Electronic Supporting Information

Synthesis, Structure and Inhibitory Activity of a Stereoisomer of Oseltamivir Carboxylate

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General

Synthesis. Unless otherwise noted, all reactions were performed in oven-dried or flame-dried glassware. Air-sensitive reagents and solutions were transferred via syringe or cannula and were introduced to the apparatus through rubber septa. Dichloromethane (ACS grade), THF (ACS grade), and toluene (HPLC grade) were used as such or dried by distillation according to standard procedures (CH_2Cl_2 on CaH_2; THF on Na/benzophenone). Solvents for chromatography and filtration including hexane, ethyl acetate, dichloromethane, petroleum ether, and methanol were HPLC grade and used as received. Ammonia-methanol mixture was prepared by bubbling liquid ammonia in methanol at 0 °C for 30 min. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 pre-coated plates with visualization under short-wavelenght UV light and by dipping the plates with molybdate reagent (aqueous H_2SO_4 solution of ceric sulphate/ammonium molybdate) followed by heating. Flash column chromatography was performed using 40-63 μm silica gel and the indicated solvent mixtures. Melting points (mp) were measured with an optical Optiphot2-Pol thermo-microscope and are uncorrected. Optical rotation data were obtained on a digital polarimeter at ambient temperature using a 100 mm cell with a 1 mL capacity and are given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded at 300 MHz or 400 MHz (^{1}H) and 75 MHz or 100 MHz (^{13}C). Spectra were referenced to tetramethylsilane (0.0 ppm, ^{1}H; 0.0 ppm, ^{13}C, in $CDCl_3$). Chemical shifts (δ) are reported in parts per million (ppm), and multiplicities are indicated as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), m (multiplet), and b (broad). Coupling constants, *J*, are reported in Hertz. ^{1}H and ^{13}C NMR assignments are corroborated by 1D and 2D experiments (gCOSY, gHSQC, DEPT, and NOESY sequences). ESI-mass spectra were recorded on API 150EX apparatus and are reported in the form of (*m/z*). High resolution mass analysis (ES) was performed on LTQ ORBITRAP XL Thermo apparatus. Elemental analyses were performed by the Microanalytical Laboratory of the Dipartimento di Farmacia, Università degli Studi di Parma.

X-ray analysis. X-ray diffraction experiments on compound **7** were carried out with a Bruker-Siemens SMART AXS 1000 diffractometer equipped with CCD detector, Mo Kα radiation (λ = 0.71069). Data were subsequently corrected for absorption effects by the *SADABS*[1] procedure. The phase problem was solved by direct methods and the structures were refined by full-matrix least-squares on all F^2 using SHELXL97,[2] as implemented in the WINGX.[3] Analytical expressions of neutral atom scattering factors were taken from the International Tables for X-Ray Crystallography.[4] The structure drawings were obtained using *ORTEPIII*.[5]

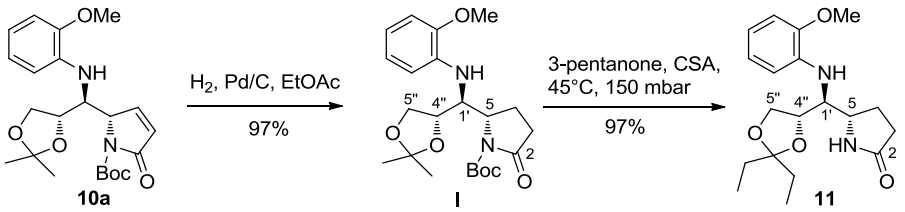
Starting Materials

Synthesis. Lactam **10a** was prepared from *N*-(*tert*-butoxycarbonyl)-2-[(*tert*-butyldimethylsilyl)oxy]pyrrole (**7**), 2,3-*O*-isopropylidene-D-glyceraldehyde (**8**), and 2-methoxyaniline (**9**) either in neat conditions or on-water according to a previously reported procedure.[6]

Biological Characterization. Reagents were purchased from Sigma Aldrich and Fluka, with the exception of 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (4-MU-NANA) and **2**, purchased from Carbosynth, UK.

Experimental Procedures and Characterization Data of Compounds in Scheme 3

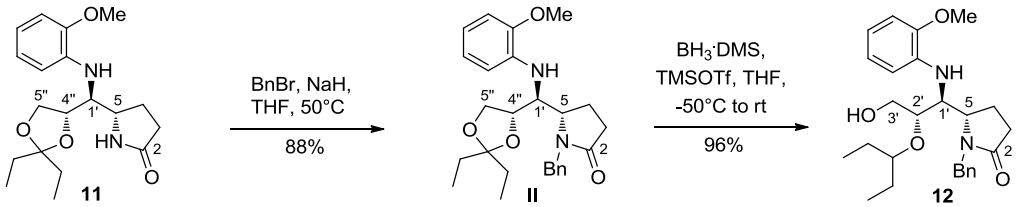
(*S*)-5-{(*S*)-[(*S*)-2,2-Diethyl-1,3-dioxolan-4-yl][(2-methoxyphenyl)amino]methyl}pyrrolidin-2-one (11).



To a solution of lactam **10a** (800 mg, 1.9 mmol) in EtOAc (20 mL), NaOAc (15 mg) and a catalytic amount of Pd/C were added. The reaction vessel was degassed under vacuum and thoroughly purged with hydrogen (three times). The mixture was stirred under hydrogen for 2.5 h, after which time the hydrogen was evacuated and the catalyst filtered off. The organic phase was extracted with water (2 × 10 mL), dried with $MgSO_4$, filtered and concentrated under vacuum to give a saturated lactam intermediate **I** (779 mg, 97%) as a colourless resin; TLC R_f 0.29 (Hex/EtOAc 70:30); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.79 (m, 1H, Ar**H**), 6.75 (m, 1H, Ar**H**), 6.65 (ddd, *J* = 7.8, 7.8, 1.6 Hz, 1H, Ar**H**), 6.60 (dd, *J* = 7.8, 1.5 Hz, 1H, Ar**H**), 4.62 (ddd, *J* = 8.7, 3.3, 3.3 Hz, 1H, **H5**), 4.19-4.08 (m, 3H, **H1'**, **H4''**, **H5''**), 3.85 (m, 1H, **H5''**), 3.84 (s, 3H, OC**H$_3$**), 2.72 (ddd, *J* = 17.7, 10.2, 10.2 Hz, 1H, **H3**), 2.39 (ddd, *J* = 17.7, 9.3, 4.0 H, 1H, **H3**), 2.22-2.08 (m, 2H, **H4**), 1.55 (s, 9H, ***t*-Bu**), 1.52 (s, 3H, C**H$_3$**), 1.36 (s, 3H, C**H$_3$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.3 (Cq, **C2**), 150.5 (Cq, ***C*=O**, Boc), 146.8, 137.1, 121.6, 117.8, 111.0, 110.3, 110.2, 83.1 (Cq, *t*-Bu), 76.7 (CH, **C4''**), 68.2 (CH_2, **C5''**), 59.0 (CH, **C5**), 57.2 (CH_3, O**C**H$_3$), 55.7 (CH, **C1'**), 32.3 (CH_2, **C3**), 28.3 (3C, CH_3, *t*-Bu, Boc), 27.0 (CH_3), 25.4 (CH_3), 18.2 (CH_2, **C4**); MS (ESI$^+$) *m*/*z* 343.3 [M−Boc + Na]$^+$; 443.3 [M + Na]$^+$. To a solution of intermediate compound **I** (750 mg, 1.78 mmol) in 3-pentanone (10 mL), camphorsulfonic acid (CSA, 435 mg, 1.87 mmol, 1.05 eq.) was added. The reaction vessel was degassed up to 150-200 mbar vacuum value and heated to 45 °C. The vacuum was monitored every hour and reapplied when necessary. After 6 hours the reaction was completed; saturated aqueous $NaHCO_3$ was added up to pH 8 and the reaction extracted with water (3 × 10 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure to give lactam **11** (601 mg, 97%) as a white solid; mp=116.8-117.5 °C; TLC R_f=0.30 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.86 (ddd, *J* = 8.2, 7.6, 1.4 Hz, 1H, Ar**H**), 6.78 (dd, *J* = 8.2, 1.5 Hz, 1H, Ar**H**), 6.70 (m, 2H, Ar**H**), 6.51 (bs, 1H, N**H**CO), 4.12-3.98 (m, 2H, **H1'**, **H4''**), 3.84 (m, 1H, **H5''**), 3.84 (s, 3H, OC**H$_3$**), 3.74 (m, 1H, **H5''**), 3.39 (ddd, *J* = 9.2, 8.8, 8.8 Hz, 1H, **H5**), 2.31-2.14 (m, 3H, **H3**, **H4**), 1.98-1.81 (m, 1H, **H4**), 1.71 (q, *J* = 7.5 Hz, 2H, C**H$_2$**), 1.63 (q, *J* = 7.5 Hz, 2H, C**H$_2$**), 0.95 (t, *J* = 7.5 Hz, 3H, C**H$_3$**), 0.89 (t, *J* = 7.5 Hz, 3H, C**H$_3$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.0 (Cq, **C2**), 146.8, 137.0, 121.5, 117.7, 114.5, 110.9, 110.2, 79.7 (CH, **C4''**), 69.2 (CH_2, **C5''**), 60.3 (CH, **C1'**), 59.1 (CH, **C5**), 55.7 (CH_3, O**C**H$_3$), 30.0 (CH_2, **C3**), 29.8 (CH_2), 29.3 (CH_2), 25.0 (CH_2, **C4**), 8.41 (CH_3), 8.39 (CH_3); MS (ESI$^+$) *m*/*z* 371.2 [M + Na]$^+$. Anal. Calcd for $C_{19}H_{28}N_2O_4$ (MW 348.44): C, 65.49; H, 8.10; N, 8.04. Found: C, 65.28; H, 8.07; N, 8.01.

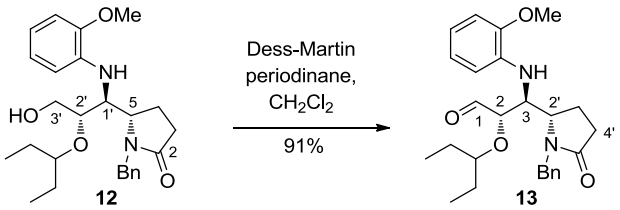
CCDC 948365 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

(*S*)-1-Benzyl-5-{(1*S*,2*S*)-3-hydroxy-1-[(2-methoxyphenyl)amino]-2-(pentan-3-yloxy)propyl}pyrrolidin-2-one (12).



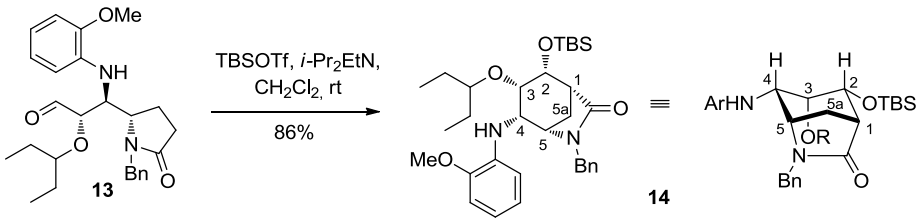
To a solution of compound **11** (580 mg, 1.66 mmol) in dry THF (10 mL) under nitrogen atmosphere, sodium hydride (60% in mineral oil, 99 mg, 2.49 mmol, 1.5 eq.) and benzyl bromide (0.30 mL, 2.49 mmol, 1.5 eq.) were sequentially added. The reaction was heated to 50 °C and kept under magnetic stirring. After 3 h the reaction was completed, aqueous NH_4Cl was added up to pH 8 and the reaction extracted with water (2 × 15 mL), dried with $MgSO_4$, filtered and concentrated under vacuum. The crude residue was purified by silica gel flash chromatography (EtOAc/petroleum ether 70:30) giving intermediate **II** (641 mg) in 88% yield as a colourless resin; $[\alpha]_D^{20}$=-8.7 (*c*=1.0, $CHCl_3$); TLC R_f=0.35 (EtOAc/Hex 70:30); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.35-7.26 (m, 3H, Ar**H**), 7.21 (m, 2H, Ar**H**), 6.90 (ddd, *J* = 7.5, 7.5, 1.5 Hz, 1H, Ar**H**), 6.80 (dd, *J* = 7.8, 1.3 Hz, 1H, Ar**H**), 6.73 (dd, *J* = 7.5, 1.4 Hz, 1H, Ar**H**), 6.69 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H, Ar**H**), 4.98 (1/2 ABq, *J* = 15.2 Hz, 1H, C**H$_2$**Ph), 4.07-3.95 (m, 3H, **H4''**, **H5**, **H5''**), 3.86 (m, 1H, **H1'**), 3.85 (s, 3H, OC**H$_3$**), 3.74 (dd, *J* = 8.9, 2.3 Hz, 1H, **H5''**), 3.58 (1/2ABq, *J* = 15.2 Hz, 1H, C**H$_2$**Ph), 2.63 (ddd, *J* = 17.2, 10.3, 6.8 Hz, 1H, **H3**), 2.45 (ddd, *J* = 17.2, 10.3, 6.0 Hz, 1H, **H3**), 2.17 (m, 1H, **H4**), 2.0 (m, 1H, **H4**), 1.60-1.49 (m, 4H, C**H$_2$**), 0.84 (t, *J* = 7.3 Hz, 3H, C**H$_3$**), 0.73 (t, *J* = 7.3 Hz, 3H, C**H$_3$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 175.4 (Cq, **C2**), 147.1, 137.1, 136.4, 128.9 (2C), 128.2 (2C), 127.7, 121.6, 117.9, 114.0, 110.7, 110.3, 76.7 (CH, **C4''**), 68.4 (CH_2, **C5''**), 57.5 (CH, **C1'**), 55.8 (CH, **C5**), 55.6 (CH_3, O**C**H_3), 44.4 (CH_2, **C**H_2Ph), 30.5 (CH_2), 30.1 (CH_2), 28.9 (CH_2), 18.6 (CH_2), 8.4 (CH_3), 8.3 (CH_3); MS (ESI^+) *m*/*z* 461.3 $[M + Na]^+$. Intermediate compound **II** (395 mg, 0.90 mmol) was dissolved in THF (25 mL) and the solution, kept under nitrogen, was cooled to -50 °C. Borohydride dimethylsulfide complex (0.60 mL, 6.3 mmol, 7.0 eq.) and TMSOTf (0.81 mL, 4.5 mmol, 5.0 eq.) were sequentially added and the temperature was allowed to raise from -50 °C to rt. After 5 h the reaction was complete, quenched by adding saturated aqueous $NaHCO_3$ up to pH 8 and extracted with water (3 × 20 mL), dried with $MgSO_4$, filtered and concentrated under vacuum. The crude residue was purified by silica gel flash chromatography (EtOAc/petroleum ether 40:60) obtaining **12** (381 mg) in 96% yield as a sticky oil; TLC R_f=0.38 (Hex/EtOAc 30/70); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34-7.21 (m, 5H, Ar**H**), 6.86 (ddd, *J* = 7.6, 7.6, 1.2 Hz, 1H, Ar**H**), 6.78 (m, 2H, Ar**H**), 6.70 (ddd, *J* = 7.8, 7.8, 1.2 Hz, 1H, Ar**H**), 4.98 (1/2 ABq, *J* = 15.0 Hz, 1H, C**H$_2$**Ph), 4.25 (bs, 1H, N**H**), 3.95 (m, 2H, **H5**, **H1'**), 3.81 (s, 3H, OC**H$_3$**), 3.67 (m, 2H, **H3'**), 3.60 (1/2 ABq, *J* = 15.0 Hz, 1H, C**H$_2$**Ph), 3.42 (ddd, *J* = 7.2, 3.9, 3.9 Hz, 1H, **H2'**), 3.17 (app. quint, *J* = 5.5 Hz, 1H, OC**H**Et_2), 2.64 (ddd, *J* = 17.2, 9.0, 9.0 Hz, 1H, **H3**), 2.40 (ddd, *J* = 17.0, 7.7, 7.7 Hz, 1H, **H3**), 2.15 (bs, 1H, O**H**), 2.01 (m, 2H, **H4**), 1.49-1.24 (m, 4H, C**H$_2$**), 0.83 (t, *J* = 7.4 Hz, 3H, C**H$_3$**), 0.75 (t, *J* = 7.4 Hz, 3H, C**H$_3$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 175.7 (Cq, **C2**), 147.3, 137.3, 136.3, 128.8 (2C), 128.5 (2C), 127.7, 121.6, 117.9, 111.7, 110.1, 80.5 (CH, **C2'**), 70.8 (CH_2, **C3'**), 61.7 (CH), 57.3 (CH), 55.7 (CH, **C5**), 54.2 (CH_3, O**C**H_3), 44.4 (CH_2, **C**H_2Ph), 30.7 (CH_2), 26.1 (CH_2), 25.3 (CH_2), 18.9 (CH_2), 9.6 (CH_3), 9.1 (CH_3); MS (ESI^+) *m*/*z* 463.28 $[M + Na]^+$. Anal. Calcd. For $C_{26}H_{36}N_2O_4$ (MW 440.58): C, 70.88; H, 8.24; N, 6.36. Found: C, 70.87; H, 8.22; N, 6.35.

(2*S*,3*S*)-3-[(*S*)-1-Benzyl-5-oxopyrrolidin-2-yl]-3-[(2-methoxyphenyl)amino]-2-(pentan-3-yloxy)propanal (13).



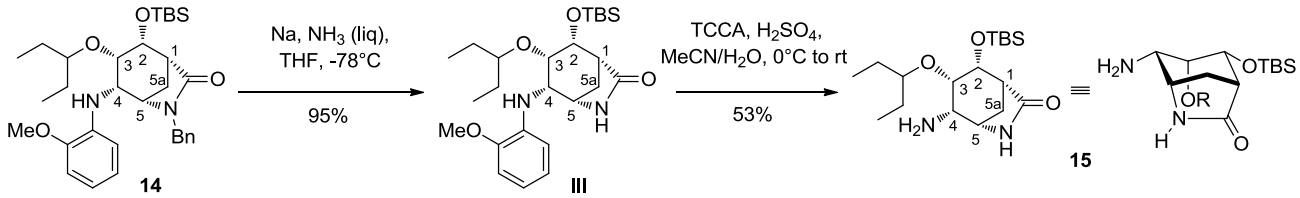
Alcohol **12** (380 mg, 0.86 mmol) was dissolved in dry CH_2Cl_2 (5 mL) under nitrogen atmosphere and solid Dess Martin Periodinane (1,1,1-tris(acetoxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one, DMP, 494 mg, 1.16 mmol, 1.35 eq.) was added. After 1 hour the reaction was completed, quenched by adding saturated aq. $NaHCO_3$ up to pH 8 and extracted with brine (3 × 5 mL), dried with $MgSO_4$, filtered and concentrated under vacuum. The crude residue was then purified by silica gel flash chromatography (EtOAc/petroleum ether 30:70) yielding aldehyde **13** (342 mg, 91%) as a colourless resin; $[\alpha]_D^{20}$=-34.5 (*c*=0.2, $CHCl_3$); TLC R_f=0.42 (EtOAc/Hex 70:30); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.65 (d, *J* = 1.8 Hz, 1H, **H1**), 7.35-7.24 (m, 5H, Ar**H**), 6.85 (ddd, *J* = 7.6, 7.6, 1.7 Hz, 1H, Ar**H**), 6.80-6.69 (m, 2H, Ar**H**), 6.51 (dd, *J* = 7.7, 0.8 Hz, 1H, Ar**H**), 4.94 (1/2 ABq, *J* = 15.2 Hz, 1H, C**H$_2$**Ph), 4.49 (bs, 1H, N**H**), 4.12 (bs, 1H, **H3**), 3.92 (1/2 ABq, *J* = 15.2 Hz, 1H, C**H$_2$**Ph), 3.84 (s, 3H, OC**H$_3$**), 3.83 (m, 1H, **H2**), 3.63 (ddd, *J* = 8.3, 2.9, 2.9 Hz, 1H, **H2'**), 3.12 (app. quint, *J* = 5.9 Hz, 1H, OC**H**Et$_2$), 2.62 (ddd, *J* = 17.2, 9.2, 9.2 Hz, 1H, **H4'**), 2.38 (ddd, *J* = 17.2, 10.3, 4.2 Hz, 1H, **H4'**), 2.32-2.21 (m, 1H, **H3'**), 2.19-2.03 (m, 1H, **H3'**), 1.49 (m, 2H, C**H$_2$**), 1.39 (m, 2H, C**H$_2$**), 0.90 (t, *J* = 7.6 Hz, 3H, C**H$_3$**), 0.79 (t, *J* = 7.7 Hz, 3H, C**H$_3$**); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 204.2 (CH, **C1**), 175.5 (Cq, **C5'**), 147.5, 136.6, 136.0, 128.9 (2C), 128.5 (2C), 127.8, 121.5, 118.3, 111.0, 110.2, 83.6, 83.0, 58.2 (CH, **C3**), 55.6 (CH, **C2'**), 54.5 (CH_3, O**C**H_3), 44.6 (CH_2, **C**H_2Ph), 30.7 (CH_2), 26.2 (CH_2), 24.9 (CH_2, **C3'**), 20.4 (CH_2, **C4'**), 9.7 (CH_3), 9.1 (CH_3); MS (ESI$^+$) *m*/*z* 461.3 $[M + Na]^+$; Anal. Calcd for $C_{26}H_{34}N_2O_4$ (MW 438.56): C, 71.21; H, 7.81; N, 6.39. Found: C, 71.10; H, 7.77; N, 6.37.

(1*R*,2*R*,3*S*,4*S*,5*S*)-6-Benzyl-2-[(*tert*-butyldimethylsilyl)oxy]-4-[(2-methoxyphenyl)amino]-3-(pentan-3-yloxy)-6-azabicyclo[3.2.1]octan-7-one (14).



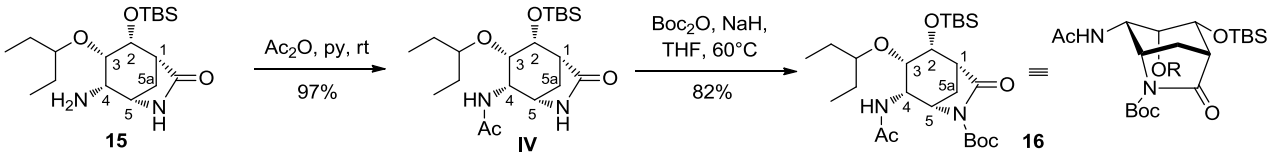
Aldehyde **13** (340 mg, 0.78 mmol) was dissolved in dry CH_2Cl_2 (16 mL) under nitrogen atmosphere and dropped in 1 hour into a preformed solution of DIPEA (*i*-Pr_2EtN, 0.68 mL, 3.88 mmol, 5.0 eq.) and TBSOTf (0.54 mL, 2.34 mmol, 3.0 eq.) in dry CH_2Cl_2 (30 mL). After the addition of the aldehyde **13** was completed, the mixture was subjected to stirring for two additional hours. After that time, it was quenched by adding brine and extracted with water (3 × 50 mL), dried with $MgSO_4$, filtered and concentrated under vacuum. The crude residue was then purified by silica gel flash chromatography (petroleum ether/EtOAc 95:5) giving bicyclic compound **14** (371 mg, 86% yield) as a colourless oil; $[\alpha]_D^{20}$=+48.5 (*c*=1.0, $CHCl_3$); TLC R_f=0.58 (Hex/EtOAc 75/25); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.13 (m, 3H, Ar**H**), 6.96 (m, 2H, Ar**H**), 6.84 (m, 2H, Ar**H**), 6.69 (ddd, *J* = 7.7, 7.7, 1.4 Hz, 1H, Ar**H**), 6.47 (dd, *J* = 7.7, 1.2 Hz, 1H, Ar**H**), 5.30 (bs, 1H, N**H**), 5.02 (1/2 ABq, *J* = 14.9 Hz, 1H, C$\mathbf{H_2}$Ph), 4.23 (bd, *J* = 4.0 Hz, 1H, **H2**), 3.92 (s, 3H, OC$\mathbf{H_3}$), 3.89 (1/2 ABq, *J* = 14.9 Hz, 1H, C$\mathbf{H_2}$Ph), 3.82 (m, 1H, **H4**), 3.75 (m, 2H, **H3**, **H5**), 3.33 (app. quint, *J* = 5.6 Hz, 1H, OC**H**Et_2), 2.62 (dd, *J* = 4.4, 4.4 Hz, 1H, **H1**), 2.40 (d, *J* = 11.1 Hz, 1H, **H5a**), 1.95 (ddd, *J* = 10.8, 5.3, 4.4 Hz, 1H, **H5a**), 1.70-1.60 (m, 4H, C$\mathbf{H_2}$), 1.00 (t, *J* = 7.2 Hz, 3H, C$\mathbf{H_3}$), 0.98 (t, *J* = 7.2 Hz, 3H, C$\mathbf{H_3}$), 0.93 (s, 9H, *t*-Bu, TBS), 0.15 (s, 3H, **C**H_3, TBS), 0.14 (s, 3H, **C**H_3, TBS); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8 (Cq, **C7**), 147.2, 137.5, 135.9, 128.5 (2C), 128.4 (2C), 127.2, 121.6, 116.7, 110.3, 110.1, 82.2 (CH), 79.1 (CH, **C3**), 68.0 (CH, **C2**), 55.7 (CH_3, O**C**H_3), 54.4 (CH, **C5**), 51.9 (CH, **C4**), 46.8 (CH, **C1**), 46.4 (CH_2, **C**H_2Ph), 29.9 (CH_2, **C5a**), 25.9 (3C, CH_3, *t*-Bu, TBS), 25.8 (2C, CH_2), 18.1 (Cq, *t*-Bu, TBS), 9.4 (CH_3), 9.1 (CH_3), -4.7 (CH_3, TBS), -4.8 (CH_3, TBS); MS (ESI^+) *m*/*z* 553.4 $[M + H]^+$; Anal. Calcd. for $C_{32}H_{48}N_2O_4Si$ (MW 552.82): C, 69.52; H, 8.75; N, 5.07. Found: C, 69.59; H, 8.78; N, 5.03.

(1*R*,2*R*,3*S*,4*S*,5*S*)-4-Amino-2-[(*tert*-butyldimethylsilyl)oxy]-3-(pentan-3-yloxy)-6-azabicyclo[3.2.1]-octan-7-one (15).



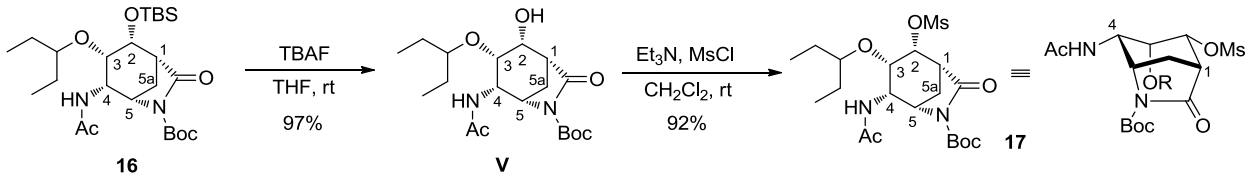
Anhydrous ammonia (10 mL) was condensed into a two-necked flask containing a solution of **14** (265 mg, 0.48 mmol) in anhydrous THF (15 mL) kept at -78 °C. Sodium was added to the mixture until the blue colour persisted. The reaction was stirred for 30 min at -78 °C monitoring by TLC, and then quenched by careful addition of solid NH_4Cl. Ammonia was evaporated and the residue was treated with saturated NH_4Cl aqueous solution and extracted with EtOAc (3 × 10 mL). The organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuum to give a *N*-deprotected bicyclic intermediate **III** (211 mg, 95%) as a colourless oil; TLC R_f=0.55 (EtOAc/Hex 80:20); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.89 (ddd, *J* = 7.7, 7.7, 1.4 Hz, 1H, Ar**H**), 6.81 (dd, *J* = 7.7, 1.4 Hz, 1H, Ar**H**), 6.71 (ddd, *J* = 7.6, 7.6, 1.4 Hz, 1H, Ar**H**), 6.60 (dd, *J* = 7.7, 1.4 Hz, 1H, Ar**H**), 5.48 (s, 1H, N**H**CO), 4.14 (m, 1H, **H2**), 3.86 (s, 3H, OC**H$_3$**), 3.80 (bd, *J* = 5.7 Hz, 1H, **H3**), 3.76 (d, *J* = 5.3 Hz, 1H, **H4**), 3.68 (bd, *J* = 5.3 Hz, 1H, **H5**), 3.24 (app. quint, *J* = 5.8 Hz, 1H, OC**H**Et$_2$), 2.47 (dd, *J* = 4.6, 4.6 Hz, 1H, **H1**), 2.36 (dd, *J* = 11.3, 1.0 Hz, 1H, **H5a**), 2.18 (ddd, *J* = 10.6, 5.3, 4.8 Hz, 1H, **H5a**), 1.64-1.50 (m, 4H, C**H$_2$**), 0.99-0.89 (m, 6H, C**H$_3$**), 0.93 (s, 9H, *t*-Bu, TBS), 0.15 (s, 3H, C**H$_3$**, TBS), 0.14 (s, 3H, C**H$_3$**, TBS); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.5 (Cq, **C7**), 147.0, 135.9, 121.2, 116.6, 109.9, 109.8, 82.7 (CH), 79.1 (CH, **C3**), 68.0 (CH, **C2**), 55.2 (CH_3, O**C**H$_3$), 53.5 (CH, **C5**), 51.0 (CH, **C4**), 45.5 (CH, **C1**), 30.3 (CH_2, **C5a**), 25.8 (CH_2), 25.7 (3CH_3, *t*-Bu, TBS), 24.4 (CH_2), 17.9 (Cq, *t*-Bu, TBS), 9.6 (CH_3), 9.0 (CH_3), -4.7 (CH_3, TBS), -4.8 (CH_3, TBS); MS (ESI$^+$) *m/z* 463.3 [M + H]$^+$. To a solution of intermediate **III** (200 mg, 0.43 mmol) in 2:1 MeCN/H_2O (23 mL) at 0 °C, aqueous H_2SO_4 (1.0 M solution, 0.43 mmol, 1.0 eq.) and trichloroisocyanuric acid (TCCA, 100 mg, 0.43 mmol, 1.0 eq.) were sequentially added. The reaction mixture was gradually warmed to rt and quenched after 12 h with saturated aq. $NaHCO_3$. When pH 10 was reached, the mixture was extracted with EtOAc (3 × 15 mL), dried with $MgSO_4$, filtered and concentrated under vacuum. The crude residue was purified by silica gel flash chromatography (from EtOAc/MeOH(NH_3) 80:20 to EtOAc/MeOH(NH_3) 60:40), yielding the primary amine **15** (81 mg, 53% yield) as a colourless resin; $[\alpha]_D^{20}$=+35.6 (*c*=0.25, $CHCl_3$); TLC R_f =0.55 (EtOAc/MeOH(NH_3) 80:20); ^{1}H NMR (400 MHz, CD_3OD) δ: 4.07 (bd, *J* = 3.6 Hz, 1H, **H2**), 3.55 (d, *J* = 5.5 Hz, 1H, **H3**), 3.49 (d, *J* = 5.5 Hz, 1H, **H5**), 3.25 (app. quint, *J* = 5.6 Hz, 1H, OC**H**Et$_2$), 3.04 (d, *J* = 5.2 Hz, 1H, **H4**), 2.35 (dd, *J* = 4.4, 4.4 Hz, 1H, **H1**), 2.28 (d, *J* = 11.2 Hz, 1H, **H5a**), 2.13 (ddd, *J* = 10.8, 5.2, 5.2 Hz, 1H, **H5a**), 1.67-1.44 (m, 4H, C**H$_2$**), 0.93 (s, 9H, *t*-Bu, TBS), 0.91 (t, *J* = 7.2 Hz, 3H, C**H$_3$**), 0.88 (t, *J* = 7.4 Hz, 3H, C**H$_3$**), 0.15 (s, 3H, C**H$_3$**, TBS), 0.13 (s, 3H, C**H$_3$**, TBS); ^{13}C NMR (100 MHz, CD_3OD) δ: 178.8 (Cq, **C7**), 82.4 (CH), 79.8 (CH, **C3**), 68.5 (CH, **C2**), 58.6 (CH, **C5**), 49.9 (CH, **C4**), 45.9 (CH, **C1**), 31.3 (CH_2, **C5a**), 25.4 (CH_2), 24.8 (3C, CH_3, *t*-Bu, TBS), 24.1 (CH_2), 17.4 (Cq, *t*-Bu, TBS), 8.4 (CH_3), 8.2 (CH_3), -6.1 (2C, CH_3, TBS); MS (ESI$^+$) *m/z* 357.6 [M + H]$^+$. Anal. Calcd for $C_{18}H_{36}N_2O_3Si$ (MW 356.58): C, 60.63; H, 10.18; N, 7.86. Found: C, 60.54; H, 10.21; N, 7.83.

(1*R*,2*R*,3*S*,4*S*,5*S*)-4-Acetamido-6-*tert*-butoxycarbonyl-2-[(*tert*-butyldimethylsilyl)oxy]-3-(pentan-3-yloxy)-6-azabicyclo[3.2.1]octan-7-one (16).



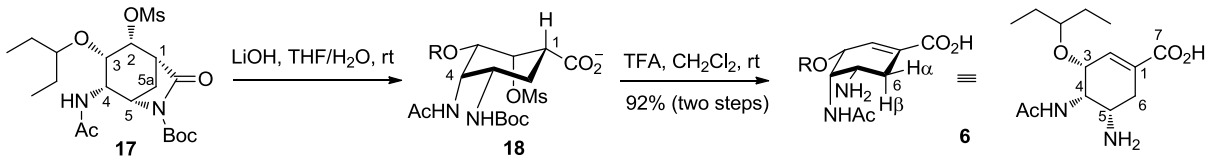
Primary amine **15** (40 mg, 0.11 mmol) was dissolved in pyridine (1.3 mL) at room temperature. Acetic anhydride was added dropwise (32 µL, 0.22 mmol, 2.0 eq.) and, after 4 h, the reaction was quenched by the addition of MeOH (0.8 mL). The mixture was diluted with toluene and concentrated under vacuum until pyridine was removed. Intermediate compound **IV** was obtained (42 mg, 97% yield) as a colourless resin; $[\alpha]_D^{20}$=+38.3 (*c*=0.2, $CHCl_3$); TLC R_f =0.60 (EtOAc/MeOH(NH_3) 80:20); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.44 (d, *J* = 8.0 Hz, 1H, N**H**Ac), 5.80 (s, 1H, N**H**C=O), 4.23 (ddd, *J* = 7.6, 5.6, 1.2 Hz, 1H, **H4**), 4.06 (bd, *J* = 4.4 Hz, 1H, **H2**), 3.68 (d, *J* = 5.6 Hz, 1H, **H5**), 3.52 (d, *J* = 5.2 Hz, 1H, **H3**), 3.17 (app. quint, *J* = 4.8 Hz, 1H, OC**H**Et_2), 2.41 (dd, *J* = 4.5, 4.5 Hz, 1H, **H1**), 2.35 (d, *J* = 11.5 Hz, 1H, **H5a**), 2.13 (ddd, *J* = 10.4, 5.2, 5.2 Hz, 1H, **H5a**), 2.01 (s, 3H, C**H**$_3$CO), 1.61-1.40 (m, 4H, C**H**$_2$), 0.94-0.86 (m, 6H, C**H**$_3$), 0.88 (s, 9H, *t*-Bu, TBS), 0.09 (s, 3H, C**H**$_3$, TBS), 0.08 (s, 3H, C**H**$_3$, TBS); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.3 (Cq, **C7**), 169.7 (Cq, **C**=O), 82.7 (CH), 78.5 (CH, **C3**), 67.7(CH, **C2**), 55.9 (CH, **C5**), 48.2 (CH, **C**4), 45.5 (CH, **C1**), 30.8 (CH_2, **C5a**), 26.2 (CH_2), 25.8 (3CH_3, *t*-Bu, TBS), 24.8 (CH_2), 23.6 (CH_3, Ac), 18.0 (Cq, *t*-Bu, TBS), 9.9 (CH_3), 9.5 (CH_3), -6.0 (2C, CH_3, TBS); MS (ESI$^+$) *m*/*z* 421.5 [M + Na]$^+$. Crude compound **IV** (40 mg, 0.10 mmol) was dissolved in dry THF (5.0 mL) under nitrogen atmosphere. Sodium hydride (60% mineral oil, 14 mg, 0.35 mmol, 3.5 eq.) and di-*tert*-butyl dicarbonate (Boc_2O, 65 mg, 0.30 mmol, 3.0 eq.) were sequentially added. The reaction mixture was warmed to 60 °C; after 2 h the mixture was cooled to rt and quenched by the addition of saturated aq. NH_4Cl until pH 7 was reached. The aqueous layer was extracted with EtOAc (3 × 5 mL) and the combined organic phases were dried, filtered and concentrated under vacuum. The crude residue was purified by silica gel flash chromatography (diethyl ether/EtOAc 80:20) giving compound **16** (40 mg, 82% yield) as a colourless resin; $[\alpha]_D^{20}$=-8.6 (*c*=0.5, $CHCl_3$); TLC R_f =0.8 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.79 (d, *J* = 8.1 Hz, 1H, N**H**Ac), 4.33 (dd, *J* = 8.4 ,4.8 Hz, 1H, **H4**), 4.29 (bd, *J* = 5.6 Hz, 1H, **H5**), 4.08 (bd, *J* = 5.6 Hz, 1H, **H2**), 3.60 (bd, *J* = 4.4 Hz, 1H, **H3**), 3.09 (app. quint, *J* = 5.2 Hz, 1H, OC**H**Et_2), 2.55 (dd, *J* = 5.4, 5.4 Hz, 1H, **H1**), 2.28 (d, *J* = 12.0 Hz, 1H, **H5a**), 2.04 (ddd, *J* = 11.2, 5.6, 5.6 Hz, 1H, **H5a**), 1.96 (s, 3H, C**H**$_3$CO), 1.50 (s, 9H, *t*-Bu, Boc), 1.47-1.35 (m, 4H, C**H**$_2$), 0.87 (s, 9H, *t*-Bu, TBS), 0.84 (t, *J* = 7.8 Hz, 3H, C**H**$_3$), 0.8 (t, *J* = 7.8 Hz, 3H, C**H**$_3$), 0.08 (s, 3H, C**H**$_3$, TBS), 0.05 (s, 3H, C**H**$_3$, TBS); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.6 (Cq, **C7**), 168.2 (Cq, **C**=O), 150.7 (Cq, Boc), 81.7 (Cq, *t*-Bu, Boc), 81.5 (CH), 77.5 (CH, **C3**), 67.6 (CH, **C2**), 57.7 (CH, **C5**), 48.5 (CH, **C4**), 47.1 (CH, **C1**), 28.1 (CH_2, **C5a**), 26.8 (3C, CH_3, *t*-Bu, Boc), 24.6 (CH_3, Ac), 24.5 (3CH_3, *t*-Bu, TBS), 23.3 (CH_2), 22.3 (CH_2), 16.8 (Cq, *t*-Bu, TBS), 8.4 (CH_3), 7.7 (CH_3), -5.9 (CH_3 TBS), -5.9 (CH_3 TBS); MS (ESI$^+$) *m*/*z* 421.5 [M – Boc + Na]$^+$; 521.5 [M + Na]$^+$. Anal. Calcd for $C_{25}H_{46}O_6Si$: C, 60.21; H, 9.30; N 5.62. Found: C, 60.09; H, 9.34; N 5.61.

(1*R*,2*R*,3*S*,4*S*,5*S*)-4-Acetamido-6-*tert*-butoxycarbonyl-7-oxo-3-(pentan-3-yloxy)-6-azabicyclo[3.2.1]-octan-2-yl methanesulfonate (17).



Bicyclic compound **16** (40 mg, 0.08 mmol) was dissolved in dry THF (4 mL). Tetra-*n*-butylammonium fluoride (TBAF, 42 mg, 0.16 mmol, 2.0 eq.) was added at room temperature. After 2 h the reaction mixture was quenched with saturated aq. NH_4Cl and extracted with EtOAc (3 × 3 mL). The combined organic phases were dried, filtered and concentrated under vacuum yielding intermediate product **V** (29 mg, 97% yield) as a colourless oil; $[\alpha]_D^{20}$=-31.8 (*c*=1.0, $CHCl_3$); TLC R_f =0.3 (70:30 EtOAc/Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.13 (d, *J* = 8.0 Hz, 1H, N**H**Ac), 4.42 (dd, *J* = 8.0, 4.8 Hz, 1H, **H4**), 4.30 (d, *J* = 5.8 Hz, 1H, **H3**), 4.16 (bd, *J* = 4.0 Hz, 1H, **H2**), 3.79 (bd, *J* = 4.3 Hz, 1H, **H3**), 3.16 (app. quint, *J* = 5.5 Hz, 1H, OC**H**Et_2), 2.74 (dd, *J* = 4.5, 4.5 Hz, 1H, **H1**), 2.36 (d, *J* = 11.8 Hz, 1H, **H5a**), 2.10 (ddd, *J* = 11.8, 5.4, 5.4 Hz, 1H, **H5a**), 1.99 (s, 3H, Ac), 1.53 (s, 9H, *t*-Bu, Boc), 1.5-1.3 (m, 4H, C**H**$_2$), 0.85 (t, *J* = 7.6 Hz, 3H, C**H**$_3$), 0.81 (t, *J* = 7.6 Hz, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.5 (Cq, **C7**), 169.5 (Cq, **C**=O), 152.1 (Cq, Boc), 82.7 (Cq, *t*-Bu, Boc), 82.0 (CH), 77.6 (CH, **C3**), 68.1 (CH, **C2**), 58.4 (CH, **C5**), 49.2 (CH, **C4**), 47.2 (CH, **C1**), 28.8 (CH_2, **C5a**), 27.8 (3C, CH_3, *t*-Bu, Boc), 25.2 (CH_3, Ac), 24.0 (CH_2), 23.0 (CH_2), 8.4 (CH_3), 8.0 (CH_3). Crude intermediate **V** (29 mg, 0.076 mmol) was dissolved in dry CH_2Cl_2 (4 mL) and triethylamine (Et_3N, 16 µL, 0.11 mmol, 1.5 eq.) and methanesulfonyl chloride (MsCl, 7 µL, 0.09 mmol, 1.2 eq.) were sequentially added at room temperature under nitrogen atmosphere. After 2 h, the reaction mixture was quenched by the addition of saturated aq. $NaHCO_3$ and extracted in CH_2Cl_2 (3 × 3 mL). The combined organic phases were dried, filtered and concentrated under vacuum; the crude so obtained was subjected to filtration on a silica pad. Protected bicyclic compound **17** was obtained (32 mg, 92% yield) as a colourless oil; $[\alpha]_D^{20}$=-32.1 (*c*=1.0, $CHCl_3$); TLC R_f =0.3 (50:50 EtOAc/Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.93 (bd, *J* = 7.8 Hz, 1H, N**H**Ac), 4.95 (d, *J* = 4.3 Hz, 1H, **H2**), 4.36 (m, 2H, **H4**, **H5**), 3.99 (m, 1H, **H3**), 3.28 (app. quint, *J* = 5.2 Hz, 1H, OC**H**Et_2), 3.11 (s, 3H, C**H**$_3$, Ms), 2.95 (dd, *J* = 3.8, 3.8 Hz, 1H, **H1**), 2.24 (m, 2H, **H5a**), 2.00 (s, 3H, Ac), 1.53 (s, 9H, *t*-Bu, Boc), 1.51–1.34 (m, 4H, C**H**$_2$), 0.87 (t, *J* = 7.4 Hz, 3H, C**H**$_3$), 0.82 (t, *J* = 7.4 Hz, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.5 (Cq, **C7**), 169.0 (Cq, **C**=O), 151.8 (Cq, Boc), 83.6 (Cq, *t*-Bu, Boc), 83.0 (CH), 75.5 (CH, **C3**), 74.6 (CH, **C2**), 58.0 (CH, **C5**), 49.6 (CH, **C4)**, 45.9 (CH, **C1**), 38.9 (CH_3, Ms), 29.6 (CH_2, **C5a**), 28.0 (3C, CH_3, *t*-Bu, Boc), 25.4 (CH_3, Ac), 24.3 (CH_2), 23.3 (CH_2), 9.4 (CH_3), 8.6 (CH_3); MS (ESI$^+$) *m/z* 485.5 [M + Na]$^+$. Anal. Calcd for $C_{20}H_{34}O_2O_8S$: C, 51.93; H, 7.41; N 6.06. Found: C, 51.88; H, 7.43; N 6.04.

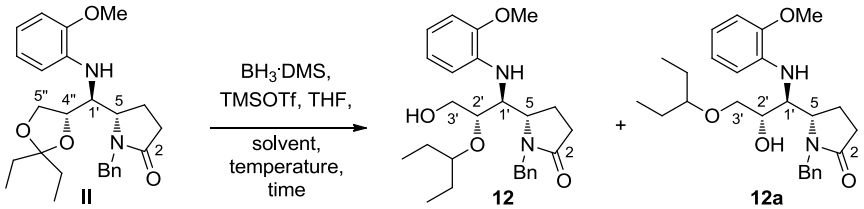
(3*R*,4*S*,5*S*)-4-Acetamido-5-amino-3-(pentan-3-yloxy)cyclohex-1-enecarboxylic acid alias 4-*epi*-Oseltamivir Carboxylate (6).



Bicyclic compound **17** (25 mg, 0.054 mmol) was dissolved in a 3:1 THF/H_2O mixture (4 mL) and solid LiOH (2.8 mg, 0.12 mmol, 2.2 eq) was added at room temperature under vigorous stirring. After the starting material was consumed (checked by silica TLC), the reaction mixture was quenched by the addition of solid NH_4Cl (3.0 eq.). The mixture was concentrated under vacuum; the crude was dissolved in EtOAc and filtered on a silica pad to remove polar impurities. Crude carboxylate **18** was obtained, which was directly dissolved in dry CH_2Cl_2 (3 mL). Trifluoroacetic acid (TFA, 1.0 mL) was added to the reaction mixture under nitrogen atmosphere at room temperature. After 3 h, the solvent was evaporated under vacuum furnishing 4-*epi*-oseltamivir carboxylate **6** (19 mg, 92% yield) as a trifluoroacetate salt. The final product was dissolved in 3 mL of H_2O (0.1% TFA), filtered with a 0.2 μm, 100 mm Anatop 10 LC filter (Whatman) and purified by preparative RP-HPLC (RP C18-10 μm, 250×21.2 mm) using MeCN (0.05% TFA) in H_2O (0.05% TFA), 0-50% linear gradient over 30 min at room temperature. A flow rate of 8.0 mL/min was used and detection was at 220 nm. R_t = 26.5 min. Purity of the final target was checked by analytical HPLC (Discovery C18-10 μm column, 250 × 4.6 mm) in acetonitrile/water using a gradient program and was found to be >98% pure. A resin; $[\alpha]_D^{20}$=-26.0 (*c*=0.05, acetone); ^{1}H NMR (400 MHz, D_2O) δ: 6.74 (m, 1H, **H2**), 4.70 (m, 1H, **H4**), 4.59 (m, 1H, **H3**), 3.67 (ddd, *J* = 10.8, 5.8, 2.2 Hz, 1H, **H5**), 3.42 (app. quint, *J* = 5.3 Hz, 1H, OC**H**Et_2), 2.71 (bdd, *J* = 17.5, 5.8 Hz, 1H, **H6α**), 2.29 (dddd, *J* = 17.2, 10.8, 3.2, 3.2 Hz, 1H, **H6β**), 1.98 (s, 3H, Ac), 1.6-1.3 (m, 4H, C**H$_2$**), 0.79 (t, *J* = 7.6 Hz, 3H, C**H$_3$**), 0.75 (t, *J* = 7.5 Hz, 3H, C**H$_3$**); ^{13}C NMR (100 MHz, D_2O) δ: 177.6 (Cq, **C7**), 170.4 (Cq, **C**=O), 139.9 (CH, **C2**), 130.1 (Cq, **C1**), 83.2 (CH, O**C**HEt_2), 72.7 (CH, **C3**), 50.3 (CH, **C5**), 48.2 (CH, **C4**), 27.3 (CH_2, OCH**Et$_2$**), 26.2 (CH_2, OCH**Et$_2$**), 26.0 (CH_2, **C6**), 23.6 (CH_3, Ac), 10.7 (CH_3, OCH**Et$_2$**), 9.5 (CH_3, OCH**Et$_2$**). HRMS (ESI) $C_{14}H_{24}N_2O_4$ calcd for $[M + H]^+$, 285.1809; found 285.1817.

Reductive Ketal Opening to 12 - Optimization Studies

Table S1. Optimization of the reductive ketal opening to alcohol **12**.



Entry	Solvent	Temp. (°C)	Time (h)	Product **12** (yield%)[a]	Product **12a** (yield %)[a]
1	CH_2Cl_2 dry	-60 to -45	0.5	-	92
2	CH_2Cl_2 dry	-60 to -30	1.5	-	95
3	CH_2Cl_2 dry	-60 to -20	2	-	98
4	CH_2Cl_2	-60 to -20	2	-	98
5	CH_2Cl_2 dry/THF dry (2.5/1)	-60 to -20	10	84	traces
6	THF dry	-60 to -20	15	89	traces
7	THF dry	-50 to rt	15	94	-
8	THF	-50 to rt	5	96	-

[a] Yield of isolated product.

Inhibitory Activity

Virus Isolates: Viral isolates of A/Wilson Smith/33 and A/Udorn/72 (H3N2) were generated by plasmid-based reverse genetics essentially as previously described.[7] Briefly, 293T cells were transfected with eight genome-sense (pHH21) plasmids and four protein expression plasmids (pcDNA3.1) encoding PB1, PB2, PA, and NP using FuGENE 6 transfection reagent. At 16h post-transfection the cells were co-cultured with MDCK cells in serum-free DMEM containing 2.5 μg/mL *N*-acetyl trypsin (Sigma). Viruses were propagated through two passages in MDCK cells followed by plaque assay titration on MDCK cells.

Kinetic Measurements: IC_{50} values were calculated in an *in vitro* sialidase activity assay. Inhibitors were dissolved in DMSO as 10 $mmolL^{-1}$ stock solutions and further diluted in tris-NaCl buffer [200 $mmolL^{-1}$ NaCl, 50 $mmolL^{-1}$ Tris-Cl, pH 7.8]. Subsequently, 95 μL reactions were arranged in opaque 96-well plates in Tris-NaCl buffer, including 5 μL viral isolate and 10 μL of the inhibitors at varying concentrations from 10^{-3} to 10^{-10} $molL^{-1}$. Reactions were initiated by the addition of 5 μL 1 $mmolL^{-1}$ 4-MU-NANA substrate and incubated at 37 °C. Fluorescence of the cleaved substrate was monitored every 15 seconds for 10 min using a Molecular Devices Spectramax M2e plate reader. IC_{50} values were calculated from reaction rates using Softmax Pro and Grafit software.

Molecular Modelling

Protein Preparation: The crystal structure of the neuraminidase N2-oseltamivir complex was retrieved from the Protein Data Bank (PDB code: 4GZP).[8] Ions and water molecules laying beyond 3 Å of the protein as well as all mannose and glucosamine units were deleted. The Protein Preparation Wizard implemented in Maestro 9.2[9] was used to add missing hydrogens and to cap the protein N- and C-termini with methylamino and acetyl groups, respectively. The orientation of the side chain amide groups of asparagine and glutamine residues, the hydroxyl and thiol groups of serines and cysteines, and the tautomeric state of histidine residues were sampled to optimize the overall hydrogen bonding networks. The resulting protein structure was submitted to a restrained energy minimization applying the OPLS2005 force field[10] to a root mean square deviation (RMSD) value of 0.3 Å, calculated on heavy atoms. The remaining water molecules and ions were removed from the prepared protein structure before docking studies.

Docking Simulations: The $^{5}H_{4}$ halfchair conformation of compound **6,** in zwitterionic form, was built in Maestro and submitted to an energy minimization procedure applying the OPLS2005 force field and the GBSA water salvation treatment, with a convergence threshold of 0.05 kJ mol^{-1} $Å^{-1}$. Docking grids were generated with Glide 5.7[11] and were centred on the co-crystallized oseltamivir molecule. The dimensions of bounding and enclosing boxes were set to 10 Å and 22 Å, respectively. Van der Waals radii scaling of 0.8 was applied to ligand non-polar atoms. Docking was performed in standard precision (SP) mode. 50 poses were collected and ranked according to their GScore. The best-ranked pose of compound **6** was selected. Ions and water molecules deleted prior to docking studies were reintroduced in the protein structure and the resulting neuraminidase-compound **6** complex was energy minimized applying the GBSA water salvation treatment and the OPLS2005 force field to a convergence threshold of 2 kJ mol^{-1} $Å^{-1}$ with all atoms free to move. The final energy-minimized complex is depicted in Figure 4 of the main text.

Metadynamics Simulations: Metadynamics simulations were performed with Desmond 3.0.[12] The dihedral angles C3-C4-C5-C6 (ϕ) and H5-C5-C4-H4 (τ) were selected as collective variables to describe the transition between the two halfchair conformations of compound **6** and the H4-H5 proton-proton coupling constant, respectively. The energy-minimized $^{5}H_{4}$ halfchair of compound **6** was placed in a cubic TIP3P water box, with at least 20 Å between the ligand molecule and its closest periodic image. Systems were first relaxed using the default relaxation protocol implemented in Desmond 3.0 applying the Langevin barostat and thermostat.[13] The equilibration stage was followed by 5 ns-long metadynamics simulations, which were conducted in the NPT ensemble at 298 K and 1 atm applying the Langevin coupling scheme.[13] The height of the Gaussian potentials and the time interval between two successive Gaussians were set to 0.01 kcal/mol and 0.09 ps for the dihedral ϕ and to 0.002 kcal/mol and 0.3 ps for the dihedral τ. The width of the Gaussian potentials was set to 2 degrees in both cases. As previously suggested for a two-basins case,[14] metadynamics simulations were stopped immediately after the so-called "recrossing event", i.e. when the system re-crosses the same saddle point between the two basins in the reverse direction. Accordingly, free energy profiles were reconstructed immediately after the recrossing event applying the Desmond Metadynamics Analysis tool available from the Schrödinger Script Center (www.schrodinger.com/scriptcenter).

Prediction of the $J_{4,5}$ Coupling Constant: For a given value of dihedral τ, the corresponding value of $J_{4,5}$ ($J_{4,5}\tau$) was calculated applying the Haasnoot equation (1):[15a,15b]

$$J_{4,5}\tau = 14.64\cos^2\tau - 0.78\cos\tau + 0.58 + \Sigma_i\{\lambda_i[0.34 - 2.31\cos^2(s_i\tau + 18.4|\lambda_i|)]\} \quad (1)$$

which takes into account substituents electronegativity. In eq. (1) λ_i is the electronegativity constant of the substituent *i* attached to the C4 or C5 carbons. According to equation (1), the substituents attached to the C4 and C5 carbons of compound **6** should be labelled as S1-S4 depending on their relative position with respect to their geminal protons. In particular, projecting the H5-C5-C4-H4 dihedral along the C5-C4 vector, the S1 group is that found at ~120° counting clockwise from H5 and it assumes a value of s_i of +1. On the contrary, the S2 group is the substituent located at ~120° counting anticlockwise from H5 and it has s_i equal to -1. Using the same procedure described for H5, the S3 and S4 groups can be identified with respect to the H4

proton. For each S_i group of compound **6**, the most similar substituent was identified among those listed in Reff. 15b and 15c and its electronegativity constant λ was assigned to the group. The final electronegativity constants assigned to the C6 carbon (S1), the amino group (S2), the amide group (S3) and the C3H-isopentyloxy fragment (S4) were 0.74 (CH_2CH_3), 1.19 (NH_2), 0.85 (NHCOR) and 0.62 (CHMeOH), respectively. Thus, $J_{4,5}\tau_D = 3.60$ and $J_{4,5}\tau_E = 2.42$.

The relative probability of a specific dihedral value τ ($P\tau$) was calculated starting from its corresponding free energy value applying the Boltzmann equation (2):

$$P\tau / P\tau_0 = \exp -(G\tau - G\tau_0)/KT \qquad (2)$$

where τ_0 is the value of dihedral τ corresponding to the lowest free energy value, G is the free energy, K is the Boltzmann constant and T is the temperature. The expected value of $J_{4,5}$ was calculated from the two minima identified in the free energy profile of the dihedral τ. Basin E (Figure 3) corresponded to τ_0 and, for basin D, $G\tau_D$ was 1.3 kcal/mol. The final $J_{4,5}$ coupling constant was obtained combining the two τ values corresponding to the two basins (τ_D and τ_E) and calculated with eq. (1), with their corresponding probabilities applying equation (3):

$$J_{4,5} = J_{4,5}\tau_D(P\tau_D/(P\tau_D + P\tau_E)) + J_{4,5}\tau_E(P\tau_E/(P\tau_D + P\tau_E)) \qquad (3)$$

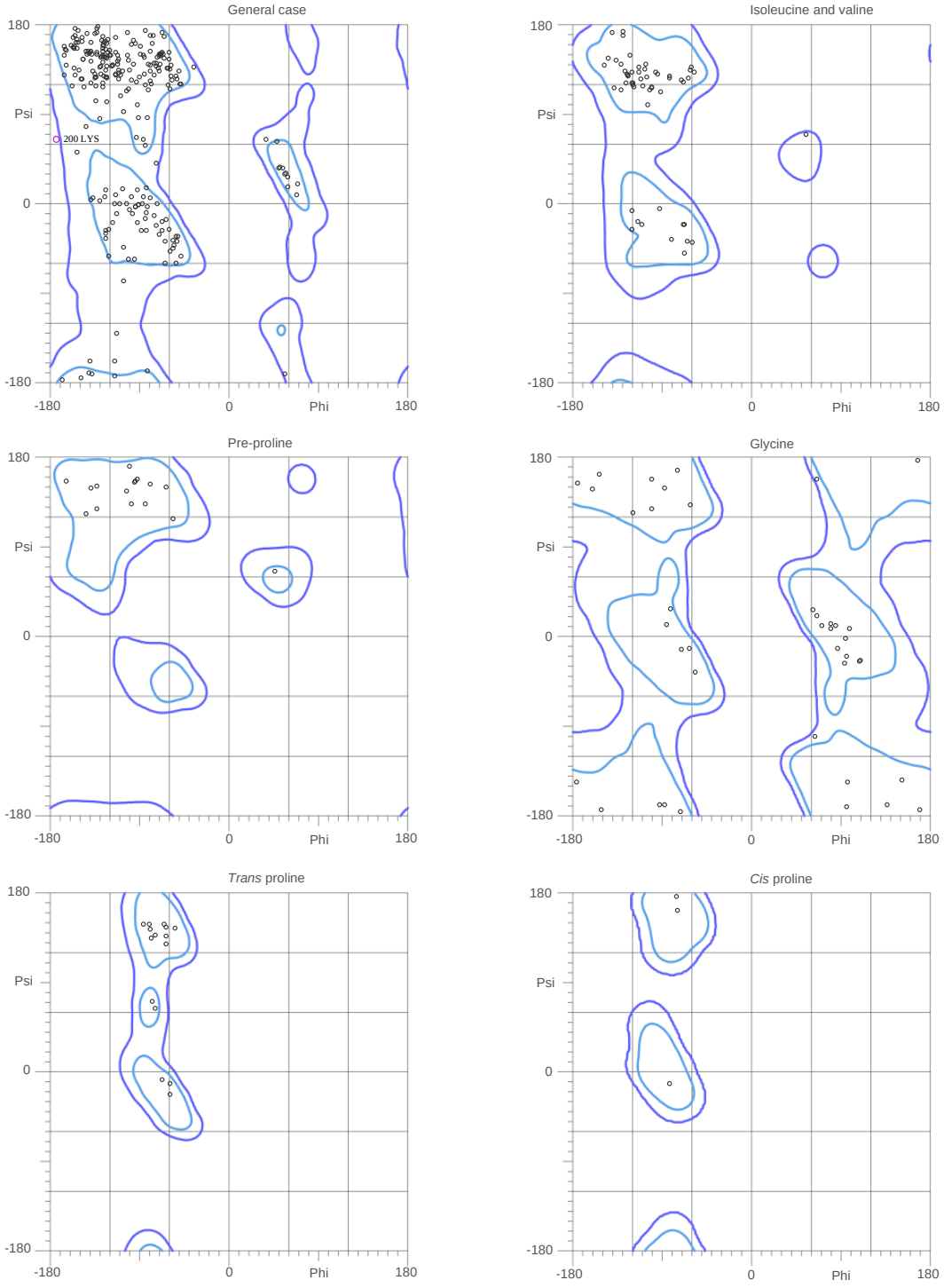
Macromolecular X-ray Crystallography

Macromolecular X-ray Crystallography: N8 sialidase from A/Duck/Ukraine/1/63 (H3N8) was prepared from virus grown in hen's eggs. Sialidase was released from purified virions by bromelain digestion, and purified as described previously.[16] Crystals of N8 protein were grown by vapour diffusion in hanging drops consisting of 1 μL concentrated protein solution (8 mg ml^{-1} in 50mM Tris-HCl pH 8.0) and 1 μL reservoir solution (200 $mmolL^{-1}$ $MgCl_2$, 100 $mmolL^{-1}$ 2-(*N*-morpholino)ethanesulfonic acid (pH 6.5), 10% (*w/v*) polyethylene glycol 4000). Crystals of N8 sialidase were soaked reservoir solution containing 10 mM compound **6** for 60 min, followed by 30 seconds in the same supplemented with 25% glycerol. Data were collected on an in-house rotating anode (RA Micro7 HFM) and a Saturn944 CCD at 100K and processed with HKL2000.[17] Refinement was carried out using PHENIX,[18] combined with manual model building with Coot.[19] Statistical support for the structures obtained is presented in Supplementary Table S2. Structural data have been deposited with the NCBI Protein Data Bank with accession code 4M3M.

Table S2: Macromolecular X-ray crystallography data collection and refinement statistics.

Protein	N8:**6**
PDB accession code	4M3M
Space group	I4
Unit-cell parameters (Å)	a=90.8, b=90.8, c=112.0
Maximum resolution (Å)*	2.10 (2.14-2.10)
Unique reflections	23659
Completeness (%) *	89.2 (51.64)
Mean I/σI *	20.4 (3.8)
Redundancy	6.4
R_{sym} (%)*	0.118 (0.417)
Refinement	
Protein atoms	3022
Water atoms	428
Ligand atoms	20
Other atoms	15
Resolution Range (Å)	30-2.10
R_{work} (%)	17.4
R_{free} (%)	21.6
R.m.s.d. bond lengths (Å)	0.009
R.m.s.d. bond angles (^{o})	1.26
Average B-factor	19.40

Ramachandran Analysis of N8:6 structure



96.4% (374/388) of all residues were in favored (98%) regions.
99.7% (387/388) of all residues were in allowed (>99.8%) regions.

There were 1 outliers (phi, psi):
200 LYS (-174.4, 65.5)

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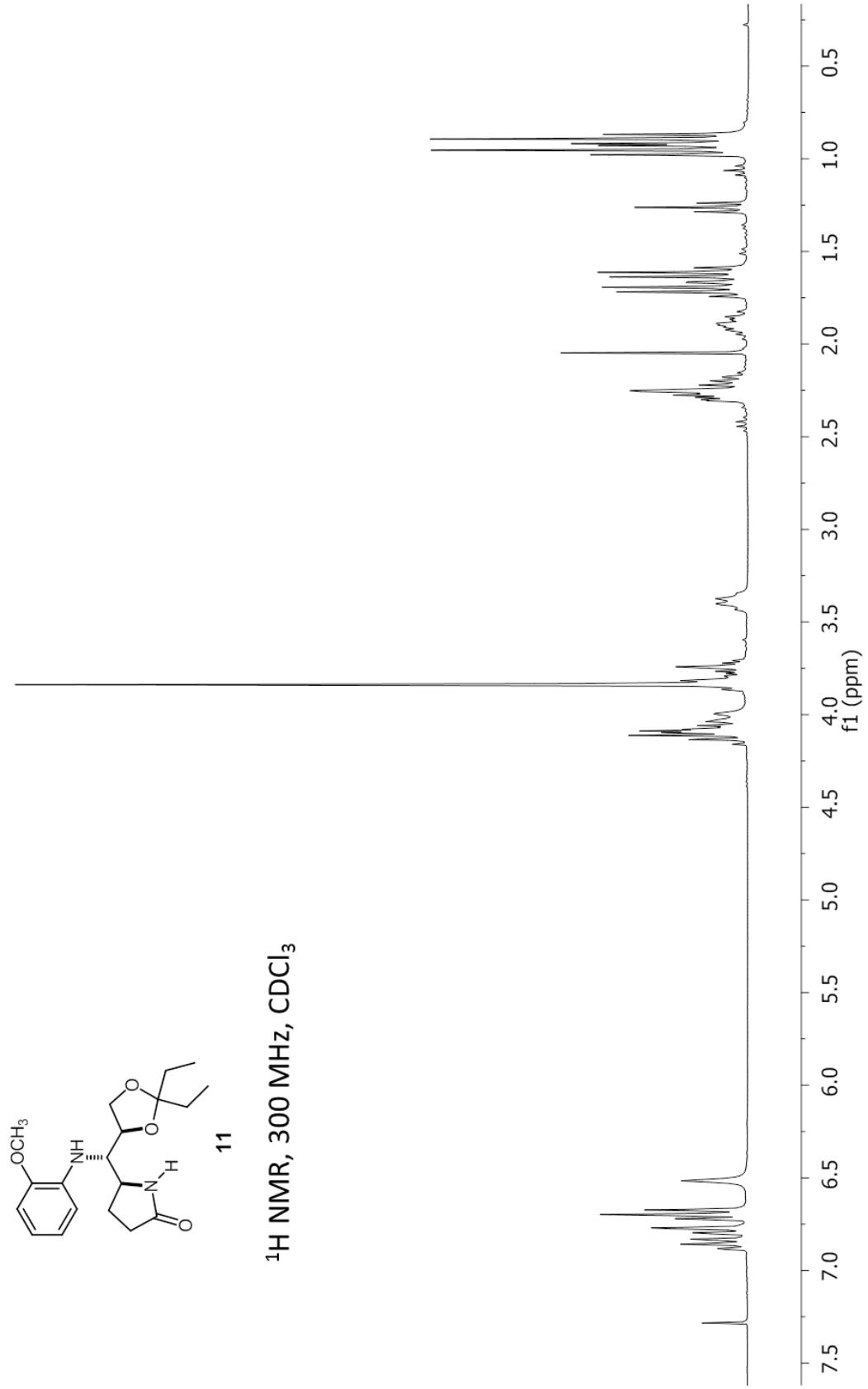
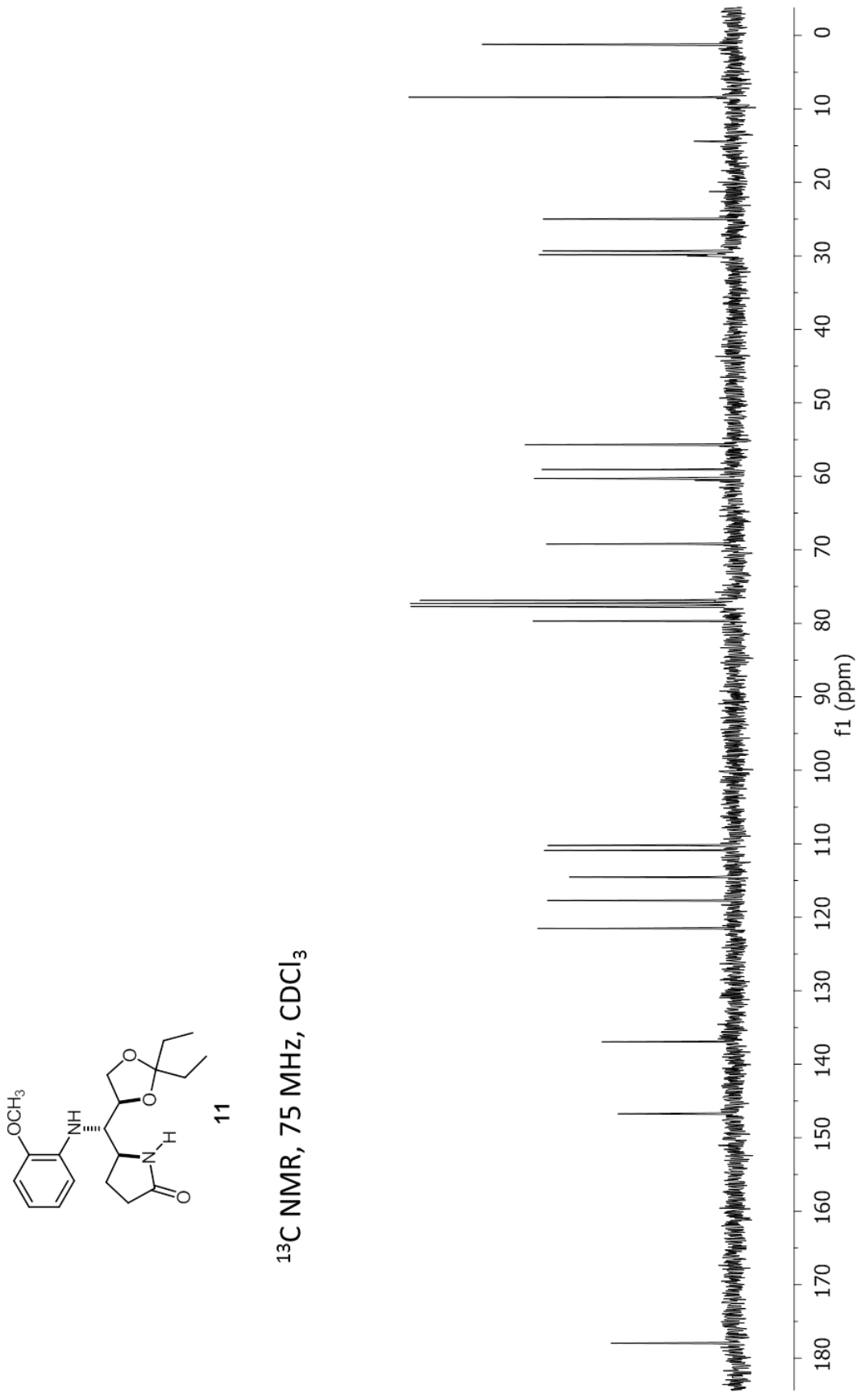
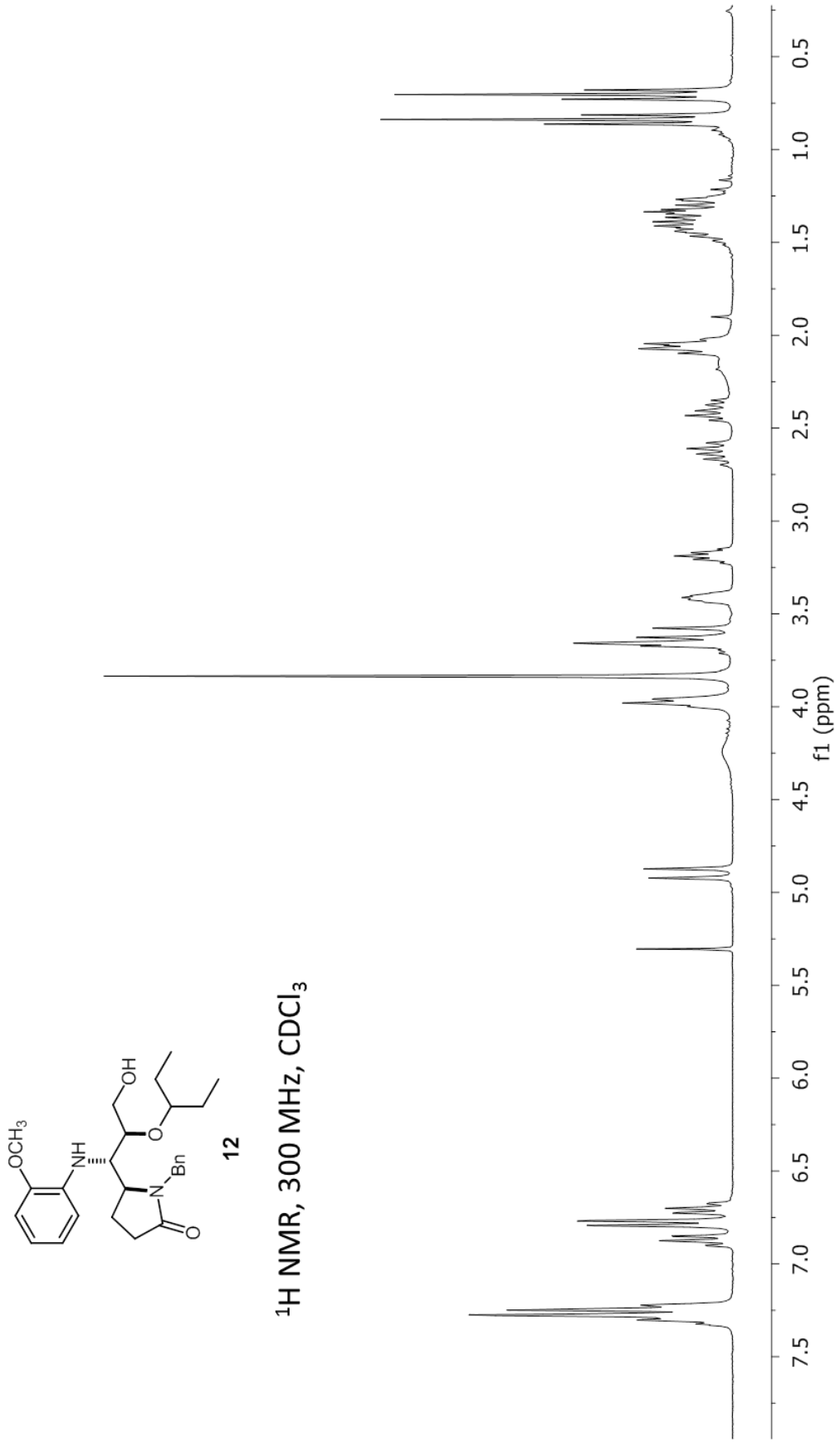
^{1}H NMR and ^{13}C NMR of Compounds in Scheme 3



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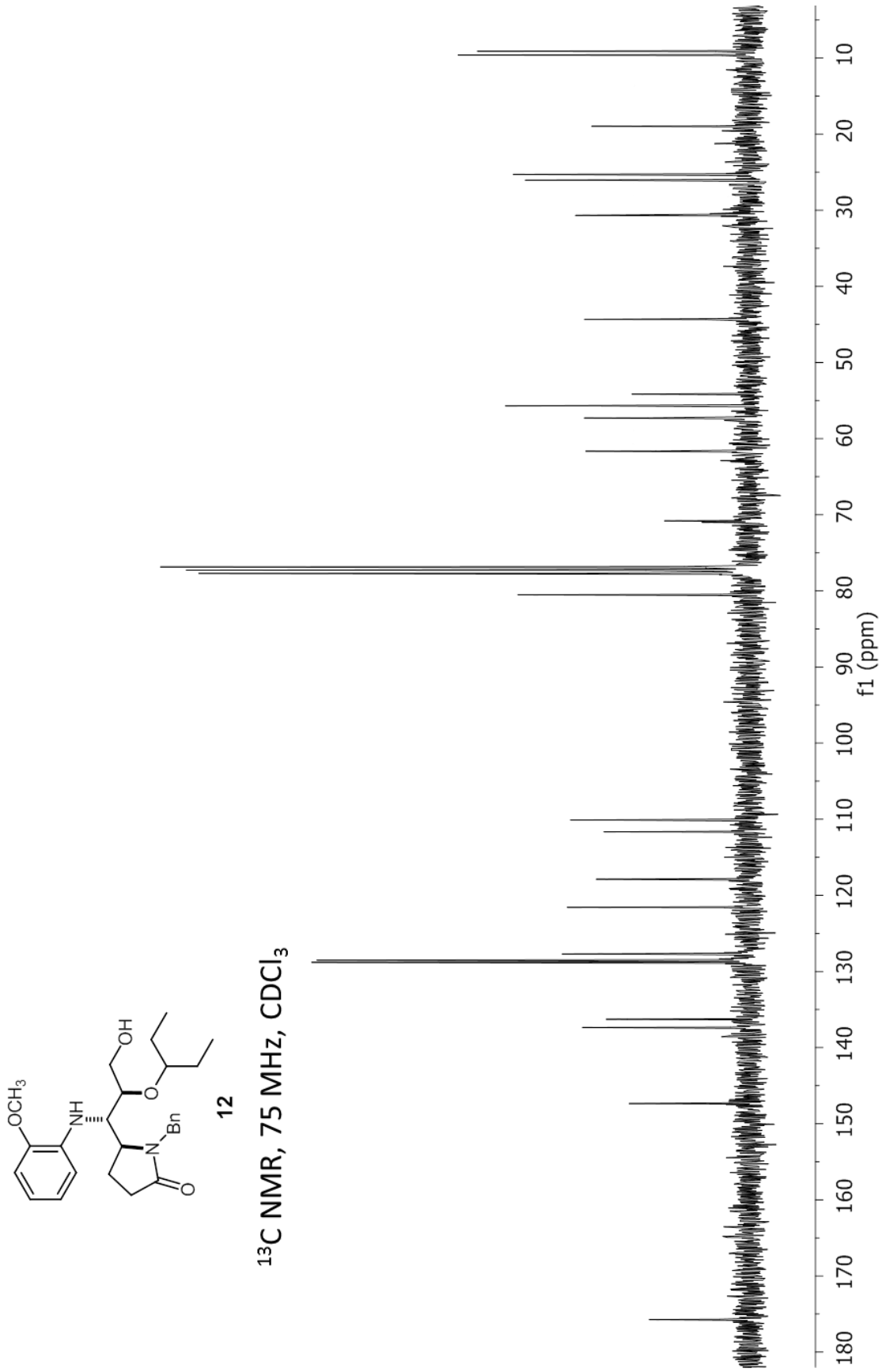
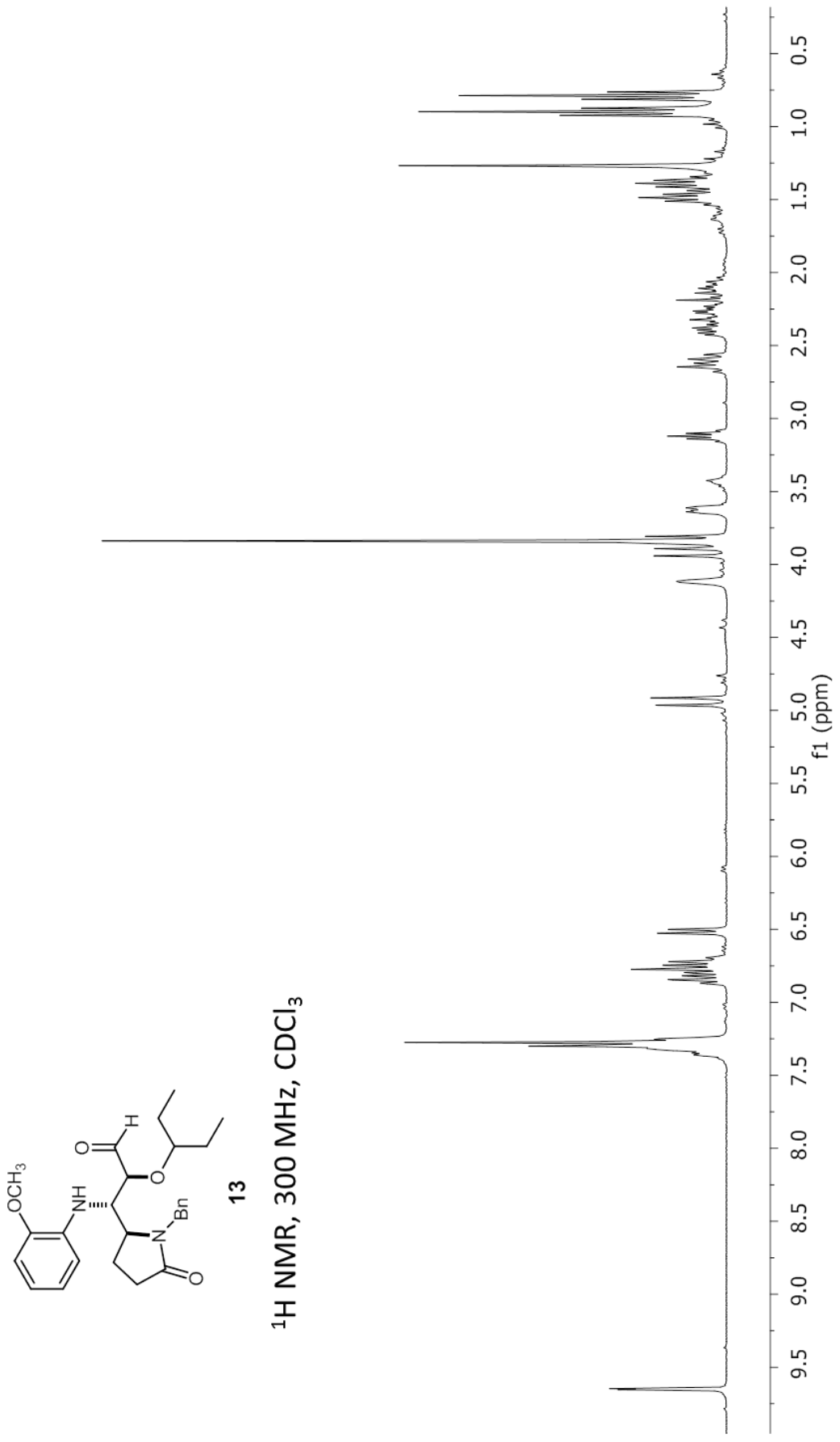
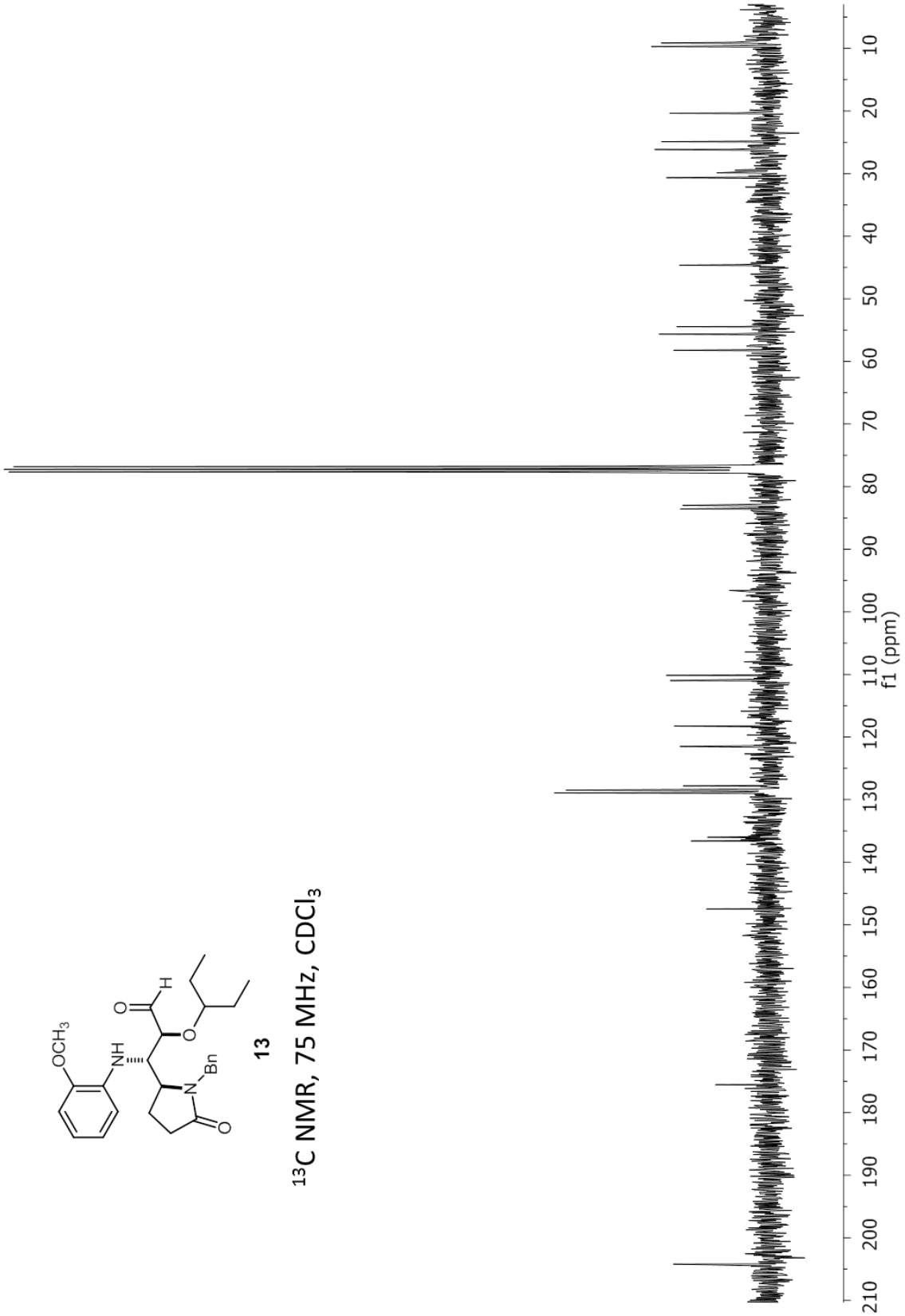
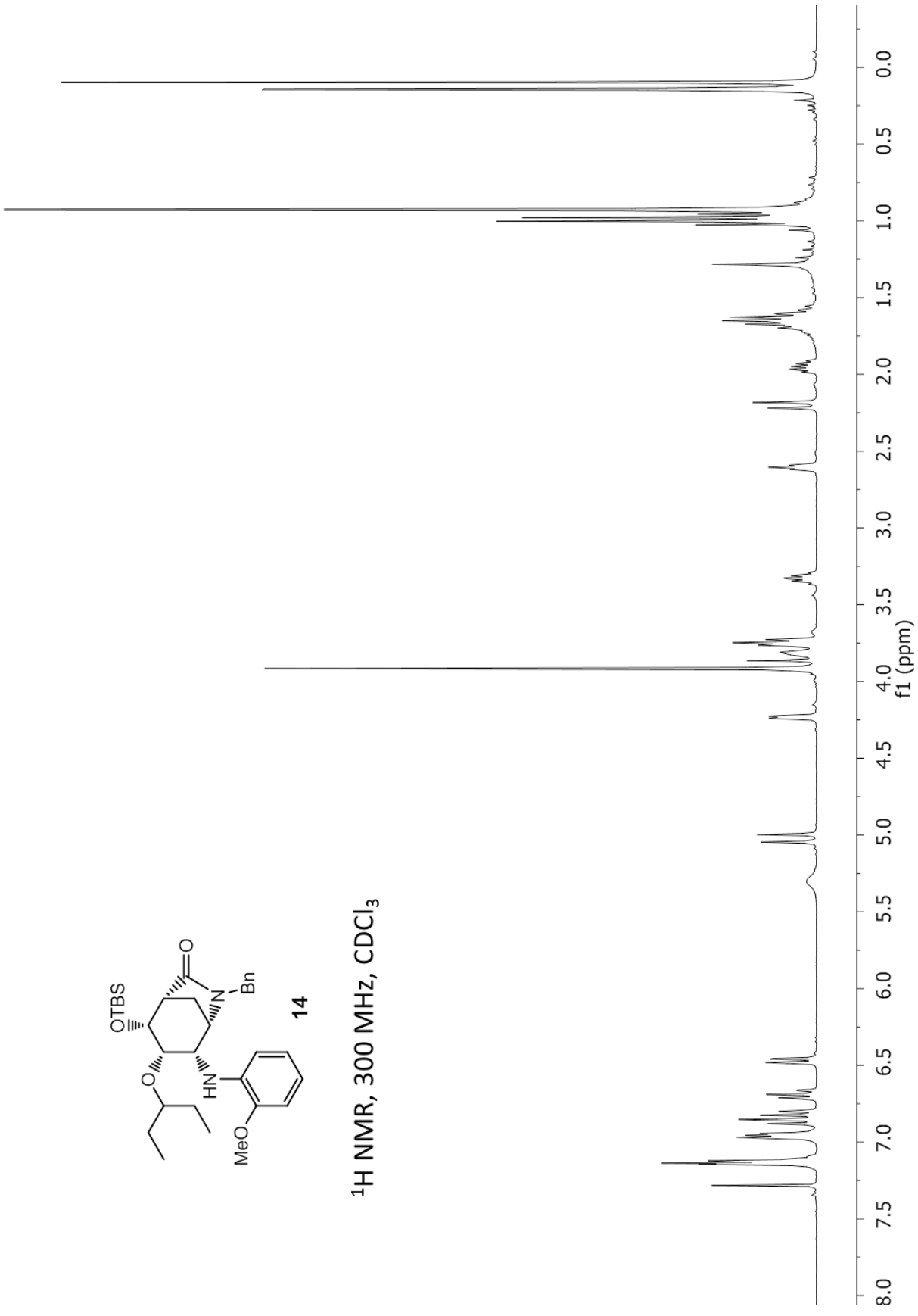
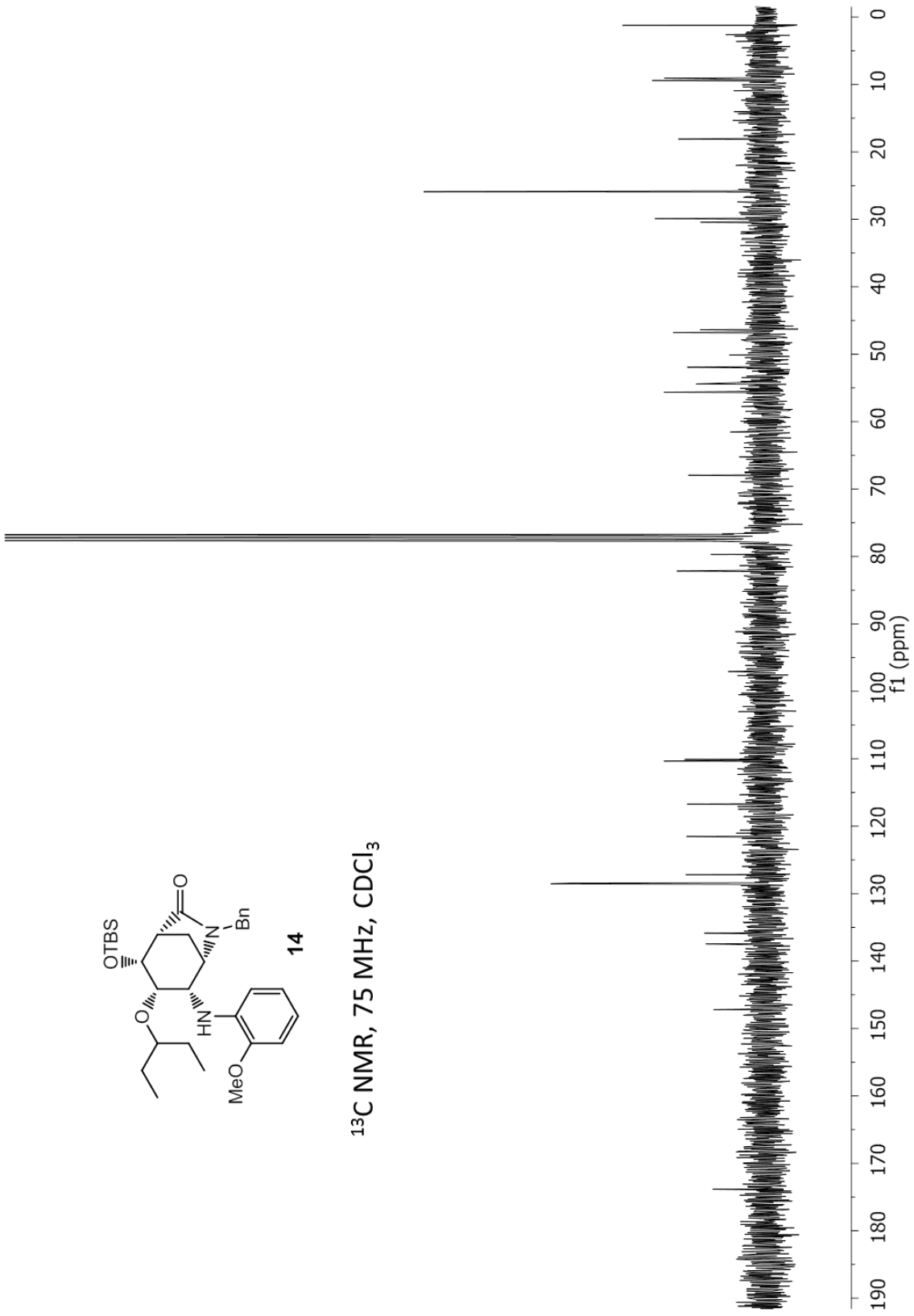
NH
OH
O
N
Bn
O
12
1H NMR, 300 MHz, CDCl3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

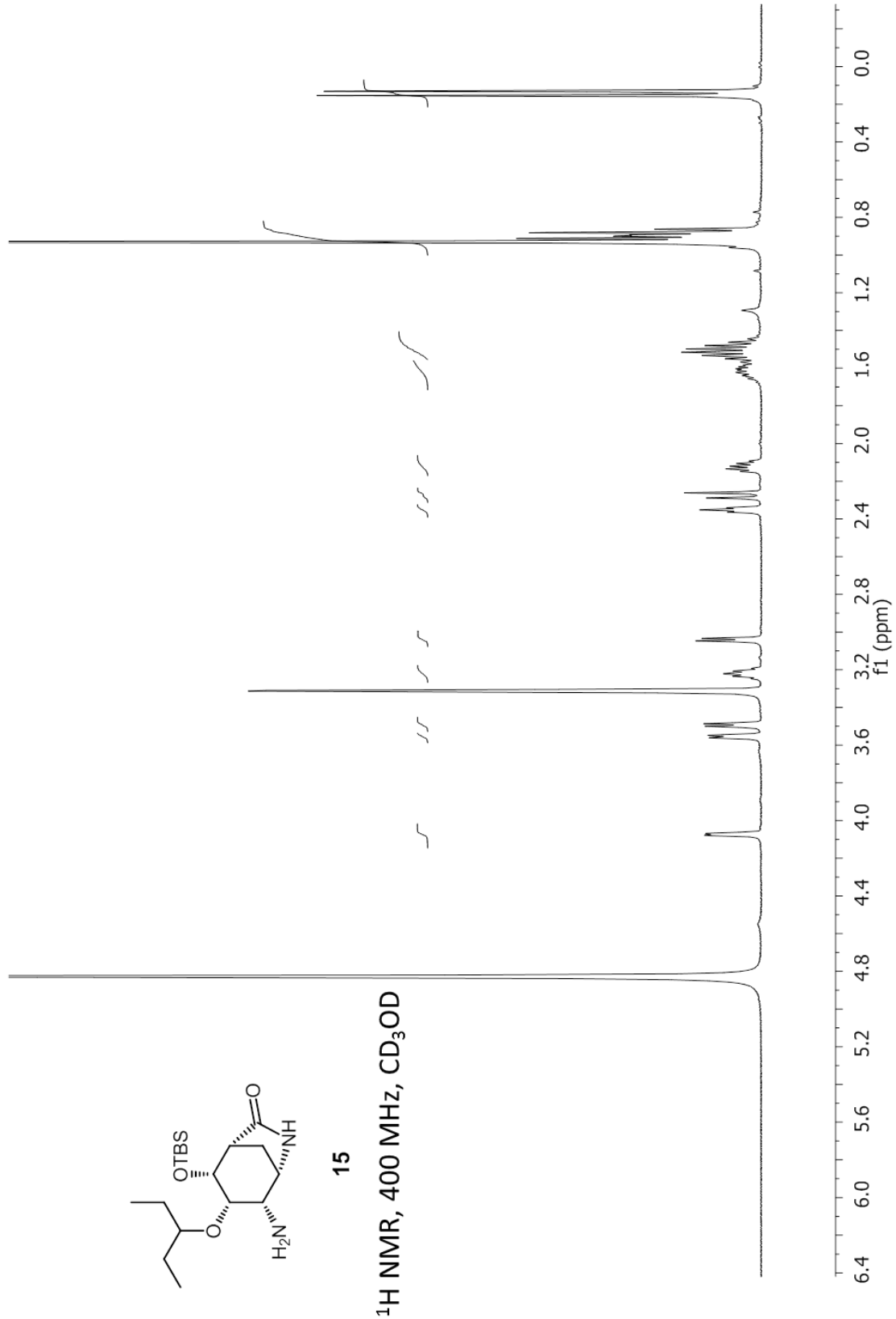


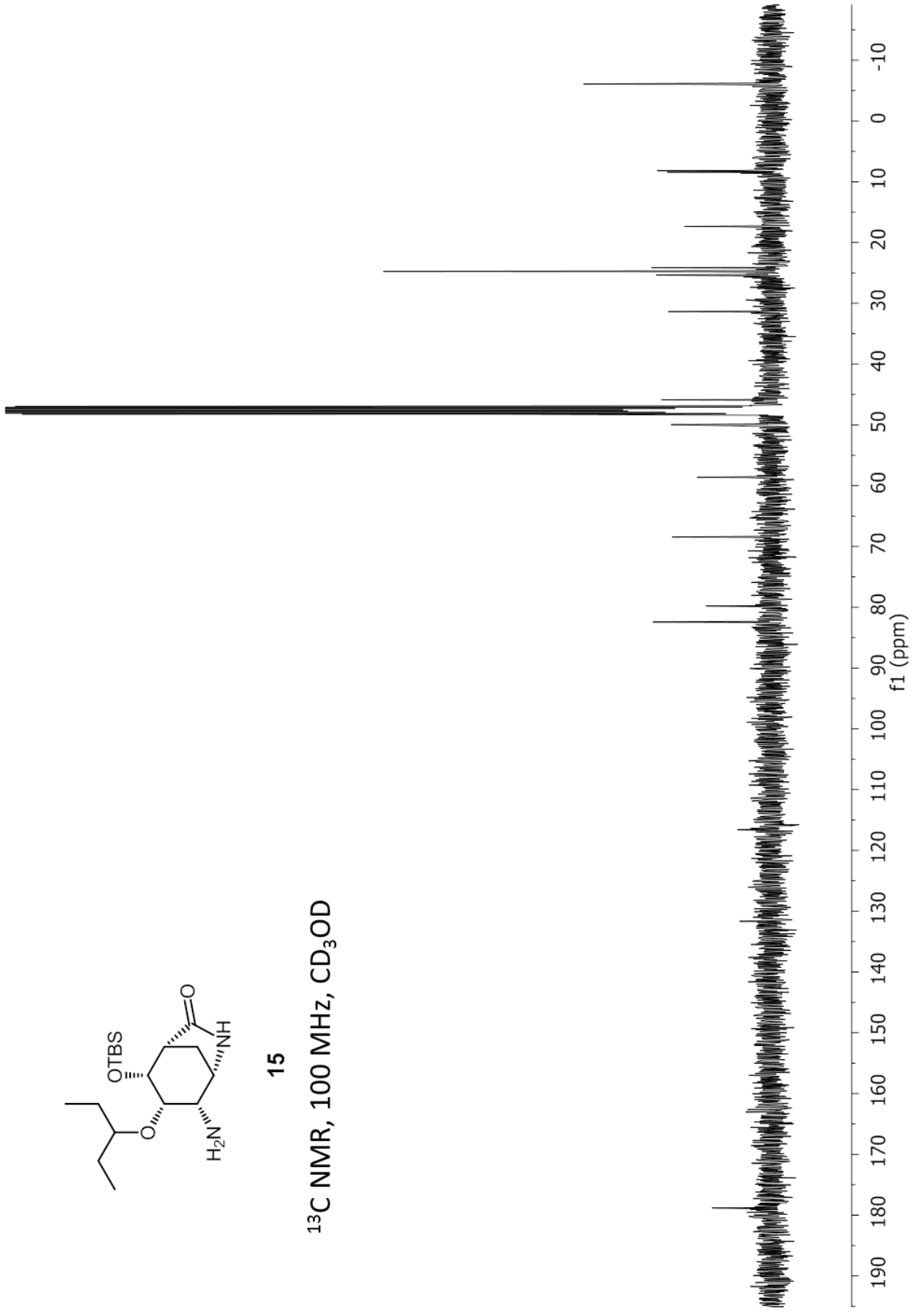


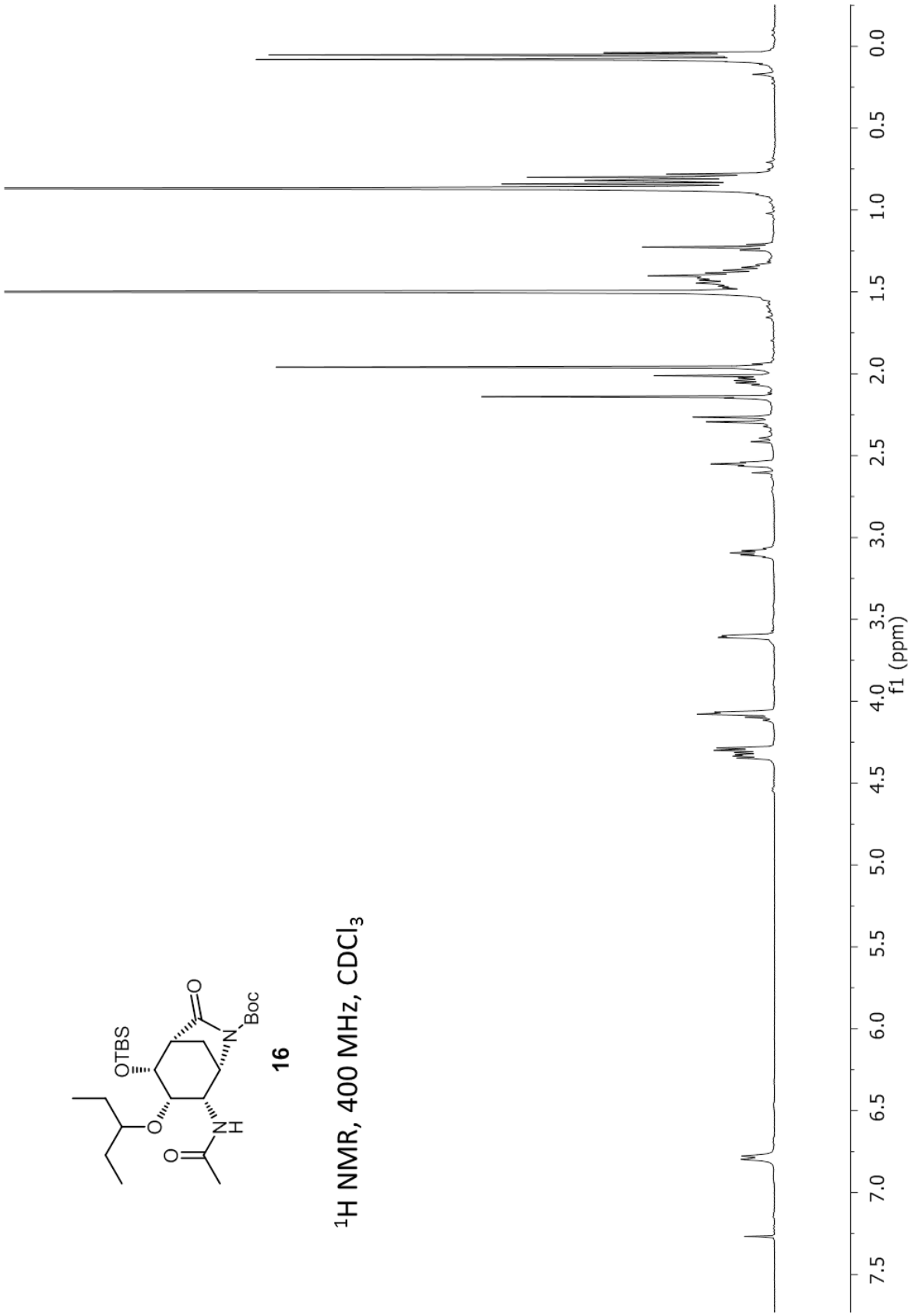


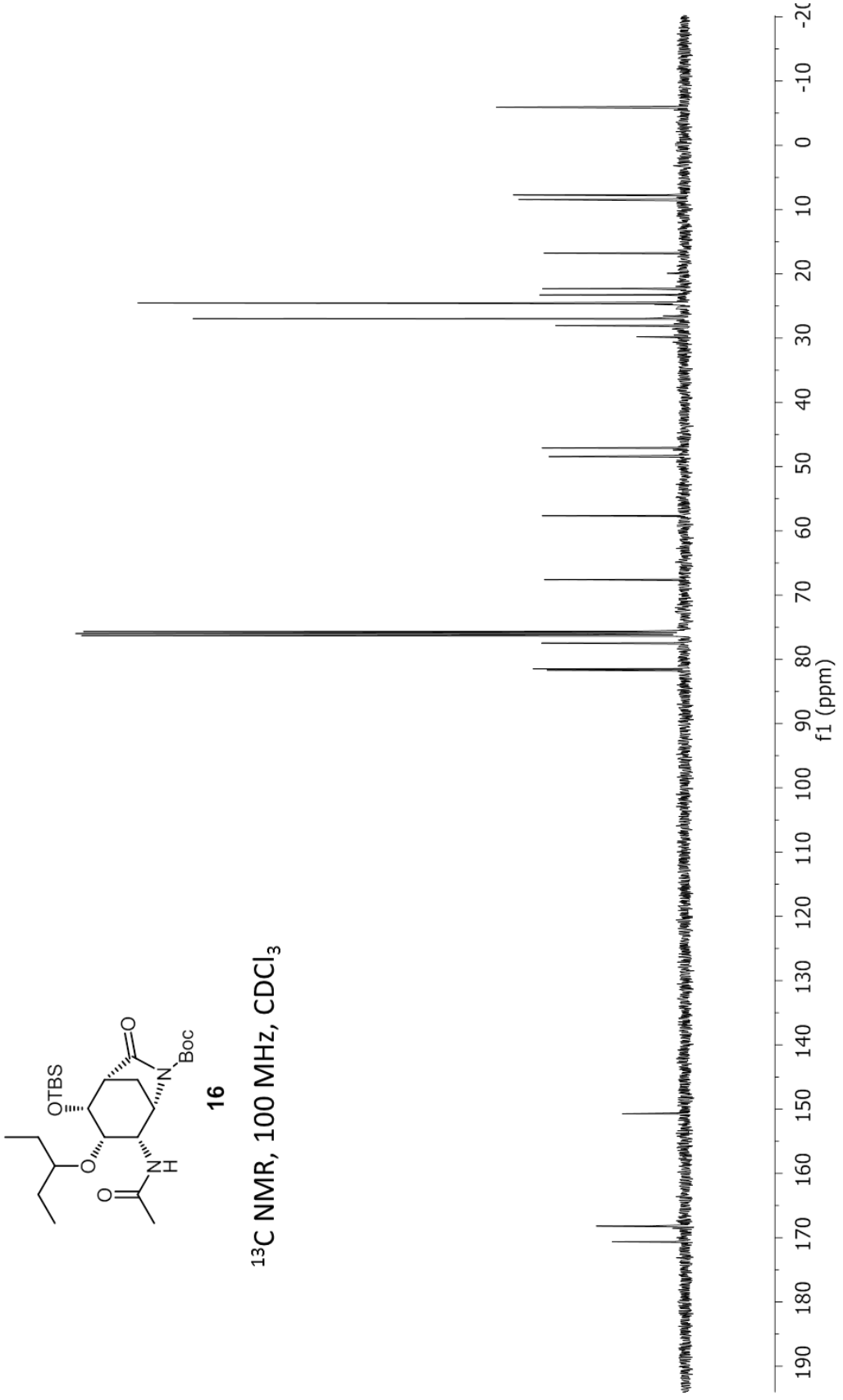


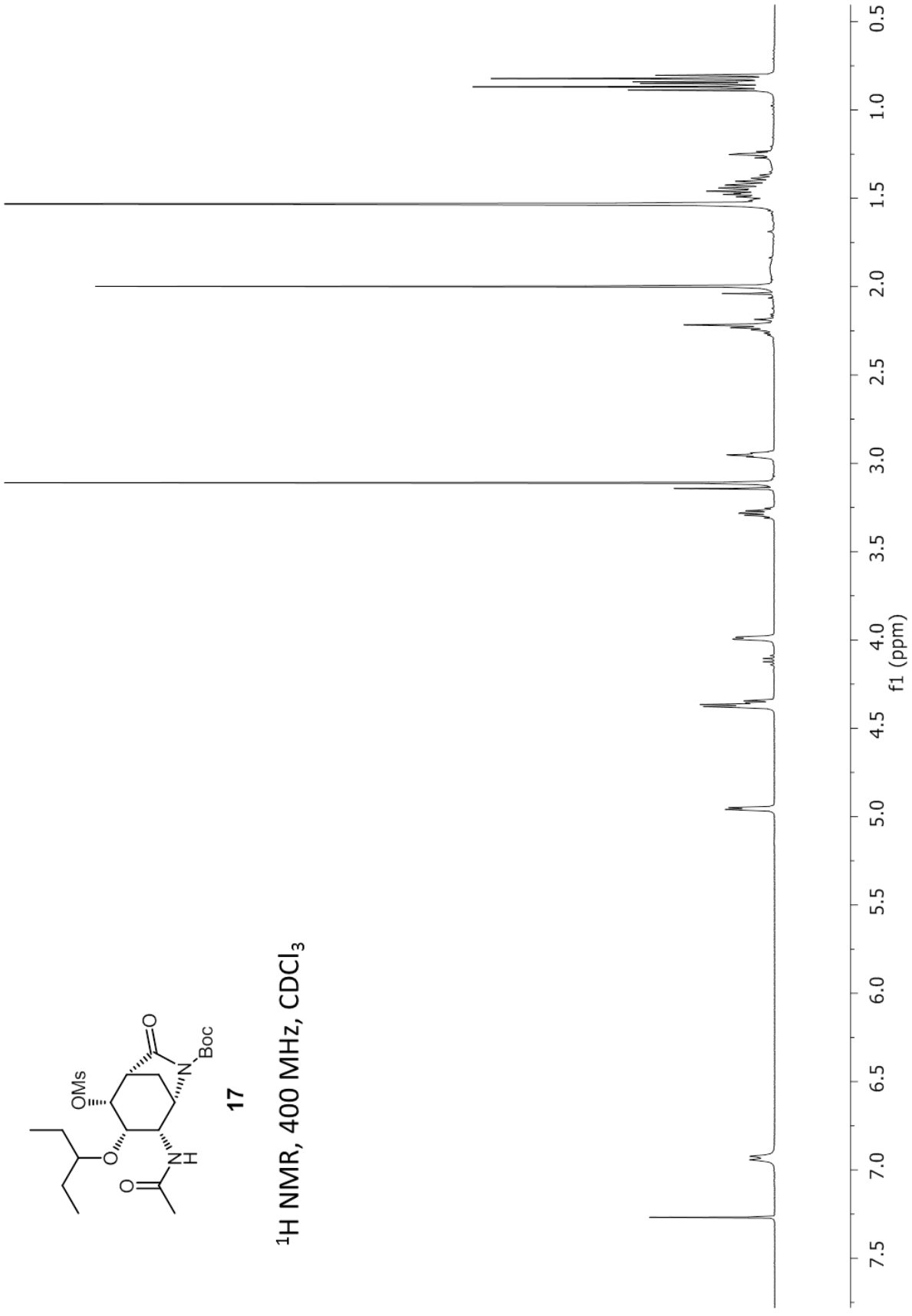







OTBS
O
N
Boc
O
O
N
H
O
16
^{1}H NMR, 400 MHz, $CDCl_3$
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)




OMs
O
N
Boc
O
O
N
H
O
17
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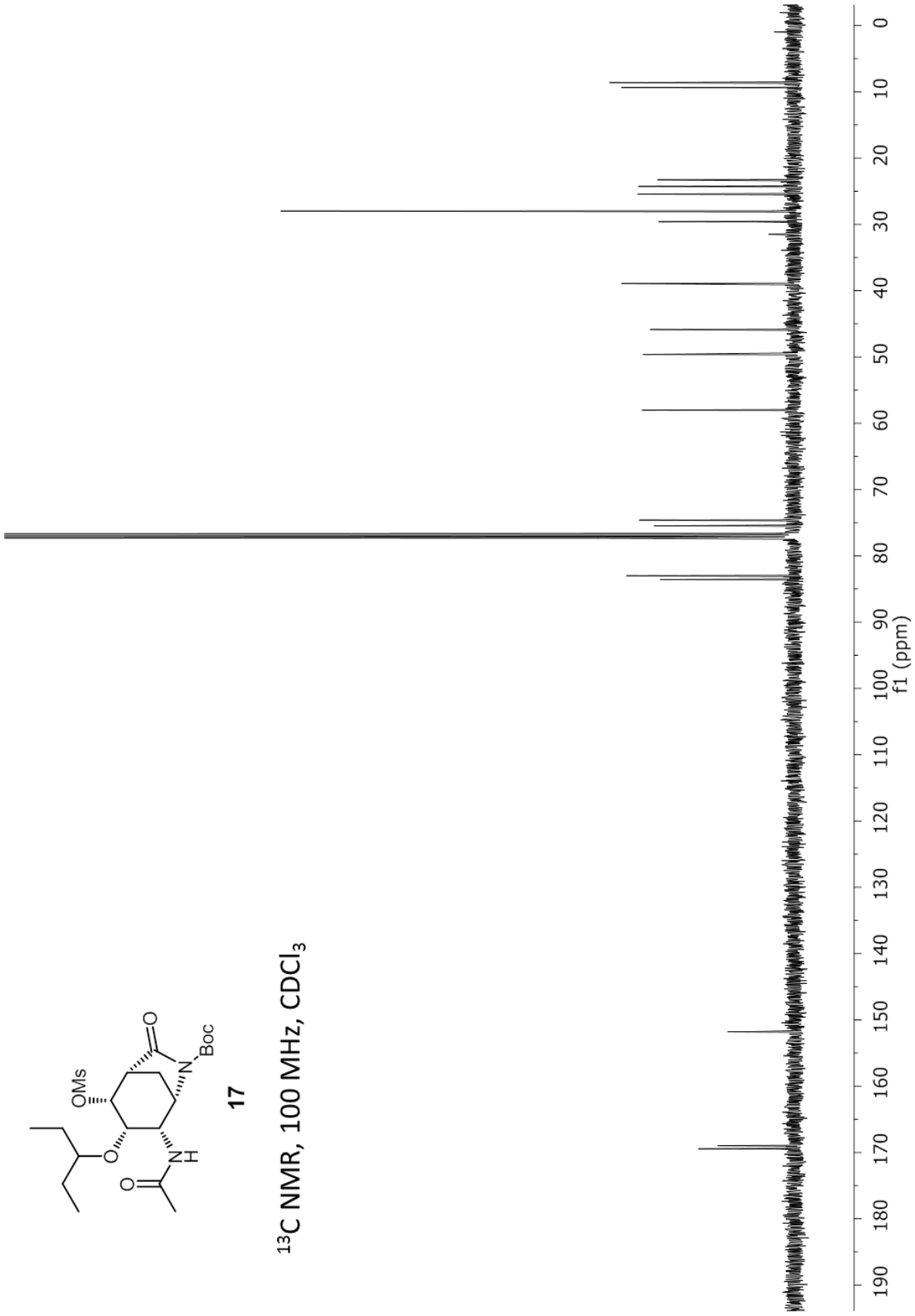
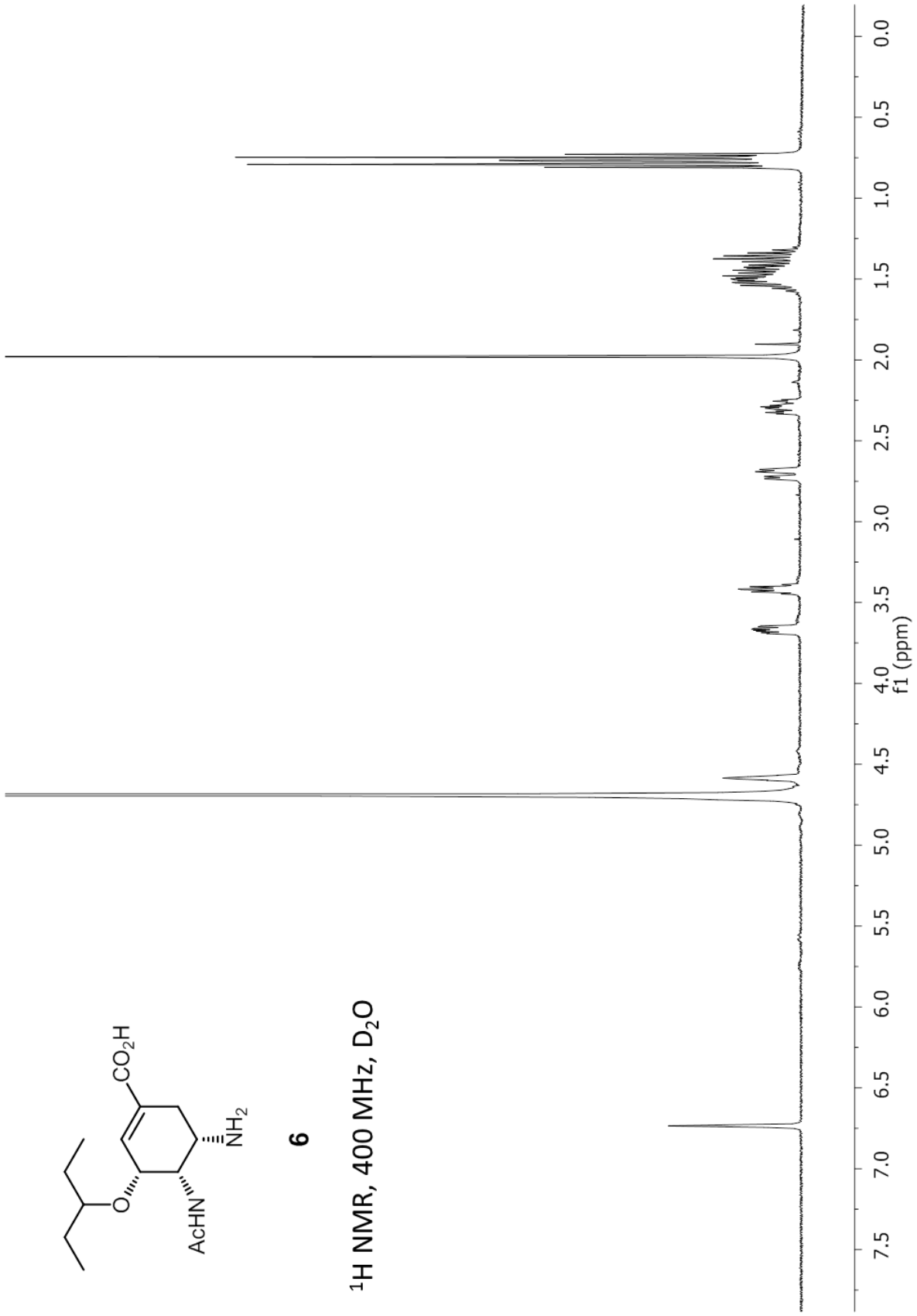
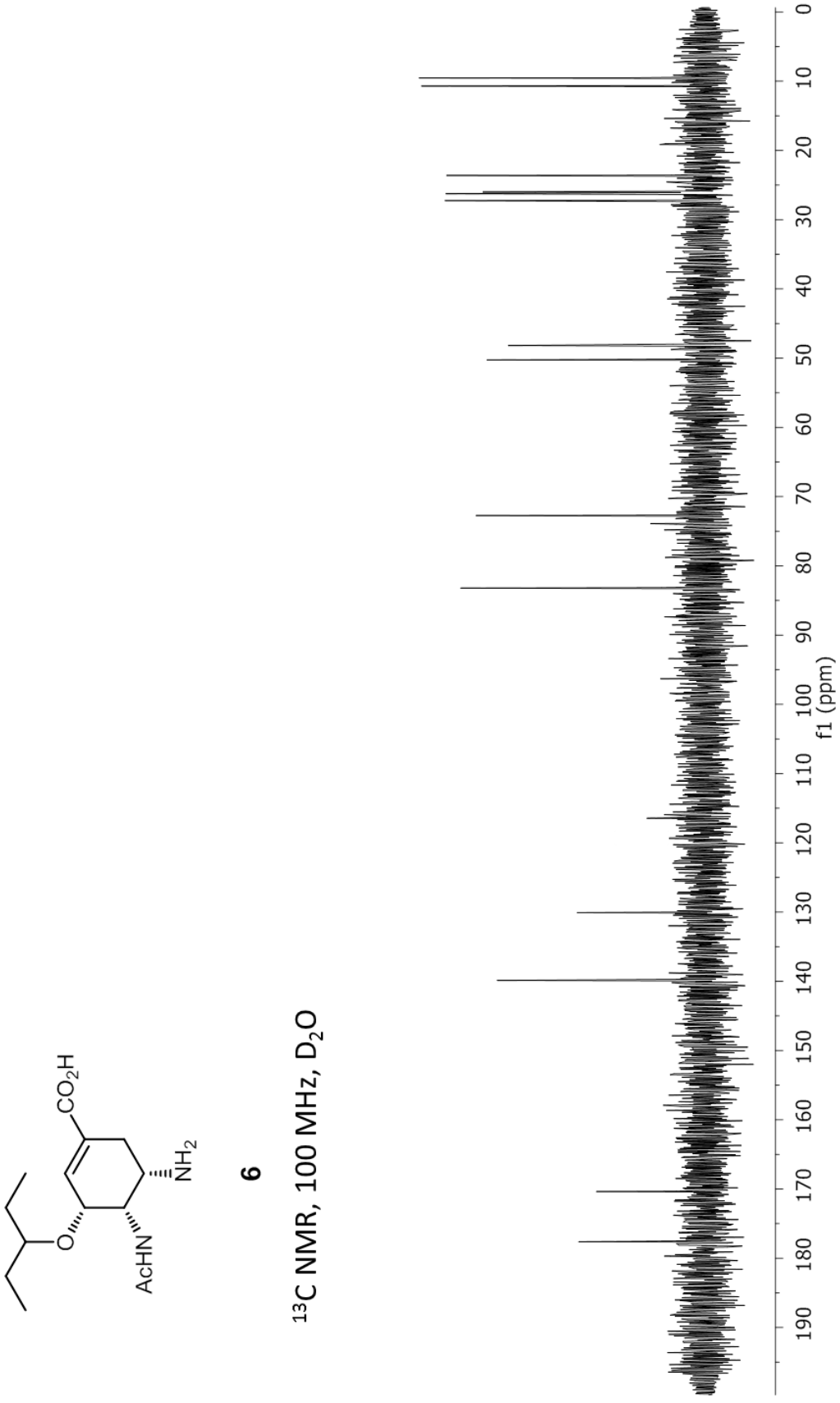
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)



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NH2
AcHN
O
6
13C NMR, 100 MHz, D2O
0
10
20
30
40
50
60
70
80
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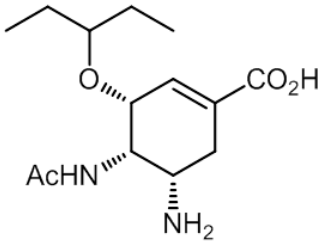
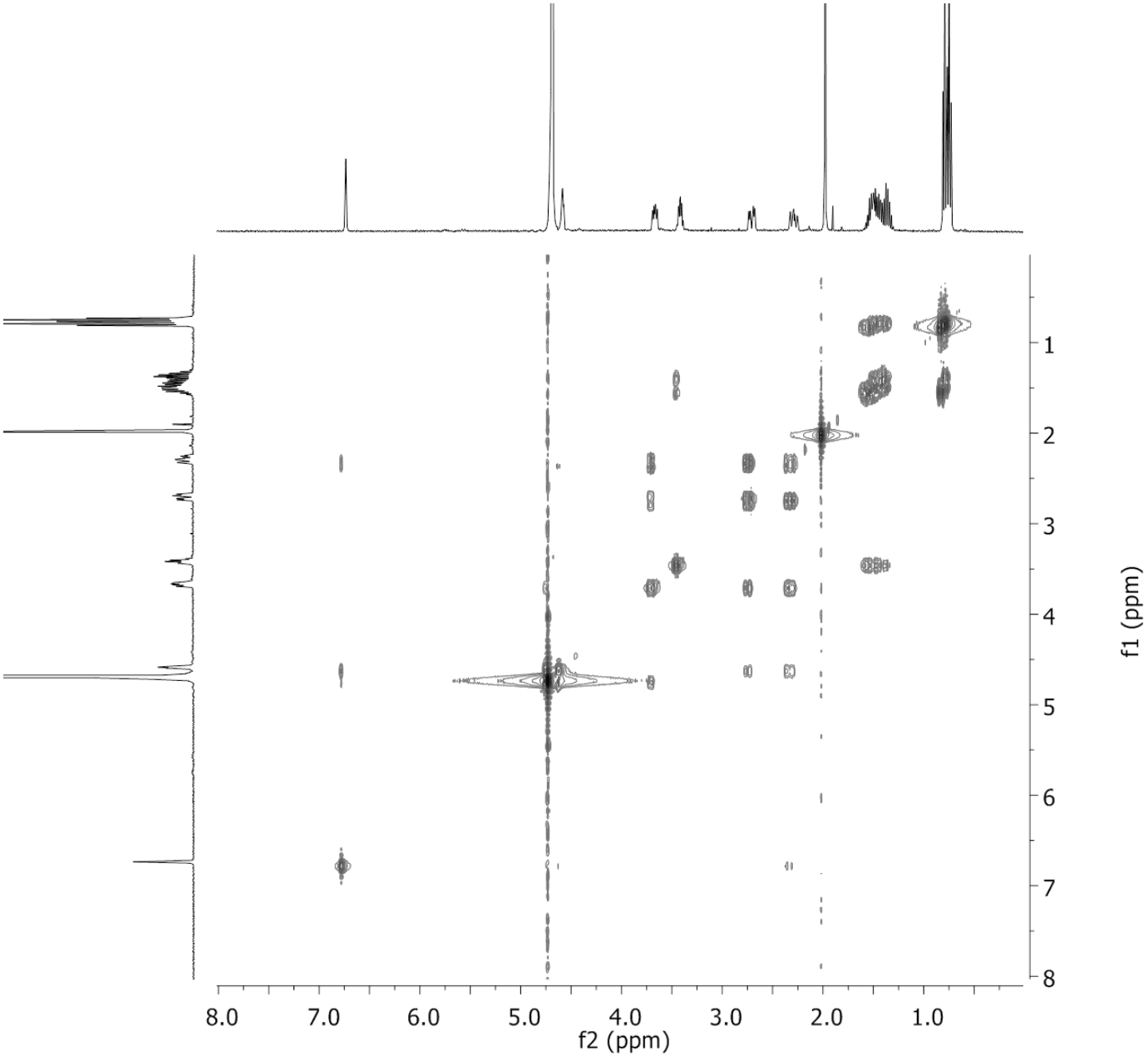
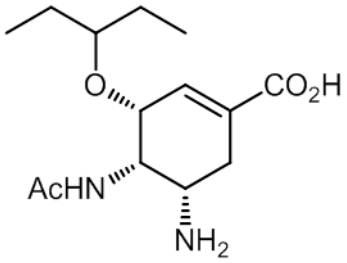
130
140
150
160
170
180
190
f1 (ppm)



6

^{1}H-^{1}H COSY NMR,
400 MHz, D_2O





6

^{1}H-^{1}H NOESY NMR,
400 MHz, D_2O