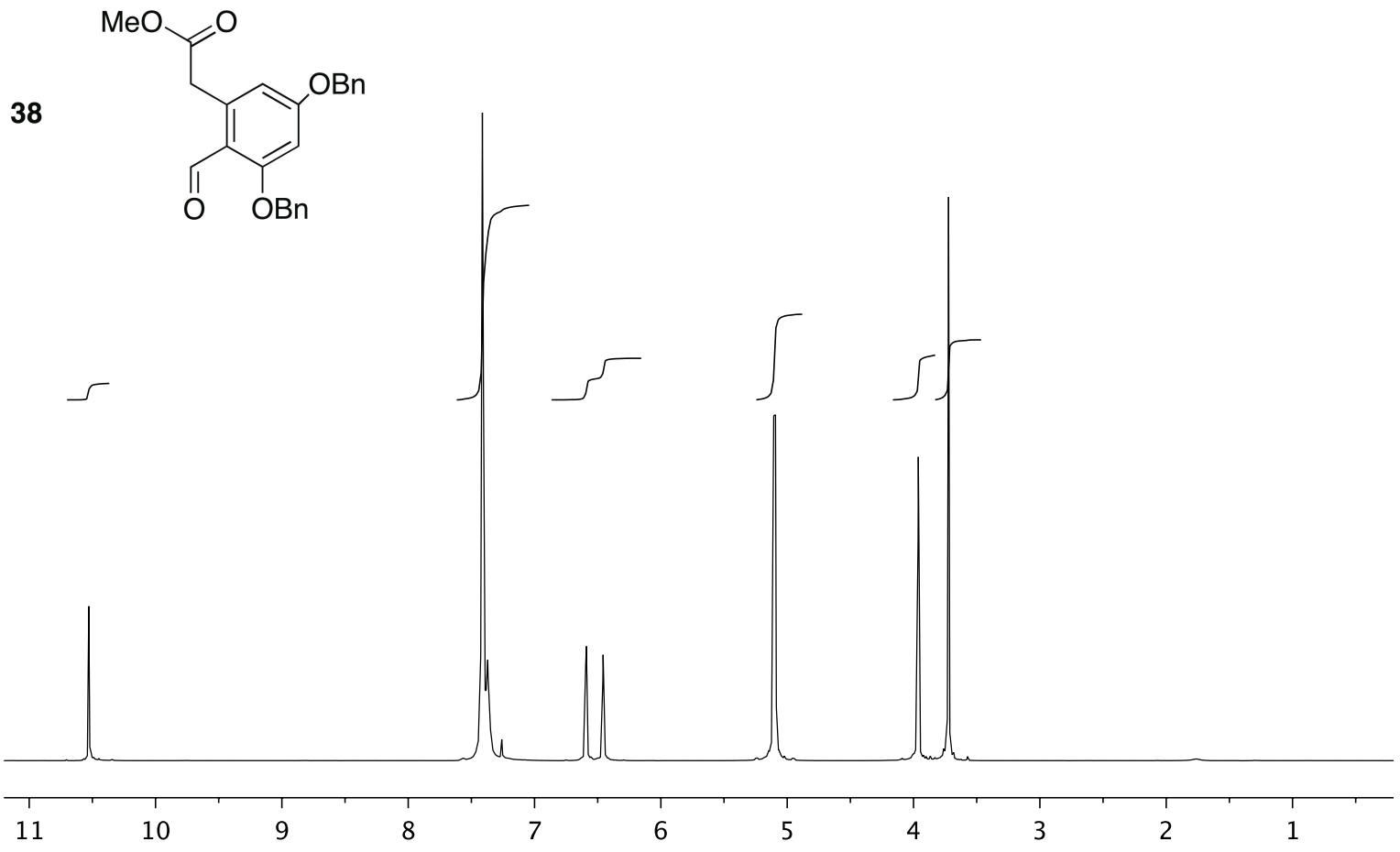


Figure S24. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **38** in $CDCl_3$



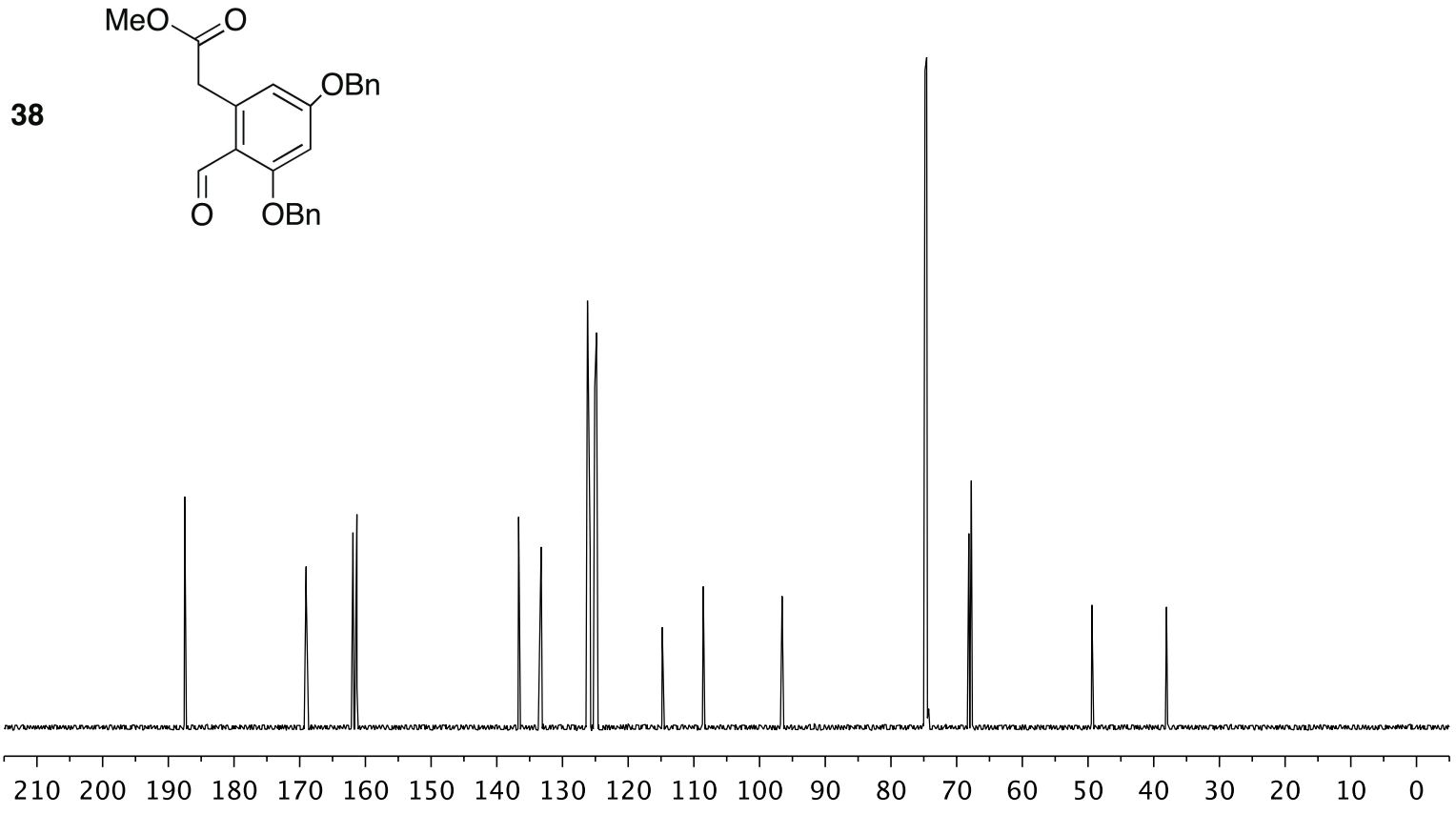
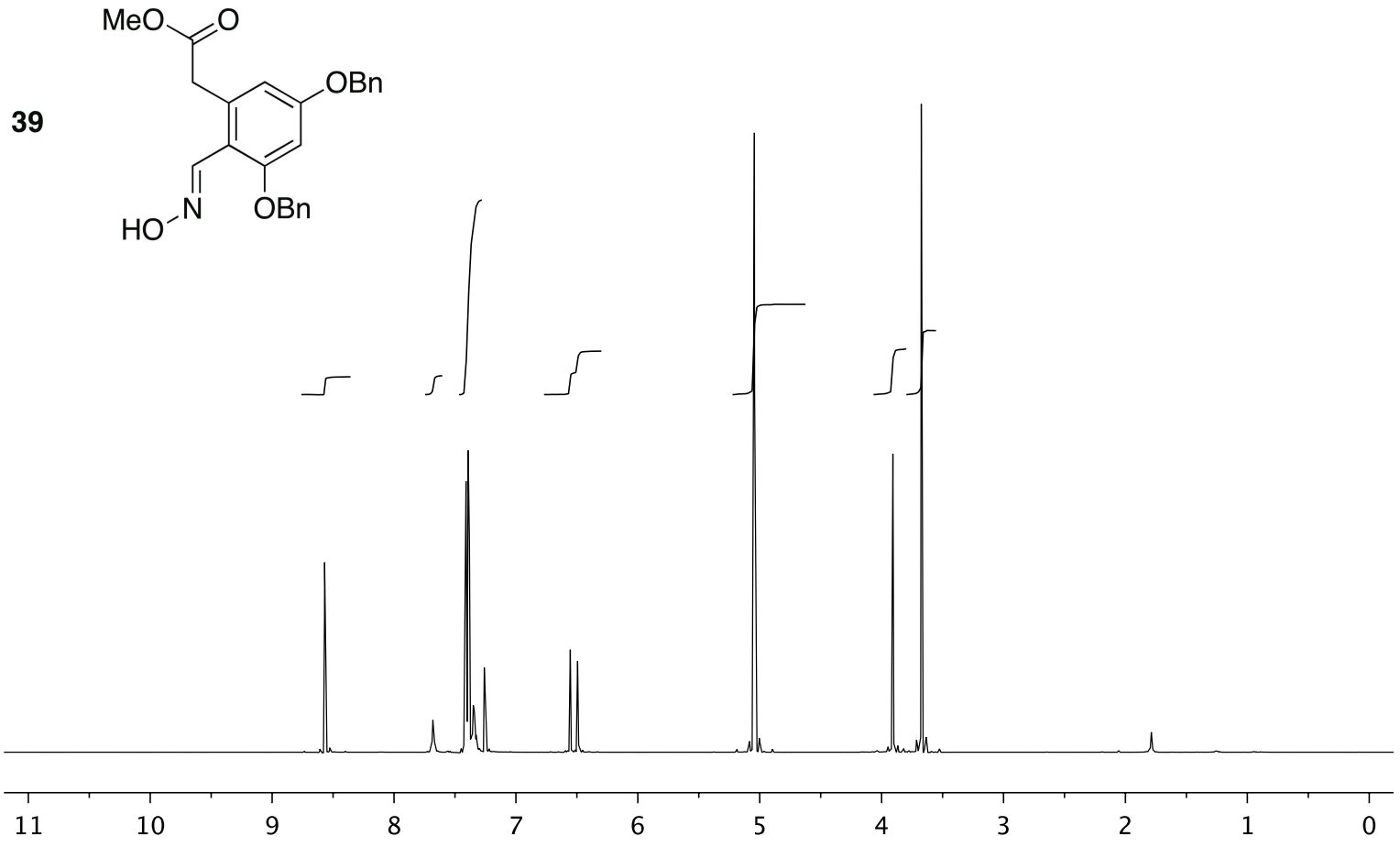


Figure S25. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **39** in $CDCl_3$



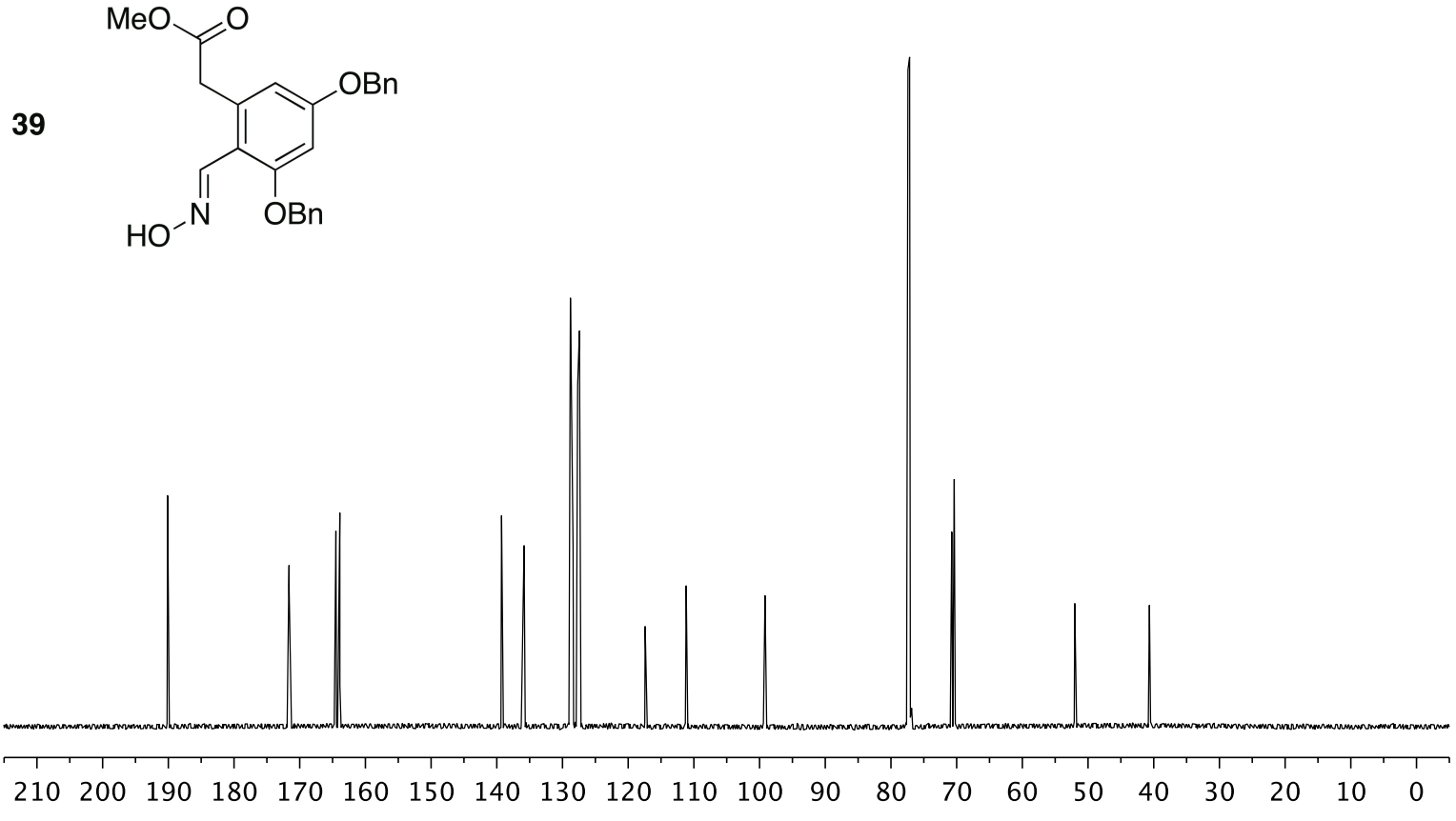
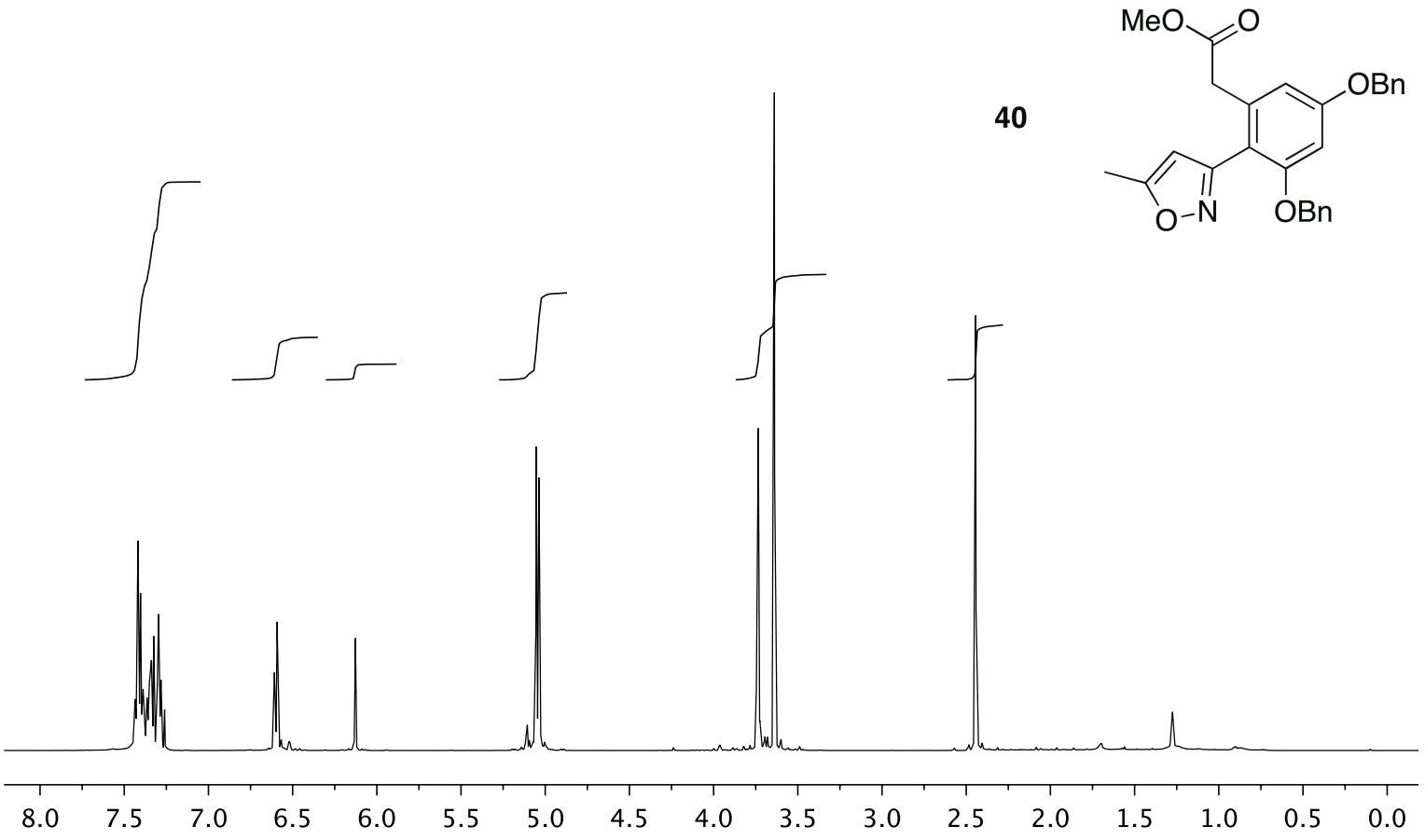


Figure S26. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **40** in $CDCl_3$



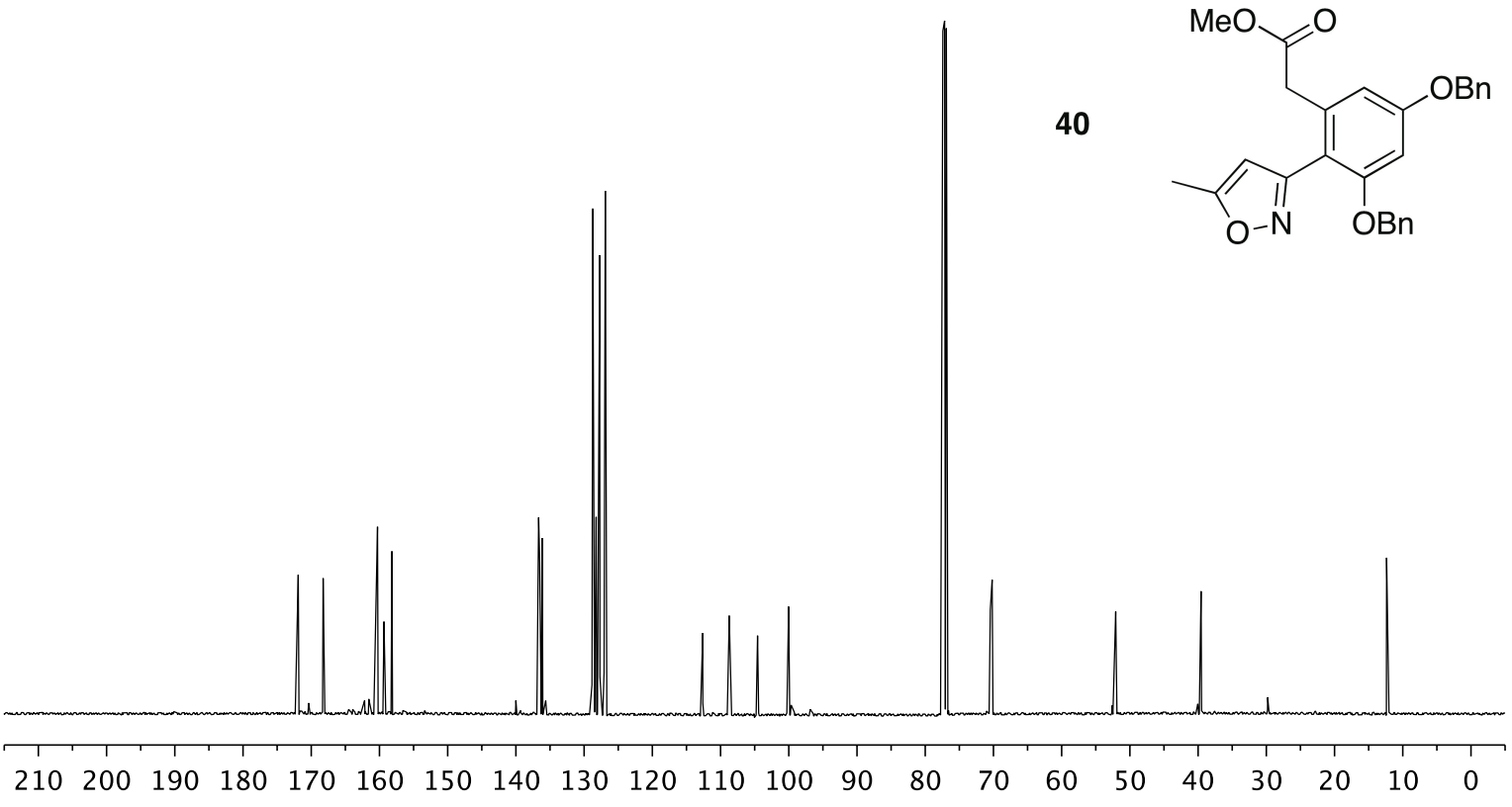
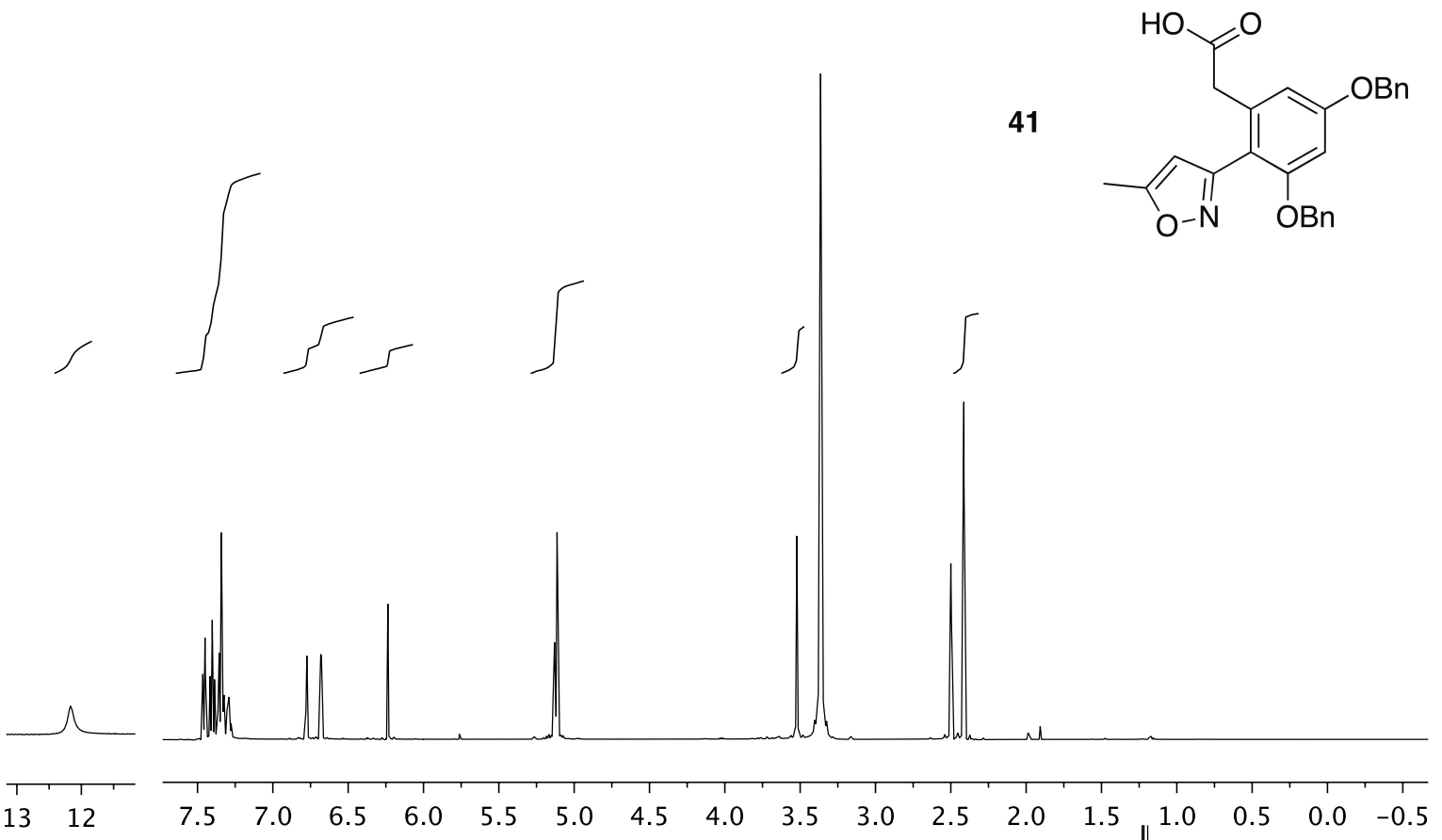


Figure S27. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **41** in DMSO-d_6



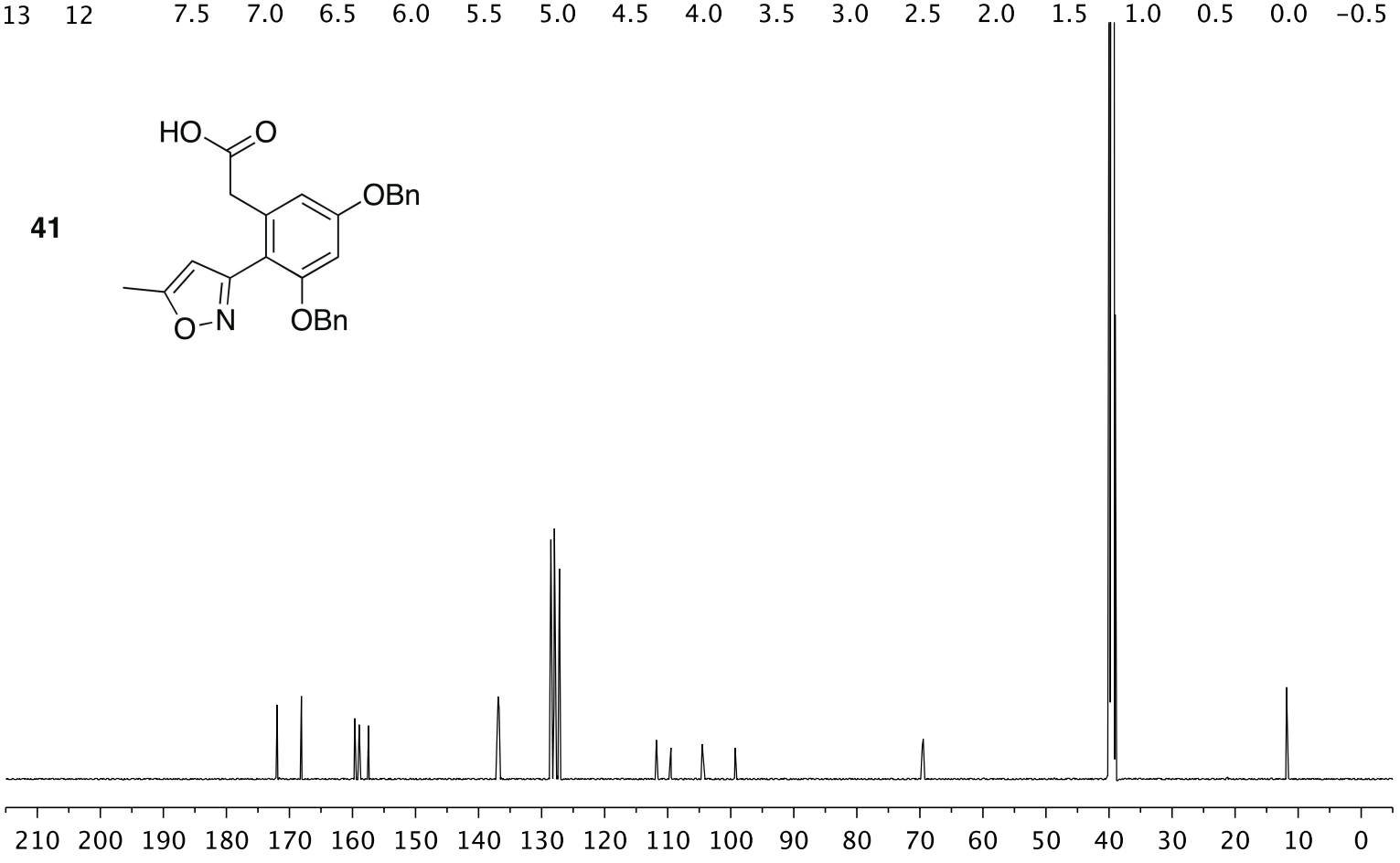


Figure S28. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **42** in acetone-d_6

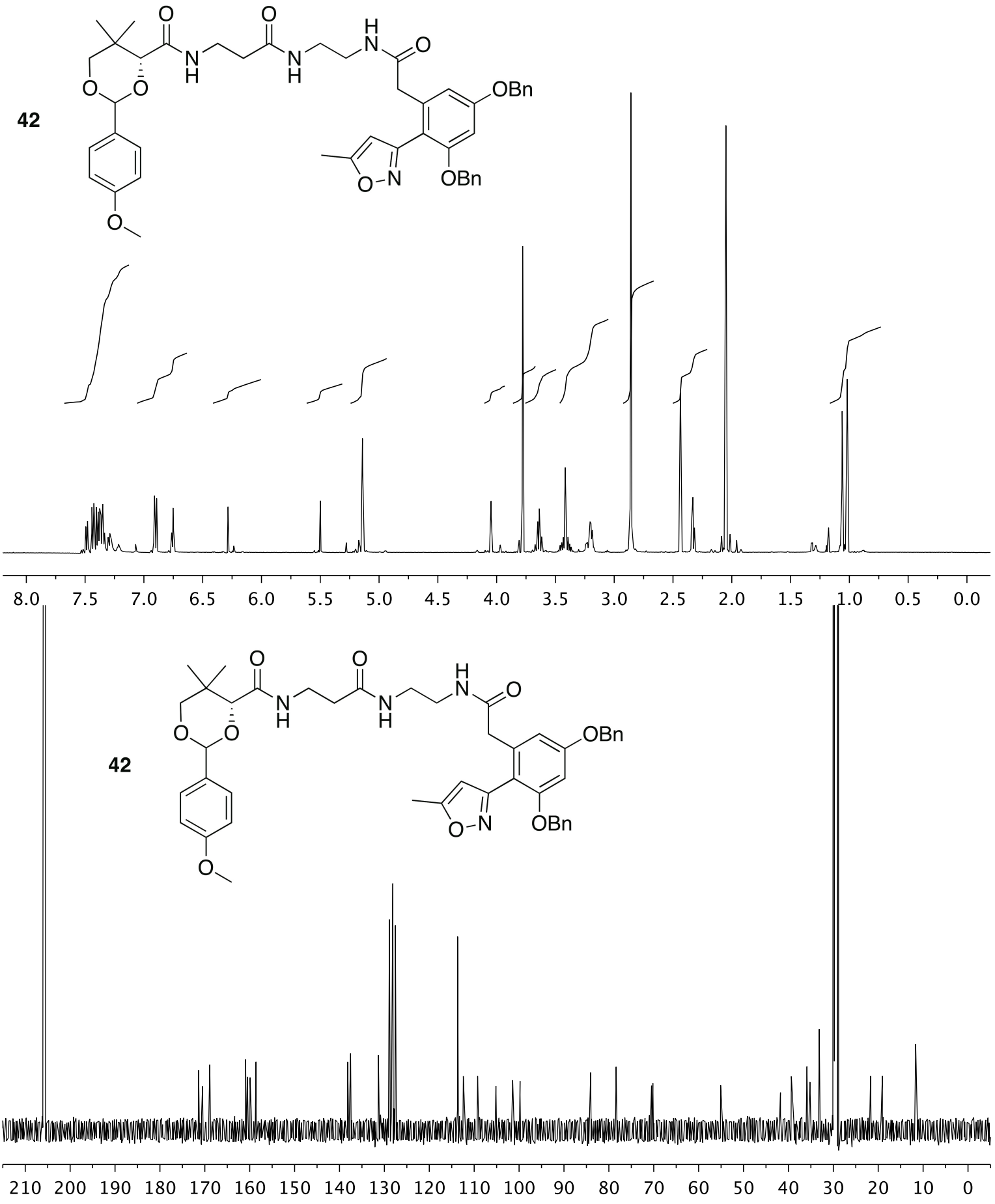
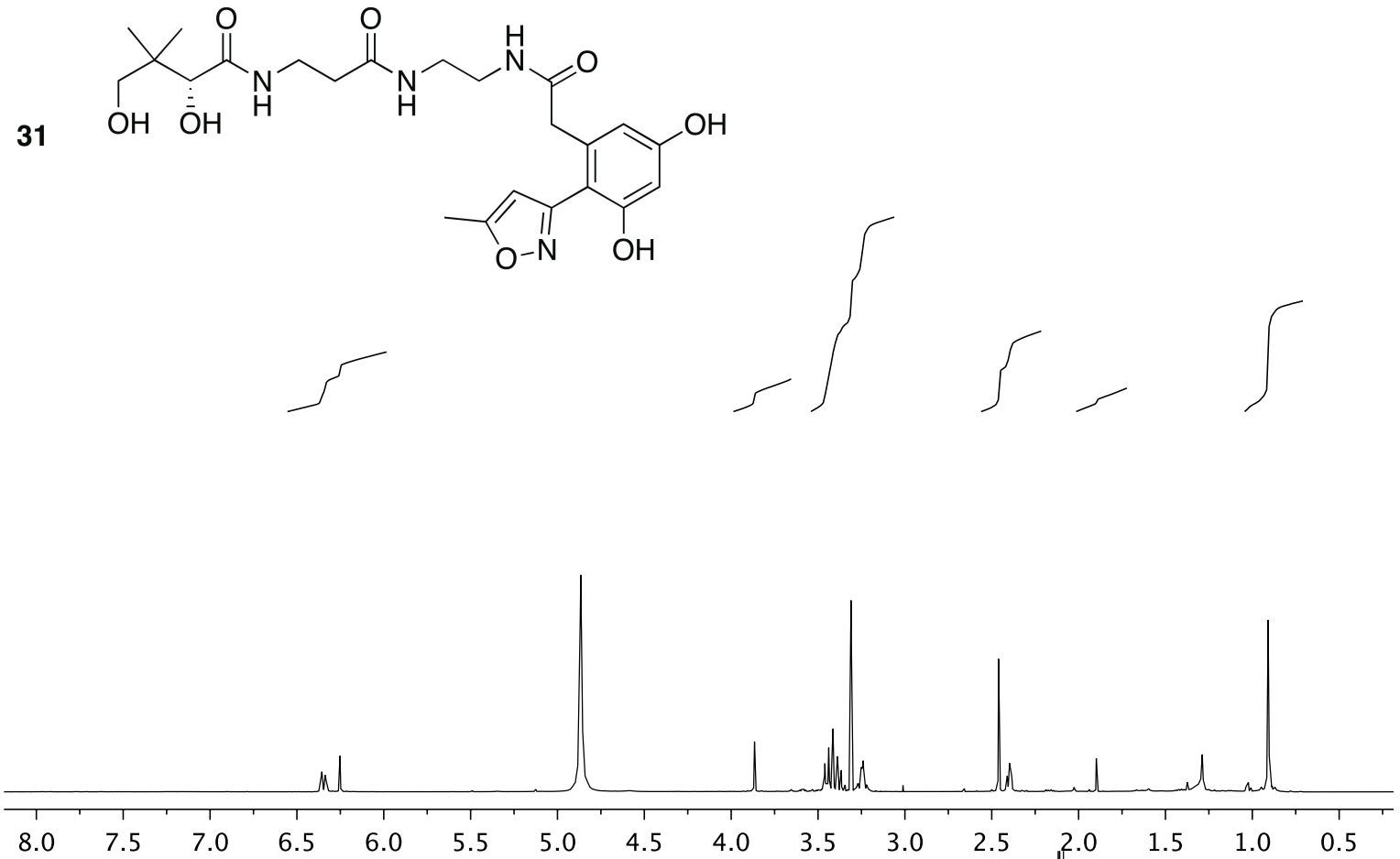


Figure S29. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of mimetic **31** in CD_3OD



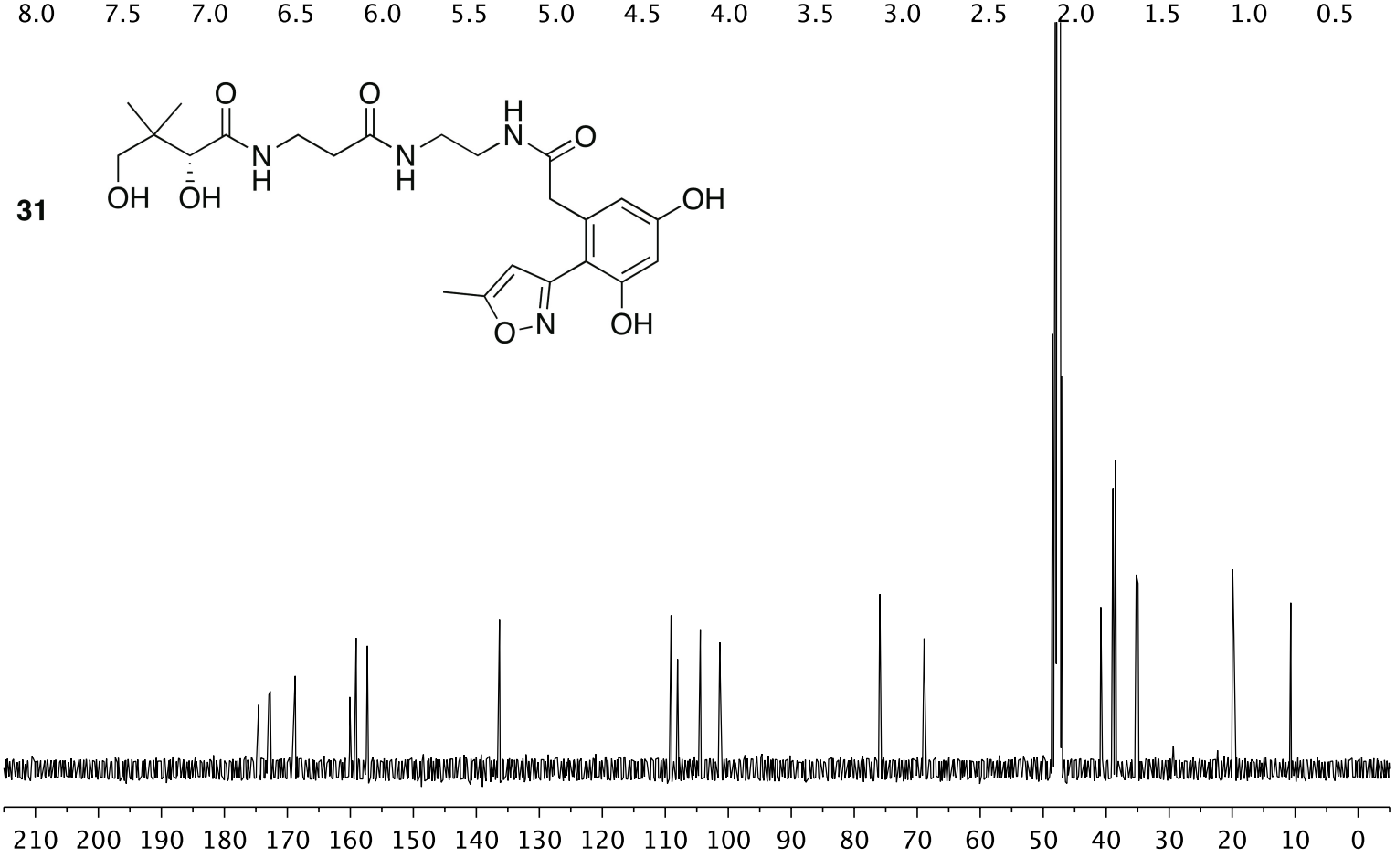
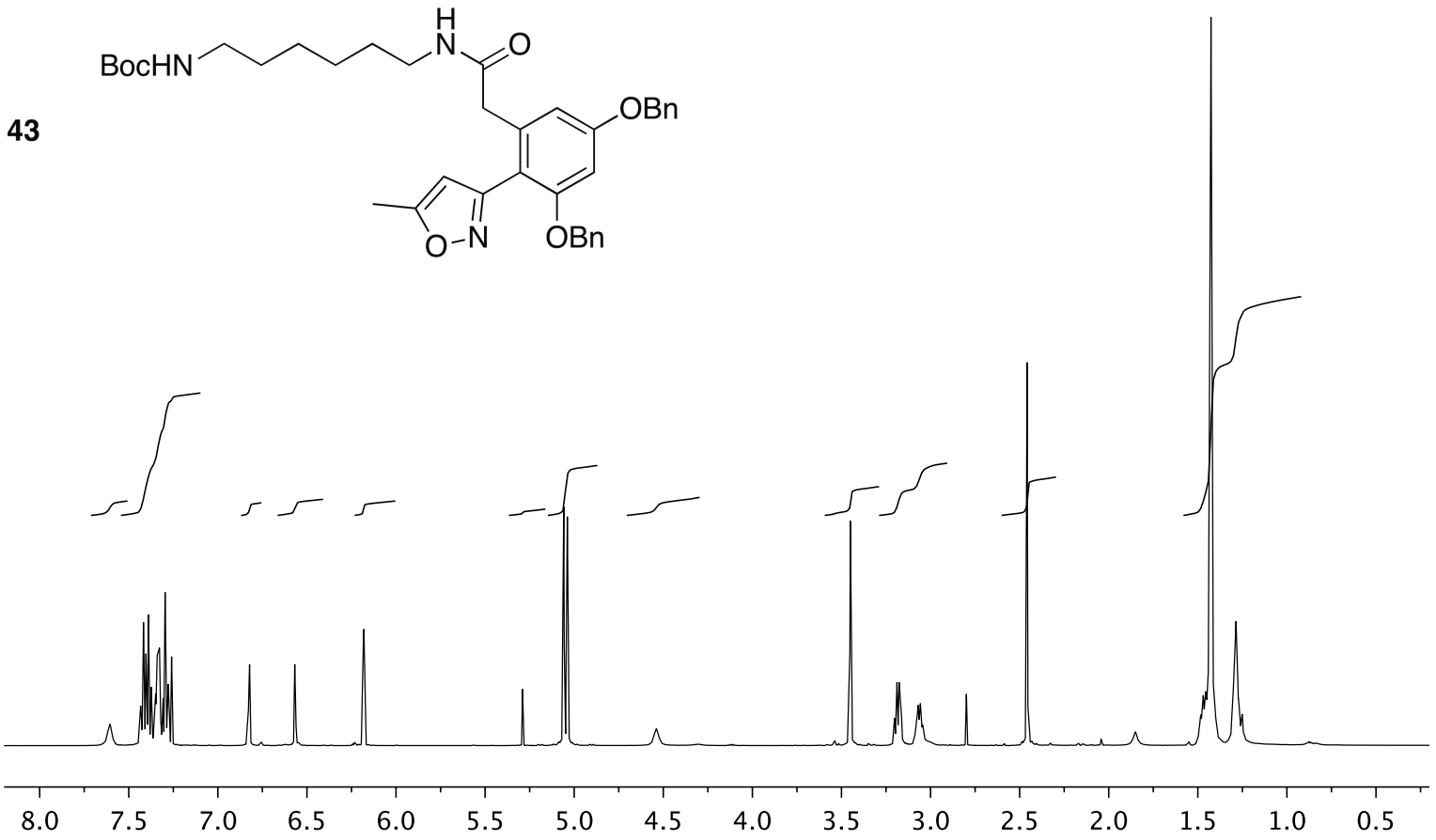


Figure S30. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **43** in $CDCl_3$



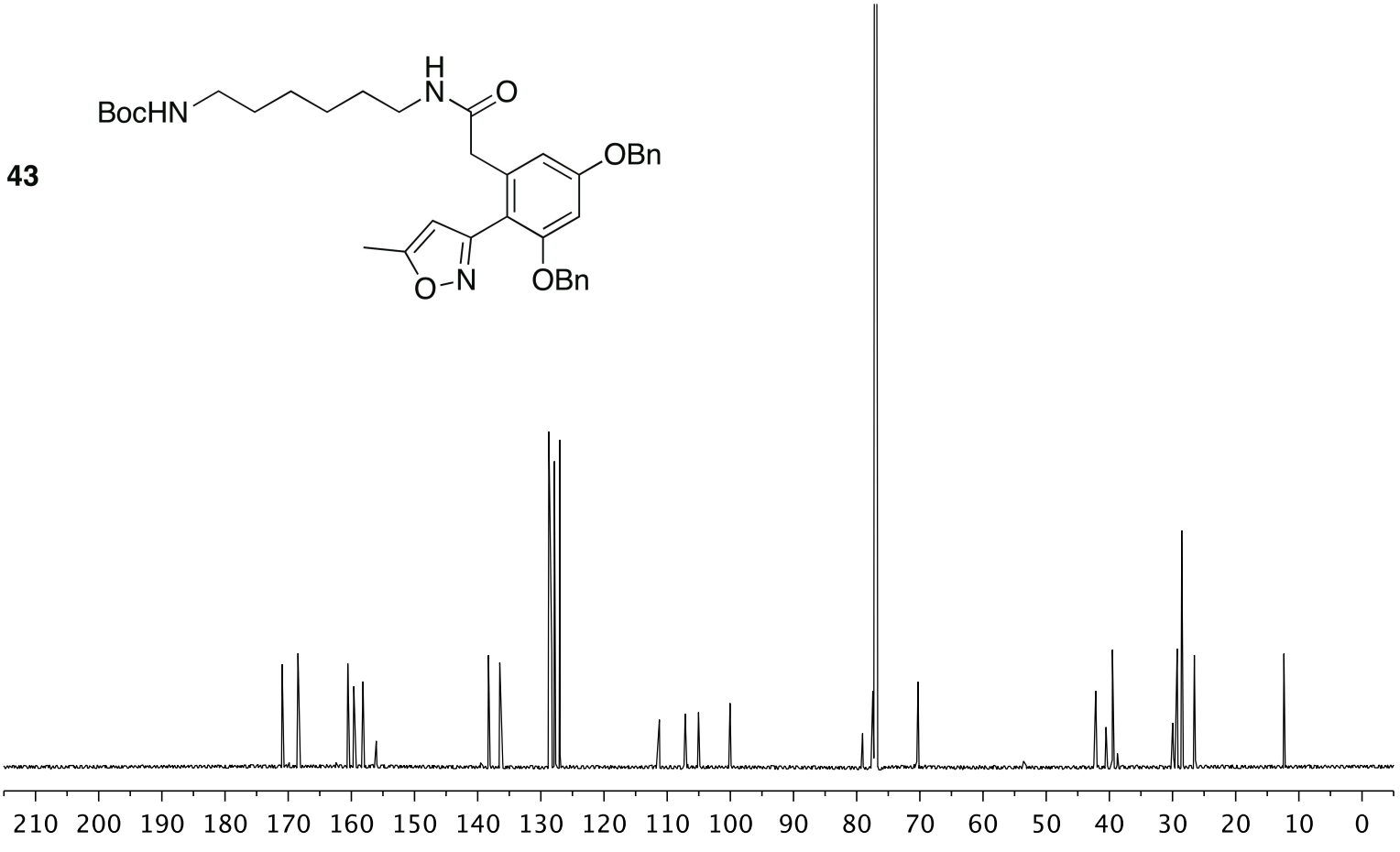
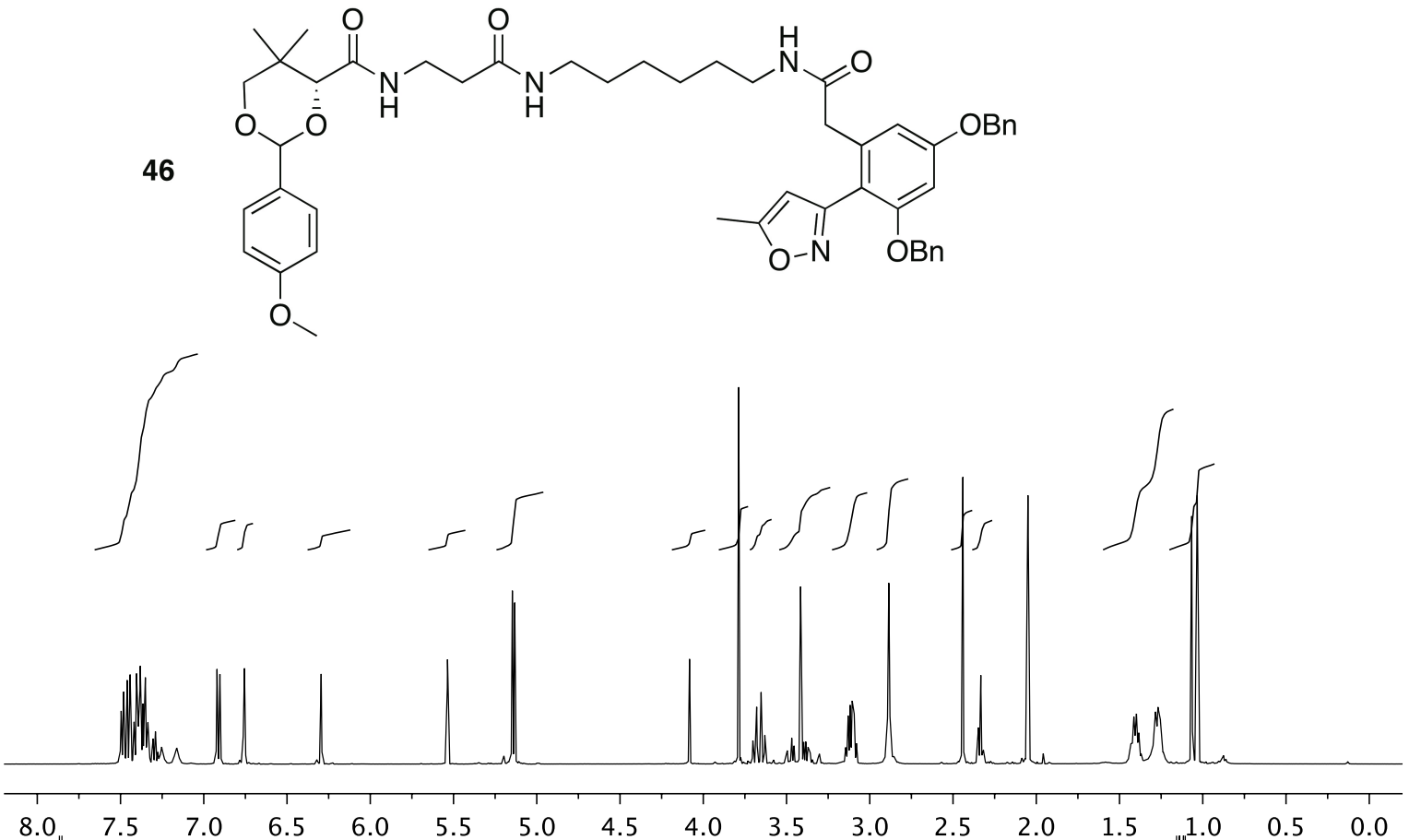


Figure S31. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **46** in acetone-d_6



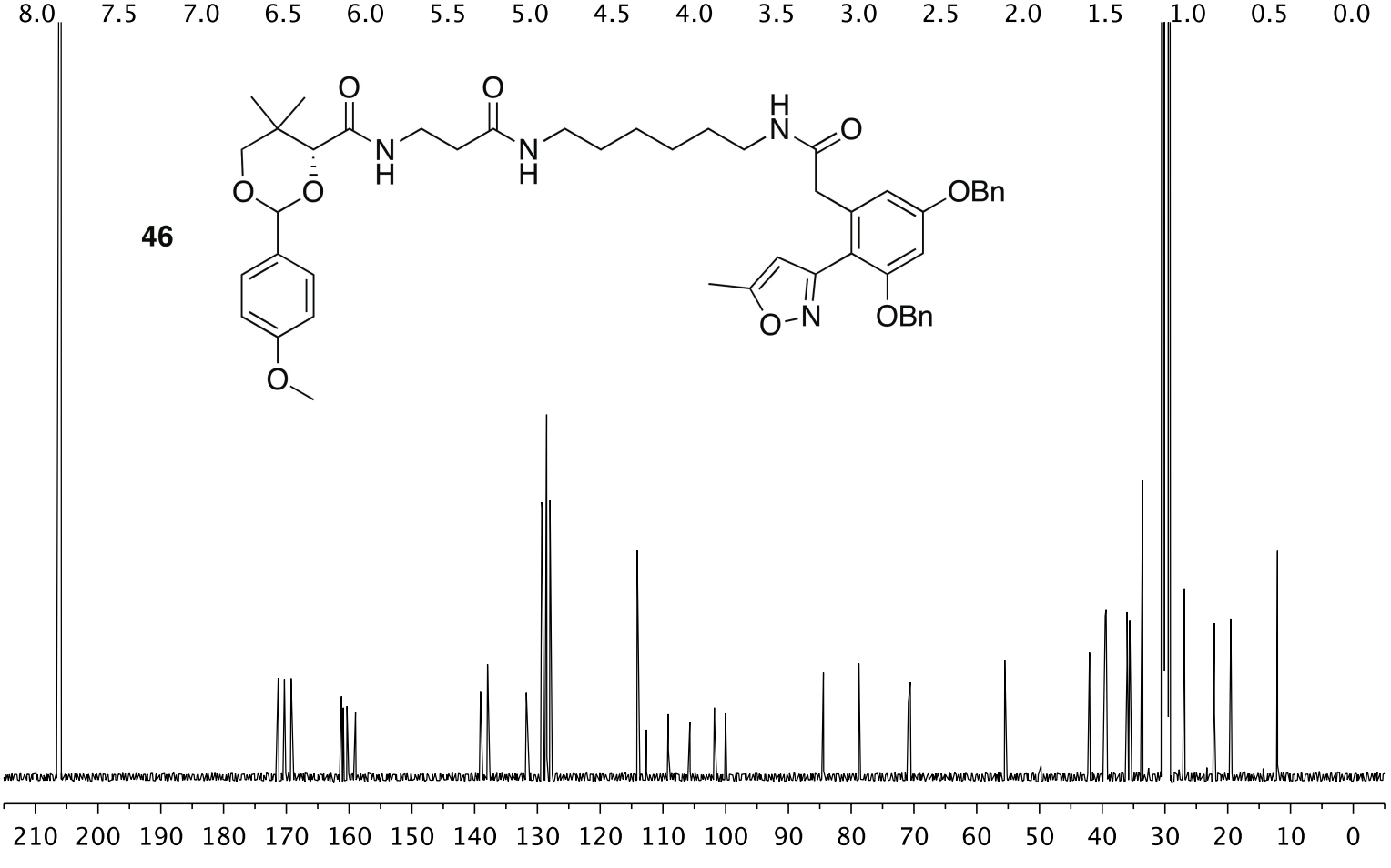


Figure S32. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **47** in acetone-d_6

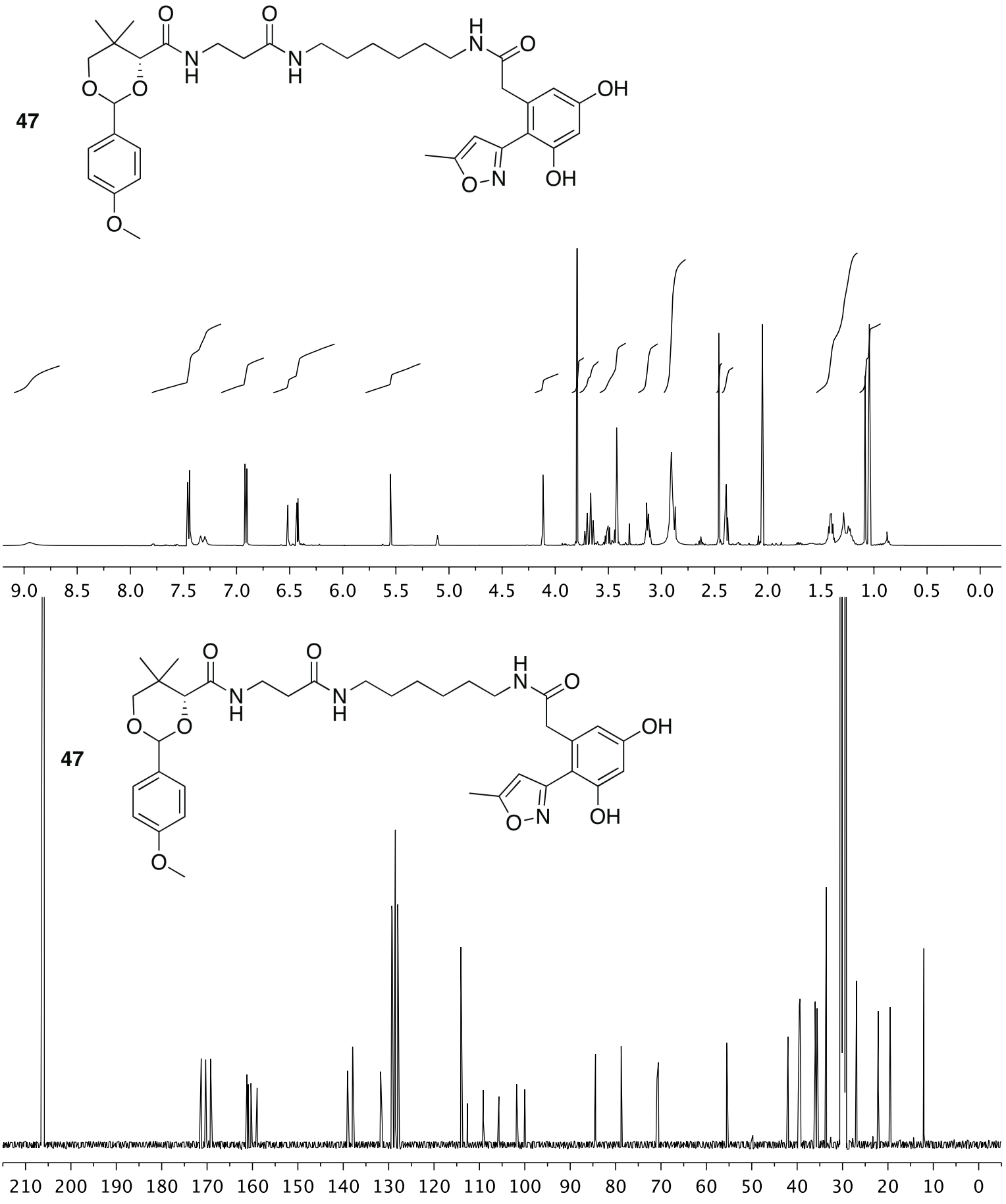


Figure S33. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of **32** in CD_3OD

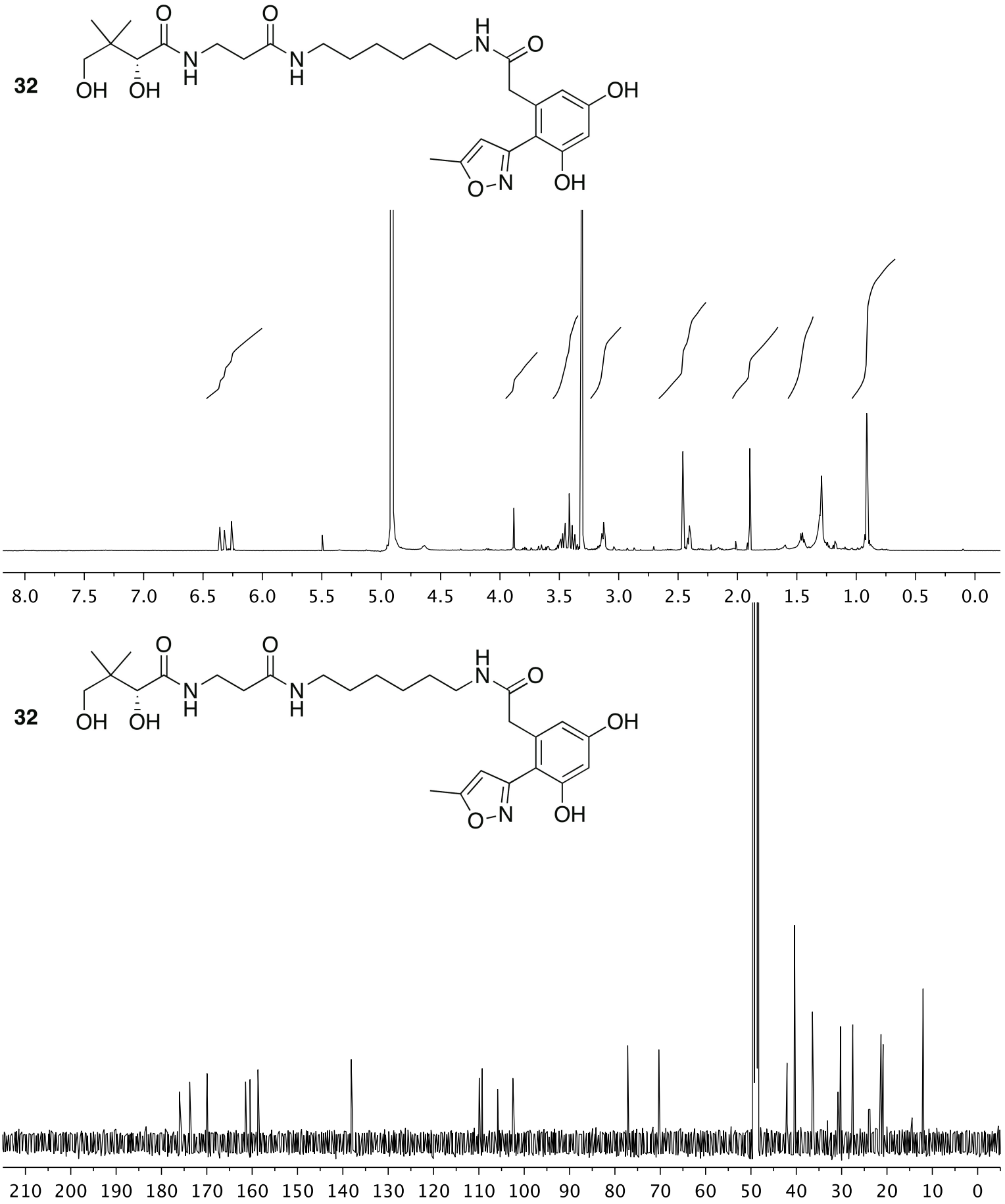


Figure S34. Docking simulations of ActACP with probes.

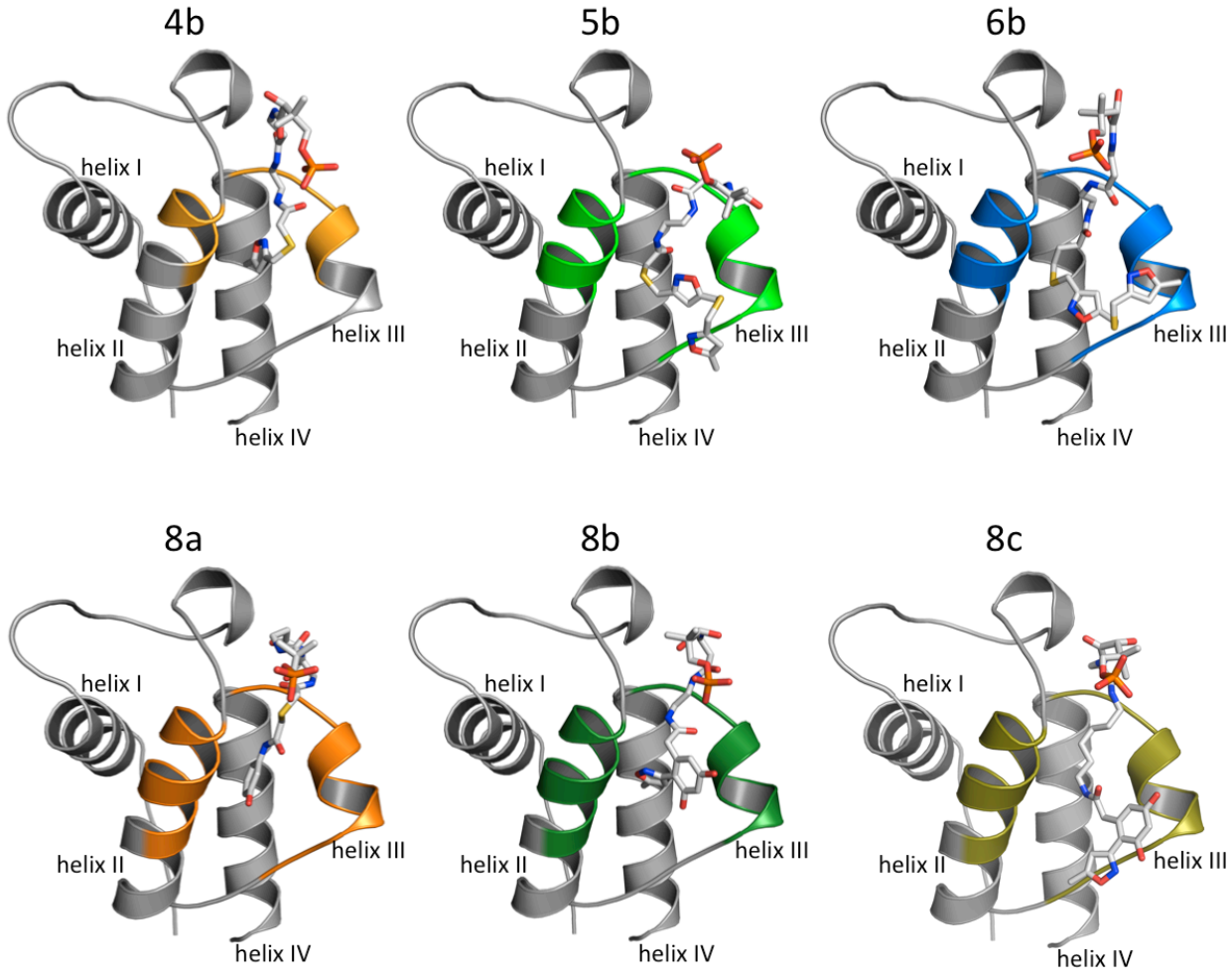
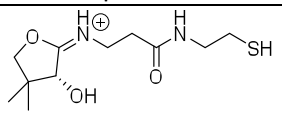
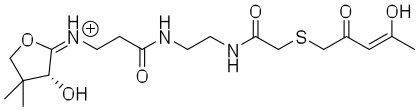
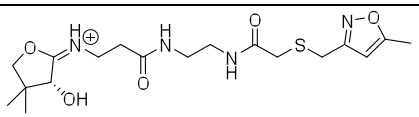
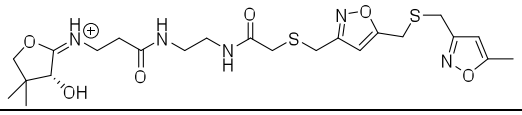
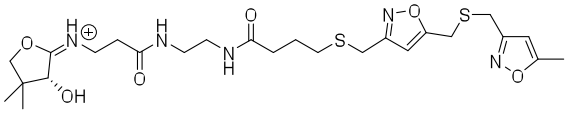
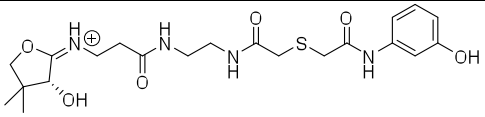
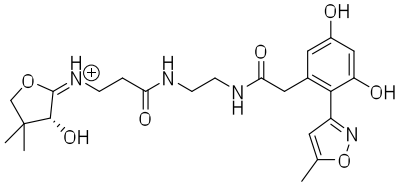
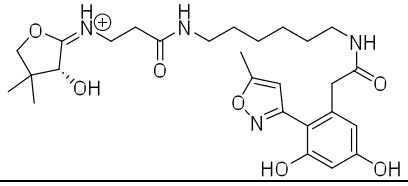
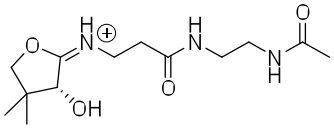


Table S1. LCMS validation of *crypto*-ACPs via PPant ejection.

ACP Species	Ejected Molecule	Expected Mass (Da)	Observed Mass (Da)
***apo*-ACP**	None expected	N/A	None observed
2 (*holo*-ACP)		261.36	261.48
4a		416.51	286.56*
4b		413.51	413.57
5b		540.67	540.56
6b		568.73	568.62
8a		467.56	467.61
8b		475.52	475.56
8c		531.63	531.60

*Ejected mass under high voltage conditions yields decomposed ion:



(Expected mass 286.35)

Loaded *crypto*-ActACP samples were subjected to LCMS analysis using high-voltage settings to eject their cargo by lactone formation (Meier JL, et al., *J Proteome Res*. **2011**, *10*, 320-9.) The observed small molecule peaks are reported here, to further confirm loading of the actACP (in addition to characteristic S42 shifts observed by NMR).