Supporting Information

The *myo*-1,2-Diaminocyclitol Scaffold Defines Potent Glucocerebrosidase Activators and Promising Pharmacological Chaperones for Gaucher Disease

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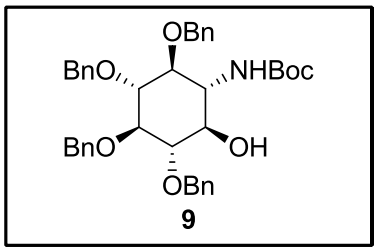
Chemistry: general methods

Solvents were distilled prior to use and dried by standard methods. FT-IR spectra are reported in υ, cm^{-1}. ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ or CD_3OD solutions at 500 MHz (for ^1H) and 100 (for ^{13}C), respectively, unless otherwise indicated. Chemical shifts (δ) are reported in parts per million (ppm) relative to the singlet at 7.26 ppm of $CDCl_3$ for ^1H and in ppm relative to the center line of a triplet at 77.16 ppm of $CDCl_3$ for ^{13}C. Optical rotations were measured with a Perkin-Elmer Model 341 polarimeter, and specific rotations are reported in 10^{-1} deg cm^2 g^{-1}. The HRMS spectra were recorded on a Waters LCT Premier Mass spectrometer.

SYNTHESIS AND COMPOUND CHARACTERIZATION

The following compounds were synthesized according to literature procedures:
(1*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-2-azido-3,4,5,6-tetrakis(benzyloxy)cyclohexanol (**7**),[1]
(1*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-2-Amino-3,4,5,6-tetrakis(benzyloxy)cyclohexanol (**8**),[2] and
(1*S*,2*R*,3*R*,4*S*)-5-cyclohexene-1,2,3,4-tetraol (**14**).[1]

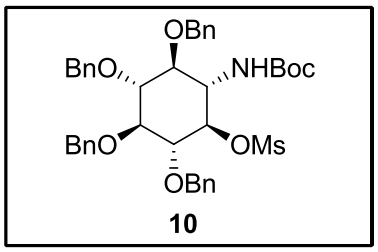
(1*R*,2*S*,3*R*,4*R*,5*S*,6*R*)-*N*-(2,3,4,5-tetrakis(benzyloxy)-6-hydroxycyclohexyl)carbamic acid *tert*-butyl ester (9)[3]



9

To a stirred solution of **8** (0.71 g, 1.3 mmol) in CH_2Cl_2 (10 mL) at 0 ºC was added Et_3N (0.3 mL, 1.7 mmol). $(Boc)_2O$ (0.40 g, 1.8 mmol) in CH_2Cl_2 (10 mL) were added dropwise, and the reaction mixture was stirred at room temperature overnight. Evaporation afforded crude **9**, which was purified by silica flash chromatography on hexane/EtOAc (3:2) to afford 0.7 g (1.10 mmol, 82%) of carbamate **9**. Oil. $[\alpha]_D^{25}$ – 21.4 (*c* 1.0, $CHCl_3$); IR (film): υ = 3342, 3063, 3030, 2976, 2901, 1687, 1534, 1497, 1453, 1365 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.47 (s, 9H), 3.45–3.67 (m, 5H), 4.50 (d, 1H, *J* =6.5 Hz), 4.70 (d, 1H, *J* =11.2 Hz), 4.87–4.94 (m, 7H), 7.28–7.35 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 28.5, 56.5, 73.4, 75.3, 75.7, 75.9, 76.0, 79.2, 80.3, 82.6, 84.0, 84.2, 127.8–128.7, 138.1, 138.4, 138.6, 156.9. HRMS calculated for $C_{39}H_{45}NO_7Na$: 662.3094 $[M+Na]^+$. Found: 662.3082.

(1*R*,2*R*,3*S*,4*R*,5*S*,6*S*)-2,3,4,5-tetrakis(benzyloxy)-6-(*tert*-butoxycarbonylamino)cyclohexyl methanesulfonate (10)[3]

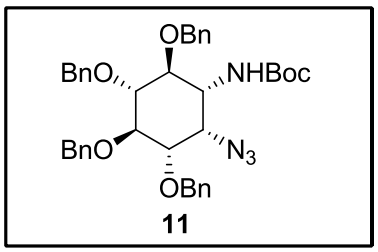


10

A solution of carbamate **9** (548 mg, 1.0 mmol) and Et_3N (400 µL, 2.9 mmol) in THF (6 mL) was treated with MsCl (100 µL, 1.2 mmol). The reaction mixture was stirred at room temperature, after 5 h the solvent was removed *in vacuo*. The residue was taken in Et_2O (10 mL), filtered and the solid was washed several times with Et_2O. The filtrate and washings were concentrated to give the crude mesylate, which was purified by filtration through a plug of silica and elution with hexane/EtOAc (6:1) to give 458 mg (0.64 mmol, 63%) of **10**.

$[\alpha]_D^{25}$ – 25.6 (*c* 1.0, $CHCl_3$); IR (film): υ = 3068, 2974, 2862, 2105, 1698, 1463 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.48 (s, 9H), 2.87 (s, 3H), 3.56–3.70 (m, 4H), 3.72–3.77 (m, 1H), 4.56–4.60 (m, 1H), 4.67–4.96 (m, 9H), 7.24–7.38 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 28.5, 39.0, 54.6, 75.6, 75.8, 76.0, 76.1, 79.1, 80.2, 80.8, 81.2, 82.5, 83.7, 127.8–128.6, 137.7, 137.9, 138.0, 138.2, 155.7. HRMS calculated for $C_{40}H_{47}NO_9NaS$: 740.2869 $[M+Na]^+$. Found: 740.2853.

(1*R*,2*S*,3*S*,4*R*,5*R*,6*S*)-*N*-(2-azido-3,4,5,6-tetrakis(benzyloxy)cyclohexyl)carbamic acid *tert*-butyl ester (11)[3]

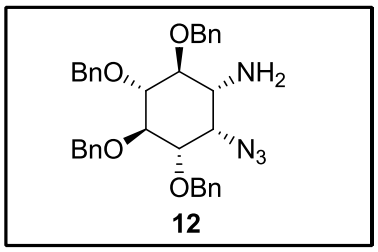


11

A solution of **10** (207 mg, 0.29 mmol) in DMF (6 mL) was treated with NaN_3 (56 mg, 0.85 mmol) and the reaction mixture was stirred at 90 ºC. After 18 h, the reaction was cooled to room temperature, diluted with H_2O (15 mL), extracted with Et_2O (4 x 20 mL), and dried over anhydrous $MgSO_4$. Filtration and evaporation afforded crude azido carbamate, which was purified by filtration through a plug of silica and elution with hexane/EtOAc (3:1) to afford 125 mg (0.19 mmol, 65%) of **11**. The ^1H NMR and ^{13}C NMR spectra were identical to those previously reported in the literature.[2] $[\alpha]_D^{25}$ – 28.5 (*c* 1.0, $CHCl_3$); IR (film): υ = 3340, 3031, 2912, 2101, 1684, 1526, 1357 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.50 (s, 9H), 3.52–3.57 (m, 2H), 3.70–3.76 (m, 2H), 3.94 (t, 1H, *J*=8.6 Hz), 4.15–4.20 (m, 1H), 4.65–4.96 (m, 8H), 7.24–7.39 (m, 20H); ^{13}C NMR (δ, 100

MHz, $CDCl_3$): 28.5, 51.5, 62.6, 73.3, 75.5, 76.0, 76.2, 79.1, 80.1, 81.0, 81.6, 84.2, 127.8–128.6, 137.6, 138.1, 138.5, 155.5. HRMS calculated for $C_{39}H_{44}N_4O_6Na$: 687.3159 $[M+Na]^+$. Found: 687.3167.

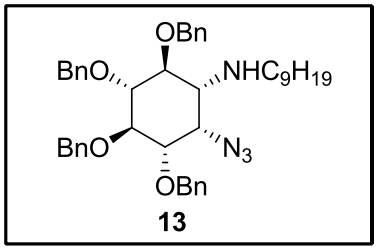
(1*R*,2*S*,3*S*,4*R*,5*R*,6*S*)-2-azido-3,4,5,6-tetrakis(benzyloxy)cyclohexanamine (12)[3]



12

To a solution of the protected azide **11** (120 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (5 mL) was added TFA (0.8 mL) and the reaction was stirred at room temperature for 4 h. Next, the solvent was removed by using N_2 stream. The residual paste was dissolved in EtOAc and neutralized with aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography (1:1 to 1:3 hexane/EtOAc gradient) to give the azido amine **12** (87 mg, 0.15 mmol, 86%). The ^1H NMR and ^{13}C NMR spectra were identical to those previously reported in the literature.[2] $[\alpha]_D^{25}$ – 14.5 (*c* 1.0, $CHCl_3$); IR (film): υ = 3377, 3063, 3030, 2914, 2101, 1697, 1585, 1496, 1454 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.50 (br s, 2H), 2.61 (d, 1H, *J* =8.8 Hz), 3.41 (t, 1H, *J* =9.4 Hz), 3.47 (t, 1H, *J* =9.2 Hz), 3.66 (d, 1H, *J* = 9.5 Hz), 3.97 (t, 1H, *J* =9.4 Hz), 4.05–4.10 (m, 1H), 4.65–5.02 (m, 8H), 7.24–7.39 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 52.7, 63.7, 73.2, 75.8, 76.0, 76.2, 81.5, 82.2, 82.5, 84.6, 127.8–128.7, 137.7, 138.4, 138.5, 138.6. HRMS calculated for $C_{34}H_{37}N_4O_4$: 565.2815 $[M+H]^+$. Found: 565.2813.

(1*R*,2*S*,3*S*,4*R*,5*R*,6*S*)-2-azido-3,4,5,6-tetrakis(benzyloxy)-*N*-nonylcyclohexanamine (13)

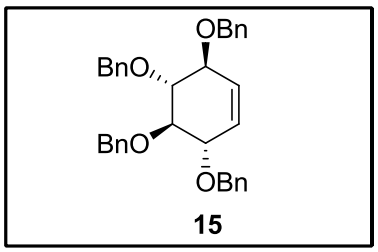


13

A solution of azido amine **12** (69 mg, 0.12 mmol) in methanol (5 mL) under an atmosphere of argon was treated successively with sodium cyanoborohydride (15 mg, 0.24 mmol), acetic acid (8 μL) and nonanal (23 μL, 0.13 mmol). After stirring for 1 h at rt, the mixture was quenched with water (0.2 mL) and concentrated *in vacuo*. The residue was taken up in diethyl oxide (20 ml), washed with water (15 mL) and the resulting aqueous phase was extracted with diethyl oxide (3 x 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated to give a yellow oil, which was purified by flash chromatography

using a mixture of hexane/AcOEt (10:1). Compound **13** was obtained as colorless oil (62 mg, 0.09 mmol, 75%). $[\alpha]_D^{25}$ – 6.4 (*c* 0.5, $CHCl_3$); IR (film): υ = 3091, 3061, 3031, 2956, 2924, 2853, 2102, 1565, 1456, 1135, 1088, 1071, 668 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.91 (t, 3H, *J* =6.9 Hz), 1.19–1.36 (m, 12H), 1.40–1.51 (m, 2H), 2.43 (td, 1H, *J* =7.2, 10.7 Hz), 2.53 (dd, 1H, *J* =2.7, 10.1 Hz), 2.67 (ddd, 1H, *J* =6.2, 7.8, 10.8 Hz), 3.52 (t, 1H, *J* =9.3 Hz), 3.57 (dd, 1H, *J* =3.1, 9.6 Hz), 3.65 (t, 1H, *J* =9.7 Hz), 3.96 (t, 1H, *J* =9.4 Hz), 4.04 (t, 1H, *J* =2.7 Hz), 4.66–4.97 (m, 8H), 7.30–7.38 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.3, 22.8, 27.3, 29.4, 29.6, 29.7, 30.0, 32.0, 47.7, 59.90, 59.92, 73.3, 75.9, 76.1, 76.2, 80.6, 81.2, 82.0, 84.9, 127.8–128.7, 137.9, 138.3, 138.56, 138.58. HRMS calculated for $C_{43}H_{55}N_4O_4$: 691.4223 $[M+H]^+$. Found: 691.4253.

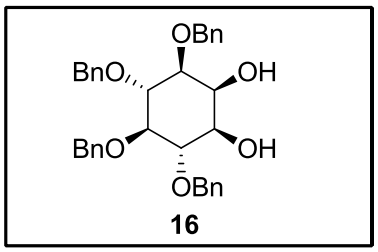
(1*S*,2*R*,3*R*,4*S*)-cyclohex-5-ene-1,2,3,4-tetrayltetrakis(oxy)tetrakis(methylene)tetrabenzene (15)



15

Sodium hydride (2.4 g, 60% dispersion in mineral oil, 60 mmol) was suspended in anhydrous DMF (30 mL) in a three-necked round bottomed flask under argon atmosphere and BnBr (7.1 mL, 60 mmol) was added dropwise at 30 ºC. In another flask under argon atmosphere, the tetraol **14** (1.5 g, 10 mmol) was dissolved in anhydrous DMF (10 mL) and then transferred *via* canula to the flask containing the hydride and BnBr. After 16 h, the mixture was cooled to 0 ºC, quenched by the addition of water (2 mL) and concentrated *in vacuo*. The residue was taken up in diethyl oxide and washed with water (10 mL). The aqueous layer was extracted with diethyl oxide (3 x 40 mL) and the combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give a yellow oil, which was purified by flash chromatography using a mixture of hexane/EtOAc (10:1). Product **15** was obtained as a white solid (3.8 g, 7.5 mmol, 75%). The ^1H NMR spectra was identical to that reported in the literature.[4] $[\alpha]_D^{25}$ + 81.8 (*c* 1.0, $CHCl_3$); IR (film): υ = 3087, 3062, 3029, 2901, 2861, 1497, 1454, 1085, 1071, 1032, 693 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 3.76 (dd, 2H, *J* =2.3, 5.3 Hz), 4.24 (dd, 2H, *J* =2.3, 5.2 Hz), 4.69–4.96 (m, 8H), 5.74 (s, 2H), 7.28–7.36 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 72.6, 75.7, 80.3, 83.8, 127.7–128.6, 138.4, 138.8. HRMS calculated for $C_{34}H_{34}O_4Na$: 529.2355 $[M+Na]^+$. Found: 529.2314.

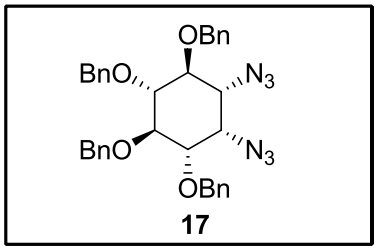
(1*R*,2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5,6-tetrakis(benzyloxy)cyclohexane-1,2-diol (16)[5]



16

To a solution of alkene **15** (1.7 g, 3.3 mmol) in 9:1 acetone: water (40 mL), NMO (0.84 g, 7.2 mmol), and osmium tetraoxide (0.05 M in *tert*-butanol stabilized with *tert*-butylhydroperoxide, 4.2 mL, 0.32 mmol) were added and the mixture was stirred overnight at room temperature. Then, sodium sulfite (0.42 g, 3.3 mmol) was added. After 1 h the mixture was diluted with H_2O (25 mL) and EtOAc (40 mL). The phases were separated and the aqueous layer were dried with MgSO4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography using a mixture of hexane/EtOAc (10:1) to yield a white solid (1.47 g, 2.71 mmol, 83%). The ^{1}H NMR spectra was identical with the literature data.[4] $[\alpha]_D^{25}$ – 20.6 (*c* 1.0, $CHCl_3$) [lit[6]:$[\alpha]_D^{25}$ – 24.3 (*c* 1.3, $CHCl_3$); IR (film): υ = 3420, 3085, 3064, 3029, 2901, 1070 cm^{-1}; ^{1}H NMR (δ, 500 MHz, $CDCl_3$): 2.40–2.44 (br s, 1H), 2.47–2.52 (br s, 1H), 3.48–3.52 (m, 3H), 3.86 (t, 1H, *J* =9.5 Hz), 3.99 (t, 1H, *J* =9.5 Hz), 4.22–4.25 (m, 1H), 4.71–4.99 (m, 8H), 7.30–7.38 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 71.8, 72.9, 75.8, 75.9, 76.1, 80.1, 81.4, 81.8, 83.3, 127.8–128.7, 137.9, 138.6, 138.7. HRMS calculated for $C_{34}H_{36}O_6Na$: 563.2410 $[M+Na]^+$. Found: 563.2371.

(1*S*,2*R*,3*R*,4*S*,5*R*,6*S*)-5,6-diazidocyclohexane-1,2,3,4-tetrayl)tetrakis(oxy)tetrakis-(methylene)tetrabenzene (17)

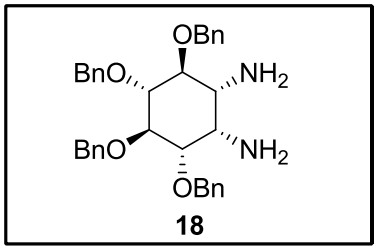


17

A solution of **16** (0.6 g, 1.1 mmol) in 6 mL of pyridine was cooled to 0 ºC. MsCl (0.3 mL, 3.1 mmol) was added, and the resulting mixture was stirred to room temperature for 16 h. The reaction mixture was then diluted with EtOAc (20 mL) and poured into ice-water (20 mL). The organic layer was washed with water (2 x 20 mL) and brine (25 mL), dried over $MgSO_4$ and filtrated. The solvent was evaporated *in vacuo* to give a yellow oil, which was treated with NaN_3 (0.8 g, 12.2 mmol) in anhydrous DMF (10 mL) at 85 ºC for 12 h. The reaction mixture was cooled to room temperature and the solvent was removed under vacuum. The residue was taken up in diethyl oxide and washed with water (10 mL). The aqueous layer was extracted with diethyl oxide

(3 x 40 mL) and the combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give a yellow oil, which was purified by flash chromatography using a mixture of hexane/EtOAc (5:1) to afford 0.50 g (0.85 mmol, 77%) of **17**. The ^{1}H NMR and IR were identical to those previously reported in the literature.[7] $[\alpha]_D^{25}$ + 18.1 (*c* 1.0, $CHCl_3$); IR (film): υ = 3083, 3063, 3031, 2923, 2872, 2101, 1091, 1065, 697 cm^{-1}; ^{1}H NMR (δ, 500 MHz, $CDCl_3$): 3.38 (dd, 1H, *J* =10.2, 3.1 Hz), 3.48 (t, 1H, *J* =9.3 Hz), 3.57 (dd, 1H, *J* =9.6, 3.2 Hz), 3.82 (t, 1H, *J* =9.7 Hz), 3.91 (t, 1H, *J* =9.5 Hz), 3.98 (t, 1H, *J* =3.1 Hz), 4.72–4.93 (m, 8H), 7.25–7.40 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 61.9, 62.0, 73.5, 76.0, 76.3, 80.1, 80.3, 81.5, 84.0, 127.7–128.7, 137.5, 137.7, 138.3. HRMS calculated for $C_{34}H_{34}N_6O_4Na$: 613.2539 $[M+Na]^+$. Found: 613.2554.

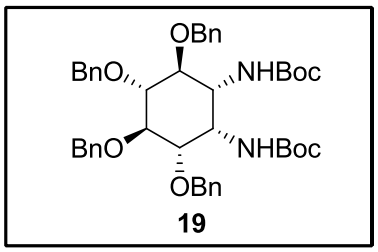
(1*S*,2*R*,3*S*,4*R*,5*R*,6*S*)-3,4,5,6-tetrakis(benzyloxy)cyclohexane-1,2-diamine (18)



18

A solution of diazide **17** (0.58 g, 0.99 mmol) in anhydrous THF (30 mL) was added dropwise under argon a solution of $LiAlH_4$ (0.11 g, 2.9 mmol) in anhydrous THF (20 mL) at 0 ºC. After stirred for 3 h at rt, the mixture was cooled back to 0 ºC, quenched by the addition of aqueous saturated Na_2SO_4 solution. The solution was diluted with ethyl acetate, dried over $MgSO_4$ and filtered through a plug of Celite. The plug of celite was washed three times with EtOAc. The filtrate and combined washings were evaporated *in vacuo* to give 0.5 g (0.93 mmol, 93%) of diamine **18**, which was used in the next step without further purification. The ^{1}H NMR and ^{13}C NMR spectra were identical to those previously reported in the literature.[8] $[\alpha]_D^{25}$ – 23.2 (*c* 1.5, $CHCl_3$); IR (film): υ = 3084, 3060, 3029, 2923, 2855, 1496, 1453, 1088, 1070, 1027, 735, 697 cm^{-1}; ^{1}H NMR (δ, 500 MHz, $CDCl_3$): 1.59 (br s, 4H), 2.69 (dd, 1H, *J* =10.0, 2.7 Hz), 3.46–3.57 (m, 3H), 3.79 (t, 1H, *J* =9.6 Hz), 4.07 (t, 1H, *J* =9.1 Hz), 4.64–5.02 (m, 8H), 7.29–7.36 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 51.7, 54.0, 72.4, 75.7, 75.8, 76.0, 81.82, 81.87, 81.99, 85.3, 127.6–128.7, 138.4, 138.7, 138.89, 138.91. HRMS calculated for $C_{34}H_{39}N_2O_4$: 539.2910 $[M+H]^+$. Found: 539.2924.

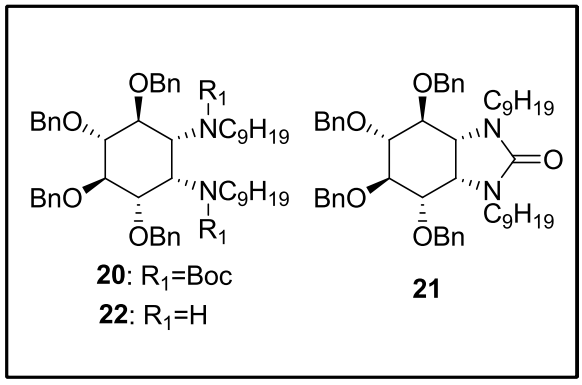
di-*tert*-butyl (1*S*,2*R*,3*S*,4*R*,5*R*,6*S*)-3,4,5,6-tetrakis(benzyloxy)cyclohexane-1,2-diyldicarbamate (19)



19

To a stirred solution of diamine **18** (269 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 ºC was added Et_3N (0.21 mL, 1.5 mmol) and $(Boc)_2O$ (0.33 g, 1.5 mmol). The reaction mixture was stirred at room temperature overnight. The solvent was evaporated to afford a yellow oil which was purified by flash chromatography (10:1 to 6:1 hexane/EtOAc gradient) to give 0.2 g (0.27 mmol, 53%) of **19**. $[\alpha]_D^{25}$ – 22.6 (*c* 1.0, $CHCl_3$). HRMS calculated for $C_{44}H_{54}N_2O_8Na$: 761.3778 $[M+Na]^+$. Found: 761.3757; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) were identical to those reported in the literature.[8]

(1*S*,2*R*,3*S*,4*R*,5*R*,6*S*)-3,4,5,6-tetrakis(benzyloxy)-N^1,N^2-dinonylcyclohexane-1,2-diamine (22) and (3a*S*,4*S*,5*R*,6*R*,7*S*,7a*R*)-4,5,6,7-tetrakis(benzyloxy)-1,3-dinonyl-hexahydro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (21)



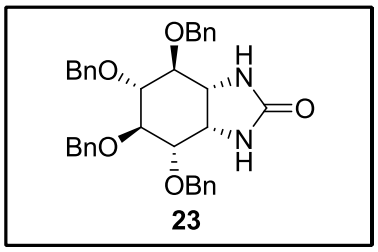
To a solution of **19** (187 mg, 0.25 mmol) in DMF (5 mL) was added NaH (25 mg, 60% dispersion in mineral oil, 0.6 mmol) at 0 ºC and stirred for 15 min. Then, nonyl iodide (200 µL, 1.00 mmol) was added, and the mixture was heated at 80 ºC for 24 h under Ar. The mixture was cooled to 23 ºC; an additional 100 µL (0.5 mmol) was added, and heating was continued for another 72 h. The reaction was quenched by addition of few drops of water. The mixture was diluted with ether and water (40 mL). The aqueous layer was extracted with ether (3 x 40 mL). The collected organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure. Chromatography on silica gel (10:1 to 5:1 hexane/EtOAc gradient) provided **20** (52 mg, 0.05 mmol, 21%), followed by **21** (50 mg, 0.06 mmol, 24%). To a solution of dicarbamate **20** (0.05 mmol) in anhydrous CH_2Cl_2 (4 mL) was added TFA (0.6 mL) and the reaction was stirred at room temperature for 4 h. Next, the

solvent was removed by using N_2 stream and dried under high vacuum. The residual paste was dissolved in EtOAc and neutralized with aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography (10:1 to 5:1 hexane/EtOAc gradient) to give **22** (34 mg, 0.04 mmol, 86%).

(**21**): $[\alpha]_D^{25}$ – 13 (*c* 1.0, $CHCl_3$); IR (film): υ = 3089, 3064, 3031, 2959, 2925, 2854, 1699, 1454, 1069, 1028, 734, 697 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.91 (dt, 6H, *J* =6.2, 6.9 Hz), 1.17–1.48 (m, 28H), 2.68–2.78 (m, 1H), 2.95–3.05 (m, 1H), 3.47–3.62 (m, 2H), 3.70 (dd, 1H, *J* =9.0, 5.6 Hz), 3.74–3.82 (m, 2H), 3.87 (dd, 1H, *J* =9.8, 2.9 Hz), 3.93 (dd, 1H, *J* =12.1, 6.8 Hz), 4.06 (dd, 1H, *J* =8.7, 7.1 Hz), 4.47–5.04 (m, 8H), 7.24–7.48 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.3, 22.8, 29.4, 29.5, 29.6, 29.71, 29.73, 32.0, 41.9, 42.7, 52.9, 56.4, 72.6, 73.1, 74.1, 75.3, 80.2, 81.0, 84.4, 127.6–128.7, 137.8, 138.0, 138.3, 138.4, 160.8. HRMS calculated for $C_{53}H_{72}N_2O_5Na$: 839.5339 $[M+Na]^+$. Found: 839.5370.

(**22**): $[\alpha]_D^{25}$ – 15 (*c* 1.0, $CHCl_3$); IR (film): υ = 3090, 3061, 3031, 2948, 2924, 2853, 1734, 1454, 1361, 1089, 1069, 1028, 732, 696 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.81–0.96 (m, 6H), 1.06–1.54 (m, 28H), 2.30–2.70 (m, 4H), 2.97–3.07 (m, 1H), 3.36–3.63 (m, 3H), 3.80 (t, 1H, *J* =9.4 Hz), 4.21 (t, 1H, *J* =9.0 Hz), 4.47–5.04 (m, 8H), 7.24–7.48 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.3, 22.8, 27.5, 29.50, 29.54, 29.73, 29.78, 29.8, 30.4, 30.8, 32.1, 47.3, 51.0, 54.5, 60.6, 72.7, 75.6, 75.7, 75.9, 81.2, 82.2, 84.3, 85.9, 127.6–128.7, 138.7, 138.9, 139.0. HRMS calculated for $C_{52}H_{75}N_2O_4$: 791.5727 $[M+H]^+$. Found: 791.5747.

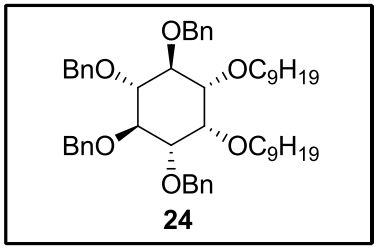
(3a*S*,4*S*,5*R*,6*R*,7*S*,7a*R*)-4,5,6,7-tetrakis(benzyloxy)-hexahydro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (23)



23

To a solution of **18** (126 mg, 0.23 mmol) in CH_2Cl_2 (7 mL), *N,N′*-carbonyldiimidazole (57 mg, 0.35 mmol) was added under Ar. The reaction mixture was heated at reflux for 2 h and concentrated. The resulting residue was purified by flash chromatography using a mixture of hexane/EtOAc (1:10) to give **23** (110 mg, 0.19 mmol, 83%). $[\alpha]_D^{25}$ – 61.8 (*c* 1.0, $CHCl_3$); IR (film): υ = 3220, 3090, 3063, 3029, 2922, 2853, 1702, 1453, 1359, 1086, 1070, 1028, 696 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 3.49–3.56 (m, 2H), 3.66 (dd, 1H, *J* =8.5, 4.6 Hz), 3.73 (t, 1H, *J* =8.1 Hz), 3.89 (t, 1H, *J* =8.3 Hz), 4.03 (dd, 1H, *J* =7.4, 4.7 Hz), 4.52–4.96 (m, 10H), 7.27–7.42 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 54.0, 56.0, 73.6, 74.9, 75.0, 75.2, 78.1, 81.2, 82.3, 82.6, 127.83–128.83, 138.0, 138.27, 138.34, 162.7. HRMS calculated for $C_{35}H_{37}N_2O_5$: 565.2702 $[M+H]^+$. Found: 565.2701.

((1*S*,2*R*,3*R*,4*S*,5*S*,6*R*)-5,6-bis(nonyloxy)cyclohexane-1,2,3,4-tetrayl)tetrakis(oxy)tetrakis(methylene)tetrabenzene (24)



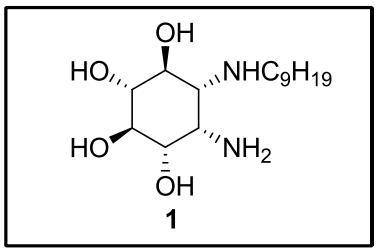
24

To a solution of diol **16** (70 mg, 0.13 mmol) in DMF (5 mL) was added NaH (21 mg, 60% dispersion in mineral oil, 0.52 mmol) at 0 ºC and stirred for 15 min. Then, nonyl iodide (256 μL, 1.3 mmol) was added, and the mixture was stirred at 0 ºC for additional 3 h. The reaction was quenched by addition of few drops of water. The residue was taken up in diethyl oxide and washed with water (10 mL). The aqueous layer was extracted with diethyl oxide (3 x 20 mL) and the combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give a yellow oil, which was purified by flash chromatography using a mixture of hexane/EtOAc (20:1) to afford 80 mg (0.10 mmol, 78%) of **24** as a pale yellow oil. $[\alpha]_D^{25}$ + 2.0 (*c* 0.7, $CHCl_3$); IR (film): υ = 3085, 3065, 3026, 2959, 2924, 2854, 1734, 1718, 1267, 1095, 1071, 1028, 728, 668 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.90–0.94 (m, 6H), 1.28–1.42 (m, 24H), 1.61–1.66 (m, 4H), 3.15–3.21 (m, 1H), 3.37–3.40 (m, 1H), 3.48 (td, 1H, *J* =2.2, 9.2 Hz), 3.53–3.58 (m, 1H), 3.61–3.66 (m, 1H), 3.78 (td, 2H, *J* =2.2, 6.5 Hz), 3.93 (d, 1H, *J* =2.05 Hz), 3.97 (td, 1H, *J* =2.3, 9.5 Hz), 4.05 (dt, 1H, *J* =2.3, 9.5 Hz), 4.75–4.97 (m, 8H), 7.30–7.42 (m, 20H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.3, 22.83, 22.84, 26.3, 26.42, 26.47, 29.52, 29.66, 29.70, 29.74, 29.8, 30.4, 30.5, 32.0, 32.1, 70.9, 72.8, 73.5, 75.0, 75.9, 76.0, 76.1, 81.1, 81.6, 81.7, 81.8, 83.7, 127.6–128.5, 138.6, 138.97, 139.02, 139.2. HRMS calculated for $C_{52}H_{72}O_6Na$: 815.5227 $[M+Na]^+$. Found: 815.5238.

Synthesis of diamines 1 and 2 by hydrogenolysis using Pd/C catalyst (MeOH/HCl)

General method: In a glass pressure flask, the benzylated azido amine **1** or diamine **2** (0.07 mmol) was dissolved in a mixture of MeOH (4 mL) and concentrated HCl (4 drops). Pd/C (40 mg, 5–15% Pd on activated C, water-wet) was then added. The flask was repeatedly filled and evacuated with hydrogen and vigorously stirred at room temperature for 24 h under H_2 (2 atm). After this period, the reaction mixture was filtered through a plug of Celite to separate the catalyst, and the filter was washed three times with MeOH. The filtrate and combined washings were concentrated to give the desired products.

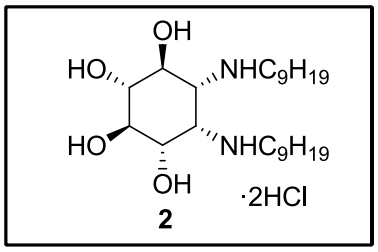
(1*S*,2*R*,3*R*,4*S*,5*S*,6*R*)-5-amino-6-(nonylamino)cyclohexane-1,2,3,4-tetraol dihydrochloride (1)



1

Obtained in 77% yield (17 mg, 0.06 mmol) as a white solid from 51 mg (0.07 mmol) of **13**. The compound was purified by flash chromatography (4:1 to 3:1 CH_2Cl_2:MeOH gradient and 1% vol NH_3). $[\alpha]_D^{25}$ – 12.5 (*c* 1.0, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 0.91 (t, 3H, *J* =6.1 Hz), 1.22–1.45 (m, 12H), 1.50–1.61 (m, 2H), 2.47–2.56 (m, 2H), 2.70–2.74 (m, 1H), 3.17 (t, 1H, *J* =8.3 Hz), 3.40–3.46 (m, 2H), 3.51 (t, 1H, *J* =9.7 Hz), 3.63 (t, 1H, *J* =9.4 Hz); ^{13}C NMR (δ, 100 MHz, CD_3SOCD_3): 13.7, 21.9, 26.7, 28.5, 28.79, 28.82, 29.6, 31.1, 46.7, 50.7, 60.9, 70.9, 72.4, 72.9, 76.4. HRMS calculated for $C_{15}H_{33}N_2O_4$: 305.2440 $[M+H]^+$. Found: 305.2427.

(1*S*,2*R*,3*R*,4*S*,5*R*,6*S*)-5,6-bis(nonylamino)cyclohexane-1,2,3,4-tetraol dihydrochloride (2)



2

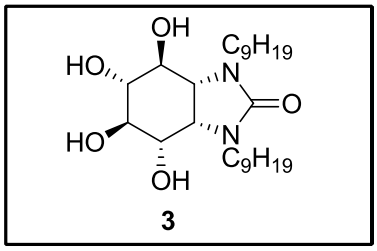
Obtained in 85% yield (15 mg, 0.03 mmol) as a white solid from 27 mg (0.03 mmol) of **22**. $[\alpha]_D^{25}$ – 9.4 (*c* 1.0, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 0.92 (t, 6H, *J* =6.7 Hz), 1.22–1.38 (m, 24H), 1.43–1.84 (m, 4H), 2.83–3.24 (m, 5H), 3.41–3.50 (m, 2H), 3.50–3.61 (m, 1H), 4.04–4.13 (m, 2H); ^{13}C NMR (δ, 100 MHz, CD_3OD): 14.3, 23.5, 27.1, 27.3, 27.4, 27.6, 30.1, 30.14, 30.22, 30.24, 30.37, 30.39, 30.6, 30.9, 32.9, 45.6, 48.9, 59.5, 62.1, 68.2, 70.9, 73.9. HRMS calculated for: $C_{24}H_{51}N_2O_4$: 431.3849 $[M+H]^+$. Found: 431.3860.

Synthesis of compounds 3, 5, and 6 by hydrogenolysis using Pd/C catalyst (MeOH)

General method: In a glass pressure flask, the benzylated compound (0.1 mmol) was dissolved in MeOH (4 mL). Pd/C (40 mg, 5–15% Pd on activated C, water-wet) was then added. The flask was repeatedly filled and evacuated with hydrogen and vigorously stirred at room temperature for 24 h under H_2 (2 atm). After this period, the reaction mixture was filtered through a plug of Celite to separate the catalyst, and the

filter was washed three times with MeOH. The filtrate and combined washings were concentrated to give the desired products.

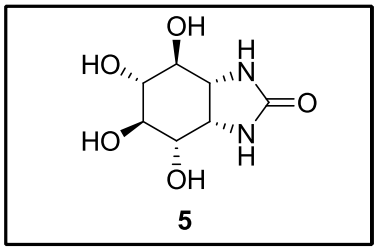
(3a*R*,4*S*,5*R*,6*R*,7*S*,7a*S*)-4,5,6,7-tetrahydroxy-1,3-dinonyl-hexahydro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (3)



3

Obtained in 93% yield (25 mg, 0.06 mmol) as a white solid from 48 mg (0.06 mmol) of **21**. $[\alpha]_D^{25}$ – 8.5 (*c* 1.0, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 0.90 (t, 6H, *J* =6.8 Hz), 1.22–1.38 (m, 24H), 1.43–1.64 (m, 4H), 3.07–3.15 (m, 1H), 3.24 (dd, 1H, *J* =10.0, 7.0 Hz), 3.30–3.39 (m, 1H), 3.46 (td, 2H, *J* =13.6, 7.6 Hz), 3.55 (t, 1H, *J* =8.2 Hz), 3.62–3.65 (m, 1H), 3.75–3.82 (m, 2H), 3.84 (dd, 1H, *J* =8.8, 2.9 Hz); ^{13}C NMR (δ, 100 MHz, CD_3OD): 14.5, 23.8, 27.8, 28.0, 30.4, 30.5, 30.8, 33.1, 43.3, 43.4, 56.2, 59.0, 71.9, 75.3, 76.1, 77.2, 163.5. HRMS calculated for $C_{25}H_{49}N_2O_5$: 457.3641 $[M+H]^+$. Found: 457.3665.

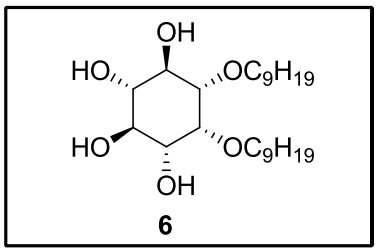
(3a*R*,4*S*,5*R*,6*R*,7*S*,7a*S*)-4,5,6,7-tetrahydroxy-hexahydro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (5)



5

Obtained in 88% yield (16 mg, 0.08 mmol) as a white solid from 50 mg (0.09 mmol) of **23**. $[\alpha]_D^{25}$ – 7.5 (*c* 1.0, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 3.13 (t, 1H, *J* =9.4 Hz), 3.39 (dd, 1H, *J* =7.9, 6.8 Hz), 3.44 (dd, 1H, *J* =9.5, 8.1 Hz), 3.49 (t, 1H, *J* = 9.3 Hz), 3.61 (dd, 1H, *J* =9.3, 4.8 Hz), 4.04 (dd, 1H, *J* =6.7, 4.8 Hz); ^{13}C NMR (δ, 100 MHz, CD_3OD): 58.3, 58.7, 72.5, 74.2, 74.8, 77.5, 166.3. HRMS calculated for $C_7H_{12}N_2O_5Na$: 227.0644 $[M+Na]^+$. Found: 227.0637.

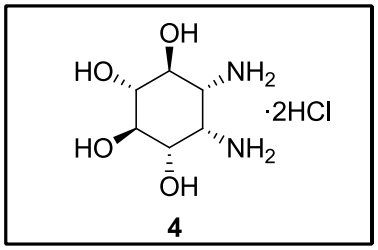
(1*R*,2*S*,3*S*,4*R*,5*R*,6*S*)-5,6-bis(nonyloxy)cyclohexane-1,2,3,4-tetraol (6)



6

Obtained in 89% yield (34 mg, 0.09 mmol) as a white solid from 70 mg (0.09 mmol) of **24**. $[\alpha]_D^{25}$ + 15.4 (*c* 1.0, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 0.90 (t, 6H, *J* =6.9 Hz), 1.25–1.42 (m, 24H), 1.53–1.65 (m, 4H), 3.07 (dd, 1H, *J* =2.3, 9.9 Hz), 3.13 (t, 1H, *J* =9.2 Hz), 3.30–3.36 (m, 1H), 3.51–3.77 (m, 6H), 3.84 (t, 1H, *J* =2.4 Hz); ^{13}C NMR (δ, 100 MHz, CD_3OD): 14.5, 23.8, 27.3, 27.4, 30.49, 30.53, 30.7, 30.82, 30.84, 31.2, 31.4, 33.1, 71.7, 73.6, 73.9, 74.6, 76.8, 79.3, 82.4. HRMS calculated for $C_{24}H_{48}O_6Na$: 455.3349 $[M+Na]^+$. Found: 455.3328.

(1*S*,2*R*,3*R*,4*S*,5*R*,6*S*)-5,6-diaminocyclohexane-1,2,3,4-tetraol dihydrochloride (4)



4

A solution of diamine **18** (86 mg, 0.16 mmol) in anhydrous CH_2Cl_2 (10 mL) at –78 ºC was treated with 1M BCl_3 in heptane (2 equiv for OBn group). The reaction mixture was allowed to room temperature and stirred for an additional 5 h. The mixture was then cooled to –78 ºC and quenched with methanol (0.5 mL). Solvents were then removed under reduced pressure and EtOAc (5 mL) was added next to the oily residue. After sonication in an ultrasonic bath for 1 min, the suspended solid was collected by filtration and dried at vacuum to give 35 mg (0.15 mmol, 87%) of **4** as a white solid. $[\alpha]_D^{25}$ – 6.4 (*c* 1.0, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 3.43 (t, 1H, *J* =7.7 Hz), 3.56 (dd, 1H, *J* =9.8, 3.1 Hz), 3.63 (t, 1H, *J* =8.2 Hz), 3.82–3.90 (m, 2H), 3.91–3.97 (m, 1H); ^{13}C NMR (δ, 100 MHz, CD_3OD): 52.5, 52.7, 69.6, 70.0, 73.4, 75.7. HRMS calculated for $C_6H_{15}N_2O_4$: 179.1032 $[M+H]^+$. Found: 179.1025.

Biological assays

Materials: The glycosidases α-glucosidase (from baker's yeast and rice), β-glucosidase (from almond), β-galactosidase (from bovine liver) and α-galactosidase (from green coffee beans) that were used in the inhibition studies, as well as 4-methylumbelliferyl-β-D-glucoside and the corresponding *p*-nitrophenyl glycoside substrates, were purchased from Sigma. Imiglucerase (Cerezyme®; recombinant human β-glucocerebrosidase analogue) was kindly provided by Genzyme.

Cell Lines and Culture. Wild-type fibroblast and lymphoblasts derived from patients with Gaucher disease homozygous for N370S GCase (GM10873) or L444P GCase (GM08752) were obtained from Eucellbank and Coriell Cell Repositories, respectively. Fibroblast cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin-streptomycin (Invitrogen) at 37 °C in 5% CO_2. Culture medium was replaced every 3-4 days and all cells used in this study were between the 14th and 30th passages. Lymphoblast cell lines were cultured in RPMI-1640 medium (Gibco) supplemented with 15% FBS and 1% penicillin-streptomycin at 37 ºC in 5% CO_2. Culture medium was replaced every 2-3 days, and all cells used in this study were between the 5th and 16th passages. Total protein was determined using the Micro BCA protein assay kit according to the manufacture's instructions (Pierce, Thermo Scientific).

Recombinant GCase (Imiglucerase, Cerezyme®) inhibition assay.[9]

Enzyme solutions (25 μL from a stock solution containing 0.1 mg mL^{-1}) in the presence of 0.25% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 in McIlvaine buffer (100 mM sodium citrate and 200 mM sodium phosphate buffer, pH 5.2 or pH 7.0) were incubated at 37 ºC without (control) or with inhibitor at a final volume of 40 μL for 30 min. After addition of 60 μL 4-methylumbelliferyl-β-D-glucopyranoside (4 mM, McIlvaine buffer, pH 5.2 or pH 7.0), the samples were incubated at 37 ºC for 10 min. Enzymatic reactions were stopped by the addition of aliquots (150 μL) of glycine/NaOH buffer (100 mM, pH 10.6). The amount of 4-methyumbelliferone formed was determined with a SpectraMax M5 (Molecular Devices Corporation) at 355 nm (excitation) and 460 nm (emission).

Inhibition parameters: The IC_{50} values were determined by plotting percent activity *versus* log [I], using at least five different inhibitor concentrations. Type of inhibition and K_i values for more active inhibitors were determined by Lineweaver-Burk or Dixon plots of assays performed with different concentrations of inhibitor and substrate.

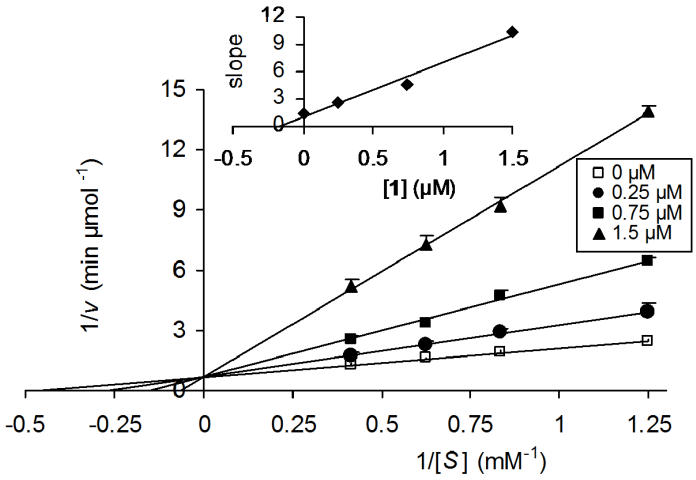


Figure S1. Lineweaver-Burk plot for the inhibition of Imiglucerase by **1** at pH 5.2 (K_i=0.17 μM)

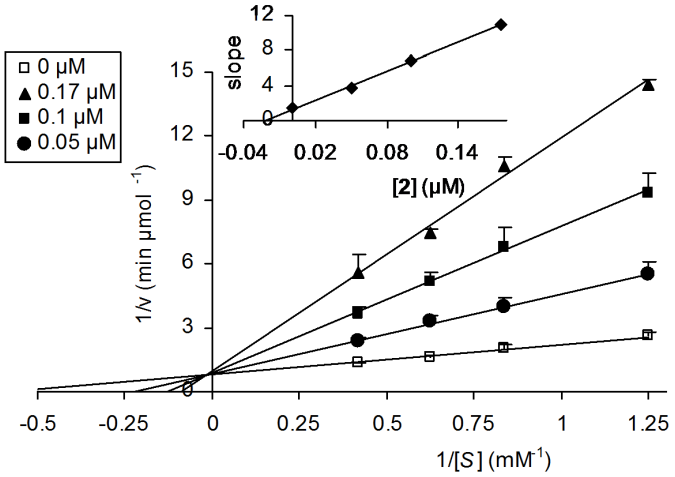


Figure S2. Lineweaver-Burk plot for the inhibition of Imiglucerase by **2** at pH 5.2 (K_i=0.026 μM)

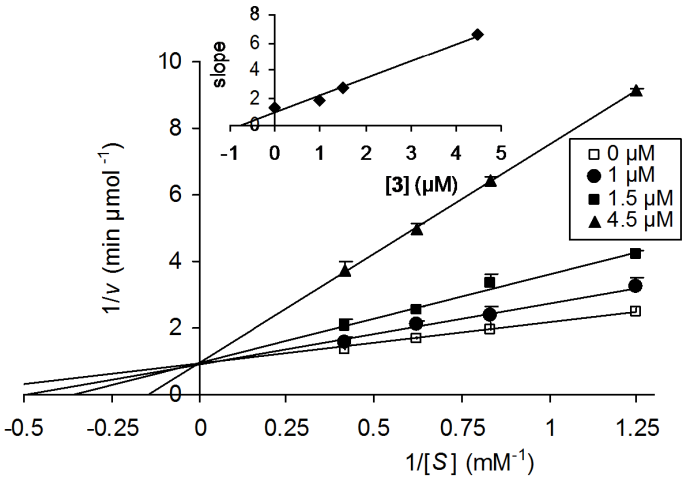


Figure S3. Lineweaver-Burk plot for the inhibition of Imiglucerase by **3** at pH 5.2 (K_i=0.70 μM)

Effects of compounds on the activity of other glycosidases and GCS

Table S1. Activity of Compounds against Commercial Glycosidases and Glucosylceramide Synthase (GCS)

Compound	α-Glucosidase (baker's yeast)[a]	α-Glucosidase (rice)[a]	β-Glucosidase (almond)[a]	α-galactosidase (from green coffee beans)[a]	β-galactosidase (from bovine liver)[a]	GCS[b]
1	5	0	31	0	92	0
2	0	0	0	0	44	73 0[c]
3	0	0	1	0	55	8
4	4	0	0	1	24	0
5	0	0	6	0	3	0
6	0	0	0	0	20	0

[a] % Inhibition at 100 μM. [b] % Inhibition at 250 μM. [c] % Inhibition at 50 μM.

Inhibition assay against commercial glycosidases.[9]

Commercial enzyme solutions were prepared with the appropriate buffer and incubated in 96-well plates at 37 ºC without (control) or with inhibitor for 5 min. After addition of the corresponding substrate solution, incubations were prolonged for different time periods: 3 min for β-glucosidase (from almond) and α-glucosidase (from baker's yeast), 5 min for β-galactosidase and 10 min for α-glucosidase (from rice), 13 min for α-galactosidase and stopped by addition of Tris solution (50 μL, 1M) or Na_2CO_3 (180 μL, 1M), depending on the enzymatic inhibition assay. The amount of *p*-nitrophenol formed was determined at 405 nm with a SpectraMax M5 (Molecular Devices Corporation) spectrophotometer. For α-glucosidase (from rice), the activity was determined with *p*-nitrophenyl-α-D-glucopyranoside (1 mM) in sodium acetate buffer (50 mM, pH 5.0). For α-glucosidase (from baker yeast), the activity was determined with *p*-nitrophenyl-α-D-glucopyranoside (1 mM) in sodium phosphate buffer (100 mM, pH 7.2). For β-glucosidase (from almond), the activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (1 mM) in sodium acetate buffer (100 mM, pH 5.0). β-galactosidase activity was determined with *p*-nitrophenyl-β-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, 0.1 mM $MgCl_2$, pH 7.2). α-galactosidase activity was determined with *p*-nitrophenyl-α-D-galactopyranoside (1 mM) in

sodium phosphate buffer (100 mM, pH 6.8). The commercial glycosidase solutions were prepared as follows: α-glucosidase (from rice) $(NH_4)_2SO_4$ suspension (30 μL) in buffer (1.8 mL); α-glucosidase (from baker's yeast): (0.1 mg mL^{-1} buffer); β-glucosidase (from almond): (0.1 mg mL^{-1} buffer); α-galactosidase (from green coffee beans): 7.4 μL in buffer (1.99 mL); β-galactosidase from bovine liver (0.5 mg mL^{-1} buffer).

Glucosylceramide Synthase assay.

A549 Cells were washed with sodium phosphate (PBS) (10 mM, 137 mM NaCl, pH=7.4) and collected by brief trypsinization. The cells were then washed twice with PBS and resuspended in 50 mM TRIS-HCl buffer (pH 7.4) and 10 mM $MgCl_2$ by sonication (three times, 30 seconds). The cell lysate (100 μL) was incubated with inhibitor (250 μM or 50 μM final concentration) for 10 min at 37 ºC. Then 25 μL of NAD (16 mM in TRIS-HCl, pH 7.4 and 10 mM $MgCl_2$), 25 μL of UDP-Glucose (2 mM in TRIS-HCl 50 mM, pH 7.4 and 10 mM $MgCl_2$) and 52 μL of NBD C_6-ceramide complexed to BSA at a 1:1 ratio (20 μM in 50 mM TRIS-HCl buffer, pH=7.4, 10 mM $MgCl_2$) were added. After 15-min incubation at 37 ºC, the reactions were stopped by adding 800 μL of MeOH and centrifuged at 10000 rpm for 3 min. The supernatant was transferred to HPLC vials. HPLC analyses were performed with a Waters 2690 Alliance System coupled to a Waters 2475 Fluorescence detector (Milford, MA) using a C18-Kromasil column and eluted with 15% water and 85% acetonitrile, both with a 0.1% of trifluoroacetic acid, flowing at 1 mL/min. The detector was set at an excitation wavelength of 465 nm and measure the emission wavelength at 530 nm. Empower Software (Waters Corporation) was utilized for data acquisition and processing.

Thermal Stabilization assay using recombinant GCase (imiglucerase).[10]

Imiglucerase aliquots (48 μL, 2 mg/mL) were incubated with three different concentrations of chemical chaperone at 48 ºC. Subsequently, 150 μL of 0.1 M acetate-phosphate buffer (pH 5.0) and 100 μL of 4-methylumbelliferyl-β-D-glucopyranoside (4 mM, 0.1% Triton X-100 and 0.2% sodium taurocholate in McIlvaine buffer, pH 5.2) were added at different times and incubated for additional 10 min at 37 ºC. Then, 300 μL of glycine/NaOH buffer (100 mM, pH 10.6) were added and liberated 4-methylumbelliferone was measured (excitation wavelength 355 nm, emission wavelength 460 nm). Enzyme activity was reported relative to unheated (37 ºC) enzyme. The effect of compounds as potential chaperones was evaluated in triplicate for each experiment.

Table S2. Stabilization Ratios of Compounds 1–4 and NN-DNJ after Thermal Denaturation (48 ºC) for 20, 40, and 60 min at the Indicated Inhibitor Concentrations.

Compound	Inhibitor concentration (μM)	Stabilization ratios		
		20 min	40 min	60 min
1	1	1.16	1.40	1.97
	10	1.83	4.29	5.50
	25	2.27	7.43	17.50
2	1	2.25	9.25	16.84
	10	3.8	17	34.75
	25	3.8	16.62	36.25
3	1	0.98	1.14	1.03
	10	1.18	1.40	1.29
	25	1.60	2.80	2.29
4	50	1.09	1.27	1.03
	100	1.22	1.50	1.14
	150	1.33	1.90	1.29
NN-DNJ	50	1.77	3.6	7.6
	100	2.01	5.34	9.22
	150	2.56	7.73	15.17

Cytotoxicity assay in wild-type human fibroblasts.

Wild-type fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; D5796; Sigma-aldrich) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in 5% CO_2/95% air. Cells used were between the 14th and 30th passage. At the time of the experiments, cells were seeded at a density of 25000 cells per well in 96-well plates. Media were renewed after 24 h and compounds were added to give final concentrations of 300-18 μM or 300-2 μM (for compounds with CC_{50} $\leq$ 15 μM). All compounds were dissolved in DMSO and control experiments were performed with DMSO. Cells were incubated at 37 °C in 5% CO_2 for 24 h. Then, the media were replaced with 100 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution and the mixture was incubated for additional 3 h at 37 °C in 5% CO_2/95% air. The number of viable cells was quantified by the estimation of its dehydrogenase activity, which reduces MTT to water-insoluble formazan, which was dissolved in 100 μL of DMSO and measured at 570 nm with SpectraMax M5 (Molecular Devices Corporation) in 96-well format.

Table S3. Cytotoxicity of Compounds 1–4 in Wild-Type Human Fibroblasts.

Compound	Cytotoxicity CC_{50} (μM)[a]
1	>300
2	16.6
3	60.7
4	>300

[a] Wild-type fibroblasts were treated with different concentrations of compounds for 24 h, and the cytotoxicity was evaluated as described in the experimental procedures. The CC_{50} values were obtained by regression analysis of the dose-response curves obtained in a single experiment with triplicates.

GCase inhibition in wild-type human fibroblasts.[10]

Fibroblasts were seeded at a density of 10^5 cells per well in 24-well plates. After 24 h, the media were replaced with fresh media with or without a test compound and incubated at 37 °C in 5% CO_2 for 24 h. The enzyme activity assay was performed after removing media supplemented with the corresponding compound. The monolayers were washed with 100 μL of PBS solution. Then, 80 μL of PBS and 80 μl of 200 mM acetate buffer (pH 4.0) were added to each well. The reactions were started by the addition of 100 μL of 5 mM 4-methylumbelliferyl-β-D-glucopyranoside (200 mM acetate buffer, pH 4.0) to each well, followed by incubation at 37 °C for 2 h. Enzymatic reactions were stopped by lysing the cells with 1.8 ml of glycine/NaOH buffer (100 mM, pH 10.6). Liberated 4-methylumbelliferone was measured (excitation 355 nm, emission 460 nm) with SpectraMax M5 fluorometer (Molecular Devices Corporation) in 24-well format. All determinations were performed in triplicate. Cells used were between the 14th and 30th passages.

Measurement of L444P or N370S GCase activity in lymphoblasts derived from patients with Gaucher disease.

Lymphoblasts were seeded at a density of $2x10^5$ cells per well in 1 mL of supplemented RPMI-1640 medium in 12-well plates. Cells were incubated in the absence or presence of various concentrations of compounds for 3 days before GCase activity was measured. After washing with phosphate buffered saline (PBS) twice, the cell pellets were lysed in water by sonication.

All enzyme activation measurements were made using aliquots of homogenate (10 μL) and 6 mM of 4-methylumbelliferyl-β-D-glucopyranoside (50 μL) in 0.1 M citrate phosphate buffer, pH 5.2 containing 0.25% sodium taurocholate, 0.1% Triton X-100 and incubated at 37 ºC for 2 h. The enzyme reactions were stopped with 150 μL of glycine/NaOH buffer (200 mM, pH 10.6) and fluorescence was measured

(excitation wavelength 355 nm, emission wavelength 460 nm) with SpectraMax M5 (Molecular Devices Corporation) in 96-well format.

The non-specific GCase activity was evaluated by addition of conduritol B epoxide[11] (500 μM) to control wells and was shown to account for about 2% of the total activity in control cells.

Supporting Information References

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(10) GCase activity assay in fibroblasts and thermal stabilization assay using imiglucerasa were performed as described in the literature with slight modifications: Sawkar, A. R.; Cheng, W.-C.; Beutler, E.; Wong, C.-H.; Balch, W. E.; Kelly, J. W. Chemical chaperones increase the cellular activity of N370S β-glucosidase: a therapeutic strategy for Gaucher disease. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15428-15433.

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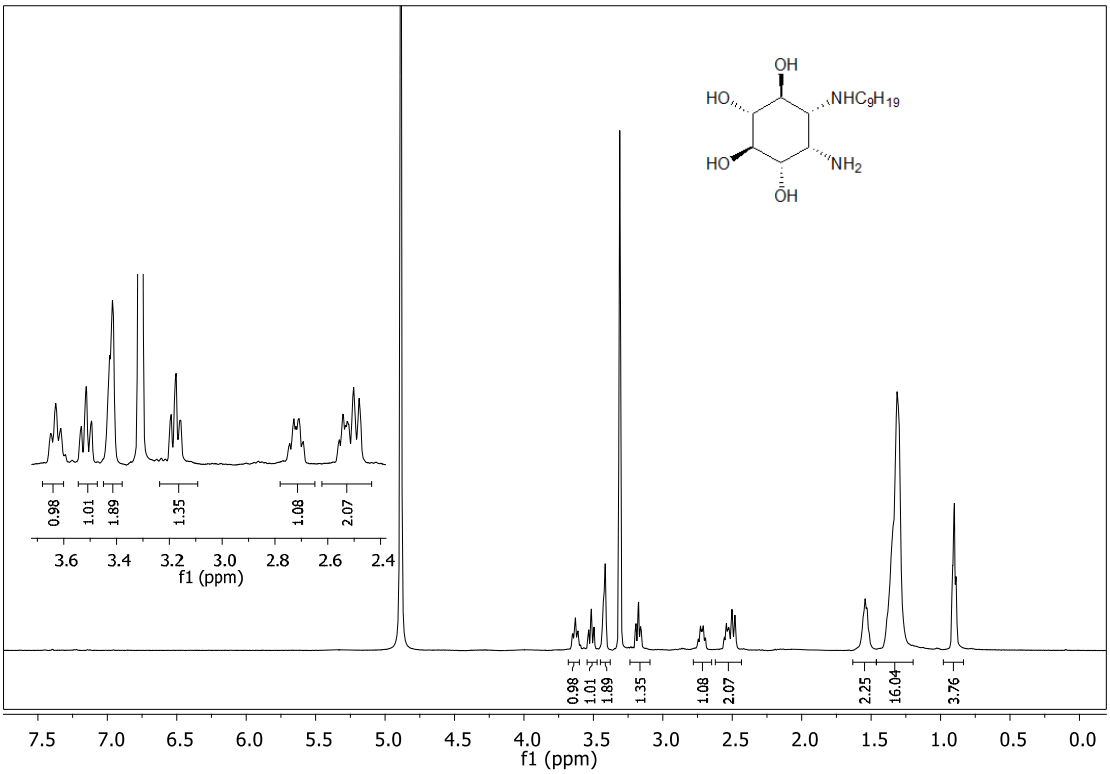
^{1}H NMR spectra (500 MHz, CD_3OD) of **1**



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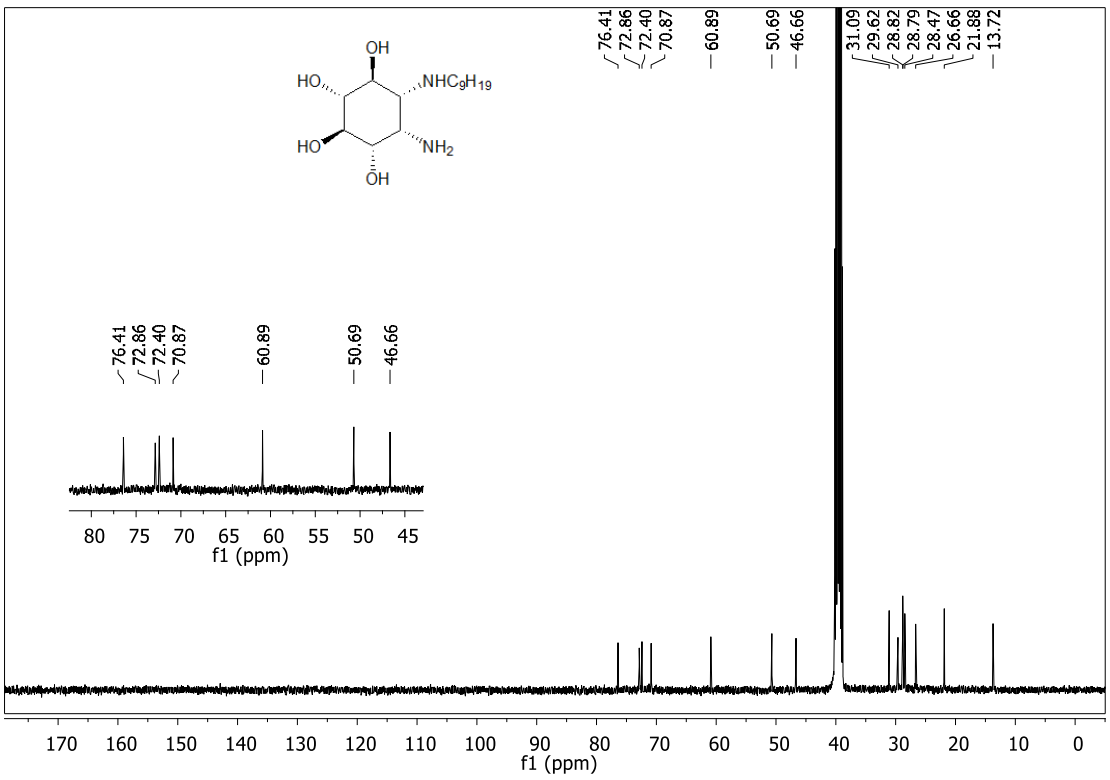

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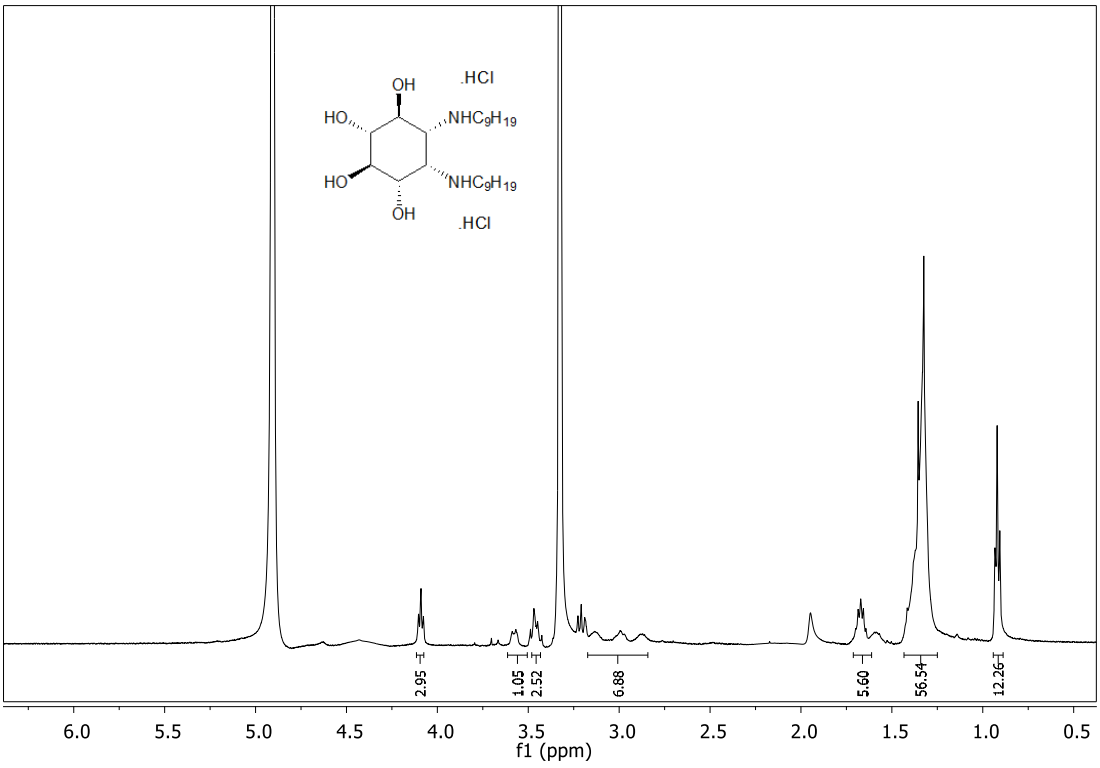

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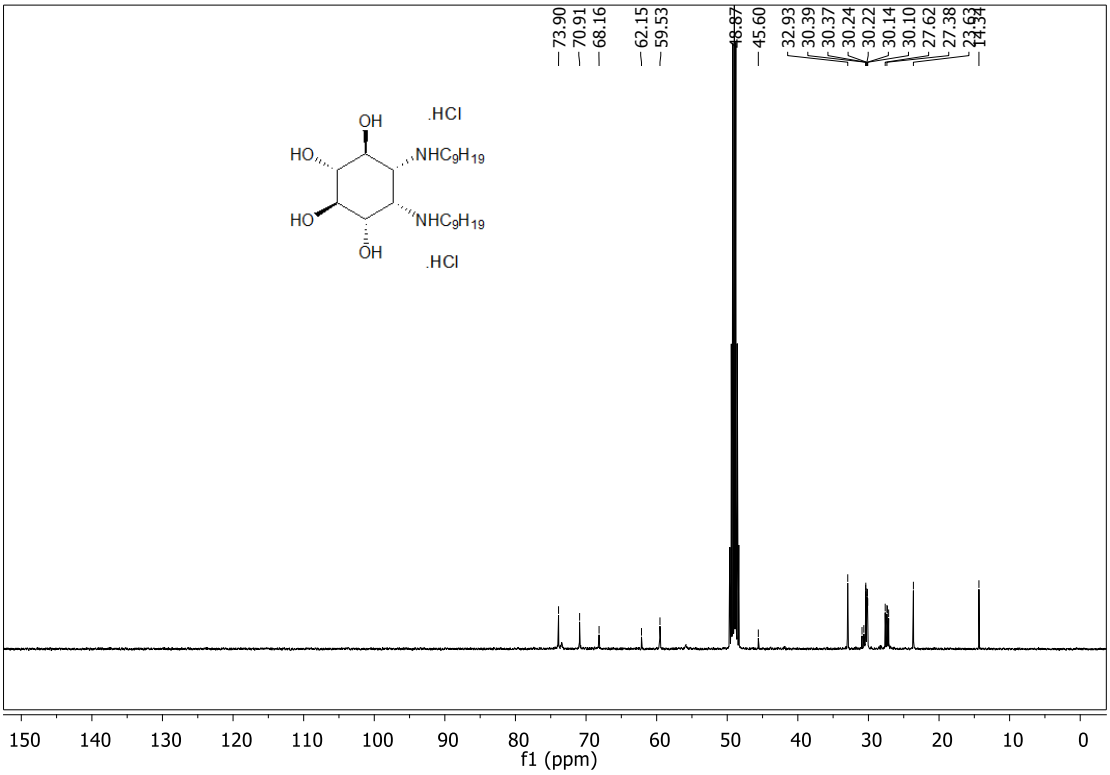

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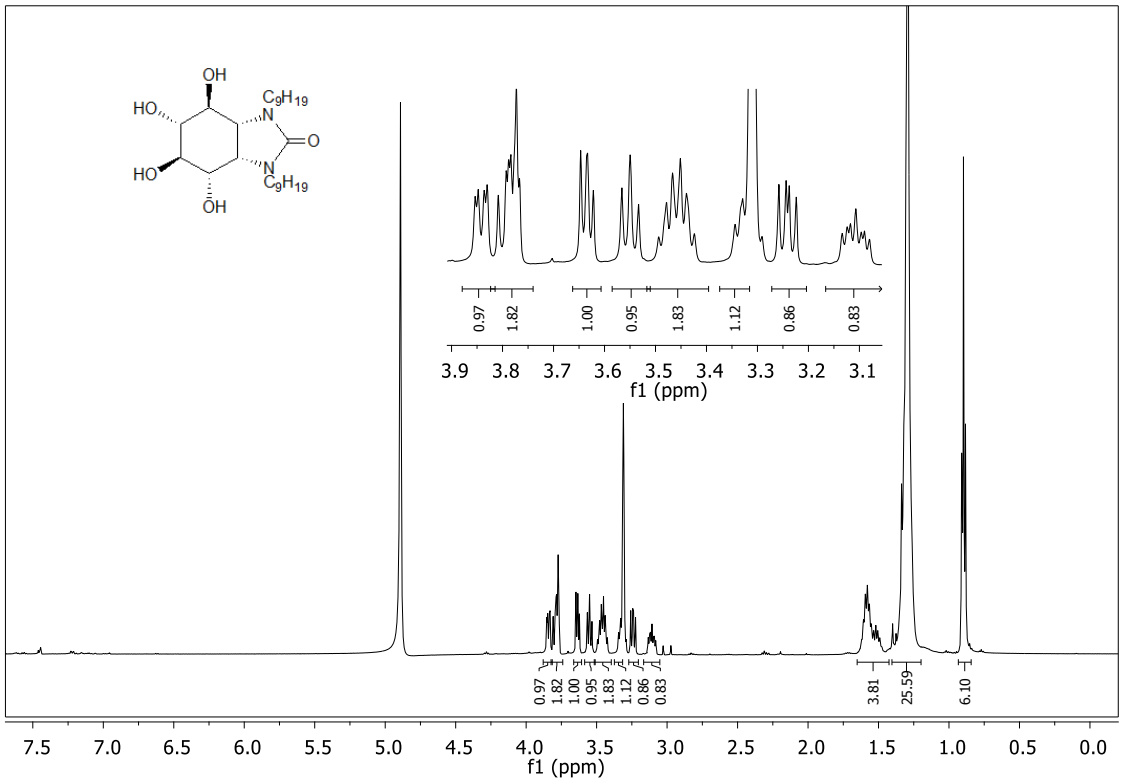

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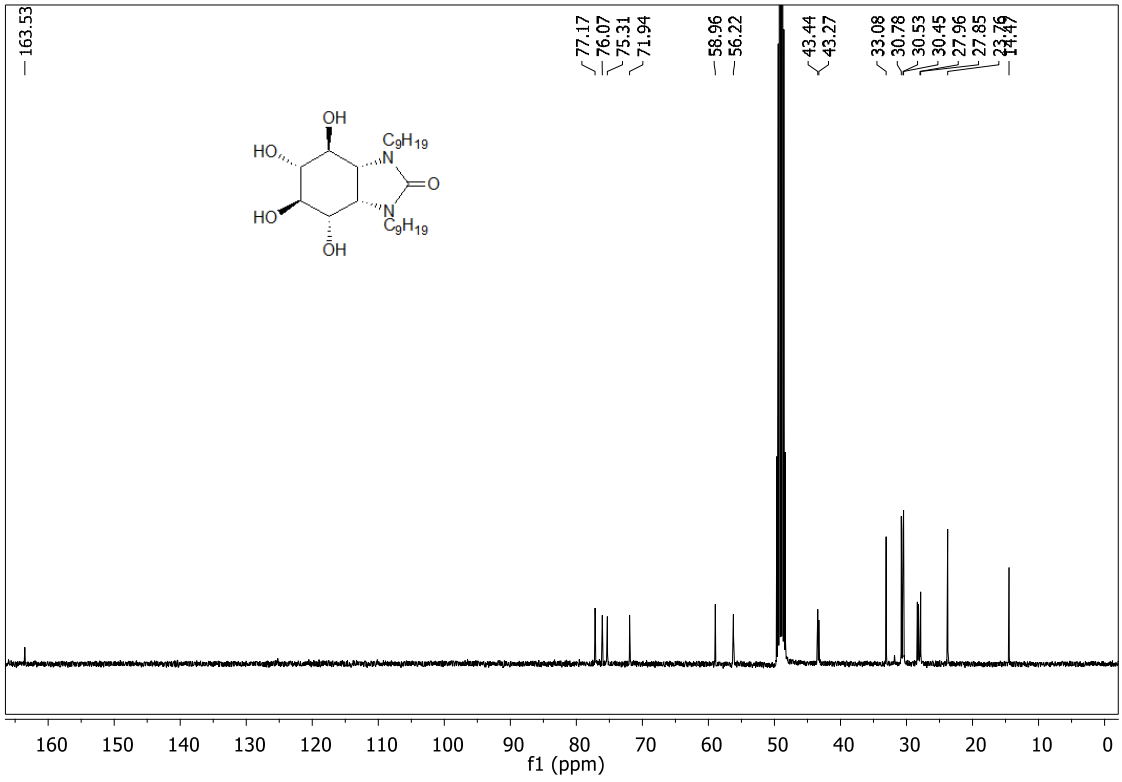

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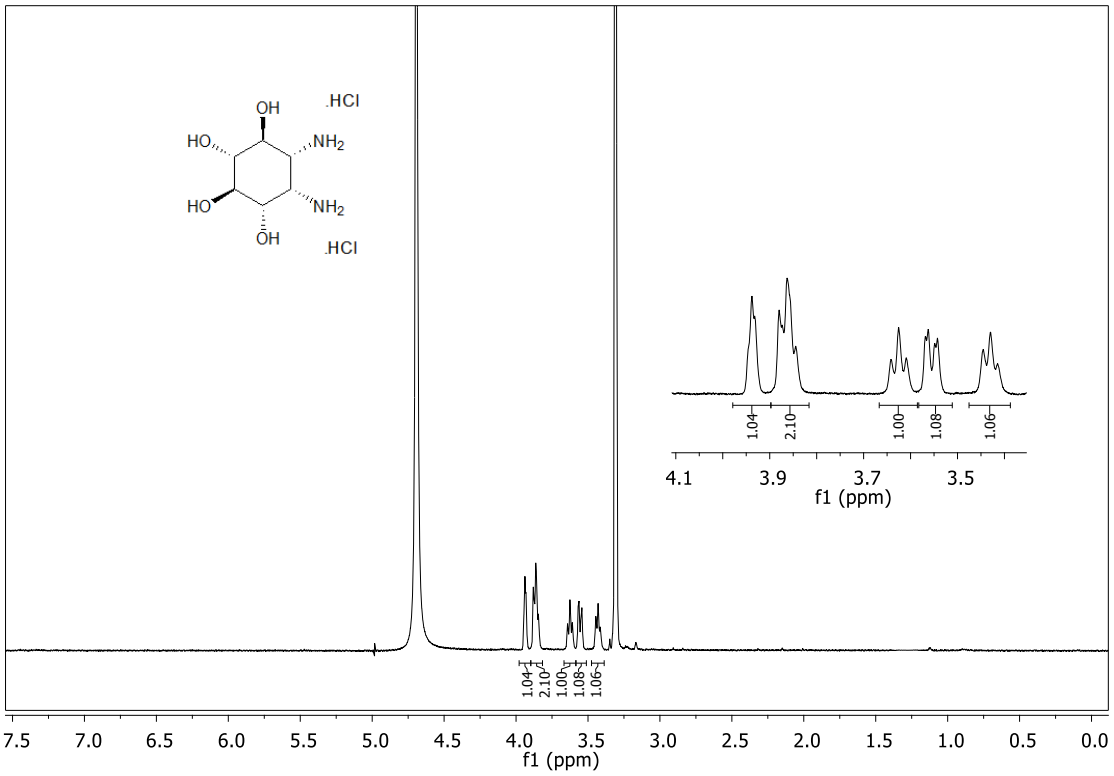

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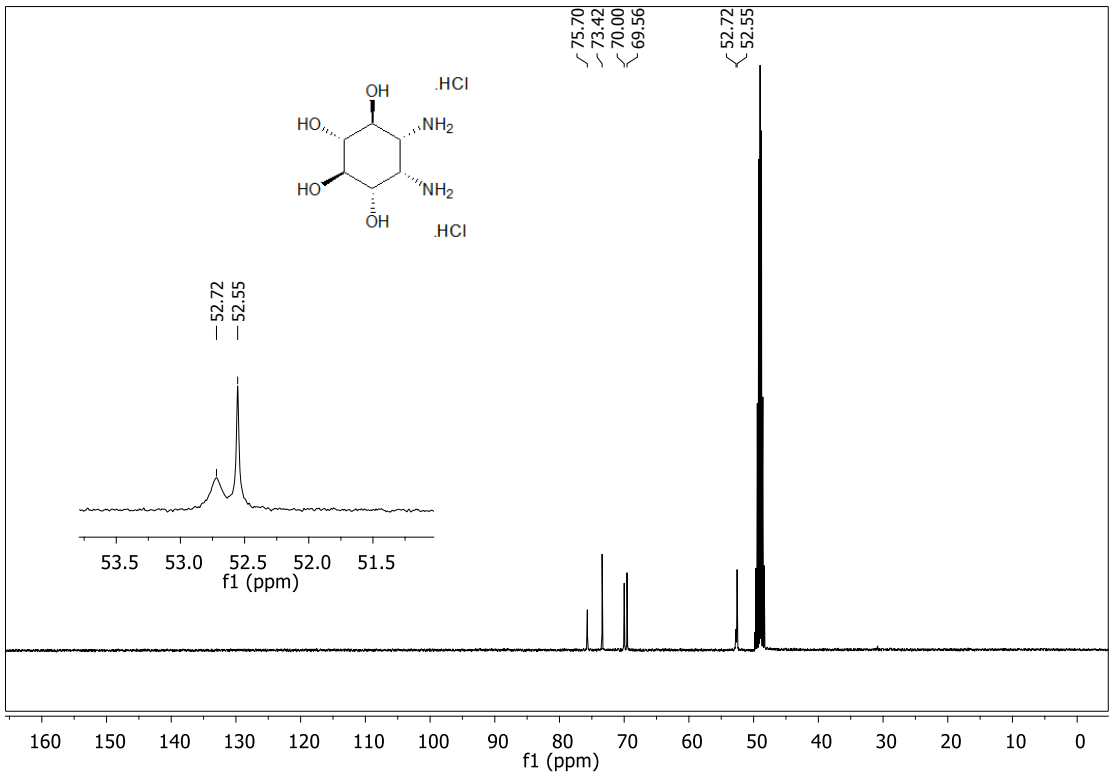

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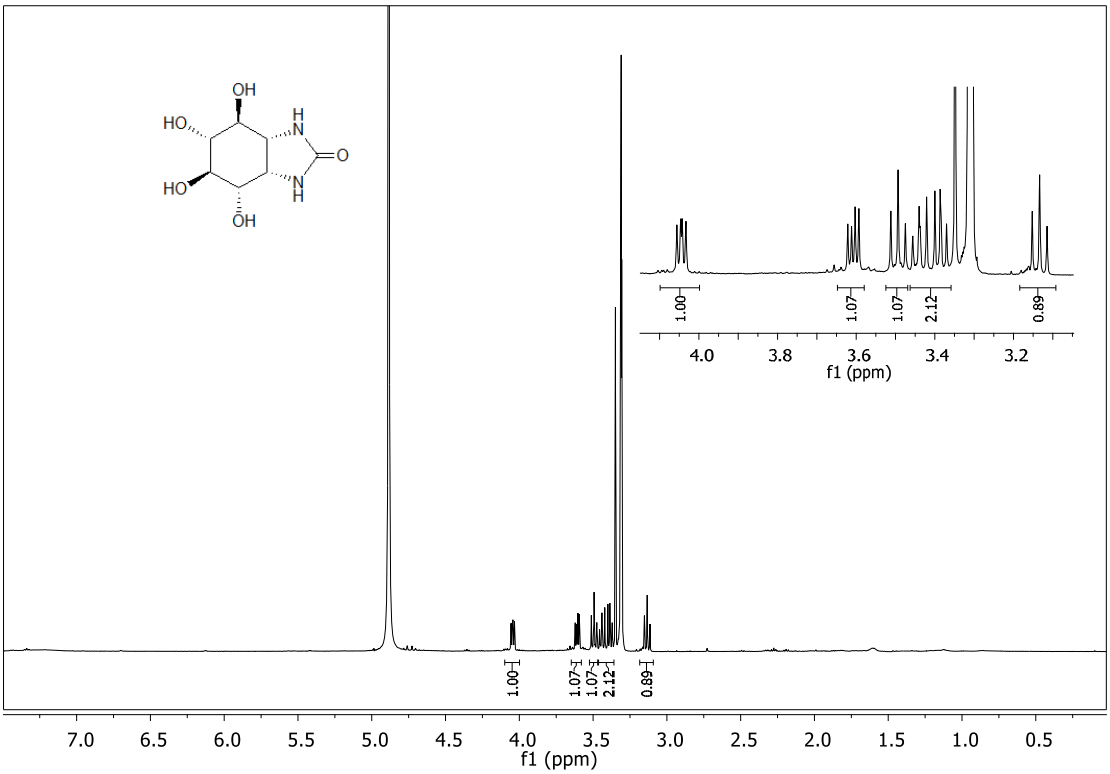

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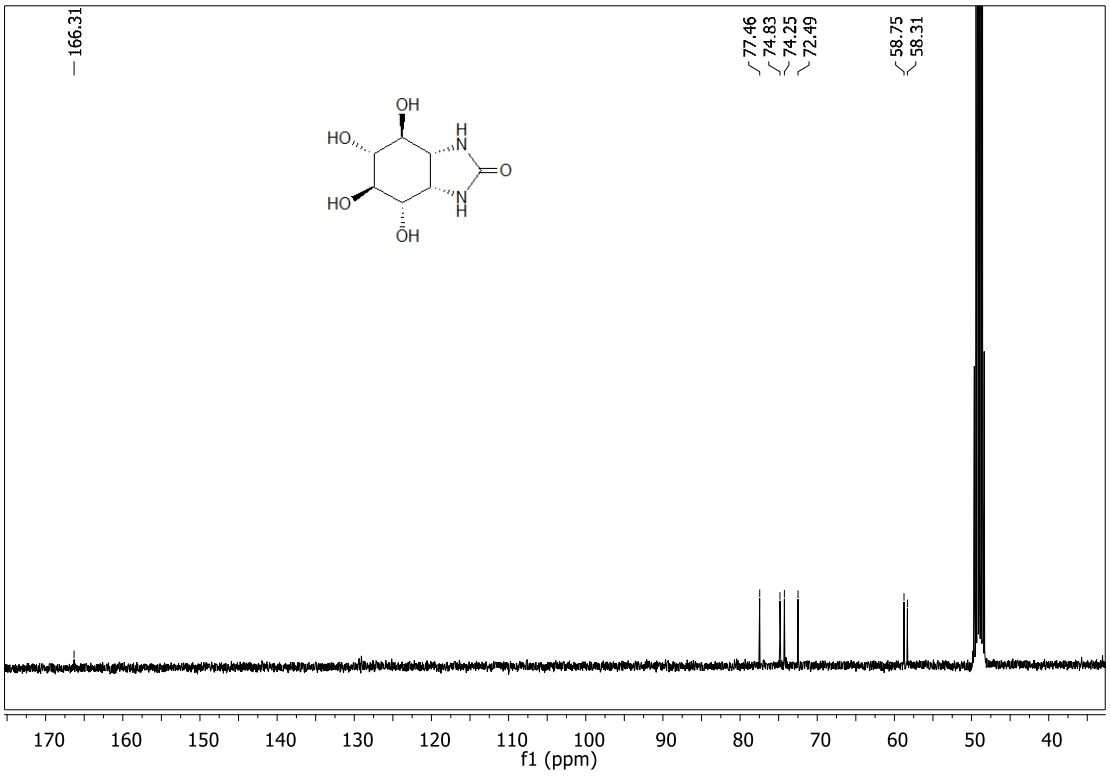

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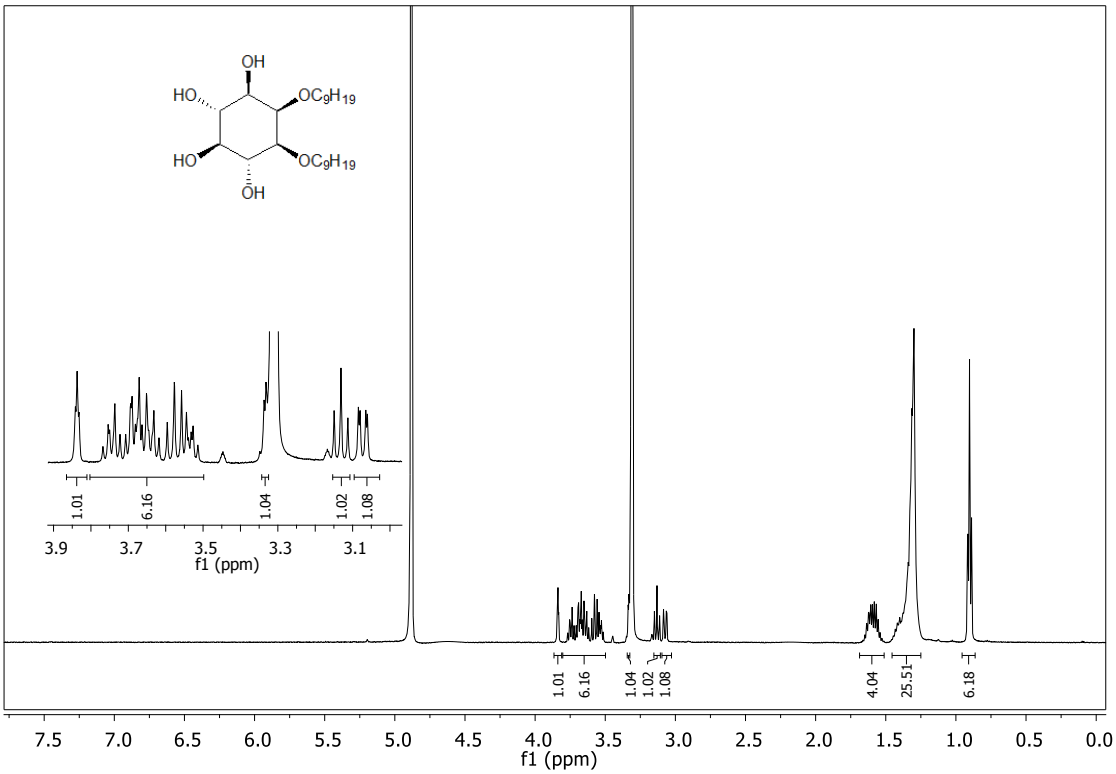

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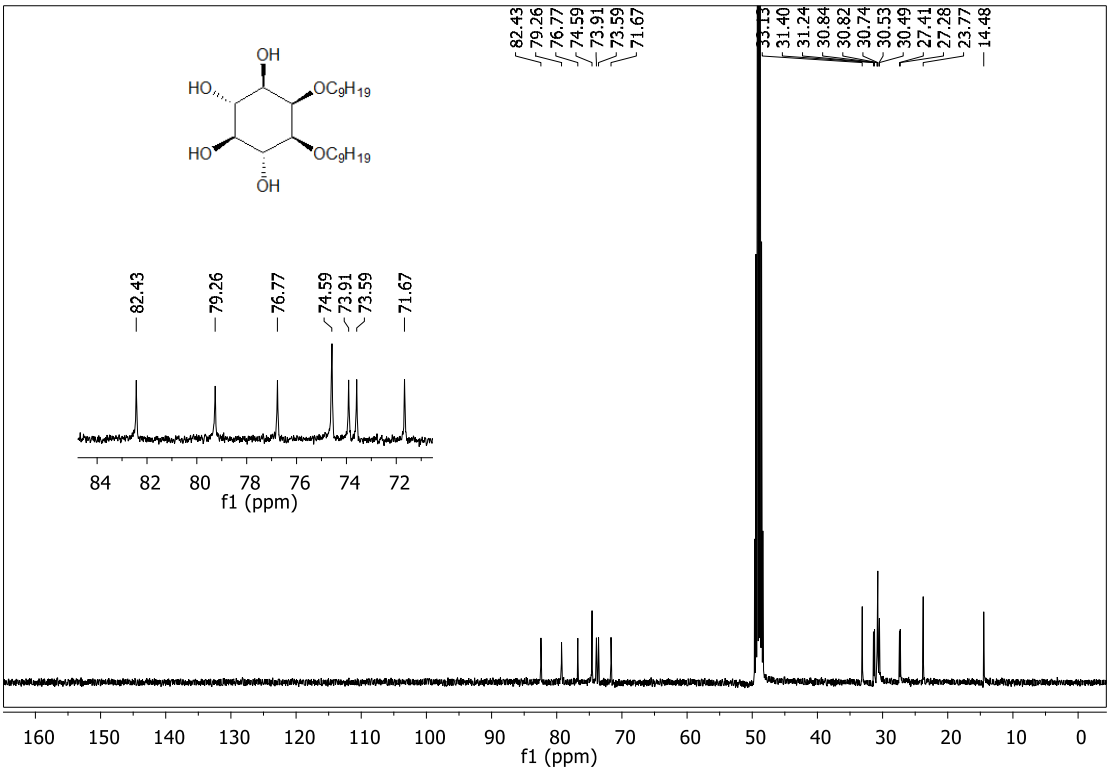

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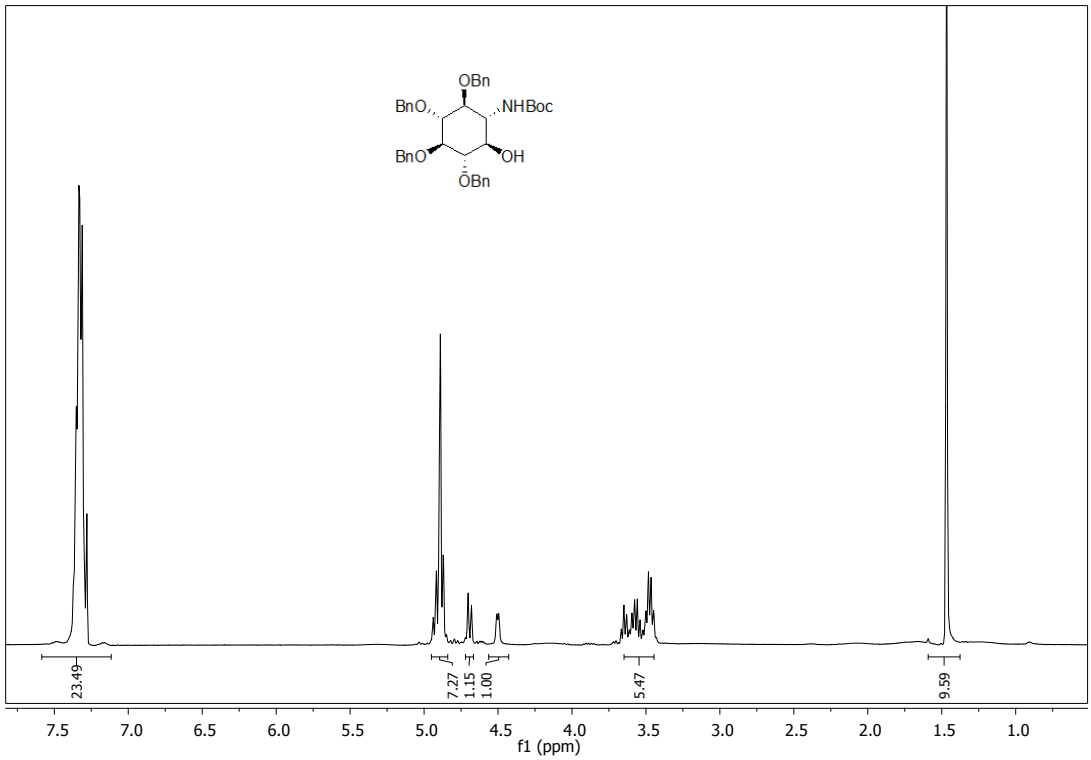

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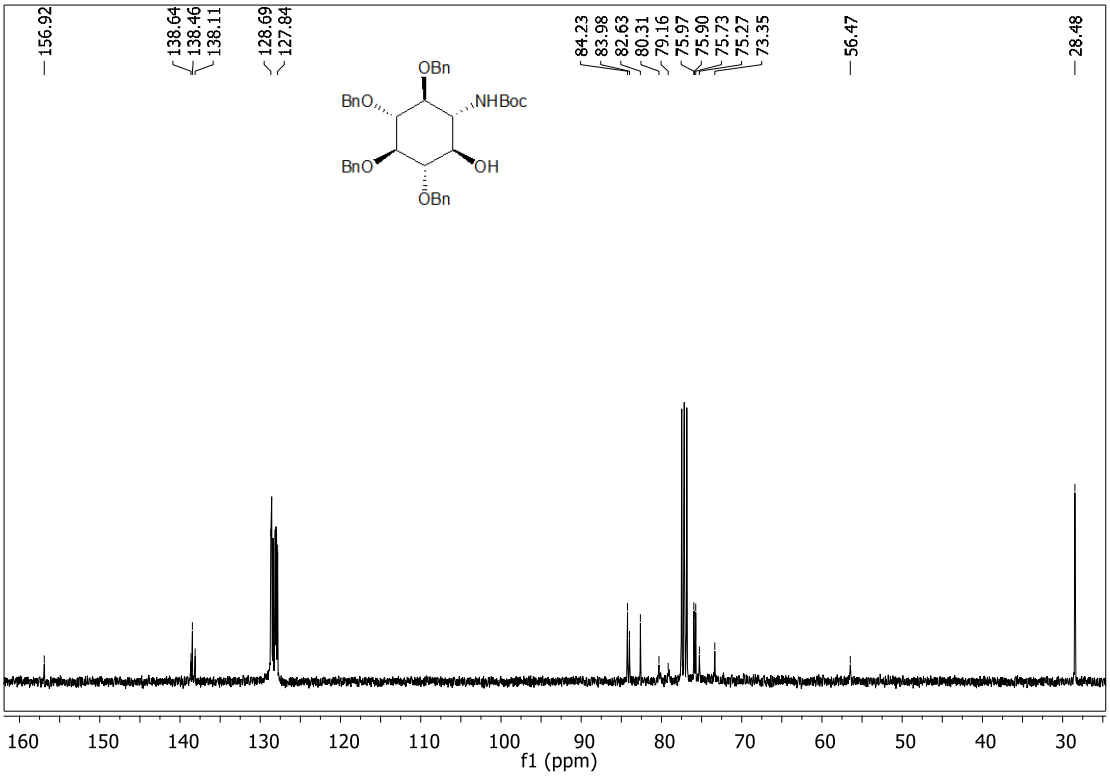

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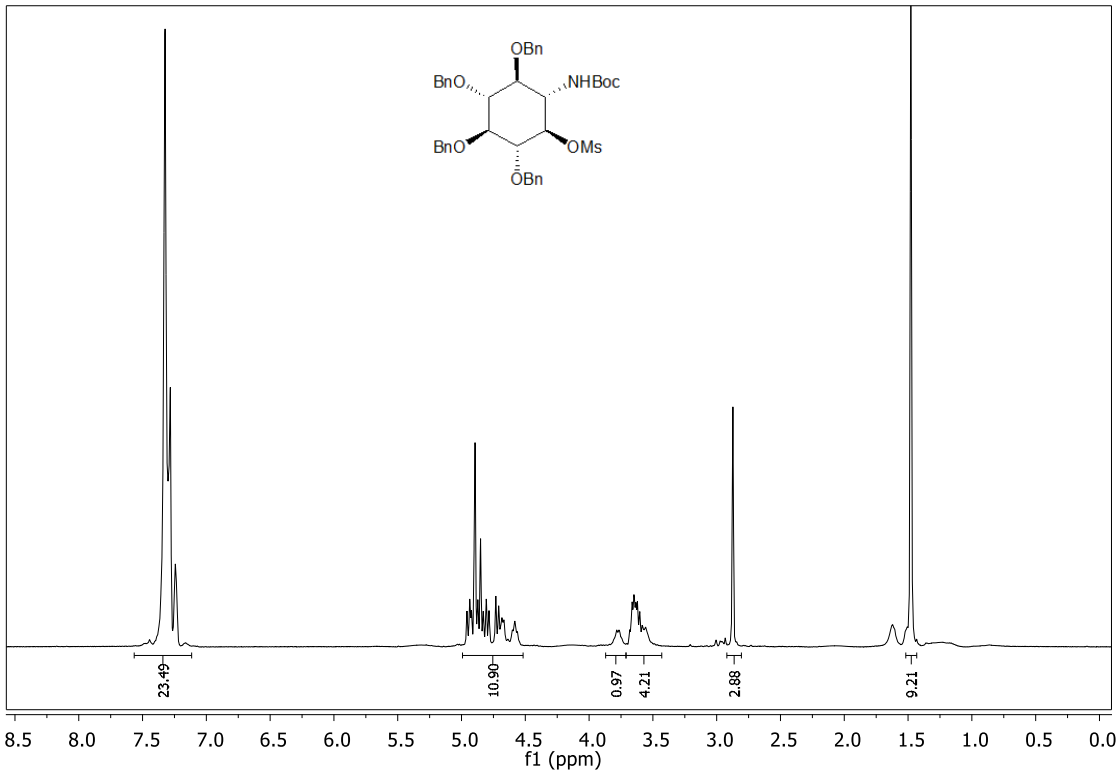

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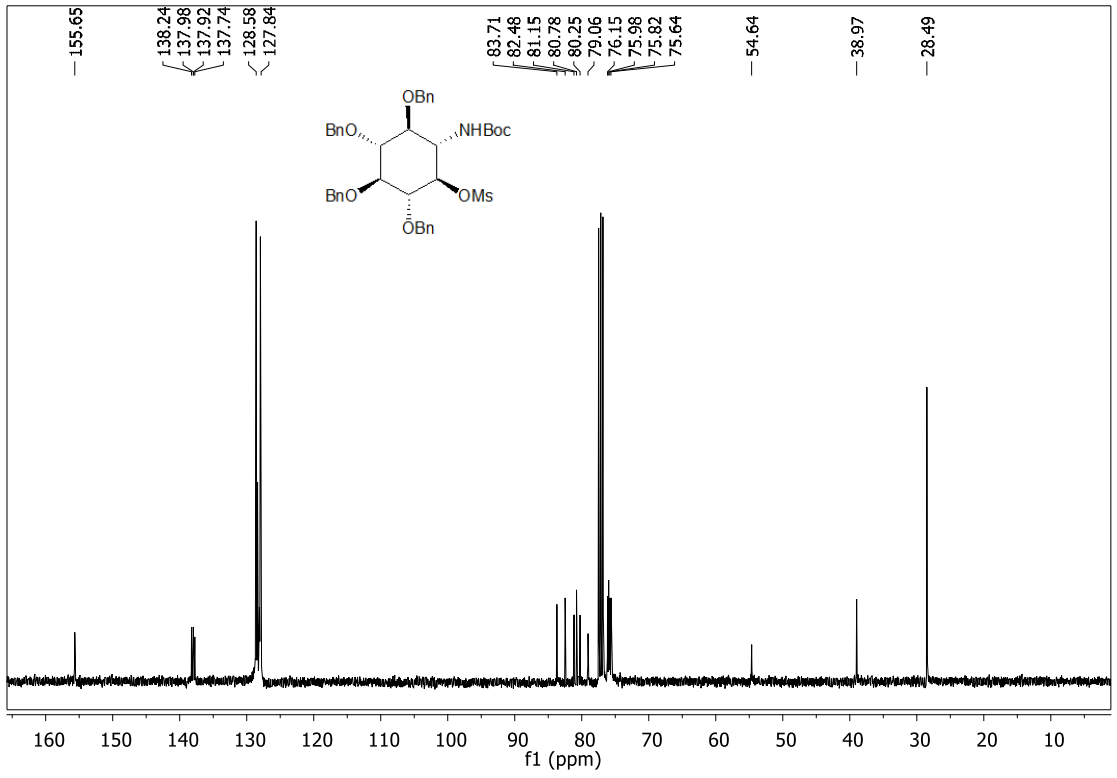

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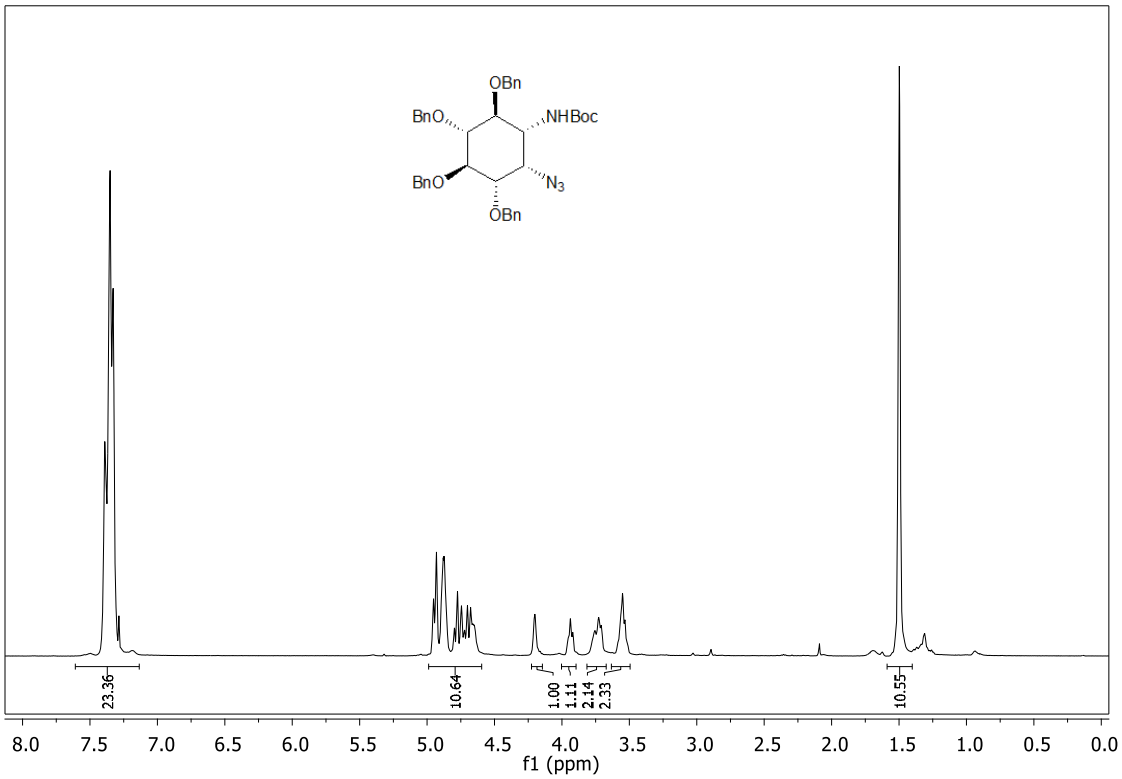

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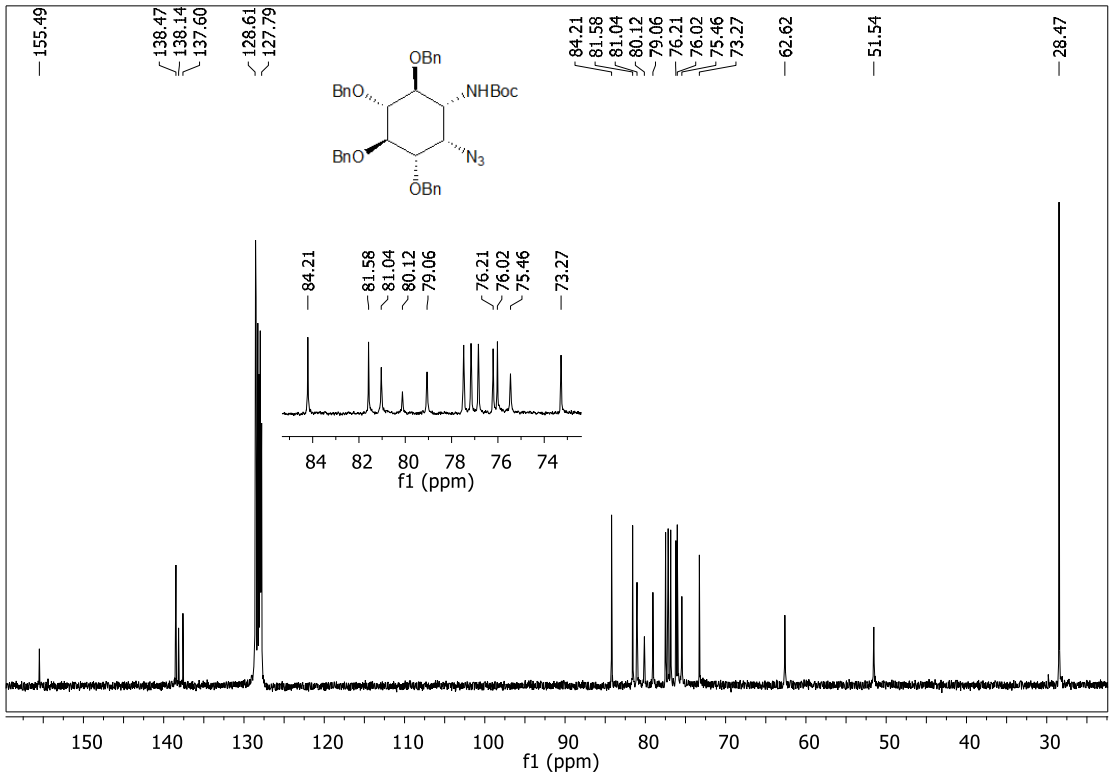

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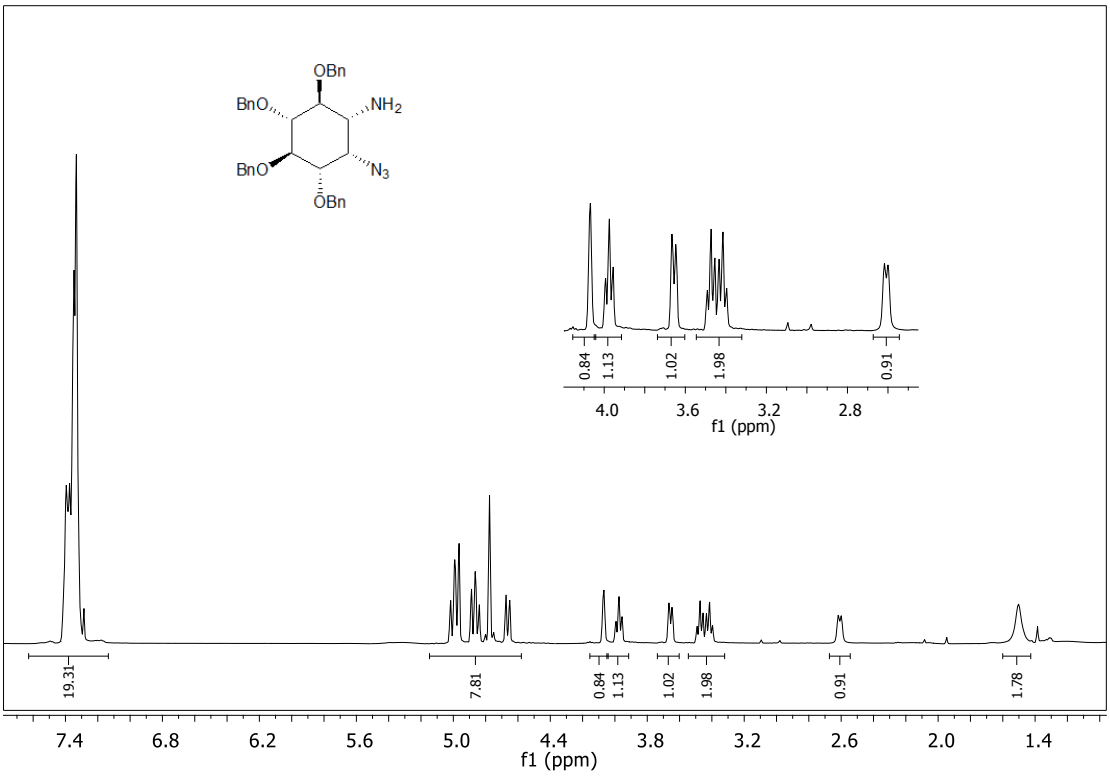

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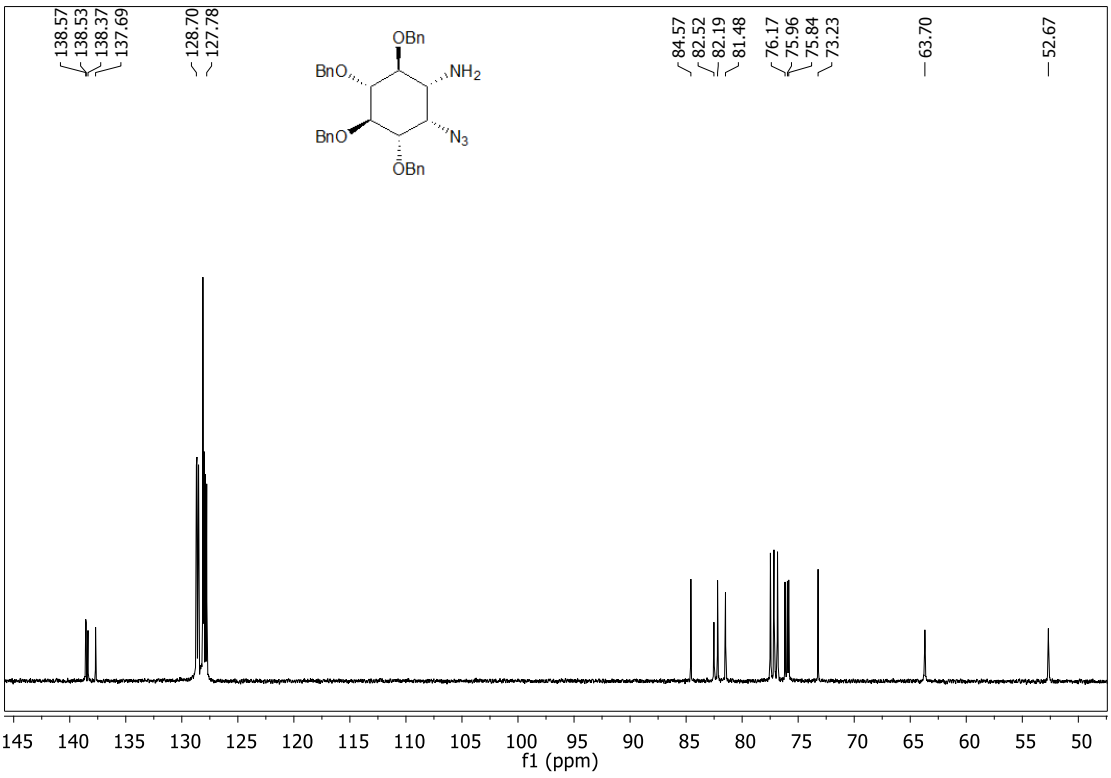

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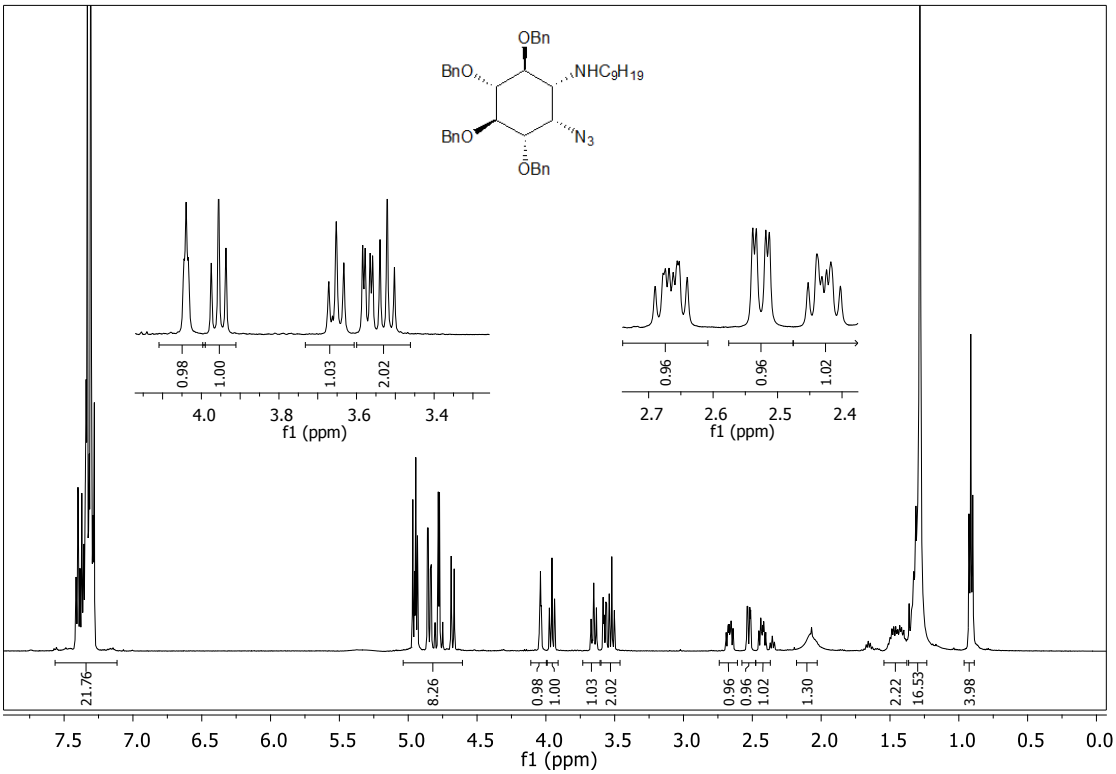

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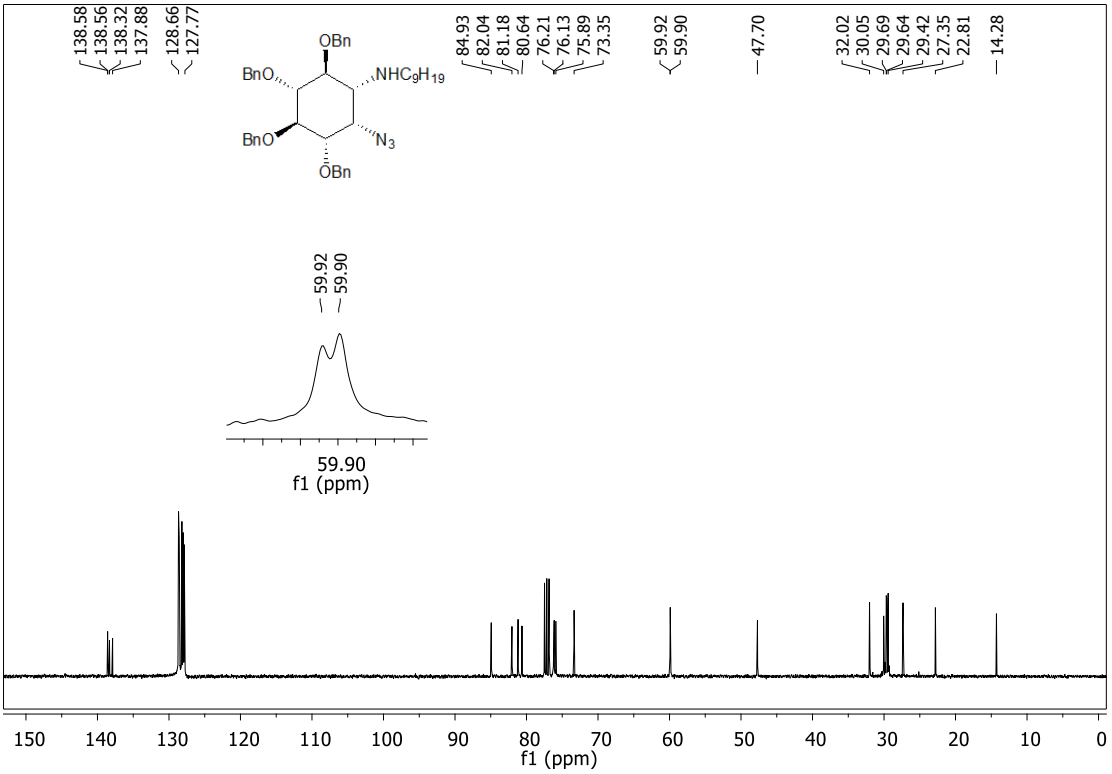

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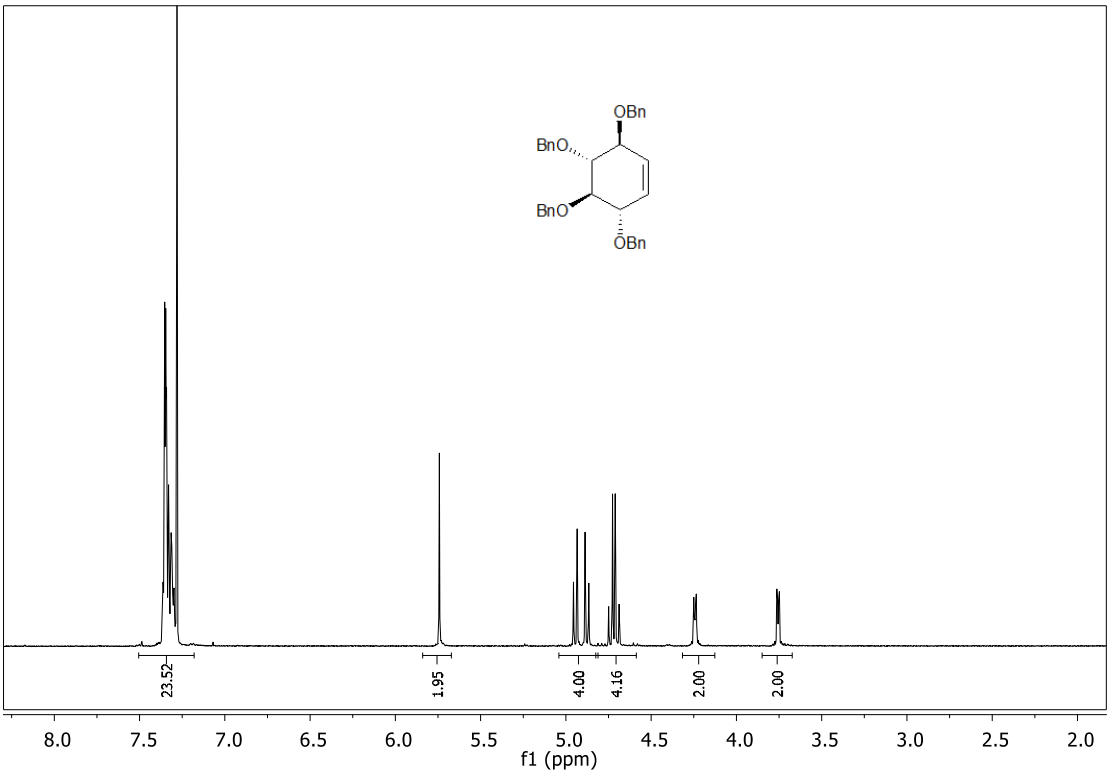

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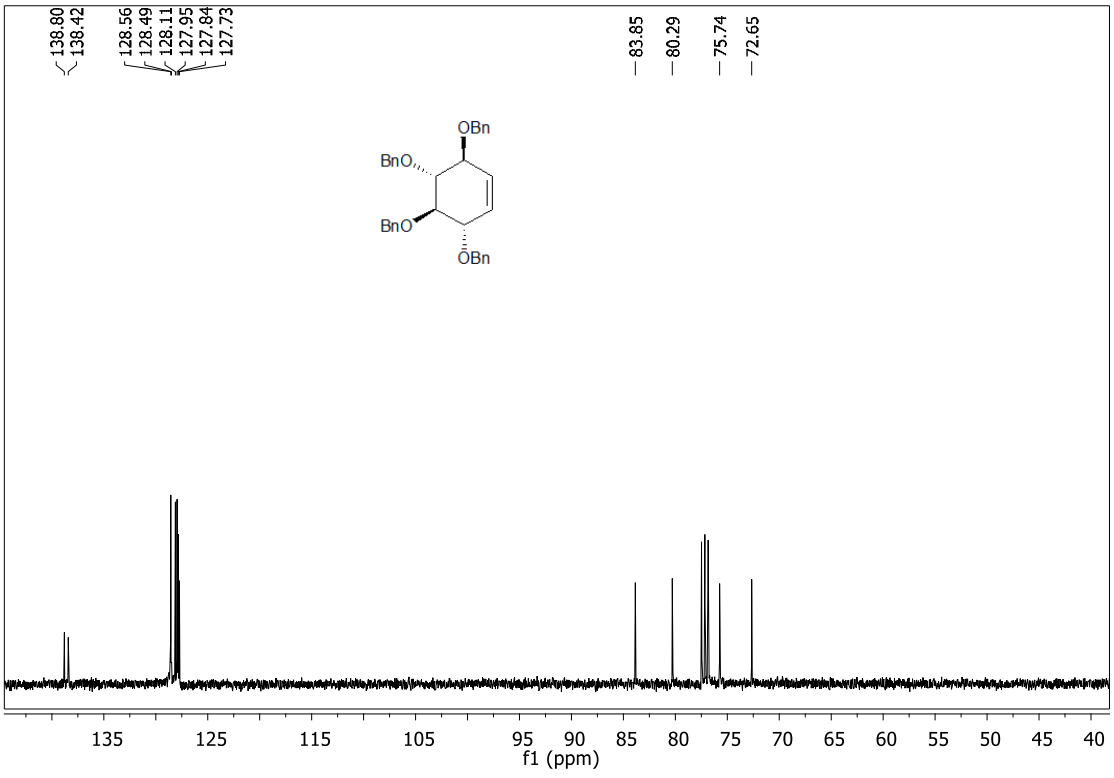

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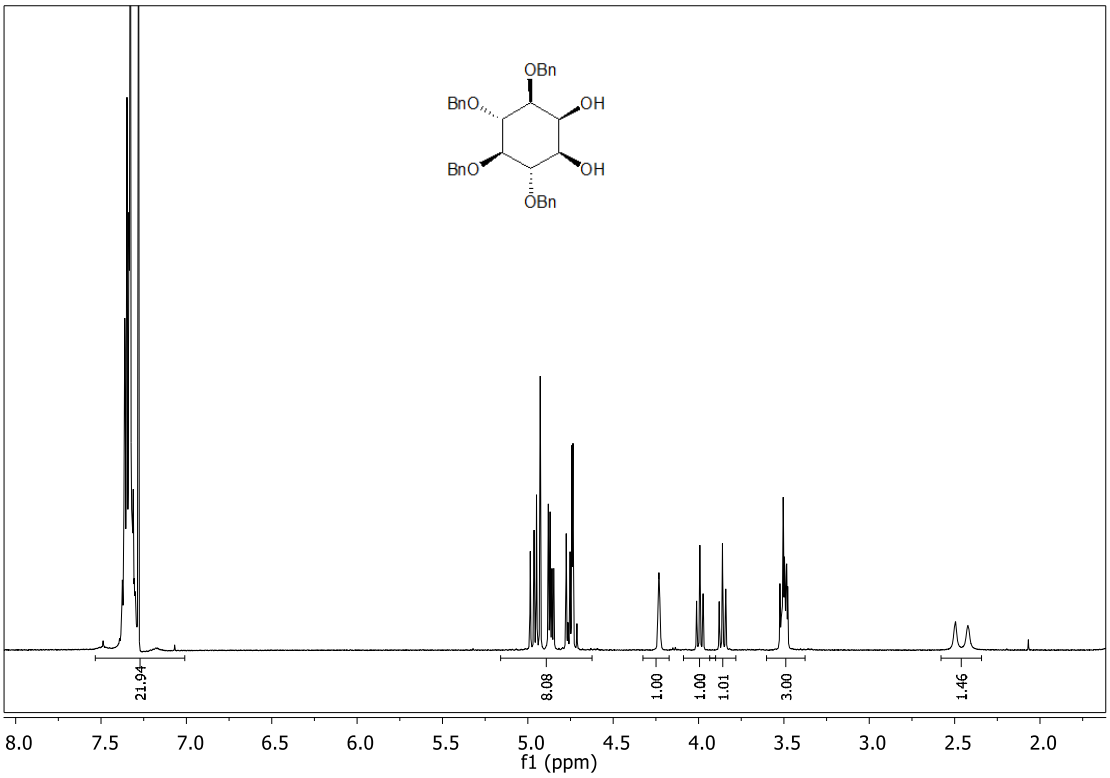

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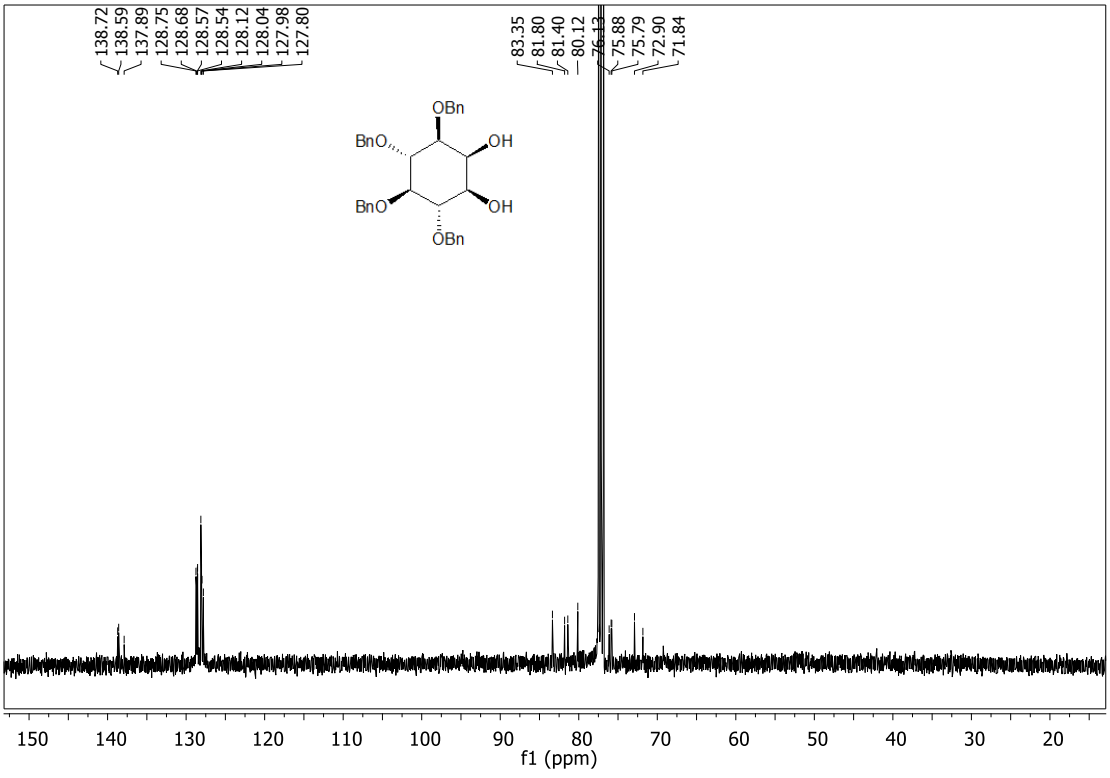

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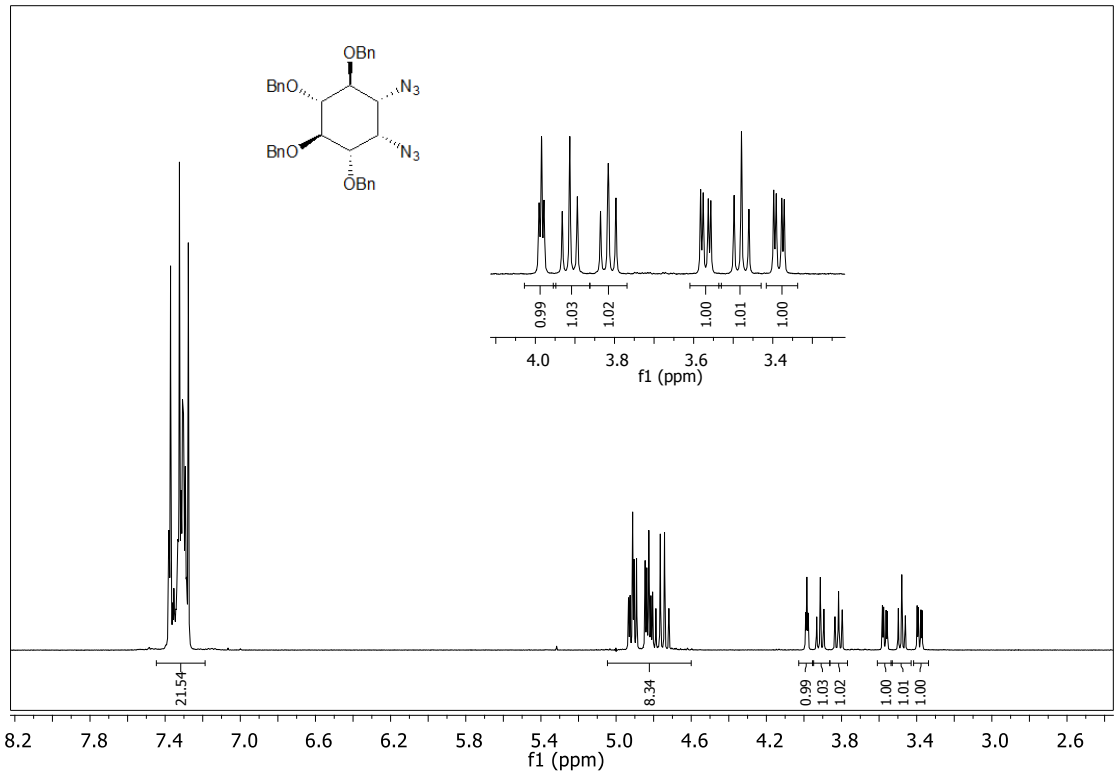

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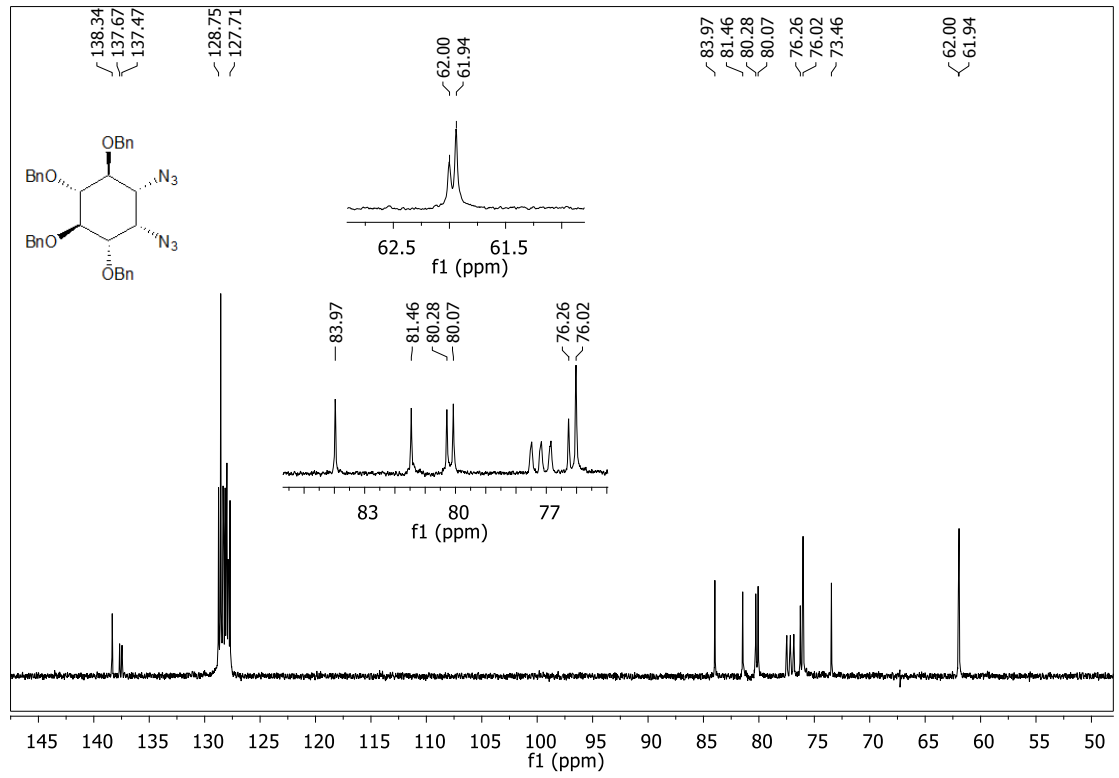

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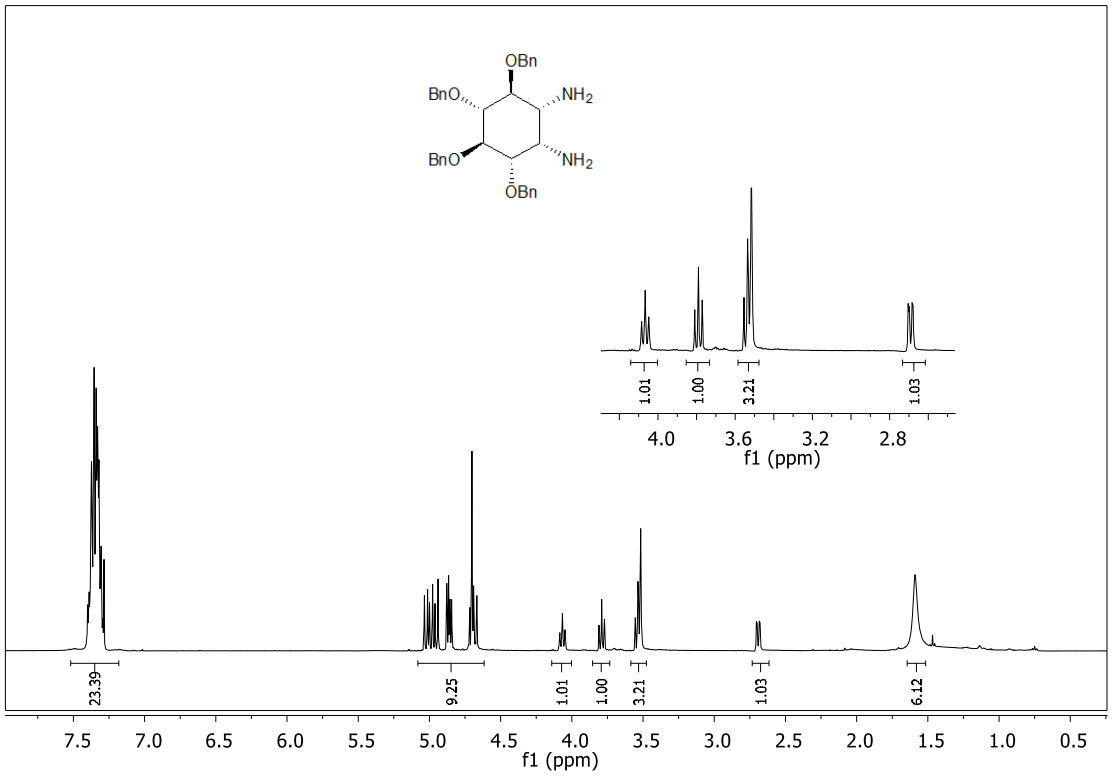

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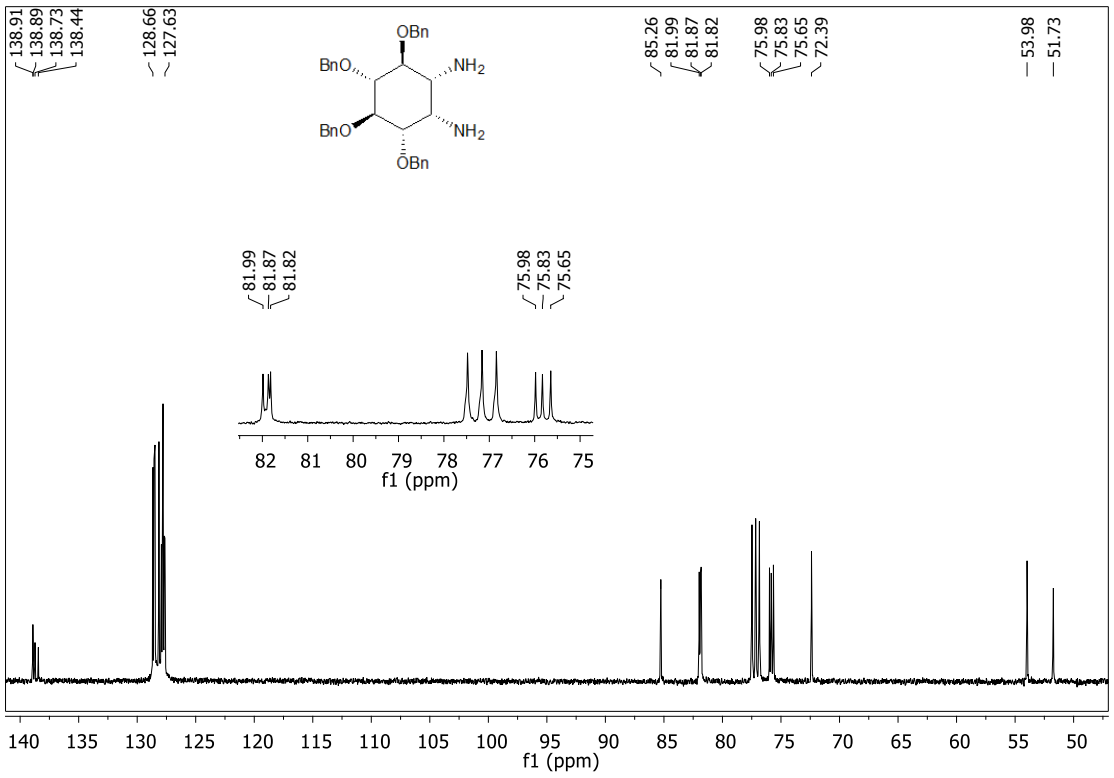

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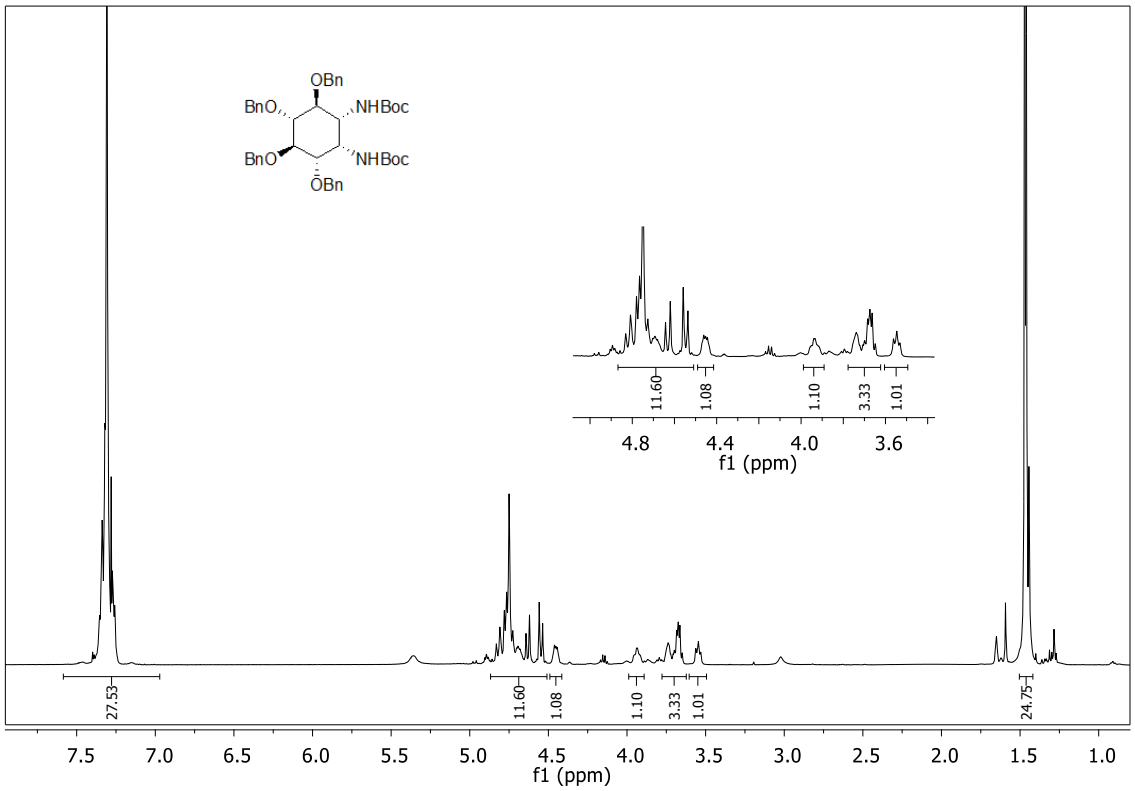
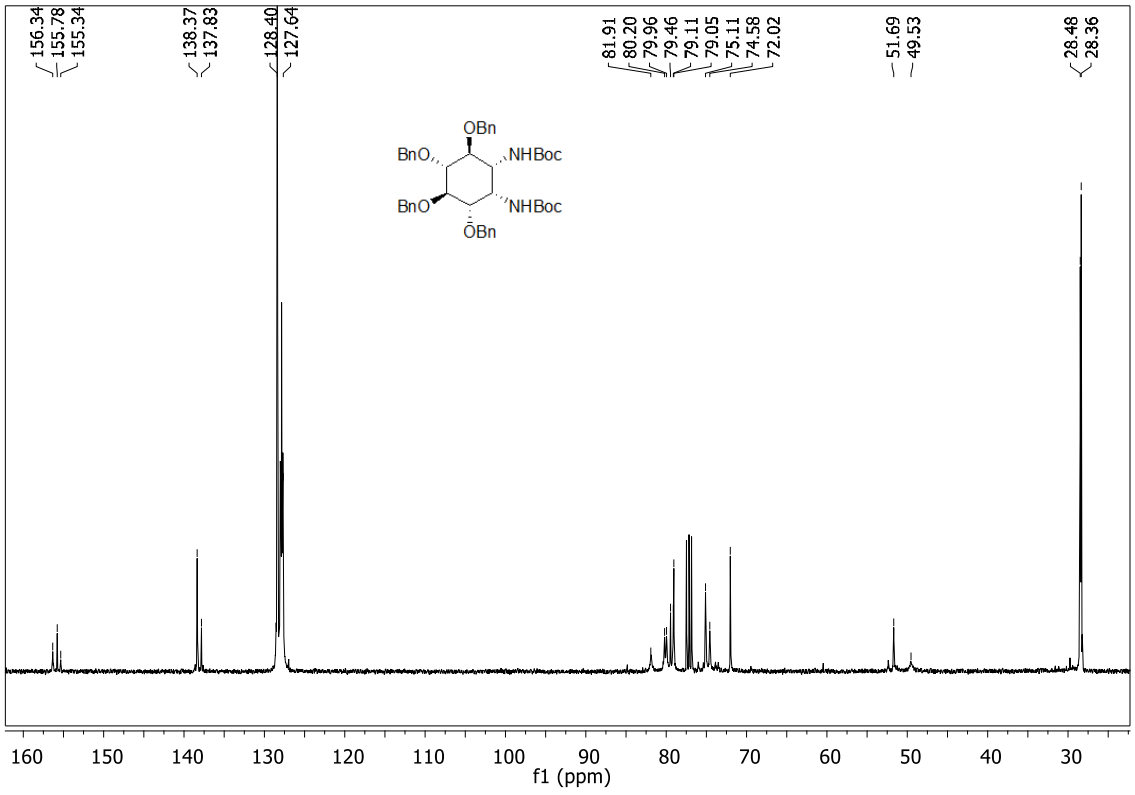
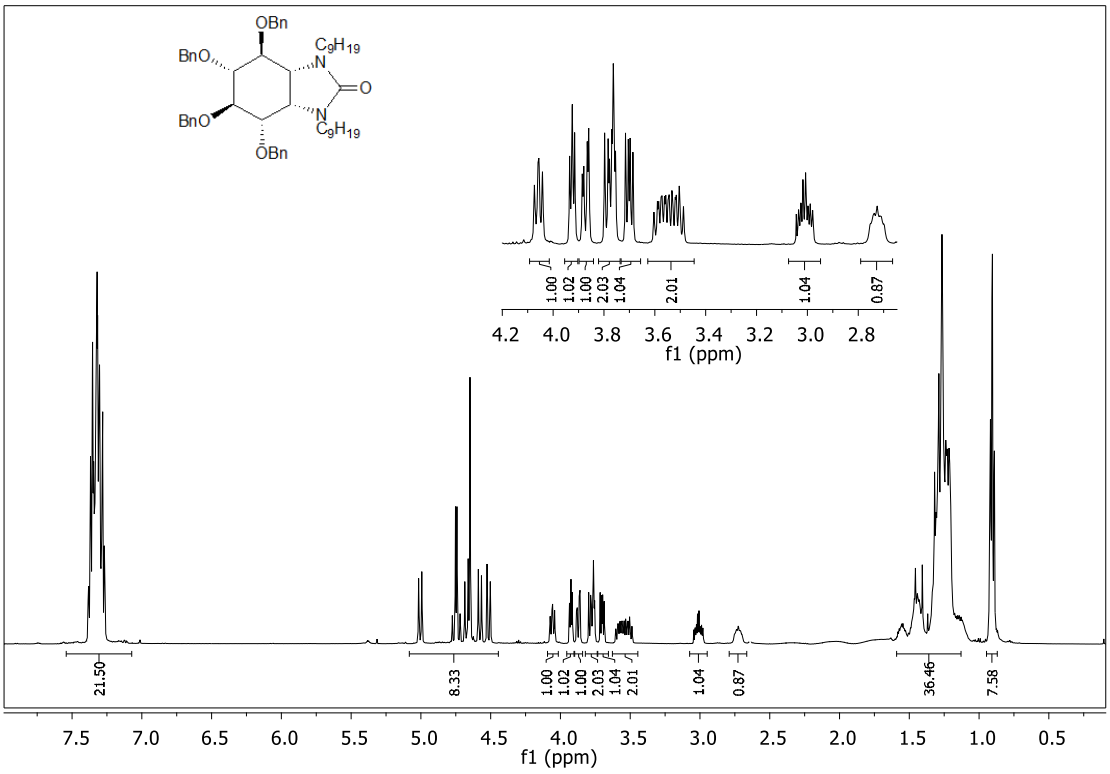


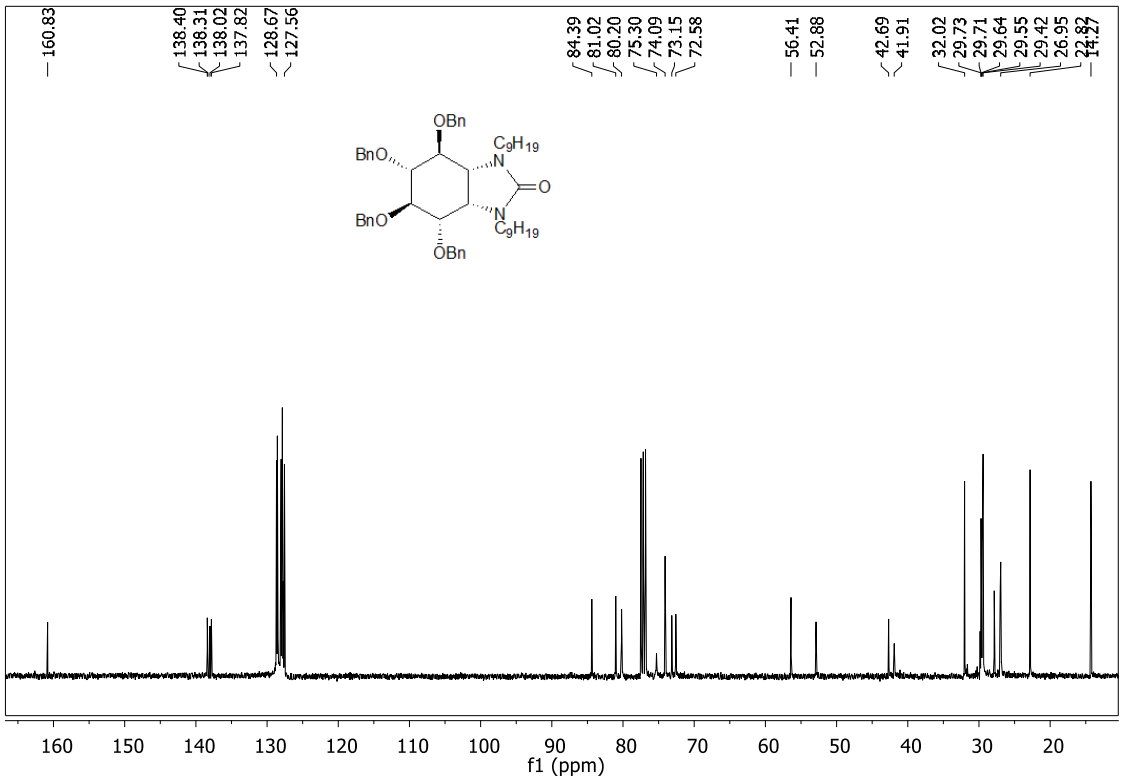
^{13}C NMR spectra (100 MHz, $CDCl_3$) of **19**



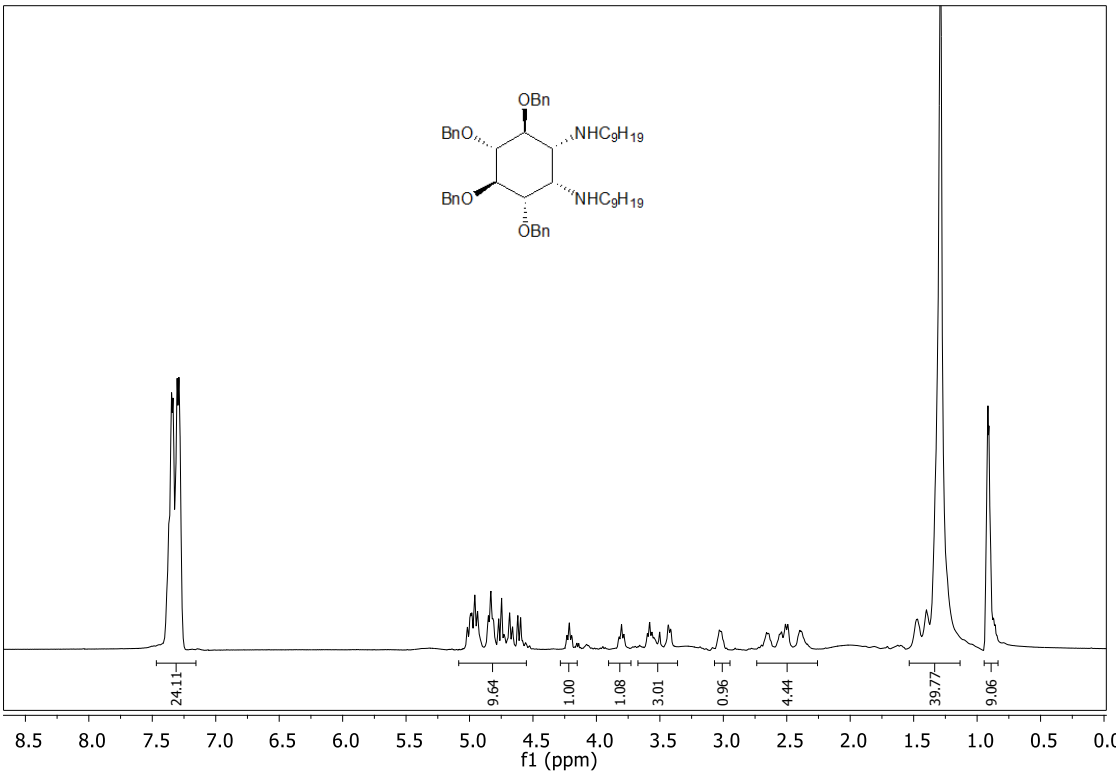
^{1}H NMR spectra (500 MHz, $CDCl_3$) of **21**



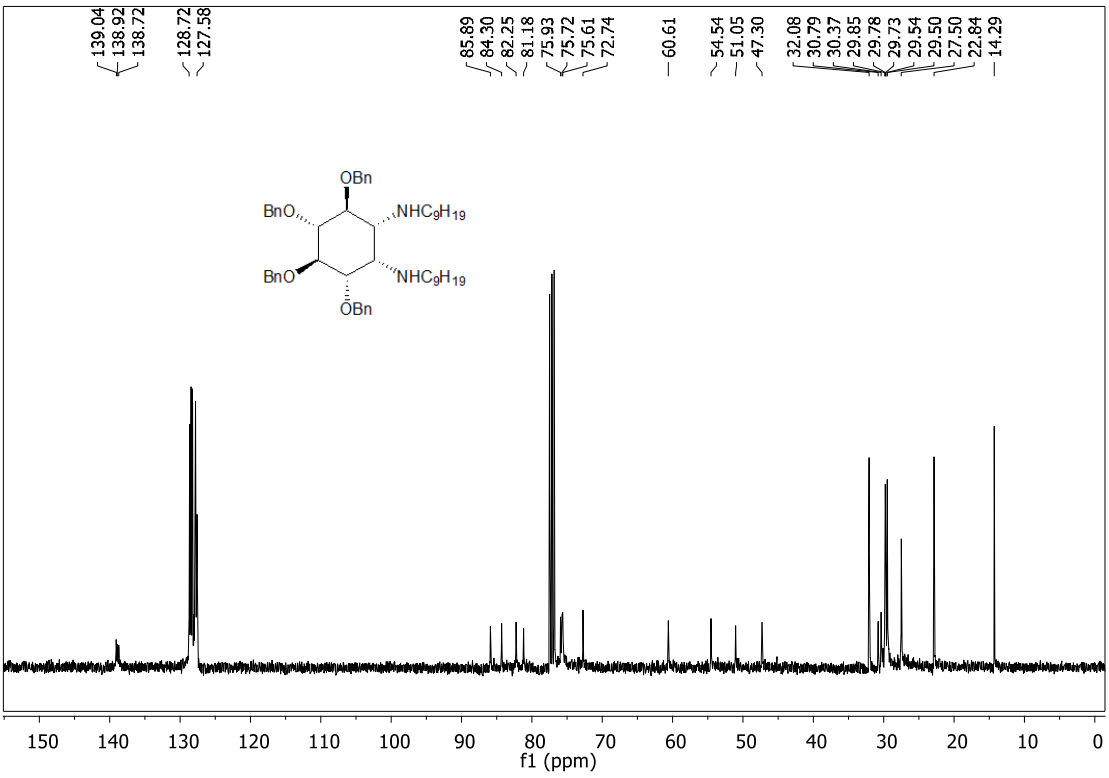
^{13}C NMR spectra (100 MHz, $CDCl_3$) of **21**



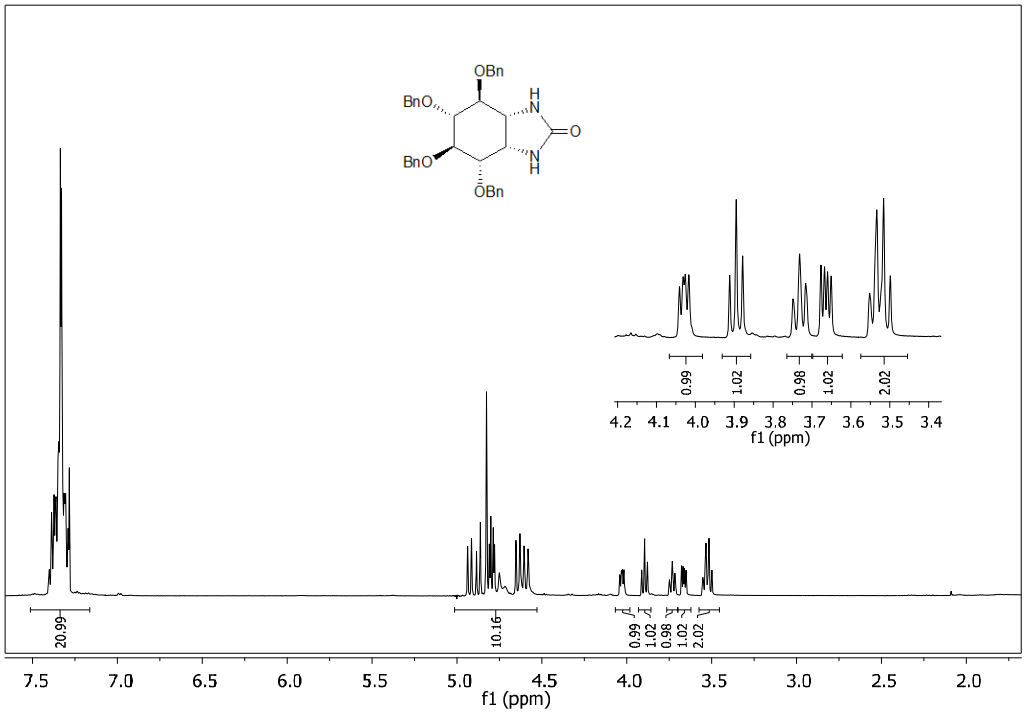
^{1}H NMR spectra (500 MHz, $CDCl_3$) of **22**



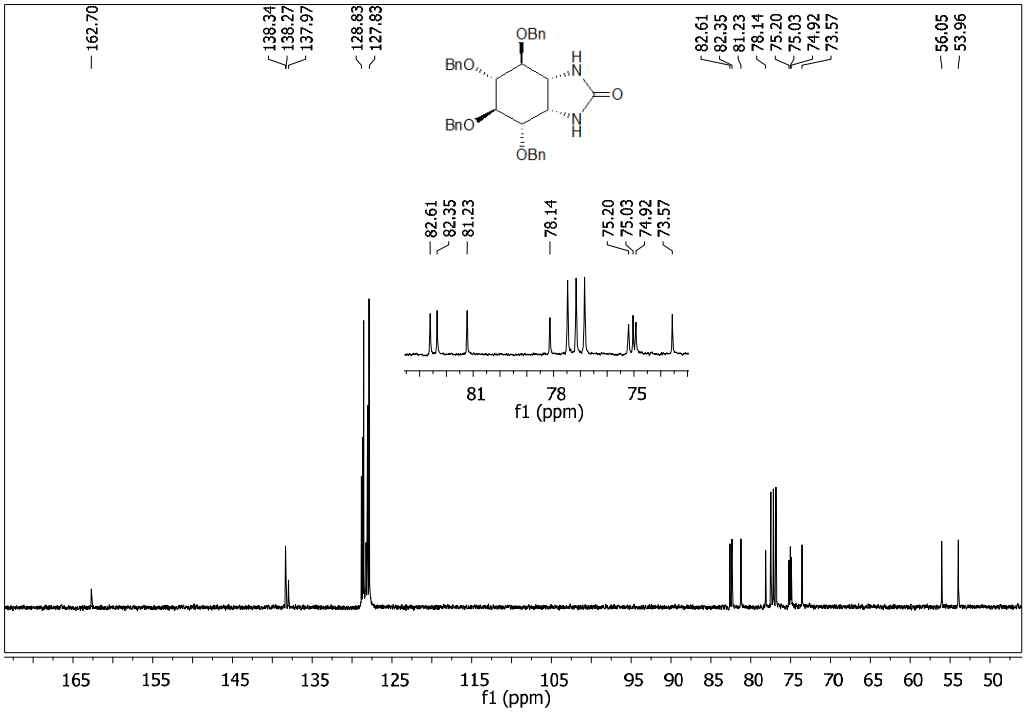
^{13}C NMR spectra (100 MHz, $CDCl_3$) of **22**



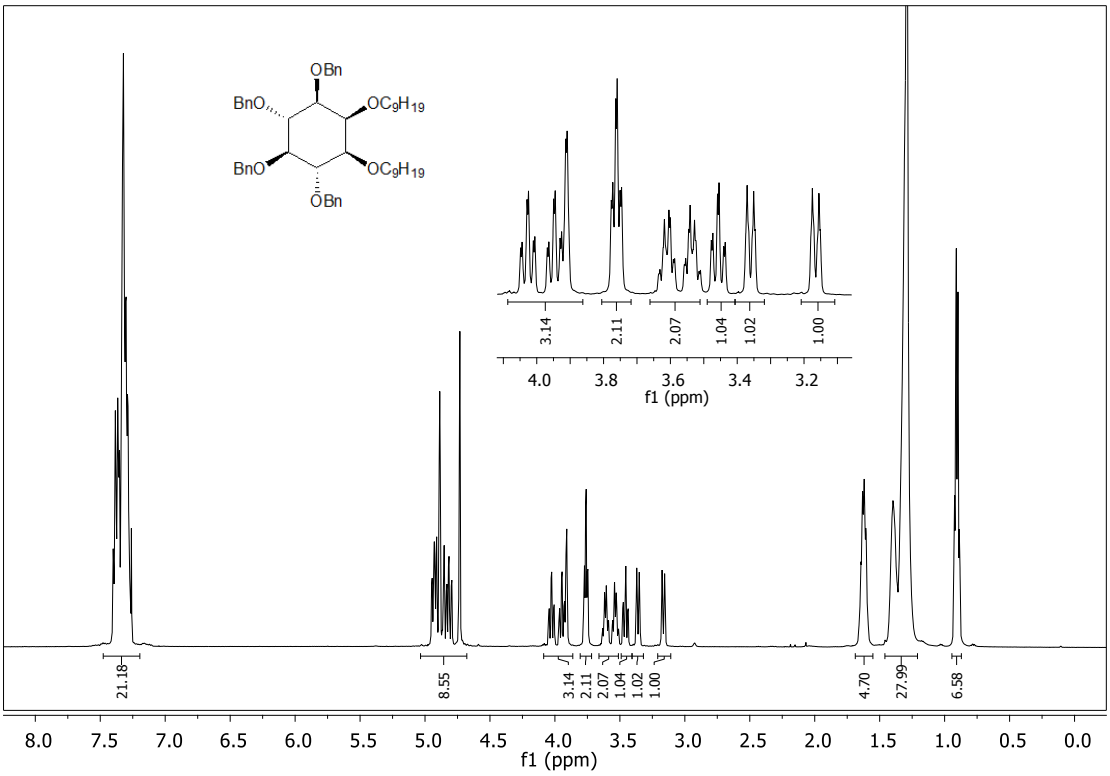
^{1}H NMR spectra (500 MHz, $CDCl_3$) of **23**



^{13}C NMR spectra (100 MHz, $CDCl_3$) of **23**



^{1}H NMR spectra (500 MHz, $CDCl_3$) of **24**



^{13}C NMR spectra (100 MHz, $CDCl_3$) of **24**