Supporting Information: Gluco-1*H*-imidazole: a new class of azole-type β-glucosidase inhibitor

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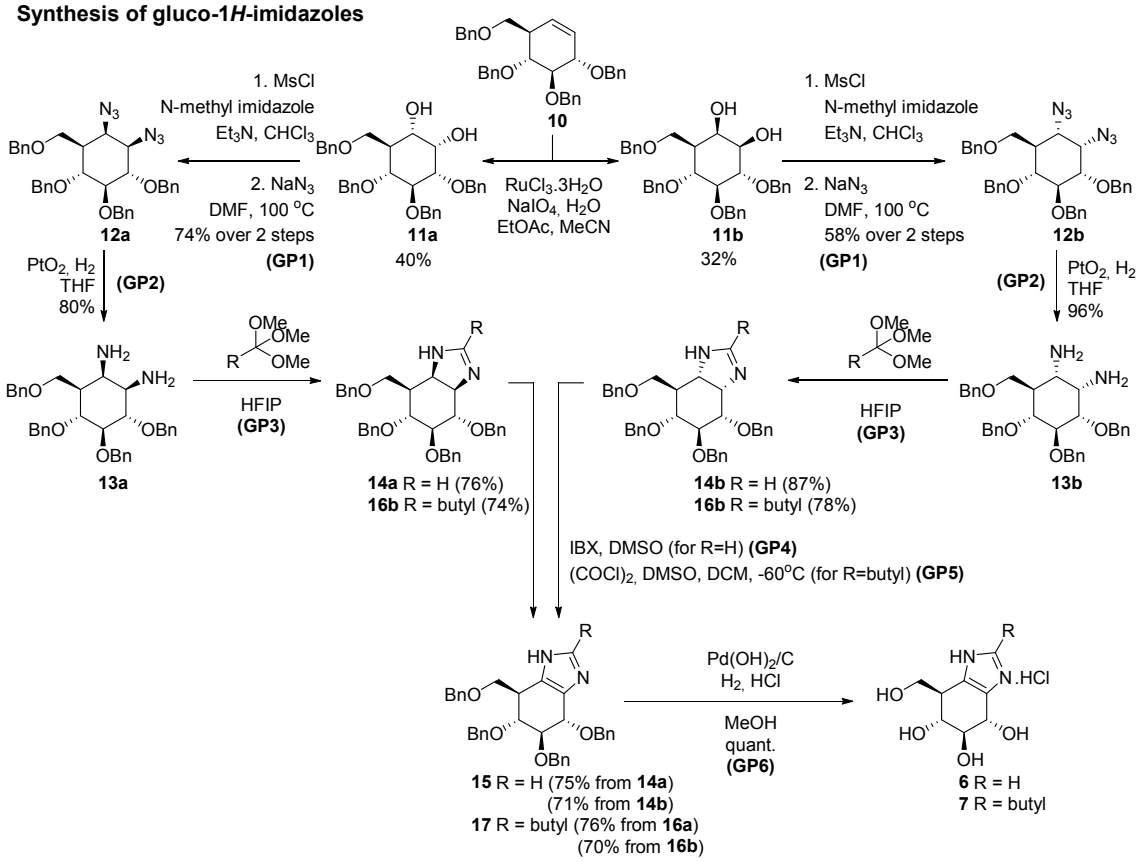
Experimental procedures and characterization data

General: Chemicals were purchased from Acros, Sigma Aldrich, Biosolve, VWR, Fluka, Merck and Fisher Scientific and used as received unless stated otherwise. Tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF) and toluene were stored over molecular sieves before use. Traces of water from reagents were removed by co-evaporation with toluene in reactions that required anhydrous conditions. All reactions were performed under an argon atmosphere unless stated otherwise. TLC analysis was conducted using Merck aluminum sheets (Silica gel 60 F_{254}) with detection by UV absorption (254 nm), by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) in 10% sulfuric acid or a solution of $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in water, followed by charring at ~150 ˚C. Column chromatography was performed using Screening Device b.v. silica gel (particle size of 40 – 63 µm, pore diameter of 60 Å) with the indicated eluents. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brüker AV-400 (400 and 101 MHz respectively) or a Brüker AV-500 (500 and 125 MHz respectively) spectrometer in the given deuterated solvent. Chemical shifts are given in ppm (δ) relative to the residual solvent peak or tetramethylsilane (0 ppm) as internal standard. Coupling constants are given in Hz. High-resolution mass spectrometry (HRMS) analysis was performed with a LTQ Orbitrap mass spectrometer (Thermo Finnigan), equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150 – 2000) and dioctyl phthalate (m/z = 391.28428) as a “lock mass”, or with a Synapt G2-Si (Waters) , equipped with an electronspray ion source in positive mode (ESI-TOF), injection via NanoEquity system (Waters), with LeuEnk (m/z = 556.2771) as “lock mass”. Eluents used: MeCN:H2O (1:1 v/v) supplemented with 0.1% formic acid. The high-resolution mass spectrometers were calibrated prior to measurements with a calibration mixture (Thermo Finnigan). Glucoimidazole **5** was prepared according to the procedure by Vasella *et al*.[1] NMR spectra of this compound are provided herein and are in agreement with those previously reported.



General procedure 1 (GP1): Bis-azidation

The diol starting material was dissolved in dry $CHCl_3$ (0.2 M), then Et_3N (3 equiv.) and *N*-methyl imidazole (10 equiv.) were added and the mixture was cooled to 0 °C. MsCl (4 equiv.) was added and the mixture was stirred 16 h at rt. The mixture was quenched with water at 0 °C, diluted with EtOAc, washed with aq. 1M HCl (2 x), H_2O and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. After co-evaporation with toluene (2 x), the crude intermediate product was dissolved in dry DMF (0.1 M). NaN_3 (10 equiv.) was added and the mixture was stirred 16 h at 100 °C. Then, the mixture was diluted with H_2O and extracted with Et_2O (3 x). The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated. The product was purified by flash column chromatography using the indicated eluent.

General procedure 2 (GP2): Azide reduction

The bisazido starting material was dissolved in THF (0.05 M) under N_2 atmosphere. PtO_2 (30 mol%) was added, the reaction mixture was purged with H_2 with a balloon, and the mixture was stirred

vigorously for 16 h. Then, the mixture was filtered over a small Celite pad and concentrated. The product was purified by flash column chromatography using the indicated eluent.

General procedure 3 (GP3): Imidazoline formation

The diamino starting material was dissolved in HFIP (0.1 M), the appropriate trimethyl orthoester (3 equiv.) was added and the mixture was stirred for 16 h at rt. The mixture was diluted with Et_2O and washed with aq. 1M NaOH (3 x), H_2O and brine, dried over $MgSO_4$, filtered and concentrated. The product was purified by flash column chromatography using the indicated eluent.

It should be noted that for both glucose and conduritol configurations, oxidation of the 2-butyl-imidazolines to the 2-butyl-imidazoles proceeded in only moderate yields when IBX/DMSO was employed. In contrast, we found that oxidation proceeded more smoothly under Swern conditions. [2,3]

General procedure 4 (GP4): Oxidation to the imidazole (IBX, DMSO)

The imidazoline starting material was dissolved in DMSO (0.1 M), IBX[4] (1.5 equiv.) was added and the mixture was stirred 16 h at 45 °C. Next, the mixture was cooled to rt, quenched with aq. 10% $Na_2S_2O_3$ and aq. 1M NaOH. The mixture was stirred for 15 min, diluted with Et_2O, washed with H_2O (3 x) and brine, dried over $MgSO_4$, filtered and concentrated. The product was purified by flash column chromatography using the indicated eluent.

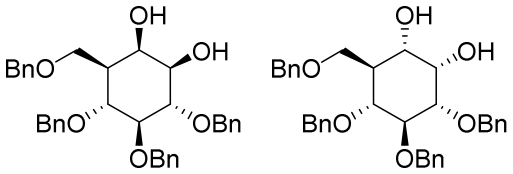
General procedure 5 (GP5): Oxidation to the imidazole (Swern conditions)

To dry DCM (0.1 M based on starting material) was added DMSO (7 equiv.) and the mixture was cooled to -60 °C. Then, oxalyl chloride (5 equiv.) was added slowly and the mixture was stirred for 30 min. The imidazoline starting material was co-evaporated with toluene (2 x), dissolved in dry DCM (1 mL) and added dropwise. The mixture was stirred for 1 h at -60 °C and subsequently quenched with Et_3N (7 equiv.). The cooling bath was removed and the mixture was allowed to reach rt. After stirring 1 h at rt, the mixture was diluted with EtOAc, washed with H_2O (3 x) and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated. The product was purified by flash column chromatography using the indicated eluent.

General procedure 6 (GP6): Hydrogenation

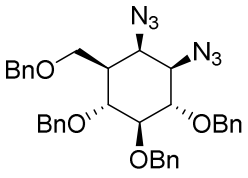
The imidazole starting material was dissolved in MeOH (0.03 M) under N_2 atmosphere, then HCl (1.25M in MeOH, 10 equiv.) and $Pd(OH)_2/C$ (20 wt%) were added and the mixture was purged with H_2 with a balloon. The mixture was stirred vigorously for 16 h, filtered over a small Celite pad and finally concentrated which afforded the pure product.

Compound 11b and **compound 11a**



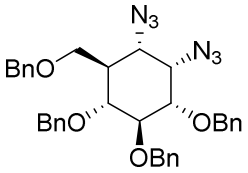
Cyclohexene **10**[5] (1.28 g, 2.46 mmol) was dissolved in EtOAc (15 mL) and MeCN (15 mL) and cooled to 0 °C. A solution of $RuCl_3.3H_2O$ (36 mg, 0.17 mmol) and $NaIO_4$ (789 mg, 3.69 mmol) in H_2O (4.9 mL) was added and the mixture was stirred vigorously at 0 °C for 90 min. The mixture was quenched by addition of aq. 10% $Na_2S_2O_3$ (20 mL) and the mixture was stirred for 15 min. Then the mixture was diluted with H_2O (100 mL) and extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated. The product was purified by flash column chromatography (pentane/EtOAc, 4:1 → 2:1) affording compound **11b** (432 mg, 32%) and **11a** (539 mg, 40%) as white solids. *Analytical data for **11b**:* ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.69 – 6.77 (m, 20H), 5.00 – 4.85 (m, 4H), 4.79 (d, *J* = 11.1 Hz, 1H), 4.59 – 4.39 (m, 3H), 4.25 (s, 1H), 3.89 (m, 3H), 3.73 (dd, *J* = 8.9, 2.9 Hz, 1H), 3.61 – 3.45 (m, 2H), 3.34 (s, OH), 2.41 (d, *J* = 4.8 Hz, OH), 1.74 (dq, *J* = 8.0, 2.3 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 138.7, 138.7, 138.4, 137.6, 128.7, 128.7, 128.6, 128.6, 128.1, 128.1, 127.9, 127.9, 127.9, 127.8, 127.7, 86.7, 82.4, 77.4, 75.8, 75.7, 75.6, 74.6, 73.7, 71.0, 68.9, 43.5. IR (neat, cm^{-1}): ν 3441, 2866, 1452, 1058. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{35}H_{39}O_6$ 555.27412, found 555.27411. *Analytical data for **11a**:* ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.15 (m, 20H), 4.94 (d, *J* = 10.8 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.82 (d, *J* = 10.8 Hz, 1H), 4.71 (s, 2H), 4.55 – 4.38 (m, 3H), 4.14 (s, 1H), 3.96 (t, *J* = 9.4 Hz, 1H), 3.84 (dd, *J* = 9.0, 2.5 Hz, 1H), 3.68 – 3.65 (m, 2H), 3.46 – 3.30 (m, 2H), 3.05 (d, *J* = 6.2 Hz, OH), 2.62 (s, OH), 2.18 (tdd, *J* = 10.9, 5.0, 2.5 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 138.8, 138.5, 138.0, 138.0, 128.5, 128.5, 128.4, 128.4, 128.0, 128.0, 127.9, 127.7, 127.7, 127.7, 127.6, 82.9, 80.0, 77.7, 75.7, 75.3, 73.3, 72.5, 70.3, 69.3, 67.7, 43.2. IR (neat, cm^{-1}): ν 3441, 2868, 1452, 1064. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{35}H_{39}O_6$ 555.27412, found 555.27374. These data are in agreement with those previously reported.[6]

Compound 12a



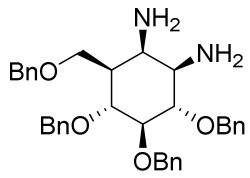
Starting from **11a** (55 mg, 0.1 mmol) and following **GP1**, the product was purified by flash column chromatography (pentane/EtOAc, 15:1) affording compound **12a** as a white solid (45 mg, 74%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.11 (m, 20H), 4.96 – 4.74 (m, 5H), 4.55 – 4.37 (m, 3H), 4.16 (t, *J* = 2.9 Hz, 1H), 3.86 – 3.75 (t, *J* = 9.6 Hz, 1H), 3.73 (dd, *J* = 8.9, 4.3 Hz, 1H), 3.58 – 3.49 (m, 2H), 3.49 – 3.41 (m, 1H), 3.38 (dd, *J* = 10.2, 9.2 Hz, 1H), 2.08 – 1.95 (m, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 138.4, 137.9, 137.8, 137.8, 128.6, 128.6, 128.6, 128.4, 128.2, 128.1, 128.1, 128.0, 127.8, 127.6, 87.0, 81.1, 78.0, 76.1, 75.9, 75.6, 73.6, 67.5, 65.9, 61.1, 43.8. IR (neat, cm^{-1}): ν 2858, 2102, 1359, 1066. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{35}H_{37}N_6O_4$ 605.28708, found 605.33734.

Compound 12b



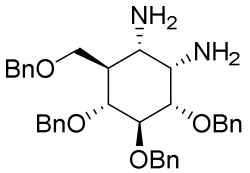
Starting from **11b** (55 mg, 0.1 mmol) and following **GP1**, the product was purified by flash column chromatography (pentane/EtOAc, 15:1) affording compound **12b** as a white solid (35 mg, 58%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.13 (m, 20H), 4.95 – 4.67 (m, 5H), 4.48 (d, *J* = 4.3 Hz, 1H), 4.45 (d, *J* = 3.5 Hz, 1H), 4.34 (d, *J* = 11.5 Hz, 1H), 4.05 (t, *J* = 2.9 Hz, 1H), 3.86 (t, *J* = 9.5 Hz, 1H), 3.82 (d, *J* = 9.2 Hz, 1H), 3.56 (ddd, *J* = 9.6, 6.5, 4.1 Hz, 2H), 3.51 (dd, *J* = 10.6, 2.4 Hz, 2H), 2.02 (t, *J* = 11.2 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 138.6, 138.4, 138.0, 137.6, 128.7, 128.5, 128.2, 128.1, 128.1, 127.9, 127.8, 127.8, 83.1, 80.4, 77.7, 76.0, 75.7, 73.3, 73.3, 65.0, 63.7, 57.7, 42.5. IR (neat, cm^{-1}): ν 2858, 2098, 1359, 1082. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{35}H_{36}N_6O_4Na$ 627.26902, found 627.26849.

Compound 13a



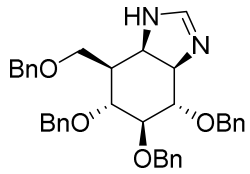
Starting from **12a** (367 mg, 0.61 mmol) and following **GP2**, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 49:1) affording compound **13a** as a colorless oil (269 mg, 80%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.11 (m, 20H), 4.99 (d, *J* = 11.1 Hz, 1H), 4.96 – 4.83 (m, 3H), 4.66 (d, *J* = 11.1 Hz, 1H), 4.55 – 4.41 (m, 3H), 3.91 (dd, *J* = 11.0, 9.3 Hz, 1H), 3.77 – 3.63 (m, 3H), 3.59 (t, *J* = 9.2 Hz, 1H), 3.38 (t, *J* = 3.0 Hz, 1H), 2.80 (dd, *J* = 10.0, 3.4 Hz, 1H), 1.87 (ddt, *J* = 11.0, 7.4, 3.2 Hz, 1H), 1.53 (s, 4H, 2 x NH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 138.9, 138.7, 138.6, 138.2, 128.6, 128.5, 128.5, 128.1, 128.0, 127.8, 127.8, 127.7, 127.5, 88.3, 82.3, 78.5, 75.8, 75.4, 75.3, 73.3, 68.8, 56.6, 51.7, 44.8. IR (neat, cm^{-1}): ν 2856, 1361, 1066. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{35}H_{41}N_2O_4$ 553.30608, found 553.30585.

Compound 13b



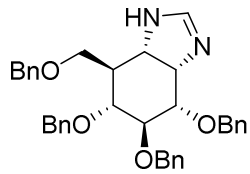
Starting from **12b** (858 mg, 1.42 mmol) and following **GP2**, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 7:3) affording compound **13b** as a colorless oil (750 mg, 96%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 20H), 4.91 (t, *J* = 11.9 Hz, 2H), 4.79 (d, *J* = 10.7 Hz, 1H), 4.69 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.46 (d, *J* = 27.6 Hz, 3H), 3.90 (t, *J* = 9.2 Hz, 1H), 3.77 (d, *J* = 7.6 Hz, 1H), 3.64 (d, *J* = 7.8 Hz, 1H), 3.50 (t, *J* = 9.6 Hz, 1H), 3.49 - 3.43 (m, 2H), 2.91 (d, *J* = 10.9 Hz, 1H), 1.97 (t, *J* = 10.9 Hz, 1H), 1.87 (s, 4H, 2 x NH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 139.1, 138.9, 138.5, 128.6, 128.5, 128.1, 128.0, 127.9, 127.9, 127.8, 127.6, 127.6, 83.1, 81.6, 78.9, 75.7, 75.4, 73.2, 72.2, 66.1, 53.3, 49.6, 43.4. IR (neat, cm^{-1}): ν 2860, 1602, 1496, 1452, 1359, 1066. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{35}H_{40}N_2O_4Na$ 575.28803, found 575.28741.

Compound 14a



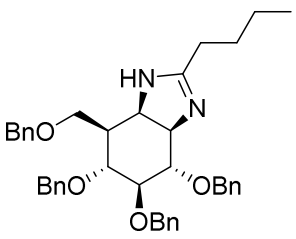
Starting from **13a** (55 mg, 0.1 mmol) and following **GP3** using trimethyl orthoformate, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 7:3) affording compound **14a** as a colorless oil (43 mg, 76%). ^{1}H-NMR (400 MHz, CD_3CN) δ 7.41 – 7.18 (m, 20H), 7.07 (s, 1H), 4.84 – 4.64 (m, 5H), 4.51 – 4.42 (m, 3H), 4.01 (dd, *J* = 9.5, 4.4 Hz, 1H), 3.86 (dd, *J* = 9.4, 6.2 Hz, 1H), 3.77 (dd, *J* = 9.2, 4.1 Hz, 1H), 3.68 (t, *J* = 8.8 Hz, 1H), 3.58 – 3.51 (m, 1H), 3.50 – 3.40 (m, 2H), 2.25 (ddt, *J* = 12.3, 8.4, 4.2 Hz, 1H). ^{13}C-NMR (101 MHz, CD_3CN) δ 156.2, 140.0, 139.9, 139.8, 139.7, 129.3, 129.2, 129.2, 128.9, 128.8, 128.7, 128.5, 128.4, 128.4, 85.1, 83.5, 78.9, 74.7, 74.5, 74.1, 73.7, 69.9, 65.6, 60.7, 41.7. IR (neat, cm^{-1}): ν 3278, 3030, 2862, 1654, 1543, 1359, 1066. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{36}H_{38}N_2O_4$ 563.29043, found 563.29022.

Compound 14b



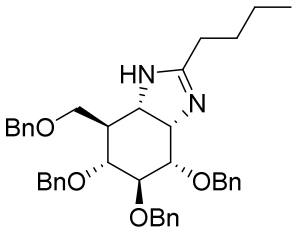
Starting from **13b** (110 mg, 0.2 mmol) and following **GP3** using trimethyl orthoformate, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 4:1) affording compound **14b** as a colorless oil (98 mg, 87%). ^{1}H-NMR (400 MHz, CD_3CN) δ 7.38 – 7.20 (m, 20H), 7.03 (s, 1H), 4.78 – 4.62 (m, 5H), 4.53 – 4.44 (m, 3H), 3.97 (dd, *J* = 9.3, 4.3 Hz, 1H), 3.86 – 3.81 (m, 1H), 3.81 – 3.78 (m, 1H), 3.70 (t, *J* = 7.0 Hz, 1H), 3.67 – 3.63 (m, 2H), 3.38 (dd, *J* = 11.3, 7.2 Hz, 1H), 1.74 – 1.66 (m, 1H). ^{13}C-NMR (101 MHz, CD_3CN) δ 155.7, 140.1, 140.0, 140.0, 139.8, 129.3, 129.2, 129.2, 128.9, 128.8, 128.7, 128.7, 128.5, 128.4, 128.4, 83.4, 79.6, 79.1, 74.5, 74.4, 73.8, 73.3, 69.2, 61.7, 60.5, 46.2. IR (neat, cm^{-1}): ν 3030, 2868, 1681, 1595, 1454, 1087. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{36}H_{39}N_2O_4$ 563.29043, found 563.29010.

Compound 16a



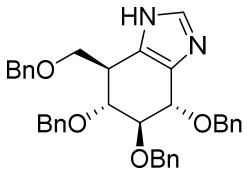
Starting from **13a** (119 mg, 0.21 mmol) and following **GP3** using trimethyl orthovalerate, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 8:2) affording compound **16a** as a colorless oil (99 mg, 74%). ^{1}H-NMR (400 MHz, CD_3CN) δ 7.39 – 7.18 (m, 20H), 4.73 (m, 5H), 4.51 – 4.43 (m, 3H), 3.99 (dd, *J* = 9.0, 4.4 Hz, 1H), 3.77 (dt, *J* = 9.2, 4.6 Hz, 2H), 3.68 (t, *J* = 8.8 Hz, 1H), 3.53 (t, *J* = 7.5 Hz, 1H), 3.46 – 3.39 (m, 2H), 2.19 (dq, *J* = 12.8, 4.3 Hz, 1H), 2.07 (td, *J* = 7.4, 3.6 Hz, 2H), 1.51 – 1.42 (m, 2H), 1.31 (dq, *J* = 14.2, 7.2 Hz, 2H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C-NMR (101 MHz, CD_3CN) δ 168.4, 140.2, 140.0, 139.9, 139.8, 129.3, 129.3, 129.2, 129.2, 128.9, 128.8, 128.7, 128.7, 128.4, 128.4, 128.3, 85.5, 83.7, 79.1, 74.8, 74.6, 73.9, 73.5, 70.0, 66.8, 61.6, 42.2, 29.7, 29.4, 23.1, 14.1. IR (neat, cm^{-1}): ν 2868, 1600, 1454, 1363, 1066. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{40}H_{47}N_2O_4$ 619.35303, found 619.35266.

Compound 16b



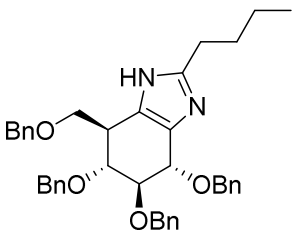
Starting from **13b** (110 mg, 0.2 mmol) and following **GP3** using trimethyl orthovalerate, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 4:1) affording compound **16b** as a colorless oil (96 mg, 78%). ^{1}H-NMR (400 MHz, CD_3CN) δ 7.40 – 7.19 (m, 20H), 4.76 (dd, *J* = 11.2, 6.3 Hz, 2H), 4.71 – 4.62 (m, 3H), 4.53 – 4.43 (m, 3H), 4.00 (dd, *J* = 9.0, 3.5 Hz, 1H), 3.83 (t, *J* = 9.4 Hz, 1H), 3.77 – 3.63 (m, 4H), 3.39 (dd, *J* = 11.2, 6.5 Hz, 1H), 2.18 (t, *J* = 7.6 Hz, 2H), 1.73 (t, *J* = 10.4 Hz, 1H), 1.52 (m, 2H), 1.31 (m, 2H), 0.87 (t, *J* = 7.3 Hz, 3H). ^{13}C-NMR (101 MHz, CD_3CN) δ 168.5, 140.1, 140.0, 139.8, 129.3, 129.2, 129.2, 129.1, 128.8, 128.7, 128.7, 128.6, 128.4, 128.4, 128.4, 128.3, 83.6, 80.0, 79.0, 74.6, 74.5, 73.8, 73.3, 69.1, 62.5, 61.0, 46.4, 29.7, 29.5, 23.1, 14.2. IR (neat, cm^{-1}): ν 2862, 1608, 1454, 1359, 1091. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{40}H_{47}N_2O_4$ 619.35303, found 619.35260.

Compound 15



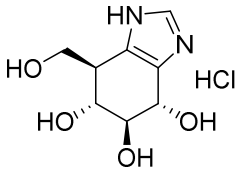
Starting from **14a** (43 mg, 76 µmol) and following **GP4**, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 67:1) affording compound **15** as a colorless oil (32 mg, 75%). Using the same conditions, product **15** could be obtained from imidazoline **14b** (82 mg, 0.15 mmol) in 71% yield (58 mg). ^{1}H-NMR (500 MHz, CD_3CN) δ 7.51 (s, 1H), 7.42 – 7.18 (m, 20H), 5.05 (d, *J* = 11.5 Hz, 1H), 4.88 – 4.81 (m, 4H), 4.67 – 4.63 (m, 1H), 4.54 – 4.44 (m, 3H), 3.96 (dd, *J* = 9.0, 6.4 Hz, 1H), 3.86 (dd, *J* = 9.0, 3.9 Hz, 1H), 3.76 (t, *J* = 8.1 Hz, 1H), 3.59 (t, *J* = 7.5 Hz, 1H), 3.09 (m, 1H). ^{13}C-NMR (125 MHz, CD_3CN) δ 140.3, 140.0, 139.8, 139.4, 136.8, 129.3, 129.2, 129.2, 129.0, 128.8, 128.8, 128.6, 128.5, 128.4, 128.3, 85.6, 79.5, 77.7, 75.5, 75.3, 73.7, 72.7, 70.0, 40.9. IR (neat, cm^{-1}): ν 3028, 2862, 1496, 1454, 1359, 1087. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{36}H_{37}N_2O_4$ 561.27478, found 561.27454.

Compound 17



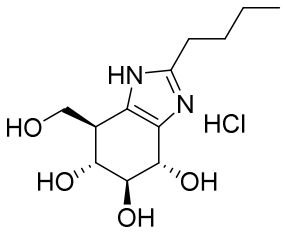
Starting from **16a** (74 mg, 0.12 mmol) and following **GP5**, the product was purified by flash column chromatography (DCM/MeOH, 199:1 → 99:1) affording compound **17** as a colorless oil (56 mg, 76%). Using the same conditions, product **17** could be obtained from imidazoline **16b** (40 mg, 64.7 µmol) in 70% yield (28 mg). ^{1}H-NMR (400 MHz, CD_3CN) δ 7.41 – 7.19 (m, 20H), 4.98 (d, *J* = 11.7 Hz, 1H), 4.87 – 4.76 (m, 4H), 4.63 (dd, *J* = 6.1, 1.5 Hz, 1H), 4.54 – 4.40 (m, 3H), 3.95 (dd, *J* = 8.8, 6.2 Hz, 1H), 3.84 (dd, *J* = 9.0, 4.1 Hz, 1H), 3.81 – 3.72 (m, 1H), 3.66 – 3.55 (m, 1H), 3.13 – 2.96 (m, 1H), 2.63 (d, *J* = 7.6 Hz, 2H), 1.68 – 1.59 (m, 2H), 1.35 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (101 MHz, CD_3CN) δ 150.4, 140.4, 140.0, 139.8, 139.5, 129.3, 129.2, 129.2, 129.1, 128.9, 128.8, 128.7, 128.5, 128.4, 128.4, 128.2, 85.4, 79.3, 77.7, 75.3, 75.2, 73.7, 72.5, 69.8, 41.3, 31.6, 29.0, 23.1, 14.1. IR (neat, cm^{-1}): ν 2862, 1454, 1359, 1089. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{40}H_{45}N_2O_4$ 617.33738, found 617.33710.

Compound 6 (gluco-1*H*-imidazole)



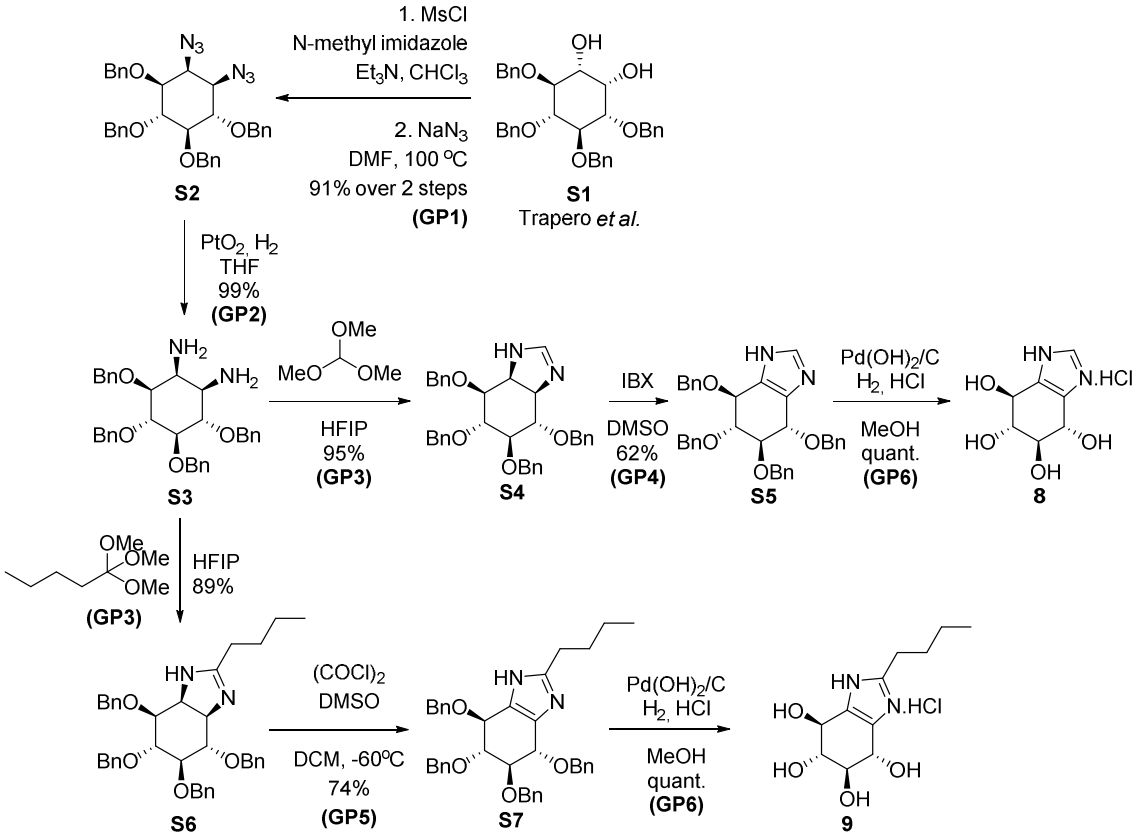
Starting from **15** (26 mg, 46.4 µmol) following **GP6**, the pure product was afforded as a colorless oil (12 mg, quant.). ^{1}H-NMR (400 MHz, D_2O) δ 8.55 (s, 1H), 4.69 (d, *J* = 7.6 Hz, 1H), 4.14 (dd, *J* = 11.4, 2.8 Hz, 1H), 3.93 (dd, *J* = 11.4, 4.8 Hz, 1H), 3.79 (t, *J* = 9.4 Hz, 1H), 3.71 (dd, *J* = 9.8, 7.4 Hz, 1H), 3.01 (s, 1H). ^{13}C-NMR (101 MHz, D_2O) δ 135.2, 128.2, 126.6, 76.9, 69.3, 66.6, 58.8, 40.9 HRMS (ESI-TOF) m/z: $[M+Na]^+$ calc for $C_8H_{12}N_2O_4$ 223.0689, found 223.0702.

Compound 7 (gluco-2-butyl-1*H*-imidazole)

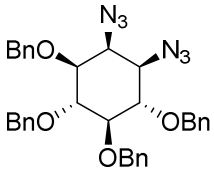


Starting from **17** (48 mg, 77.8 µmol) following **GP6**, the pure product was afforded as a colorless oil (24 mg, quant.). ^{1}H-NMR (400 MHz, MeOD) δ 4.53 (d, *J* = 7.4 Hz, 1H), 4.12 (dd, *J* = 10.8, 3.1 Hz, 1H), 3.89 (dd, *J* = 10.8, 5.0 Hz, 1H), 3.72 (t, *J* = 9.0 Hz, 1H), 3.60 (dd, *J* = 9.3, 7.4 Hz, 1H), 2.94 (t, *J* = 7.6 Hz, 2H), 2.87 (s, 1H), 1.69 – 1.58 (m, 2H), 1.29 (m, 2H), 0.86 (t, *J* = 7.3 Hz, 3H). ^{13}C-NMR (101 MHz, MeOD) δ 150.5, 129.7, 127.6, 78.9, 71.1, 68.2, 60.5, 43.0, 31.0, 26.6, 23.1, 13.8. HRMS (ESI-TOF) m/z: $[M+H]^+$ calc for $C_{12}H_{20}N_2O_4$ 257.1496, found 257.1510.

Synthesis of conduritol B-1*H*-imidazoles

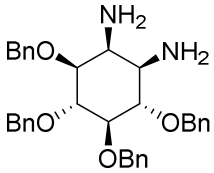


Compound S2



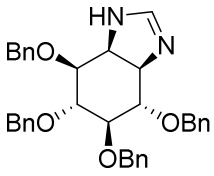
Starting from **S1**[7] (1.88 g, 3.48 mmol) and following **GP1**, the product was purified by flash column chromatography (pentane/EtOAc, 9:1) affording compound **S2** as a white solid (1.87 g, 91%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.27 (m, 20H), 4.98 – 4.91 (m, 3H), 4.89 – 4.82 (m, 3H), 4.82 – 4.72 (m, 2H), 4.01 (t, *J* = 3.1 Hz, 1H), 3.95 (t, *J* = 9.5 Hz, 1H), 3.85 (t, *J* = 9.7 Hz, 1H), 3.60 (dd, *J* = 9.6, 3.2 Hz, 1H), 3.51 (t, *J* = 9.3 Hz, 1H), 3.41 (dd, *J* = 10.2, 3.1 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 138.3, 137.6, 137.4, 128.7, 128.5, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 83.9, 81.4, 80.2, 80.0, 76.2, 75.9, 73.4, 62.0, 61.9. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{34}H_{35}N_6O_4$ 613.25337, found 613.25281. This analytical data is in accordance with literature.[7]

Compound S3



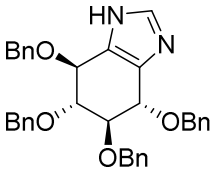
Starting from **S2** (1.8 g, 3.0 mmol) and following **GP2**, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 9:1) affording compound **S3** as a white solid (1.63 g, 99%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.13 (m, 20H), 5.03 – 4.87 (m, 3H), 4.82 (dd, *J* = 10.8, 4.4 Hz, 2H), 4.65 (m, 3H), 4.03 (t, *J* = 9.2 Hz, 1H), 3.76 (t, *J* = 9.6 Hz, 1H), 3.50 (m, 3H), 2.66 (dd, *J* = 10.0, 2.7 Hz, 1H), 1.53 (s, 4H, 2xNH_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 138.9, 138.8, 138.5, 128.6, 128.6, 128.5, 128.5, 128.1, 128.1, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 85.3, 82.0, 81.9, 81.9, 76.0, 75.8, 75.6, 72.4, 54.0, 51.8. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{34}H_{39}N_2O_4$ 539.29043, found 539.29007. This analytical data is in accordance with literature.[7]

Compound S4



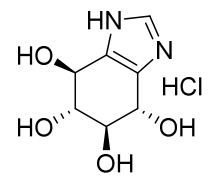
Starting from **S3** (400 mg, 0.74 mmol) and following **GP3** using trimethyl orthoformate, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 7:3) affording compound **S4** as a colorless oil (387 mg, 95%). ^{1}H-NMR (400 MHz, CD_3CN) δ 7.34 (m, 20H), 7.14 (s, 1H), 4.76 (s, 2H), 4.75 – 4.63 (m, 6H), 4.16 (dd, *J* = 10.7, 4.2 Hz, 1H), 3.97 – 3.87 (m, 2H), 3.82 – 3.73 (m, 2H), 3.57 (dd, *J* = 7.7, 5.6 Hz, 1H). ^{13}C-NMR (101 MHz, CD_3CN) δ 155.2, 139.1, 138.9, 138.8, 138.8, 128.3, 128.3, 128.3, 128.2, 127.9, 127.9, 127.8, 127.8, 127.6, 127.6, 127.5, 127.4, 83.2, 81.3, 80.5, 77.0, 73.0, 72.9, 72.9, 72.4, 64.0, 59.5. IR (neat, cm^{-1}): ν 3030, 2866, 1670, 1452, 1066. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{35}H_{37}N_2O_4$ 549.27478, found 549.27526.

Compound S5



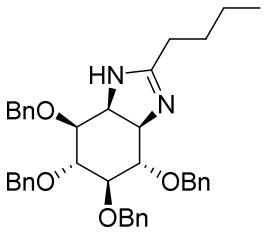
Starting from **S4** (55 mg, 0.1 mmol) and using **GP4**, the product was purified by flash column chromatography (DCM/MeOH, 199:1 → 67:1) affording compound **S5** as a colorless oil (34 mg, 62%). ^{1}H-NMR (400 MHz, CD_3CN) δ 7.57 (s, 1H), 7.44 – 7.14 (m, 20H), 4.92 – 4.83 (m, 4H), 4.80 (d, *J* = 11.2 Hz, 2H), 4.74 – 4.70 (m, 2H), 3.91 (dd, *J* = 4.4, 2.2 Hz, 2H). ^{13}C-NMR (101 MHz, CD_3CN) δ 139.9, 137.9, 129.2, 129.2, 128.8, 128.4, 128.4, 84.5, 78.2 (broad, assigned with HSQC), 75.7, 73.2. IR (neat, cm^{-1}): ν 3030, 2866, 1585, 1496, 1452, 1344, 1053. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{35}H_{35}N_2O_4$ 547.25913, found 547.25897.

Compound 8 (conduritol B-1*H*-imidazole)



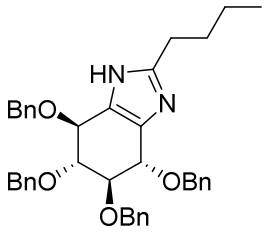
Starting from **S5** (18 mg, 32.9 µmol) and following **GP6**, the pure product was afforded as a colorless oil (8.0 mg, quant.). ^{1}H-NMR (400 MHz, D_2O) δ 8.70 (s, 1H), 4.76 (m, 2H), 3.74 – 3.58 (d, *J* = 2.7 Hz, 2H). ^{13}C-NMR (101 MHz, D_2O) δ 135.8. 127.5, 75.9, 66.5. HRMS (ESI-TOF) m/z: $[M+Na]^+$ calc for $C_7H_{10}N_2O_4$ 209.0533, found 209.0542.

Compound S6



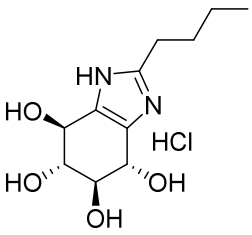
Starting from **S3** (53 mg, 0.1 mmol) and following **GP3** using trimethyl orthovalerate, the product was purified by flash column chromatography (DCM/MeOH, 99:1 → 8:2) affording compound **S6** as a colorless oil (53 mg, 89%). ^{1}H-NMR (400 MHz, CD_3CN) δ 7.42 – 7.18 (m, 20H), 4.77 – 4.56 (m, 8H), 4.11 (dd, *J* = 10.2, 4.1 Hz, 1H), 3.89 – 3.77 (m, 2H), 3.75 – 3.66 (m, 2H), 3.51 (dd, *J* = 7.8, 5.7 Hz, 1H), 2.19 – 2.06 (t, *J* = 7.6 Hz, 2H), 1.55 – 1.42 (m, 2H), 1.29 (m, 2H), 0.84 (t, *J* = 7.3 Hz, 3H). ^{13}C-NMR (101 MHz, CD_3CN) δ 168.9, 140.2, 139.9, 139.8, 129.2, 129.2, 128.9, 128.8, 128.6, 128.5, 128.4, 84.2, 82.3, 81.8, 78.5, 73.9, 73.8, 73.3, 65.6, 61.5, 29.5, 29.4, 23.1, 14.1. IR (neat, cm^{-1}): ν 2870, 1606, 1454, 1357, 1064. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{39}H_{45}N_2O_4$ 605.33738, found 605.33722.

Compound S7



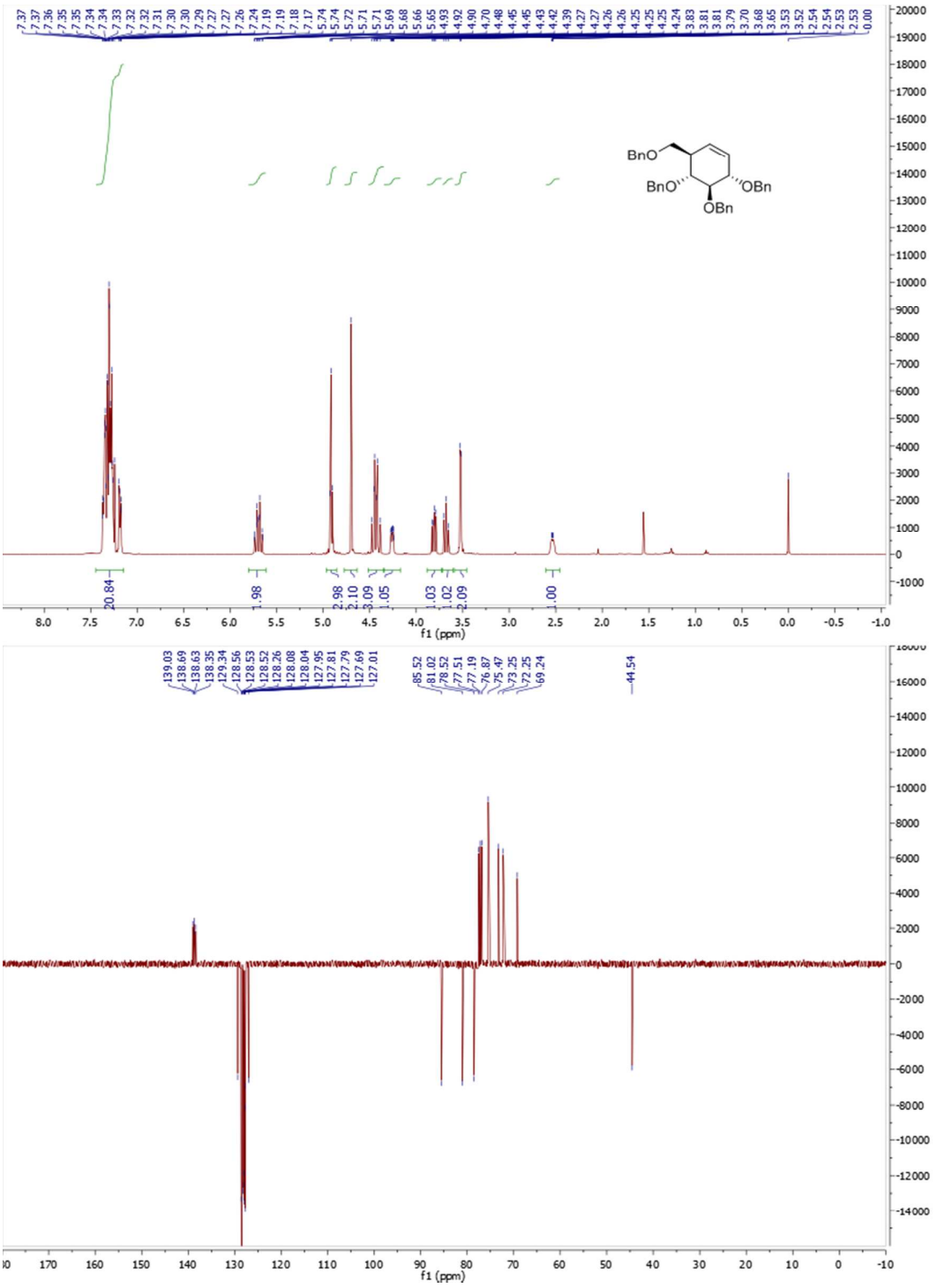
Starting from **S6** (53 mg, 0.088 mmol) and following **GP5**, the product was purified by flash column chromatography (DCM/MeOH, 99:1) affording compound **S7** as a colorless oil (39 mg, 74%). ^{1}H-NMR (400 MHz, CD_3CN) δ 7.49 – 7.17 (m, 20H), 4.87 (m, 8H), 4.70 (dd, *J* = 4.3, 2.1 Hz, 2H), 3.90 (dd, *J* = 4.3, 2.1 Hz, 2H), 2.63 (dd, *J* = 8.2, 7.4 Hz, 2H), 1.64 (m, 2H), 1.35 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (101 MHz, CD_3CN) δ 151.6, 140.1, 139.9, 129.2, 128.8, 128.7, 128.4, 128.3, 84.3, 77.0, 75.6, 73.1, 31.5, 29.0, 23.1, 14.1. IR (neat, cm^{-1}): ν 3030, 2870, 1454, 1355, 1058. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{39}H_{43}N_2O_4$ 603.32173, found 603.32178.

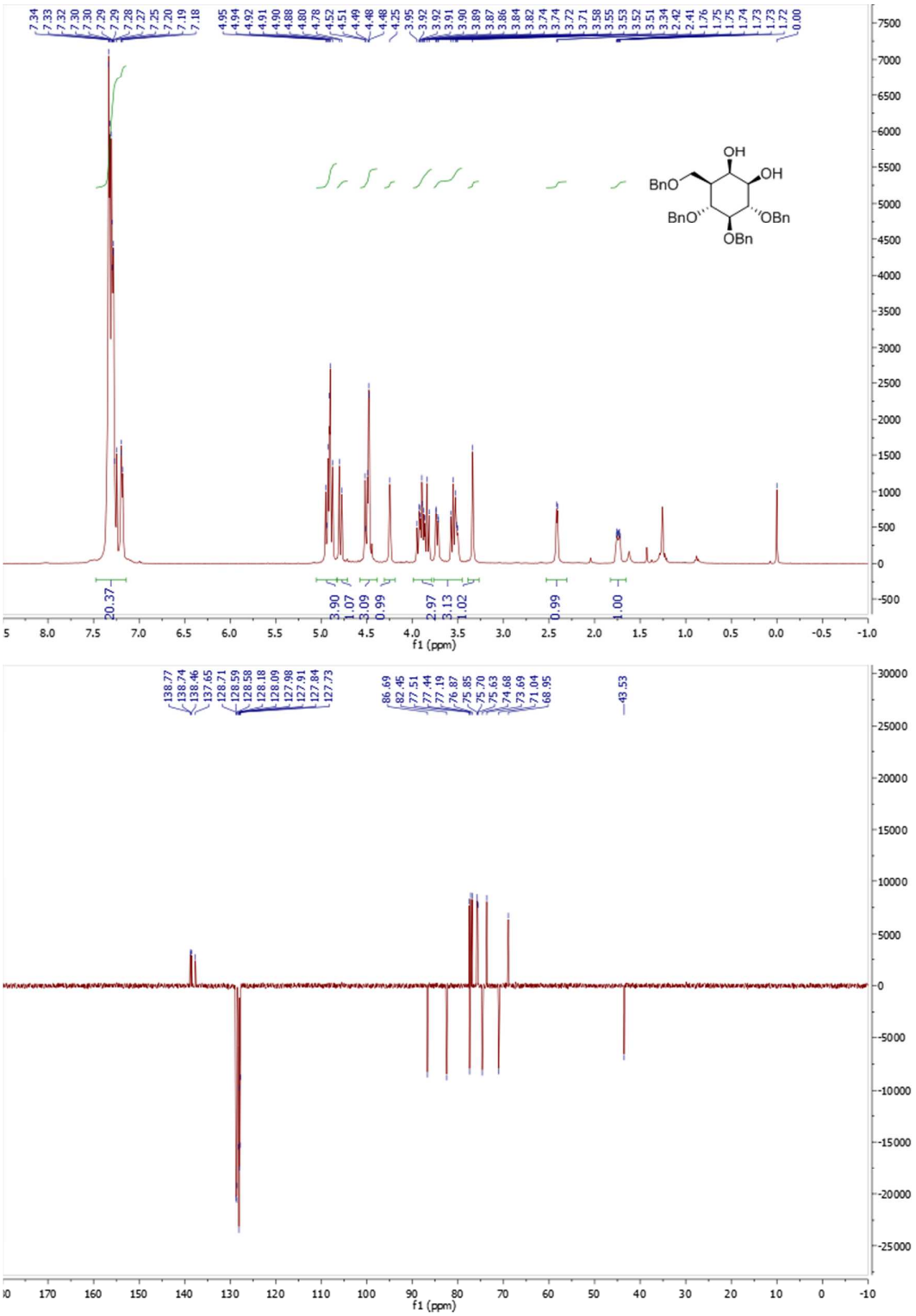
Compound 9 (conduritol B-2-butyl-1*H*-imidazole)



Starting from **S7** (46 mg, 76.3 µmol) and following **GP6**, the pure product was afforded as a white solid (23 mg, quant.). ^{1}H-NMR (400 MHz, MeOD) δ 4.48 (s, 2H), 3.45 (s, 2H), 2.82 (t, *J* = 7.3 Hz, 2H), 1.70 – 1.54 (m, 2H), 1.27 (q, *J* = 7.1 Hz, 2H), 0.86 (t, *J* = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, MeOD) δ 151.3, 129.1, 78.1, 68.4, 30.8, 26.6, 23.0, 13.8. HRMS (ESI-TOF) m/z: $[M+Na]^+$ calc for $C_{11}H_{18}N_2O_4$ 265.1159, found 265.1172.

^{1}H- and ^{13}C-NMR spectra

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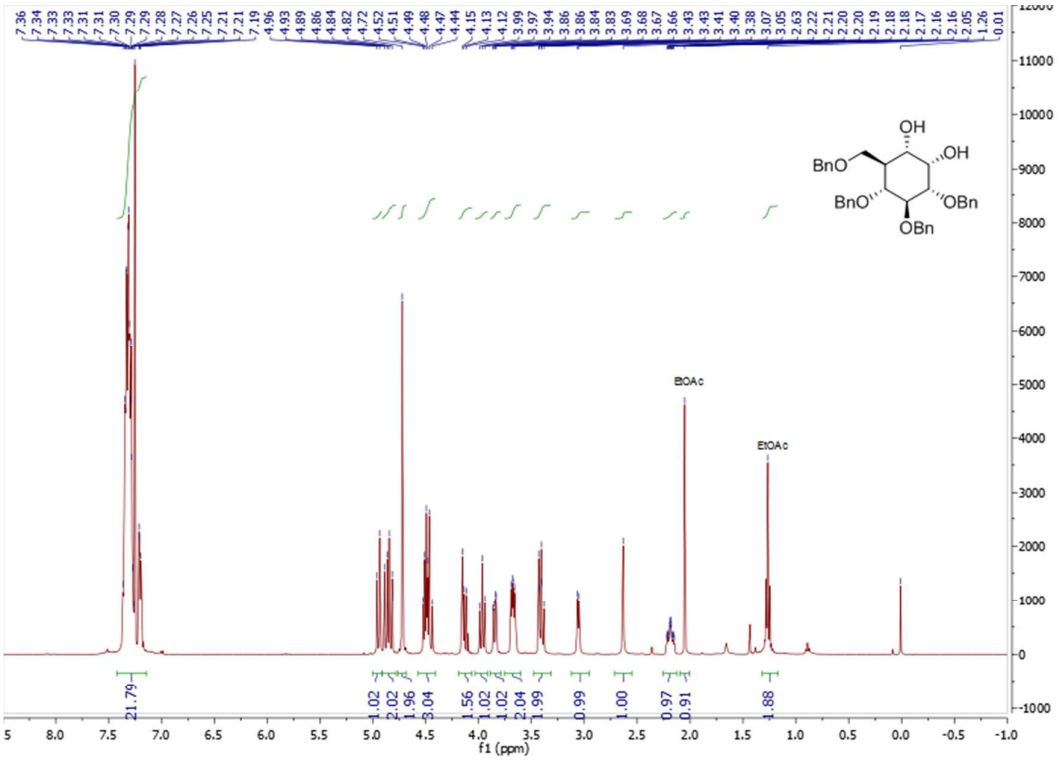
BnO
OBn
OBn
f1 (ppm)
f1 (ppm)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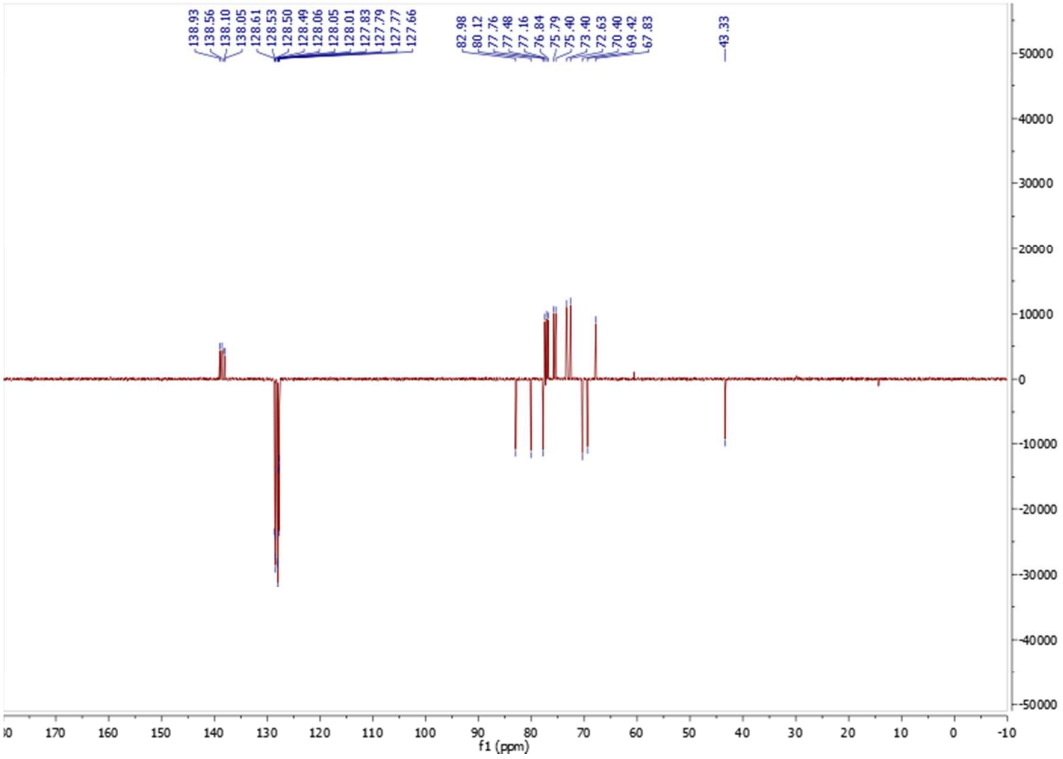
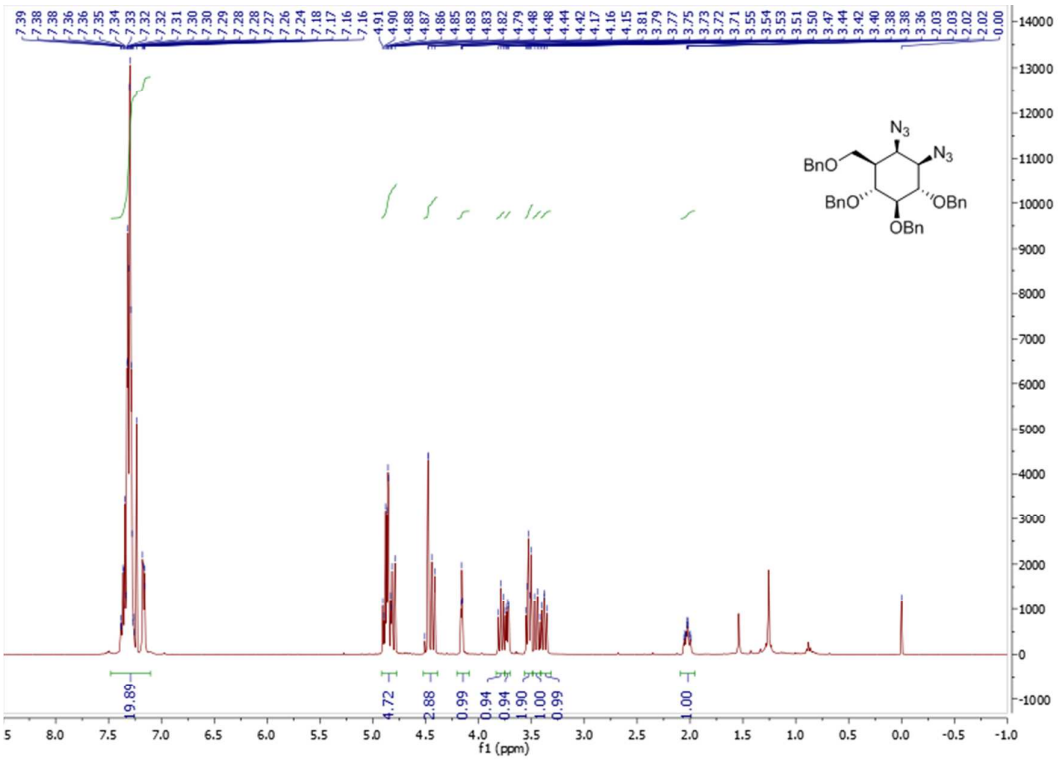
OH
BnO
BnO
OBn
OBn
EtOAc
EtOAc
f1 (ppm)


f1 (ppm)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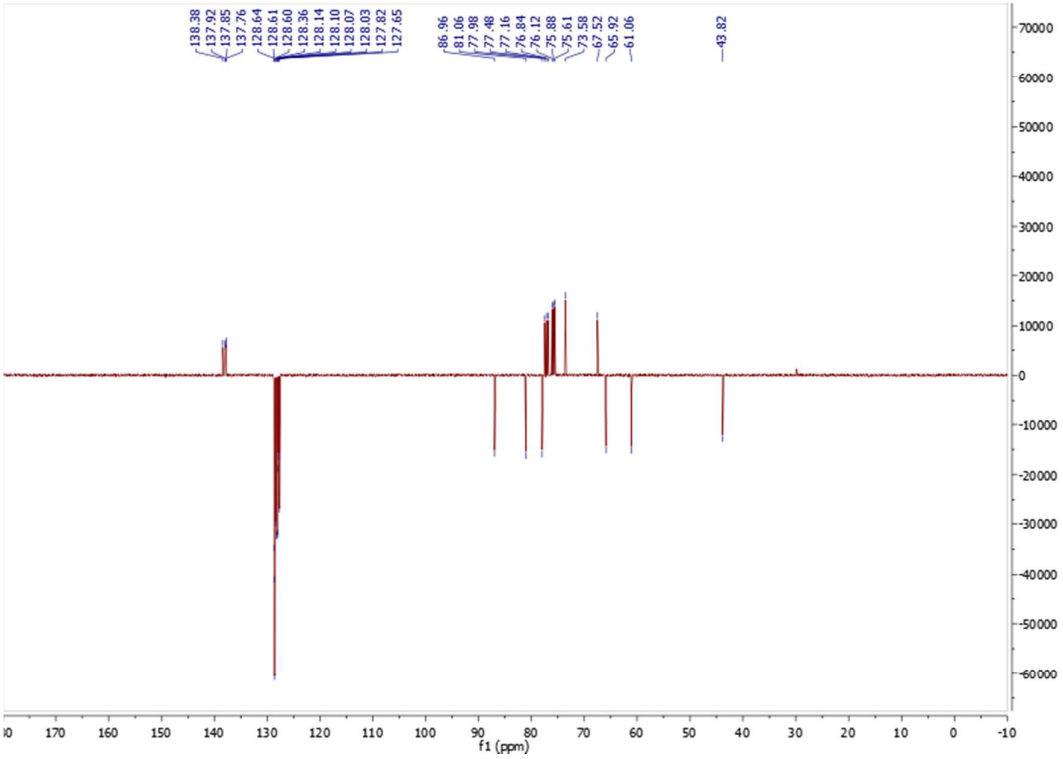
f1 (ppm)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61.06
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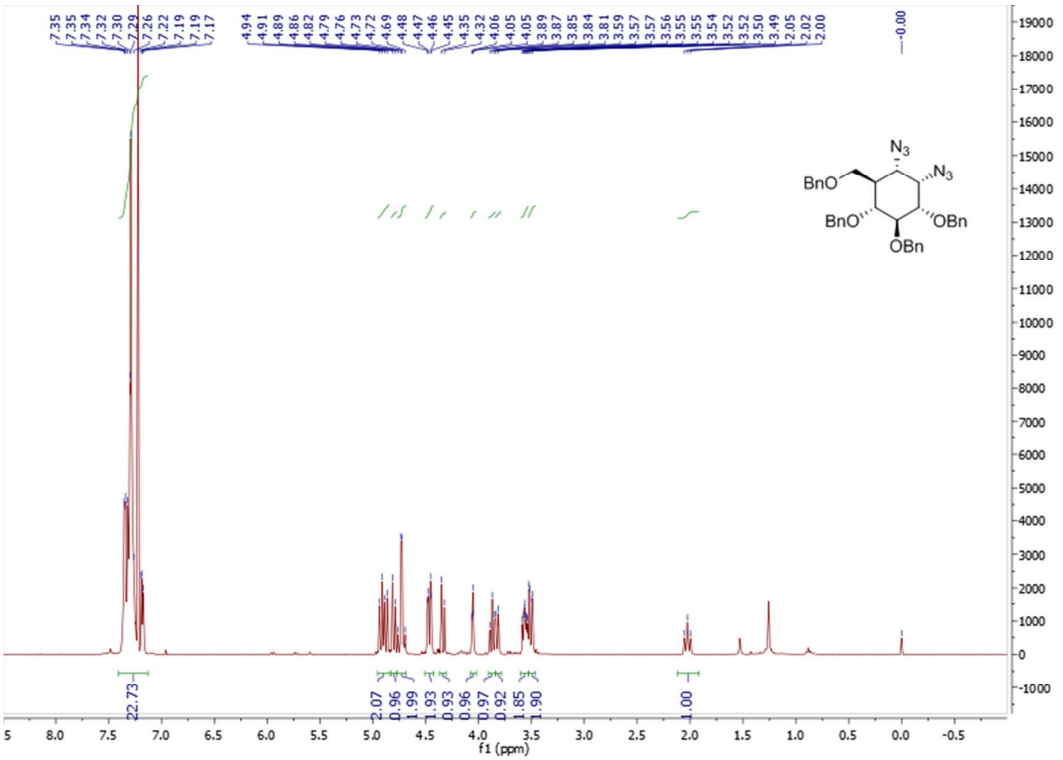
f1 (ppm)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3.54
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2.05
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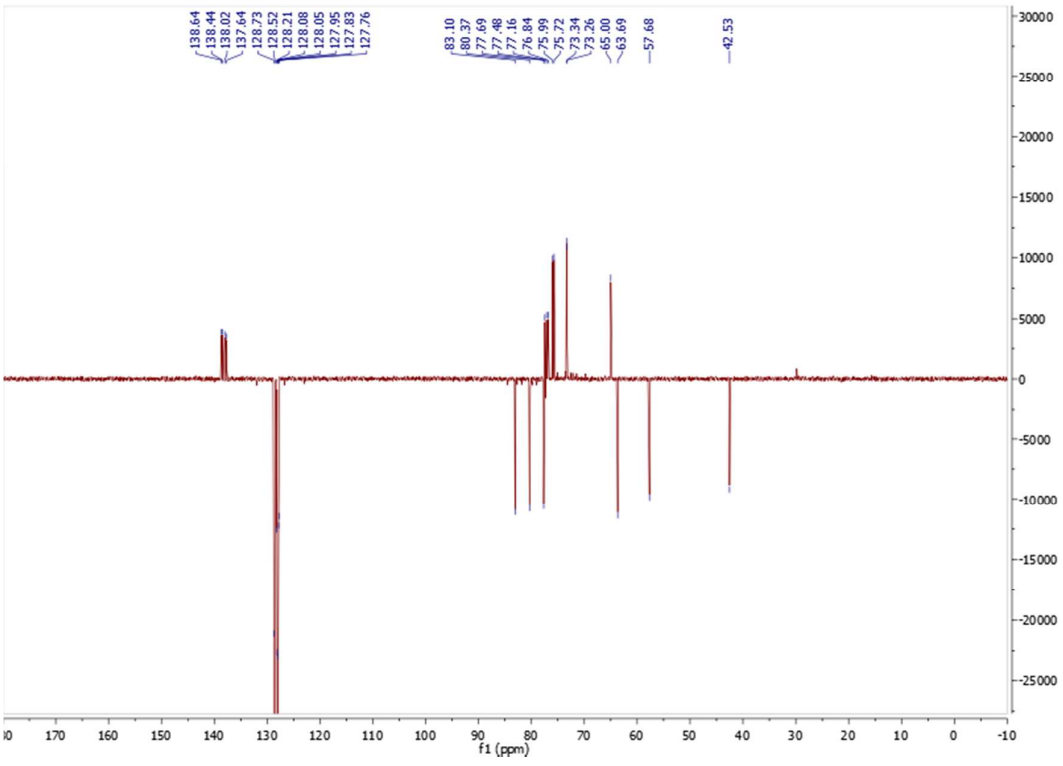
2.00
0.00
N3
N3
BnO
BnO
OBn
OBn
22.73
2.07
0.96
1.99
1.93
0.93
0.96
0.97
0.92
1.85
1.90
1.00
f1 (ppm)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128.52
128.21
128.08
128.05
127.95
127.83
127.76
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80.37
77.69
77.48
77.16
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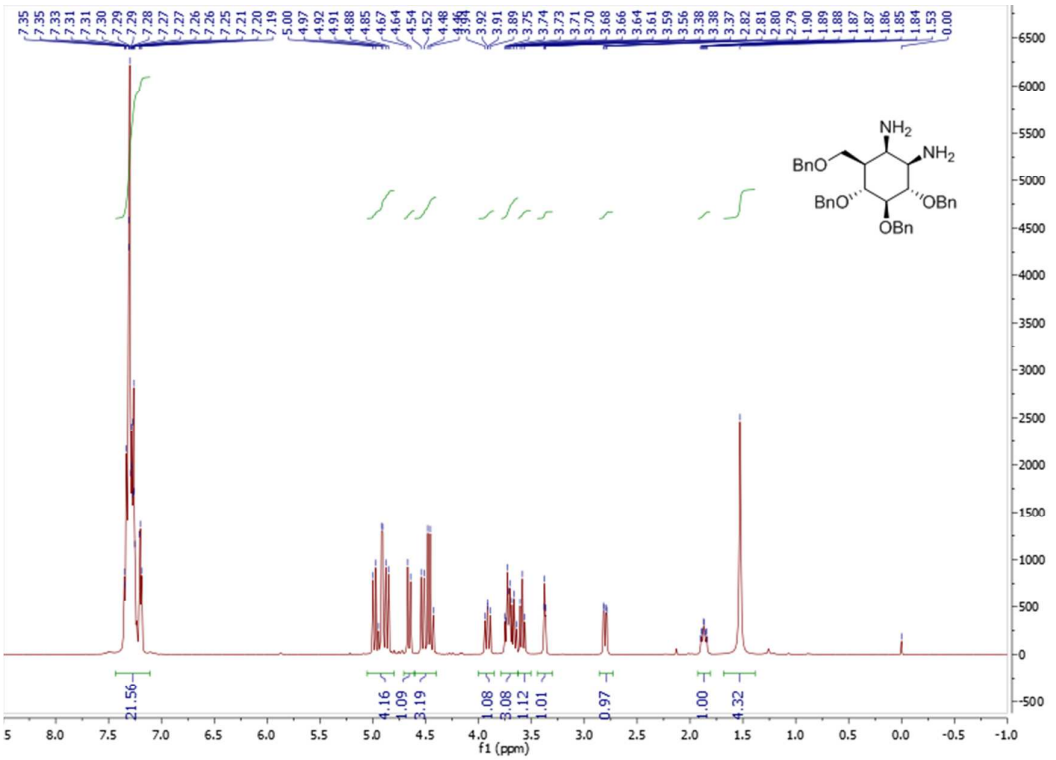
75.99
75.72
73.34
73.26
65.00
63.69
57.68
42.53
f1 (ppm)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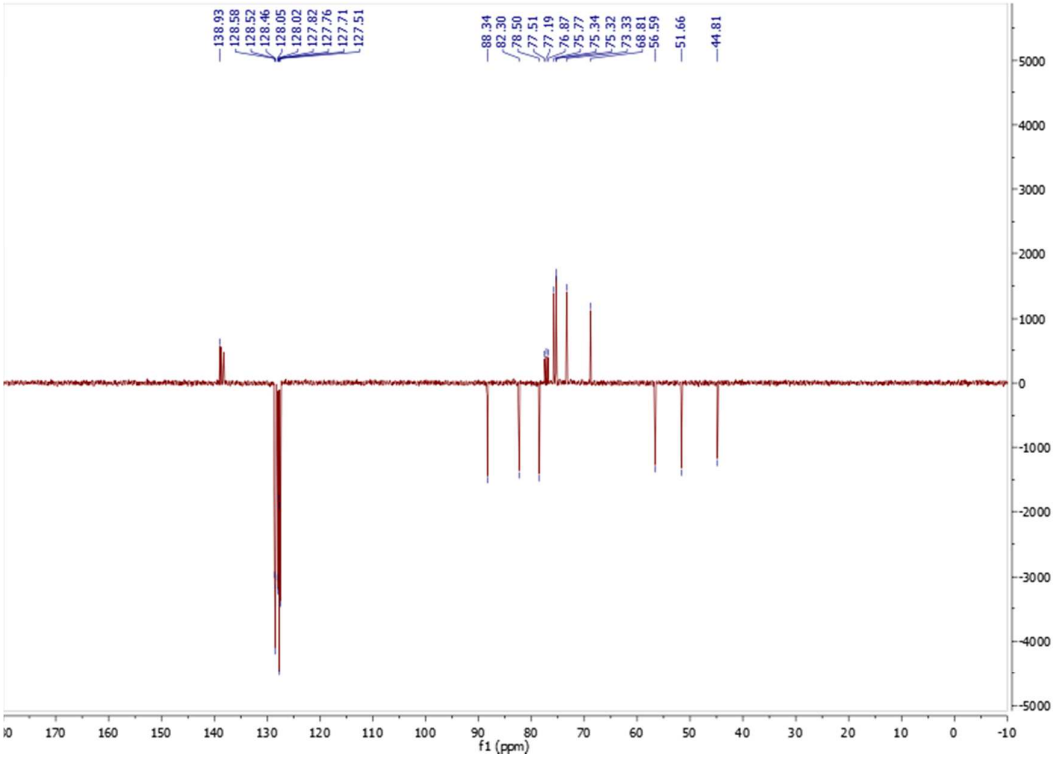
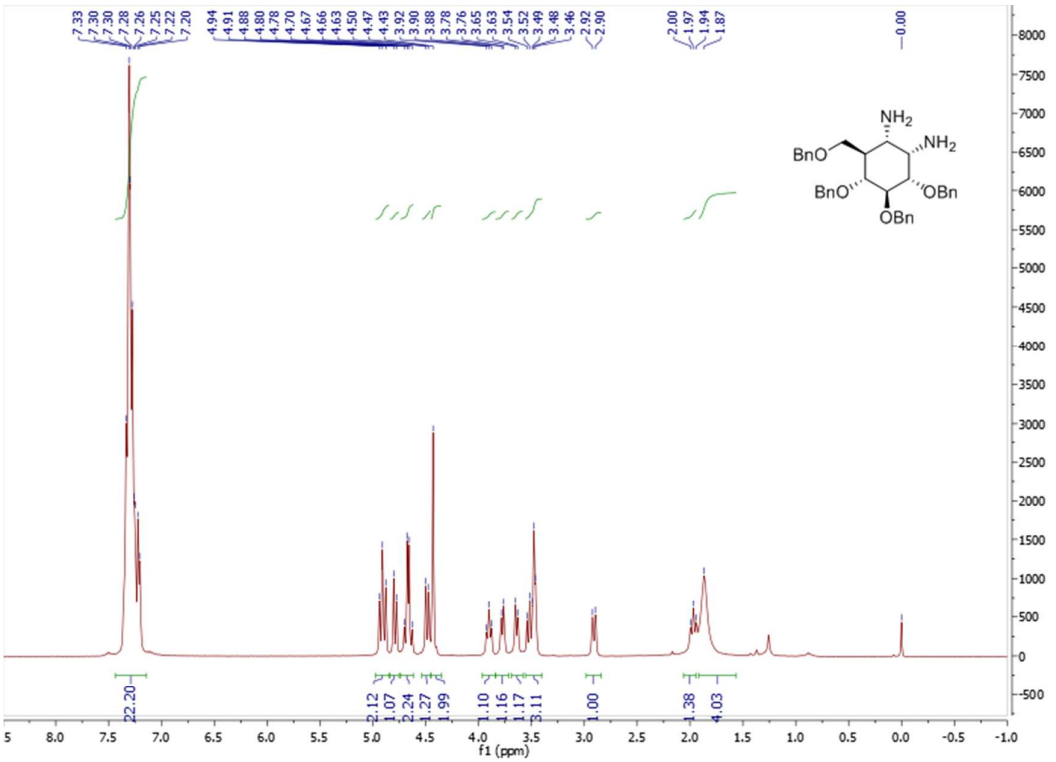
NH2
BnO
BnO
OBn
OBn
f1 (ppm)


f1 (ppm)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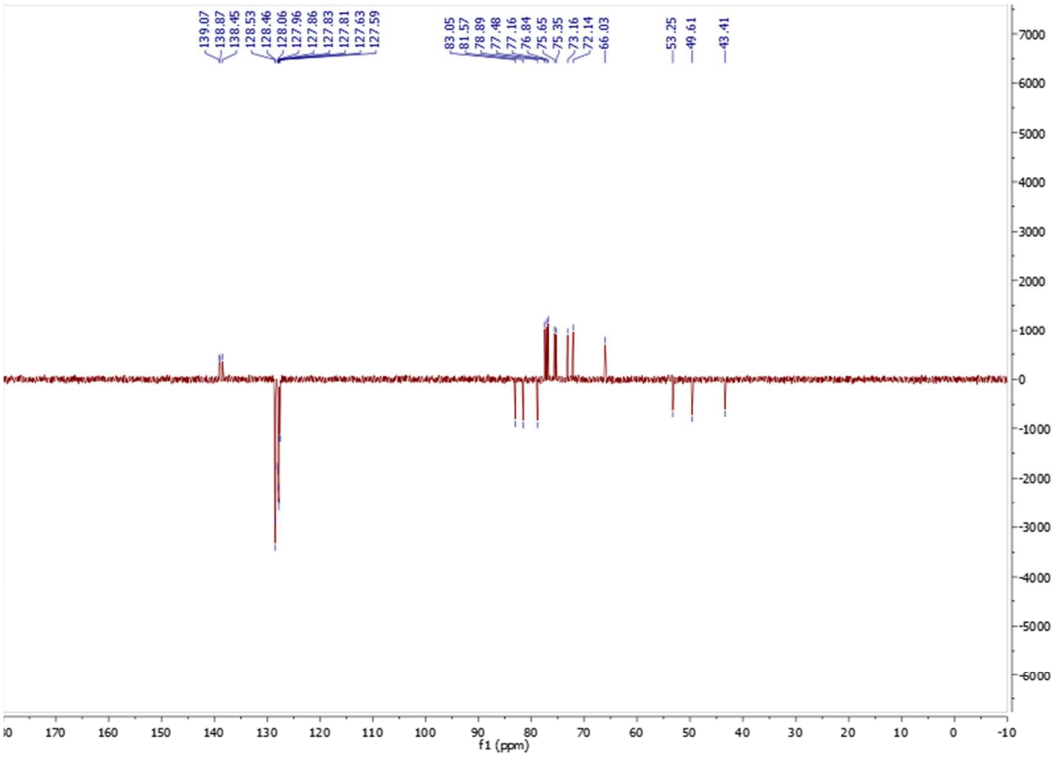
4.91
4.88
4.80
4.78
4.70
4.67
4.66
4.63
4.50
4.47
4.43
3.92
3.90
3.88
3.78
3.76
3.65
3.63
3.54
3.52
3.49
3.48
3.46
2.92
2.90
2.00
1.97
1.94
1.87
0.00
NH2
NH2
BnO
BnO
OBn
OBn
22.20
2.12
1.07
2.24
1.27
1.99
1.10
1.16
1.17
3.11
1.00
1.38
4.03
f1 (ppm)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127.63
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83.05
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78.89
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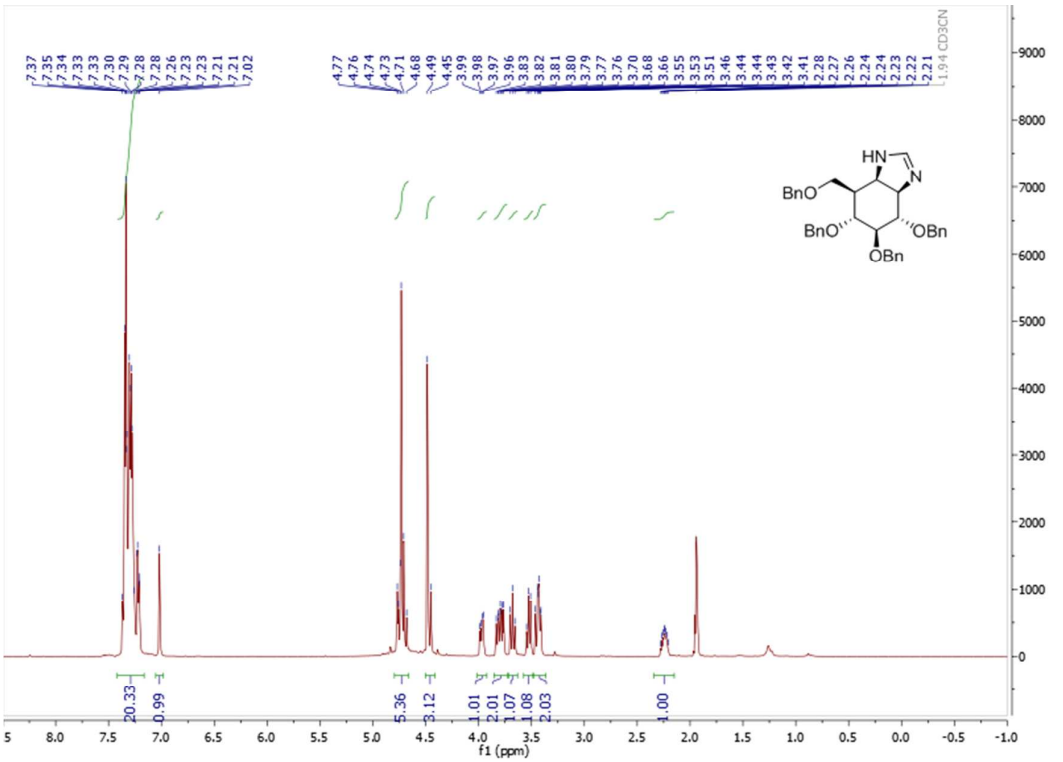
75.35
73.16
72.14
66.03
53.25
49.61
43.41
f1 (ppm)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7.21
7.02
4.77
4.76
4.74
4.73
4.71
4.68
4.49
4.45
3.99
3.98
3.97
3.96
3.83
3.82
3.81
3.80
3.79
3.77
3.76
3.70
3.68
3.66
3.55
3.53
3.51
3.46
3.44
3.44
3.43
3.42
3.41
2.28
2.27
2.26
2.24
2.24
2.23
2.22
2.21
1.94 CD3CN
HN
N
BnO
BnO
OBn
OBn
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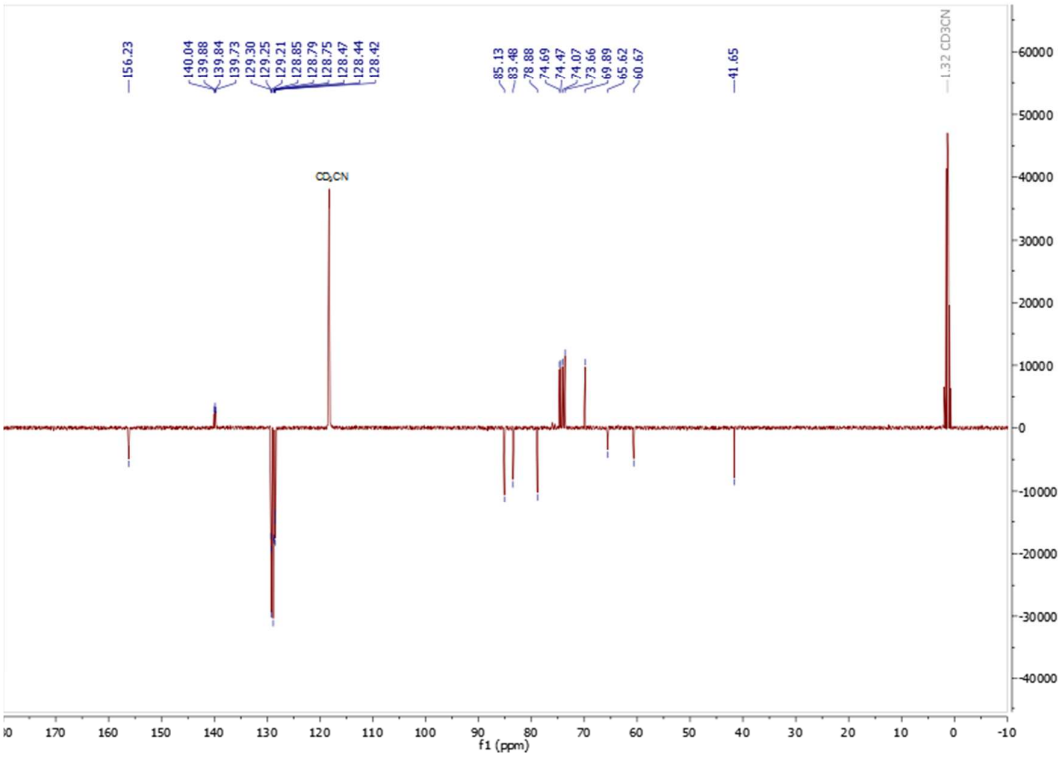
5.36
3.12
1.01
2.01
1.07
1.08
2.03
1.00
f1 (ppm)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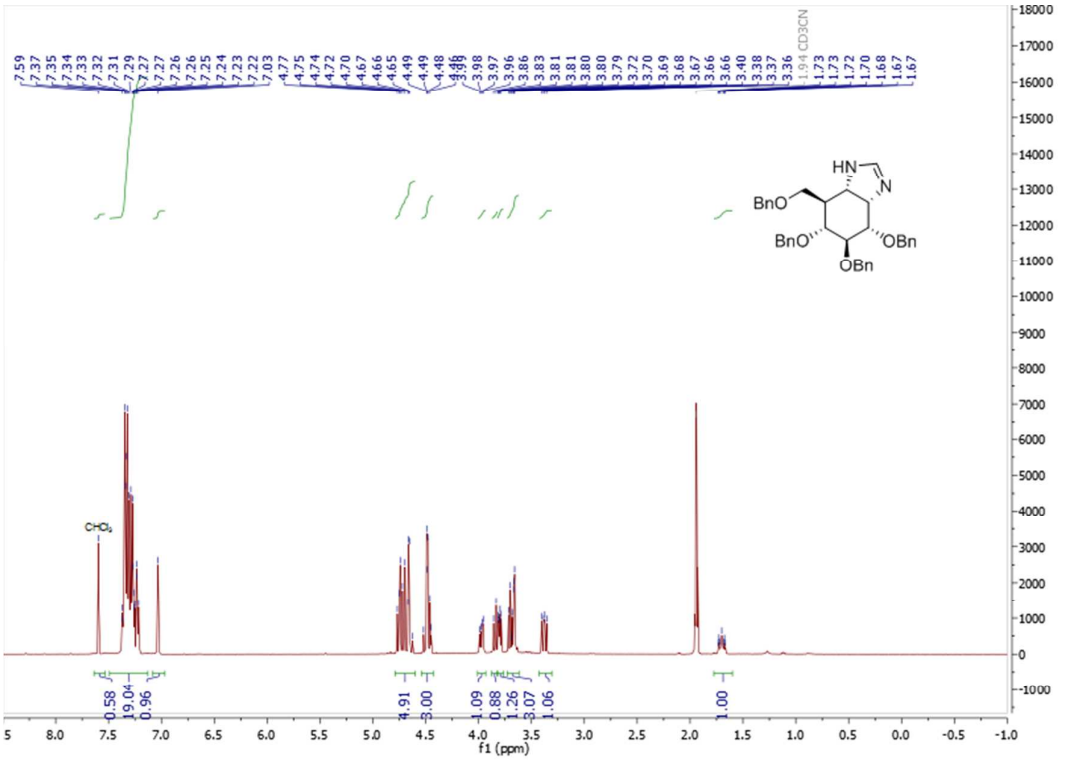
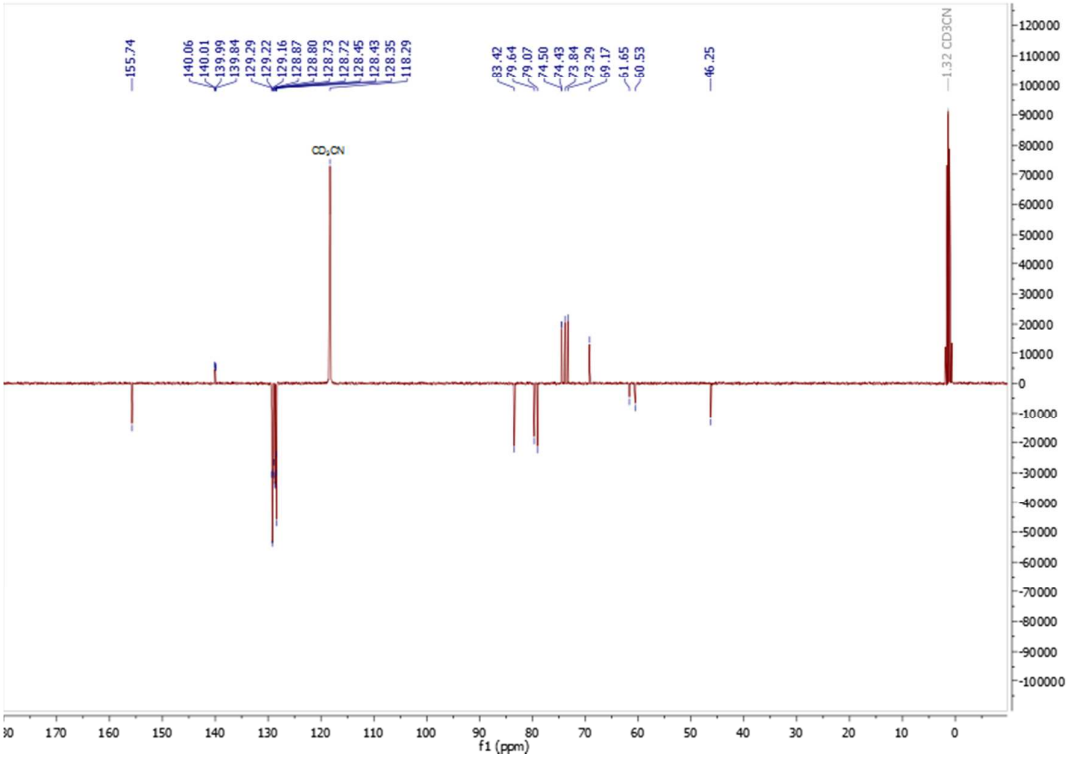
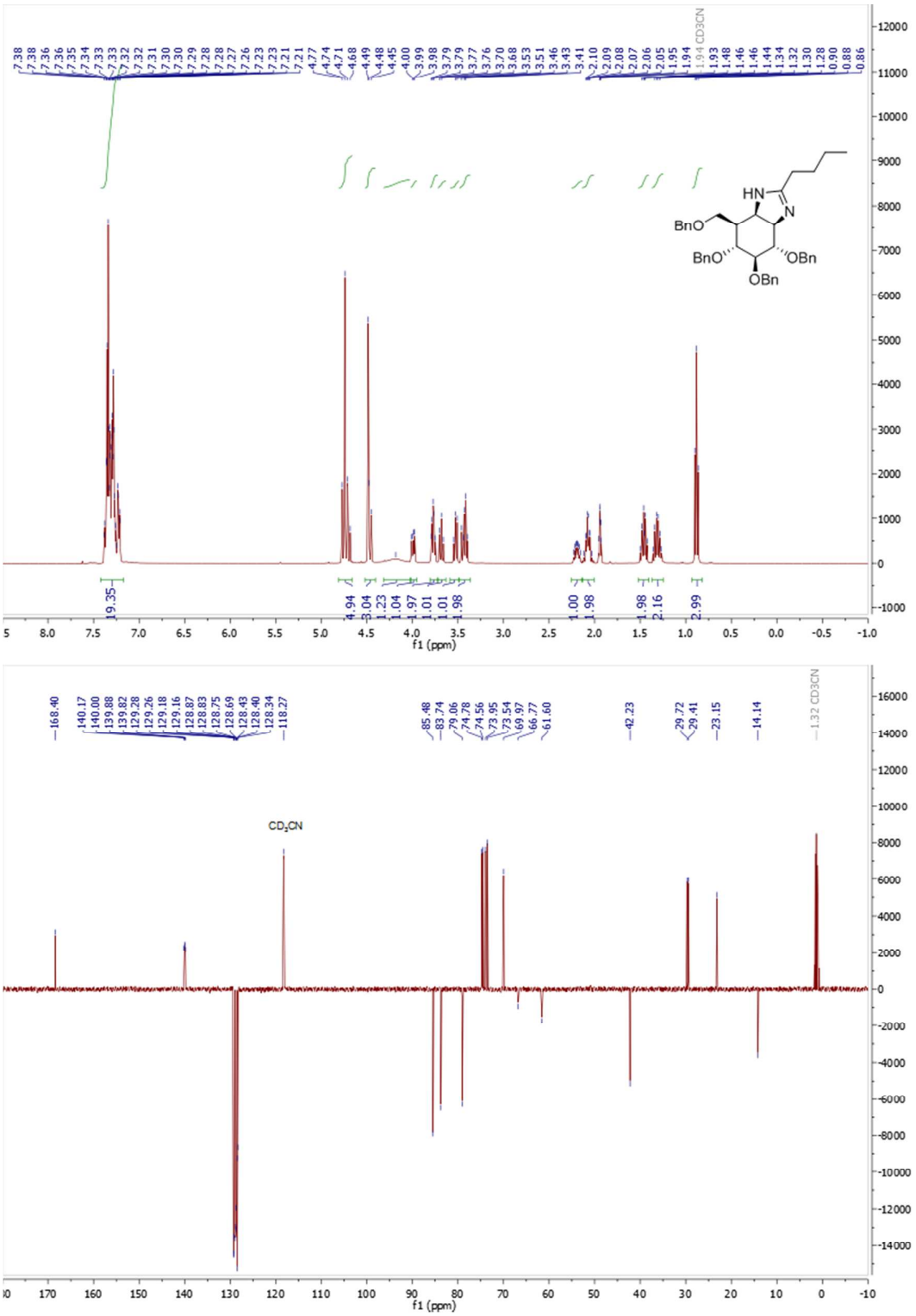
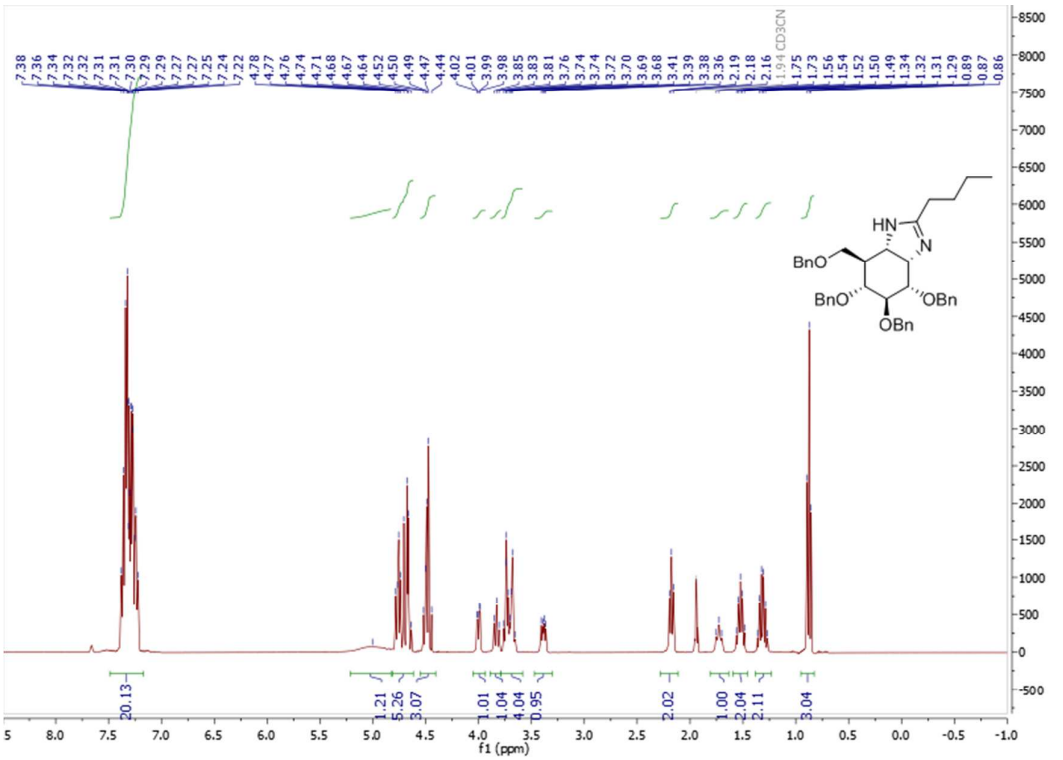
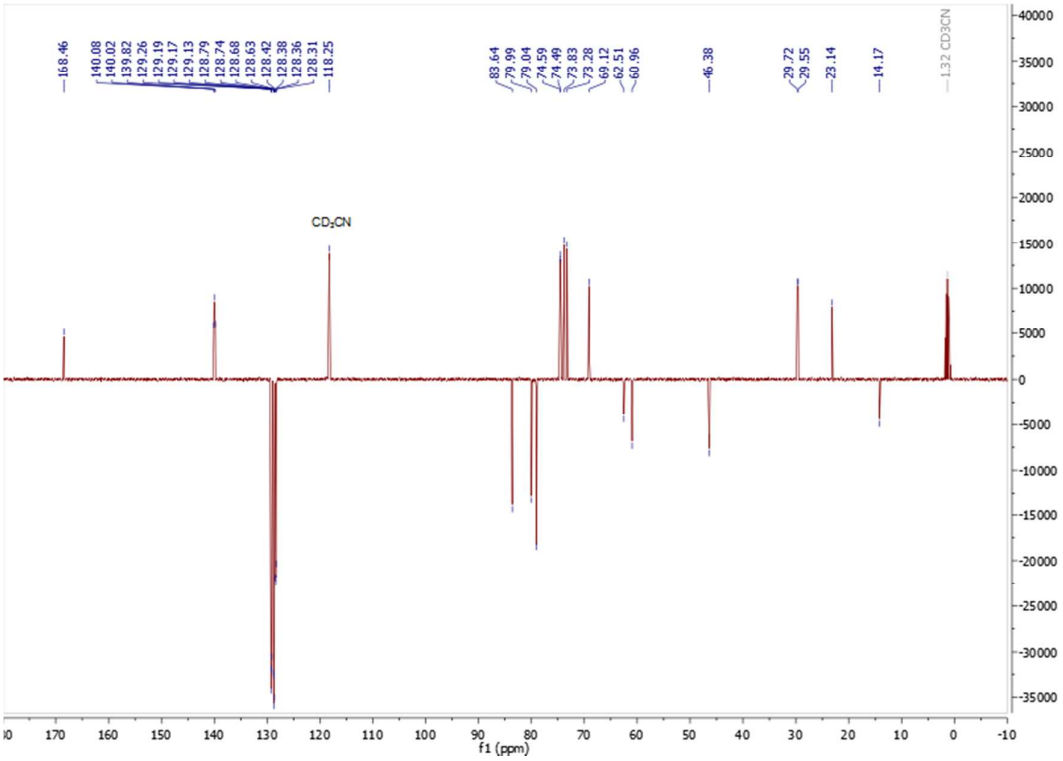
128.47
128.44
128.42
85.13
83.48
78.88
74.69
74.47
74.07
73.66
69.89
65.62
60.67
41.65
1.32 CD3CN
CD3CN
f1 (ppm)

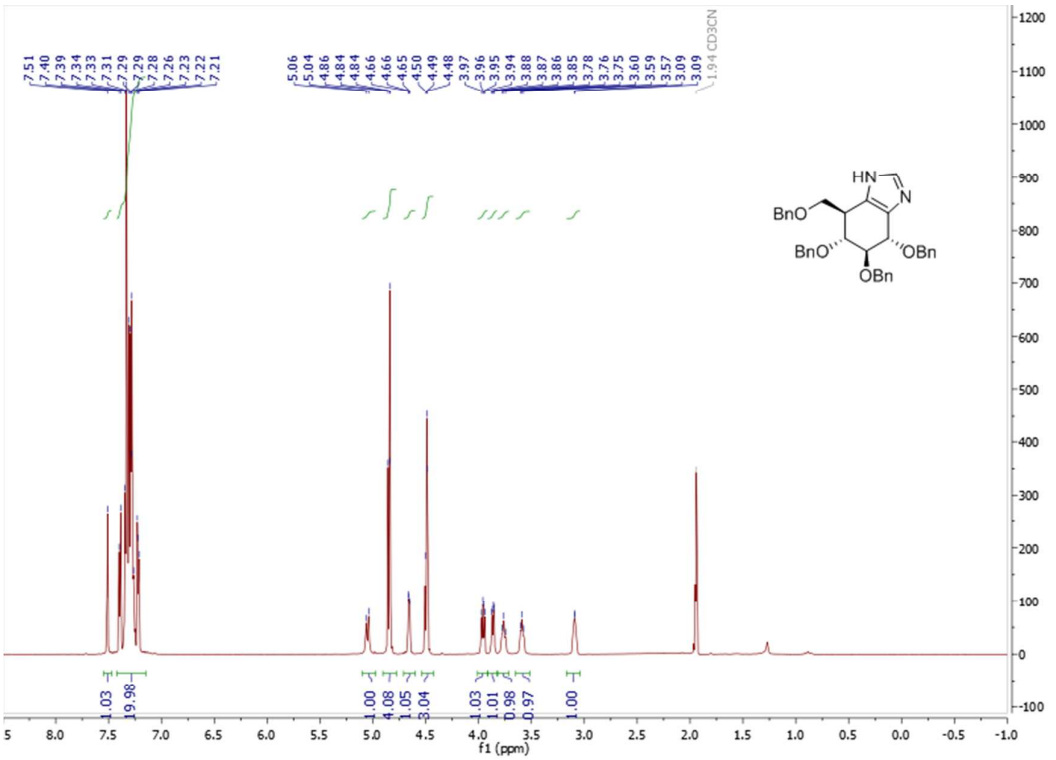

CHCl3
f1 (ppm)


CD3CN
f1 (ppm)


7.38 7.38 7.36 7.36 7.35 7.34 7.33 7.33 7.32 7.32 7.31 7.30 7.30 7.29 7.28 7.28 7.27 7.26 7.23 7.23 7.21 7.21 4.77 4.74 4.71 4.68 4.49 4.48 4.45 4.00 3.99 3.98 3.79 3.79 3.77 3.76 3.70 3.68 3.53 3.51 3.46 3.43 3.41 2.10 2.09 2.08 2.07 2.06 2.05 1.95 1.94 1.94 CD3CN 1.93 1.48 1.46 1.46 1.44 1.34 1.32 1.30 1.28 0.90 0.88 0.86
HN
N
BnO
BnO
OBn
OBn
19.35 4.94 3.04 1.23 1.04 1.97 1.01 1.01 1.98 1.00 1.98 1.98 2.16 2.99
f1 (ppm)
168.40 140.17 140.00 139.88 139.82 129.28 129.26 129.18 129.16 128.87 128.83 128.75 128.69 128.43 128.40 128.34 118.27
85.48 83.74 79.06 74.78 74.56 73.95 73.54 69.97 66.77 61.60
42.23
29.72 29.41
23.15
14.14
1.32 CD3CN
CD3CN
f1 (ppm)


HN
N
BnO
BnO
OBn
OBn
f1 (ppm)


CD3CN
f1 (ppm)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3.96
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3.94
3.88
3.87
3.86
3.85
3.78
3.76
3.75
3.60
3.59
3.57
3.09
3.09
1.94 CD3CN
HN
N
BnO
BnO
OBn
OBn
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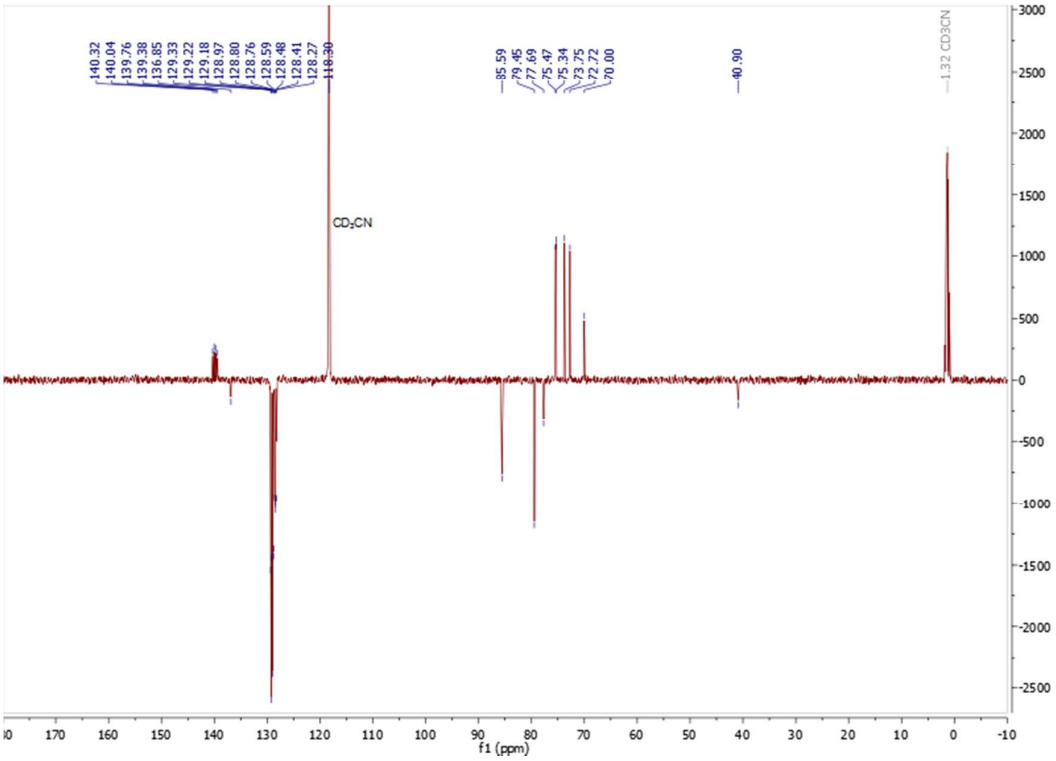
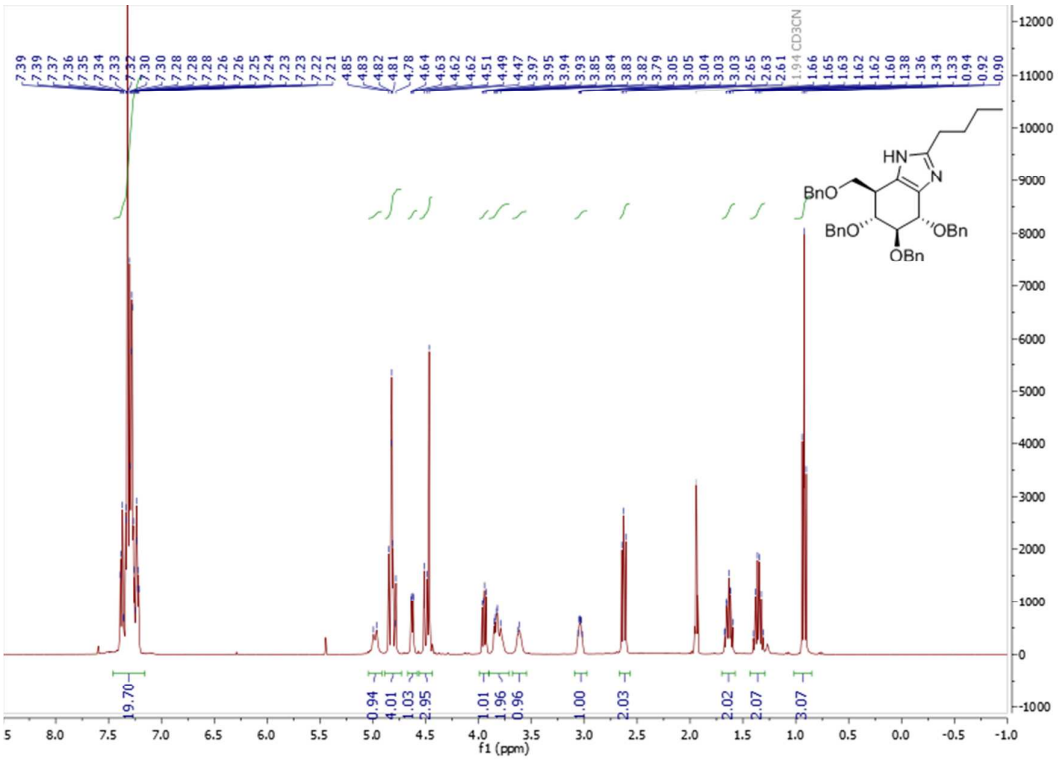
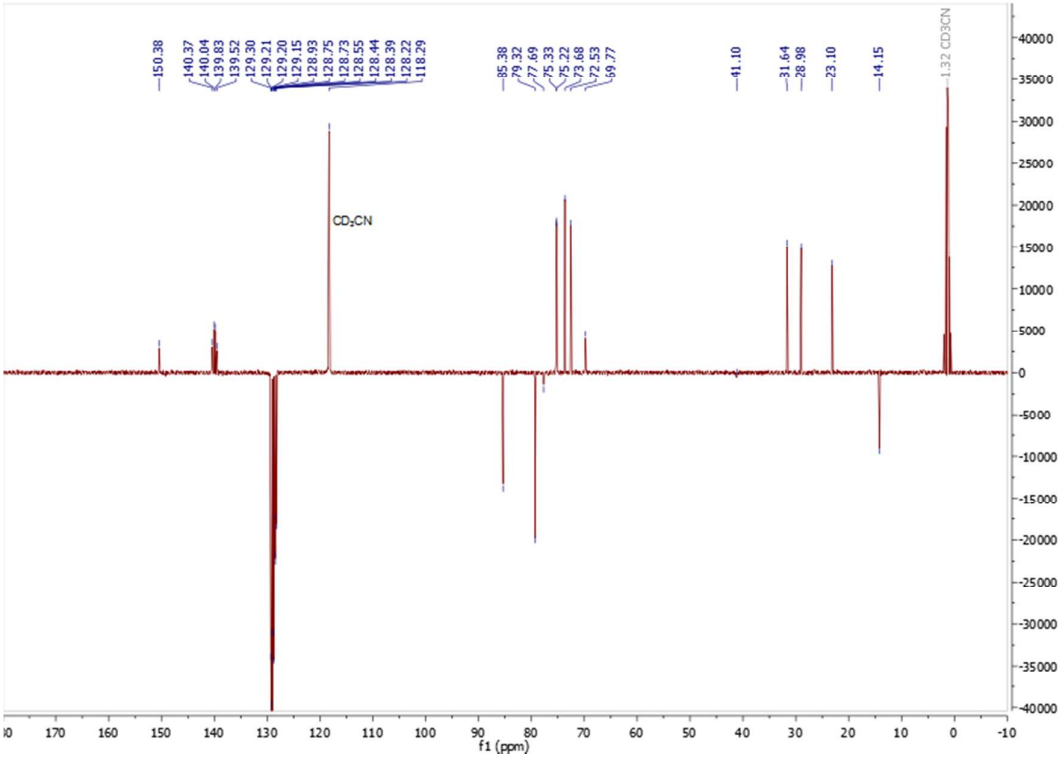
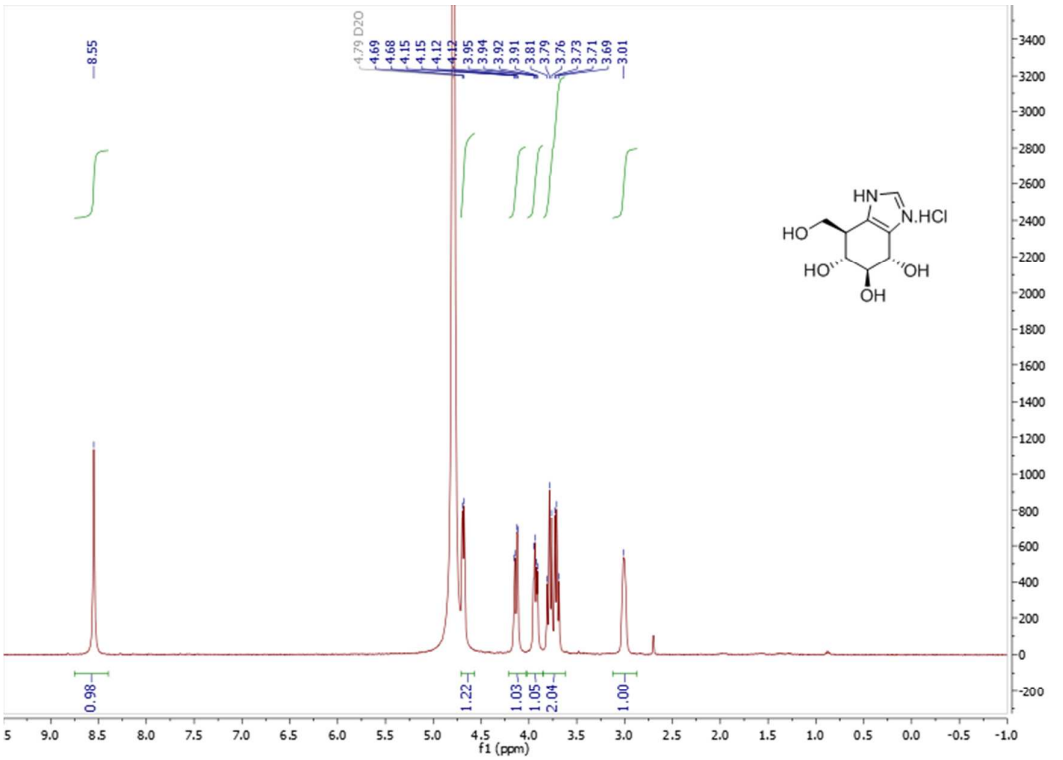
19.98
1.00
4.08
1.05
3.04
1.03
1.01
0.98
0.97
1.00
f1 (ppm)


140.32
140.04
139.76
139.38
136.85
129.33
129.22
129.18
128.97
128.80
128.76
128.59
128.48
128.41
128.27
118.30
85.59
79.45
77.69
75.47
75.34
73.75
72.72
70.00
40.90
1.32 CD3CN
CD3CN
f1 (ppm)


HN
N
BnO
BnO
OBn
OBn
19.70
0.94
4.01
1.03
2.95
1.01
1.96
0.96
1.00
2.03
2.02
2.07
3.07
f1 (ppm)


CD3CN
CD2CN
f1 (ppm)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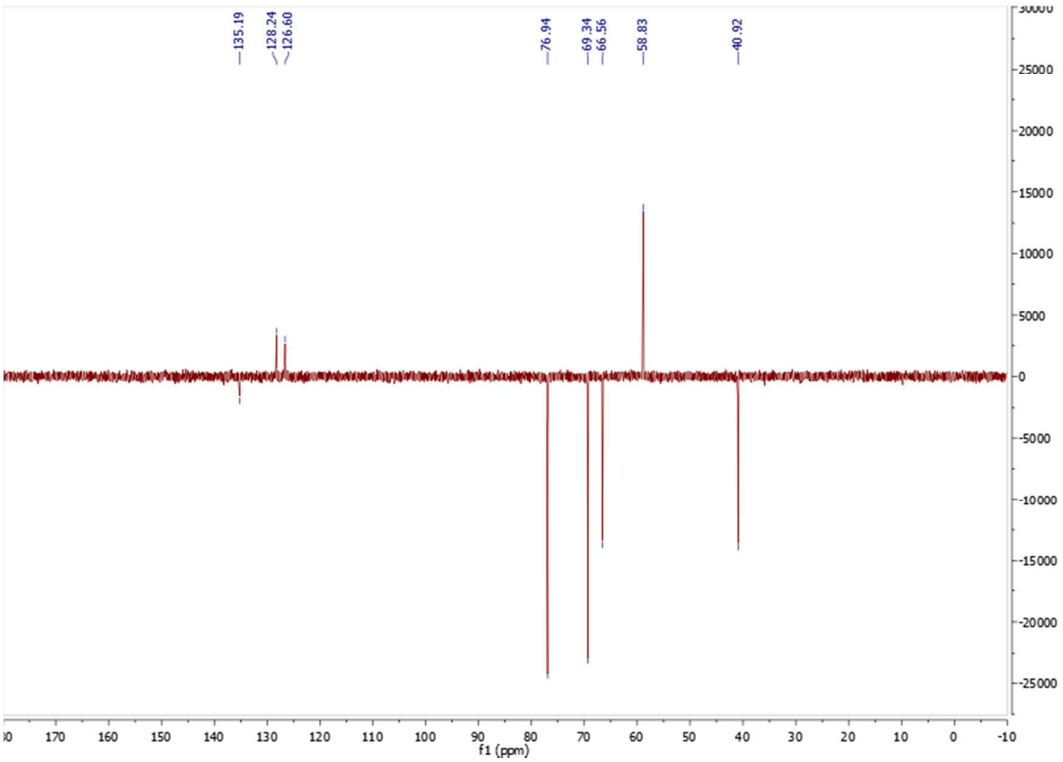
4.79 D2O
4.69
4.68
4.15
4.15
4.12
4.12
3.95
3.94
3.92
3.91
3.81
3.79
3.76
3.73
3.71
3.69
3.01
HN
N.HCl
HO
HO
OH
OH
0.98
1.22
1.03
1.05
2.04
1.00
f1 (ppm)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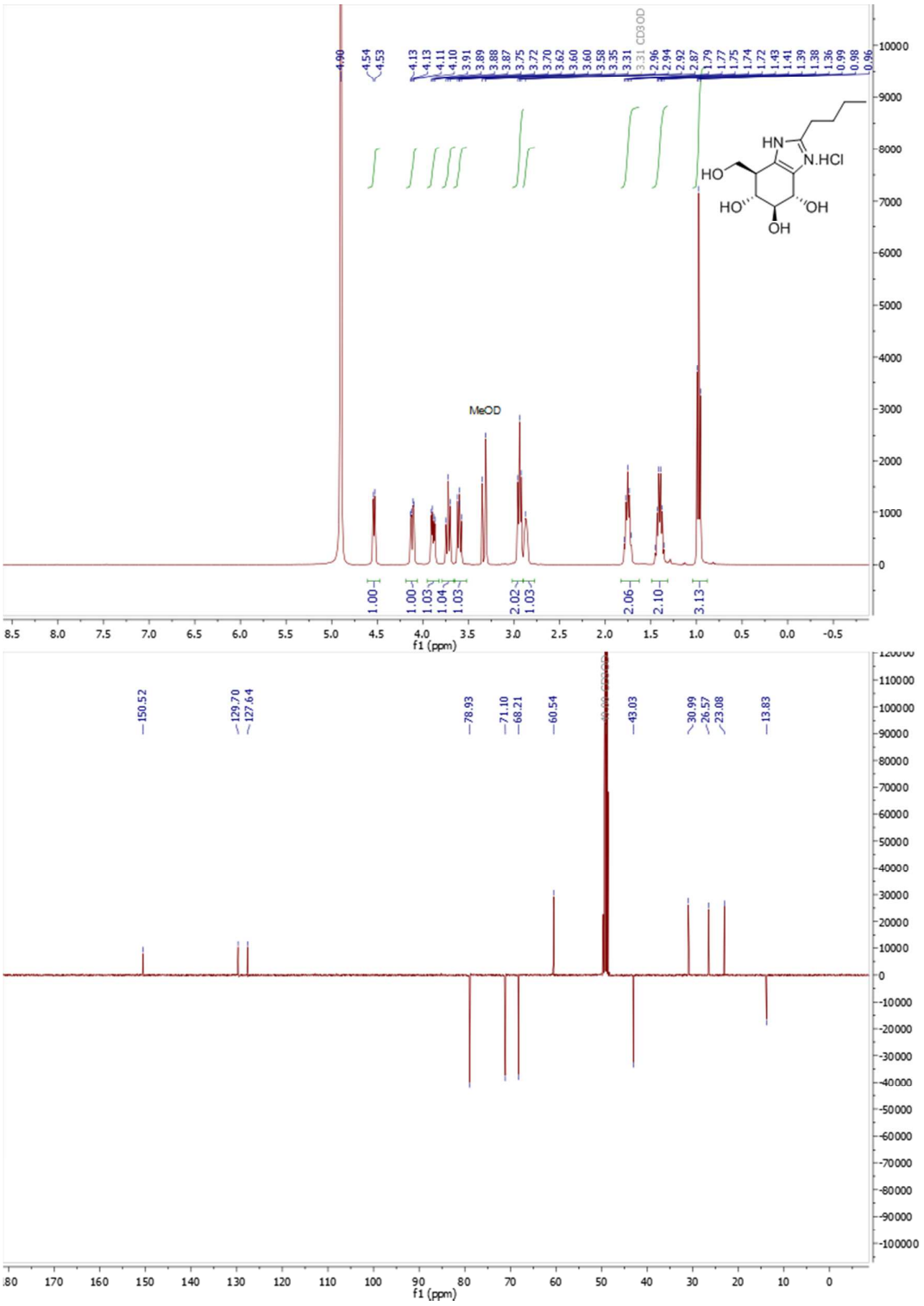
126.60
76.94
69.34
66.56
58.83
40.92
f1 (ppm)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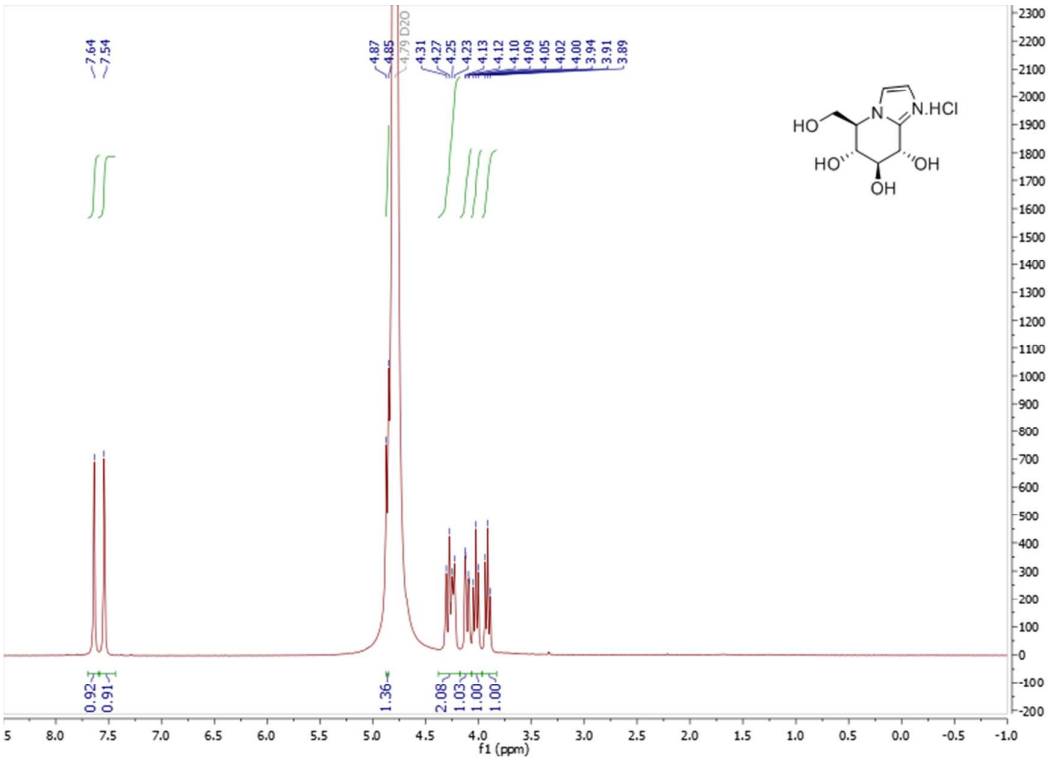
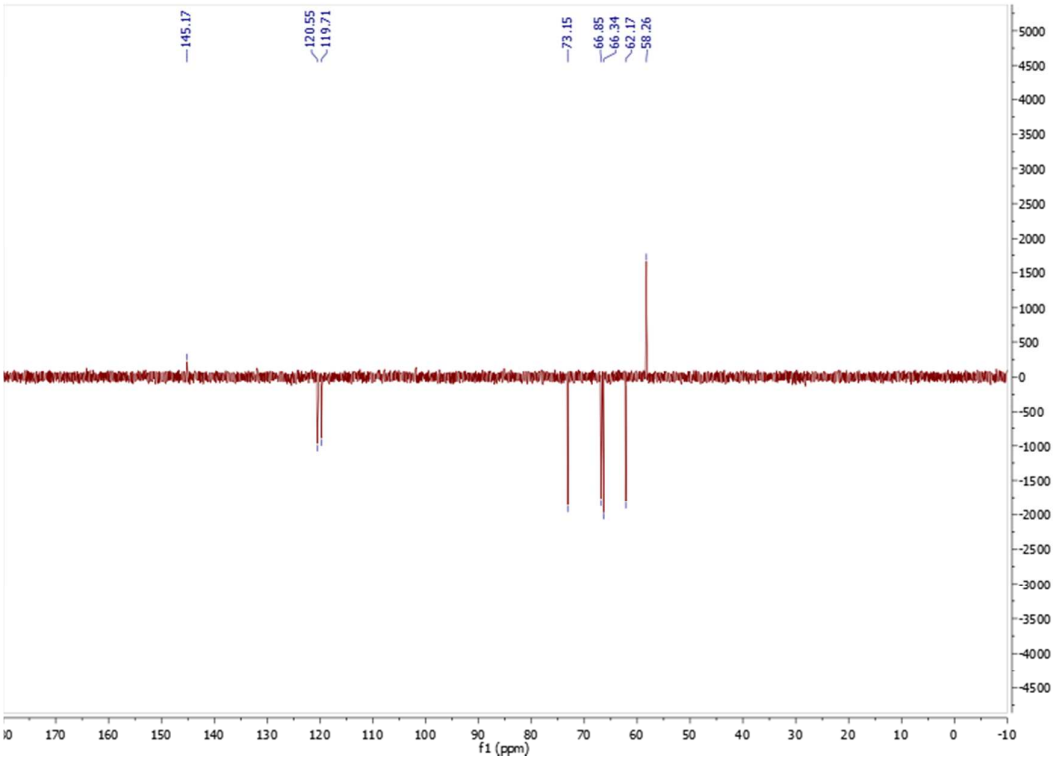
4.13
4.11
4.10
3.91
3.89
3.88
3.87
3.75
3.72
3.70
3.62
3.60
3.60
3.58
3.35
3.31
CD3OD
2.96
2.94
2.92
2.87
1.79
1.77
1.75
1.74
1.72
1.43
1.41
1.39
1.38
1.36
0.99
0.98
0.96
MeOD
HN
N.HCl
HO
HO
OH
OH
1.00
1.00
1.03
1.04
1.03
2.02
1.03
2.06
2.10
3.13
f1 (ppm)
150.52
129.70
127.64
78.93
71.10
68.21
60.54
49.00
43.03
30.99
26.57
23.08
13.83
f1 (ppm)


7.64
7.54
4.87
4.85
4.79 D2O
4.31
4.27
4.25
4.23
4.13
4.12
4.10
4.09
4.05
4.02
4.00
3.94
3.91
3.89
HO
N
N.HCl
HO
OH
OH
0.92
0.91
1.36
2.08
1.03
1.00
1.00
f1 (ppm)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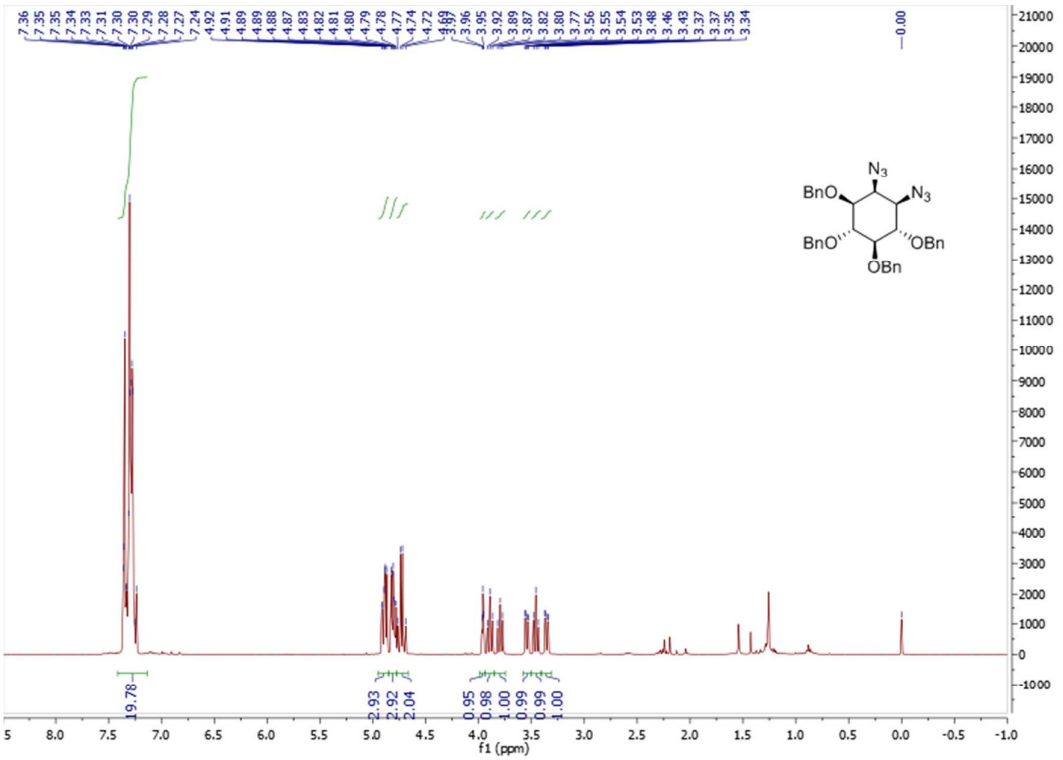
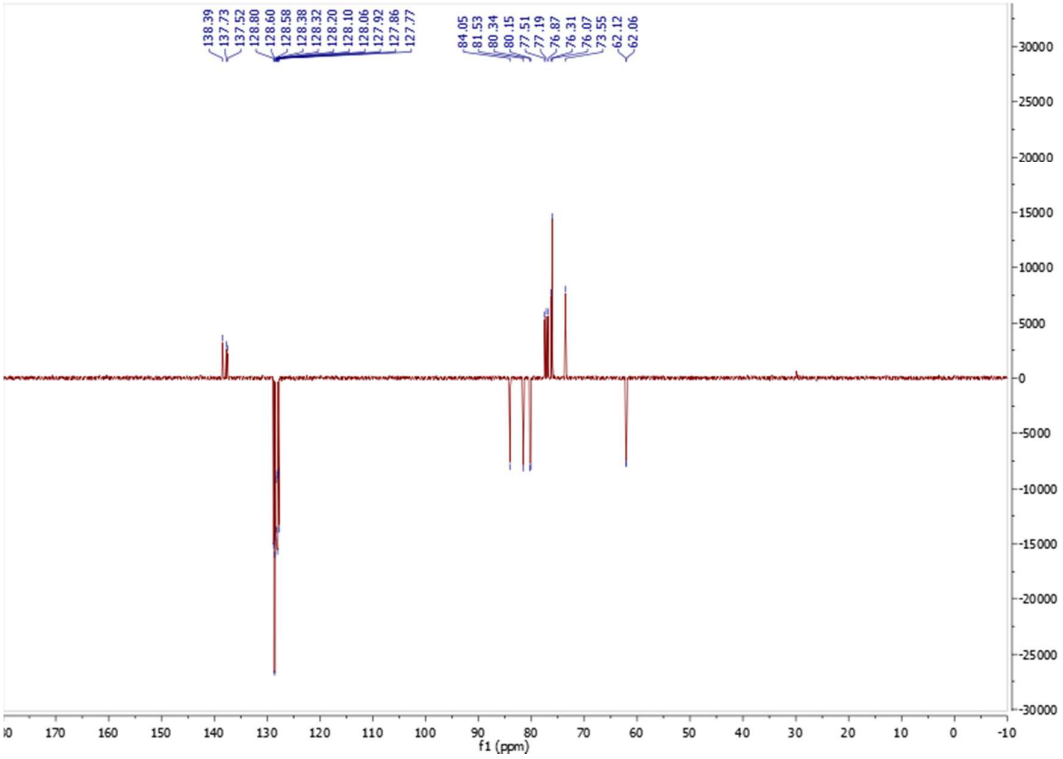
120.55
119.71
73.15
66.85
66.34
62.17
58.26
f1 (ppm)


7.36 7.35 7.35 7.34 7.33 7.31 7.30 7.30 7.29 7.28 7.27 7.24 4.92 4.91 4.89 4.89 4.88 4.87 4.83 4.82 4.81 4.80 4.79 4.78 4.77 4.74 4.72 3.96 3.95 3.92 3.89 3.87 3.82 3.80 3.77 3.56 3.55 3.54 3.53 3.48 3.46 3.43 3.37 3.37 3.35 3.34
0.00
N3
BnO
N3
BnO
OBn
OBn
19.78
2.93 2.92 2.04 0.95 0.98 1.00 0.99 0.99 1.00
f1 (ppm)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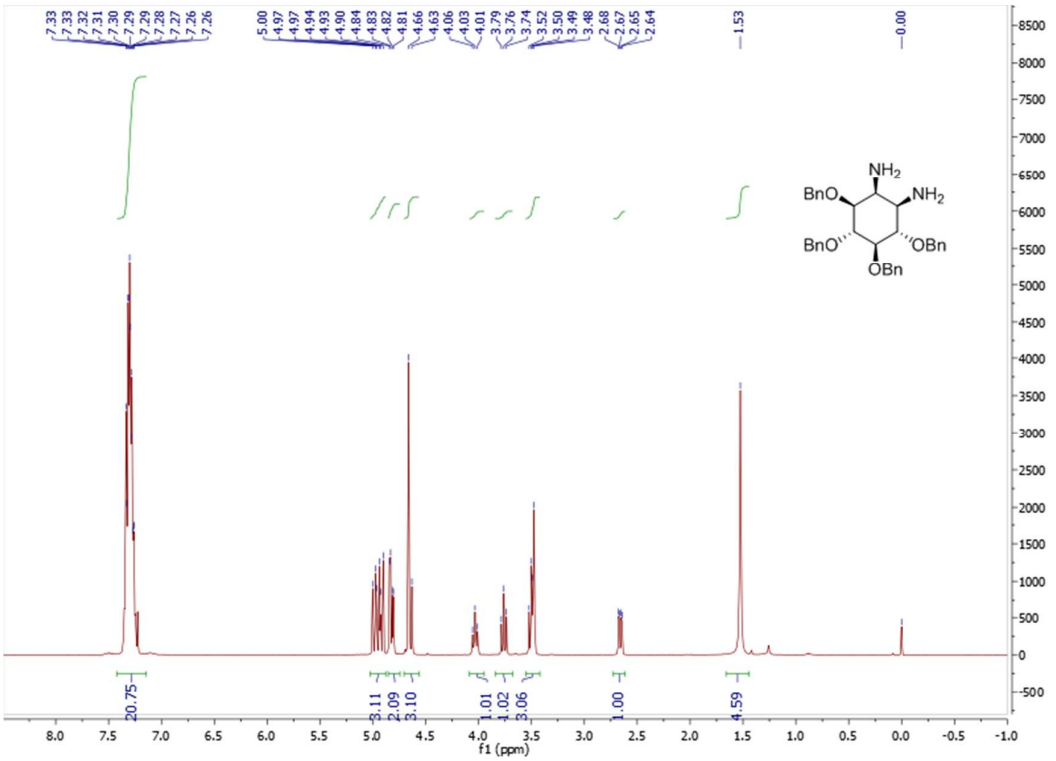
84.05 81.53 80.34 80.15 77.51 77.19 76.87 76.31 76.07 73.55 62.12 62.06
f1 (ppm)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7.33
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7.30
7.29
7.29
7.28
7.27
7.26
7.26
5.00
4.97
4.97
4.94
4.93
4.90
4.84
4.83
4.82
4.81
4.66
4.63
4.06
4.03
4.01
3.79
3.76
3.74
3.52
3.50
3.49
3.48
2.68
2.67
2.65
2.64
1.53
0.00
NH2
NH2
BnO
BnO
OBn
OBn
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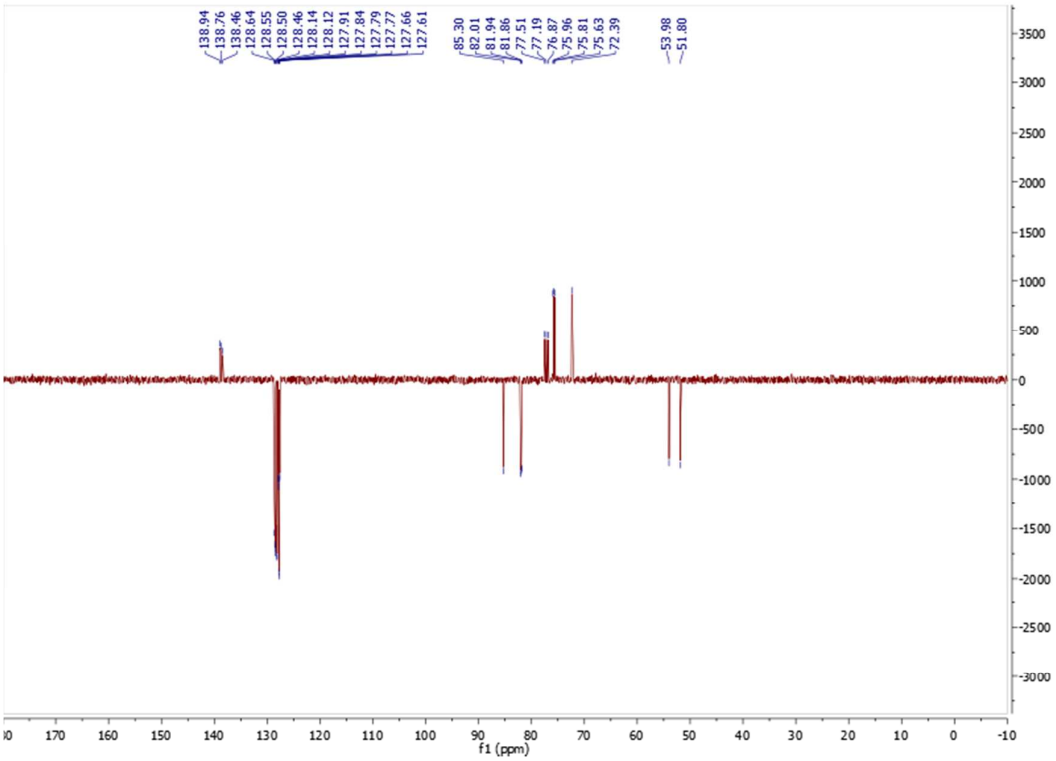
3.11
2.09
3.10
1.01
1.02
3.06
1.00
4.59
f1 (ppm)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127.91
127.84
127.79
127.77
127.66
127.61
85.30
82.01
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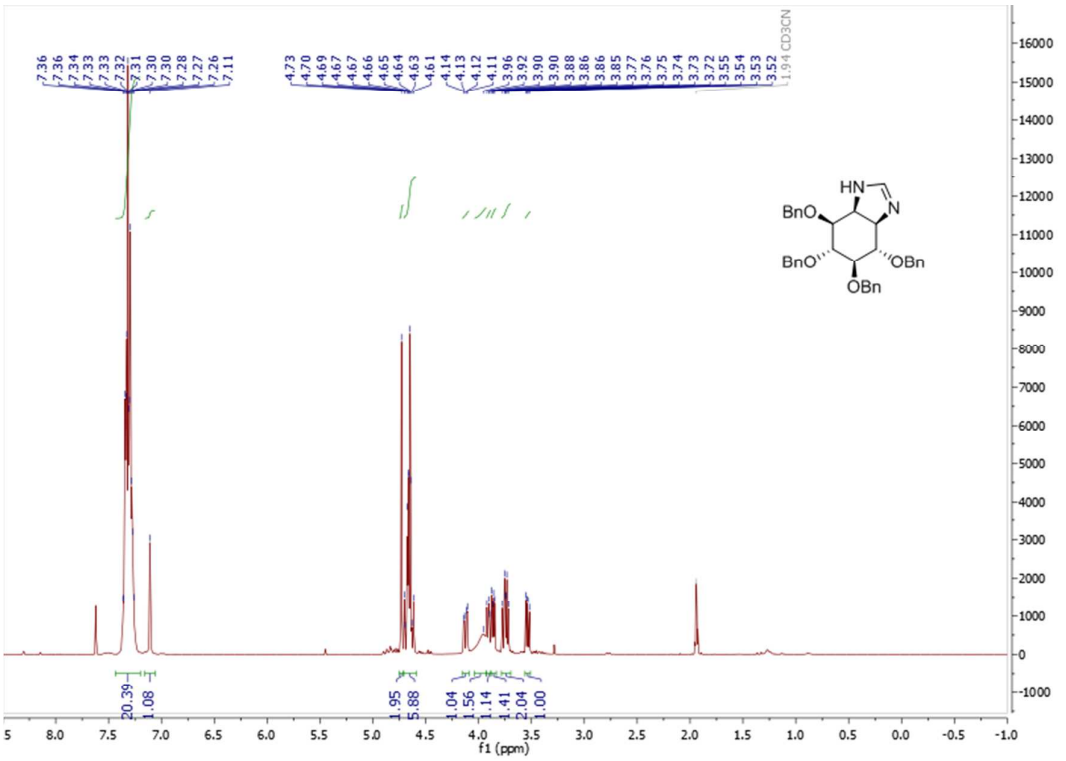
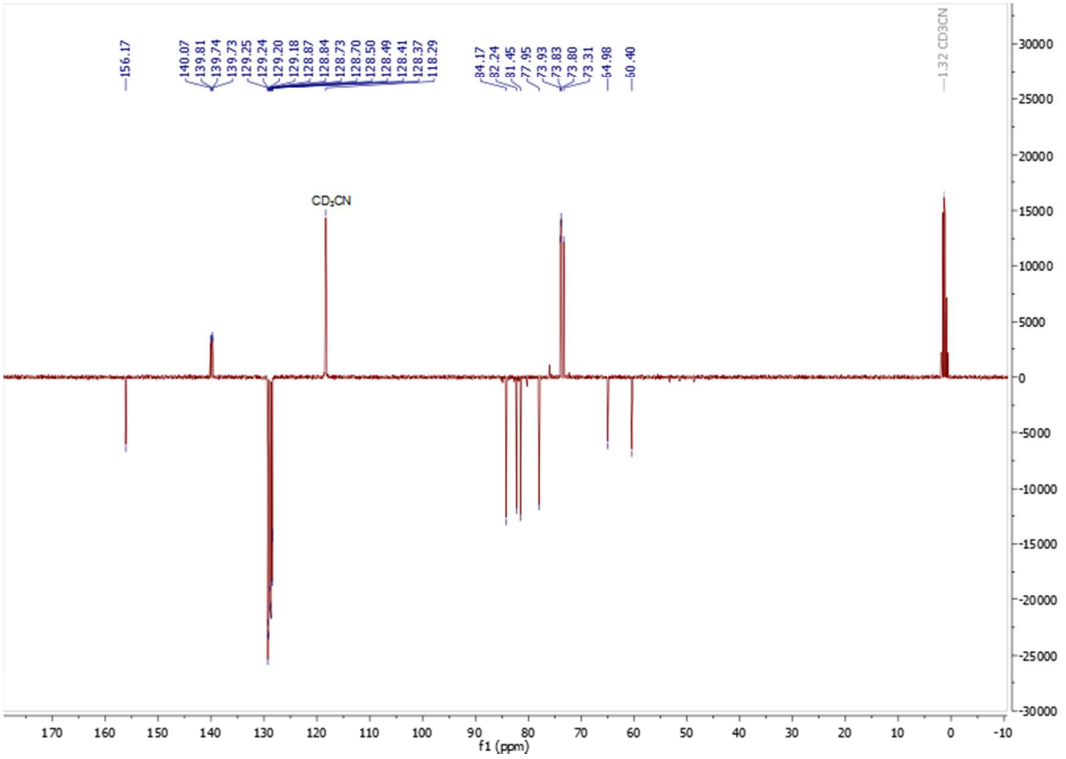
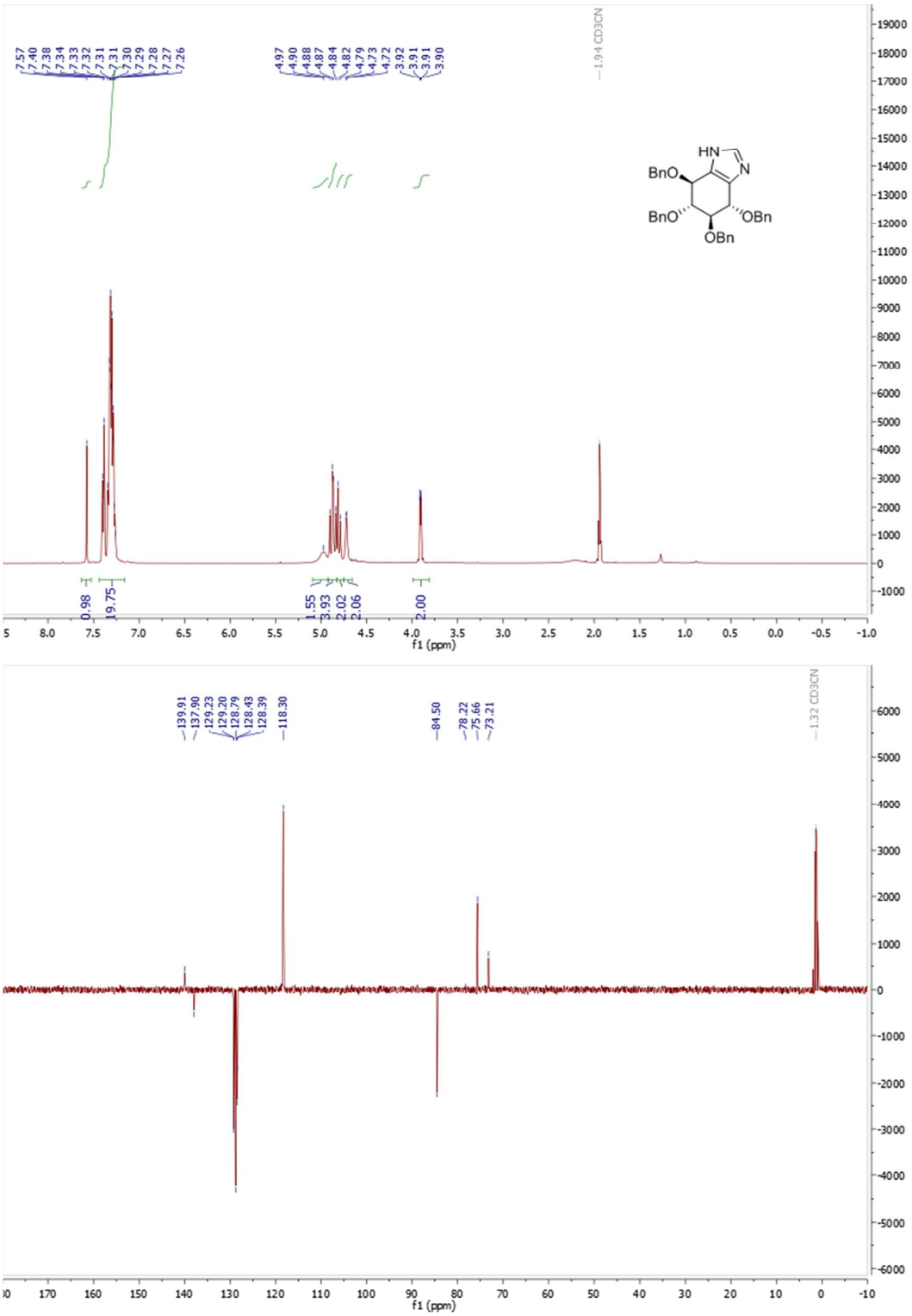
81.86
77.51
77.19
76.87
75.96
75.81
75.63
72.39
53.98
51.80
f1 (ppm)


HN
N
BnO
BnO
OBn
OBn
f1 (ppm)


CD3CN
f1 (ppm)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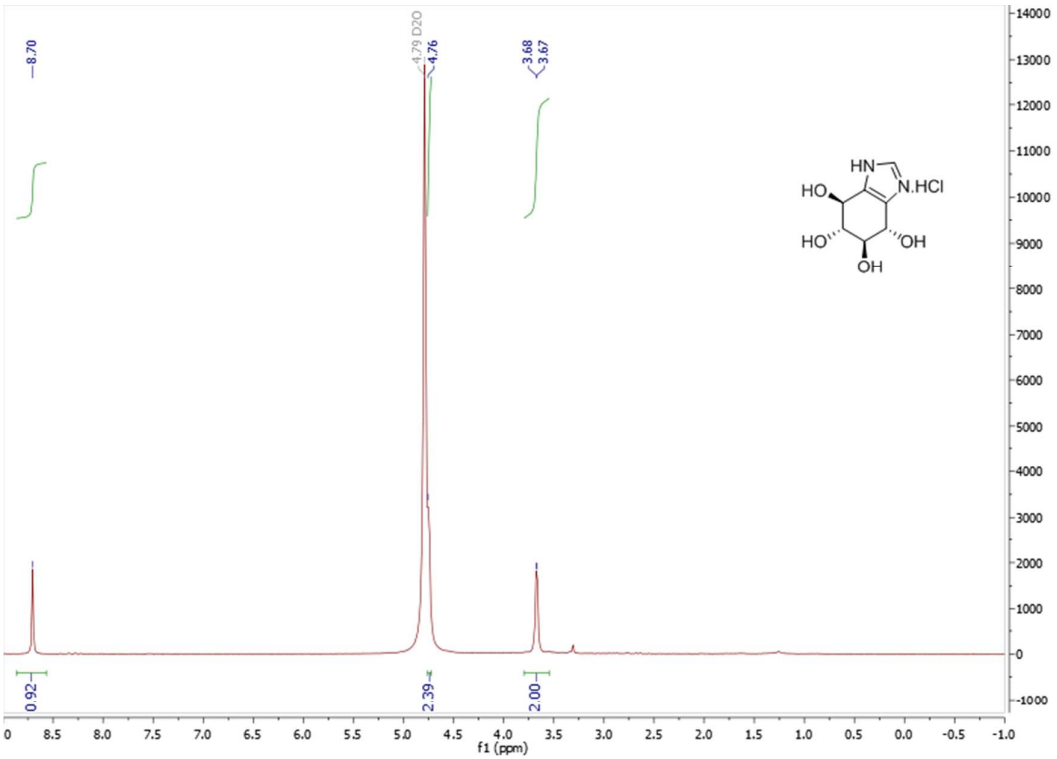
7.31
7.31
7.30
7.29
7.28
7.27
7.26
4.97
4.90
4.88
4.87
4.84
4.82
4.79
4.73
4.72
3.92
3.91
3.91
3.90
1.94 CD3CN
HN
N
BnO
BnO
OBn
OBn
0.98
19.75
1.55
3.93
2.02
2.06
2.00
f1 (ppm)
139.91
137.90
129.23
129.20
128.79
128.43
128.39
118.30
84.50
78.22
75.66
73.21
1.32 CD3CN
f1 (ppm)


8.70
4.79 D2O
4.76
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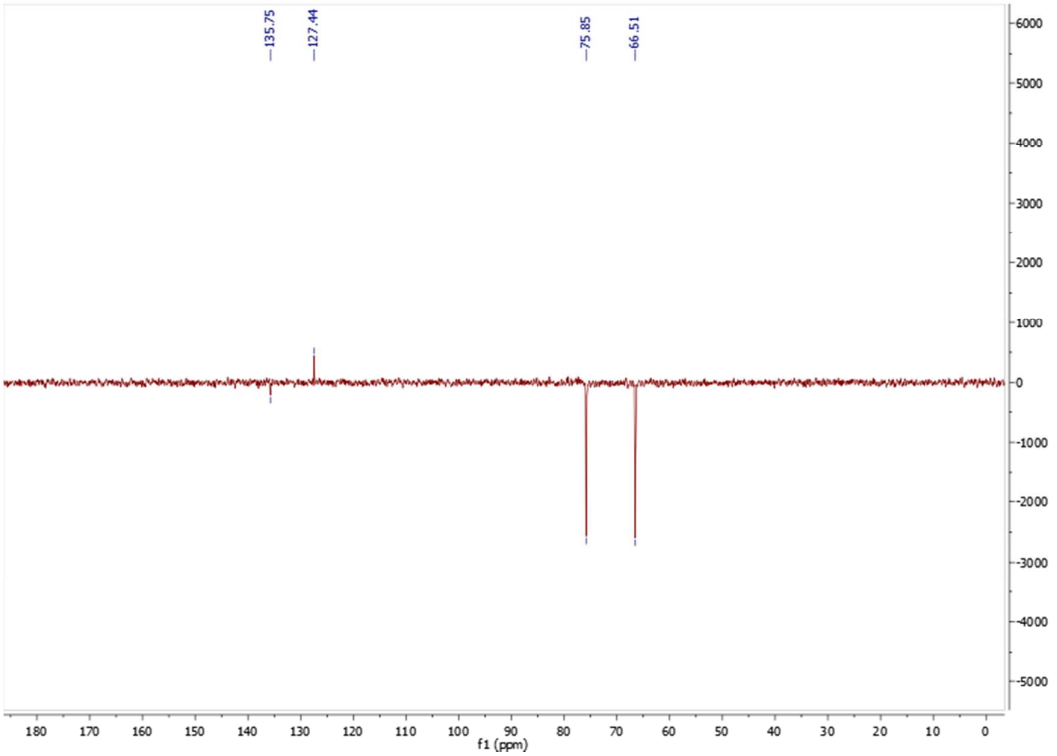
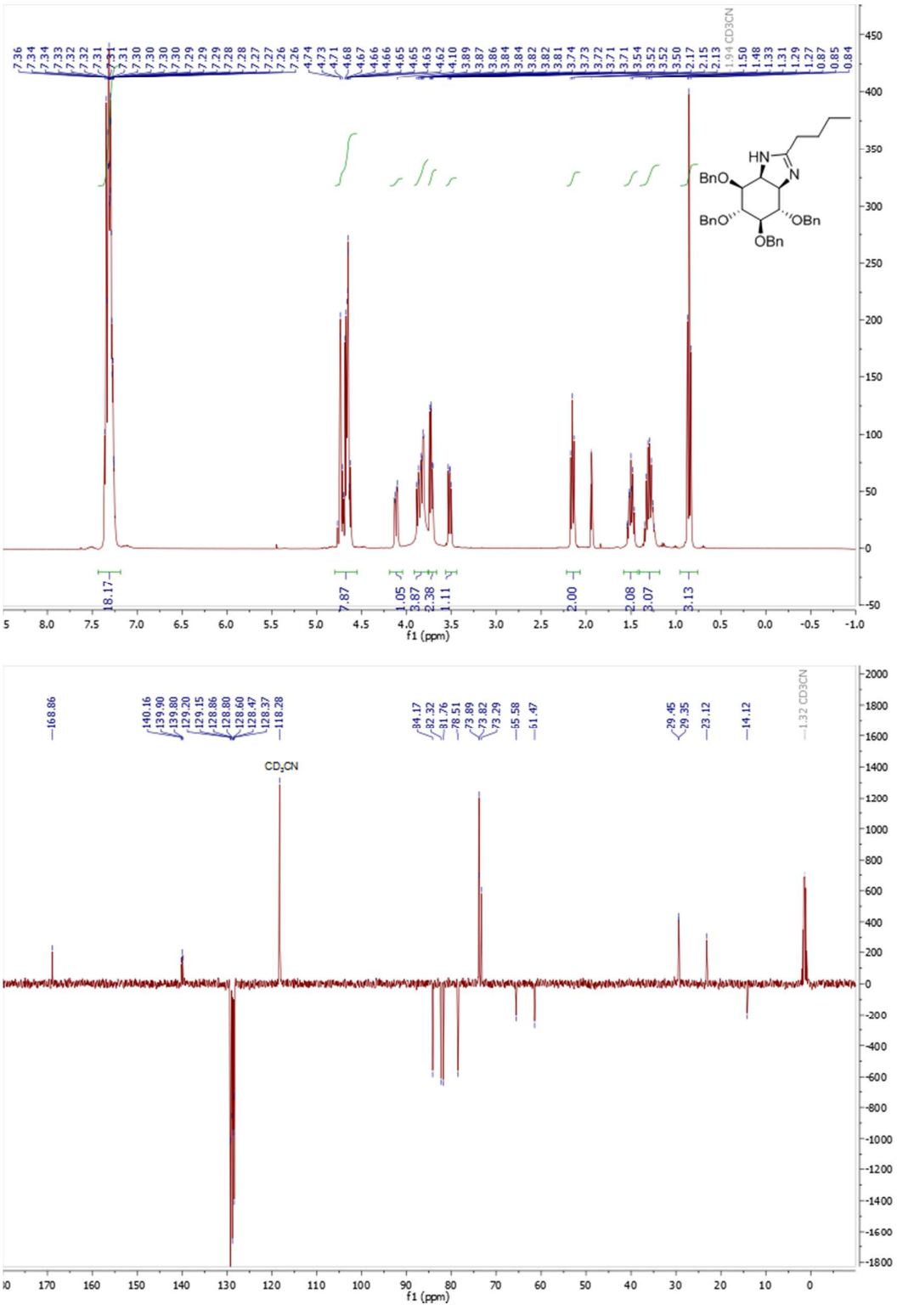
3.67
HN
N.HCl
HO
HO
OH
OH
0.92
2.39
2.00
f1 (ppm)


135.75
127.44
75.85
66.51
f1 (ppm)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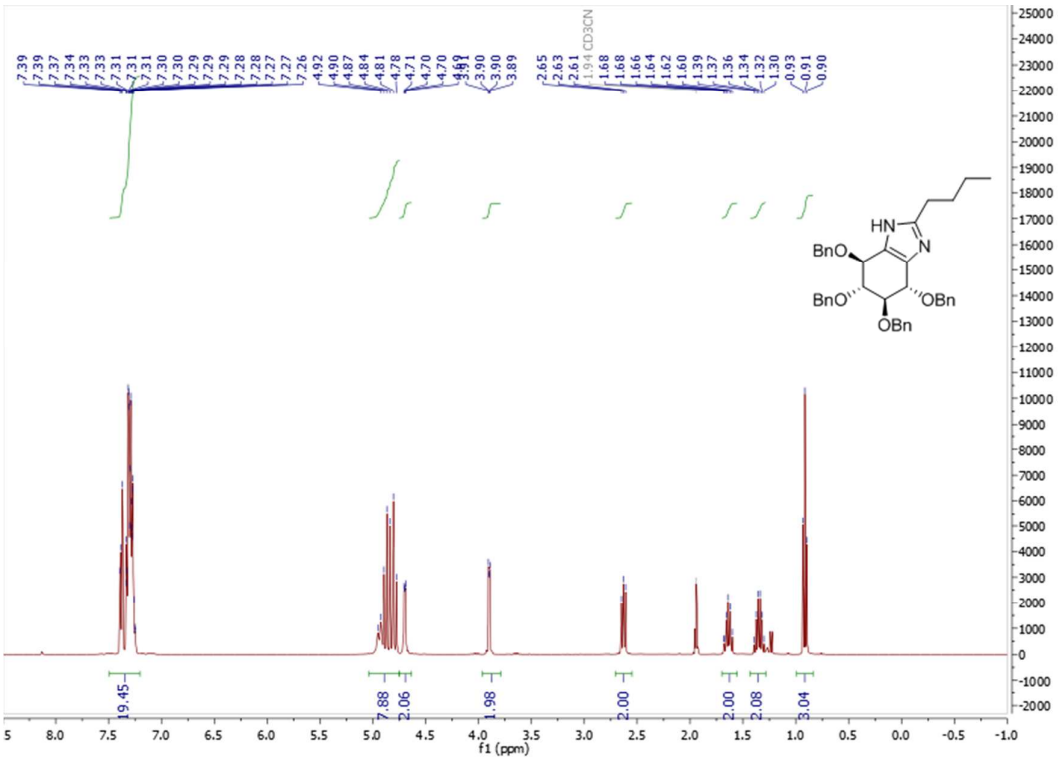
BnO
BnO
OBn
OBn
CD3CN
CD3CN
CD3CN
f1 (ppm)
f1 (ppm)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N
BnO
BnO
OBn
OBn
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7.88
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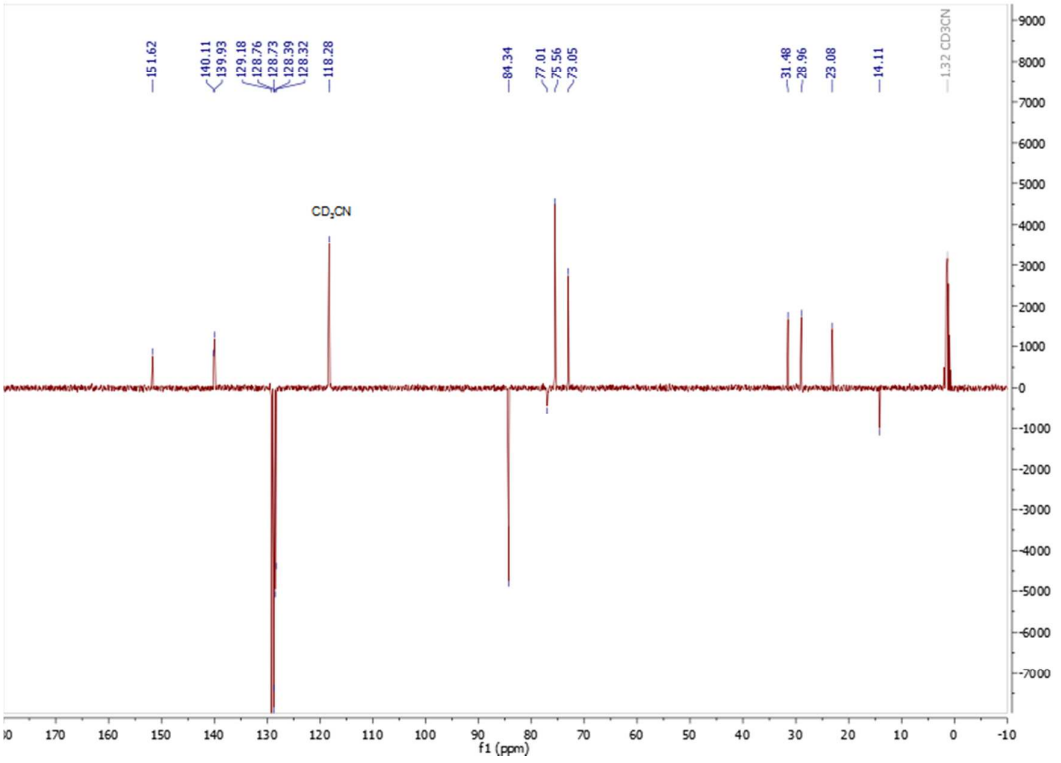
1.98
2.00
2.00
2.08
3.04
f1 (ppm)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140.11
139.93
129.18
128.76
128.73
128.39
128.32
118.28
84.34
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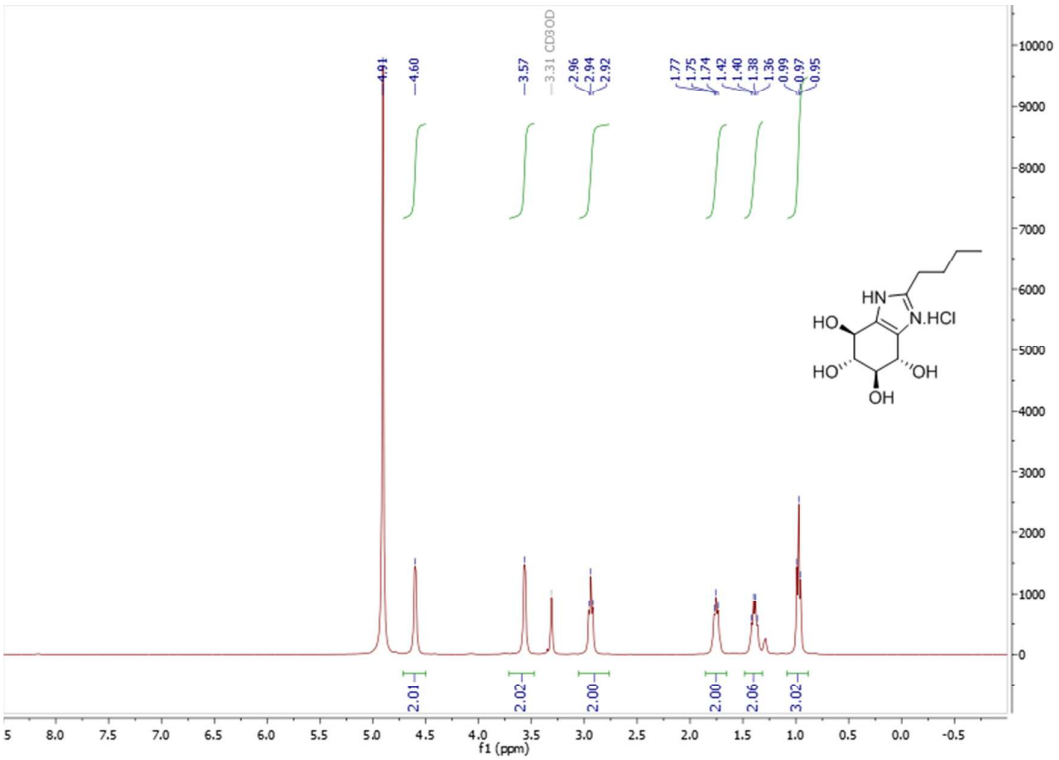
75.56
73.05
31.48
28.96
23.08
14.11
CD3CN
CD3CN
f1 (ppm)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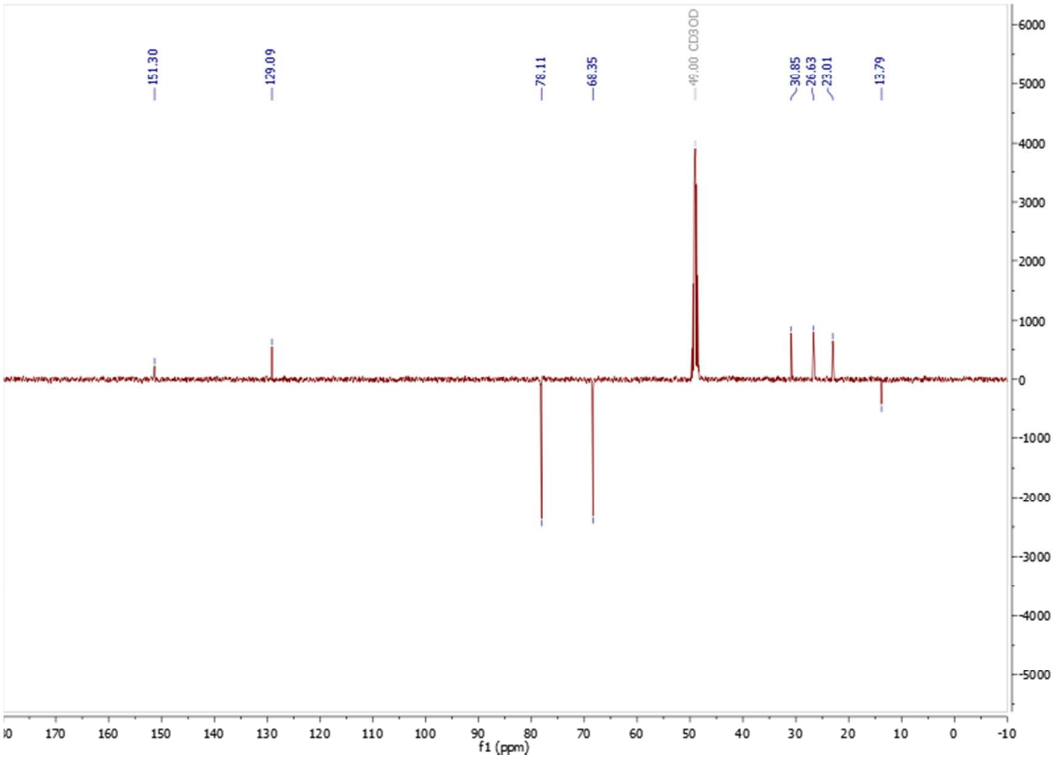
4.60
3.57
3.31 CD3OD
2.96
2.94
2.92
1.77
1.75
1.74
1.42
1.40
1.38
1.36
0.99
0.97
0.95
HN
N.HCl
HO
HO
OH
OH
2.01
2.02
2.00
2.00
2.06
3.02
f1 (ppm)


151.30
129.09
78.11
68.35
49.00 CD3OD
30.85
26.63
23.01
13.79
f1 (ppm)

pK_{AH} determination

The pK_{AH} of the imidazoles were determined with the method described by Gift *et al.*[8] using a Metrohm 691 pH-meter and Hamilton spintrode. The compound was dissolved in D_2O (0.6 mL) and basified with NaOD (0.1 M in D_2O) to pH > 8. Then, the mixture was acidified by stepwise addition of DCl (0.1 M in D_2O) and a ^{1}H-NMR spectrum (Brüker DMX-300) was recorded after each addition. A correction for determination in D_2O instead of H_2O was applied according to Krężel *et al*.[9]

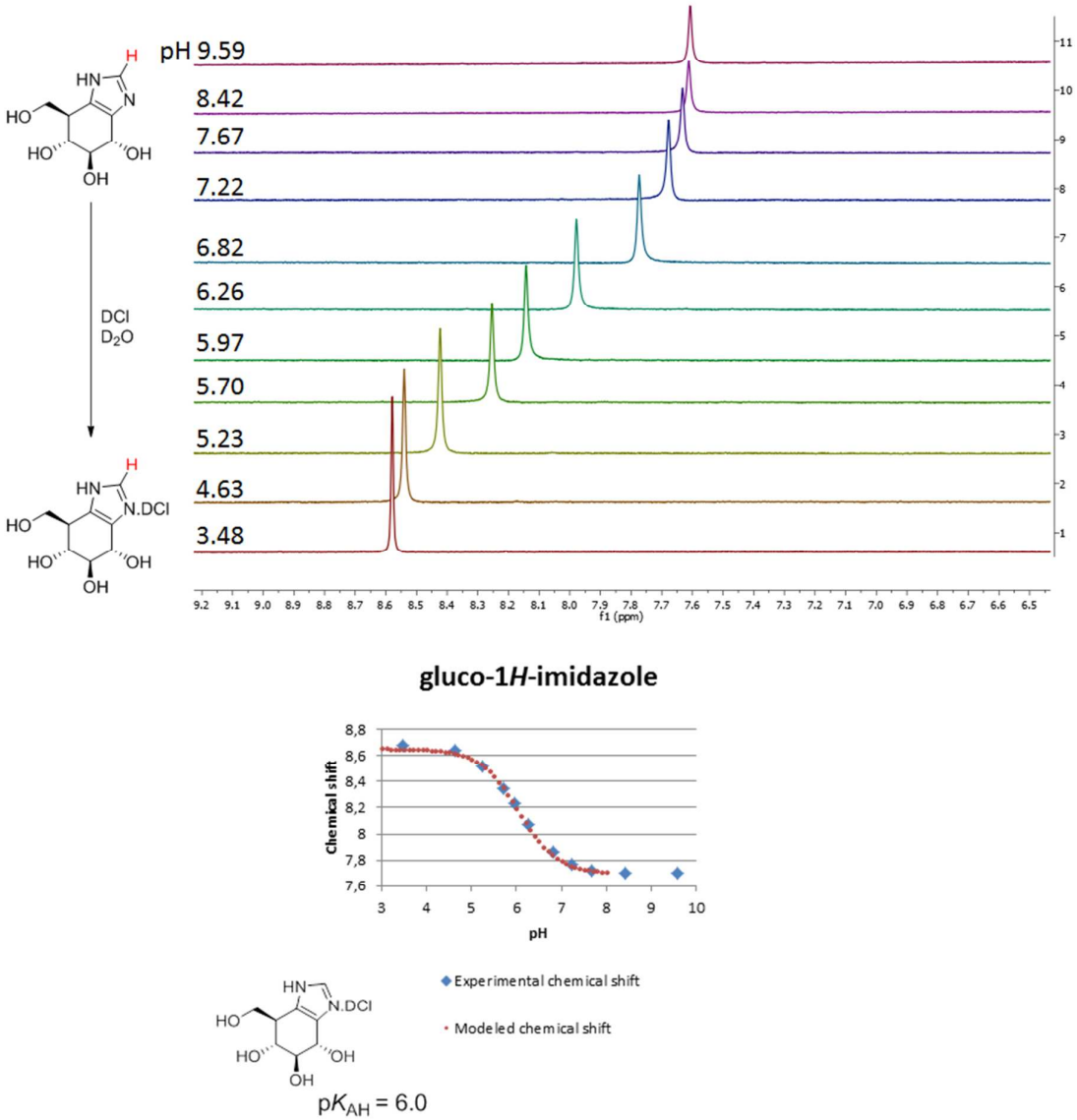


Figure S1 pK_{AH} determination of gluco-1*H*-imidazole by ^{1}H-NMR.

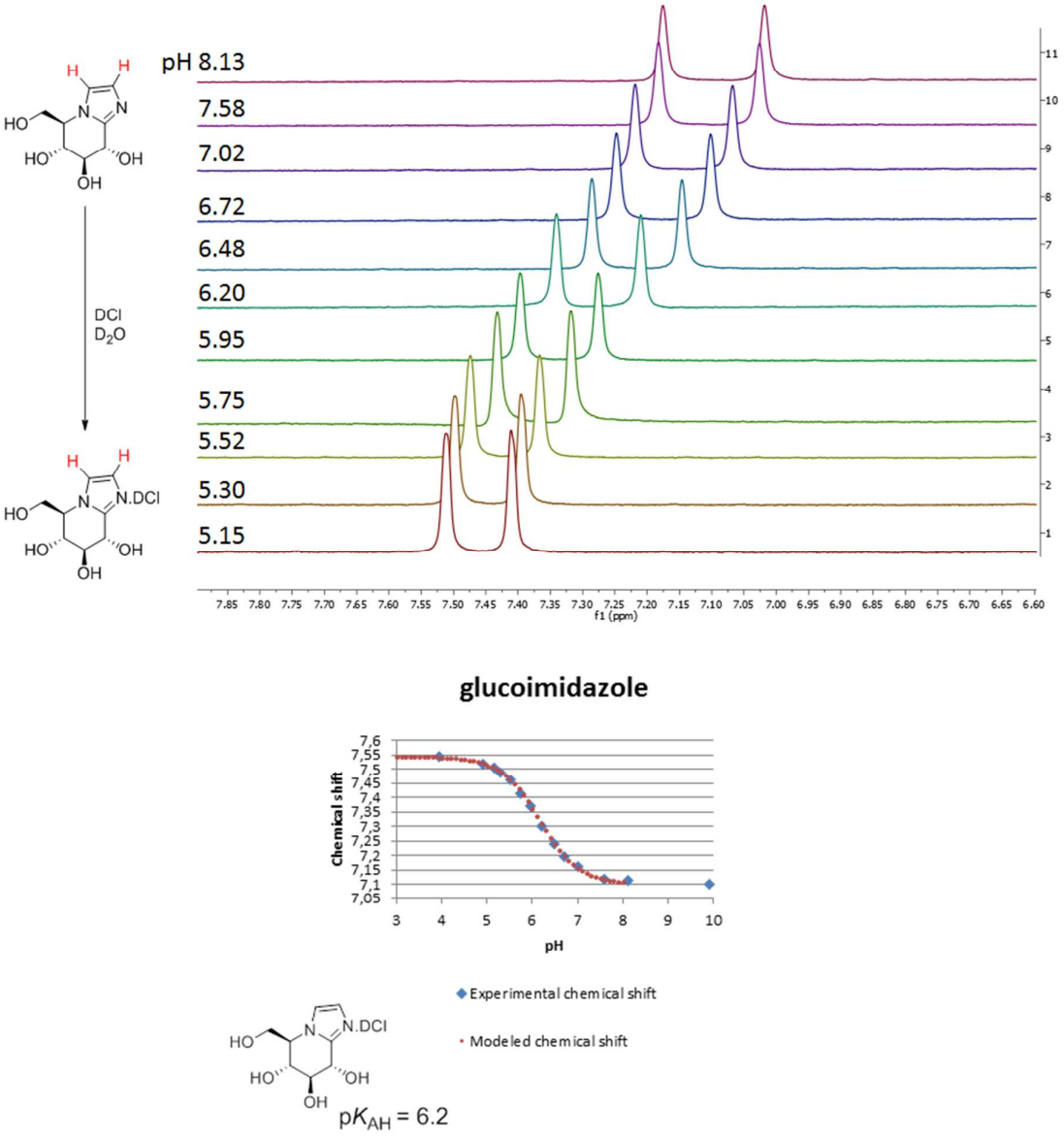


Figure S2 pK_{AH} determination of glucoimidazole by ^{1}H-NMR.

Determination of kinetic constant (K_i) values

Biochemical and Biological Methods

Enzyme preparations used for IC_{50} and kinetics measurements were as follows: Recombinant human β-glucosidase GBA1 (Cerezyme) and α-glucosidase recombinant human GAA (Myozyme) were obtained from Genzyme, USA. Bacterial β-glucosidase enzymes *Tm*GH1[10] and *Tx*GH116[11] were expressed as previously described. β-Glucosidase from almonds was purchased from Sigma Aldrich as lyophilized powder (7.9 U/mg solid). Cellular homogenates of a stable HEK293 over-expressing GBA2 cell line were obtained as previously described[12] and were pre-incubated for 30 min with 1mM CBE. Proteins were stored in small aliquots at -80 °C until use. *p*-nitrophenyl-β-D-glucopyranoside was purchased from Sigma Aldrich, 4-MU-β-d-glucopyranoside was purchased from Glycosynth, and C6-NBD-ceramide (6-[N-methyl-N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)aminododecanoyl]sphingosine) from Molecular probes, GBA1 inhibitor Conduritol-β-Epoxide (CBE) was purchased from Enzo. 2,4-dinitrophenyl-β-D-glucopyranoside[13] and 2,4-dinitrophenyl-α-D-glucopyranoside[14] were synthesized following synthetic procedures previously described and their spectroscopic data are in agreement with those previously reported.

In vitro apparent IC_{50} measurements

To determine *in vitro* apparent IC_{50} values, 25 µL of enzyme solution was pre-incubated with 25 µL of a range of 6 inhibitor dilutions for 30 min in a 96 well plate, using the following buffers: GBA1 in 150 mM McIlvaine buffer pH 5.2, 0.2% taurocholate (w/v), 0.1% Triton X-100 (v/v) and 0.1% bovine serum albumin (BSA) (w/v); GAA in 150 mM McIlvaine buffer pH 4.8 and 0.1% BSA (w/v); *Tm*GH1, *Tx*GH116 and β-glucosidase from sweet almonds in 50 mM $NaHPO_4$ pH 6.8 and 0.1% BSA (w/v).

After 30 min of pre-incubation, 50 µL of substrate solution in the same buffer was added to this E (25 µL) + I (25 µL) mixture (total reaction volume 100 µL). GBA1 residual activity was measured using final 24 nM concentration of enzyme (Cerezyme) and 200 µM of 2,4-dinitrophenyl-β-D-glucopyranoside substrate, incubated for 30 min at 37 °C. GAA activity was measured using final concentrations of 156 nM and 200 µM of 2,4-dinitrophenyl-α-D-glucopyranoside substrate, for 30 min at 37 °C. *Tm*GH1, *Tx*GH116 and β-glucosidase from sweet almonds residual activity was measured using final concentrations of 37 nM, 82 nM and 0.125 U/mL respectively and 400 µM of *p*-nitrophenyl-β-D-glucopyranoside, for 30 min at 37 °C. Finally, all enzyme reactions were monitored for 10 minutes and the release of 2,4-dinitrophenolate or *p*-nitrophenolate and UV-absorbance was measured at 420 nm in a Tecan GENios Microplate Reader. Values plotted for [I] are those in the final reaction mixture, containing E + I + S. Data was corrected for background absorbance, then normalized to the untreated control condition and finally curve-fitted via one phase exponential decay function (GraphPad Prism 5.0). Apparent i*n vitro* IC_{50} values were determined in technical triplicates.

For GBA2, 12.5 µL of lysate was pre-incubated with 12.5 µL of a range of 7 inhibitor dilutions for 30 min at 37°C. Afterwards, 100 µL of 3.7 mM 4-MU-β-d-glucopyranoside in 150 mM McIlvaine buffer pH 5.8 and 0.1% BSA (w/v) were added and incubated for 1h at 37°C. After stopping the substrate reaction with 200 µL 1M NaOH-Glycine (pH 10.3), liberated 4-MU fluorescence was measured with a fluorimeter LS55 (Perkin Elmer) using λ_{Ex} 366 nm and λ_{Em} 445 nm. All IC_{50} values were determined in duplicate.

***In situ* apparent IC_{50} measurements**

IC50 values for GCS were determined with NBD-ceramide as substrate as previously described[15]. RAW 264.7 (American Type culture collection) were cultured in RPMI medium (Gibco) supplemented with 10% FCS, 1 mM GlutaMAX™ and 100 units/mL penicillin/streptomycin (Gibco) at 37°C and 5% CO2. The RAW 264.7 cells were grown to confluence in 12-well plates and pre-incubated for 1h with 300 µM CBE, followed by 1h incubation at 37°C in the presence of a range of 6 inhibitor concentrations and with 1 nmol C6-NBD-ceramide. The cells were washed 3x with PBS and harvested by scraping. After lipid extraction[16], the C6-NBD lipids were separated and detected by HPLC (λ_{Ex} 470 nm and λ_{Em} 530 nm). IC_{50} values were determined in duplicate from the titration curves of observed formed C6-NBD-glucosylceramide.

Table S1 – Apparent IC_{50} values in µM for azoles **5**-**9**.

	*Tm*GH1	*Tx*GH116	Sweet almonds	GBA1	GBA2	GCS	GAA
6	107	93	14	6.1	> 50	> 50	> 100
7	9.8	72	0.042	0.069	> 50	> 50	> 100
8	> 100	> 100	> 100	21.8	> 50	> 50	> 100
9	> 100	> 100	0.350	0.192	> 50	> 50	> 100
5	0.014	0.165	0.065	0.050	> 50	> 50	> 100

All reported values are the mean from two or three technical replicates.

Kinetic studies

The kinetic studies of reversible imidazole inhibitors in *Tm*GH1, *Tx*GH116 and β-glucosidase from sweet almonds were performed by monitoring the UV-absorbance of *p*-nitrophenolate released from *p*-nitrophenyl β-D-glucopyranoside. *Tm*GH1, *Tx*GH116 and β-glucosidase from sweet almonds (25 µL) at 37 nM, 82 nM and 0.125 U/mL respectively in 50 mM phosphate buffer (pH 6.8) and 0.1% BSA (w/v) were pre-incubated with a range of inhibitor dilutions (25 µL) for 30 min at 37 °C in a 96 well plate. The reaction was then started by adding 50 µL of different *p*-nitrophenyl β-D-glucopyranoside substrate concentrations (0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 2.5 and 5 mM) in 50 mM phosphate buffer (pH 6.8) to the 50 µL enzyme-inhibitor mixture. For kinetic studies in human recombinant β-glucosidase, 25 µL of 24 nM Cerezyme in 150 mM McIlvaine buffer pH 5.2 supplemented with 0.2% taurocholate (w/v), 0.1% Triton X-100 (v/v) and 0.1% bovine serum albumin (BSA) (w/v), was incubated with a range of inhibitor dilutions (25 µL) for 30 min at 37 °C in a 96 well plate. The reaction was then started by adding 50 µL of different 2,4-dinitrophenyl-α-D-glucopyranoside

substrate concentrations (0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 mM) in the previously described 150 mM McIlvaine buffer (pH 5.2) to the 50 µL enzyme-inhibitor mixture.

The release of *p*-nitrophenolate or 2,4-dinitrophenolate was monitored by absorbance at 420 nm for 10 min (at 25 °C for *Tm*GH1, *Tx*GH116 and β-Glucosidase from almonds or 37 °C for human Cerezyme and Myozyme) in a Tecan GENios Microplate Reader to determine the hydrolysis rate. The K_i values of reversible competitive or linear mixed inhibition were determined by Michaelis-Menten model using standard nonlinear regression (GraphPad Prism 5.0). K_i values were determined in technical triplicates.

Protein expression and crystallography

*Tm*GH1

*Tm*GH1 was produced by expression of the construct pET-28a-*Tm*GH1-His_6 and purified as described by Zechel *et al*.[17] TmGH1 was crystallized by sitting drop vapour diffusion, with the protein at 10 mg/ml in 50 mM imidazole pH 7.0 and the well solution comprised of 11 % polyethylene glycol (PEG) 4000, 0.1 M imidazole pH 7.0, 50 mM calcium acetate, 100 mM trimethylamine *N*-oxide. The protein drop was seeded with a seed stock grown under similar conditions. To generate the ligand complex, a crystal of *Tm*GH1 was soaked with 10 mM gluco-1*H*-imidazole **6** for 4 days, and fished into liquid nitrogen via a cryoprotectant solution comprised of the well solution supplemented with 25 % (v/v) ethylene glycol. Data were collected at Diamond beamline I03, processed using *DIALS*[18] and scaled using *AIMLESS*[19] to a resolution of 1.7 Å. The structure was solved using 1OD0 without the water molecules as the starting model for *REFMAC*[20], and refined by manual rebuilding in *Coot*[21] combined with further cycles of refinement using *REFMAC*. Crystal structure figures were generated using Pymol.

There are two molecules in the asymmetric unit of the *Tm*GH1 crystal structure. **6** is modelled in the active site of chain B only at an occupancy of 0.8, whilst the equivalent site in chain A has been modelled with ethylene glycol in two alternative conformations and two water molecules. The authors have observed that crystal structures of ligand complexes obtained with *Tm*GH1 crystals sometimes yield ligand in only one out of two molecules in the asymmetric unit. It may be that some of the active sites are blocked by N-terminal residues on adjacent chains, as observed for 1OD0.pdb (where 5 residues at the start of chain B extend into the active site of mol A), but for this complex it has not been possible to definitively model N-terminal residues before Val3.

*Tx*GH116

*Tx*GH116 was produced by expression of construct pET30a-*Tx*GH116 Δ1-18 with a C-terminal His_6 tag and purified as described by Charoenwattanasatien *et al*.[22] *Tx*GH116 was crystallized by the sitting drop vapour diffusion method, with a well solution of 0.2M ammonium sulfate, 20 % (v/v) PEG 3350,

0.1 M Bis-Tris pH 6.8. To generate ligand complexes, crystals of *Tx*GH116 were soaked with 10 mM gluco-1*H*-imidazole **6** for 20 hours, before fishing via a cryoprotectant solution with 25 % (v/v) ethylene glycol. Data were collected at Diamond beamline I03, processed using *DIALS* and scaled using *AIMLESS* to a resolution of 2.1 Å. The structure was solved using *MOLREP*[23], with 5BVU as the model, and the solved structure refined by cycles of manual rebuilding in *Coot* and refinement using *REFMAC*. Crystal structure figures were generated using Pymol.

Table S2 - Data collection and refinement statistics

	***Tm*GH1**	***Tx*GH116**
Data collection		
Space group	$P2_12_12_1$	$P2_12_12$
Cell dimensions		
a, *b*, *c* (Å)	94.3, 94.6, 113.4	177.6, 54.1, 83.6
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	72.77-1.70 (1.73-1.70)	8.97-2.10 (2.16-2.10)
R_{sym} or R_{merge}	0.080 (2.894)	0.152 (0.692)
R_{pim}	0.023 (0.831)	0.104 (0.462)
$CC_{1/2}$	0.998 (0.610)	0.957 (0.596)
I / σI	13.8 (1.5)	5.0 (1.5)
Completeness (%)	100.0 (99.8)	98.4 (97.7)
Redundancy	12.6 (12.9)	3.0 (3.1)
Refinement		
No. reflections	111884	47048
R_{work} / R_{free}	0.19/0.22	0.20/0.25
No. atoms		
Protein	7231	6111
Ligand/ion	60	52
Water	369	268
B-factors		
Protein	37.5	26.4
Ligand/ion	39.5	30.7
Water	42.4	29.3
R.m.s deviations		
Bond lengths (Å)	0.018	0.016
Bond angles (°)	1.77	1.71
Ramachandran plot residues		
In most favorable regions (%)	98.3	95.8
In allowed regions (%)	1.5	3.7
PDB code	5OSS	5OST

Electron density and B-factor comparisons for complexes of 5 and 6

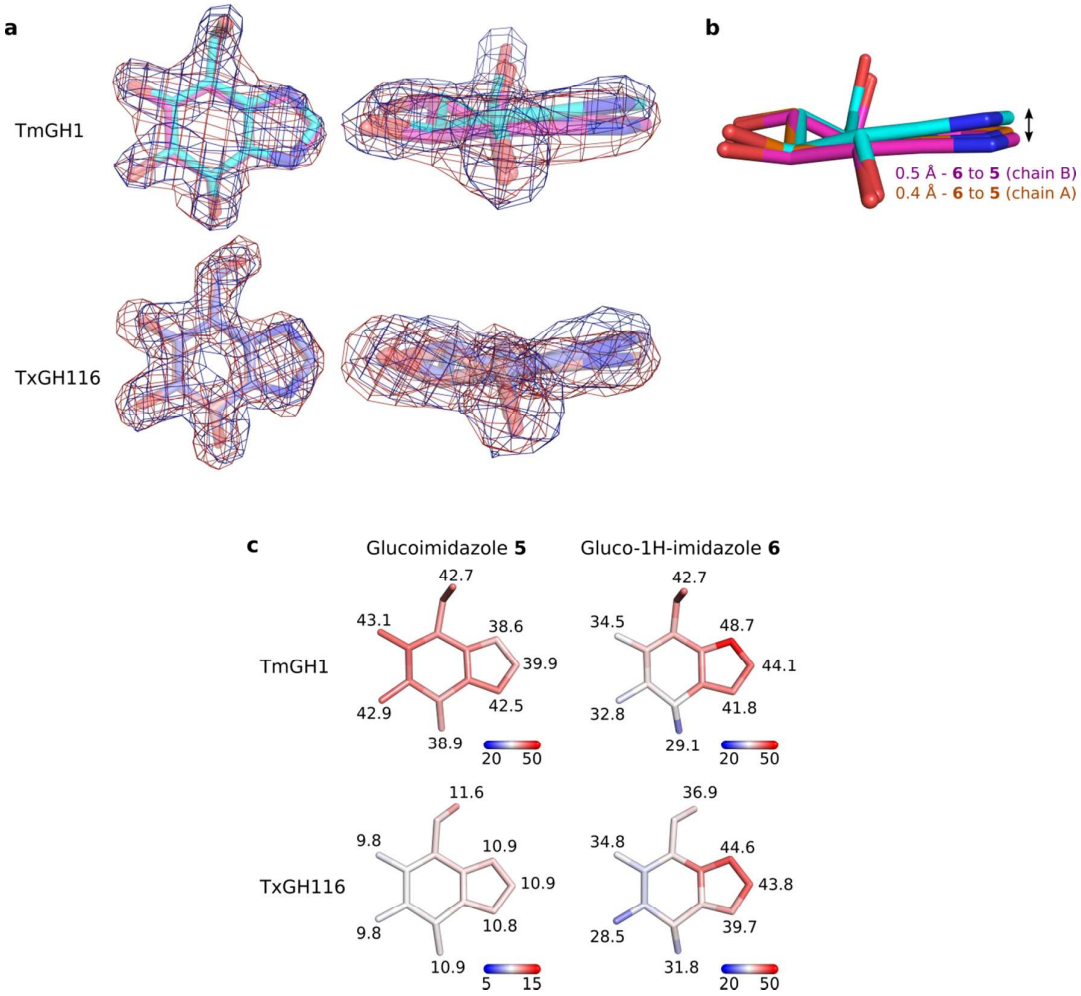


Figure S3 Electron density and B-factor comparisons for complexes of **5** and **6**. **a** Superposed electron densities for **5** and **6** in complex with *Tm*GH1 (pink ligand for **5**, cyan for **6**; chain B of each structure) and *Tx*GH116 (salmon for **5**, blue for **6**). The small 'upwards' shift at the apical carbon of the imidazole in complexes of **6** compared to **5** is well supported by the diverging electron densities at this region. In contrast, electron densities overlay well in the 'glucose' portion of the ligands. Densities shown are REFMAC maximum-likelihood/σ_A weighted 2Fo−Fc contoured between 1.5–2.0 r.m.s.d (0.38–0.48 $e^-/Å^3$ for *Tm*GH1-**5**, *Tm*GH1–**6**, *Tx*GH116-**6**; 0.89 $e^-/Å^3$ for *Tx*GH116-**5**). **b** Superposition of *Tm*GH1-**6** (cyan), against **5** from chains A (orange) and B (pink) of the *Tm*GH1-**5** complex. **6** shows a clear 'upwards' shift compared to both molecules of **5**, which overlay well with each other. **c** Ligands in complex with *Tm*GH1 and *Tx*GH116 colored by B-factor, with B-factors of peripheral atoms annotated. B-factors increase substantially towards the imidazole portion of **6** in both *Tm*GH1 and *Tx*GH116 complexes, indicating greater crystallographic disorder at this region of the ligand. B-factors are more consistent in complexes with **5**. The *Tm*GH1-**5** ligand shown is from chain B; the ligand from chain A shows a similar B-factor trend.

ITC

ITC experiments were carried out using a MicroCal AutoITC200 (Malvern Instruments, formerly GE Healthcare). All titrations were run at 25 °C in 50 mM Sodium Phosphate, pH 5.8 or 6.8. Proteins were buffer exchanged into ITC buffer via at least 3 rounds of dilution/concentration using an Amicon Ultra spin concentrator (Millipore), and further degassed under vacuum prior to use. Cell concentrations of 100 μM (protein) and syringe concentrations of 2 mM (ligand) were used for titrations using gluco-1*H*-Imidazoles **6** and **7**. Cell concentrations of 50 μM and syringe concentrations of 500 μM were used for titrations using **5**. Analyses were carried out using the MicroCal PEAQ-ITC analysis software (Malvern Instruments).

Table S3 ITC calculated parameters of binding for **5**-**7** with *Tm*GH1 or *Tx*GH116 at pH 5.8 or 6.8.

pH 6.8	***Tm*GH1**			***Tx*GH116**		
	6	**7**	**5**	**6**	**7**	**5**
N (sites)	0.98 ± 0.06	0.96 ± 0.2	0.96 ± 0.03	0.98 ± 0.09	0.96 ± 0.02	0.98 ± 0.1
K_D (μM)	28.3 ± 6.9	5.02 ± 1.0	0.14 ± 0.02	27.3 ± 0.4	19.6 ± 2.6	0.075 ± 0.01
ΔH (kJmol^{-1})	–18.4 ± 1.4	14.2 ± 0.2	–52.7 ± 1.2	–22.5 ± 0.3	–7.4 ± 0.1	–41.8 ± 2.3
$-\Delta S$ (kJmol^{-1})	–7.6 ± 2.0	–44.6 ± 0.7	13.6 ± 1.3	–3.6 ± 0.2	–19.5 ± 0.4	1.04 ± 2.4
ΔG (kJmol^{-1})	–26.0 ± 0.6	–30.3 ± 0.6	–39.1 ± 0.34	–26.1 ± 0.06	–26.9 ± 0.4	–40.7 ± 0.47

pH 5.8	***Tm*GH1**			***Tx*GH116**		
	6	**7**	**5**	**6**	**7**	**5**
N (sites)	0.99 ± 0.04	0.99 ± 0.09	1.02 ± 0.07	1.13 ± 0.02	0.97 ± 0.01	1.04 ± 0.02
K_D (μM)	36.6 ± 13.9	19.1 ± 0.9	0.055 ± 0.004	12.2 ± 0.8	16.0 ± 3.4	0.032 ± 0.002
ΔH (kJmol^{-1})	–13.1 ± 0.6	17.1 ± 1.1	–40.7 ± 3.9	–15.4 ± 0.4	–2.3 ± 0.1	–32.9 ± 0.4
$-\Delta S$ (kJmol^{-1})	–12.3 ± 1.4	–44.1 ± 1.2	–0.79 ± 4.0	–12.6 ± 0.6	–25.9 ± 1.1	–9.9 ± 0.5
ΔG (kJmol^{-1})	–25.4 ± 0.9	–26.9 ± 0.2	–41.5 ± 0.17	–28.1 ± 0.15	–26.6 ± 1.8	–42.8 ± 0.21

All reported values are the mean ± standard deviation from three (ligands **6**, **7**) or four (ligand **5**) technical replicates.

Representative ITC traces pH 6.8

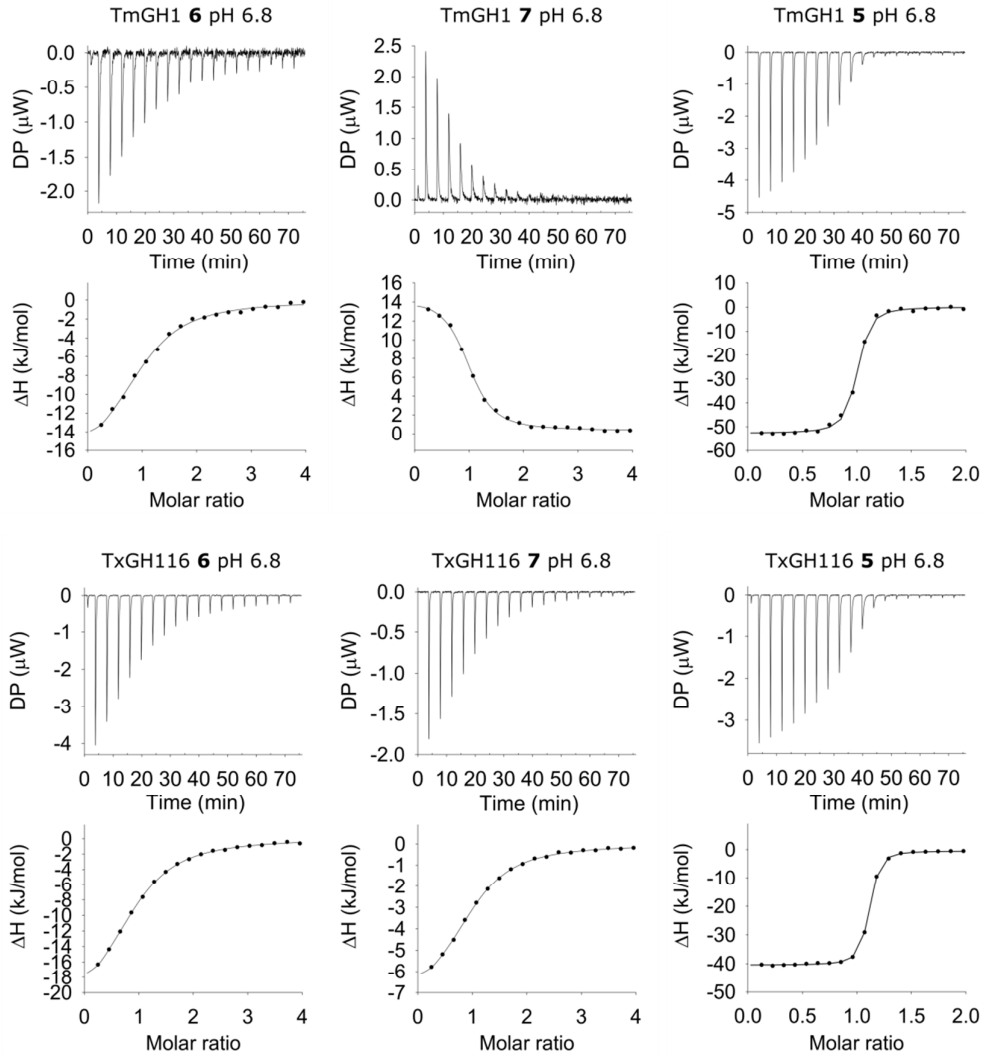


Figure S4

Representative ITC traces pH 5.8

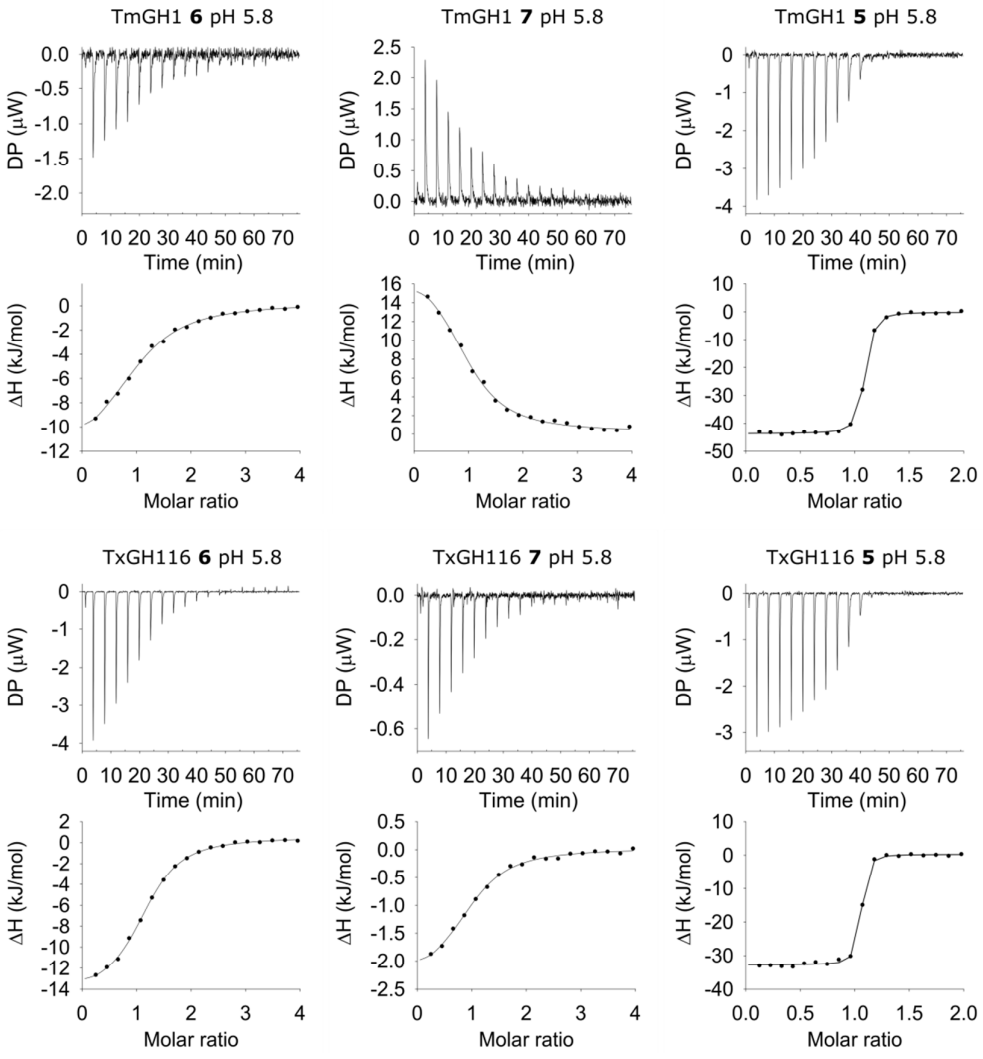


Figure S5

DFT calculations

Geometry optimization

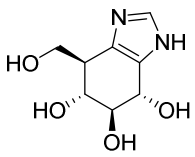
All calculations were performed with DFT as level of theory in combination with the B3LYP hybrid functional. A conformer distribution search option included in the Spartan 04 program[24], in gas-phase with the use of 6-31G(d) as basis set, was used as starting point for the geometry optimization. All generated structures were further optimized with Gaussian 03[25] at 6-311G(d,p). Optimization was done in gas-phase and subsequently corrections for solvent effects were done by the use of a polarizable continuum model using water as solvent parameter. The free Gibbs energy of the computed conformations was calculated using Equation (1) in which ΔE_{gas} is the gas-phase energy (electronic energy), ΔG^{T}_{RRHO} (T= 298.15 K and pressure= 1 atm.) is the sum of corrections from the electronic energy to free Gibbs energy in the rigid-rotor-harmonic-oscillator approximation (RRHO) also including zero-point-vibrational energy, and ΔG^{T}_{solv} is their corresponding free solvation Gibbs energy.

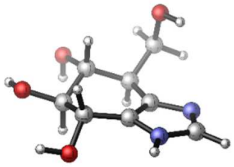
$$\Delta G^{T}_{aq} = \Delta E_{gas} + \Delta G^{T}_{gas,RRHO} + \Delta G_{solv} \qquad (1)$$

$$= \Delta G^{T}_{gas} + \Delta G_{solv}$$

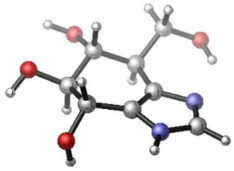
The denoted free energies include unscaled zero-point vibrational energies. Visualisation of the conformations of interest was done with CYLview.[25] The three lowest energy geometries for **6** were all calculated to adopt a $^{4}H_{3}$ conformation, differing only in the rotation angle around the C5-C6 axis: *gt*, *gg* and *tg* respectively.

Gluco-1*H*-imidazole (both tautomers)

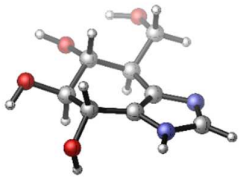




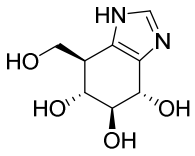
ΔG^{T}_{aq} = 0.0 kcal/mol
$^{4}H_{3}$ ***gg***

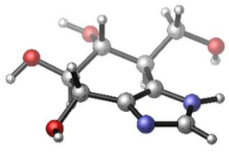


ΔG^{T}_{aq} = +0.1 kcal/mol
$^{4}H_{3}$ ***gt***

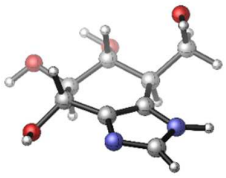


ΔG^{T}_{aq}= +0.3 kcal/mol
$^{4}H_{3}$ ***tg***

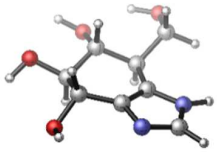




ΔG^{T}_{aq} = +0.3 kcal/mol
$^{4}H_{3}$ ***gt***

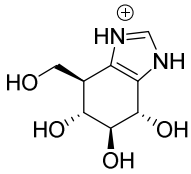


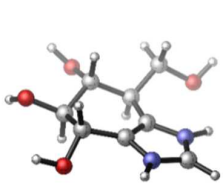
ΔG^{T}_{aq} = +0.8 kcal/mol
$^{4}H_{3}$ ***gg***



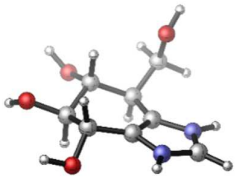
ΔG^{T}_{aq} = +1.9 kcal/mol
$^{4}H_{3}$ ***tg***

Gluco-1*H*-imidazole (protonated)

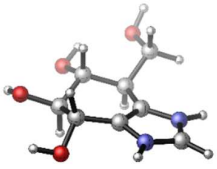




ΔG^{T}_{aq} = 0.0 kcal/mol
$^{4}H_{3}$ ***gt***

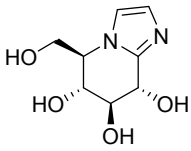


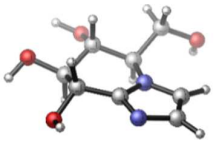
ΔG^{T}_{aq} = +0.0 kcal/mol
$^{4}H_{3}$ ***gg***



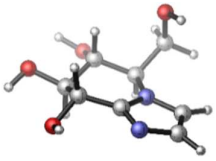
ΔG^{T}_{aq} = +3.2 kcal/mol
$^{4}H_{3}$ ***tg***

Glucoimidazole

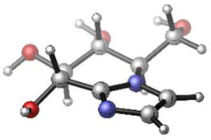




ΔG^{T}_{aq} = 0.0 kcal/mol
$^{4}H_{3}$ ***gt***

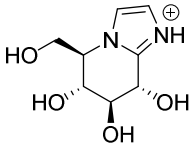


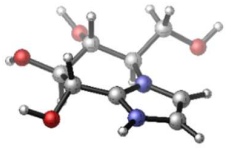
ΔG^{T}_{aq} = +0.3 kcal/mol
$^{4}H_{3}$ ***gg***



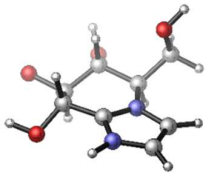
ΔG^{T}_{aq} = +2.0 kcal/mol
$^{4}H_{3}$ ***tg***

Glucoimidazole (protonated)

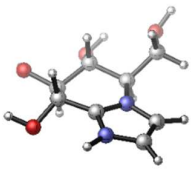




ΔG^{T}_{aq} = 0.0 kcal/mol
$^{4}H_{3}$ ***gt***



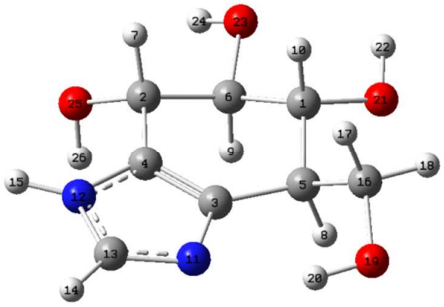
ΔG^{T}_{aq} = +0.2 kcal/mol
$^{4}H_{3}$ ***gg***



ΔG^{T}_{aq} = +3.5 kcal/mol
$^{4}H_{3}$ ***tg***

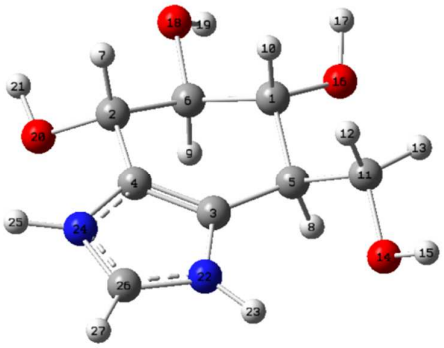
Mulliken charges

Mulliken atomic charges of lowest energy geometry of **6** (Gluco-1*H*-imidazole)



1	C	0.061369
2	C	0.104400
3	C	0.042436
4	**C**	**0.106302**
5	C	-0.195326
6	C	0.055654
7	H	0.143586
8	H	0.136044
9	H	0.121522
10	H	0.125417
11	N	-0.429194
12	N	-0.386821
13	**C**	**0.145851**
14	H	0.152469
15	H	0.306533
16	C	0.043782
17	H	0.092401
18	H	0.107852
19	O	-0.463283
20	H	0.244617
21	O	-0.463231
22	H	0.283708
23	O	-0.472542
24	H	0.297766
25	O	-0.452488
26	H	0.291176

Mulliken atomic charges of lowest energy geometry of **6** (Gluco-1*H*-imidazole (protonated))

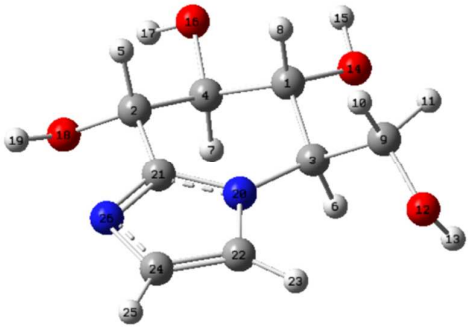


1	C	0.052501
2	C	0.125295
3	C	0.177257
4	**C**	**0.112704**
5	C	-0.186080
6	C	0.049958
7	H	0.162319
8	H	0.172471
9	H	0.135404
10	H	0.143009
11	C	0.037413

12 H 0.117263
13 H 0.119184
14 O -0.475253
15 H 0.314409
16 O -0.463192
17 H 0.302591
18 O -0.470192
19 H 0.306331
20 O -0.462279
21 H 0.307617
22 N -0.356638
23 H 0.319731
24 N -0.361151
25 H 0.343516
26 C 0.263310
27 H 0.212501

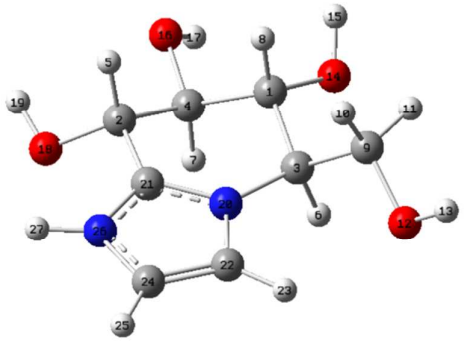
Mulliken atomic charges of lowest energy geometry of **5** (Glucoimidazole)

1 C 0.003047
2 C 0.078107
3 C 0.007354
4 C 0.061292
5 H 0.143840
6 H 0.168935
7 H 0.127926
8 H 0.141160
9 C 0.017720
10 H 0.126707
11 H 0.124005
12 O -0.453463
13 H 0.300757
14 O -0.452985
15 H 0.293432
16 O -0.471394
17 H 0.303466
18 O -0.463576
19 H 0.295509
20 N -0.395317
21 C 0.306442
22 C -0.000203



23 H 0.132330
24 C -0.117443
25 H 0.125825
26 N -0.403472

Mulliken atomic charges of lowest energy geometry of **5** (Glucoimidazole (protonated))



1 C -0.008704
2 C 0.083032
3 C 0.019480
4 C 0.067236
5 H 0.186544
6 H 0.193093
7 H 0.147109
8 H 0.159518
9 C 0.024460
10 H 0.129999
11 H 0.127428
12 O -0.461056
13 H 0.313881
14 O -0.453391
15 H 0.315280
16 O -0.464524
17 H 0.310720
18 O -0.455800
19 H 0.319187
20 N -0.409436
21 C 0.471773
22 C 0.019759
23 H 0.171271
24 C 0.022793
25 H 0.187550
26 N -0.358208
27 H 0.341005

Restricted conformational energy surface calculations

The geometry with the lowest free Gibbs energy was selected as the starting point for the partial conformational energy surface calculation. A survey of the possible neighbouring conformational space was made by scanning two dihedral angles, including the C1-C2-C3-C4 (D1), C3-C4-C5-O (D3) ranging from −60° to −20°. The C5-O-C1-C2 (D5) was fixed at 0° since this is highly favoured. The resolution of this survey is determined by the step size which was set to 5° per puckering parameter. These structures were calculated with Gaussian 03 with a 6-311G(d,p) as basis set. Furthermore, solvation effects of H_2O were taken into account with a polarizable continuum model function.

Gluco-1*H*-imidazole (6) (both tautomers)

Name	Q	Theta	Phi	ΔG_{aq}^{T}
Glucose-1H-imidazole_D1_-40_D3_-45_D5_0	0.463	49.8	216.1	0.0
Glucose-1H-imidazole_D1_-45_D3_-45_D5_0	0.492	49.7	210.0	0.0
Glucose-1H-imidazole_D1_-45_D3_-40_D5_0	0.464	49.8	204.0	0.0
Glucose-1H-imidazole_D1_-40_D3_-50_D5_0	0.497	50.1	221.2	0.2
Glucose-1H-imidazole_D1_-40_D3_-40_D5_0	0.432	49.6	210.1	0.2
Glucose-1H-imidazole-2_D1_-45_D3_-40_D5_0	0.461	49.7	204.9	0.2
Glucose-1H-imidazole_D1_-35_D3_-45_D5_0	0.438	50.2	222.8	0.3
Glucose-1H-imidazole_D1_-50_D3_-40_D5_0	0.498	50.2	198.9	0.3
Glucose-1H-imidazole_D1_-45_D3_-50_D5_0	0.524	49.8	215.2	0.3
Glucose-1H-imidazole-2_D1_-45_D3_-45_D5_0	0.491	49.7	211.0	0.4
Glucose-1H-imidazole_D1_-45_D3_-35_D5_0	0.439	50.4	197.4	0.4
Glucose-1H-imidazole-2_D1_-40_D3_-45_D5_0	0.463	49.9	217.0	0.4
Glucose-1H-imidazole_D1_-35_D3_-50_D5_0	0.475	50.9	227.6	0.4
Glucose-1H-imidazole-2_D1_-45_D3_-35_D5_0	0.435	50.1	198.1	0.4
Glucose-1H-imidazole_D1_-50_D3_-45_D5_0	0.524	49.8	204.6	0.5
Glucose-1H-imidazole_D1_-50_D3_-35_D5_0	0.476	51.0	192.5	0.5
Glucose-1H-imidazole-2_D1_-40_D3_-40_D5_0	0.431	49.7	211.1	0.5
Glucose-1H-imidazole-2_D1_-50_D3_-40_D5_0	0.495	50.1	199.4	0.5
Glucose-1H-imidazole_D1_-35_D3_-40_D5_0	0.405	49.8	217.0	0.6
Glucose-1H-imidazole-2_D1_-50_D3_-35_D5_0	0.471	50.8	193.1	0.6
Glucose-1H-imidazole_D1_-40_D3_-35_D5_0	0.405	49.8	203.2	0.6
Glucose-1H-imidazole-2_D1_-40_D3_-50_D5_0	0.499	50.4	222.1	0.6
Glucose-1H-imidazole-2_D1_-35_D3_-45_D5_0	0.440	50.6	223.7	0.7
Glucose-1H-imidazole-2_D1_-50_D3_-45_D5_0	0.523	49.8	205.4	0.7
Glucose-1H-imidazole_D1_-30_D3_-50_D5_0	0.457	52.1	234.4	0.7
Glucose-1H-imidazole-2_D1_-45_D3_-50_D5_0	0.524	49.9	216.2	0.7
Glucose-1H-imidazole_D1_-30_D3_-45_D5_0	0.418	51.2	230.1	0.7
Glucose-1H-imidazole-2_D1_-35_D3_-50_D5_0	0.477	51.3	228.4	0.8

Glucose-1H-imidazole-2_D1_-40_D3_-35_D5_0	0.403	49.7	204.2	0.8
Glucose-1H-imidazole_D1_-45_D3_-30_D5_0	0.419	51.4	190.2	0.8
Glucose-1H-imidazole_D1_-40_D3_-55_D5_0	0.534	50.6	225.6	0.8
Glucose-1H-imidazole-2_D1_-45_D3_-30_D5_0	0.414	51.1	190.8	0.8
Glucose-1H-imidazole-2_D1_-35_D3_-40_D5_0	0.405	50.0	218.0	0.8
Glucose-1H-imidazole_D1_-50_D3_-30_D5_0	0.457	52.3	185.9	0.9
Glucose-1H-imidazole_D1_-35_D3_-55_D5_0	0.513	51.5	231.6	0.9
Glucose-1H-imidazole_D1_-50_D3_-50_D5_0	0.555	49.7	209.9	0.9
Glucose-1H-imidazole_D1_-35_D3_-35_D5_0	0.375	49.6	210.1	0.9
Glucose-1H-imidazole_D1_-55_D3_-40_D5_0	0.535	50.8	194.5	1.0
Glucose-1H-imidazole-2_D1_-50_D3_-30_D5_0	0.453	51.9	186.2	1.1
Glucose-1H-imidazole_D1_-45_D3_-55_D5_0	0.559	50.1	219.8	1.1
Glucose-1H-imidazole_D1_-30_D3_-40_D5_0	0.382	50.4	224.7	1.1
Glucose-1H-imidazole_D1_-55_D3_-35_D5_0	0.514	51.7	188.5	1.1
Glucose-1H-imidazole-2_D1_-55_D3_-40_D5_0	0.531	50.5	195.1	1.1
Glucose-1H-imidazole-2_D1_-55_D3_-35_D5_0	0.509	51.4	189.0	1.1
Glucose-1H-imidazole-2_D1_-30_D3_-45_D5_0	0.421	51.7	230.8	1.1
Glucose-1H-imidazole_D1_-30_D3_-55_D5_0	0.497	52.9	238.0	1.1
Glucose-1H-imidazole_D1_-40_D3_-30_D5_0	0.382	50.6	195.6	1.1
Glucose-1H-imidazole-2_D1_-30_D3_-50_D5_0	0.460	52.5	235.0	1.1
Glucose-1H-imidazole-2_D1_-35_D3_-35_D5_0	0.374	49.6	211.0	1.2
Glucose-1H-imidazole-2_D1_-40_D3_-30_D5_0	0.379	50.3	196.3	1.2
Glucose-1H-imidazole_D1_-25_D3_-50_D5_0	0.444	53.8	241.4	1.3
Glucose-1H-imidazole-2_D1_-50_D3_-50_D5_0	0.553	49.7	210.8	1.3
Glucose-1H-imidazole_D1_-25_D3_-45_D5_0	0.403	52.8	237.7	1.3
Glucose-1H-imidazole-2_D1_-40_D3_-55_D5_0	0.536	51.0	226.3	1.3
Glucose-1H-imidazole_D1_-55_D3_-45_D5_0	0.559	50.2	200.2	1.3
Glucose-1H-imidazole-2_D1_-35_D3_-55_D5_0	0.516	52.0	232.2	1.3
Glucose-1H-imidazole-2_D1_-30_D3_-40_D5_0	0.383	50.8	225.6	1.4
Glucose-1H-imidazole-2_D1_-45_D3_-25_D5_0	0.398	52.6	183.1	1.4
Glucose-1H-imidazole_D1_-45_D3_-25_D5_0	0.403	53.0	182.7	1.4
Glucose-1H-imidazole_D1_-55_D3_-30_D5_0	0.498	53.1	182.3	1.4
Glucose-1H-imidazole-2_D1_-55_D3_-45_D5_0	0.556	50.0	200.9	1.4
Glucose-1H-imidazole_D1_-50_D3_-25_D5_0	0.444	54.0	179.0	1.5
Glucose-1H-imidazole_D1_-30_D3_-35_D5_0	0.348	49.8	218.1	1.5
Glucose-1H-imidazole-2_D1_-45_D3_-55_D5_0	0.560	50.3	220.6	1.5
Glucose-1H-imidazole-2_D1_-55_D3_-30_D5_0	0.493	52.7	182.5	1.6
Glucose-1H-imidazole_D1_-35_D3_-30_D5_0	0.349	49.9	202.2	1.6
Glucose-1H-imidazole-2_D1_-30_D3_-55_D5_0	0.501	53.4	238.3	1.6
Glucose-1H-imidazole-2_D1_-50_D3_-25_D5_0	0.439	53.6	179.2	1.6

Glucose-1H-imidazole_D1_-25_D3_-40_D5_0	0.364	51.7	233.0	1.6
Glucose-1H-imidazole_D1_-25_D3_-55_D5_0	0.486	54.6	244.4	1.6
Glucose-1H-imidazole-2_D1_-25_D3_-45_D5_0	0.406	53.3	238.1	1.7
Glucose-1H-imidazole-2_D1_-25_D3_-50_D5_0	0.448	54.3	241.7	1.7
Glucose-1H-imidazole-2_D1_-35_D3_-30_D5_0	0.346	49.7	203.0	1.8
Glucose-1H-imidazole-2_D1_-30_D3_-35_D5_0	0.348	50.0	219.0	1.8
Glucose-1H-imidazole_D1_-55_D3_-50_D5_0	0.588	49.9	205.2	1.8
Glucose-1H-imidazole_D1_-50_D3_-55_D5_0	0.588	49.8	214.5	1.8
Glucose-1H-imidazole_D1_-55_D3_-25_D5_0	0.487	54.9	176.1	1.9
Glucose-1H-imidazole_D1_-35_D3_-60_D5_0	0.553	52.2	234.9	1.9
Glucose-1H-imidazole_D1_-40_D3_-25_D5_0	0.364	51.9	187.4	1.9
Glucose-1H-imidazole_D1_-20_D3_-50_D5_0	0.436	55.9	248.3	1.9
Glucose-1H-imidazole_D1_-20_D3_-45_D5_0	0.393	54.9	245.3	1.9
Glucose-1H-imidazole-2_D1_-25_D3_-40_D5_0	0.366	52.2	233.6	2.0
Glucose-1H-imidazole_D1_-40_D3_-60_D5_0	0.572	51.2	229.1	2.0
Glucose-1H-imidazole-2_D1_-55_D3_-25_D5_0	0.481	54.4	176.1	2.0
Glucose-1H-imidazole-2_D1_-60_D3_-35_D5_0	0.548	52.1	185.6	2.0
Glucose-1H-imidazole_D1_-30_D3_-60_D5_0	0.539	53.6	240.8	2.0
Glucose-1H-imidazole-2_D1_-40_D3_-25_D5_0	0.360	51.5	187.9	2.0
Glucose-1H-imidazole-2_D1_-25_D3_-55_D5_0	0.491	55.2	244.5	2.1
Glucose-1H-imidazole-2_D1_-60_D3_-40_D5_0	0.568	51.1	191.4	2.1
Glucose-1H-imidazole-2_D1_-60_D3_-30_D5_0	0.533	53.5	179.6	2.1
Glucose-1H-imidazole-2_D1_-50_D3_-20_D5_0	0.431	55.7	172.1	2.1
Glucose-1H-imidazole_D1_-20_D3_-55_D5_0	0.480	56.8	250.6	2.1
Glucose-1H-imidazole-2_D1_-45_D3_-20_D5_0	0.388	54.7	175.2	2.1
Glucose-1H-imidazole_D1_-20_D3_-40_D5_0	0.351	53.8	241.6	2.2
Glucose-1H-imidazole_D1_-25_D3_-35_D5_0	0.327	50.7	227.0	2.2
Glucose-1H-imidazole_D1_-45_D3_-20_D5_0	0.393	55.2	175.2	2.2
Glucose-1H-imidazole_D1_-50_D3_-20_D5_0	0.437	56.2	172.3	2.2
Glucose-1H-imidazole_D1_-30_D3_-30_D5_0	0.319	49.5	210.1	2.2
Glucose-1H-imidazole-2_D1_-20_D3_-45_D5_0	0.397	55.5	245.5	2.2
Glucose-1H-imidazole-2_D1_-55_D3_-50_D5_0	0.586	49.8	206.1	2.2
Glucose-1H-imidazole-2_D1_-50_D3_-55_D5_0	0.587	49.9	215.4	2.2
Glucose-1H-imidazole_D1_-60_D3_-35_D5_0	0.554	52.4	185.3	2.2
Glucose-1H-imidazole-2_D1_-20_D3_-50_D5_0	0.440	56.5	248.2	2.2
Glucose-1H-imidazole-2_D1_-55_D3_-20_D5_0	0.474	56.6	169.7	2.3
Glucose-1H-imidazole-2_D1_-60_D3_-25_D5_0	0.523	55.2	173.6	2.3
Glucose-1H-imidazole_D1_-25_D3_-60_D5_0	0.529	55.4	246.7	2.4
Glucose-1H-imidazole_D1_-60_D3_-40_D5_0	0.573	51.4	190.9	2.4
Glucose-1H-imidazole_D1_-35_D3_-25_D5_0	0.328	50.9	193.2	2.4

Glucose-1H-imidazole-2_D1_-30_D3_-30_D5_0	0.318	49.6	211.0	2.4
Glucose-1H-imidazole_D1_-45_D3_-60_D5_0	0.596	50.5	223.6	2.4
Glucose-1H-imidazole-2_D1_-25_D3_-35_D5_0	0.328	51.1	227.8	2.4
Glucose-1H-imidazole_D1_-60_D3_-30_D5_0	0.539	53.8	179.6	2.4
Glucose-1H-imidazole-2_D1_-35_D3_-60_D5_0	0.557	52.7	235.4	2.4
Glucose-1H-imidazole-2_D1_-35_D3_-25_D5_0	0.324	50.6	193.9	2.4
Glucose-1H-imidazole-2_D1_-20_D3_-40_D5_0	0.355	54.4	241.9	2.5
Glucose-1H-imidazole_D1_-40_D3_-20_D5_0	0.352	54.0	178.9	2.5
Glucose-1H-imidazole-2_D1_-20_D3_-55_D5_0	0.486	57.3	250.5	2.5
Glucose-1H-imidazole_D1_-55_D3_-20_D5_0	0.481	57.1	169.9	2.5
Glucose-1H-imidazole-2_D1_-30_D3_-60_D5_0	0.544	54.1	241.1	2.5
Glucose-1H-imidazole-2_D1_-40_D3_-60_D5_0	0.575	51.5	229.8	2.5
Glucose-1H-imidazole-2_D1_-40_D3_-20_D5_0	0.347	53.6	179.1	2.6
Glucose-1H-imidazole_D1_-20_D3_-35_D5_0	0.311	52.5	236.7	2.6
Glucose-1H-imidazole-2_D1_-60_D3_-20_D5_0	0.518	57.3	167.8	2.7
Glucose-1H-imidazole-2_D1_-60_D3_-45_D5_0	0.592	50.4	196.9	2.7
Glucose-1H-imidazole_D1_-20_D3_-60_D5_0	0.524	57.5	252.5	2.8
Glucose-1H-imidazole_D1_-60_D3_-45_D5_0	0.597	50.6	196.2	2.8
Glucose-1H-imidazole_D1_-25_D3_-30_D5_0	0.294	49.9	219.6	2.9
Glucose-1H-imidazole-2_D1_-25_D3_-60_D5_0	0.535	55.9	246.8	2.9
Glucose-1H-imidazole_D1_-60_D3_-25_D5_0	0.529	55.6	173.7	2.9
Glucose-1H-imidazole_D1_-30_D3_-25_D5_0	0.294	50.0	200.7	2.9
Glucose-1H-imidazole-2_D1_-20_D3_-35_D5_0	0.314	53.0	237.1	2.9
Glucose-1H-imidazole-2_D1_-45_D3_-60_D5_0	0.597	50.7	224.4	2.9
Glucose-1H-imidazole-2_D1_-25_D3_-30_D5_0	0.294	50.1	220.5	3.0
Glucose-1H-imidazole_D1_-35_D3_-20_D5_0	0.312	52.7	183.7	3.0
Glucose-1H-imidazole-2_D1_-35_D3_-20_D5_0	0.308	52.3	184.1	3.1
Glucose-1H-imidazole-2_D1_-30_D3_-25_D5_0	0.292	49.8	201.6	3.1
Glucose-1H-imidazole_D1_-55_D3_-55_D5_0	0.620	49.8	209.8	3.1
Glucose-1H-imidazole_D1_-20_D3_-30_D5_0	0.274	51.2	230.3	3.2
Glucose-1H-imidazole-2_D1_-20_D3_-60_D5_0	0.530	58.0	252.3	3.2
Glucose-1H-imidazole-2_D1_-20_D3_-30_D5_0	0.275	51.6	230.9	3.4
Glucose-1H-imidazole_D1_-50_D3_-60_D5_0	0.623	50.0	218.4	3.4
Glucose-1H-imidazole_D1_-60_D3_-20_D5_0	0.525	57.8	168.1	3.5
Glucose-1H-imidazole_D1_-25_D3_-25_D5_0	0.264	49.5	210.1	3.5
Glucose-1H-imidazole-2_D1_-55_D3_-55_D5_0	0.619	49.8	210.6	3.6
Glucose-1H-imidazole_D1_-30_D3_-20_D5_0	0.275	51.4	190.0	3.6
Glucose-1H-imidazole-2_D1_-25_D3_-25_D5_0	0.263	49.6	210.9	3.6
Glucose-1H-imidazole_D1_-60_D3_-50_D5_0	0.624	50.2	201.3	3.7
Glucose-1H-imidazole-2_D1_-30_D3_-20_D5_0	0.272	51.0	190.6	3.7

Glucose-1H-imidazole-2_D1_-60_D3_-50_D5_0	0.621	50.0	202.0	4.3
Glucose-1H-imidazole-2_D1_-50_D3_-60_D5_0	0.624	50.2	219.3	4.4
Glucose-1H-imidazole_D1_-20_D3_-25_D5_0	0.240	50.0	221.6	4.8
Glucose-1H-imidazole-2_D1_-20_D3_-25_D5_0	0.240	50.3	222.3	4.9
Glucose-1H-imidazole_D1_-25_D3_-20_D5_0	0.240	50.1	198.6	4.9
Glucose-1H-imidazole-2_D1_-25_D3_-20_D5_0	0.238	49.9	199.4	4.9
Glucose-1H-imidazole-2_D1_-20_D3_-20_D5_0	0.210	49.5	210.8	5.2
Glucose-1H-imidazole_D1_-20_D3_-20_D5_0	0.211	49.5	210.0	5.2
Glucose-1H-imidazole_D1_-55_D3_-60_D5_0	0.654	49.9	213.8	5.4
Glucose-1H-imidazole_D1_-60_D3_-55_D5_0	0.654	49.9	206.0	5.5
Glucose-1H-imidazole-2_D1_-60_D3_-55_D5_0	0.653	49.9	206.6	5.8
Glucose-1H-imidazole-2_D1_-55_D3_-60_D5_0	0.654	50.0	214.5	6.0
Glucose-1H-imidazole_D1_-60_D3_-60_D5_0	0.688	49.9	209.9	7.3
Glucose-1H-imidazole-2_D1_-60_D3_-60_D5_0	0.687	49.9	210.5	7.8

Gluco-1*H*-imidazole-protonated (6)

Name	**Q**	**Theta**	**Phi**	ΔG_{aq}^{T}
Glucose-1H-imidazole-protonated_D1_-45_D3_-40_D5_0	0.465	49.7	204.3	0.0
Glucose-1H-imidazole-protonated_D1_-45_D3_-45_D5_0	0.495	49.6	210.3	0.0
Glucose-1H-imidazole-protonated_D1_-40_D3_-45_D5_0	0.466	49.7	216.4	0.1
Glucose-1H-imidazole-protonated_D1_-40_D3_-40_D5_0	0.434	49.6	210.4	0.1
Glucose-1H-imidazole-protonated_D1_-50_D3_-40_D5_0	0.500	50.1	199.0	0.2
Glucose-1H-imidazole-protonated_D1_-30_D3_-45_D5_0	0.422	51.3	230.4	0.3
Glucose-1H-imidazole-protonated_D1_-50_D3_-45_D5_0	0.527	49.7	205.0	0.3
Glucose-1H-imidazole-protonated_D1_-35_D3_-45_D5_0	0.442	50.3	223.1	0.4
Glucose-1H-imidazole-protonated_D1_-40_D3_-35_D5_0	0.407	49.7	203.6	0.4
Glucose-1H-imidazole-protonated_D1_-40_D3_-50_D5_0	0.501	50.1	221.6	0.4
Glucose-1H-imidazole-protonated_D1_-45_D3_-35_D5_0	0.440	50.2	197.5	0.4
Glucose-1H-imidazole-protonated_D1_-35_D3_-40_D5_0	0.407	49.8	217.3	0.4
Glucose-1H-imidazole-protonated_D1_-35_D3_-35_D5_0	0.377	49.5	210.5	0.5
Glucose-1H-imidazole-protonated_D1_-45_D3_-50_D5_0	0.527	49.7	215.6	0.5
Glucose-1H-imidazole-protonated_D1_-45_D3_-30_D5_0	0.420	51.3	190.2	0.5
Glucose-1H-imidazole-protonated_D1_-35_D3_-50_D5_0	0.479	50.9	228.0	0.5
Glucose-1H-imidazole-protonated_D1_-50_D3_-35_D5_0	0.476	50.9	192.6	0.6
Glucose-1H-imidazole-protonated_D1_-30_D3_-40_D5_0	0.385	50.5	225.0	0.6
Glucose-1H-imidazole-protonated_D1_-50_D3_-30_D5_0	0.459	52.1	185.8	0.7
Glucose-1H-imidazole-protonated_D1_-55_D3_-40_D5_0	0.537	50.6	194.6	0.8
Glucose-1H-imidazole-protonated_D1_-45_D3_-25_D5_0	0.404	52.8	182.6	0.8
Glucose-1H-imidazole-protonated_D1_-55_D3_-30_D5_0	0.499	53.0	182.2	0.8

Glucose-1H-imidazole-protonated_D1_-30_D3_-35_D5_0	0.351	49.8	218.5	0.9
Glucose-1H-imidazole-protonated_D1_-50_D3_-50_D5_0	0.558	49.6	210.3	1.0
Glucose-1H-imidazole-protonated_D1_-40_D3_-30_D5_0	0.383	50.4	195.6	1.0
Glucose-1H-imidazole-protonated_D1_-50_D3_-25_D5_0	0.445	53.9	178.8	1.0
Glucose-1H-imidazole-protonated_D1_-55_D3_-35_D5_0	0.516	51.6	188.6	1.0
Glucose-1H-imidazole-protonated_D1_-50_D3_-20_D5_0	0.437	56.1	172.0	1.1
Glucose-1H-imidazole-protonated_D1_-40_D3_-55_D5_0	0.538	50.7	225.9	1.2
Glucose-1H-imidazole-protonated_D1_-35_D3_-30_D5_0	0.350	49.8	202.4	1.2
Glucose-1H-imidazole-protonated_D1_-35_D3_-55_D5_0	0.518	51.6	232.0	1.2
Glucose-1H-imidazole-protonated_D1_-55_D3_-25_D5_0	0.488	54.7	175.9	1.3
Glucose-1H-imidazole-protonated_D1_-45_D3_-55_D5_0	0.563	50.1	220.2	1.3
Glucose-1H-imidazole-protonated_D1_-55_D3_-45_D5_0	0.562	50.0	200.3	1.4
Glucose-1H-imidazole-protonated_D1_-30_D3_-50_D5_0	0.460	52.1	234.5	1.4
Glucose-1H-imidazole-protonated_D1_-30_D3_-30_D5_0	0.320	49.5	210.5	1.6
Glucose-1H-imidazole-protonated_D1_-55_D3_-20_D5_0	0.481	56.9	169.7	1.7
Glucose-1H-imidazole-protonated_D1_-40_D3_-25_D5_0	0.365	51.8	187.3	1.8
Glucose-1H-imidazole-protonated_D1_-30_D3_-55_D5_0	0.501	53.0	238.0	1.8
Glucose-1H-imidazole-protonated_D1_-25_D3_-35_D5_0	0.330	50.8	227.4	1.8
Glucose-1H-imidazole-protonated_D1_-35_D3_-25_D5_0	0.328	50.7	193.4	1.9
Glucose-1H-imidazole-protonated_D1_-50_D3_-55_D5_0	0.592	49.8	214.9	2.0
Glucose-1H-imidazole-protonated_D1_-60_D3_-40_D5_0	0.575	51.2	190.9	2.0
Glucose-1H-imidazole-protonated_D1_-60_D3_-35_D5_0	0.556	52.3	185.2	2.0
Glucose-1H-imidazole-protonated_D1_-55_D3_-50_D5_0	0.591	49.8	205.6	2.0
Glucose-1H-imidazole-protonated_D1_-45_D3_-20_D5_0	0.394	55.0	175.1	2.0
Glucose-1H-imidazole-protonated_D1_-60_D3_-25_D5_0	0.531	55.5	173.5	2.0
Glucose-1H-imidazole-protonated_D1_-60_D3_-20_D5_0	0.525	57.6	167.9	2.1
Glucose-1H-imidazole-protonated_D1_-25_D3_-50_D5_0	0.447	53.9	241.4	2.1
Glucose-1H-imidazole-protonated_D1_-60_D3_-30_D5_0	0.541	53.7	179.3	2.1
Glucose-1H-imidazole-protonated_D1_-20_D3_-35_D5_0	0.314	52.6	236.9	2.2
Glucose-1H-imidazole-protonated_D1_-20_D3_-50_D5_0	0.439	56.1	248.2	2.2
Glucose-1H-imidazole-protonated_D1_-40_D3_-20_D5_0	0.352	53.8	179.0	2.2
Glucose-1H-imidazole-protonated_D1_-30_D3_-60_D5_0	0.545	53.8	241.1	2.2
Glucose-1H-imidazole-protonated_D1_-35_D3_-60_D5_0	0.559	52.3	235.3	2.3
Glucose-1H-imidazole-protonated_D1_-25_D3_-45_D5_0	0.407	52.9	238.0	2.3
Glucose-1H-imidazole-protonated_D1_-40_D3_-60_D5_0	0.577	51.2	229.5	2.4
Glucose-1H-imidazole-protonated_D1_-25_D3_-30_D5_0	0.296	49.9	220.0	2.4
Glucose-1H-imidazole-protonated_D1_-25_D3_-55_D5_0	0.489	54.7	244.3	2.5
Glucose-1H-imidazole-protonated_D1_-30_D3_-25_D5_0	0.295	49.8	201.0	2.5
Glucose-1H-imidazole-protonated_D1_-20_D3_-55_D5_0	0.484	56.9	250.6	2.5
Glucose-1H-imidazole-protonated_D1_-60_D3_-45_D5_0	0.599	50.5	196.4	2.6

Glucose-1H-imidazole-protonated_D1_-20_D3_-40_D5_0	0.355	53.9	241.8	2.7
Glucose-1H-imidazole-protonated_D1_-45_D3_-60_D5_0	0.601	50.5	223.9	2.8
Glucose-1H-imidazole-protonated_D1_-35_D3_-20_D5_0	0.313	52.6	183.8	2.8
Glucose-1H-imidazole-protonated_D1_-20_D3_-45_D5_0	0.397	55.1	245.5	2.9
Glucose-1H-imidazole-protonated_D1_-20_D3_-30_D5_0	0.276	51.3	230.5	3.1
Glucose-1H-imidazole-protonated_D1_-25_D3_-60_D5_0	0.533	55.5	246.7	3.2
Glucose-1H-imidazole-protonated_D1_-55_D3_-55_D5_0	0.624	49.7	210.2	3.2
Glucose-1H-imidazole-protonated_D1_-30_D3_-20_D5_0	0.275	51.2	190.3	3.3
Glucose-1H-imidazole-protonated_D1_-25_D3_-25_D5_0	0.266	49.5	210.4	3.3
Glucose-1H-imidazole-protonated_D1_-20_D3_-60_D5_0	0.528	57.6	252.4	3.3
Glucose-1H-imidazole-protonated_D1_-20_D3_-25_D5_0	0.242	50.1	221.9	3.4
Glucose-1H-imidazole-protonated_D1_-60_D3_-50_D5_0	0.627	50.0	201.5	3.6
Glucose-1H-imidazole-protonated_D1_-50_D3_-60_D5_0	0.628	50.0	218.8	3.6
Glucose-1H-imidazole-protonated_D1_-25_D3_-20_D5_0	0.241	50.0	198.9	3.9
Glucose-1H-imidazole-protonated_D1_-55_D3_-60_D5_0	0.659	49.8	214.1	5.2
Glucose-1H-imidazole-protonated_D1_-60_D3_-55_D5_0	0.658	49.8	206.1	5.2
Glucose-1H-imidazole-protonated_D1_-20_D3_-20_D5_0	0.212	49.5	210.4	6.1
Glucose-1H-imidazole-protonated_D1_-60_D3_-60_D5_0	0.692	49.8	209.9	7.7

Glucoimidazole (5)

Name	**Q**	**Theta**	**Phi**	ΔG_{aq}^{T}
Glucose-imidazole_D1_-50_D3_-40_D5_0	0.493	50.2	198.6	0.0
Glucose-imidazole_D1_-45_D3_-40_D5_0	0.459	49.9	203.8	0.0
Glucose-imidazole_D1_-45_D3_-45_D5_0	0.488	49.8	209.9	0.1
Glucose-imidazole_D1_-50_D3_-35_D5_0	0.471	50.9	192.1	0.1
Glucose-imidazole_D1_-50_D3_-45_D5_0	0.519	49.9	204.5	0.1
Glucose-imidazole_D1_-45_D3_-35_D5_0	0.434	50.3	197.1	0.2
Glucose-imidazole_D1_-50_D3_-30_D5_0	0.453	52.1	185.4	0.3
Glucose-imidazole_D1_-40_D3_-40_D5_0	0.428	49.8	209.9	0.4
Glucose-imidazole_D1_-40_D3_-45_D5_0	0.459	50.0	216.0	0.4
Glucose-imidazole_D1_-55_D3_-30_D5_0	0.493	52.9	181.8	0.4
Glucose-imidazole_D1_-55_D3_-35_D5_0	0.509	51.6	188.1	0.4
Glucose-imidazole_D1_-45_D3_-50_D5_0	0.520	50.0	215.3	0.5
Glucose-imidazole_D1_-40_D3_-50_D5_0	0.494	50.5	221.2	0.5
Glucose-imidazole_D1_-55_D3_-40_D5_0	0.529	50.7	194.1	0.5
Glucose-imidazole_D1_-40_D3_-35_D5_0	0.401	49.9	203.0	0.6
Glucose-imidazole_D1_-45_D3_-30_D5_0	0.415	51.3	189.8	0.7
Glucose-imidazole_D1_-50_D3_-25_D5_0	0.440	53.7	178.5	0.7
Glucose-imidazole_D1_-35_D3_-45_D5_0	0.435	50.6	222.6	0.7

Glucose-imidazole_D1_-35_D3_-50_D5_0	0.472	51.3	227.5	0.8
Glucose-imidazole_D1_-35_D3_-40_D5_0	0.402	50.0	216.8	0.8
Glucose-imidazole_D1_-55_D3_-25_D5_0	0.483	54.6	175.5	0.8
Glucose-imidazole_D1_-25_D3_-50_D5_0	0.442	54.3	240.9	0.8
Glucose-imidazole_D1_-50_D3_-50_D5_0	0.549	49.9	209.9	0.8
Glucose-imidazole_D1_-55_D3_-45_D5_0	0.554	50.2	199.9	0.9
Glucose-imidazole_D1_-55_D3_-20_D5_0	0.477	56.7	169.3	0.9
Glucose-imidazole_D1_-40_D3_-55_D5_0	0.530	51.0	225.5	1.0
Glucose-imidazole_D1_-60_D3_-30_D5_0	0.535	53.6	178.9	1.0
Glucose-imidazole_D1_-35_D3_-55_D5_0	0.511	52.0	231.5	1.0
Glucose-imidazole_D1_-60_D3_-25_D5_0	0.526	55.4	173.1	1.0
Glucose-imidazole_D1_-50_D3_-20_D5_0	0.432	55.8	171.7	1.0
Glucose-imidazole_D1_-30_D3_-50_D5_0	0.455	52.6	234.2	1.1
Glucose-imidazole_D1_-35_D3_-35_D5_0	0.371	49.7	209.9	1.1
Glucose-imidazole_D1_-60_D3_-35_D5_0	0.549	52.3	184.8	1.1
Glucose-imidazole_D1_-25_D3_-55_D5_0	0.485	55.3	243.9	1.1
Glucose-imidazole_D1_-30_D3_-45_D5_0	0.416	51.7	229.8	1.1
Glucose-imidazole_D1_-30_D3_-55_D5_0	0.496	53.5	237.7	1.1
Glucose-imidazole_D1_-45_D3_-25_D5_0	0.399	52.8	182.3	1.1
Glucose-imidazole_D1_-25_D3_-45_D5_0	0.401	53.3	237.2	1.2
Glucose-imidazole_D1_-60_D3_-20_D5_0	0.521	57.4	167.5	1.2
Glucose-imidazole_D1_-40_D3_-30_D5_0	0.378	50.5	195.3	1.2
Glucose-imidazole_D1_-45_D3_-55_D5_0	0.554	50.4	219.8	1.2
Glucose-imidazole_D1_-30_D3_-40_D5_0	0.379	50.8	224.5	1.3
Glucose-imidazole_D1_-60_D3_-40_D5_0	0.568	51.2	190.5	1.3
Glucose-imidazole_D1_-45_D3_-20_D5_0	0.389	54.8	174.7	1.4
Glucose-imidazole_D1_-25_D3_-35_D5_0	0.325	51.1	226.7	1.5
Glucose-imidazole_D1_-25_D3_-60_D5_0	0.529	56.0	246.2	1.5
Glucose-imidazole_D1_-30_D3_-60_D5_0	0.537	54.2	240.5	1.5
Glucose-imidazole_D1_-30_D3_-35_D5_0	0.345	50.1	217.8	1.5
Glucose-imidazole_D1_-25_D3_-40_D5_0	0.362	52.2	232.6	1.5
Glucose-imidazole_D1_-55_D3_-50_D5_0	0.582	49.9	205.1	1.6
Glucose-imidazole_D1_-35_D3_-60_D5_0	0.551	52.8	234.8	1.6
Glucose-imidazole_D1_-40_D3_-25_D5_0	0.360	51.8	187.0	1.7
Glucose-imidazole_D1_-50_D3_-55_D5_0	0.582	50.0	214.5	1.7
Glucose-imidazole_D1_-35_D3_-30_D5_0	0.345	49.9	201.9	1.8
Glucose-imidazole_D1_-40_D3_-60_D5_0	0.569	51.6	229.1	1.8
Glucose-imidazole_D1_-60_D3_-45_D5_0	0.591	50.6	196.0	1.9
Glucose-imidazole_D1_-40_D3_-20_D5_0	0.348	53.7	178.4	1.9
Glucose-imidazole_D1_-30_D3_-30_D5_0	0.316	49.7	209.8	2.0

Glucose-imidazole_D1_-35_D3_-25_D5_0	0.324	50.8	192.9	2.2
Glucose-imidazole_D1_-45_D3_-60_D5_0	0.591	50.8	223.6	2.2
Glucose-imidazole_D1_-30_D3_-25_D5_0	0.291	50.0	200.3	2.4
Glucose-imidazole_D1_-35_D3_-20_D5_0	0.308	52.5	183.3	2.5
Glucose-imidazole_D1_-55_D3_-55_D5_0	0.614	49.9	209.7	2.8
Glucose-imidazole_D1_-60_D3_-50_D5_0	0.618	50.2	201.1	2.9
Glucose-imidazole_D1_-20_D3_-55_D5_0	0.479	57.4	249.9	3.1
Glucose-imidazole_D1_-50_D3_-60_D5_0	0.618	50.3	218.5	3.1
Glucose-imidazole_D1_-20_D3_-60_D5_0	0.524	58.1	251.7	3.2
Glucose-imidazole_D1_-20_D3_-50_D5_0	0.435	56.6	247.6	3.3
Glucose-imidazole_D1_-20_D3_-45_D5_0	0.392	55.5	244.7	3.6
Glucose-imidazole_D1_-20_D3_-40_D5_0	0.349	54.3	241.0	3.9
Glucose-imidazole_D1_-60_D3_-55_D5_0	0.648	50.0	205.7	4.4
Glucose-imidazole_D1_-25_D3_-30_D5_0	0.291	50.2	219.2	4.4
Glucose-imidazole_D1_-55_D3_-60_D5_0	0.648	50.1	213.9	4.5
Glucose-imidazole_D1_-20_D3_-35_D5_0	0.309	53.0	236.1	4.6
Glucose-imidazole_D1_-25_D3_-25_D5_0	0.262	49.7	209.8	5.1
Glucose-imidazole_D1_-30_D3_-20_D5_0	0.271	51.2	189.7	5.1
Glucose-imidazole_D1_-20_D3_-30_D5_0	0.272	51.6	229.6	5.3
Glucose-imidazole_D1_-25_D3_-20_D5_0	0.238	50.1	198.3	5.9
Glucose-imidazole_D1_-20_D3_-25_D5_0	0.238	50.3	221.1	5.9
Glucose-imidazole_D1_-20_D3_-20_D5_0	0.208	49.7	209.7	6.5
Glucose-imidazole_D1_-60_D3_-60_D5_0	0.682	50.0	209.8	6.6

Glucoimidazole-protonated (5)

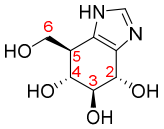
Name	**Q**	**Theta**	**Phi**	ΔG_{aq}^{T}
Glucose-imidazole-protonated_D1_-40_D3_-45_D5_0	0.463	49.7	215.3	0.0
Glucose-imidazole-protonated_D1_-45_D3_-40_D5_0	0.464	49.8	203.1	0.6
Glucose-imidazole-protonated_D1_-40_D3_-40_D5_0	0.432	49.6	209.2	0.7
Glucose-imidazole-protonated_D1_-50_D3_-35_D5_0	0.478	51.1	191.4	0.8
Glucose-imidazole-protonated_D1_-50_D3_-40_D5_0	0.500	50.3	197.8	0.8
Glucose-imidazole-protonated_D1_-50_D3_-30_D5_0	0.461	52.4	184.9	0.8
Glucose-imidazole-protonated_D1_-55_D3_-35_D5_0	0.518	51.9	187.4	1.0
Glucose-imidazole-protonated_D1_-55_D3_-30_D5_0	0.503	53.2	181.4	1.0
Glucose-imidazole-protonated_D1_-45_D3_-45_D5_0	0.492	49.6	209.1	1.0
Glucose-imidazole-protonated_D1_-50_D3_-45_D5_0	0.525	49.8	203.7	1.1
Glucose-imidazole-protonated_D1_-45_D3_-35_D5_0	0.440	50.4	196.4	1.1
Glucose-imidazole-protonated_D1_-55_D3_-40_D5_0	0.538	50.8	193.4	1.1
Glucose-imidazole-protonated_D1_-40_D3_-35_D5_0	0.406	49.8	202.2	1.2

Glucose-imidazole-protonated_D1_-45_D3_-50_D5_0	0.524	49.7	214.6	1.2
Glucose-imidazole-protonated_D1_-40_D3_-50_D5_0	0.497	50.1	220.6	1.2
Glucose-imidazole-protonated_D1_-50_D3_-20_D5_0	0.441	56.3	171.5	1.3
Glucose-imidazole-protonated_D1_-45_D3_-30_D5_0	0.421	51.5	189.1	1.3
Glucose-imidazole-protonated_D1_-55_D3_-20_D5_0	0.486	57.1	169.3	1.3
Glucose-imidazole-protonated_D1_-50_D3_-25_D5_0	0.449	54.1	178.2	1.4
Glucose-imidazole-protonated_D1_-55_D3_-45_D5_0	0.561	50.2	199.1	1.5
Glucose-imidazole-protonated_D1_-60_D3_-25_D5_0	0.536	55.8	172.9	1.6
Glucose-imidazole-protonated_D1_-60_D3_-35_D5_0	0.559	52.6	184.2	1.6
Glucose-imidazole-protonated_D1_-40_D3_-55_D5_0	0.533	50.6	225.1	1.7
Glucose-imidazole-protonated_D1_-50_D3_-50_D5_0	0.554	49.7	209.3	1.7
Glucose-imidazole-protonated_D1_-60_D3_-30_D5_0	0.546	54.0	178.5	1.7
Glucose-imidazole-protonated_D1_-45_D3_-25_D5_0	0.406	53.1	181.8	1.7
Glucose-imidazole-protonated_D1_-35_D3_-45_D5_0	0.438	50.2	222.4	1.7
Glucose-imidazole-protonated_D1_-35_D3_-55_D5_0	0.512	51.5	231.1	1.8
Glucose-imidazole-protonated_D1_-40_D3_-30_D5_0	0.383	50.6	194.6	1.8
Glucose-imidazole-protonated_D1_-45_D3_-55_D5_0	0.558	50.0	219.2	1.9
Glucose-imidazole-protonated_D1_-60_D3_-40_D5_0	0.577	51.4	189.9	1.9
Glucose-imidazole-protonated_D1_-35_D3_-40_D5_0	0.404	49.7	216.1	1.9
Glucose-imidazole-protonated_D1_-60_D3_-20_D5_0	0.532	57.8	167.5	1.9
Glucose-imidazole-protonated_D1_-55_D3_-25_D5_0	0.492	55.0	175.2	2.0
Glucose-imidazole-protonated_D1_-45_D3_-20_D5_0	0.397	55.3	174.4	2.0
Glucose-imidazole-protonated_D1_-35_D3_-50_D5_0	0.474	50.8	227.1	2.1
Glucose-imidazole-protonated_D1_-35_D3_-20_D5_0	0.314	52.8	182.9	2.1
Glucose-imidazole-protonated_D1_-30_D3_-50_D5_0	0.456	52.1	234.1	2.3
Glucose-imidazole-protonated_D1_-55_D3_-50_D5_0	0.589	49.8	204.3	2.3
Glucose-imidazole-protonated_D1_-30_D3_-45_D5_0	0.417	51.2	229.7	2.4
Glucose-imidazole-protonated_D1_-25_D3_-45_D5_0	0.402	52.8	237.4	2.4
Glucose-imidazole-protonated_D1_-35_D3_-35_D5_0	0.374	49.5	209.3	2.4
Glucose-imidazole-protonated_D1_-40_D3_-25_D5_0	0.366	52.0	186.4	2.4
Glucose-imidazole-protonated_D1_-25_D3_-50_D5_0	0.443	53.8	241.1	2.5
Glucose-imidazole-protonated_D1_-35_D3_-30_D5_0	0.349	49.9	201.1	2.5
Glucose-imidazole-protonated_D1_-50_D3_-55_D5_0	0.587	49.7	213.8	2.5

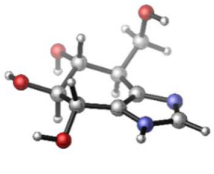
NMR calculations

Based on the optimized structures the spin-spin coupling constants were calculated according to the work of Rablen and Bally[26] with the use of 6-311g(d,p) u+1s as basis set and PCM(H_2O) as solvent model. The calculated total nuclear spin-spin coupling terms were used as calculated spin-spin coupling constants. The calculated $^3J_{(H,H)}$ coupling constants for these low energy $^4H_3$ rotamers of **6** matched well with experimental $^3J_{(H,H)}$ coupling constants, suggesting that that **6** most likely adopts a $^4H_3$ conformation in solution.

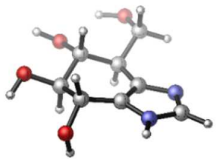
Gluco-1*H*-imidazole



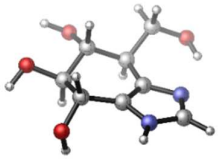
6



gg (0.0 kcal/mol)



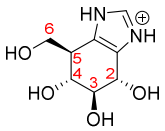
gt (+0.1 kcal/mol)



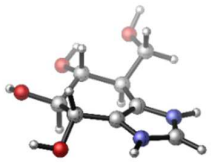
tg (+0.3 kcal/mol)

H-H Coupling	**Exp. $^3J_{(H,H)}$ (Hz)**	**DFT calculated $^3J_{(H,H)}$ (Hz)**	**DFT calculated $^3J_{(H,H)}$ (Hz)**	**DFT calculated $^3J_{(H,H)}$ (Hz)**
H2-H3	7.6	7.1	7.3	7.2
H3-H4	9.8	10.1	9.5	9.8
H4-H5	9.4	9.5	9.5	9.2
H5-H6a	2.8	2.5	4.5	10.0
H5-H6b	4.8	2.6	10.7	3.2
H6a-H6b	11.4	12.8	11.1	11.9

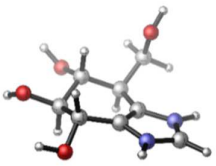
Gluco-1*H*-imidazole (protonated)



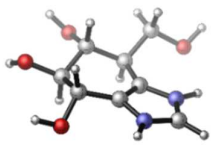
6



gt (0.0 kcal/mol)



gg (+0.0 kcal/mol)



tg (+2.3 kcal/mol)

H-H Coupling	**Exp. $^3J_{(H,H)}$ (Hz)**	**DFT calculated $^3J_{(H,H)}$ (Hz)**	**DFT calculated $^3J_{(H,H)}$ (Hz)**	**DFT calculated $^3J_{(H,H)}$ (Hz)**
H2-H3	7.6	8.0	8.0	7.9
H3-H4	9.8	9.9	10.2	10.0
H4-H5	9.4	10.0	9.8	7.8
H5-H6a	2.8	4.9	3.2	12.5
H5-H6b	4.8	11.7	2.2	4.8
H6a-H6b	11.4	8.8	10.4	8.8

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