Supporting Information

Design, Synthesis and X-ray Studies of Potent HIV-1 Protease Inhibitors with P2-Carboxamide Functionalities

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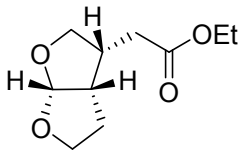
Contents:

General Methods: Anhydrous reactions are performed under argon atmosphere using oven-dried glass ware. All chemicals and reagents were purchased from commercial suppliers and used without further purification. Anhydrous solvents were obtained as follows: anhydrous tetrahydrofuran, diethyl ether, and benzene were distilled from sodium metal under argon. Anhydrous dichloromethane, toluene, methanol, and acetonitrile was dried via distillation from CaH_2 under argon. All other solvents were reagent grade. TLC analysis was carried out with SiliCycle 60A-F_{254} plates. Flash chromatography was performed using SiliCycle 230-400 mesh, 60 Å pore diameter silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova-300 or, Bruker Avance ARX-400spectrophotometer. NMR data is reported as: δ value (chemical shift, *J*-value (Hz), integration, where s = singlet, d = doublet, t = triplet, q = quartet, brs = broad singlet). HPLC data was collected using a system composed of an Agilent 1100 series degasser, quaternary pump, thermostatable column compartment, variable wavelength detector. Mass spectra were recorded at the Purdue University Mass Spectrometry Center. The purity of all test compounds was determined by HPLC analysis. All test compounds showed ≥95% purity.

Synthesis of Inhibitors:

Ethyl 2-((3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl)acetate (6):



6

To a stirred solution of **5** (250 mg, 1.92 mmol) in dichloromethane (10 mL) were added Na_2HPO_4 (163 mg, 1.15 mmol) and DMP (1.22 g, 2.88 mmol) in portion wise at 0 °C under argon atmosphere. The mixture was stirred at 23 °C for 2 h. After completion of starting material, the reaction mixture was quenched with saturated $Na_2S_2O_3$ and saturated $NaHCO_3$ in the ration of 1:1 and stirred vigorously for 15 min, then it was extracted with CH_2Cl_2 (2 x 30 mL). The combined extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the crude ketone which was used for the next reaction.

To a suspension of NaH (80 mg, 1.99 mmol) in THF (10 mL) was added $(EtO)_2OPCH_2CO_2Et$ (0.44 mL, 1.99 mmol) at 0 °C and stirred for 10 minutes at same temperature, to that suspension was added a solution of above ketone (250 mg, 1.95 mmol) in THF (3 mL) at 0 °C, then the reaction mixture temperature was allowed to room temperature and stirred for 4 h. The reaction mixture was quenched with water and extracted with EtOAc. The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified *via* silica gel chromatography to afford *α*, *β*-unsaturated ester (270 mg, 71% (70:30 *dr*) over two steps) as a colourless oil. R_f = 0.5 (40% EtOAc/hexanes).

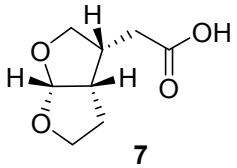
Major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 (td, *J* = 2.6, 2.1 Hz, 1H), 5.82 (dd, *J* = 4.9, 0.5 Hz, 1H), 5.07 – 4.79 (m, 2H), 4.29 – 4.00 (m, 2H), 3.95 (ddd, *J* = 9.3, 7.9, 1.5 Hz, 1H), 3.82 – 3.68 (m, 1H), 3.44 (ddt, *J* = 6.7, 4.6, 2.1 Hz, 1H), 2.27 (dddd, *J* = 12.5, 11.3, 8.8, 7.9 Hz, 1H), 1.95 (ddtd, *J* = 12.5, 5.3, 1.6, 0.6 Hz, 1H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 164.1, 113.1, 108.1, 72.3, 67.2, 60.3, 48.8, 34.6, 14.2. LRMS-ESI *(m/z)*: calcd for $C_{10}H_{14}O_4$ 198.0; found 199.0 $[M+H]^+$.

Minor isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (d, *J* = 4.9 Hz, 1H), 5.76 (d, *J* = 2.1 Hz, 1H), 4.73 – 4.37 (m, 2H), 4.18 (dd, *J* = 7.1, 2.3 Hz, 2H), 4.02 – 3.86 (m, 2H), 3.78 (ddd, *J* = 10.8, 8.8, 5.9 Hz, 1H), 2.41 (dddd, *J* = 13.0, 10.7, 10.0, 8.0 Hz, 1H), 2.10 –1.87 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 162.9, 111.3, 109.8, 72.4, 67.8, 60.1, 46.8, 33.7, 14.1. LRMS-ESI *(m/z)*: calcd for $C_{10}H_{14}O_4$ 198.0; found 199.0 $[M+H]^+$.

To a stirred solution of above *α*, *β*-unsaturated ester (150 mg, 0.75 mmol) in EtOAc (5 mL) was added PtO_2 (30 mg) under argon atmosphere. The resulting mixture was stirred at 23 °C under 1 atm H_2 balloon atmosphere over 2 h. Upon completion, the reaction mixture was filtered through a plug of celite and solvent was removed under reduced pressure to afford saturated ester **6** (150 mg, 99% (91:9 *dr*)) as a colorless oil. R_f = 0.46 (50% EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (d, *J* = 4.9 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.07 – 3.97 (m, 1H), 3.86 (td, *J* = 6.5, 5.6, 3.5 Hz, 2H), 3.45 (dd, *J* = 11.2, 8.5 Hz, 1H), 2.92 (d, *J* = 4.7 Hz, 1H), 2.72 (dp, *J* = 11.1, 7.7 Hz, 1H), 2.47 – 2.30 (m, 2H), 1.92 – 1.76 (m, 2H), 1.25 (t,

J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 109.4, 71.6, 68.9, 60.6, 45.2, 37.9, 32.5, 25.2, 14.0. LRMS-ESI *(m/z)*: calcd for $C_{10}H_{16}O_4$ 200.1; found 201.0 $[M+H]^+$.

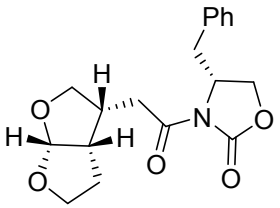
2-((3*R*,3a*S*,6a*R*)-Hexahydrofuro[2,3-b]furan-3-yl)acetic acid (**7**):



7

To a solution of **6** (50 mg, 0.25 mmol) in THF/H_2O (2 mL, 1:1) was added LiOH.H_2O (32 mg, 0.75 mmol) at 0 °C and the resulting mixture was stirred at 23 °C for 3 h. Upon completion, the reaction mixture was acidified to pH 4 with 1 N HCl at 0 °C, the residue was extracted with CH_2Cl_2 (3×10 mL), the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford acid **7** (41 mg, 96%) as a colorless oil. R_f = 0.2 (100% EtOAc) was used for next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (d, J = 4.9 Hz, 1H), 4.14 – 3.95 (m, 1H), 4.02 – 3.78 (m, 2H), 3.48 (dd, J = 11.2, 8.5 Hz, 1H), 2.96 (ddt, J = 9.9, 7.8, 5.2 Hz, 1H), 2.73 (dp, J = 11.1, 7.7 Hz, 1H), 2.62 – 2.34 (m, 2H), 1.98 – 1.73 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 109.4, 71.4, 68.9, 45.1, 37.6, 32.1, 25.2. LRMS-ESI *(m/z)*: calcd for $C_8H_{12}O_4$ 172.0; found 173.1 $[M+H]^+$.

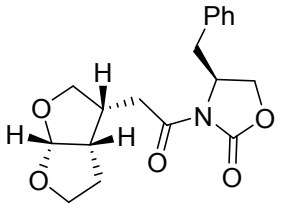
(*R*)-4-Benzyl-3-(2-((3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl)acetyl)oxazolidin-2-one (8):



8

To a suspension of acid **7** (40 mg, 0.23 mmol) in THF (3 mL) was added triethylamine (0.97 mL, 0.69 mmol) at 0 °C and stirred for 5 minutes at the same temperature, to that suspension were added (*R*)-4-benzyloxazolidin-2-one (45.2 mg, 0.25 mmol) and lithium chloride(19.5 mg, 0.46 mmol) at 0 °C. After 5 min, Trimethylacetyl chloride (0.069 mL, 0.58 mmol) was added at 0 °C, then the reaction mixture temperature was allowed to room temperature and stirred for 4 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified *via* silica gel chromatography to afford **8** (55 mg, 72%) as a solid. R_f = 0.5 (60% EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 3H), 7.23 – 7.15 (m, 2H), 5.76 (d, J = 5.0 Hz, 1H), 4.68 (ddt, J = 9.6, 7.5, 3.3 Hz, 1H), 4.28 – 4.15 (m, 2H), 4.13 – 4.04 (m, 1H), 3.93 – 3.81 (m, 2H), 3.51 (dd, J = 11.3, 8.5 Hz, 1H), 3.28 (dd, J = 13.4, 3.5 Hz, 1H), 3.18 – 2.96 (m, 3H), 2.86 – 2.69 (m, 2H), 1.95 – 1.79 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 153.4, 134.9, 129.2, 128.9, 127.3, 109.4, 71.6, 68.9, 66.3, 55.0, 45.2, 37.8, 37.3, 33.8, 25.5. LRMS-ESI *(m/z)*: calcd for $C_{18}H_{21}NO_5$ 331.1; found 354.0 $[M+Na]^+$.

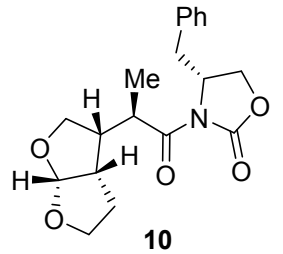
(*S*)-4-Benzyl-3-(2-((3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl)acetyl)oxazolidin-2-one (9):



9

The title compound **9** (40 mg, 70%) was obtained by following the procedure outlined for compound **8**, with Acid **7** (30 mg, 0.174) and (*S*)-4-benzyl oxazolidin-2-one (33 mg, 0.191 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.27 (m, 3H), 7.22 – 7.16 (m, 2H), 5.76 (dd, J = 5.0, 1.8 Hz, 1H), 4.67 (tdd, J = 7.6, 3.3, 1.6 Hz, 1H), 4.20 (dtdt, J = 8.4, 5.0, 3.8, 1.7 Hz, 2H), 4.07 (ddd, *J* = 8.6, 7.2, 1.6 Hz, 1H), 3.88 (ddt, *J* = 7.4, 5.7, 1.9 Hz, 2H), 3.50 (ddd, *J* = 11.1, 8.5, 1.6 Hz, 1H), 3.32 – 3.24 (m, 1H), 3.19 (ddd, *J* = 17.8, 7.4, 1.4 Hz, 1H), 3.08 – 3.00 (m, 1H), 2.94 (ddd, *J* = 17.8, 7.3, 1.3 Hz, 1H), 2.85 – 2.74 (m, 2H), 1.94 – 1.79 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 153.4, 134.9, 129.2, 128.9, 127.3, 109.4, 71.6, 68.9, 66.3, 55.0, 45.2, 37.8, 37.2, 33.9, 25.5. LRMS-ESI *(m/z)*: calcd for $C_{18}H_{21}NO_5$ 331.1; found 332.1 $[M+H]^+$.

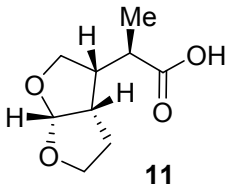
(*R*)-4-Benzyl-3-((*R*)-2-((3*S*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl)propanoyl)oxazolidin-2-one (10):



10

To a stirred solution of **8** (15 mg, 0.045 mmol) in THF (2 mL) was added Sodium bis(trimethylsilyl)amide solution (0.14 mL, 0.14 mmol, 1.0 M in THF) at –78 °C. The reaction mixture was stirred at –78 °C for 45 minutes. After this period, to the reaction mixture methyl iodide (0.016 mL, 0.271 mmol) was added at –78 °C and stirred for 4 h at the same temperature. Upon completion, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified *via* silica gel chromatography to afford **10** (10 mg, 64%) as a semisolid. R_f = 0.57 (60% EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.27 (m, 3H), 7.23 – 7.16 (m, 2H), 5.75 (d, *J* = 5.0 Hz, 1H), 4.73 (ddt, *J* = 9.5, 7.1, 3.5 Hz, 1H), 4.30 – 4.18 (m, 2H), 4.04 (dd, *J* = 8.4, 7.3 Hz, 1H), 3.90 – 3.77 (m, 3H), 3.56 (dd, *J* = 11.6, 8.4 Hz, 1H), 3.25 (dd, *J* = 13.4, 3.4 Hz, 1H), 2.99 – 2.88 (m, 1H), 2.84 – 2.71 (m, 2H), 1.75 – 1.68 (m, 2H), 1.23 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 152.8, 134.8, 129.3, 128.9, 127.4, 109.8, 70.7, 68.9, 66.2, 55.0, 44.5, 44.2, 37.8, 37.6, 25.6, 17.7. LRMS-ESI *(m/z)*: calcd for $C_{19}H_{23}NO_5$ 345.1; found 368.1 $[M+Na]^+$.

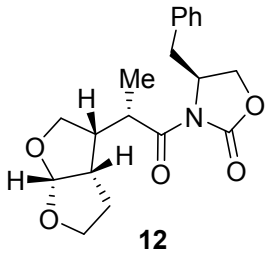
(*R*)-2-((3*S*,3a*S*,6a*R*)-Hexahydrofuro[2,3-b]furan-3-yl)propanoic acid (11):



11

To a stirred solution of **10** (15 mg, 0.04 mmol) in THF/H_2O (2 mL, 1:1) at 0 °C temperature were added hydrogen peroxide (0.02 mL, 0.17 mmol) and Lithium hydroxide (3.6 mg, 0.08 mmol). The resulting mixture was warmed to 23 °C and stirred for 3 h. Upon completion, the reaction mixture was acidified to pH 4-5 with 1 N HCl at 0 °C, the residue was extracted with EtOAc (3×10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford acid **11** (7 mg, 87%) as a colorless oil. R_f = 0.2 (100% EtOAc) was used for next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (d, *J* = 4.8 Hz, 1H), 4.06 – 3.97 (m, 1H), 3.96 – 3.81 (m, 2H), 3.58 – 3.49 (m, 1H), 2.96 (d, *J* = 9.6 Hz, 1H), 2.53 (t, *J* = 6.3 Hz, 2H), 1.90 (tt, *J* = 13.3, 6.8 Hz, 2H), 1.23 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.1, 109.8, 70.3, 68.9, 45.1, 38.7, 36.0, 25.2, 16.7. LRMS-ESI *(m/z)*: calcd for $C_9H_{14}O_4$ 186.0; found 187.1 $[M+H]^+$.

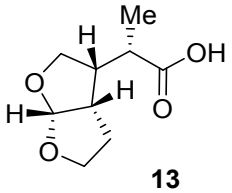
(*S*)-4-Benzyl-3-((*S*)-2-((3*S*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl)propanoyl)oxazolidin-2-one (12):



12

The title compound **12** (25 mg, 61%) was obtained by following the procedure outlined for compound **10**, with compound **9** (40 mg, 0.12 mmol) and methyl iodide (0.045 mL, 0.725 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.26 (m, 3H), 7.24 – 7.14 (m, 2H), 5.75 (d, *J* = 4.9 Hz, 1H), 4.73 – 4.63 (m, 1H), 4.26 – 4.17 (m, 2H), 3.95 – 3.86 (m, 3H), 3.78 (dq, *J* = 11.0, 6.9 Hz, 1H), 3.43 (dd, *J* = 11.3, 8.1 Hz, 1H), 3.22 (dd, *J* = 13.4, 3.4 Hz, 1H), 2.93 – 2.85 (m, 1H), 2.81 – 2.68 (m, 2H), 1.99 – 1.85 (m, 2H), 1.34 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 153.0, 134.8, 129.3, 128.9, 127.4, 109.2, 70.3, 68.8, 66.1, 55.0, 45.1, 44.3, 37.7, 36.9, 24.9, 17.4. LRMS-ESI *(m/z)*: calcd for $C_{19}H_{23}NO_5$ 345.1; found 346.1 $[M+H]^+$.

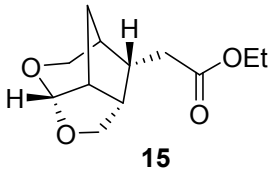
(*S*)-2-((3*S*,3a*S*,6a*R*)-Hexahydrofuro[2,3-b]furan-3-yl)propanoic acid (13):



13

The title compound **13** (7 mg, 86%) was obtained by following the procedure outlined for compound **11**, from compound **12** (15 mg, 0.04). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (d, *J* = 4.9 Hz, 1H), 4.04 – 3.83 (m, 3H), 3.58 (dd, *J* = 10.6, 8.6 Hz, 1H), 2.87 (dd, *J* = 10.3, 5.3 Hz, 1H), 2.54 – 2.39 (m, 2H), 1.92 (ddt, *J* = 12.8, 9.9, 7.3 Hz, 1H), 1.86 – 1.76 (m, 1H), 1.30 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.6, 109.1, 70.7, 68.7, 45.3, 44.4, 38.6, 24.4, 16.4. LRMS-ESI *(m/z)*: calcd for $C_9H_{14}O_4$ 186.0; found 187.0 $[M+H]^+$.

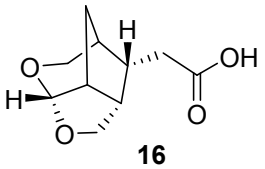
Ethyl 2-((3*S*,7a*S*,8*S*)-hexahydro-4*H*-3,5-methanofuro[2,3-b]pyran-8-yl)acetate (15):



15

By following the procedure outlined for compound **6,** the title compound **15** (30 mg, 0.132 mmol, 71% over 3 steps) was obtained from **14** (29 mg, 0.185 mmol). R_f = 0.2 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = –16.0 (c 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 (d, *J* = 6.5 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 4.00 (d, *J* = 9.7 Hz, 1H), 3.77 – 3.64 (m, 3H), 2.71 – 2.60 (m, 2H), 2.57 – 2.39 (m, 3H), 2.15 (q, *J* = 5.4 Hz, 1H), 1.86 (d, *J* = 11.5 Hz, 1H), 1.51 (dddd, *J* = 11.6, 4.8, 3.9, 1.1 Hz, 1H), 1.24 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.81, 103.79, 68.91, 61.54, 60.34, 45.43, 41.95, 39.09, 37.91, 31.73, 27.39, 14.11. LRMS-ESI *(m/z)*: calcd for $C_{12}H_{18}O_4$ 226.1; found 227.1 $[M+H]^+$.

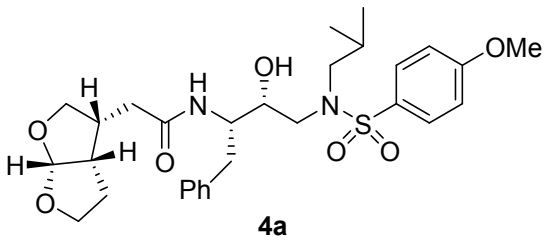
2-((3*S*,7a*S*,8*S*)-Hexahydro-4*H*-3,5-methanofuro[2,3-b]pyran-8-yl)acetic acid (16):



16

By following the procedure outlined for compound **7,** the title compound **16** (24 mg, 0.121 mmol, 91%) was obtained from **15** (30 mg, 0.132 mmol). R_f = 0.5 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = –22.1 (c 0.74, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (d, *J* = 6.4 Hz, 1H), 4.03 (d, *J* = 9.8 Hz, 1H), 3.78 – 3.66 (m, 3H), 2.73 – 2.63 (m, 2H), 2.62 – 2.50 (m, 2H), 2.44 (m, 1H), 2.21 (m, 1H), 1.89 (d, *J* = 11.6 Hz, 1H), 1.53 (dt, *J* = 11.7, 4.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.12, 103.76, 68.88, 61.52, 45.43, 41.93, 38.86, 37.89, 31.37, 27.39. LRMS-ESI *(m/z)*: calcd for $C_{10}H_{14}O_4$ 198.0; found 199.0 $[M+H]^+$.

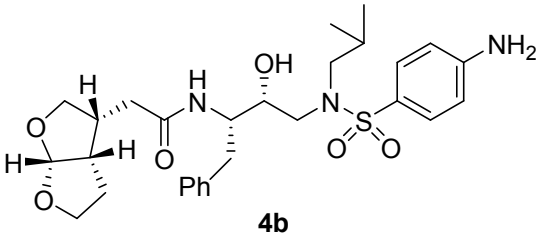
2-((3*R*,3a*S*,6a*R*)-Hexahydrofuro[2,3-b]furan-3-yl)-*N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)acetamide (4a):



4a

To a suspension of acid **7** (8 mg, 0.046 mmol) and Isostere **17** (22.6 mg, 0.055 mmol) in DMF (1 ml) was added *N*,*N*-Diisopropylethylamine (0.048 mL, 0.279 mmol) at 23 °C and stirred for 5 min. To that suspension, HATU (17.6 mg, 0.06 mmol) was added and stirred for 24 h. The reaction mixture was quenched with aq. $NaHCO_3$ solution and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was purified *via* silica gel chromatography to afford Inhibitor **4a** (20.5 mg, 77%) as an amorphous solid, R_f = 0.26 (100% EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.9 Hz, 2H), 7.32 – 7.26 (m, 2H), 7.25 – 7.17 (m, 3H), 6.98 (d, *J* = 8.9 Hz, 2H), 5.76 (d, *J* = 8.5 Hz, 1H), 5.64 (d, *J* = 4.9 Hz, 1H), 4.30 – 4.19 (m, 1H), 4.04 (d, *J* = 3.1 Hz, 1H), 3.87 (s, 4H), 3.86 – 3.78 (m, 1H), 3.78 – 3.71 (m, 1H), 3.42 – 3.33 (m, 1H), 3.12 (dd, *J* = 15.1, 8.1 Hz, 3H), 3.07 – 2.99 (m, 2H), 2.98 – 2.89 (m, 1H), 2.82 (dd, *J* = 13.4, 6.9 Hz, 1H), 2.72 – 2.63 (m, 1H), 2.61 – 2.50 (m, 1H), 2.24 – 2.05 (m, 2H), 1.84 (ddd, *J* = 14.6, 13.2, 7.0 Hz, 1H), 1.58 – 1.49 (m, 2H), 0.89 (dd, *J* = 11.6, 6.6 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 163.0, 137.7, 129.7, 129.3, 129.1, 128.5, 126.6, 114.3, 109.4, 72.7, 71.4, 68.9, 58.7, 55.5, 53.6, 53.3, 45.0, 38.5, 38.3, 34.6, 34.3, 27.2, 24.9, 20.0, 19.8. LRMS-ESI *(m/z)*: calcd for $C_{29}H_{40}N_2O_7S$ 560.2; found 561.2 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{29}H_{40}N_2O_7SNa$, 583.2448; found 583.2443.

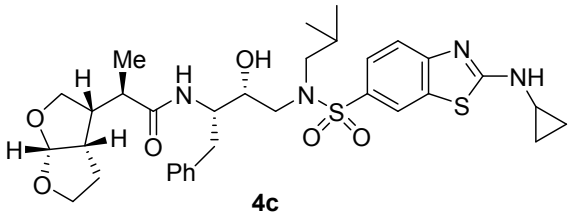
_N_-((2_S_,3_R_)-4-((4-Amino-_N_-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)-2-((3_R_,3a_S_,6a_R_)-hexahydrofuro[2,3-b]furan-3-yl)acetamide (4b):



4b

Compound **7** (6 mg, 0.034 mmol) was treated with Isostere **18** (16.3 mg, 0.05 mmol) by following the procedure outlined for inhibitor **4a** to give inhibitor **4b** (15 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.50 (m, 2H), 7.29 (dt, *J* = 6.8, 1.3 Hz, 2H), 7.25 – 7.16 (m, 3H), 6.68 (d, *J* = 8.8 Hz, 2H), 5.82 (d, *J* = 8.5 Hz, 1H), 5.63 (d, *J* = 4.9 Hz, 1H), 4.24 (ddd, *J* = 10.0, 7.2, 4.4 Hz, 1H), 4.16 (s, 2H), 4.05 (s, 1H), 3.93 – 3.86 (m, 1H), 3.85 – 3.68 (m, 3H), 3.41 – 3.28 (m, 1H), 3.14 – 2.97 (m, 3H), 2.96 – 2.86 (m, 2H), 2.80 (dd, *J* = 13.4, 6.9 Hz, 1H), 2.71 – 2.61 (m, 1H), 2.59 – 2.49 (m, 1H), 2.22 – 2.02 (m, 2H), 1.84 (dq, *J* = 7.8, 6.4 Hz, 1H), 1.59 – 1.45 (m, 2H), 0.89 (dd, *J* = 10.2, 6.6 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 150.7, 137.7, 129.3, 129.1, 128.5, 126.5, 126.1, 114.0, 109.4, 72.7, 71.4, 68.9, 58.8, 53.5, 53.4, 45.0, 38.3, 34.6, 34.3, 27.2, 24.9, 20.1, 19.9. LRMS-ESI *(m/z)*: calcd for $C_{28}H_{39}N_3O_6S$ 545.2; found 546.2 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{40}N_3O_6S$, 546.2632; found 546.2637.

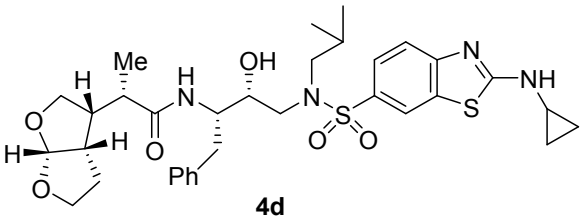
(_R_)-_N_-((2_S_,3_R_)-4-((2-(Cyclopropylamino)-_N_-isobutylbenzo[d]thiazole)-6-sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)-2-((3_S_,3a_S_,6a_R_)-hexahydrofuro[2,3-b]furan-3-yl)propanamide (4c):



4c

Compound **11** (7 mg, 0.037 mmol) was treated with Isostere **19** (22 mg, 0.045 mmol) by following the procedure outlined for inhibitor **4a** to give inhibitor **4c** (16 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 1.9 Hz, 1H), 7.68 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.30– 7.17 (m, 5H), 6.89 (s, 1H), 5.88 (d, *J* = 8.3 Hz, 1H), 5.56 (d, *J* = 4.6 Hz, 1H), 4.41 – 4.20 (m, 2H), 4.01 – 3.85 (m, 2H), 3.76 (td, *J* = 7.7, 6.1 Hz, 1H), 3.57 (dt, *J* = 8.6, 6.9 Hz, 1H), 3.45 – 3.33 (m, 1H), 3.18 – 3.07 (m, 3H), 3.01 – 2.86 (m, 3H), 2.76 (tt, *J* = 6.8, 3.6 Hz, 1H), 2.49 – 2.38 (m, 2H), 2.12 – 2.01 (m, 1H), 1.95 – 1.83 (m, 1H), 1.35 (dq, *J* = 13.0, 6.7 Hz, 1H), 1.16 – 1.08 (m, 1H), 1.05 (d, *J* = 6.8 Hz, 3H), 0.98 – 0.85 (m, 8H), 0.80 (q, *J* = 3.7, 3.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 172.8, 155.7, 137.8, 131.3, 130.3, 129.0, 128.5, 126.6, 125.2, 120.7, 118.6, 109.7, 73.1, 69.9, 68.8, 58.9, 53.6, 53.3, 44.8, 44.7, 40.5, 34.7, 27.2, 26.6, 24.5, 20.0, 19.9, 17.8, 7.9. LRMS-ESI *(m/z)*: calcd for $C_{33}H_{44}N_4O_6S_2$ 656.2; found 657.2 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{33}H_{45}N_4O_6S_2$, 657.2775; found 657.2769.

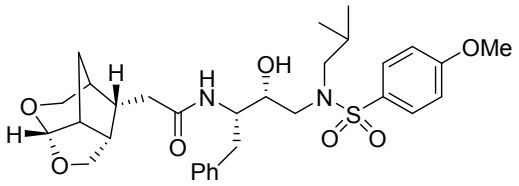
(*S*)-*N*-((2*S*,3*R*)-4-((2-(Cyclopropylamino)-*N*-isobutylbenzo[d]thiazole)-6-sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)-2-((3*S*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-yl)propanamide (4d):



4d

Compound **13** (7 mg, 0.037 mmol) was treated with Isostere **19** (22 mg, 0.045 mmol) by following the procedure outlined for inhibitor **4a** to give inhibitor **4d** (17 mg, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.70 – 7.64 (m, 1H), 7.54 (d, *J* = 20.8 Hz, 1H), 7.28 (d, *J* = 6.9 Hz, 2H), 7.23 (d, *J* = 7.2 Hz, 3H), 5.78 (d, *J* = 8.5 Hz, 1H), 5.67 (d, *J* = 4.8 Hz, 1H), 4.21 (s, 1H), 3.86 (dd, *J* = 11.6, 6.4 Hz, 3H), 3.60 (t, *J* = 7.7 Hz, 1H), 3.41 – 3.30 (m, 1H), 3.18 – 2.70 (m, 9H), 2.34 (dd, *J* = 13.5, 6.1 Hz, 2H), 2.02 (dd, *J* = 11.3, 6.6 Hz, 1H), 1.83 (h, *J* = 8.9, 7.9 Hz, 2H), 1.69 (dd, *J* = 12.7, 6.4 Hz, 1H), 1.00 (d, *J* = 6.8 Hz, 5H), 0.88 (dd, *J* = 11.0, 6.6 Hz, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 173.0, 155.7, 137.6, 131.3, 130.2, 129.1, 128.5, 126.6, 125.2, 120.7, 118.5, 109.3, 72.8, 70.5, 68.6, 58.8, 53.5, 53.3, 45.6, 44.1, 40.6, 34.8, 27.2, 26.6, 24.5, 20.0, 19.8, 17.0, 7.9. LRMS-ESI *(m/z)*: calcd for $C_{33}H_{44}N_4O_6S_2$ 656.2; found 657.2 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{33}H_{45}N_4O_6S_2$, 657.2775; found 657.2772.

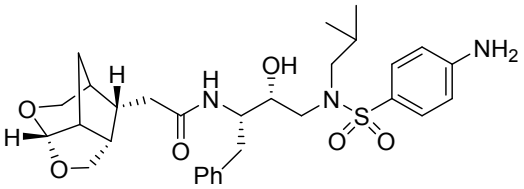
2-((3*S*,7a*S*,8*S*)-Hexahydro-4*H*-3,5-methanofuro[2,3-b]pyran-8-yl)-*N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)acetamide (**4e**):



4e

Following the procedure outlined for inhibitor **4a**, the reaction of amine **17** (11 mg, 0.027 mmol) and acid **16** (5 mg, 0.025 mmol) afforded inhibitor **4e** (11.5 mg, 73%) as an amorphous solid. ^{1}H NMR (800 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.9 Hz, 2H), 7.32 – 7.28 (m, 2H), 7.25 – 7.21 (m, 3H), 7.00 (d, *J* = 8.9 Hz, 2H), 5.86 (d, *J* = 8.5 Hz, 1H), 5.34 (d, *J* = 6.8 Hz, 1H), 4.23 (m, 1H), 3.90 (m, 1H), 3.89 (s, 3H), 3.67 (dd, *J* = 23.6, 10.6 Hz, 2H), 3.60 (dd, *J* = 11.4, 7.3 Hz, 1H), 3.48 (dd, *J* = 9.8, 5.6 Hz, 1H), 3.12 (dd, *J* = 15.0, 8.1 Hz, 1H), 3.09 – 3.03 (m, 2H), 2.97 – 2.88 (m, 2H), 2.85 (dd, *J* = 13.4, 7.0 Hz, 1H), 2.62 (q, *J* = 6.2 Hz, 1H), 2.47 (dt, *J* = 9.9, 6.1 Hz, 1H), 2.32 (ddd, *J* = 9.5, 7.6, 4.0 Hz, 1H), 2.27 – 2.24 (m, 2H), 1.96 (q, *J* = 5.8 Hz, 1H), 1.89 – 1.84 (m, 1H), 1.81 (d, *J* = 11.5 Hz, 1H), 1.44 (dt, *J* = 11.5, 4.4 Hz, 1H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 172.28, 163.00, 137.72, 129.81, 129.36, 129.10, 128.52, 126.56, 114.30, 103.74, 72.72, 68.80, 61.79, 58.67, 55.57, 53.72, 53.33, 45.41, 41.83, 39.04, 37.82, 34.72, 33.45, 27.31, 27.22, 20.06, 19.88. LRMS-ESI *(m/z)*: calcd for $C_{31}H_{42}N_2O_7S$ 586.2; found 587.2 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{31}H_{42}N_2O_7SNa$, 609.2605; found 609.2598.

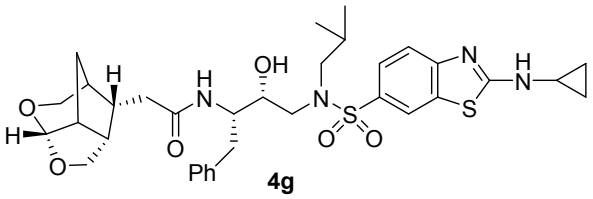
_N_-((2_S_,3_R_)-4-((4-Amino-_N_-isobutylphenyl)sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)-2-((3_S_,7a_S_,8_S_)-hexahydro-4_H_-3,5-methanofuro[2,3-b]pyran-8-yl)acetamide (4f):



4f

Following the procedure outlined for inhibitor **4a**, the reaction of amine **18** (18 mg, 0.044 mmol) and acid **16** (8 mg, 0.04 mmol) afforded inhibitor **4f** (17.5 mg, 77%) as an amorphous solid. ^{1}H NMR (800 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.7 Hz, 2H), 7.32 – 7.28 (m, 2H), 7.25 – 7.20 (m, 3H), 6.69 (d, *J* = 8.6 Hz, 2H), 5.88 (d, *J* = 8.6 Hz, 1H), 5.34 (d, *J* = 6.8 Hz, 1H), 4.23 (m, 1H), 4.19 – 4.09 (m, 2H), 3.89 (dq, *J* = 7.5, 3.6 Hz, 1H), 3.69 – 3.59 (m, 3H), 3.48 (dd, *J* = 9.8, 5.6 Hz, 1H), 3.12 – 3.02 (m, 3H), 2.94 – 2.87 (m, 2H), 2.83 (dd, *J* = 13.5, 7.0 Hz, 1H), 2.62 (m, 1H), 2.47 (dt, *J* = 9.8, 6.1 Hz, 1H), 2.31 (ddd, *J* = 15.7, 9.2, 6.1 Hz, 1H), 2.28 – 2.23 (m, 1H), 1.96 (q, *J* = 5.8 Hz, 1H), 1.86 (dt, *J* = 13.9, 6.9 Hz, 1H), 1.80 (d, *J* = 11.6 Hz, 1H), 1.64 (br*s*, 1H), 1.44 (dt, *J* = 11.5, 4.5 Hz, 1H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (200 MHz, $CDCl_3$) δ 172.2, 150.6, 137.8, 129.4, 129.1, 128.5, 126.5, 126.2, 114.0, 103.7, 72.8, 68.8, 61.8, 58.8, 53.6, 53.4, 45.4, 41.8, 39.1, 37.8, 34.7, 33.5, 29.6, 27.3, 27.2, 20.1, 19.9. LRMS-ESI *(m/z)*: calcd for $C_{30}H_{41}N_3O_6S$ 571.2; found 572.3 $[M+H]^+$.

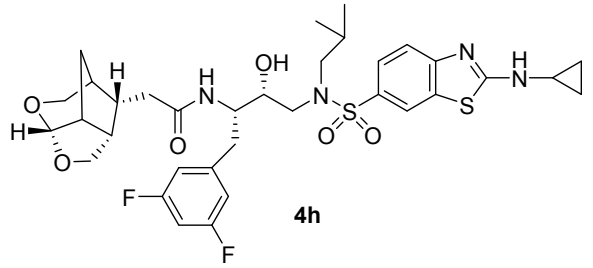
_N_-((2_S_,3_R_)-4-((2-(Cyclopropylamino)-_N_-isobutylbenzo[d]thiazole)-6-sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)-2-((3_S_,7a_S_,8_S_)-hexahydro-4_H_-3,5-methanofuro[2,3-b]pyran-8-yl)acetamide (4g):



4g

Following the procedure outlined for inhibitor **4a**, the reaction of amine **19** (19 mg, 0.038 mmol) and acid **16** (7 mg, 0.035 mmol) afforded inhibitor **4g** (16.5 mg, 70%) as an amorphous solid. ^{1}H NMR (800 MHz, $CDCl_3$) δ 8.08 (d, *J* = 1.9 Hz, 1H), 7.69 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.31 – 7.28 (m, 2H), 7.26 – 7.21 (m, 3H), 6.00 (d, *J* = 8.5 Hz, 1H), 5.33 (d, *J* = 6.8 Hz, 1H), 4.24 (m, 1H), 4.14 (m, 1H), 3.92 (dt, *J* = 8.2, 4.1 Hz, 1H), 3.66 (dd, *J* = 23.3, 10.5 Hz, 2H), 3.60 (dd, *J* = 11.3, 7.3 Hz, 1H), 3.47 (dd, *J* = 9.8, 5.6 Hz, 1H), 3.16 – 3.09 (m, 2H), 3.06 (dd, *J* = 14.3, 4.9 Hz, 1H), 2.97 (dd, *J* = 13.5, 7.9 Hz, 1H), 2.89 (dt, *J* = 13.4, 8.3 Hz, 2H), 2.77 (tt, *J* = 6.8, 3.6 Hz, 1H), 2.61 (m, 1H), 2.47 (dt, *J* = 9.8, 6.1 Hz, 1H), 2.32 (m, 1H), 2.26 (d, *J* = 7.9 Hz, 2H), 1.96 (m, 1H), 1.89 (dt, *J* = 13.8, 7.6 Hz, 1H), 1.80 (d, *J* = 11.5 Hz, 1H), 1.44 (dt, *J* = 11.5, 4.5 Hz, 1H), 0.98 – 0.94 (m, 2H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.81 (m, 2H). ^{13}C NMR (200 MHz, $CDCl_3$) δ 172.90, 172.25, 155.45, 137.79, 131.11, 130.45, 129.10, 128.51, 127.71, 126.56, 125.29, 120.80, 118.53, 113.85, 103.74, 72.80, 68.79, 61.80, 58.71, 53.73, 53.38, 45.42, 41.82, 39.05, 37.84, 34.74, 33.44, 29.62, 27.31, 27.23, 26.64, 20.08, 19.90, 7.91. LRMS-ESI *(m/z)*: calcd for $C_{34}H_{44}N_4O_6S_2$ 668.2; found 669.2 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{34}H_{45}N_4O_6S_2$, 669.2775; found 669.2780.

***N*-((2*S*,3*R*)-4-((2-(Cyclopropylamino)-*N*-isobutylbenzo[d]thiazole)-6-sulfonamido)-1-(3,5-difluorophenyl)-3-hydroxybutan-2-yl)-2-((3*S*,7a*S*,8*S*)-hexahydro-4H-3,5-methanofuro[2,3-b]pyran-8-yl)acetamide (4h):**



4h

Following the procedure outlined for inhibitor **4a**, the reaction of amine **20** (20.9 mg, 0.042 mmol) and acid **16** (7 mg, 0.035 mmol) afforded inhibitor **4h** (17 mg, 68%) as an amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 1.9 Hz, 1H), 7.68 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 6.83 – 6.73 (m, 3H), 6.65 (tt, *J* = 9.0, 2.3 Hz, 1H), 6.09 (d, *J* = 8.7 Hz, 1H), 5.33 (d, *J* = 6.8 Hz, 1H), 4.18 (td, *J* = 9.4, 8.8, 4.4 Hz, 1H), 3.91 (q, *J* = 5.7 Hz, 1H), 3.76 (d, *J* = 9.7 Hz, 1H), 3.68 – 3.50 (m, 3H), 3.10 (d, *J* = 6.3 Hz, 2H), 3.04 (dd, *J* = 14.4, 4.5 Hz, 1H), 2.96 – 2.84 (m, 3H), 2.75 (tt, *J* = 6.8, 3.5 Hz, 1H), 2.63 (d, *J* = 5.4 Hz, 1H), 2.50 (dq, *J* = 12.3, 6.1 Hz, 1H), 2.42 – 2.32 (m, 1H), 2.28 (dd, *J* = 7.7, 2.7 Hz, 2H), 1.99 (d, *J* = 5.8 Hz, 1H), 1.92 – 1.77 (m, 2H), 1.46 (dt, *J* = 11.6, 4.3 Hz, 1H), 0.92 (dt, *J* = 21.5, 6.6 Hz, 8H), 0.79 (qd, *J* = 5.0, 3.6 Hz, 2H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 172.7, 172.3, 163.5, 162.3, 155.8, 142.1, 131.5, 130.2, 125.2, 120.8, 118.8, 112.0, 111.9, 103.8, 102.0, 72.8, 68.8, 61.8, 58.9, 53.4, 45.5, 41.9, 39.1, 37.9, 34.3, 33.5, 29.6, 27.3, 26.6, 20.1, 19.9, 14.1, 8.0. LRMS-ESI *(m/z)*: calcd for $C_{34}H_{42}F_2N_4O_6S_2$ 704.2; found 705.2 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{34}H_{43}F_2N_4O_6S_2$, 705.2587; found 705.2583.

Methods: Determination of X-ray structure of HIV-1 protease-inhibitor complex

The HIV-1 protease was expressed and purified as described [1]. The protease-inhibitor complex was crystallized by the hanging drop vapor diffusion method with well solutions of 1.8 M NaCl, 0.1 M Na Acetate, pH 6.2. Diffraction data were collected on a single crystal cooled to 90 K at SER-CAT (22-ID beamline), Advanced Photon Source, Argonne National Lab (Chicago, USA) with X-ray wavelength of 1.0 Å, and processed by HKL-2000 [2] to give an Rmerge of 8.2%. The crystal structure was solved by PHASER [3] in CCP4i Suite [4, 5, 6] using one of the previously reported isomorphous structures [7] as the initial model, and refined by Refmac5 in CCP4 [8] with 1.18 Å resolution diffraction data. JLigand [9] was used to construct the inhibitor and the restraints for refinement. COOT [10, 11] was used for modification of the model. Alternative conformations were modeled, isotropic atomic displacement parameters (B factors) were applied for all atoms including solvent molecules. The final refined solvent structure comprised one Na^+ ion, six Cl^- ions, two glycerol and 225 water molecules. The crystallographic statistics are listed in Table 1. The coordinates and structure factors of the protease complex with compound **4h** (GRL-026-18A) have been deposited in the Protein Data Bank [12] with code: 6VCE.

Table: Crystallographic Data Collection and Refinement Statistics

Space group	$P2_12_12$
Unit cell dimensions: (Å)	
a	58.90
b	86.21
c	46.09
Resolution range (Å)	50-1.18 (1.22-1.18)
Unique reflections	5.6 (2.1)
R_{merge} (%) overall (final shell)	72509 (4238)
I/σ(I) overall (final shell)	93.6 (55.5)
Completeness (%) overall (final shell)	8.2 (35.8)
Redundancy overall (final shell)	17.7 (2.1)
Refinement	50-1.18
R (%)	13.6
R_{free} (%)	15.5
No. of solvent atoms (total occupancies)	225
RMS deviation from ideality	
Bonds (Å)	0.021
Angle distance (Å)	0.026
Average B-factors ($Å^2$)	
Wilson Plot B factor	11.8
Main-chain atoms	13.2
Side-chain atoms	18.1
Whole chain atoms	15.6
Inhibitor	11.1
Solvent	24.2

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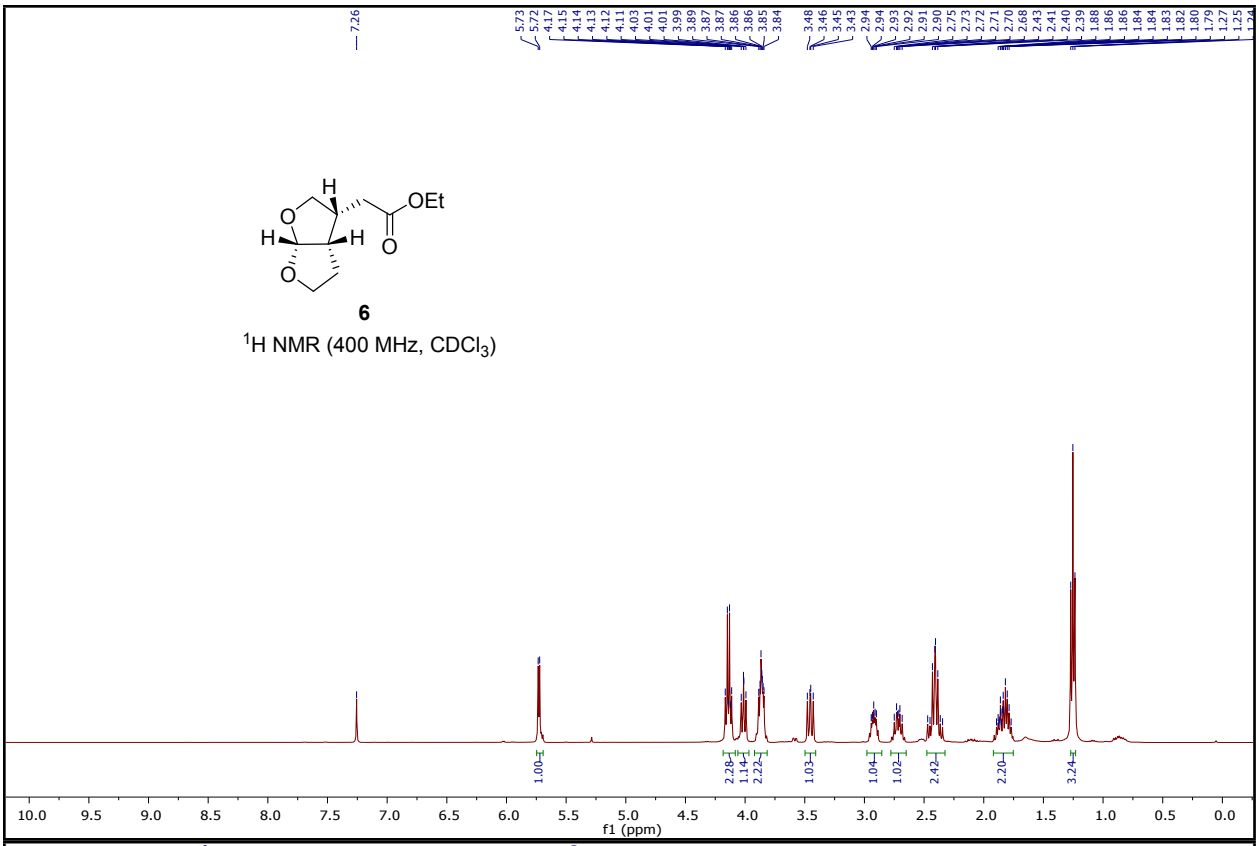
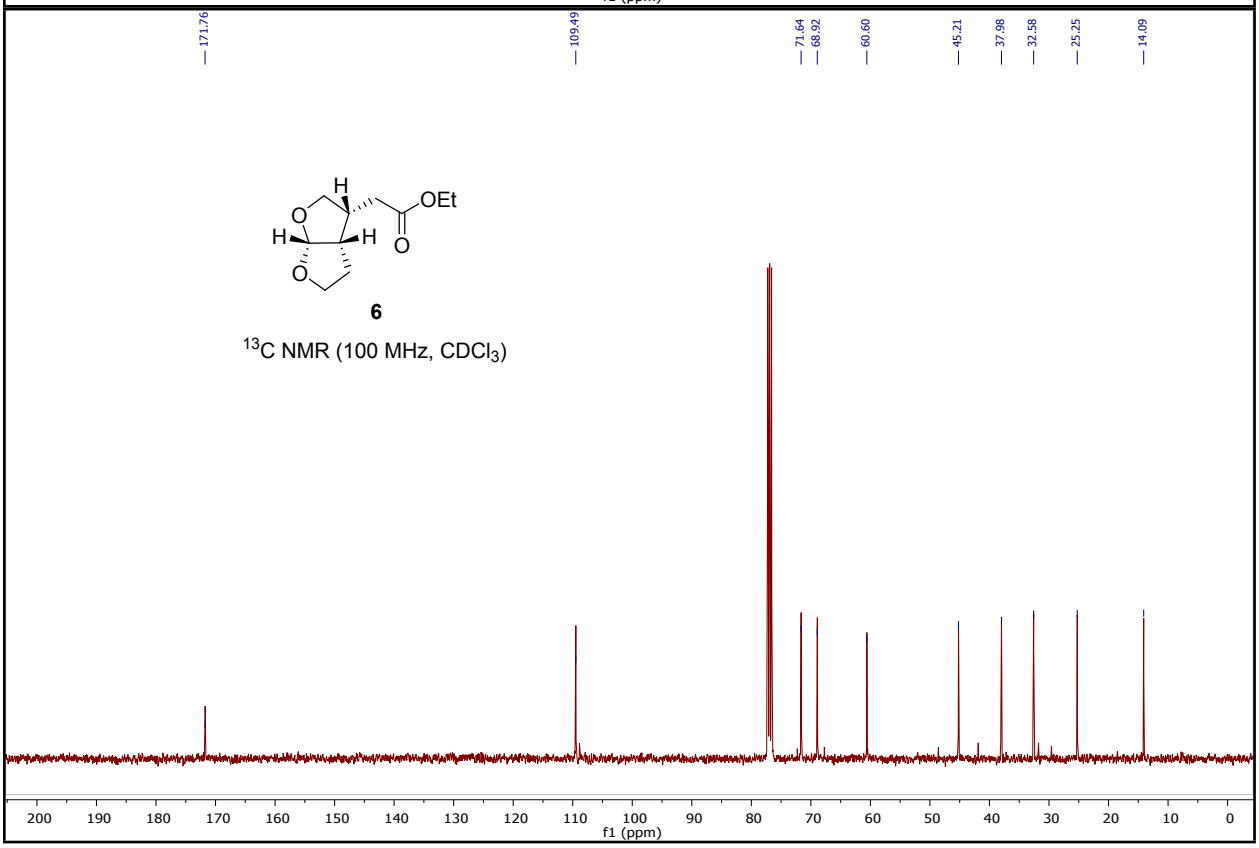


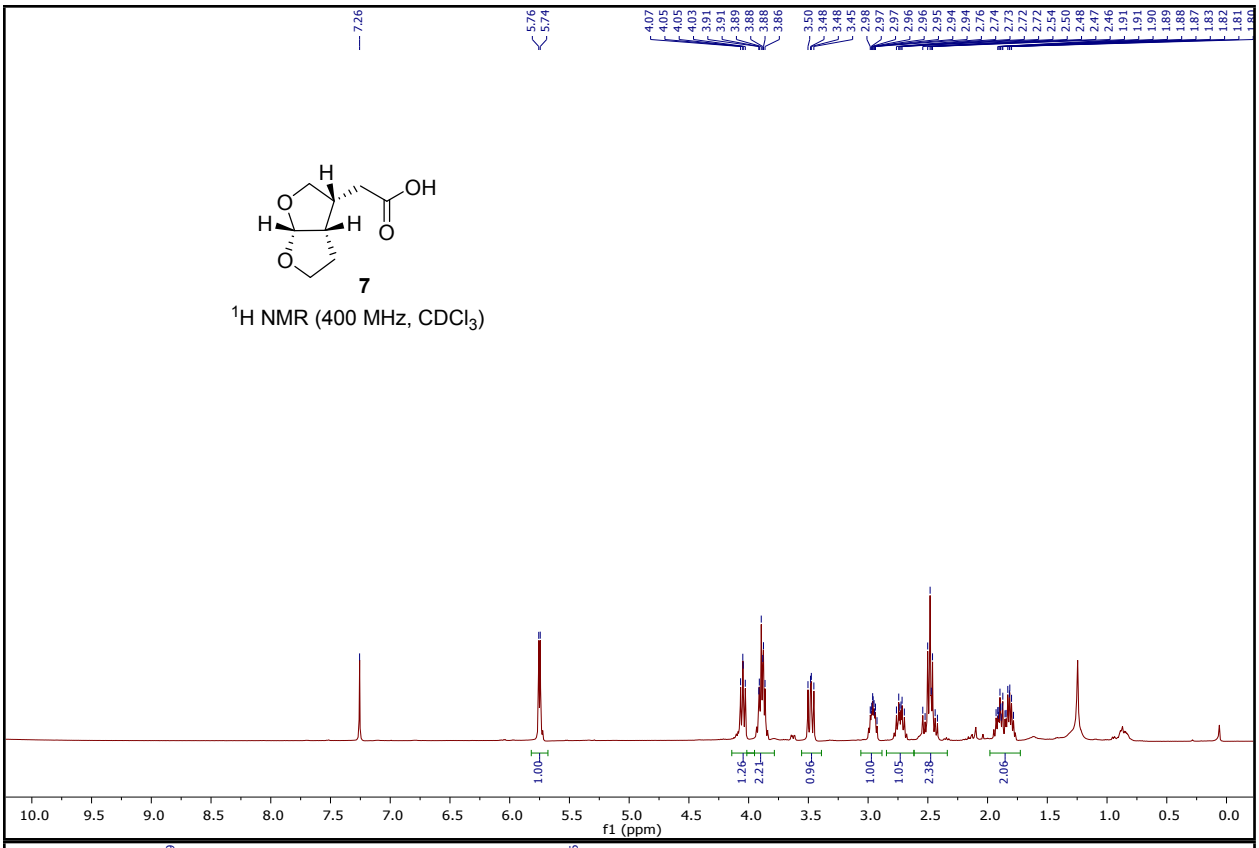
6

^{1}H NMR (400 MHz, $CDCl_3$)



6

^{13}C NMR (100 MHz, $CDCl_3$)



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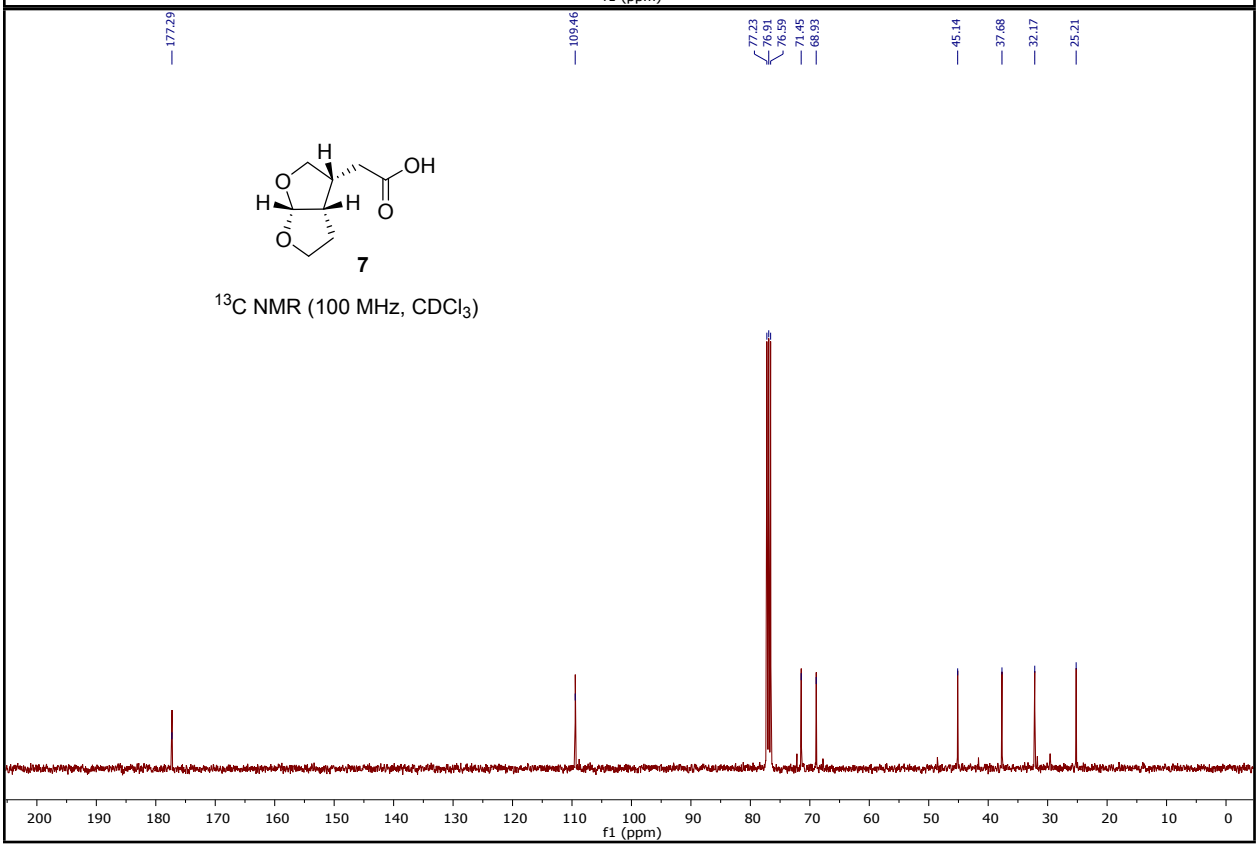

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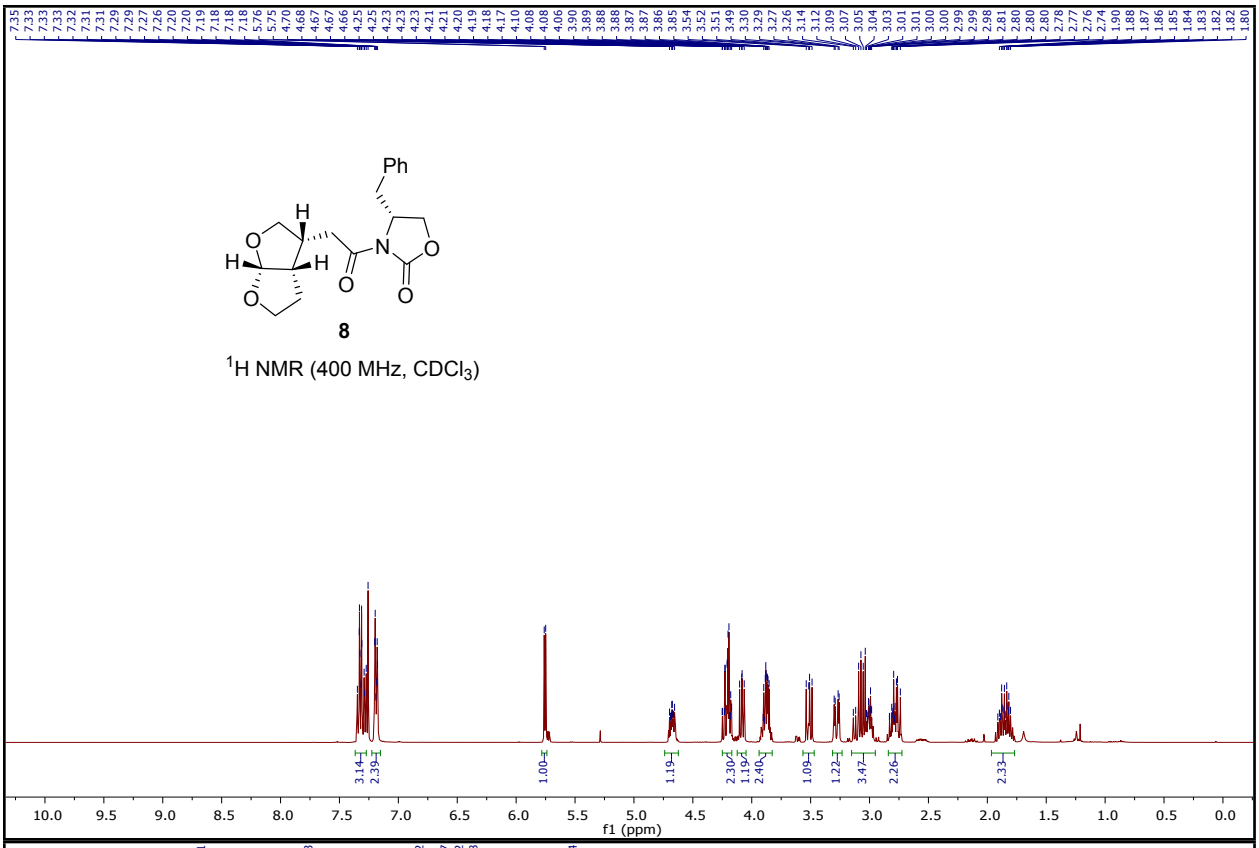

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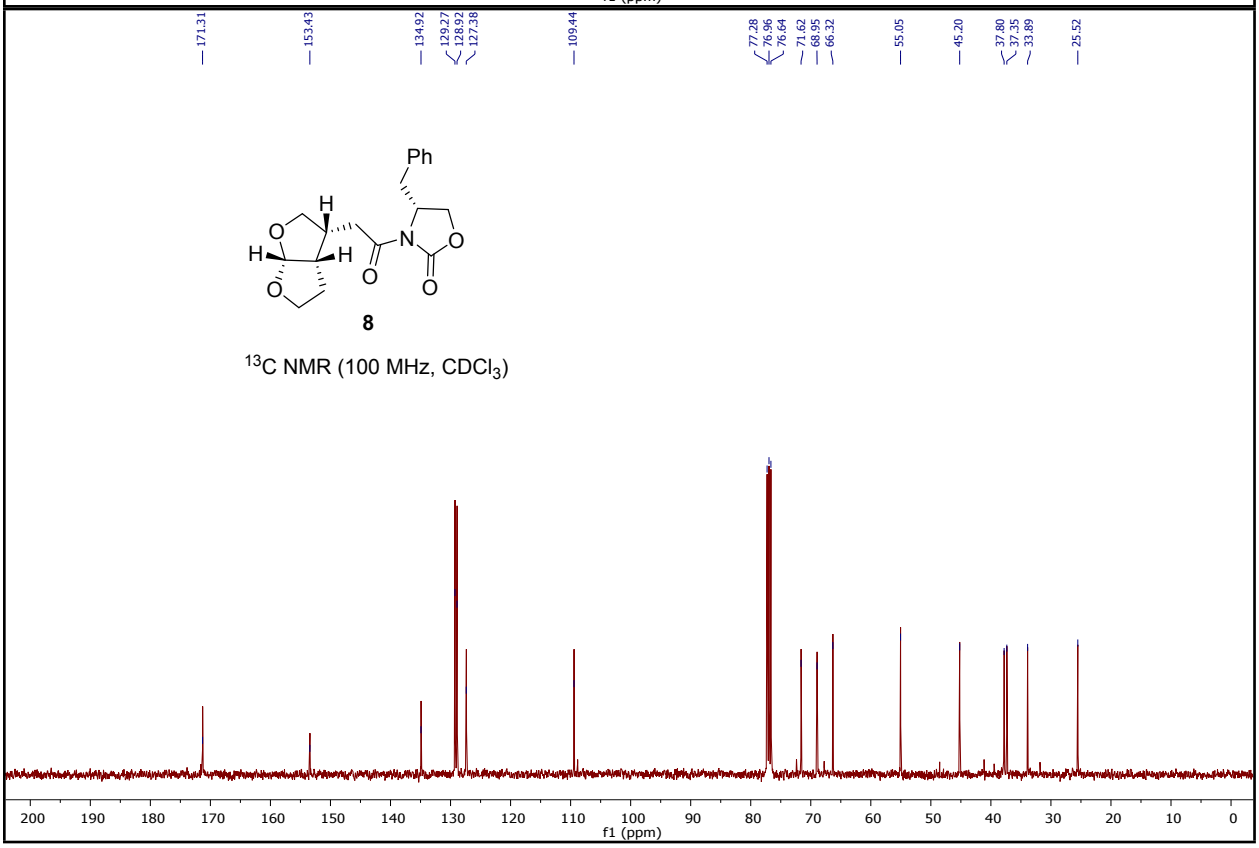

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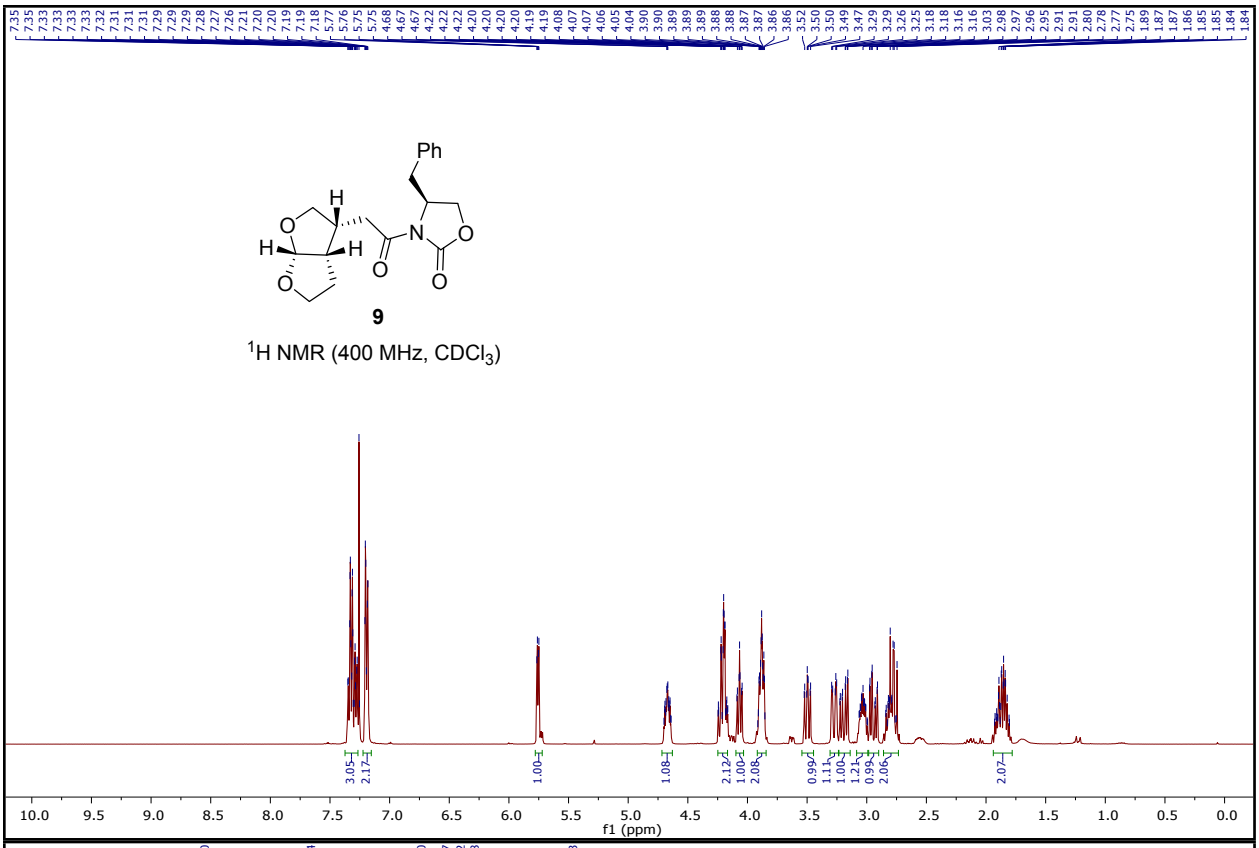

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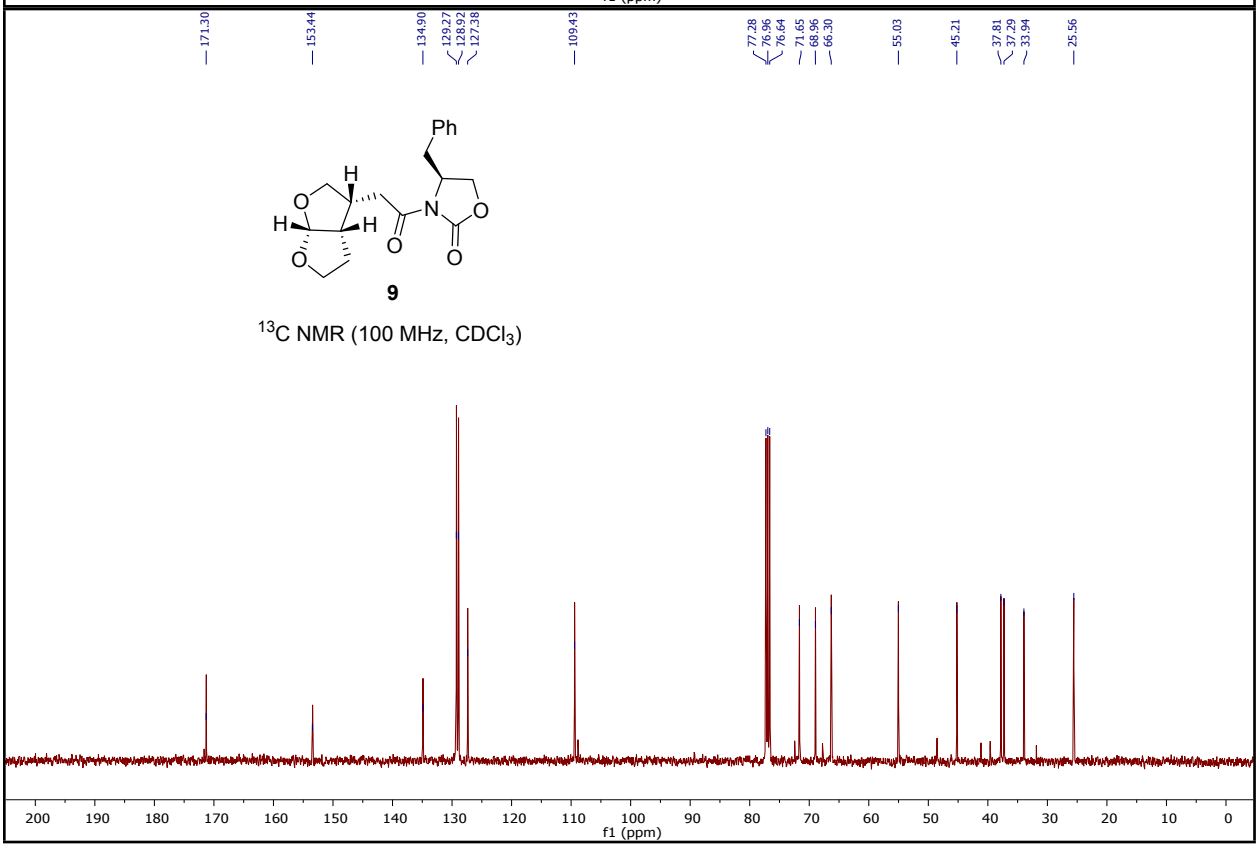

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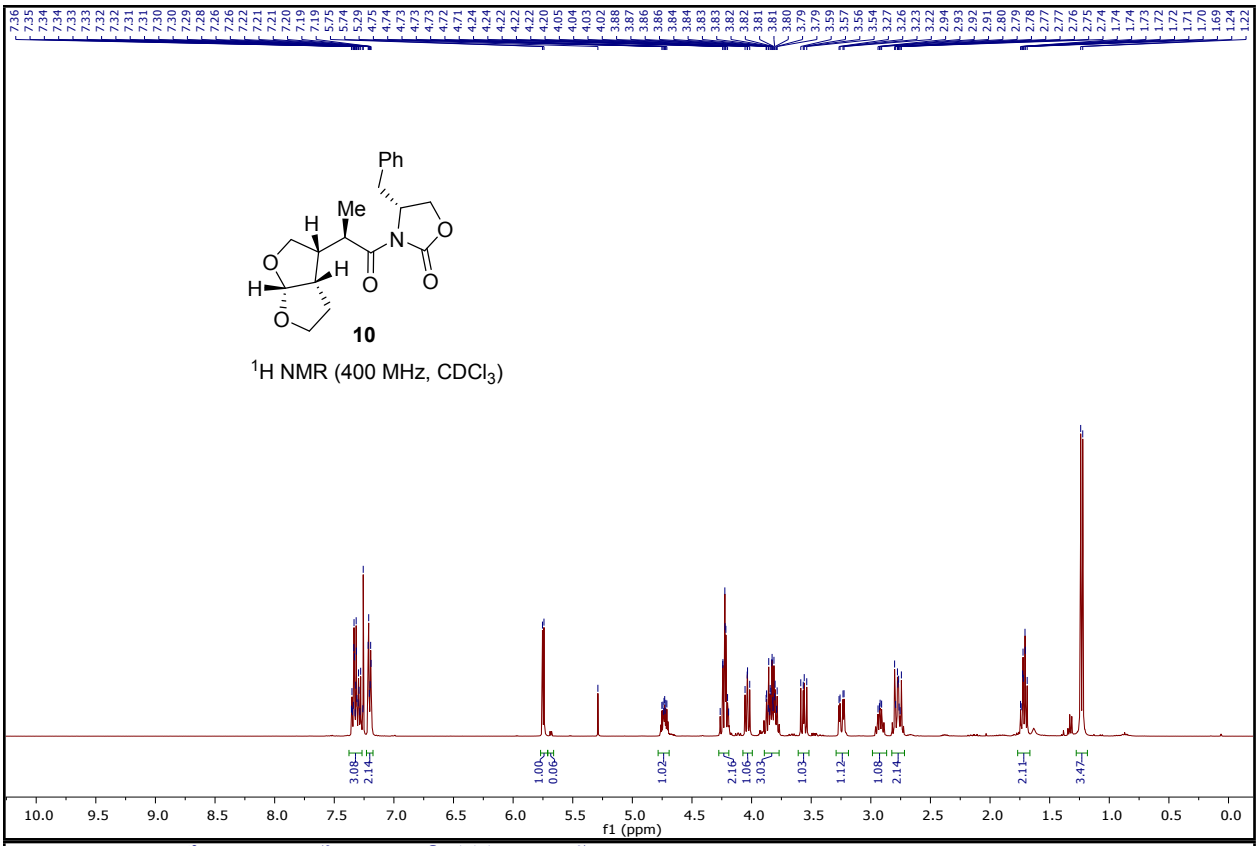
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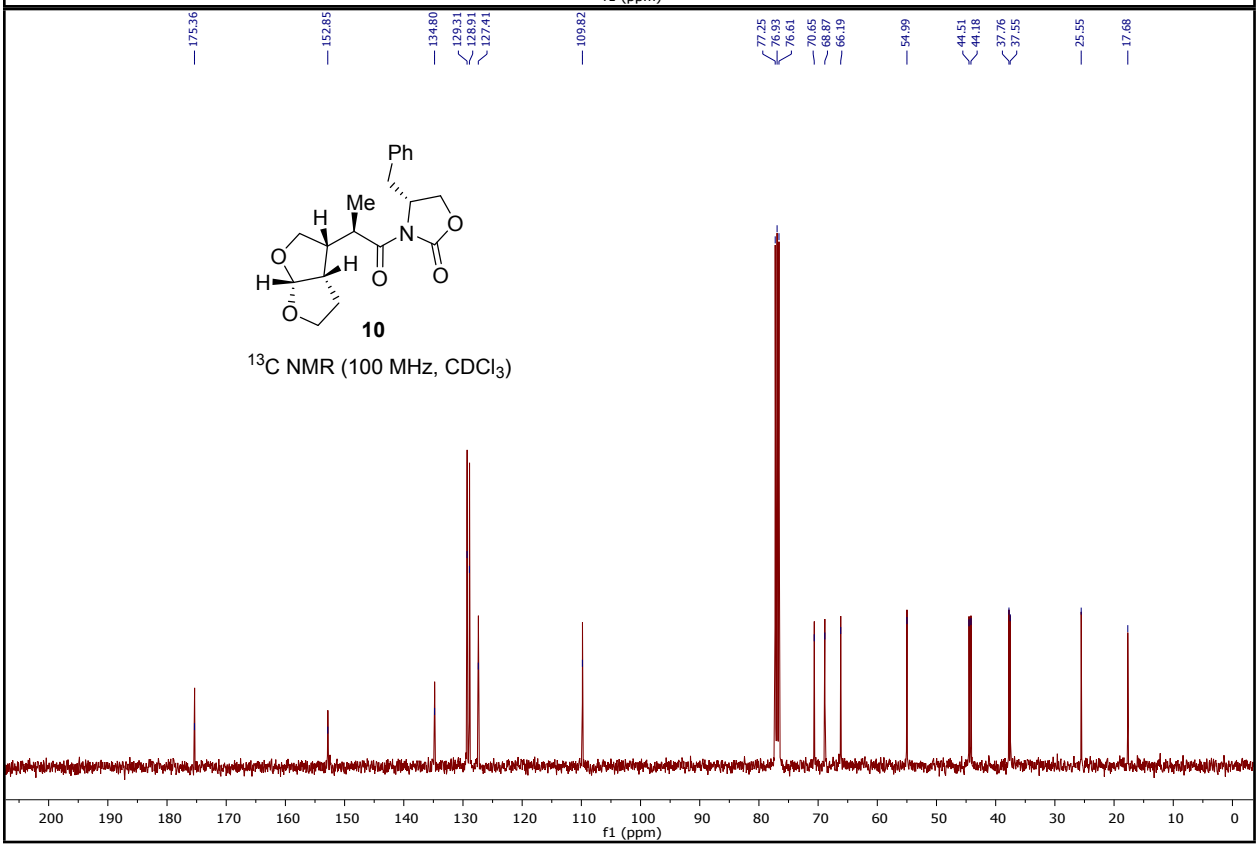
H
O
N
O
O
H
H
O
O
10
1H NMR (400 MHz, CDCl3)
f1 (ppm)


Ph
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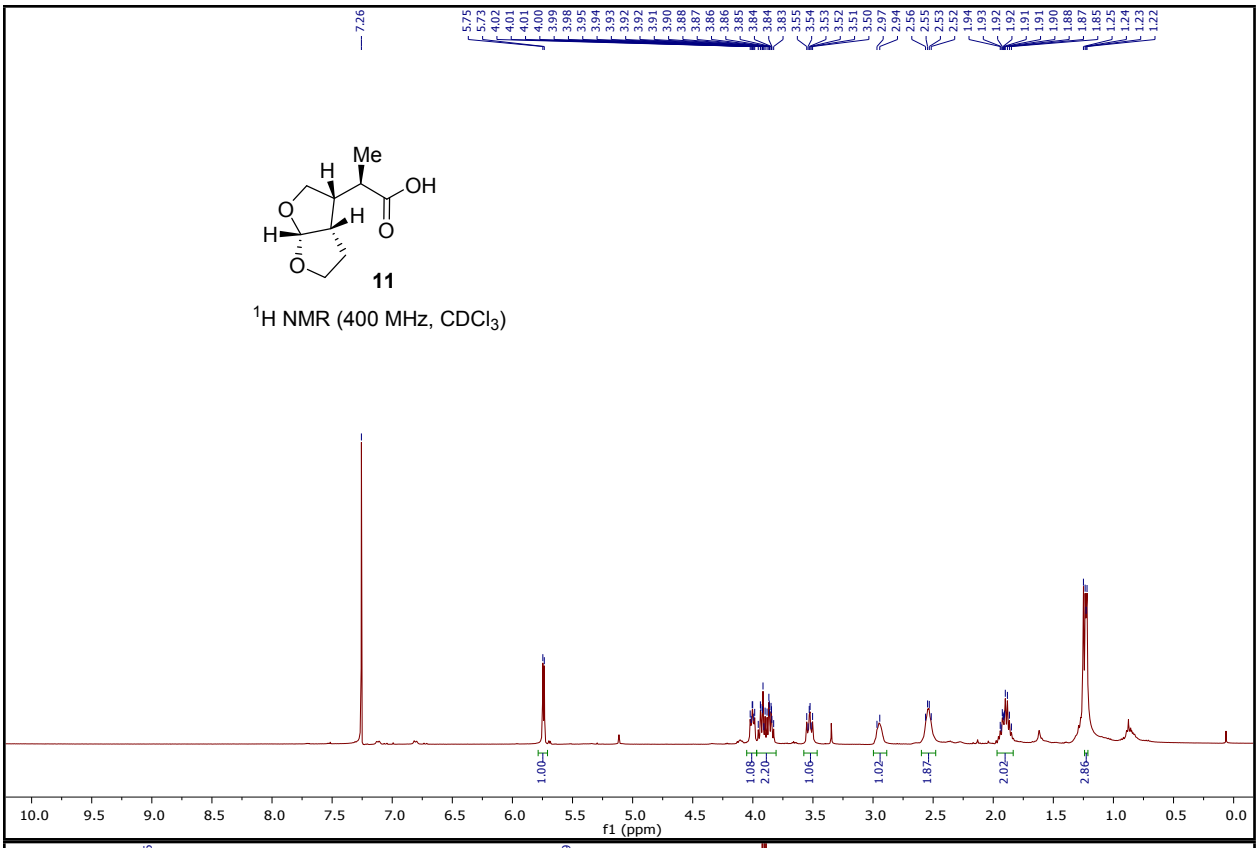
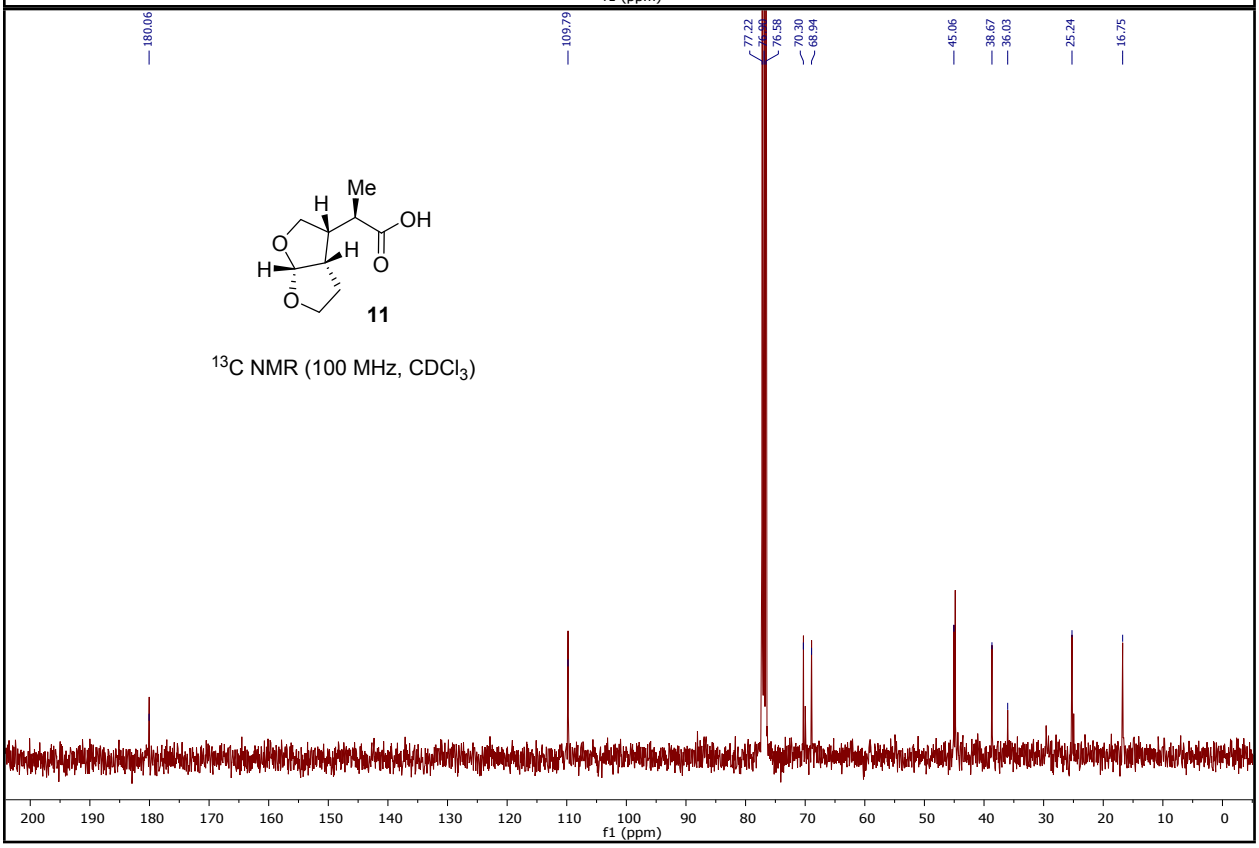
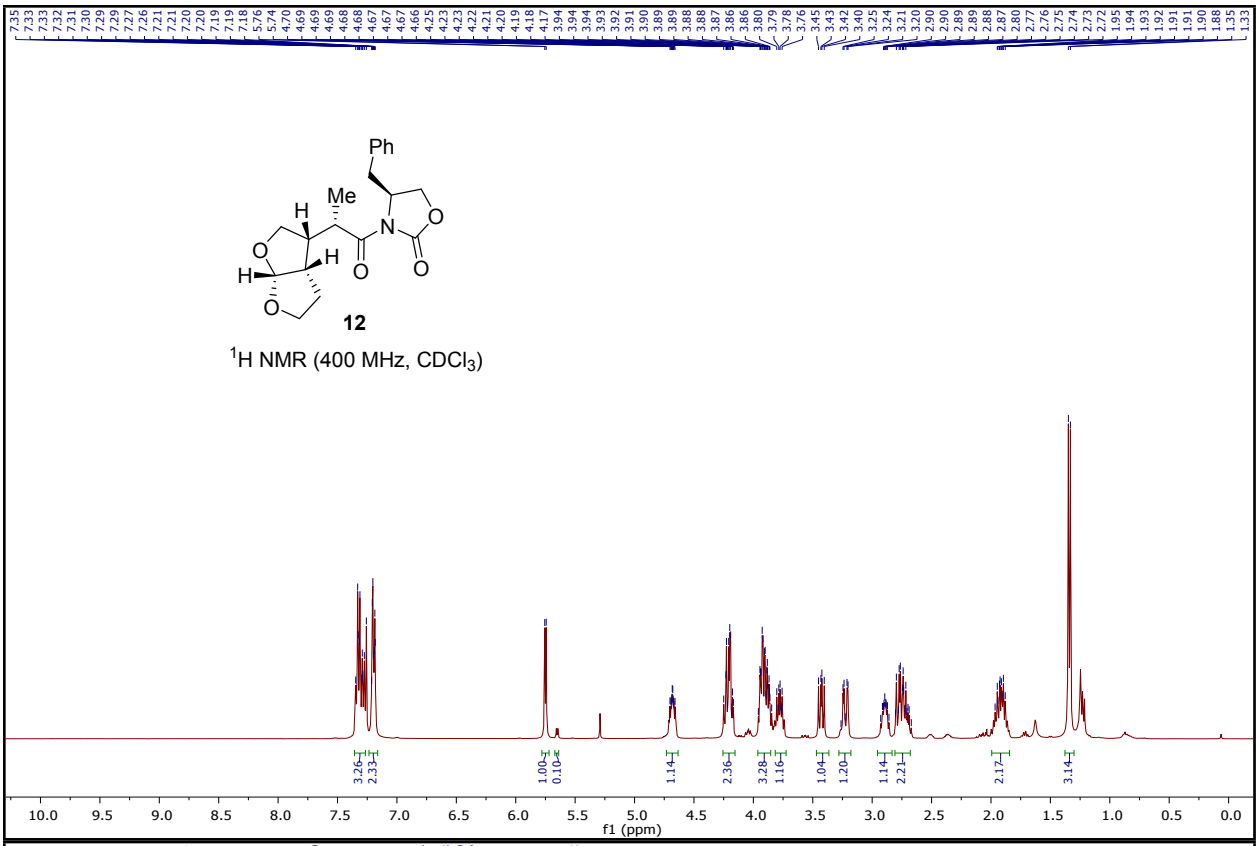
H
O
N
O
O
H
H
O
O
10
13C NMR (100 MHz, CDCl3)
f1 (ppm)


7.26
5.75 5.73 4.02 4.01 4.01 4.00 3.99 3.98 3.95 3.94 3.93 3.92 3.92 3.91 3.90 3.88 3.87 3.86 3.86 3.85 3.84 3.84 3.83 3.55 3.54 3.53 3.52 3.51 3.50 2.97 2.94 2.56 2.55 2.53 2.52 1.94 1.93 1.92 1.92 1.91 1.91 1.90 1.88 1.87 1.85 1.25 1.24 1.23 1.22
Me
H
OH
O
H
H
O
O
11
^{1}H NMR (400 MHz, $CDCl_3$)
1.00
1.08
2.20
1.06
1.02
1.87
2.02
2.86
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


180.06
109.79
77.22
76.58
70.30
68.94
45.06
38.67
36.03
25.24
16.75
Me
H
OH
O
H
H
O
O
11
^{13}C NMR (100 MHz, $CDCl_3$)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



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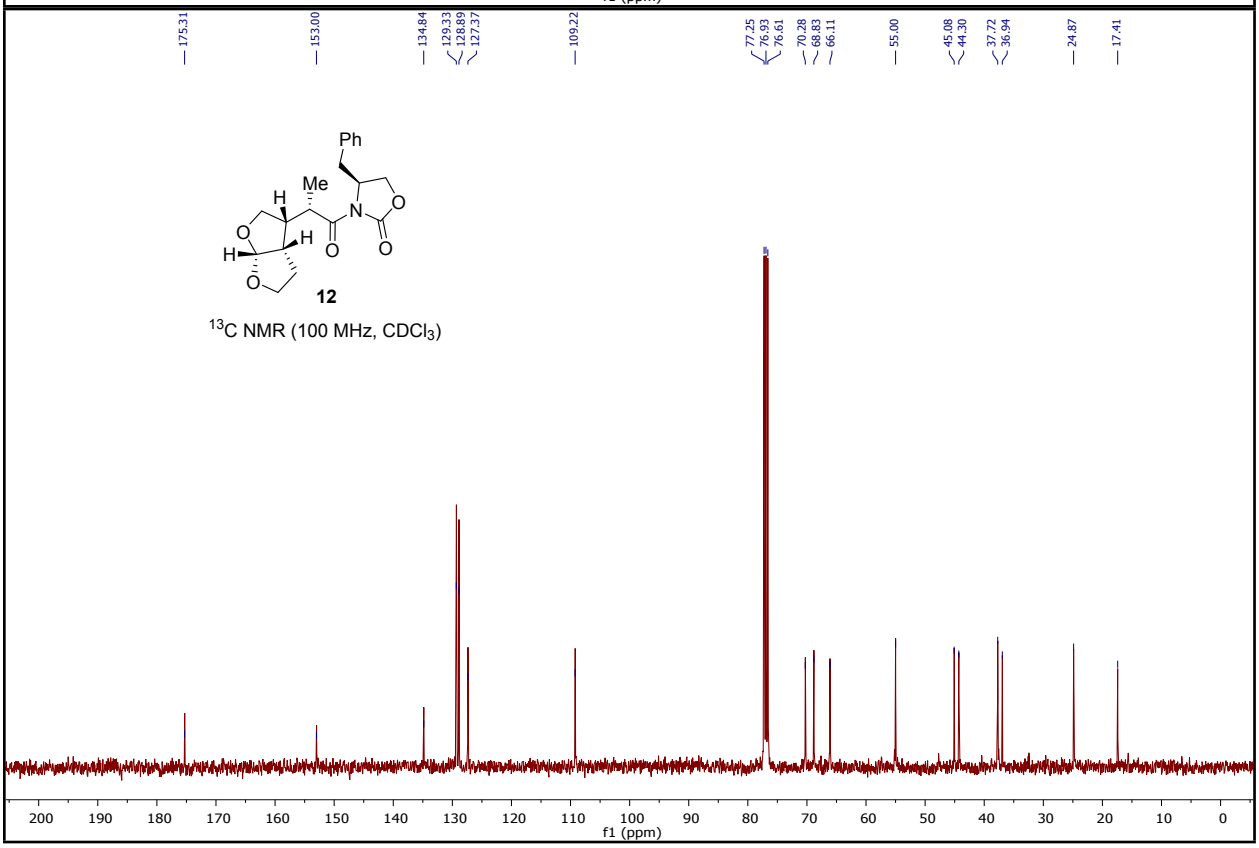

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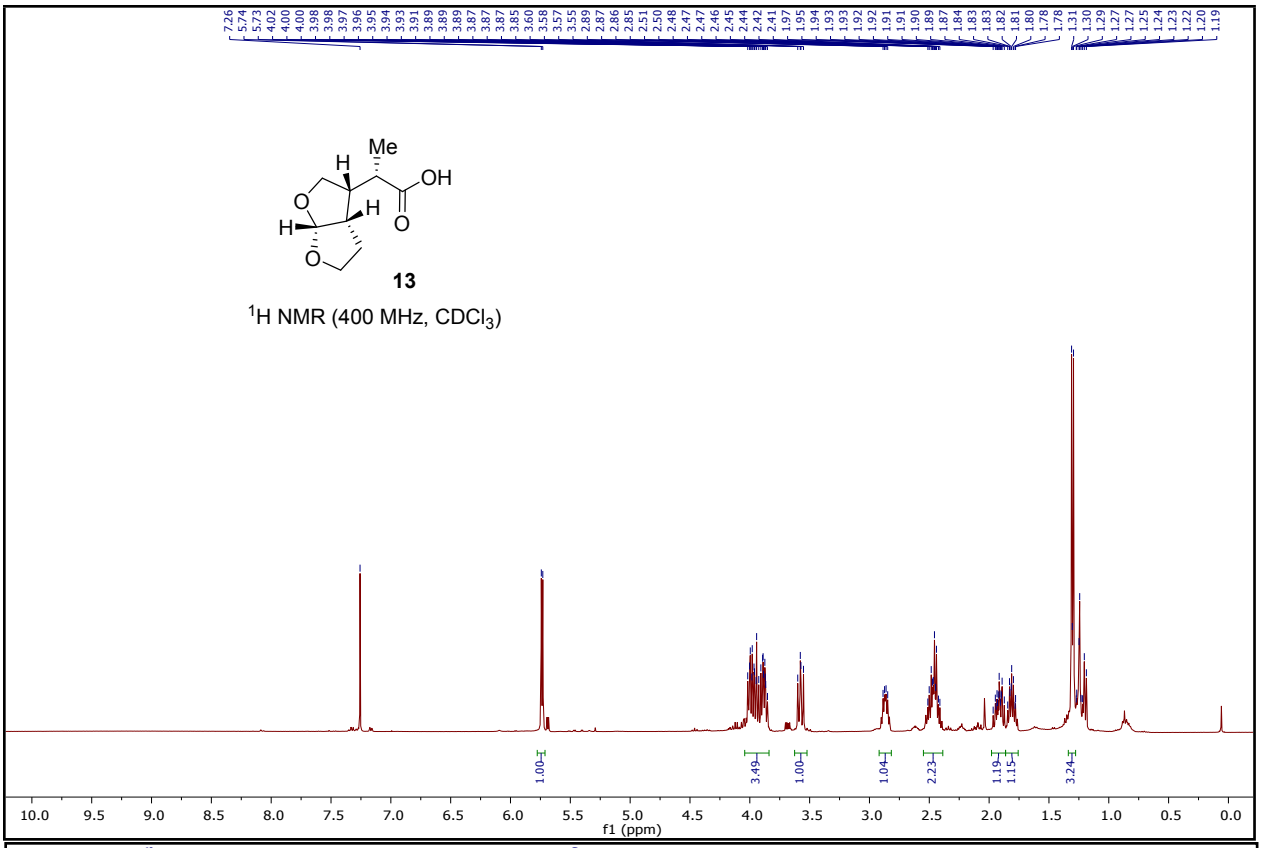
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H
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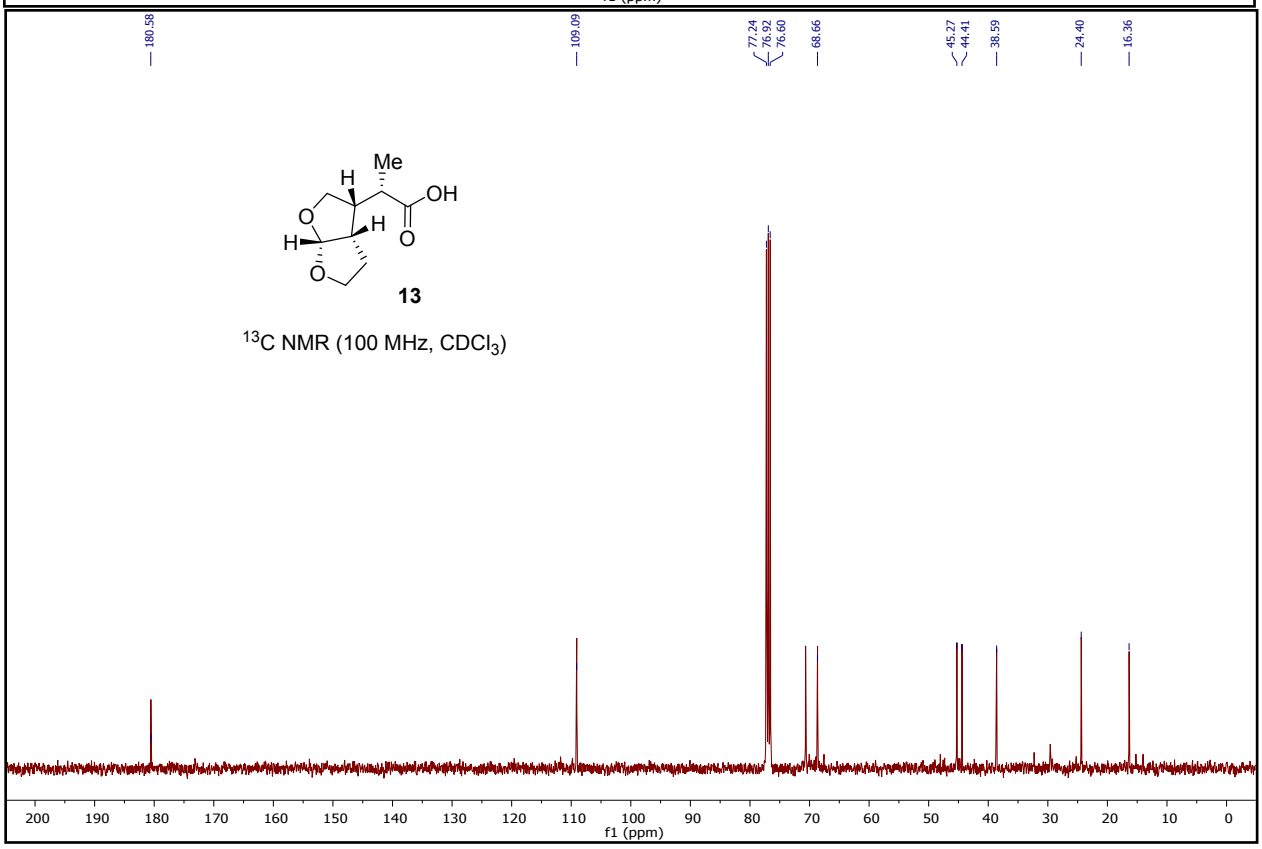
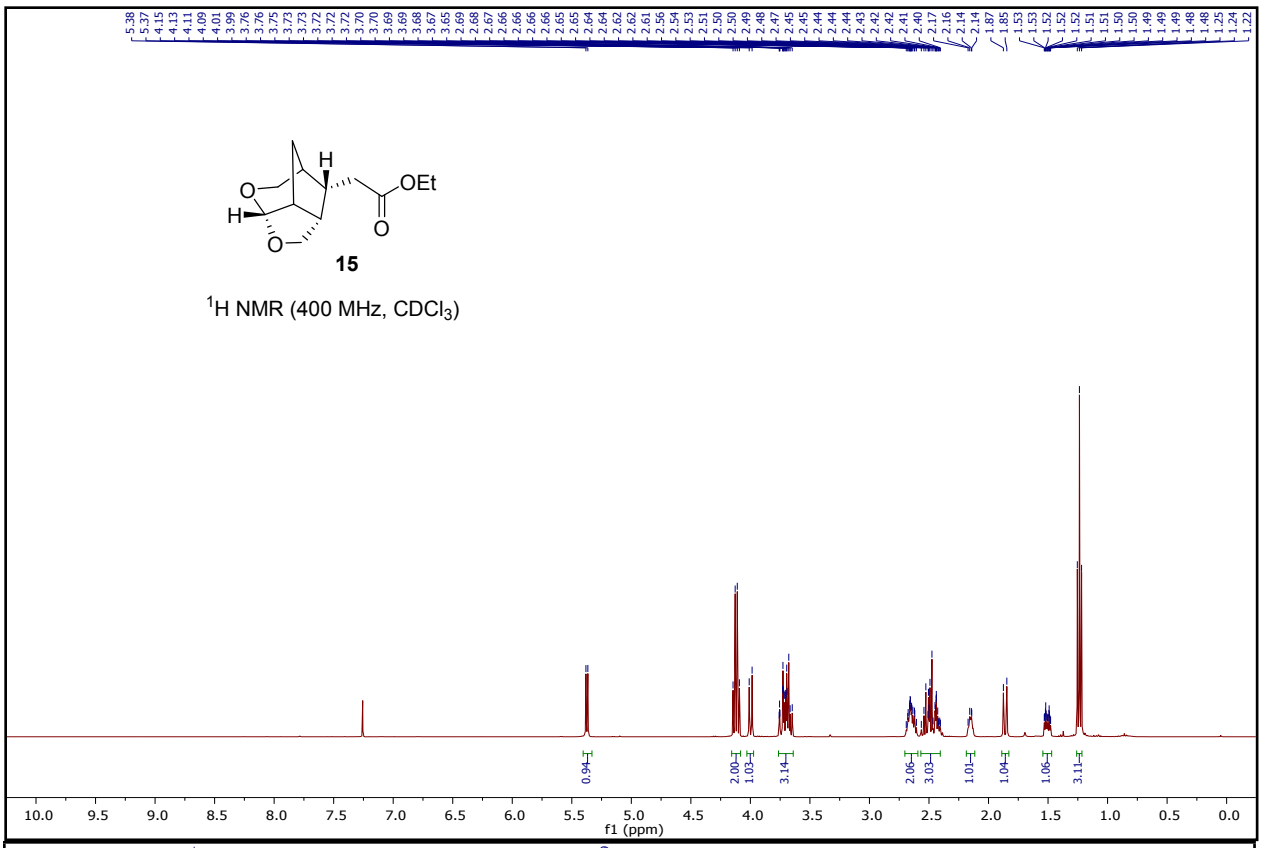
H
H
O
O
13
1H NMR (400 MHz, CDCl3)
f1 (ppm)


Me
H
OH
O
H
H
O
O
13
13C NMR (100 MHz, CDCl3)
f1 (ppm)



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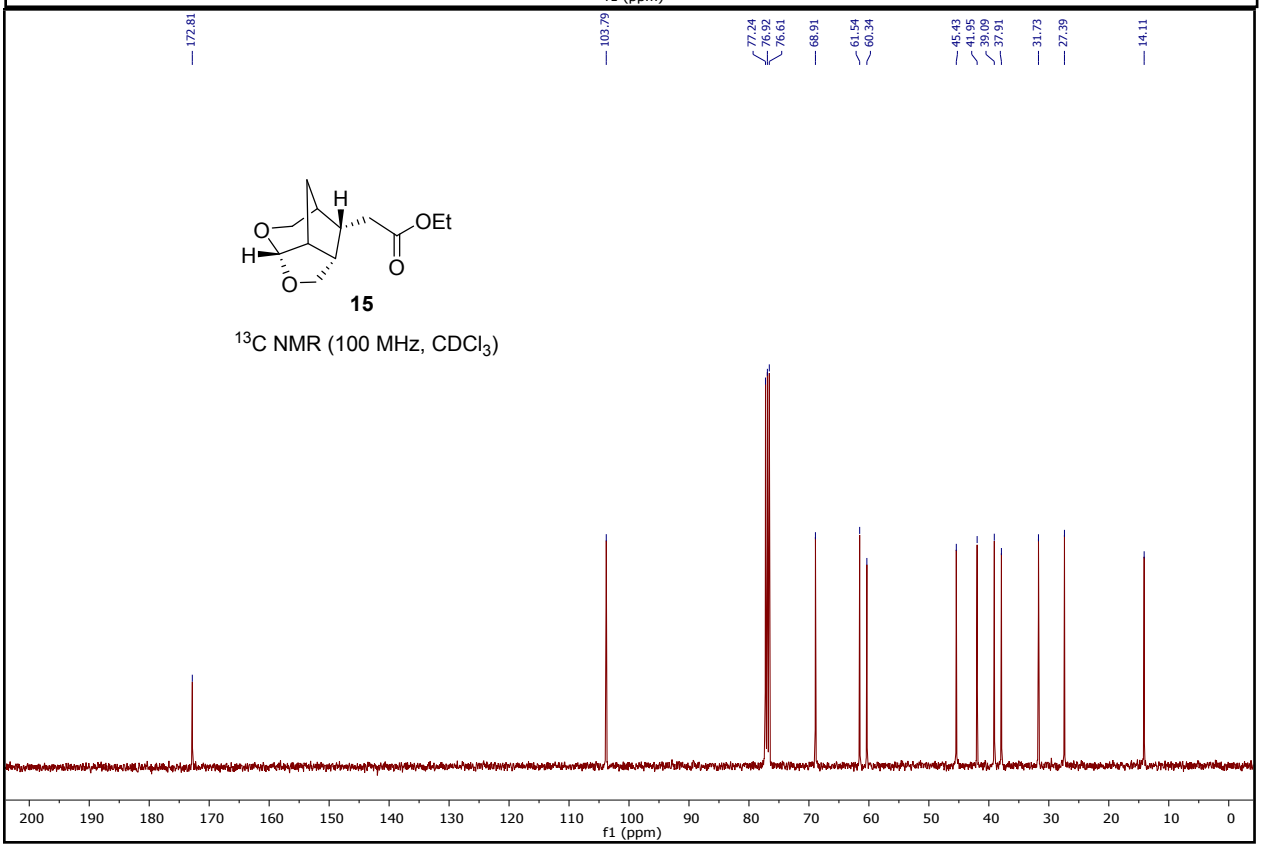

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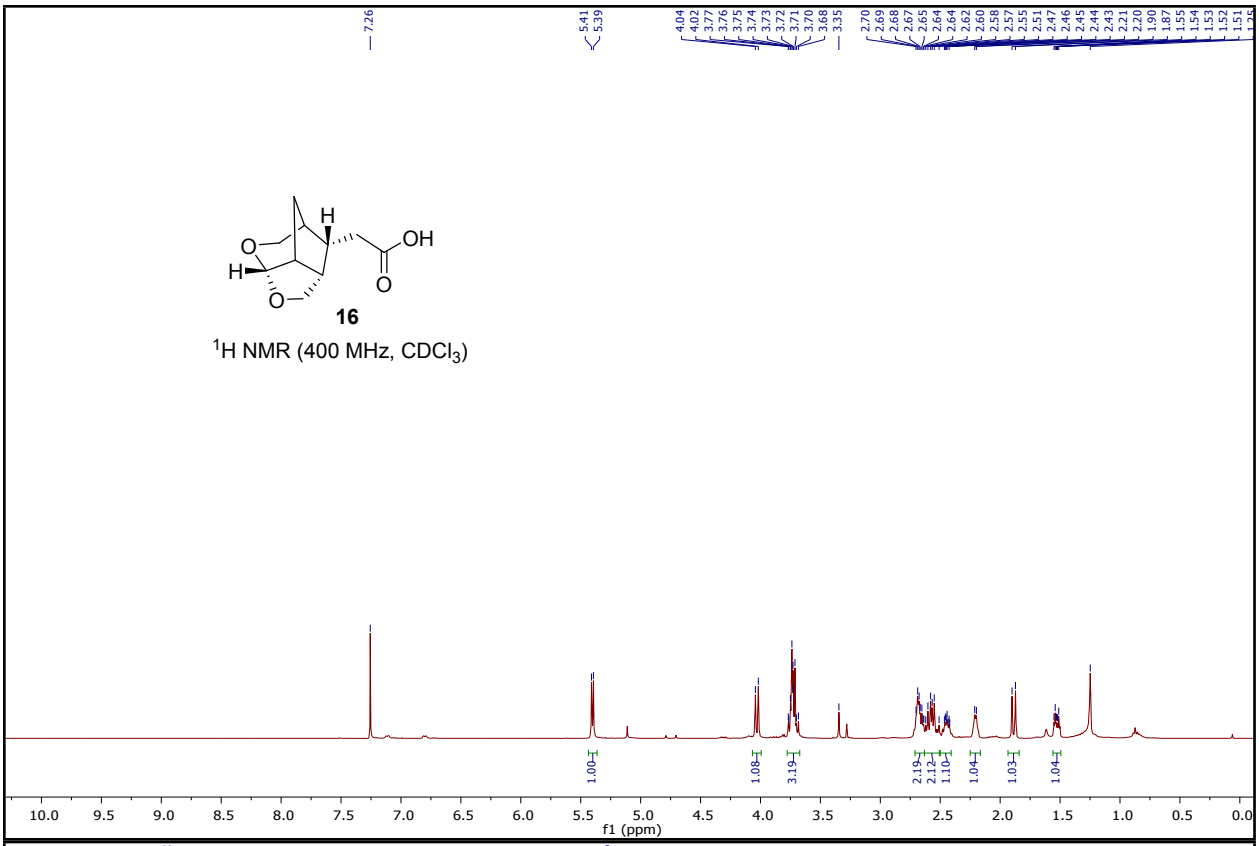

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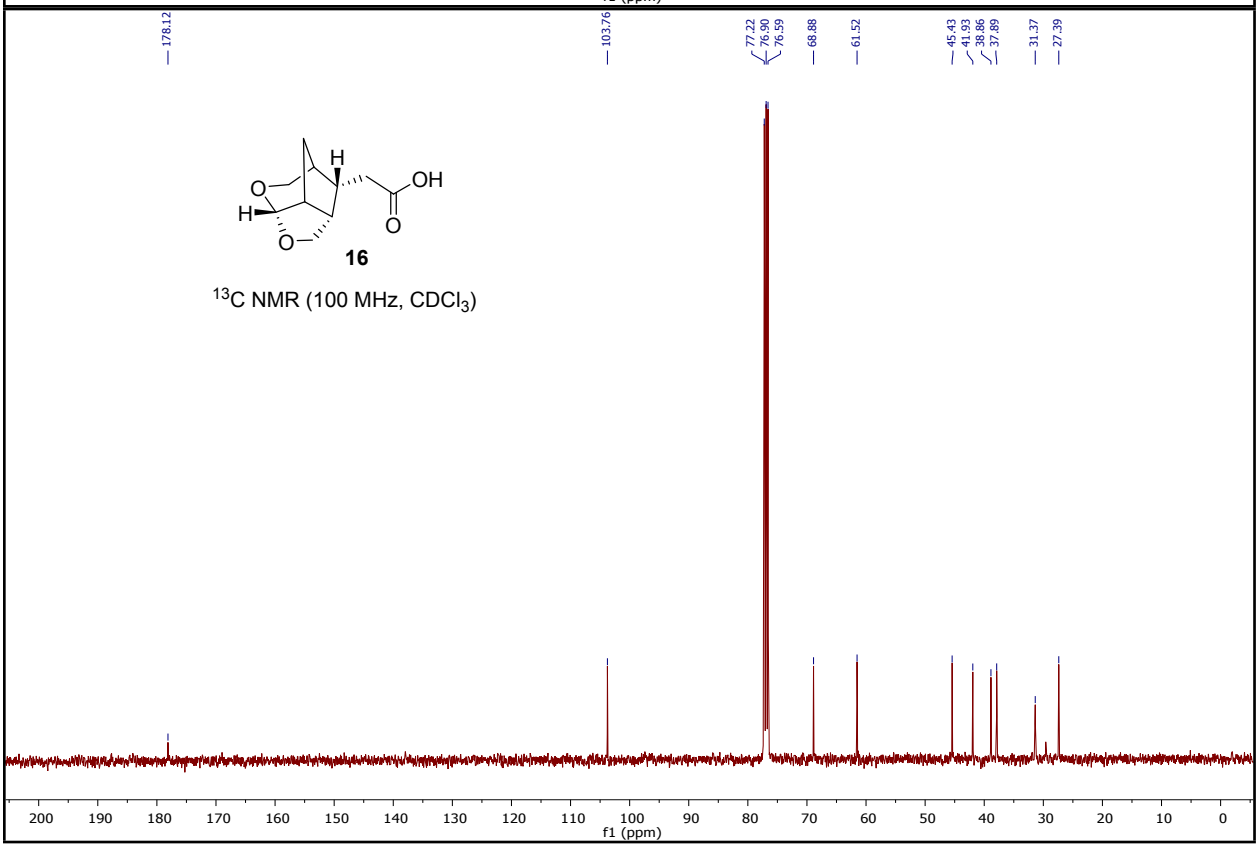

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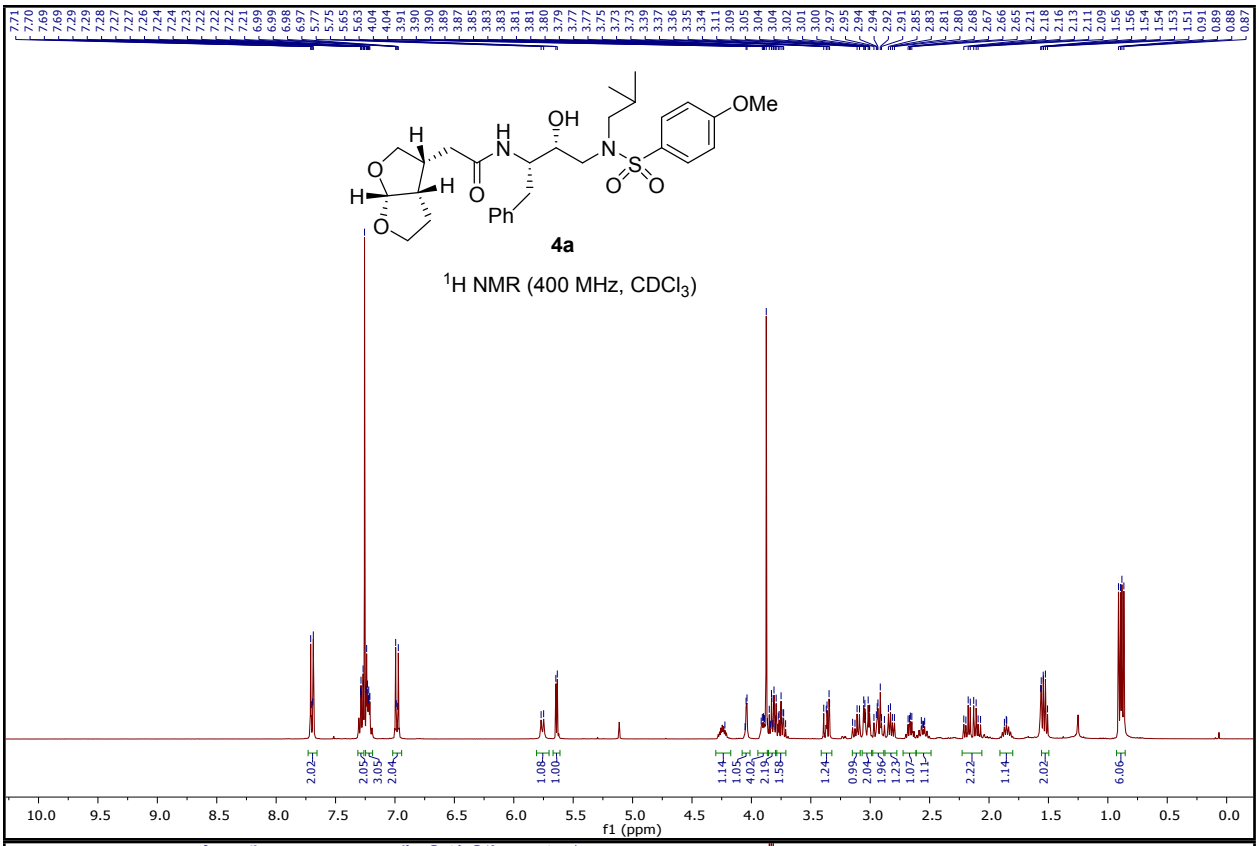
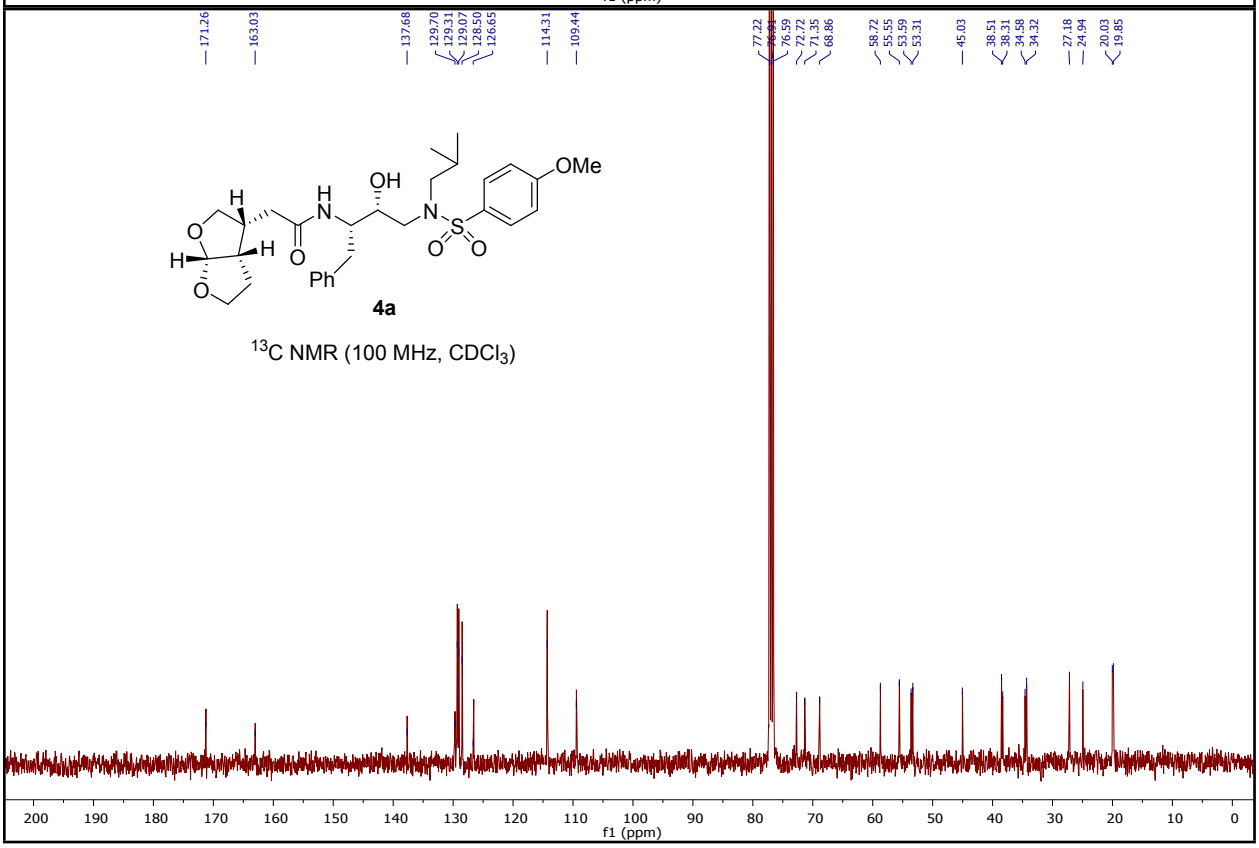
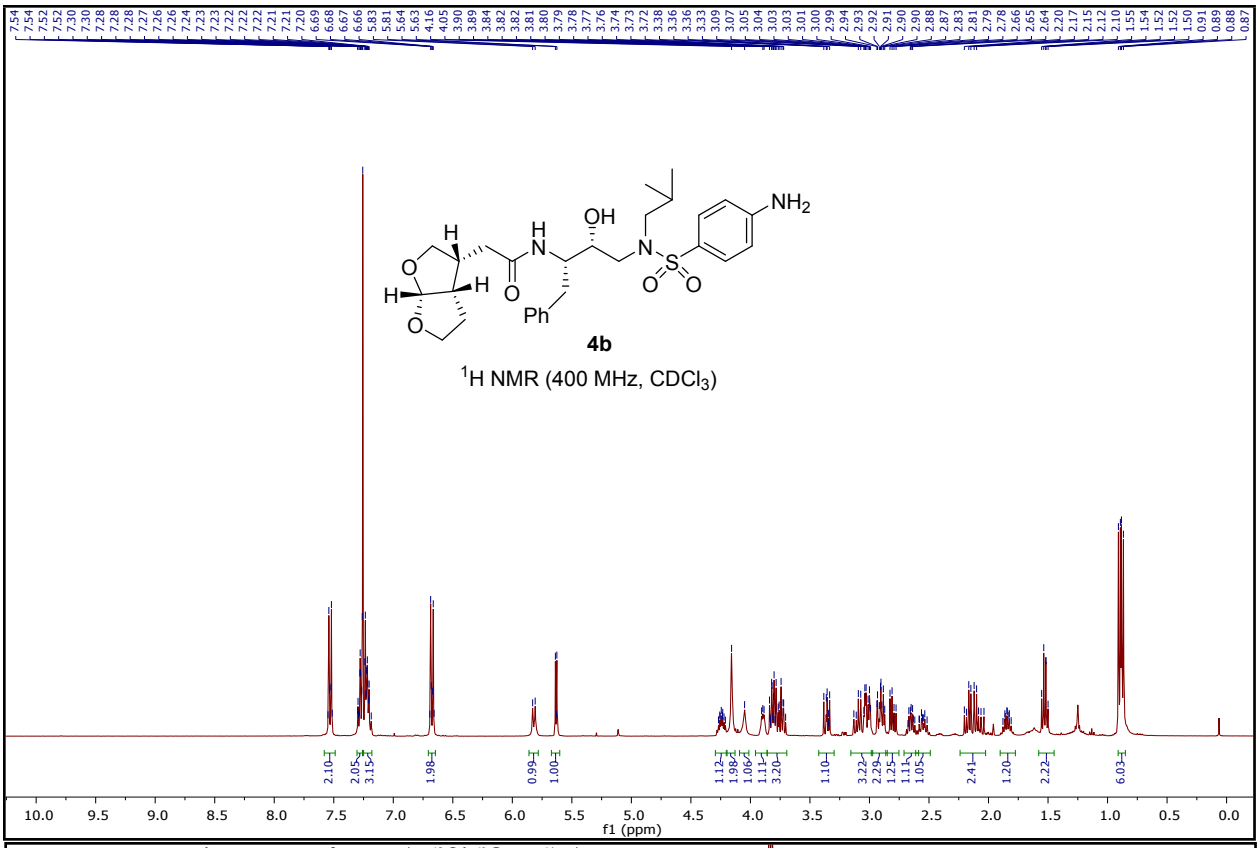

OMe
OH
H
N
O
S
Ph
4a
^{1}H NMR (400 MHz, $CDCl_3$)
f1 (ppm)


OMe
OH
H
N
O
S
Ph
4a
^{13}C NMR (100 MHz, $CDCl_3$)
f1 (ppm)


4b
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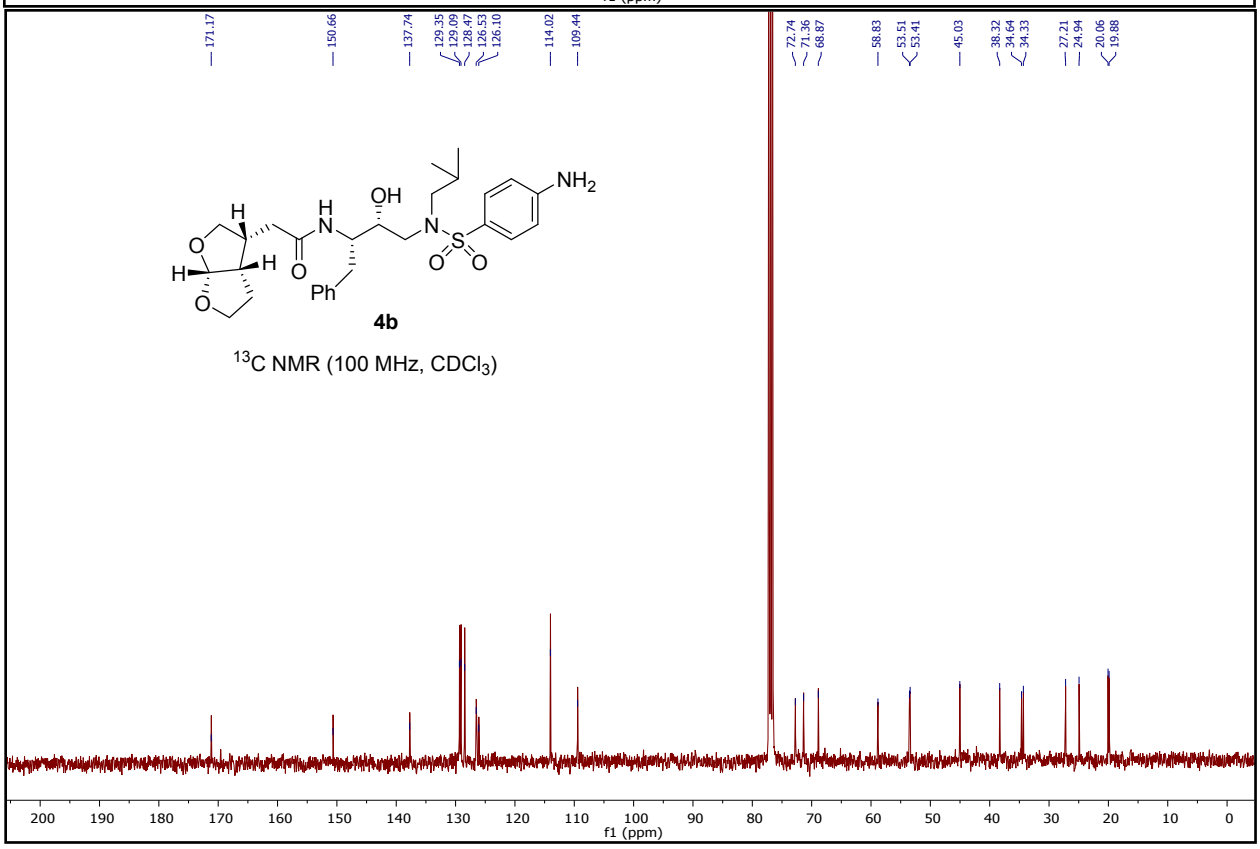
f1 (ppm)


4b
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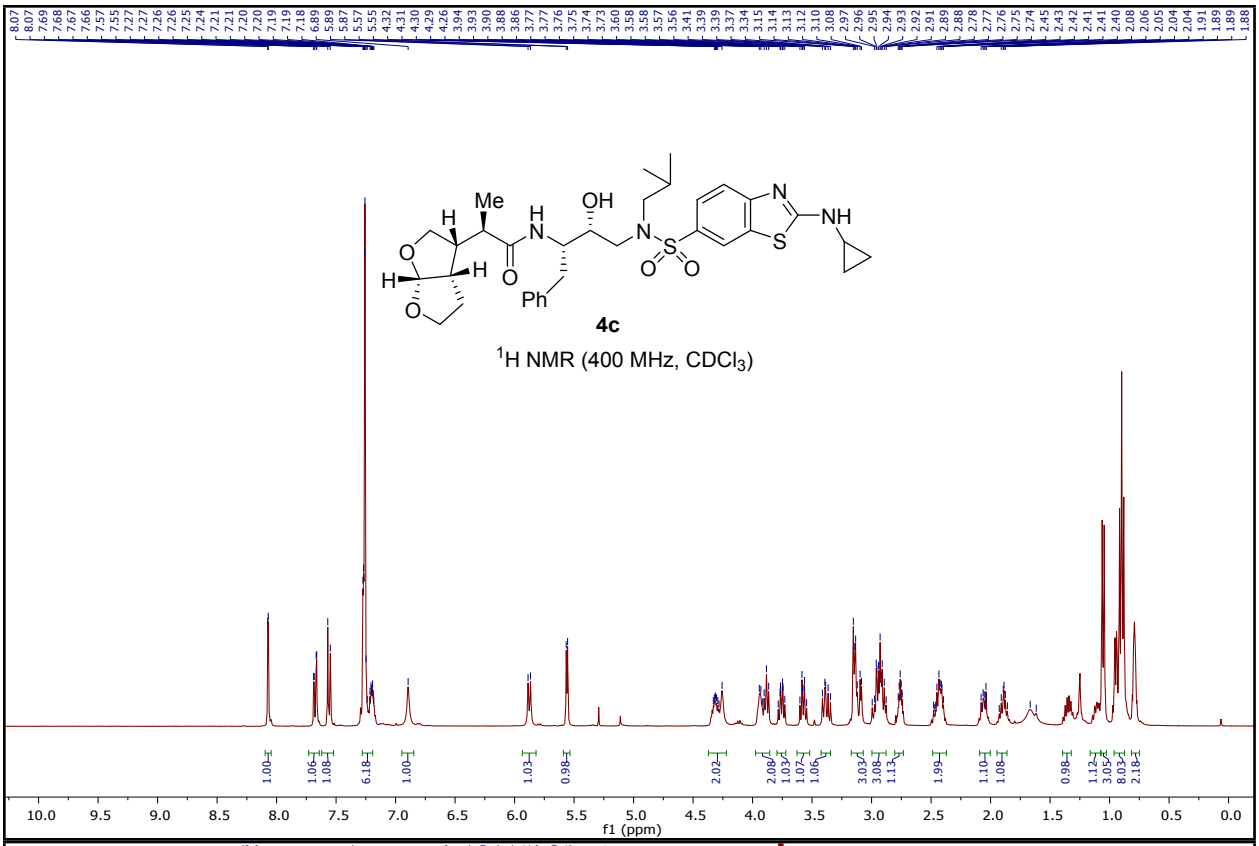
f1 (ppm)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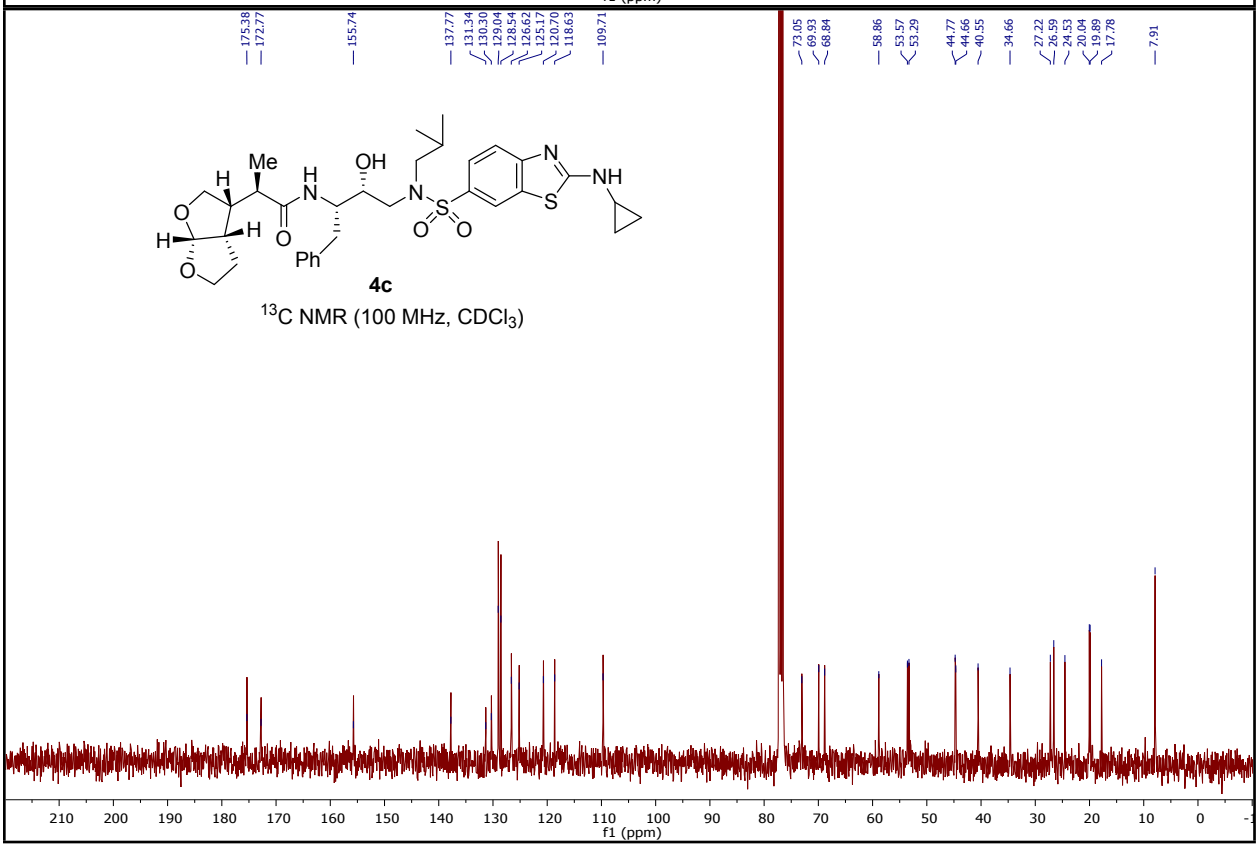
f1 (ppm)


4c
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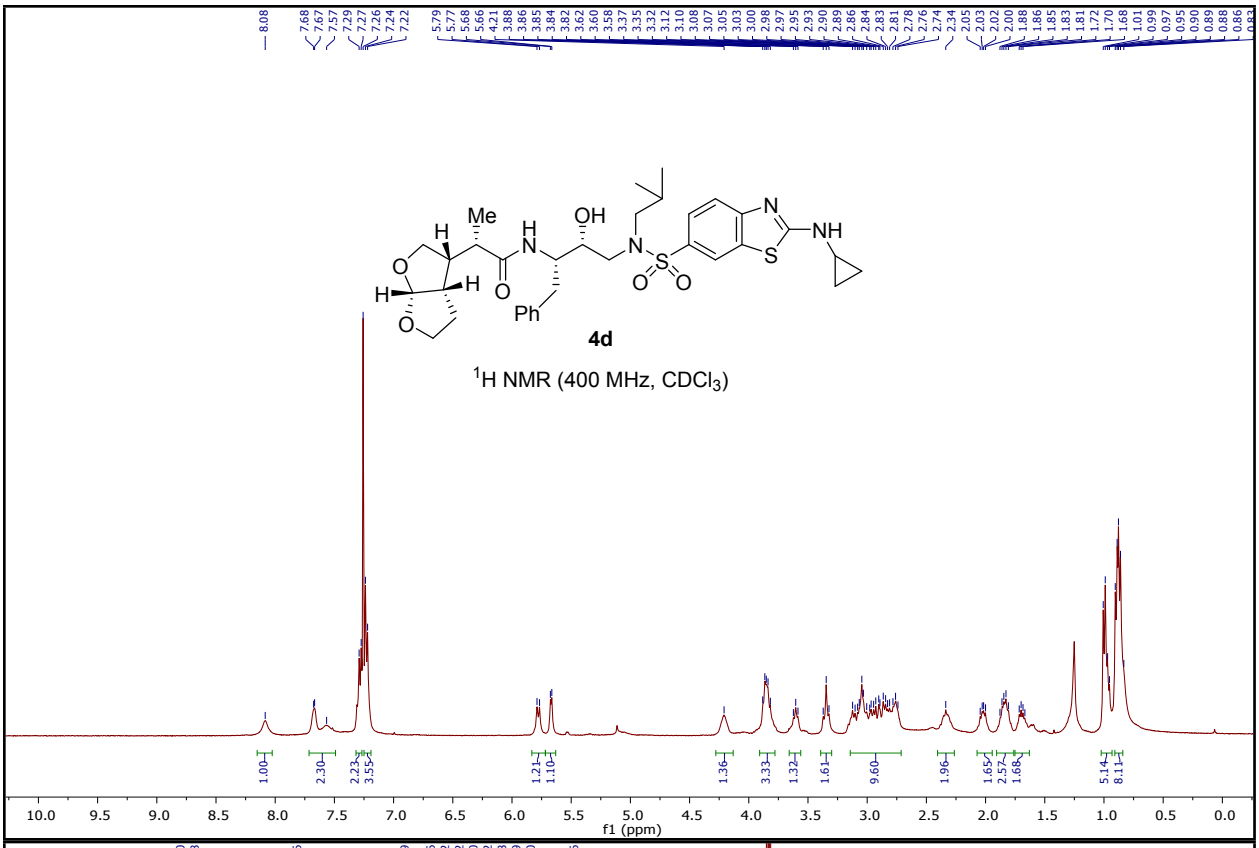
f1 (ppm)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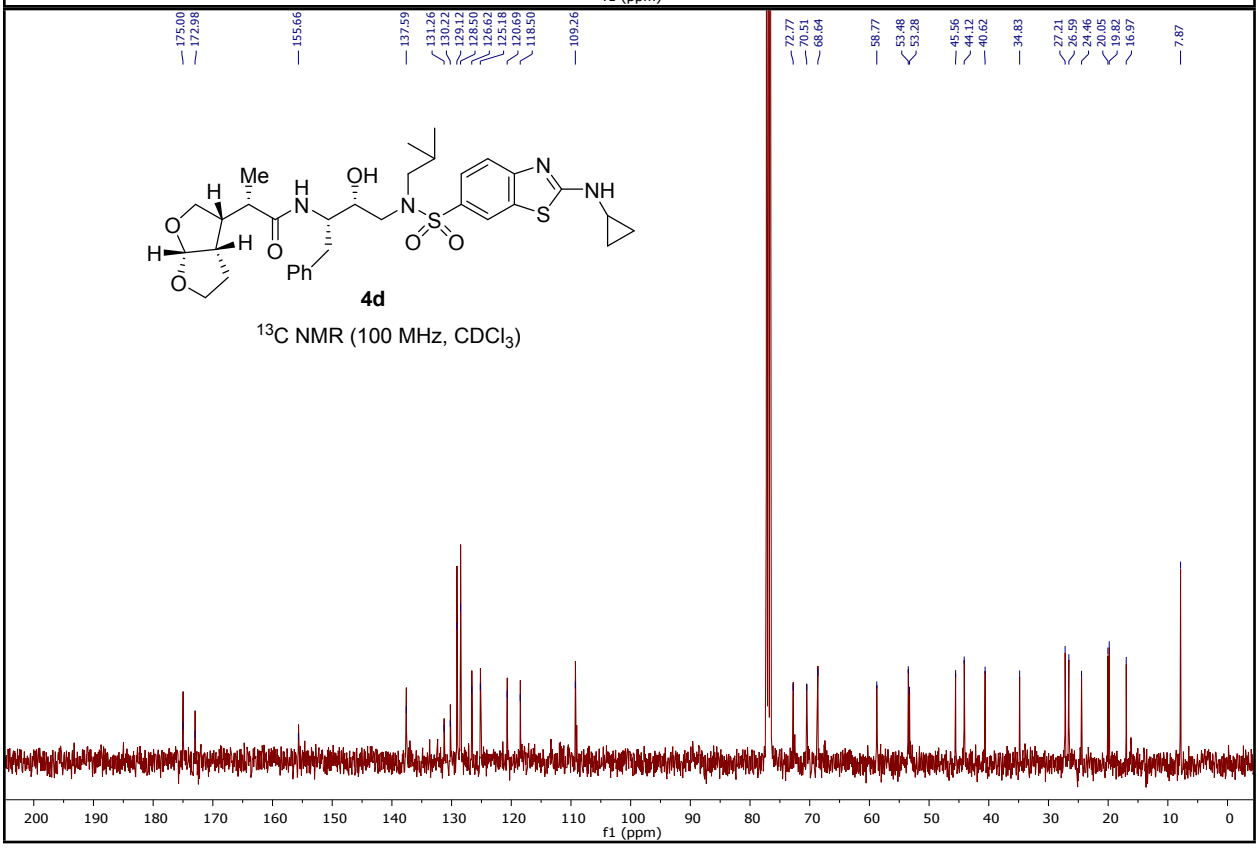
f1 (ppm)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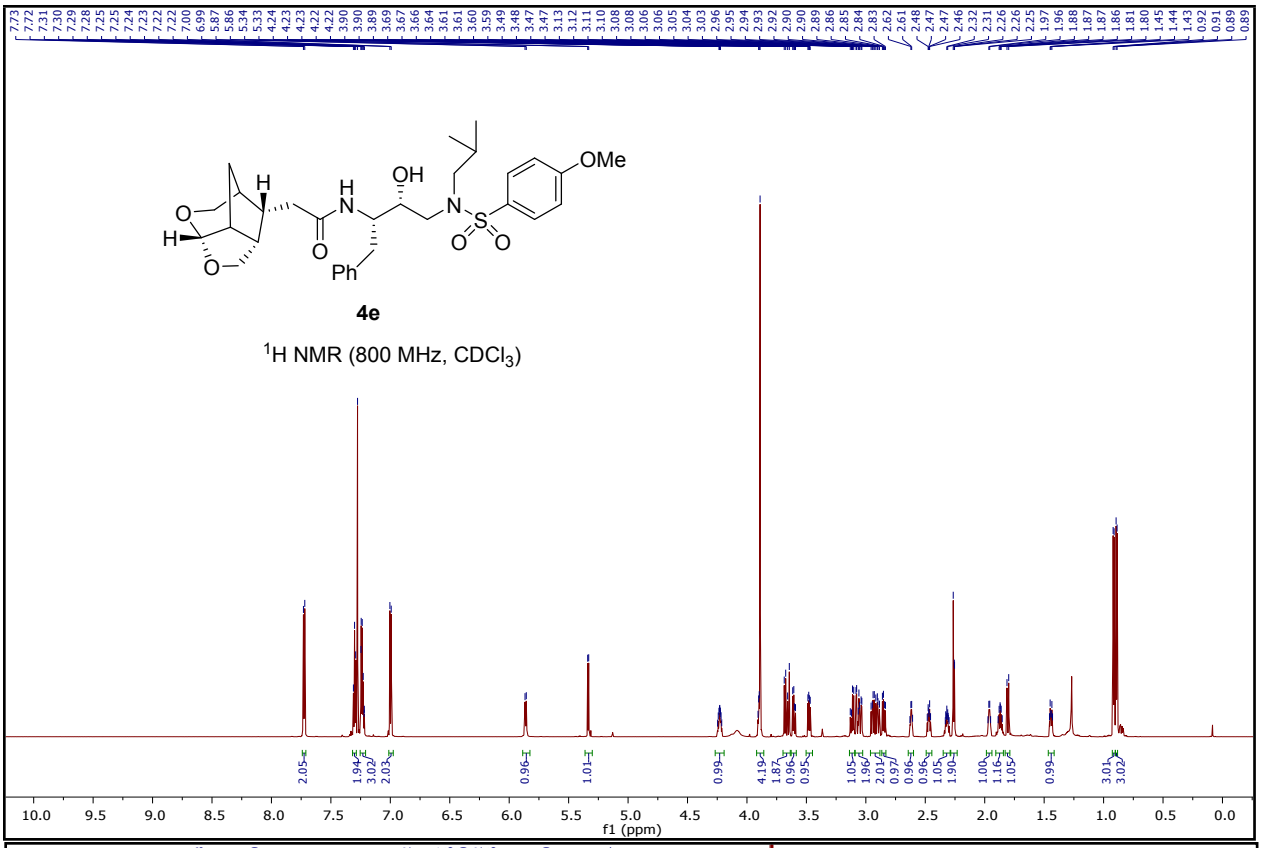
f1 (ppm)


OMe
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O
S
Ph
4e
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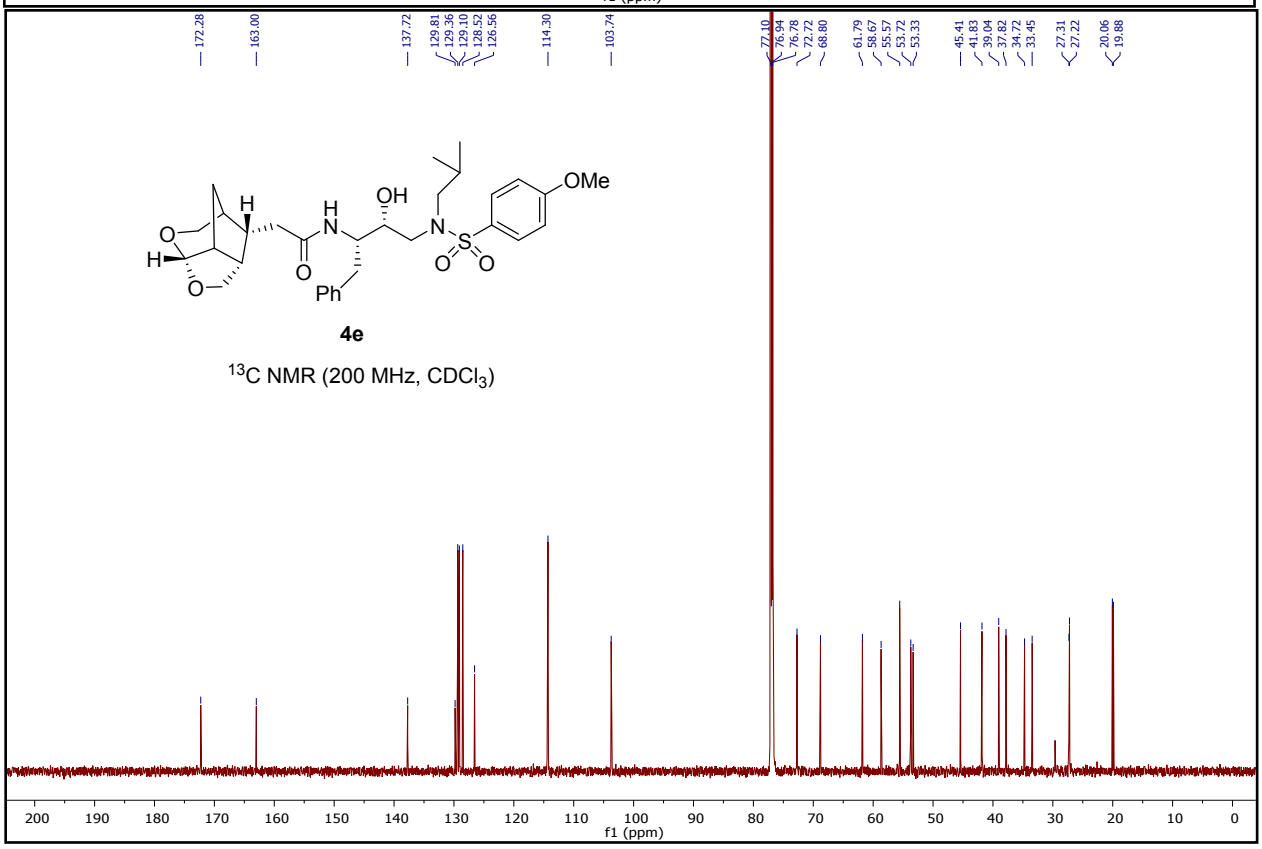
f1 (ppm)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O
S
Ph
4e
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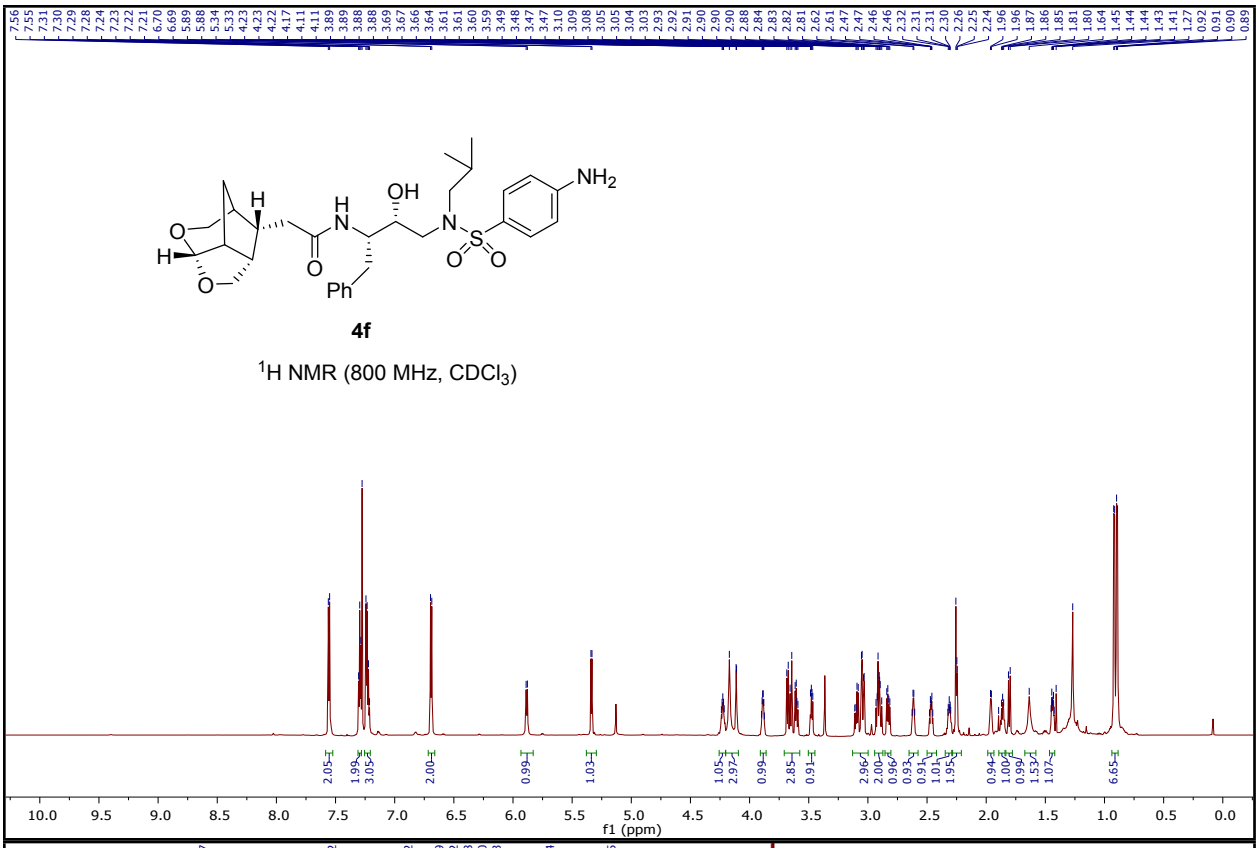
f1 (ppm)



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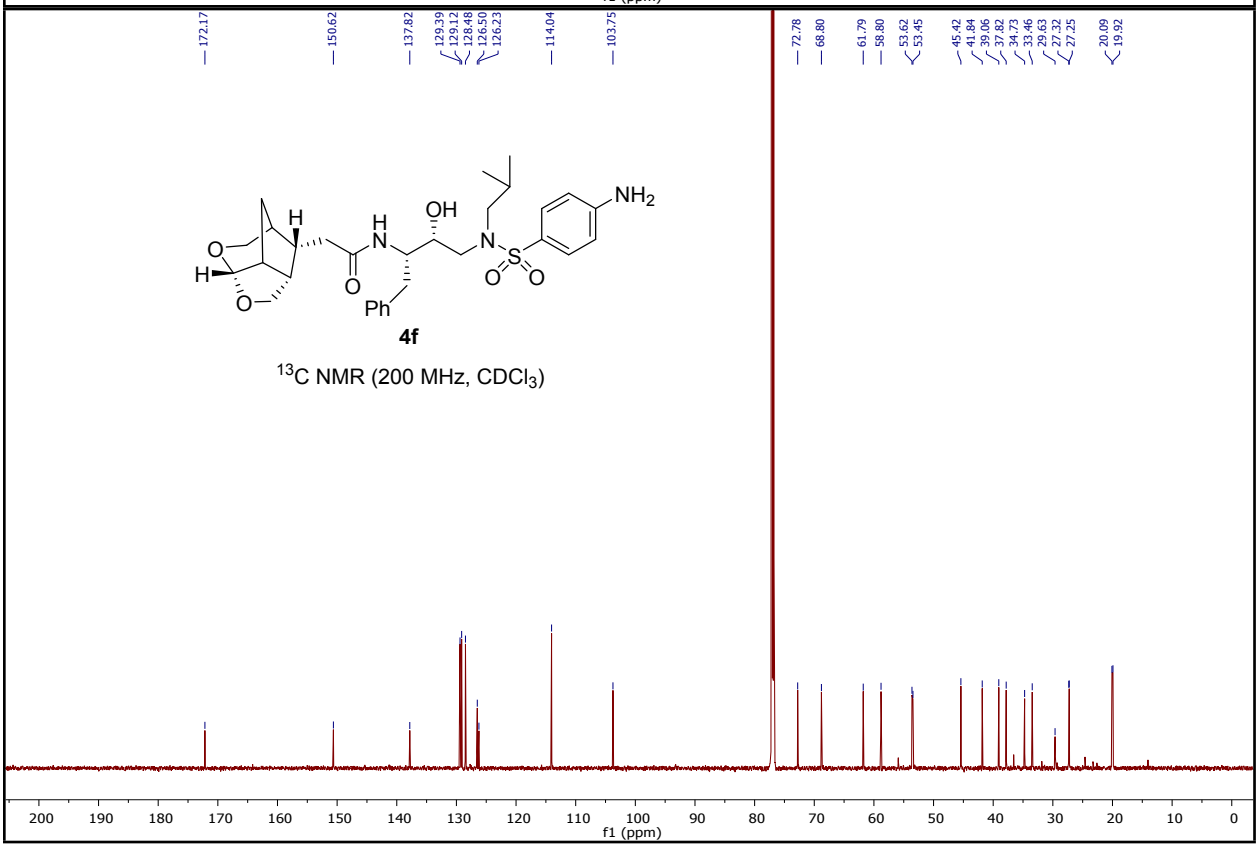

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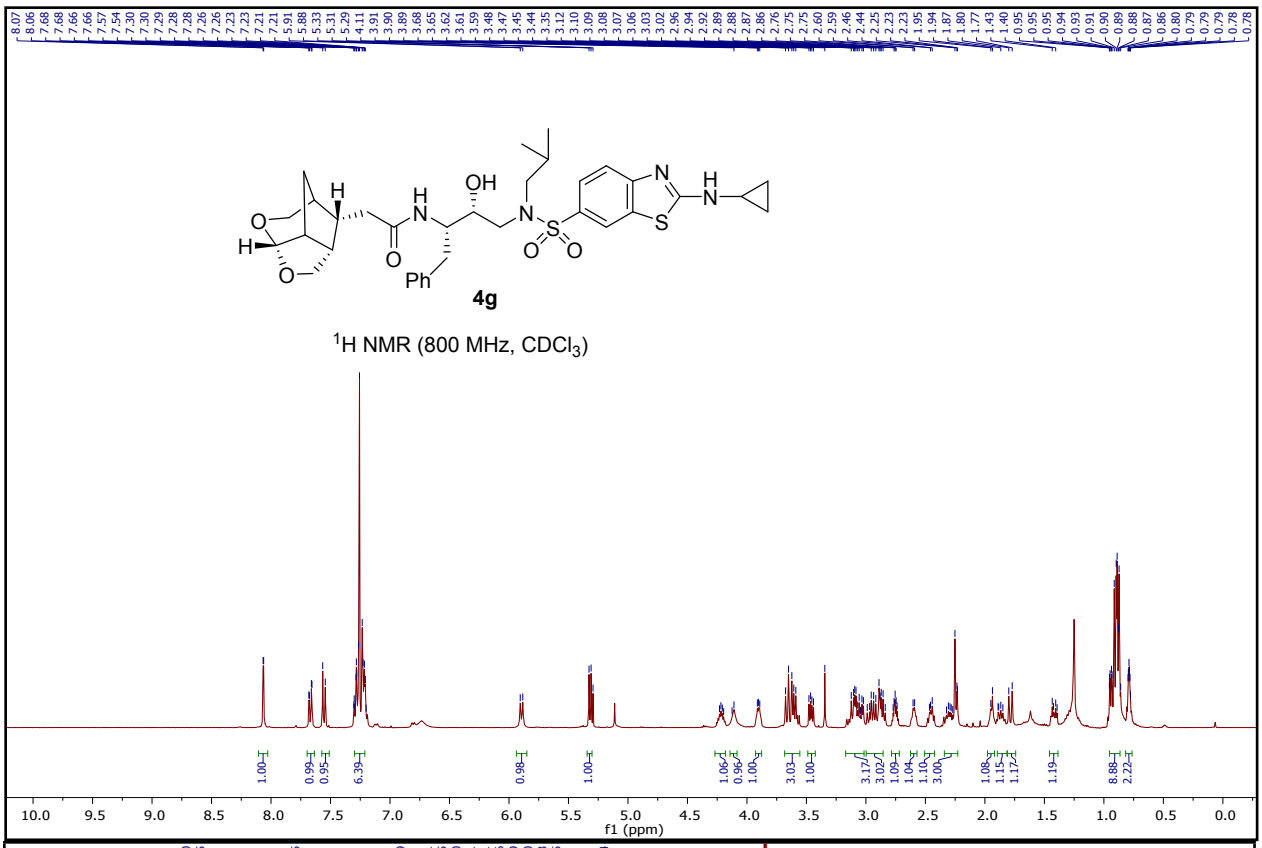

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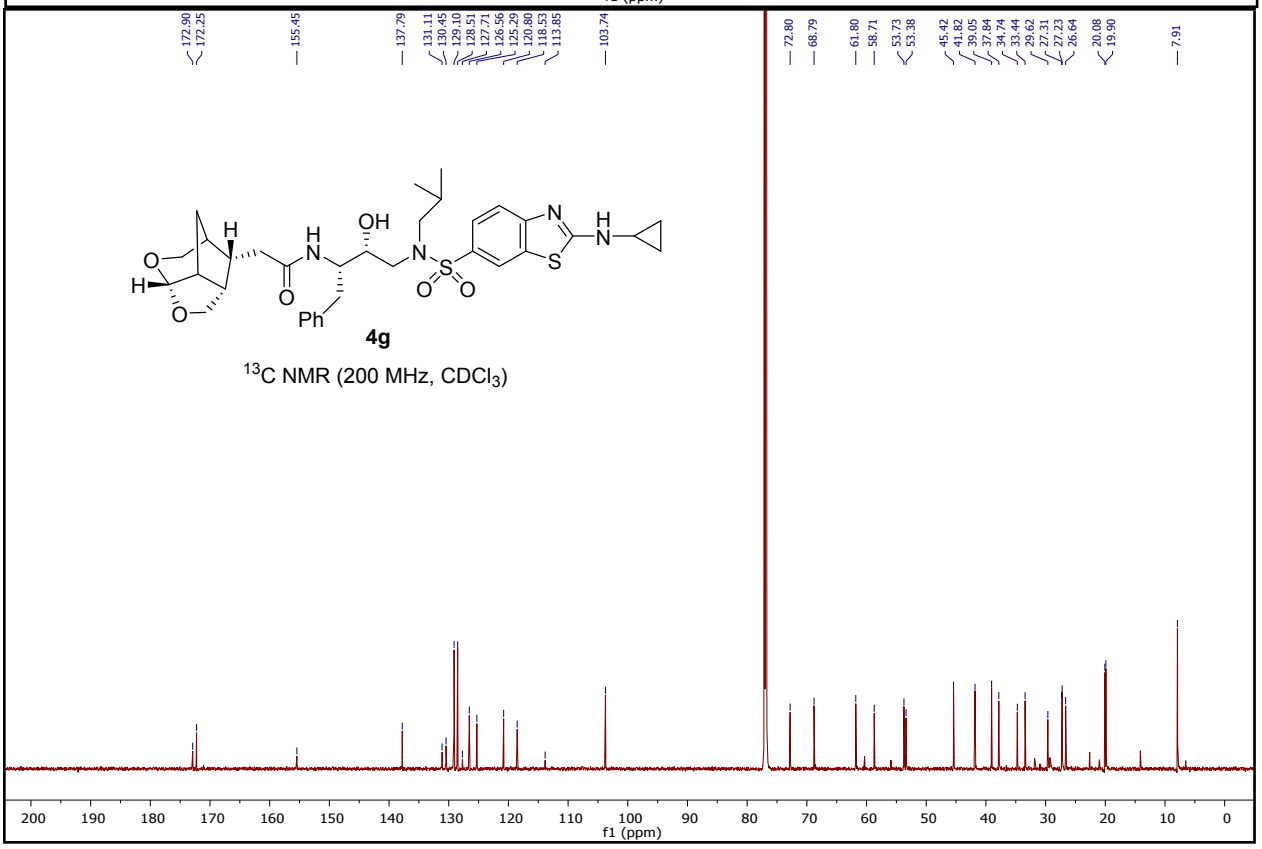

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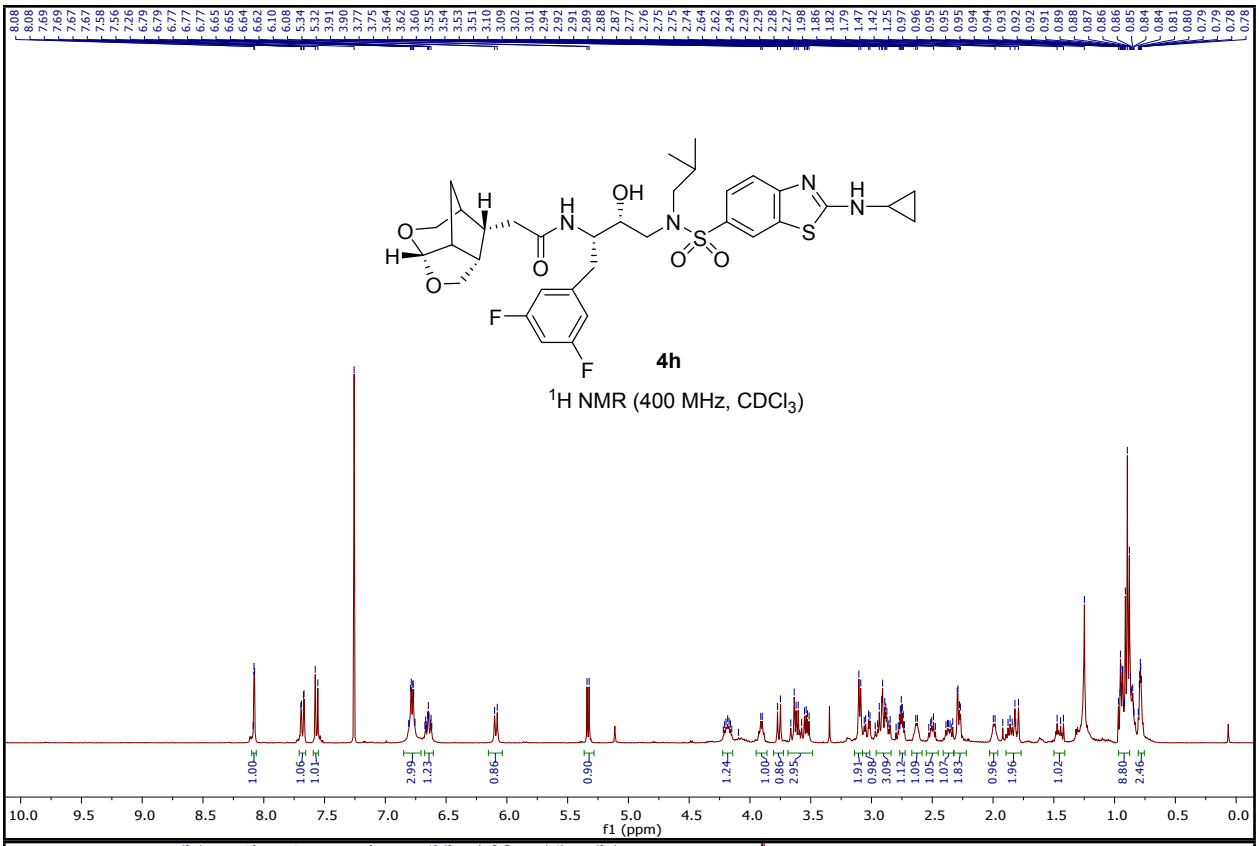

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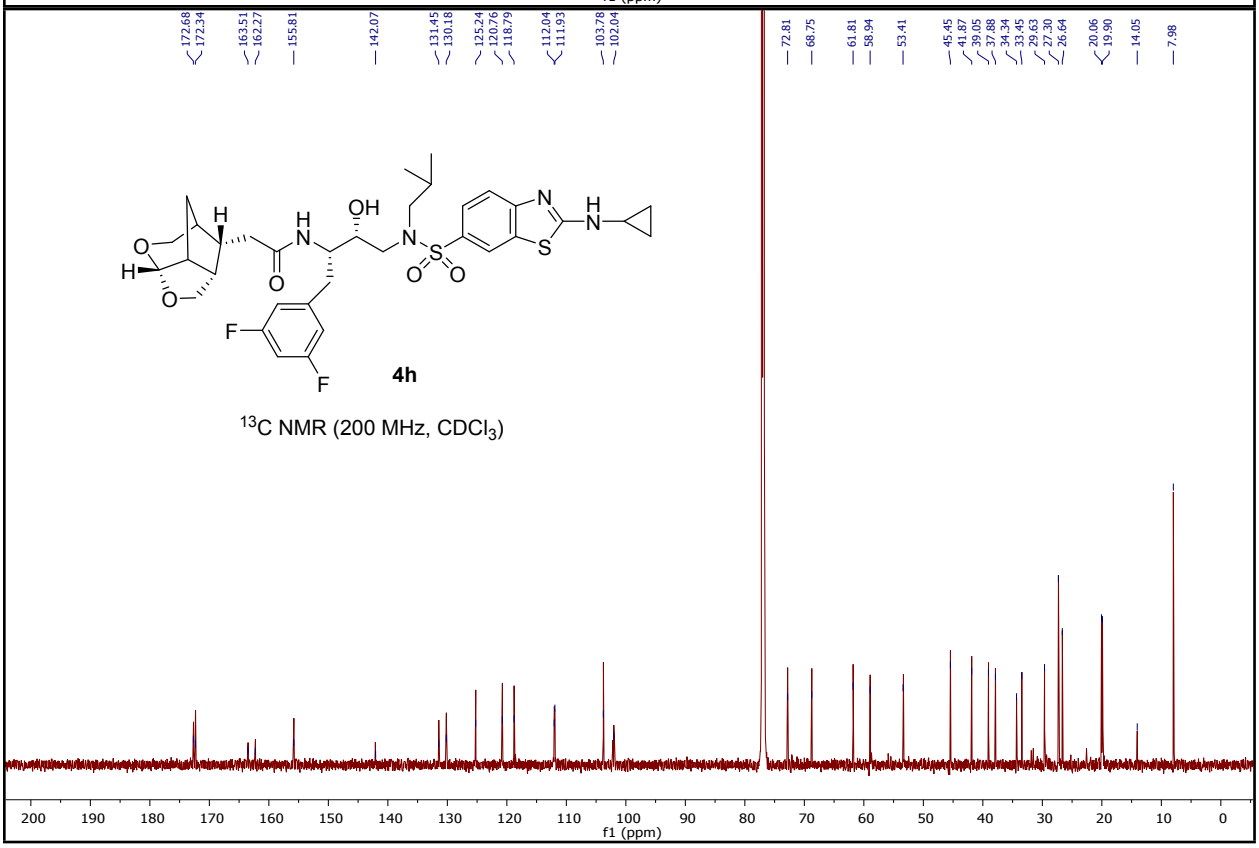

^{13}C NMR (200 MHz, $CDCl_3$)

Cells, viruses, and antiviral agents. Human CD4$^+$ MT-2 cells were grown in RPMI-1640-based culture medium supplemented with 10% fetal calf serum (FCS: JRH Biosciences, Lenexa, MD), 50 unit/mL penicillin, and 100 μg/mL of kanamycin. The following HIV-1 viruses were employed for the drug susceptibility assay (see below): a laboratory HIV-1strain (HIV-1_{LAI}), a clinical HIV-1 strain isolated from drug-naive patients with AIDS (HIV-$1_{ERS104pre}$) (1), and six HIV-1 clinical isolates which were originally isolated from patients with AIDS, who had received 9 to 11 anti-HIV-1 drugs over the past 32 to 83 months, and were genotypically and phenotypically characterized as multi-PI-resistant HIV-1 variants (1, 2). All such primary HIV-1 strains were passaged once or twice in 3-day old phytohemagglutinin-activated peripheral blood mononuclear cells (PHA-PBM), and the culture supernatants were stored at –80 °C until use. Amprenavir (APV) was received as a gift from Glaxo-Wellcome, Research Triangle Park, NC. Darunavir (DRV) was synthesized as previously described (3).

1. Yoshimura, K., et al. *Proc. Natl. Acad. Sci.* USA **96**, 8675-8680 (1999).
2. Koh, Y., et al. *Antimicrob. Agents Chemother.* **53**, 987-996 (2009).
3. Koh Y, et al *J Mol Biol* **282**, 28709-28720 (2007)

Molecular Formula Strings (please revise)

Compound	SMILES	K_i (nM)	IC_{50} (nM)
4a	O=C(N[C@@H](CC1=CC=CC=C1)[C@H](O)CN(CC(C)C)S(C2=CC=C(OC)C=C2)(=O)=O)C[C@]3([H])[C@@]4([H])[C@@](OCC4)([H])OC3	0.28	>1000
4b	O=C(N[C@@H](CC1=CC=CC=C1)[C@H](O)CN(CC(C)C)S(C2=CC=C(N)C=C2)(=O)=O)C[C@]3([H])[C@@]4([H])[C@@](OCC4)([H])OC3	17.4	>1000
4c	O=C(N[C@@H](CC1=CC=CC=C1)[C@H](O)CN(CC(C)C)S(=O)(C2=CC(SC(NC3CC3)=N4)=C4C=C2)=O)[C@H](C)[C@]5([H])[C@@]6([H])[C@@](OCC6)([H])OC5	284	>1000
4d	O=C(N[C@@H](CC1=CC=CC=C1)[C@H](O)CN(CC(C)C)S(=O)(C2=CC(SC(NC3CC3)=N4)=C4C=C2)=O)[C@@H](C)[C@]5([H])[C@@]6([H])[C@@](OCC6)([H])OC5	1.45	164
4e	O=C(C[C@@]1([H])C(C2)CO[C@]3([H])C2C1CO3)N[C@@H](CC4=CC=CC=C4)[C@H](O)CN(CC(C)C)S(C5=CC=C(OC)C=C5)(=O)=O	0.032	2.9
4f	O=C(C[C@@]1([H])C(C2)CO[C@]3([H])C2C1CO3)N[C@@H](CC4=CC=CC=C4)[C@H](O)CN(CC(C)C)S(C5=CC=C(N)C=C5)(=O)=O	0.022	2.6
4g	O[C@H](CN(CC(C)C)S(C1=CC(SC(NC2CC2)=N3)=C3C=C1)(=O)=O)[C@@H](NC(C[C@@]4([H])C(C5)CO[C@]6([H])C5C4CO6)=O)CC7=CC=CC=C7	0.066	2.6
4h	O[C@H](CN(CC(C)C)S(C1=CC(SC(NC2CC2)=N3)=C3C=C1)(=O)=O)[C@@H](NC(C[C@@]4([H])C(C5)CO[C@]6([H])C5C4CO6)=O)CC7=CC(F)=CC(F)=C7	0.030	0.079