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Sugar hydrazide imides: a new family of glycosidase inhibitors

Emil Lindbäck,*[a] Óscar Lopéz,*[b], Ådne Tobiesen,[a] José G. Fernández-Bolaños,[b] and Magne O. Sydnes*[a]

[a]Faculty of Science and Technology, Department of Mathematics and Natural Science, University of Stavanger, NO-4036 Stavanger, Norway

[b]Department of Organic Chemistry, University of Seville, Professor García González 1, 41012 Seville, Spain

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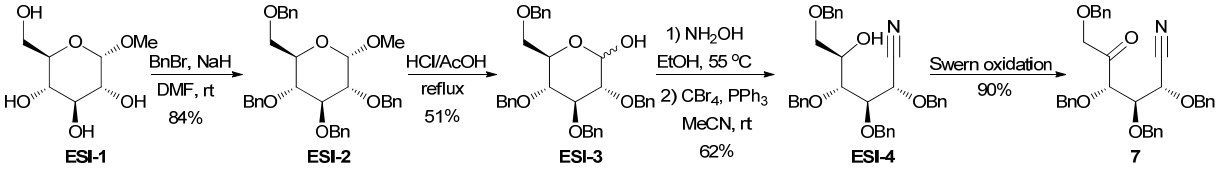
General Methods

Acetonitrile and methylene chloride were both dried over 4 Å molecular sieves (oven dried). For petroleum ether (PE), the 40-65 °C fraction was used. All reactions were carried out under a N_2 atmosphere if not otherwise specified. Glassware used for anhydrous reactions were dried in an oven for 24 h at 140 °C before use. TLC analyses were performed on Merck silica gel 60 F254 plates using UV light for detection. Silica gel NORMASIL 60® 40-63 µm was used for flash column chromatography. NMR spectra were recorded with a Bruker Avance NMR spectrometer; ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra were recorded at 100 MHz in $CDCl_3$, CD_3OD or DMSO-d_6. Chemical shifts are reported in ppm relative to an internal standard of residual chloroform (δ = 7.26 ppm for ^{1}H NMR; δ = 77.00 ppm for ^{13}C NMR), residual methanol (δ = 3.31 ppm for ^{1}H NMR; δ = 49.00 ppm for ^{13}C NMR), and residual DMSO (δ = 2.50 ppm for ^{1}H NMR; δ = 29.84 ppm for ^{13}C NMR). High-resolution mass spectra (HRMS) were recorded from MeOH solutions on a JMS-T100LC AccuTOF™ in positive electrospray ionization (ESI) mode.



Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (ESI-2). A solution of methyl α-D-glucopyranoside (**ESI-1**) (2.5 g, 12.9 mmol) in DMF (40 mL) at 0 °C under a nitrogen atmosphere was in portions added NaH (60% dispersion in mineral oil, 3.09 g, 77.2 mmol). After addition, the resulting suspension was kept stirring for 30 min. After this time, benzyl bromide (9.2 mL, 77.2 mmol) was added dropwise at 0 °C and the resulting mixture was stirred overnight at rt. Water (50 mL) was slowly added at 0 °C and the aqueous mixture was extracted with EtOAc (3 × 40 mL). The combined organic extract was concentrated under reduced pressure to afford a concentrate, which underwent purification by flash silica column chromatography (EtOAc/PE 1:9 → 2:5) to provide the title compound (6.0 g, 84%) as a colourless syrup. The observed NMR data are in full agreement with reported data:[1] ^{1}H-NMR δH (400 MHz, $CDCl_3$) 3.41 (3 H, s), 3.57–3.79 (5 H, m), 4.01 (1 H, d, *J* 9.0 Hz), 4.49–4.52 (2 H, m), 4.61–4.71 (3 H, m), 4.81–4.87 (3 H, m), 5.01 (1 H, d, *J* 11.0), 7.15–7.40 (20 H, m). ^{13}C-NMR δC (100 MHz, $CDCl_3$) 55.1, 68.5, 70.0, 73.4, 73.5, 75.0, 75.7, 77.7, 79.8, 82.1, 98.2, 127.6–128.4 (Ar), 137.9, 138.2, (2Ar), 138.8.

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (ESI-3). To a solution of methyl glucoside **ESI-2** (6.40 g, 11.5 mmol) in glacial acetic acid (68 mL) at 85 °C was added dropwise aqueous HCl (26 mL, 2 M). After addition, the mixture was kept stirring at 124 °C for 45 min. The mixture was allowed to reach rt and the product precipitated overnight. The precipitate was collected on a glass filter funnel and was consecutively washed with H_2O (3 × 40 mL) and MeOH (3 × 40 mL). The precipitate of **ESI-3** (2.7 g, 47%) was used directly in the next reaction without any further purification.

2,3,4,6-Tetra-*O*-benzyl-D-glucononitrile (ESI-4).[2] Small pieces of sodium (505.4 mg, 22.0 mmol) were dissolved in absolute EtOH (120 mL) under a nitrogen atmosphere at rt. When the sodium was completely dissolved, the temperature was elevated to 60 °C and $NH_2OH{\cdot}HCl$ (2.98 g, 42.9 mmol) was added and the resulting suspension was stirred for 5 min. Pyranose **ESI-3** (2.90 g, 5.36 mmol) was added and the reaction mixture was kept stirring for 7 h at 60 °C. The mixture

was then allowed to reach rt and the solvent was removed under reduced pressure. The residue was dissolved in H_2O (100 mL) and EtOAc (75 mL). The phases were separated and the aqueous phase was extracted with EtOAc (2 × 75 mL). The combined organic fractions were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue together with PPh_3 (2.81 g, 10.7 mmol) was dissolved in anhydrous MeCN (42 mL). The resultant solution was added dropwise a solution of CBr_4 (4.44 g, 13.4 mmol) in anhydrous MeCN (20 mL) at rt and the resulting mixture was then kept stirring for 20 min. The reaction was quenched by adding a solution of PPh_3 (703.0 mg, 2.68 mmol) in a 11:30 MeCN/MeOH mixture (82 mL) and stirring for 15 min. After this time, the solvent was removed under reduced pressure and the residue was purified by flash silica column chromatography (EtOAc/PE 3:17 → 1:3) to provide title compound (1.79 g, 62%) as a light yellow syrup. The observed NMR data are in full agreement with reported data:[2] ^{1}H-NMR δH (400 MHz, $CDCl_3$) 2.48 (1 H, d, *J* 6.6 Hz), 3.59 (1 H, dd, *J* 9.6 Hz, *J* 4.7 Hz), 3.63 (1 H, dd, *J* 9.7 Hz, *J* 3.6 Hz), 3.90 (1 H, dd, *J* 7.7 Hz, *J* 3.0 Hz), 3.96–4.02 (1 H, m), 4.08 (1 H, dd, *J* 6.8 Hz, *J* 3.0 Hz), 4.45 (1 H, d, *J* 6.8 Hz), 4.52–4.57 (4 H, m), 4.68 (1 H, d, *J* 11.3 Hz), 4.71 (1 H, d, *J* 11.1 Hz), 4.78 (1 H, d, *J* 11.1 Hz), 4.88 (1 H, d, *J* 11.3), 7.23–7.40 (20 H, m). ^{13}C-NMR δC (100 MHz, $CDCl_3$) 69.2, 69.7, 70.5, 72.9, 73.4, 74.3, 75.3, 77.8, 78.7, 117.0, 127.8–128.7 (Ar), 135.6, 137.4, 137.6 (2Ar).

2,3,4,6-Tetra-*O*-benzyl-D-*xylo*-5-hexulosononitrile (7).[2] To a solution of DMSO (0.15 mL, 2.15 mmol) in CH_2Cl_2 (1.2 mL) at -78 °C was dropwise added oxalyl chloride (0.092 mL, 1.07 mmol). The resulting mixture was kept stirring at -78 °C for 10 min. A solution of alcohol **ESI-4** (76.7 mg, 0.143 mmol) in CH_2Cl_2 (1.2 mL) was added dropwise. After the addition, the mixture was kept stirring at -78 °C for 90 min before the slow addition of Et_3N (0.80 mL). The mixture was kept stirring at -78 °C for 15 min and then at rt for 30 min. Water (10 mL) was then added and the aqueous mixture was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic fractions were concentrated *in vacuo* and the residue was purified by flash silica column chromatography (EtOAc/PE 10:90 → 15:85) to provide the title compound (69.2 mg, 90%) as an oil. The observed NMR data are in full agreement with reported data:[2] ^{1}H-NMR δH (400 MHz, $CDCl_3$) 4.10–4.23 (3 H, m), 4.34–4.44 (4 H, m), 4.49–4.65 (5 H, m), 4.80 (1 H, d, *J* 11.3 Hz), 7.17–7.36 (20 H, m). ^{13}C-NMR δC (100 MHz, $CDCl_3$) 68.4, 73.1, 73.3, 74.5, 74.9, 75.2, 79.1, 82.2, 116.4, 127.7–128.7 (Ar), 135.3, 136.3, 136.6, 137.1 (Ar), 206.8.

2,3,4,6-Tetra-*O*-benzyl-5-(2-(*tert*-butoxycarbonyl)hydrazinyl)-5-deoxy-D-glucononitrile (8) and 2,3,4,6-tetra-*O*-benzyl-5-(2-(*tert*-butoxycarbonyl)hydrazinyl)-5-deoxy-L-idononitrile (9). A stirred solution of ketone **7** (118 mg, 0.220 mmol) and *tert*-butyl carbazate (58.0 mg, 0.441 mmol) in EtOH/THF (1:1, 1.3 mL) at rt under a nitrogen atmosphere was added AcOH (79.3 mg, 1.32 mmol). After stirring for 14 h, AcOH (79.3 mg, 1.32 mmol) and $NaBH_3CN$ (110.6 mg, 1.76 mmol) were added. The mixture was kept stirring at rt for 6 h before the slow addition of saturated aqueous $NaHCO_3$ (10 mL). The aqueous mixture was extracted with EtOAc (3 × 10 mL) and the combined organic fractions were concentrated under reduced pressure. Purification of the residue by flash silica column chromatography (EtOAc/PE 1:9 → 1:7) provided compounds **8** (44.2 mg, 31%) and **9** (28.1 mg, 20%) as colourless syrups. **Data of compound 8**: R_f 0.46 (EtOAc/PE 7:13). $[\alpha]_D^{26}$ +19 (*c* 0.21, EtOAc). ^{1}H-NMR δH (400 MHz, $CDCl_3$) 1.43 (9 H, s, Boc), 3.26 (1 H, brs, 5-H), 3.55 (1 H, dd, $J_{6b,6a}$ 9.8 Hz, $J_{6b,5}$ 6.2 Hz, 6-H_b), 3.67 (1 H, dd, $J_{6a,6b}$ 9.8 Hz, $J_{6a,5}$ 4.4 Hz, 6-H_a), 3.91 (1 H, dd, $J_{4,5}$ 6.1 Hz, $J_{4,3}$ 3.5 Hz, 4-H), 4.15 (1 H, dd, $J_{3,2}$ 6.7 Hz, $J_{3,4}$ 3.5 Hz, 3-H), 4.24 (1 H, brs, NH), 4.41-4.48 (3 H, m, 2-H, 2CHPh), 4.52 (1 H, d, $J_{H,H}$ 11.6 Hz, CHPh), 4.64 (2 H, s, 2CHPh), 4,69 (1 H, d, $J_{H,H}$ 11.1 Hz, CHPh), 4.76 (1 H, d, $J_{H,H}$ 11.1 Hz, CHPh), 4.84 (1 H, d, $J_{H,H}$ 11.1 Hz, CHPh), 5.83 (1 H, brs, NH), 7.25-7.35 (20 H, m, ArH). ^{13}C-NMR δC (100 MHz, $CDCl_3$) 28.3 (Boc), 60.8 (5-C), 68.0 (6-C), 69.4 (2-C), 72.8 (CH_2Ph), 73.3 (CH_2Ph), 74.4 (CH_2Ph), 75.2 (CH_2Ph), 77.2 (4-C), 79.2 (3-C), 80.2 (Boc), 116.9 (1-C), 127.7-128.6 (Ar), 135.7 (Ar), 137.6 (Ar), 137.8 (Ar), 137.9 (Ar), 156.4 (Boc). HRMS (ESI); Calcd for $C_{39}H_{46}N_3O_6^+$ 652.3387; found 652.4059. **Data of compound 9**: R_f 0.42 (EtOAc/PE 7:13). $[\alpha]_D^{26}$ +24 (*c* 0.34, EtOAc). ^{1}H-NMR δH (400 MHz, $CDCl_3$) 1.45 (9 H, s, Boc), 3.10 (1 H, brs, 5-H), 3.51-3.59 (2 H, m, 6-H_a, 6-H_b), 3.96 (1 H, dd, $J_{4,3}$ 6.0 Hz, $J_{4,5}$ 3.6 Hz, 4-H), 4.11 (1 H, brs, NH), 4.15 (1 H, dd, $J_{3,4}$ 6.0 Hz, $J_{3,2}$ 4.9 Hz, 3-H), 4.42 (1 H, d, $J_{H,H}$ 12.0 Hz, CHPh), 4.45 (1 H, d, $J_{H,H}$ 12.0 Hz, CHPh), 4.54 (1 H, d, $J_{H,H}$ 11.3 Hz, CHPh), 4.55 (1 H, d, $J_{H,H}$ 11.5 Hz, CHPh), 4.69-4.76 (3 H, m, 2-H, 2CHPh), 4.84 (1 H, d, $J_{H,H}$ 11.0 Hz, CHPh), 4.90 (1 H, d, $J_{H,H}$ 11.3 Hz, CHPh), 5.96 (1 H, brs, NH), 7.25-7.36 (20 H, m, ArH). ^{13}C-NMR δC (100 MHz, $CDCl_3$) 28.3 (Boc), 60.1 (5-C), 69.0 (2-C, 6-C), 72.6 (CH_2Ph), 73.2 (CH_2Ph), 74.6 (2CH_2Ph), 77.3 (4-C), 79.3 (3-C), 80.3 (Boc), 117.0 (1-C), 127.7-128.6 (Ar), 135.7 (Ar), 137.6 (Ar), 137.9 (2Ar), 156.4 (Boc). HRMS (ESI); Calcd for $C_{39}H_{46}N_3O_6^+$ 652.3387; found 652.3389.

2,3,4,6-Tetra-*O*-benzyl-5-(2-(*tert*-butoxycarbonyl)-2-methylhydrazinyl)-5-deoxy-D-glucononitrile (10) and 2,3,4,6-tetra-*O*-benzyl-5-(2-(*tert*-butoxycarbonyl)-2-hydrazinyl)-5-deoxy-L-idononitrile (11). A solution of ketone **7** (108 mg, 0.202 mmol), $NH_2NMeBoc$ (59.0 mg, 0.404 mmol), and AcOH (72.8 mg, 1.21 mmol) in EtOH/THF (1:1, 1.2 mL) under a nitrogen atmosphere and at rt was kept stirring for 14 h. The crude reaction was then added AcOH (72.8 mg, 1.21 mmol) and $NaBH_3CN$ (101.5 mg, 1.62 mmol), and the resulting mixture was kept stirring for 6 h. Saturated aqueous $NaHCO_3$ (10

mL) was added and the resultant aqueous mixture was extracted with EtOAc (3 × 10 mL). The combined organic fractions were concentrated *in vacuo* and the residue was subjected to purification by flash silica column chromatography (EtOAc/PE 3:37 → 1:9) to provide **10** (49.8 mg, 38%) and **11** (18.1 mg, 14%) as colourless syrups. **Data of compound 10:** R_f 0.35 (EtOAc/PE 1:3). $[\alpha]_D^{26}$ +20 (*c* 0.72, EtOAc). ^{1}H-NMR δH (400 MHz, $CDCl_3$) 1.37 (9 H, s, Boc) 2.87 (3 H, s, Me), 3.21-3.25 (1 H, m, 5-H), 3.48 (1 H, dd, $J_{6b,6a}$ 9.7 Hz, $J_{6b,5}$ 5.1 Hz, 6-H_b), 3.65 (1 H, dd, $J_{6a,6b}$ 9.7 Hz, $J_{6a,5}$ 5.1 Hz, 6-H_a), 3.89-3.92 (1 H, m, 3-H), 3.93-3.96 (1 H, m, 4-H), 4.32 (1 H, d, $J_{H,H}$ 12.0 Hz, CHPh), 4.39-4.45 (3 H, m, 2-H, 2CHPh), 4.61 (1 H, d, $J_{H,H}$ 10.9 Hz, CHPh), 4.65-4.73 (3 H, m, 3CHPh), 4.77 (1 H, d, $J_{H,H}$ 11.4 Hz, CHPh), 7.19-7.27 (20 H, m, ArH). ^{13}C-NMR δC (100 MHz, $CDCl_3$) 28.4, (Boc), 37.7 (Me), 59.5 (5-C), 67.7 (6-C), 69.5 (2-C), 72.8 (CH_2Ph), 73.4 (CH_2Ph), 74.7 (CH_2Ph), 75.2 (CH_2Ph), 77.6 (3-C), 80.0 (4-C), 80.6 (Boc), 116.8 (1-C), 127.6-128.6 (Ar), 135.7 (Ar), 137.5 (Ar), 138.0 (Ar), 138.1 (Ar), 156.5 (Boc). HRMS (ESI); Calcd for $C_{40}H_{47}N_3O_6Na^+$ 688.3363; found 688.3368. **Data of compound 11:** R_f 0.31 (EtOAc/PE 1:3) 0.31. $[\alpha]_D^{26}$ +13 (*c* 0.15, EtOAc). ^{1}H-NMR δH (400 MHz, $CDCl_3$) 1.43 (9 H, s, Boc), 2.73 (3 H, s, Me), 3.10-3.12 (1 H, m, 5-H), 3.40 (1 H, dd, $J_{6b,6a}$ 9.4 Hz, $J_{6b,5}$ 4.8 Hz, 6-H_b), 3.55 (1 H, dd, $J_{6a,6b}$ 9.4 Hz, $J_{6a,5}$ 7.3 Hz, 6-H_a), 4.09 (1 H, dd, $J_{4,3}$ 6.0 Hz, $J_{4,5}$ 3.3 Hz, 4-H), 4.12-4.15 (1 H, m, 3-H), 4.40 (2 H, s, 2CHPh), 4.47 (1 H, d, $J_{2,3}$ 4.8 Hz, 2-H), 4.54-4.58 (2 H, m, 2CHPh), 4.68 (1 H, d, $J_{H,H}$ 11.1 Hz, CHPh), 4.77 (1 H, d, $J_{H,H}$ 11.4 Hz, CHPh), 4.82 (1 H, d, $J_{H,H}$ 11.1 Hz, CHPh), 4.92 (1 H, d, $J_{H,H}$ 11.8 Hz, CHPh), 7.28-7.35 (20 H, m, ArH). ^{13}C-NMR δC (100 MHz, $CDCl_3$) 28.3 (Boc), 36.6 (Me), 58.0 (5-C), 67.4 (6-C), 68.4 (2-C), 72.4 (CH_2Ph), 73.1 (CH_2Ph), 74.7 (CH_2Ph), 75.2 (CH_2Ph), 77.2 (4-C), 78.8 (3-C), 80.6 (Boc), 116.8 (1-C), 127.6-128.8 (Ar), 135.4 (Ar), 137.5 (Ar), 138.0 (Ar), 138.1 (Ar), 156.2 (Boc). $C_{40}H_{47}N_3NaO_6^+$ 688.3363; found 688.3615.

(3*S*,4*S*,5*R*,6*R*)-1-Amino-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-2-iminopiperidine hydrochloride (12). A solution of hydrazide **8** (27.0 mg, 41.5 µmol) in anhydrous CH_2Cl_2 (1.2 mL) under a nitrogen atmosphere at rt was added TFA (0.8 mL). After the addition, the reaction mixture was kept stirring for 2 h. The volatiles were removed under reduced pressure at rt and the resulting residue was dissolved in CH_2Cl_2 (10 mL) and evaporated to dryness at rt (this process was repeated three times). The residue was purified by silica column chromatography ($CHCl_3$/methanolic HCl (0.1 M) 39:1→ 19:1) to provide the title compound **12** (17.4 mg, 71%) as a colourless syrup. R_f 0.36 ($CHCl_3$/methanolic HCl (0.1 M) 9:1). $[\alpha]_D^{26}$ +15 (*c* 0.14, $CHCl_3$). ^{1}H-NMR δH (400 MHz, CD_3OD) 3.54 (1 H, dd, $J_{C6CHb,C6CHa}$ 10.5 Hz, $J_{C6CHb,6}$ 3.8 Hz, C6-H_bC), 3.84 (1 H, dd, $J_{C6CHa,C6CHb}$ 10.5 Hz, $J_{C6CHa,6}$ 3.4 Hz, C6-H_aC), 3.90-3.97 (3 H, m, 4-H, 5-H, 6-H), 4.37 (1 H, d, $J_{H,H}$ 11.8 Hz, CHPh), 4.47 (1 H, d, $J_{H,H}$ 11.8 Hz, CHPh), 4.54 (1 H, d, $J_{H,H}$ 11.6 Hz, CHPh), 4.56 (1 H, d, $J_{3,4}$ 7.8 Hz, 3-H), 4.61 (1 H, d, $J_{H,H}$ 11.6 Hz, CHPh), 4.69 (2 H, s, 2CHPh), 4.77 (1 H, d, $J_{H,H}$ 11.6Hz, CHPh), 4.81 (1 H, d, $J_{H,H}$ 11.6 Hz, CHPh), 7.25-7.35 (20 H, m, ArH). ^{13}C-NMR δC (100 MHz, CD_3OD) 67.5 (C6-CH_2), 69.2 (6-C), 73.8 (CH_2Ph), 74.2 (CH_2Ph), 74.7 (3-C), 74.9 (CH_2Ph), 75.9 (CH_2Ph), 77.4, 81.5 (4-C, 5-C), 129.1-129.7 (Ar), 137.8 (Ar), 138.7 (2Ar), 138.8 (Ar), 167.2 (2-C). HRMS (ESI); Calcd for $C_{34}H_{38}N_3O_4^+$ 552.2862; found 552.2866.

(3*S*,4*S*,5*R*,6*S*)-1-Amino-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-2-iminopiperidine hydrochloride (13). To a solution of hydrazide **9** (15 mg, 23.0 µmol) in anhydrous CH_2Cl_2 (0.72 mL) under a nitrogen atmosphere at rt was added TFA (0.58 mL). The resulting mixture was kept stirring at rt for 80 min. Toluene (5 mL) was added and the solvent was removed under reduced pressure at rt. The concentrate was purified by flash silica column chromatography (CH_2Cl_2/methanolic HCl (0.1 M) 39:1 → 19:1 → 15:1) to provide the title compound **13** (10 mg, 74%) as a colourless syrup. R_f 0.53 ($CHCl_3$/methanolic HCl (0.1 M) 9:1). $[\alpha]_D^{26}$ -54 (*c* 0.15, $CHCl_3$). ^{1}H-NMR δH (400 MHz, CD_3OD) 3.94 (1 H, dd, $J_{C6CHb,C6CHa}$ 10.5 Hz, $J_{C6CHb,6}$ 3.4 Hz, C6-H_bC), 4.02-4.09 (3 H, m, 5-H, 6-H, C6-H_aC), 4.40 (1 H, t, $J_{4,3} = J_{4,5}$ 7.9 Hz, 4-H), 4.54-4.61 (6 H, m, 3-H, 5CHPh), 4.65-4.72 (2 H, m, 2CHPH), 4.81 (1 H, d, $J_{H,H}$ 11.9 Hz, CHPh), 7.18-7.30 (20 H, m, ArH). ^{13}C-NMR δC (100 MHz, CD_3OD) 64.8 (C6-CH_2), 64.9 (6-C), 73.8 (CH_2Ph), 74.2 (CH_2Ph), 74.4 (CH_2Ph), 75.2 (CH_2Ph), 76.9 (3-C), 77.0 (5-C), 78.2 (4-C), 128.9-129.6 (Ar), 138.3 (Ar), 138.8 (2Ar), 139.2 (Ar), 167.1 (2-C). HRMS (ESI); Calcd for $C_{34}H_{38}N_3O_4^+$ 552.2862; found 552.2866.

(3*S*,4*S*,5*R*,6*R*)-3,4,5-Tris(benzyloxy)-6-((benzyloxy)methyl)-1-(methyl)amino-2-iminopiperidine hydrochloride (14). To a solution of hydrazide **10** (9.5 mg, 14.3 µmol) in anhydrous CH_2Cl_2 (0.41 mL) at rt under a nitrogen atmosphere was added TFA (0.27 mL). The reaction mixture was kept stirring at rt for 80 min. The reaction mixture was then diluted with toluene (5 mL) and the solvent was removed under reduced pressure at rt. The concentrate was dissolved in $CDCl_3$ (1 mL) and transferred to a NMR tube and the cyclization into **14** was followed by ^{1}H-NMR (a singlet appeared at 2.76 ppm upon disappearance of a singlet at 2.61 ppm) for 24 h. The content in the NMR tube was transferred to a round bottom flask by the aid of CH_2Cl_2 and the solvent was removed under reduced pressure. The residue was purified by flash silica column chromatography (CH_2Cl_2/methanolic HCl (0.1 M) 39:1 → 19:1 → 15:1) to provide the title compound **14** (5.0 mg, 58%) as a colourless syrup. R_f 0.40 ($CHCl_3$/methanolic HCl (0.1 M) 9:1). $[\alpha]_D^{26}$ +16 (*c* 0.12, MeOH). ^{1}H-NMR δH (400 MHz, CD_3OD) 2.54 (3 H, s, Me), 3.59 (1 H, dd, $J_{C6CHb,C6CHa}$ 10.5 Hz, $J_{C6CHb,6}$ 5.1 Hz, C6-H_bC), 3.73(1 H, dd, $J_{C6CHa,C6CHb}$ 10.5 Hz, $J_{C6CHa,6}$ 4.4 Hz, C6-H_aC), 3.87 (1 H, dd, $J_{4,3}$ 7.6 Hz, $J_{4,5}$ 4.0 Hz, 4-H), 3.99 (1 H, dd, $J_{5,4}$ 4.0 Hz, $J_{5,6}$ 2.9 Hz, 5-H), 4.14-4.17 (1 H, m, 6-

H), 4.40 (1 H, d, $J_{H,H}$ 11.8 Hz, CHPh), 4.46 (1 H, d, $J_{H,H}$ 11.8 Hz, CHPh), 4.56-4.63 (5 H, m, 3-H, 4CHPh), 4.77 (2 H, s, 2CHPh), 7.23-7.33 (20 H, m, ArH). ^{13}C-NMR δC (100 MHz, CD_3OD) 34.2 (Me), 63.2 (6-C), 68.4 (C6-CH_2), 73.2 (CH_2Ph), 74.2 (CH_2Ph), 74.3 (CH_2Ph), 74.9 (3-C), 75.8 (CH_2Ph), 76.8 (5-C), 82.7 (4-C), 129.1-129.7 (Ar), 137.8 (Ar), 138.6 (Ar), 138.7 (2Ar), 166.7 (2-C). HRMS (ESI); Calcd for $C_{35}H_{40}N_3O_4^+$ 566.3019; found 566.3020.

(3*S*,4*S*,5*R*,6*S*)-3,4,5-Tris(benzyloxy)-6-((benzyloxy)methyl)-1-(methyl)amino-2-iminopiperidine hydrochloride (15). Compound **15** was obtained in two parallel reactions. Hence, to two round bottom flasks containing solutions of compound **11** (9 mg, 13.5 µmol) in anhydrous CH_2Cl_2 (0.41 mL) and compound **11** (12 mg, 18.0 µmol) in anhydrous CH_2Cl_2 (0.55 mL), both under a nitrogen atmosphere at rt, were added 0.27 mL and 0.36 mL of TFA, respectively. The mixtures were kept stirring at rt for 30 min. After this time, the mixtures were diluted with toluene (5 mL) and the solvent was removed under reduced pressure. The residue in each round bottom flask was taken up in $CDCl_3$ (1 mL) and was transferred to two separate NMR tubes. The cyclization into hydrazide imide **15** was followed by ^{1}H-NMR (a singlet appeared at 2.51 ppm upon disappearance of a singlet 2.40 ppm) for 24 h. The content of both NMR tubes was combined into one common round bottom flask with the aid of CH_2Cl_2. The volatiles were removed *in vacuo* and the concentrate underwent flash silica column chromatography (CH_2Cl_2/methanolic HCl (0.1 M) 39:1 → 19:1) to provide the title compound (9.5 mg, 50%) as a colourless syrup. R_f 0.44 ($CHCl_3$/methanolic HCl (0.1 M) 9:1). $[\alpha]_D^{26}$ +29 (*c* 0.14, MeOH). ^{1}H-NMR δH (700 MHz, DMSO-d_6) 2.53 (3 H, d, $J_{H,H}$ 5.4 Hz, Me), 3.89-3.93 (2 H, m, C6-H_aC, C6-H_bC), 4.07 (1 H, dd, $J_{5,4}$ 9.1 Hz, $J_{5,6}$ 5.6 Hz, 5-H), 4.34 (1 H, dd, $J_{4,5}$ 9.1 Hz, $J_{4,3}$ 7.4 Hz, 4-H), 4.36-4.37 (1 H, m, 6-H), 4.52-4.55 (3 H, m, 3CHPh), 4.57 (1 H, d, $J_{H,H}$ 11.3 Hz, CHPh), 4.66-4.69 (3 H, m, 3-H, 2CHPh), 4.76 (1 H, d, $J_{H,H}$ 11.9 Hz, CHPh), 4.79 (1 H, d, $J_{H,H}$ 11.2 Hz, CHPh), 5.80-5.82 (1 H, m, NH), 7.20-7.36 (20 H, m, ArH), 9.24 (1 H, s, NH), 9.29 (1 H, s, NH). ^{13}C-NMR δC (125 MHz, DMSO-d_6) 33.7 (Me), 56.9 (6-C) 63.7 (C6-CH_2), 72.0 (2CH_2Ph), 72.5 (CH_2Ph), 73.4 (CH_2Ph), 75.3 75.5 (3-C, 5-C), 77.0 (4-C), 127.4-128.3 (Ar), 137.3 (Ar), 137.6 (Ar), 137.7 (Ar), 137.8 (Ar), 164.2 (2-C). HRMS (ESI); Calcd for $C_{35}H_{40}N_3O_4^+$ 566.3019; found 566.3021.

(3*S*,4*S*,5*R*,6*R*)-1-Amino-3,4,5-trihydroxy-6-(hydroxymethyl)-2-iminopiperidine acetate (1:2.7) (5). A degassed suspension of compound **12** (19.5 mg, 33.2 µmol) and Pd/C (39 mg, 10% Pd on charcoal) in EtOH/TFA (9:1, 5 mL) was hydrogenated at 1 atm and rt. The mixture was stirred overnight and was then filtered through a pad of Celite® by the aid of EtOH. The collected filtrate was concentrated under reduced pressure to afford a residue, which was purified by gravity silica column chromatography (MeCN/25% aqueous AcOH 7:1) to afford the title compound (9.4 mg, 80%) as a colourless syrup. R_f 0.22 (MeCN/25% aq AcOH 7:3). ^{1}H-NMR δH (400 MHz, D_2O) 1.83 (8 H, s, Ac), 3.49-3.51 (1 H, m, 6-H), 3.68 (1 H, t, $J_{4,5} = J_{4,3}$ 9.8 Hz, 4-H), 3.78 (1 H, dd, $J_{5,4}$ 9.8 Hz, $J_{5,6}$ 7.8 Hz, 5-H), 3.78 (1 H, dd, $J_{C6CHb,C6CHa}$ 13.0 Hz, $J_{C6CHb,6}$ 2.1 Hz, C6-H_bC), 4.08 (1 H, dd, $J_{C6CHa,C6CHb}$ 13.0 Hz, $J_{C6CHa,6}$ 2.7 Hz, C6-H_aC), 4.29 (1 H, d, $J_{3,4}$ 10.1 Hz, 3-H). ^{13}C-NMR δC (100 MHz, D_2O) 23.2 (Ac), 56.5 (C6-CH_2), 67.0, 67.2 (3-C, 5-C), 68.9 (6-C), 71.1 (4-C), 167.7 (2-C), 181.5 (Ac). HRMS (ESI); Calcd for $C_6H_{14}N_3O_4^+$ 192.0984; found 192.0989.

(3*S*,4*S*,5*R*,6*S*)-1-Amino-3,4,5-trihydroxy-6-(hydroxymethyl)-2-iminopiperidine acetate (1:3) (16). The same experimental procedure was followed as for compound **5**. Thus, 9.5 mg (16.2 µmol) of compound **13** provided the title compound (5.4 mg, 90%) as a colourless syrup. R_f 0.19 (MeCN/25% aq AcOH 7:3). ^{1}H-NMR δH (400 MHz, D_2O) 1.84 (9 H, s, Ac), 3.85-3.87 (1 H, m, 6-H), 3.96-3.97 (2 H, m, C6-H_bC, C6-H_aC), 4.01 (1 H, dd $J_{5,4}$ 10.0 Hz, $J_{5,6}$ 5.6 Hz, 5-H), 4.06 (1 H, dd $J_{4,5}$ 10.0 Hz, $J_{4,3}$ 8.1 Hz, 4-H), 4.33 (1 H, d, $J_{3,4}$ 8.1 Hz, 3-H). ^{13}C-NMR δC (100 MHz, D_2O) 23.2 (Ac), 55.1 (C6-CH_2), 66.7 (6-C), 67.1 (5-C), 69.1 (3-C), 70.8 (4-C), 167.0 (2-C), 181.5 (Ac). HRMS (ESI); Calcd for $C_6H_{14}N_3O_4^+$ 192.0984; found 192.0976.

(3*S*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(hydroxymethyl)-1-(methylamino)-2-iminopiperidine acetate (1:2.7) (6). The same experimental procedure was followed as for compound **5**. Thus, 26.3 mg (43.7 µmol) of compound **13** provided the title compound (13.6 mg, 85%) as a colourless syrup. R_f 0.33 (MeCN/25% aq AcOH 7:3). ^{1}H-NMR δH (400 MHz, D_2O) 1.84 (8 H, s, Ac), 2.51 (3 H, s, Me), 3.64 (1 H, t, $J_{4,5} = J_{4,3}$ 9.4 Hz, 4-H), 3.77-3.82 (3 H, m, 5-H, 6-H, C6-H_bC), 3.93 (1 H, br d, $J_{C6CHa,C6CHb}$ 12.0 Hz, C6-H_aC), 4.30 (1 H, d, $J_{3,4}$ 10.1 Hz, 3-H). ^{13}C-NMR δC (100 MHz, D_2O) 23.2 (Ac), 32.8 (Me), 59.9 (C6-CH_2), 62.8 (6-C), 67.2, 67.6 (3-C, 5-C), 71.4 (4-C), 167.2 (2-C), 181.4 (Ac). HRMS (ESI); Calcd for $C_7H_{16}N_3O_4^+$ 206.1141; found 206.1135.

(3*S*,4*S*,5*R*,6*S*)-3,4,5-Trihydroxy-6-(hydroxymethyl)-1-(methylamino)-2-iminopiperidine acetate (1:8) (17). The same experimental procedure was followed as for compound **5**. Thus, 9.5 mg (15.8 µmol) of compound **15** provided the title compound (3.4 mg, 31%) as a colourless syrup. R_f 0.36 (MeCN/25% aq AcOH 7:3). ^{1}H-NMR δH (400 MHz, D_2O) 1.84 (s, 24 H, Ac), 2.53 (3 H, s, Me), 3.85-4.00 (3 H, m, 5-H, C6-H_bC, C6-H_aC), 4.03-4.09 (2 H, m, 4-H, 6-H), 4.34 (1 H, d, $J_{3,4}$ 8.3 Hz, 3-H). ^{13}C-NMR δC (100 MHz, D_2O) 23.2 (Ac), 33.0 (Me), 55.4 (C6-CH_2), 59.9 (6-C), 67.3 (5-C), 69.2 (3-C), 70.7 (4-C), 167.0 (2-C), 181.5 (Ac). HRMS (ESI); Calcd for $C_7H_{16}N_3O_4^+$ 206.1141; found 206.1143.

Glycosidase inhibition assays. The glycosidase inhibition assays were carried out using the methodology reported by Bols and co-workers.[3] The percentage of inhibition was measured by preparing two 1.2-mL samples in PS cuvettes containing 0.1 M phosphate buffer (pH 6.8 or 5.6 for mannosidases) and the corresponding nitrophenyl glycopyranoside at a concentration equal to the expected value of K_M. Water or inhibitor solution (500 or 250 µM final concentration) were added up to a constant volume of 1.14 mL (control or inhibitor solution, respectively). Reaction was started by adding 60 µL of properly diluted enzyme solution at 25 °C or 35 °C (for mannosidases), and the formation of the *o*- or *p*-nitrophenolate was monitored for 125 s (300 s for mannosidases) by measuring the increase of absorbance at 400 nm or 420 nm (β-galactosidases). For mannosidases, an aliquot (200 µL) was taken every a certain time and added to a 1M Na_2CO_3 solution (1.8 mL), and the absorbance was measured at 400 nm.

Initial rates were calculated from the slopes of the plots (Abs *vs.* t) and were used for calculating the percentage of inhibition:

$$\%\ \text{Inhibition} = \frac{v_o - v}{v_o} \times 100$$

v_o and v refer to reaction rates for the enzyme and enzyme plus inhibitor solutions.

For the calculation of K_i values, the same procedure was used, but using 5 different substrate concentrations, ranging from 0.25 to 4.0 of the expected K_M value, keeping constant the inhibitor concentration. The enzyme was calibrated in such a way that for the cuvette containing the highest substrate concentration, whe initial rate was kept within the range 0.12-0.15 Abs/min. For estimating the mode of inhibition, the Lineweaver-Burk, or double reciprocal plot, was used (1/V *vs.* 1/[S]), with 2-3 different inhibitor concentrations.

In case the Linweaver-Burk plot did not provide undoubtedly the mode of inhibition, the double plot suggested by Cornish-Bowden[4] was used.

The following equations are used for calculating the inhibition constants (K_i's):

- Competitive inhibition (inhibitor only binds the free enzyme):

$$K_{ia} = \frac{[I]}{\frac{K_{M\,app}}{K_M} - 1}$$

- Mixed inhibition (inbitor binds both, the free enzyme and the ES complex):

$$K_{M_{app}} = K_M \frac{1 + \frac{[I]}{K_{ia}}}{1 + \frac{[I]}{K_{ib}}} \qquad V_{max\ app} = \frac{V_{max}}{1 + \frac{[I]}{K_{ib}}}$$

- Uncompetitive inhibition (inhibitor only binds the ES complex):

$$K_{M\ app} = \frac{K_M}{1 + \frac{[I]}{K_{ib}}} \qquad V_{max\ app} = \frac{V_{max}}{1 + \frac{[I]}{K_{ib}}}$$

, where K_M and V_{max} represent the Michaelis constant and maximum rate, respectively, of the enzyme, and $K_{M\ app}$ and $V_{max\ app}$ represent the same kinetic parameters in the presence of an inhibitor.

IC_{50} values were calculated by plotting %Inhibition (for [S] = K_M) *vs.* log[I], and adjusted to a second-order polynomial equation.

References

[1] B. Dasari, S. Jogula, R. Borhade, S. Balasubramanian, G. Chandrasekar, S. S. Kitambi and Prabhat Arya, *Org. Lett*., 2013, **15**, 432.
[2] P. Ermert and A. Vasella, *Helv. Chim. Acta*., 1991, **74**, 2043.
[3] M. Bols, R. G. Hazell and I. B. Thomsen, *Chem. Eur. J.,* 1997, **3**, 940.
[4] A. Cornish-Bowden, *Biochem. J.*, 1974, **137**, 143.

Table S1. Percentage of inhibition of hydrazide imides **5**, **6**, **16**, **17** against a panel of glycosidases (pH 6.8, [I]= 250 µM)[a]

Enzyme	5	16	6	17
α-Glucosidase (*Saccharomyces cerevisiae*)	**94**	22	**88**	20
β-Glucosidase (almonds)	**91** **99**[a]	15	26	12
α-Mannosidase (Jack beans)	65	N.I.[b]	20	N.I.[b]
β-Mannosidase (*Helix pomatia*)	66	21	25	15
α-Galactosidase (green coffee beans)	4	9	22	26
β-Galactosidase (*Asp. oryzae*)	14	18	15	13
β-Galactosidase (*E.coli*)	N.I.[b]	12	10	2

[a]pH 8.0; [b]No inhibition at the maximum inhibitor concentration tested (250 µM)

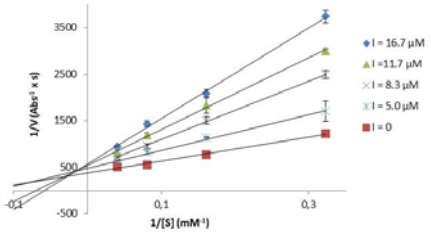


Figure S1. Lineweaver-Burk plot for the inhibition of β-glucosidase by 5 (pH 8.0)

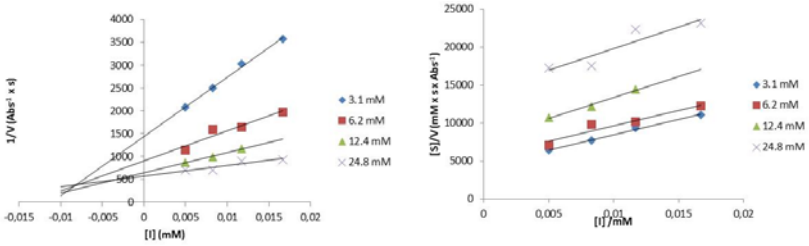


Figure S2. Cornish-Bowden plots for the inhibition of β-glucosidase by 5 (pH 8.0)

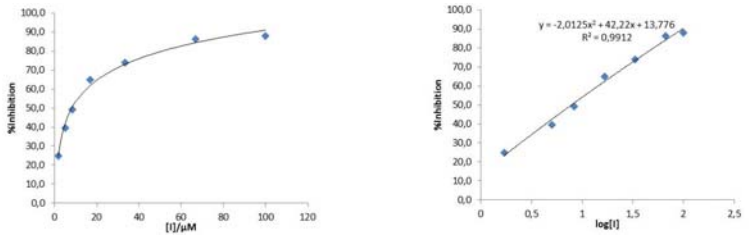
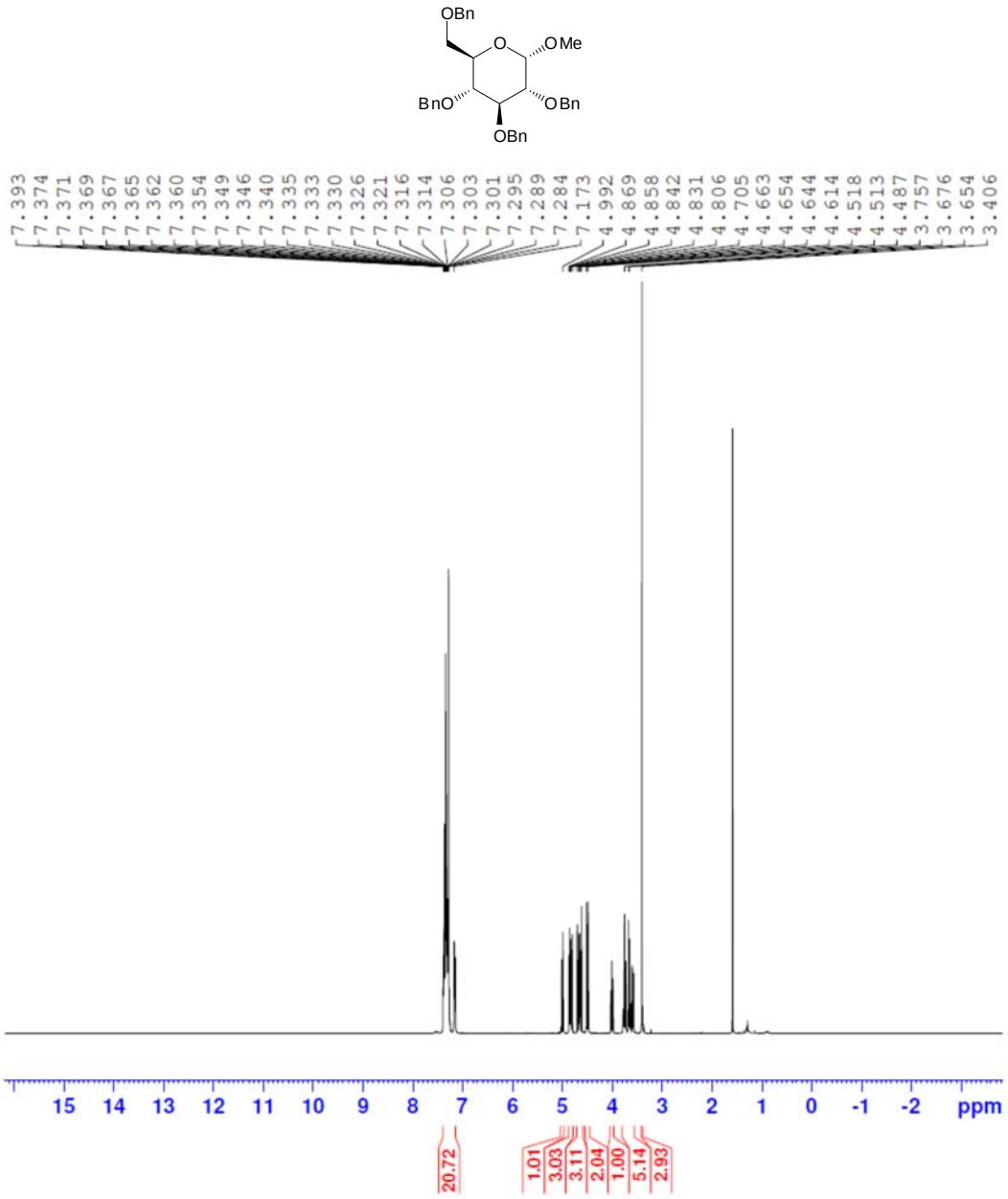
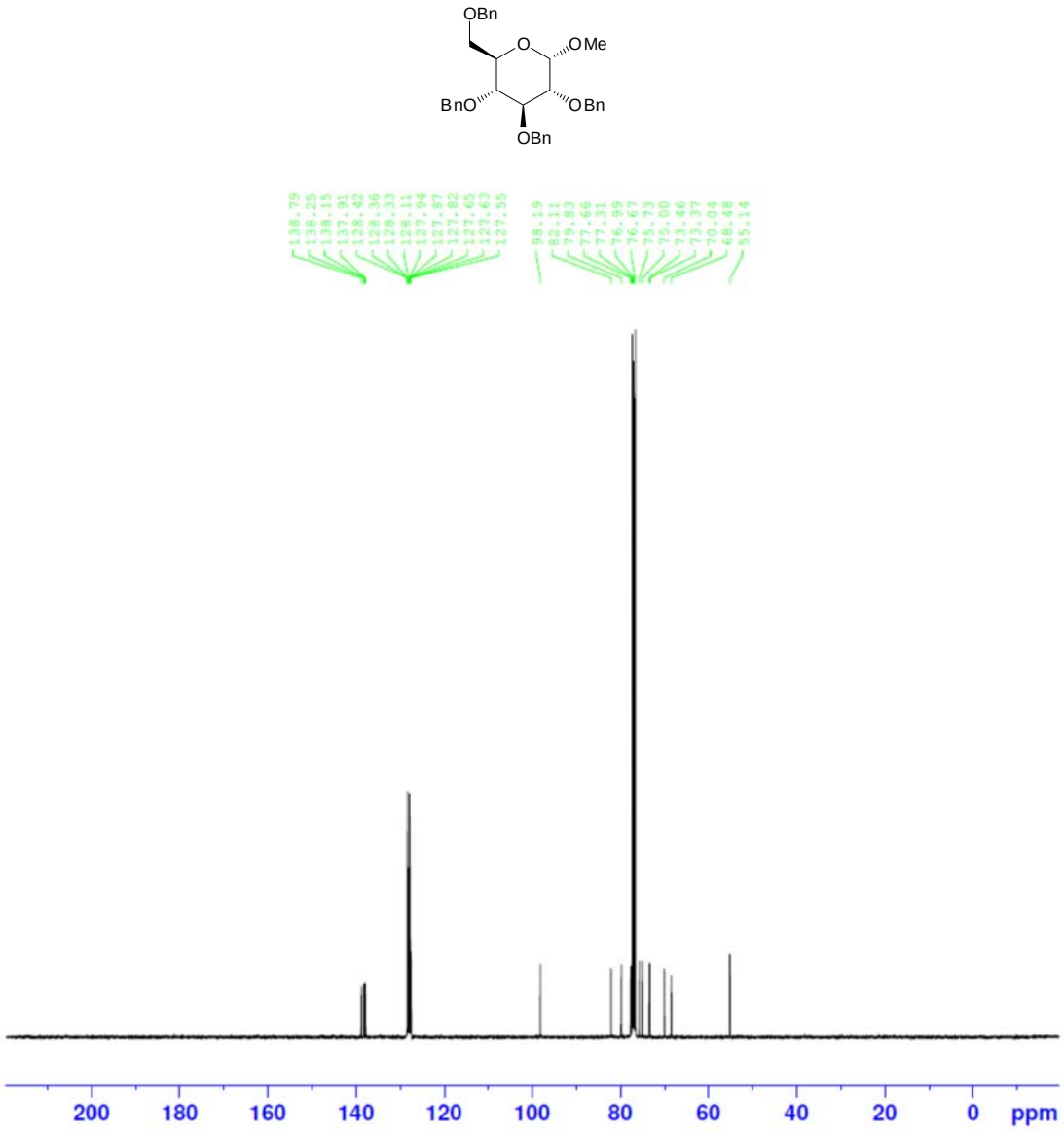


Figure S3. Calculation of IC_{50} for the inhibition of β-glucosidase by 5 (pH 8.0)

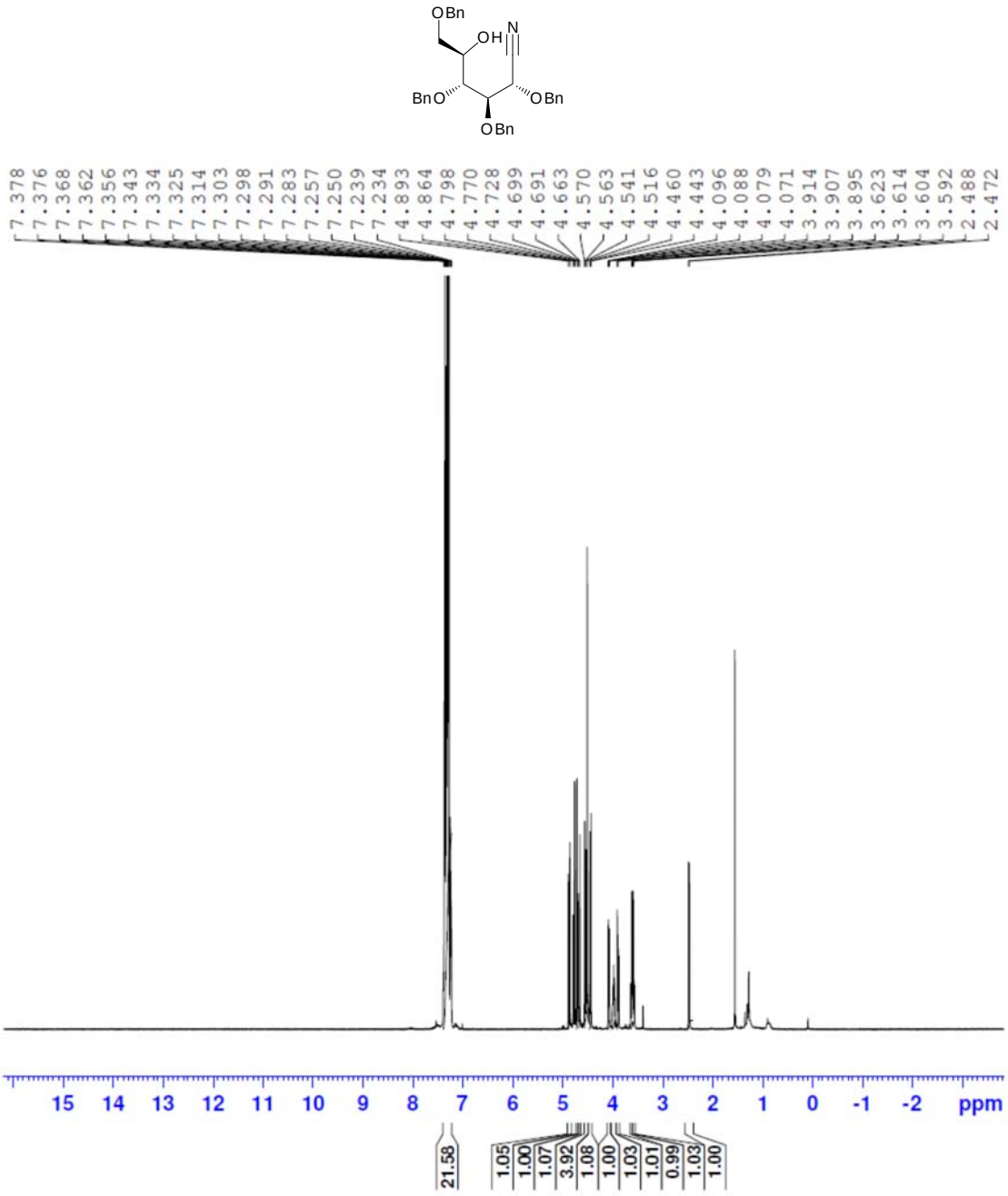
^{1}H-NMR of compound **ESI-2** ($CDCl_3$, 400 MHz)



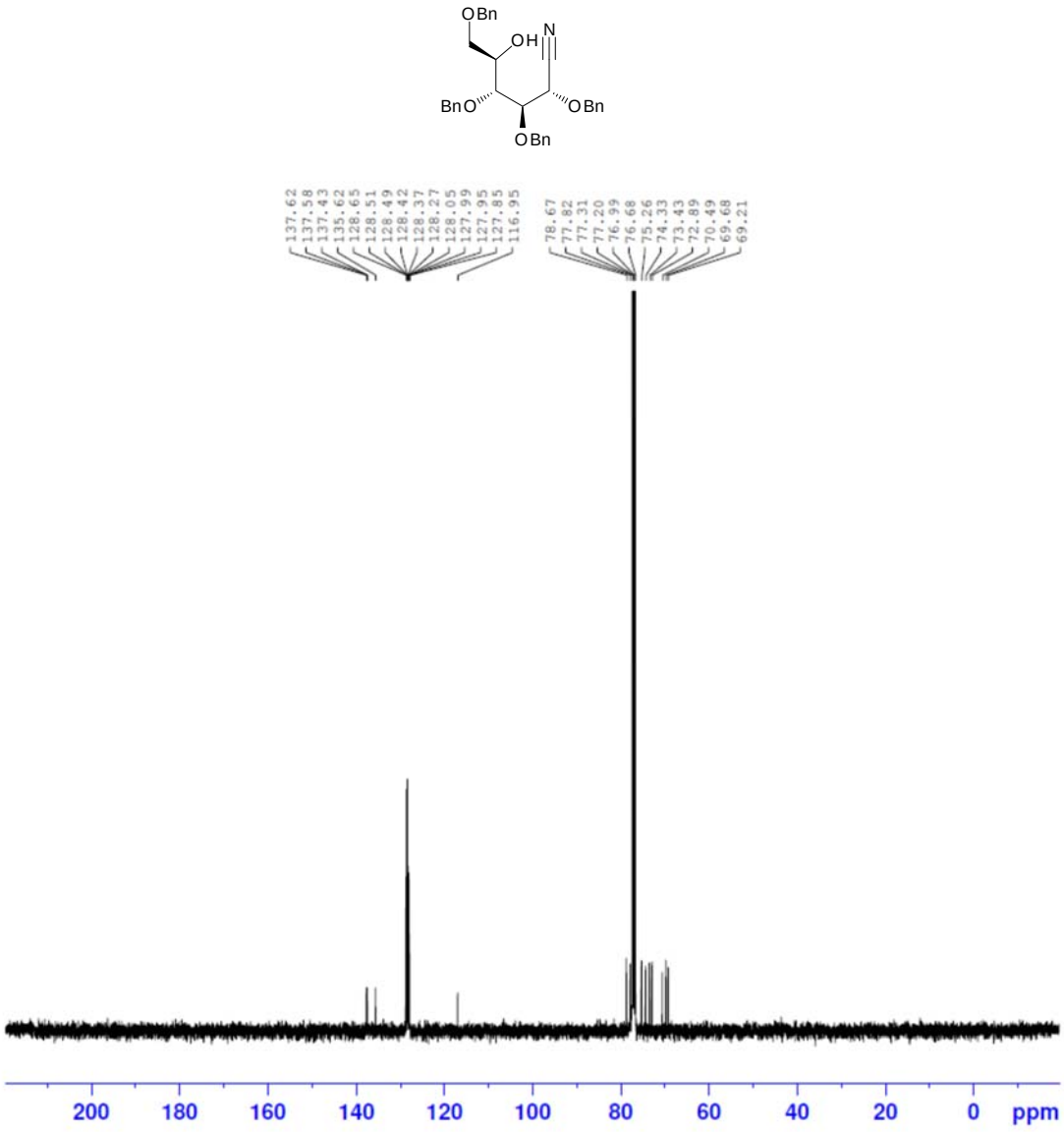
^{13}C-NMR of compound **ESI-2** ($CDCl_3$, 100 MHz)



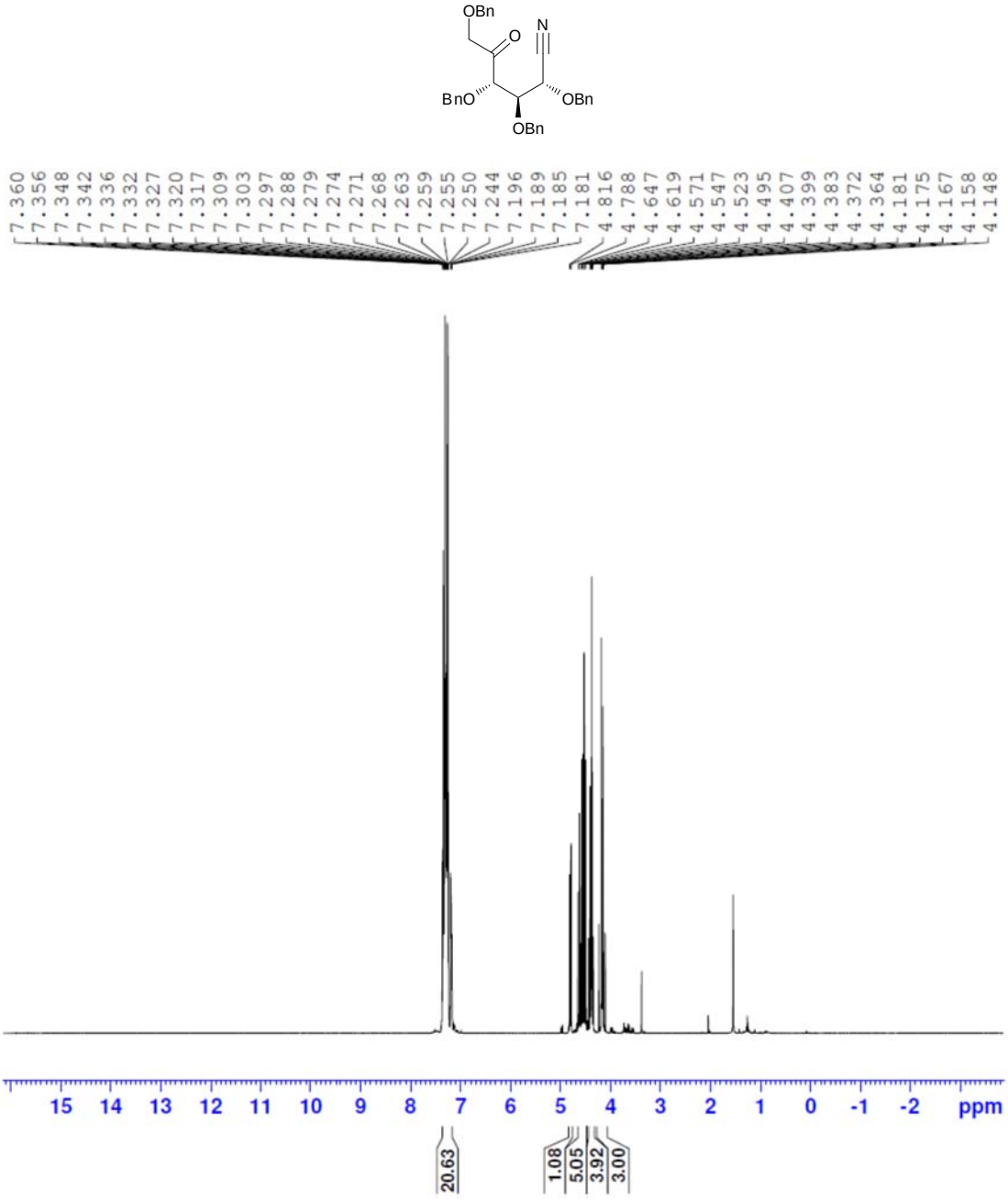
^{1}H-NMR of compound **ESI-4** ($CDCl_3$, 400 MHz)



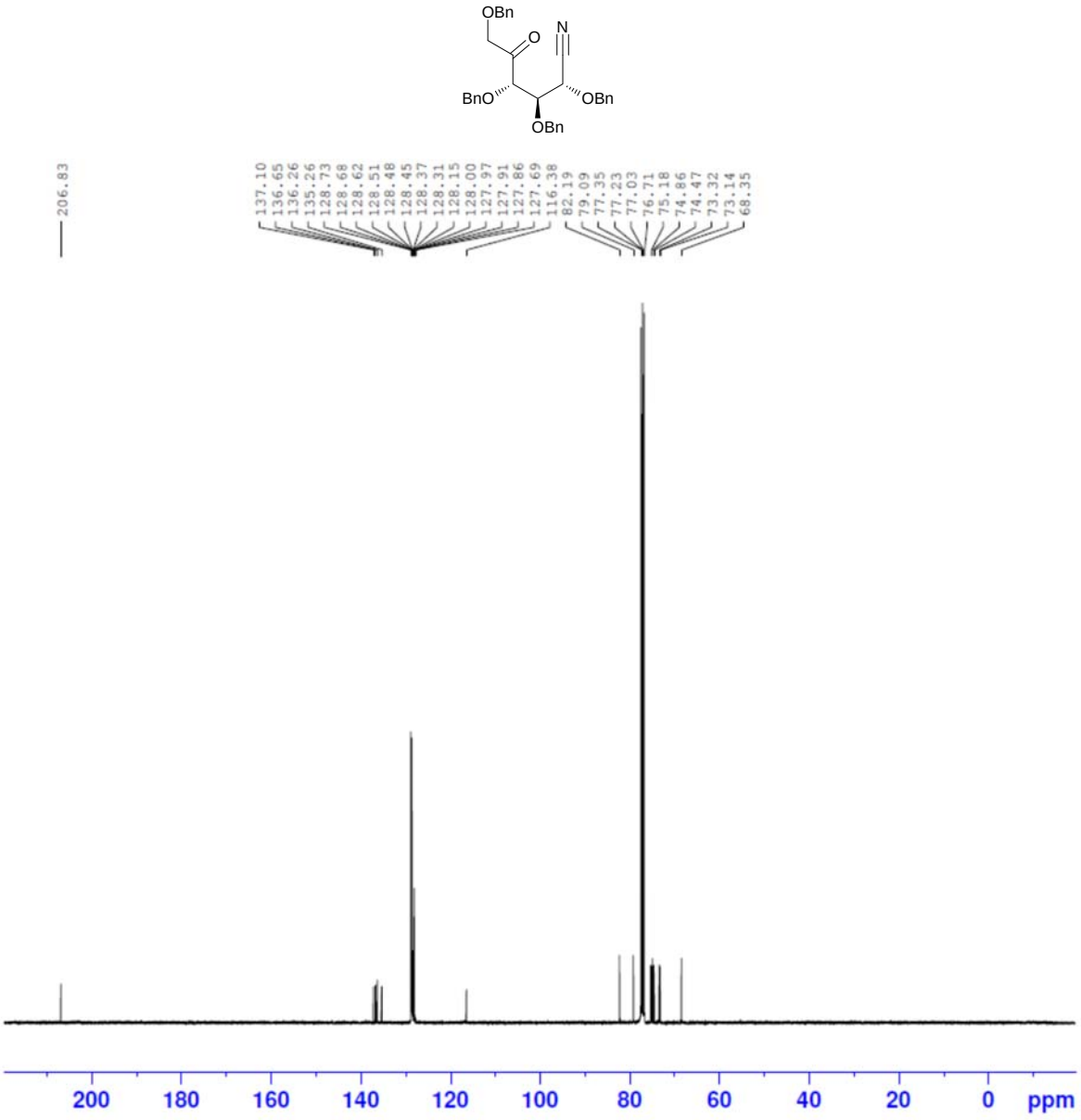
^{13}C-NMR of compound **ESI-4** ($CDCl_3$, 100 MHz)



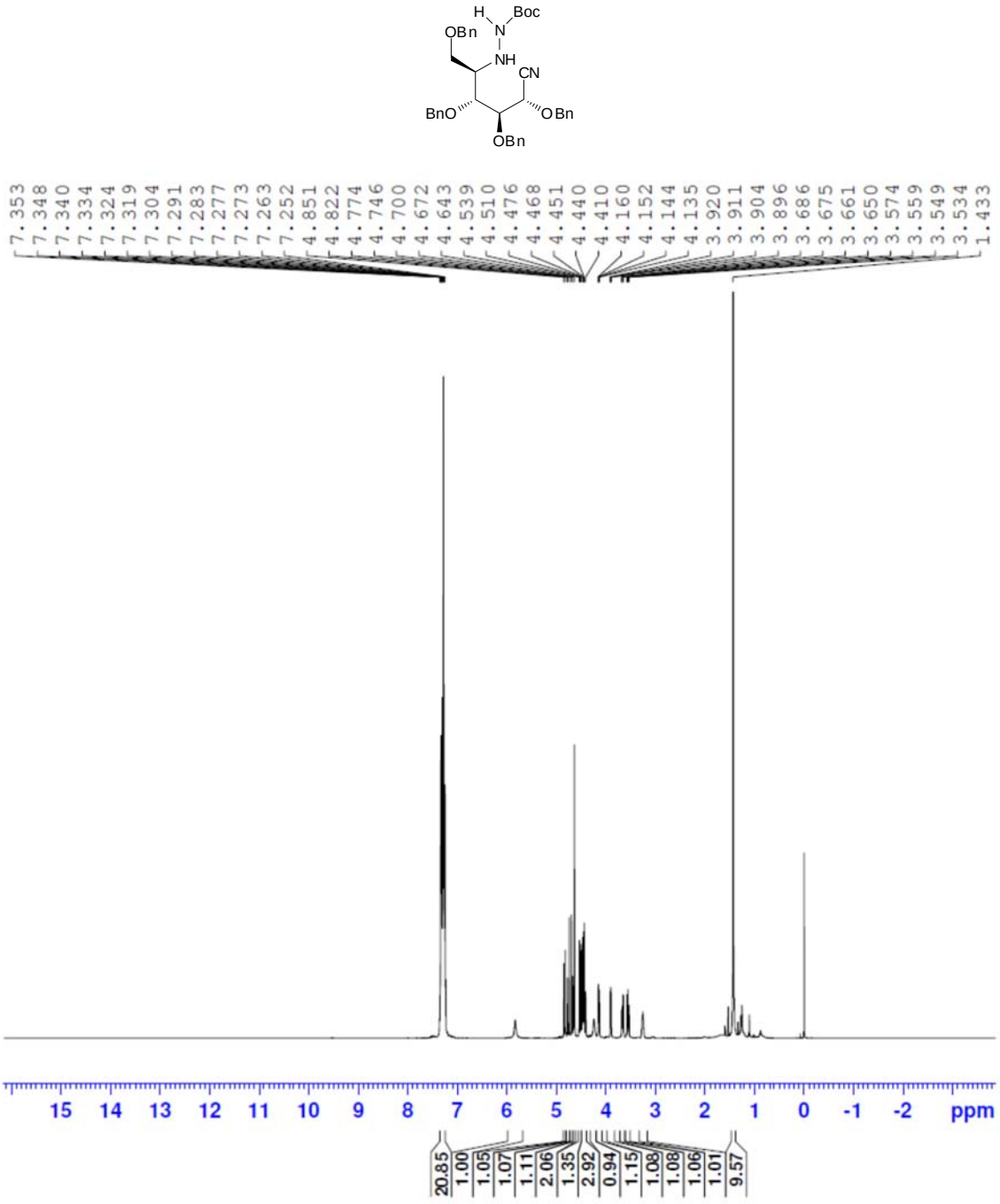
^{1}H-NMR of compound **7** ($CDCl_3$, 400 MHz)



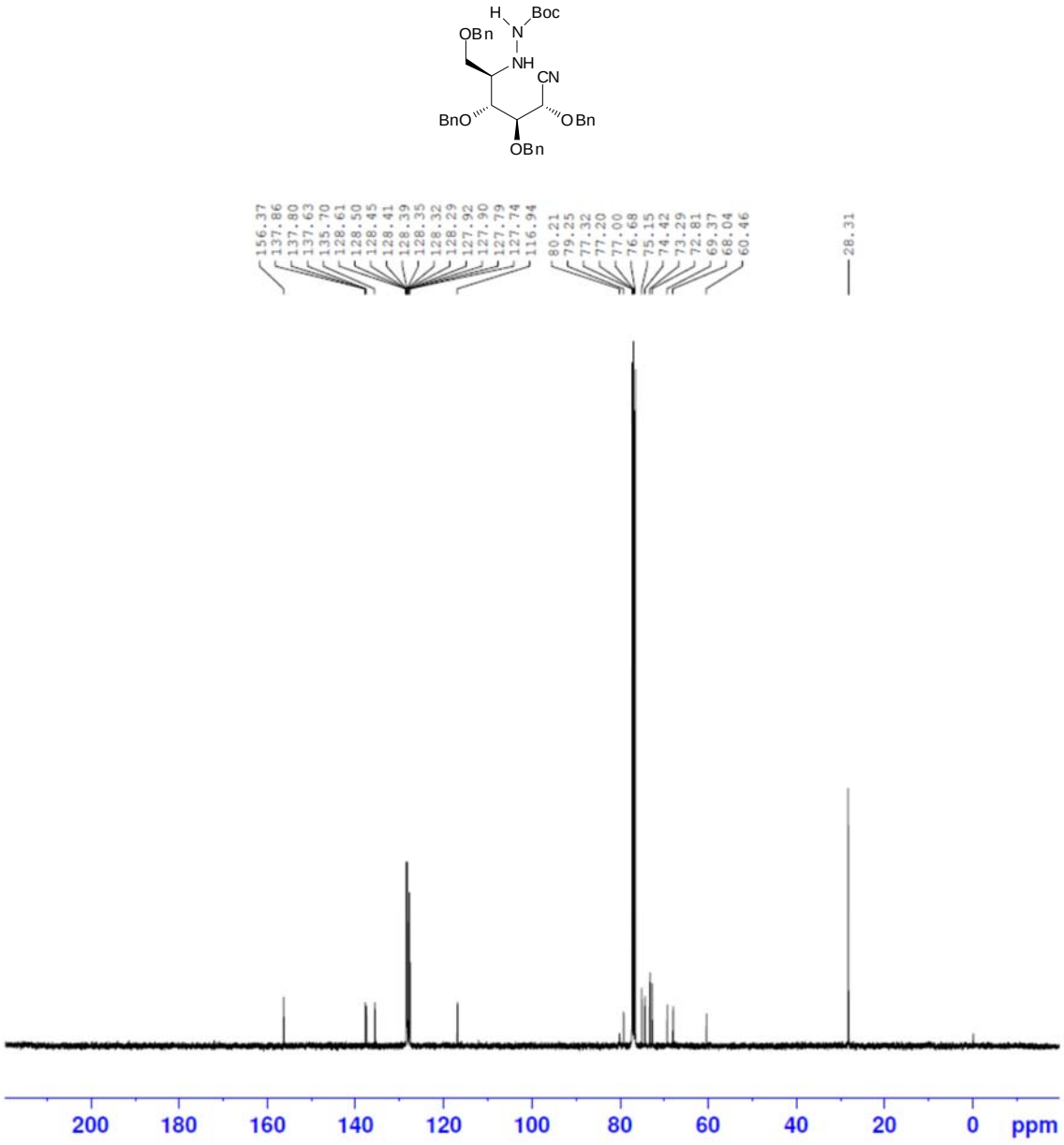
^{13}C-NMR of compound **7** ($CDCl_3$, 100 MHz)



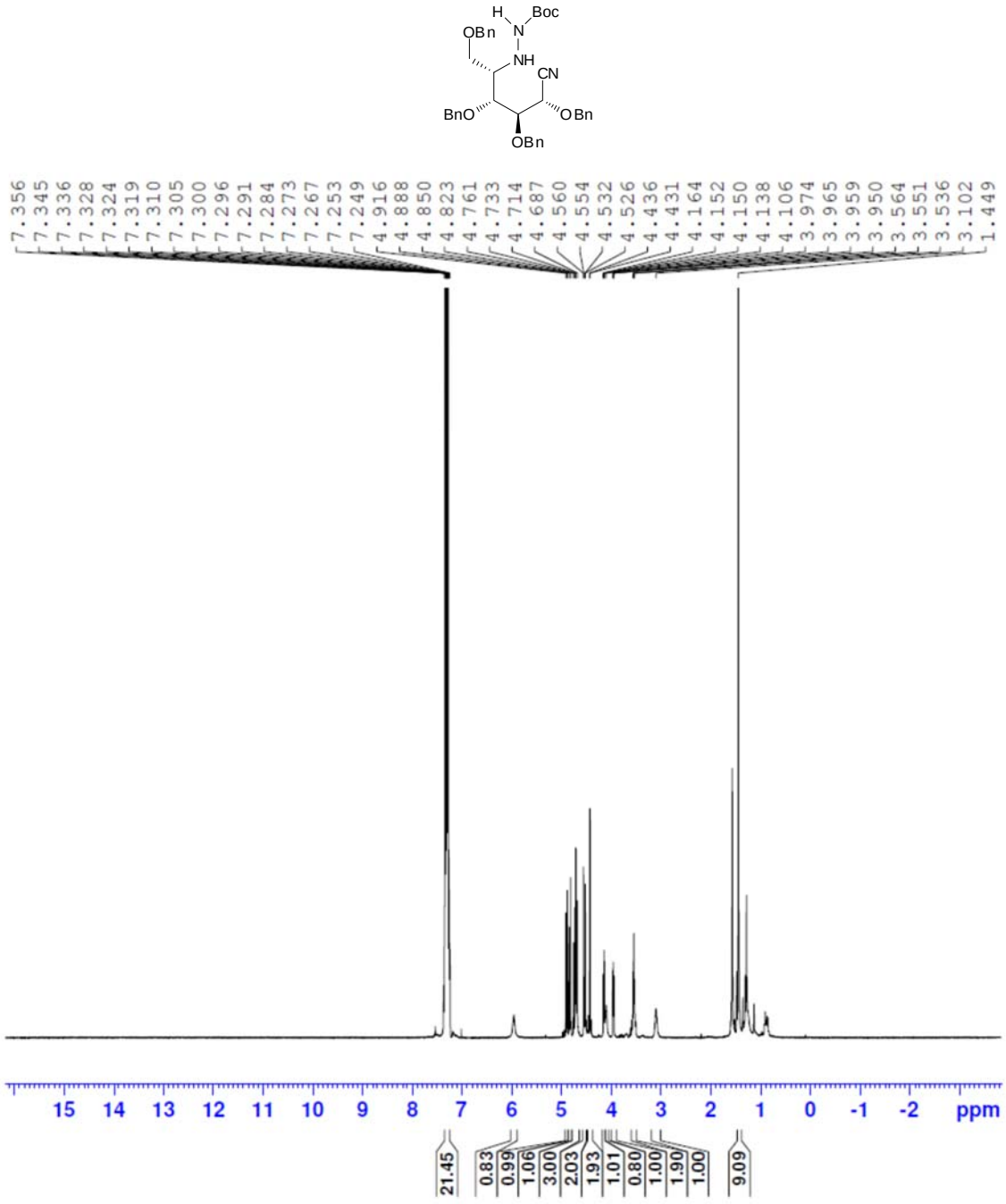
^{1}H-NMR of compound **8** ($CDCl_3$, 400 MHz)



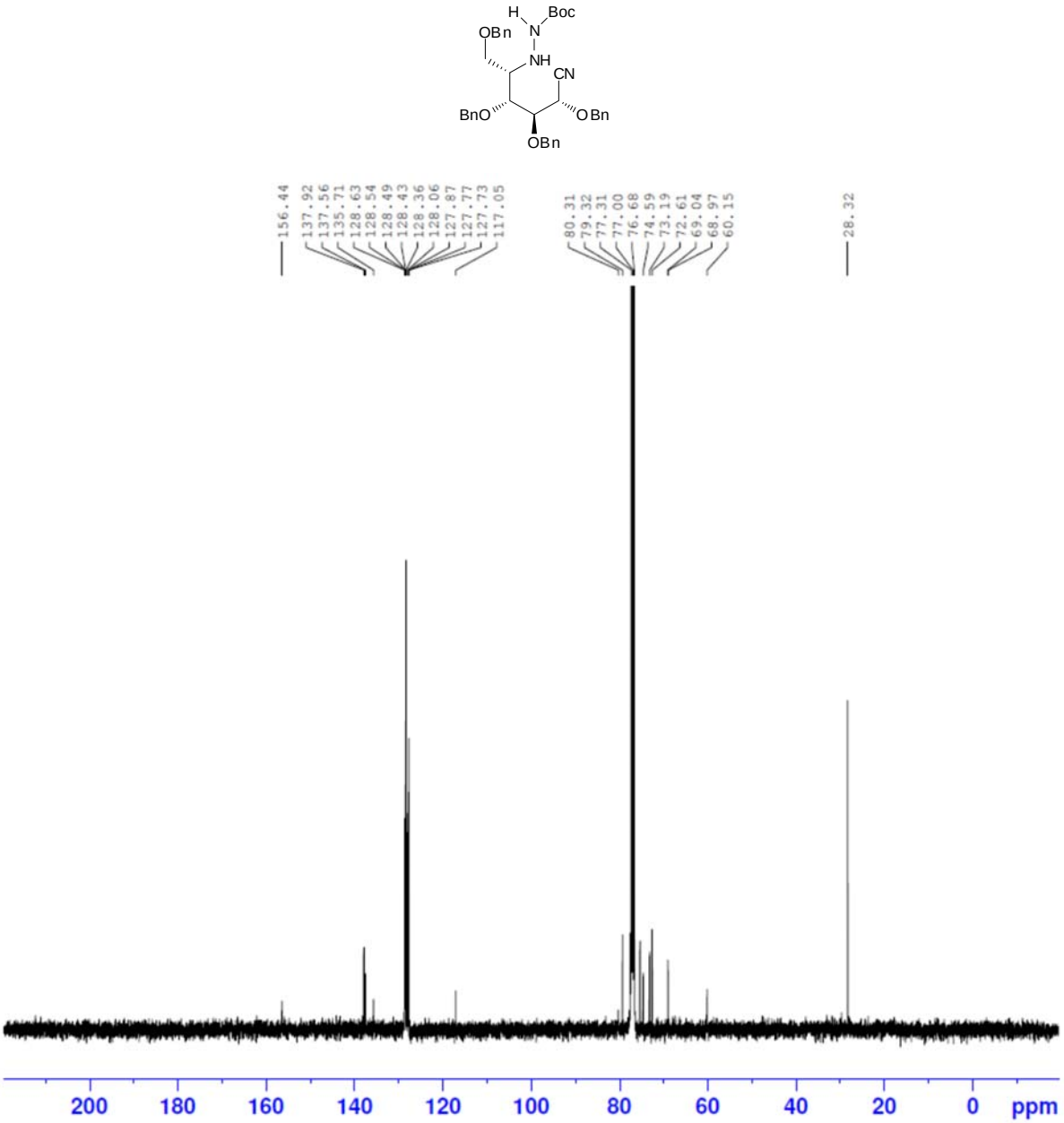
^{13}C-NMR of compound **8** ($CDCl_3$, 100 MHz)



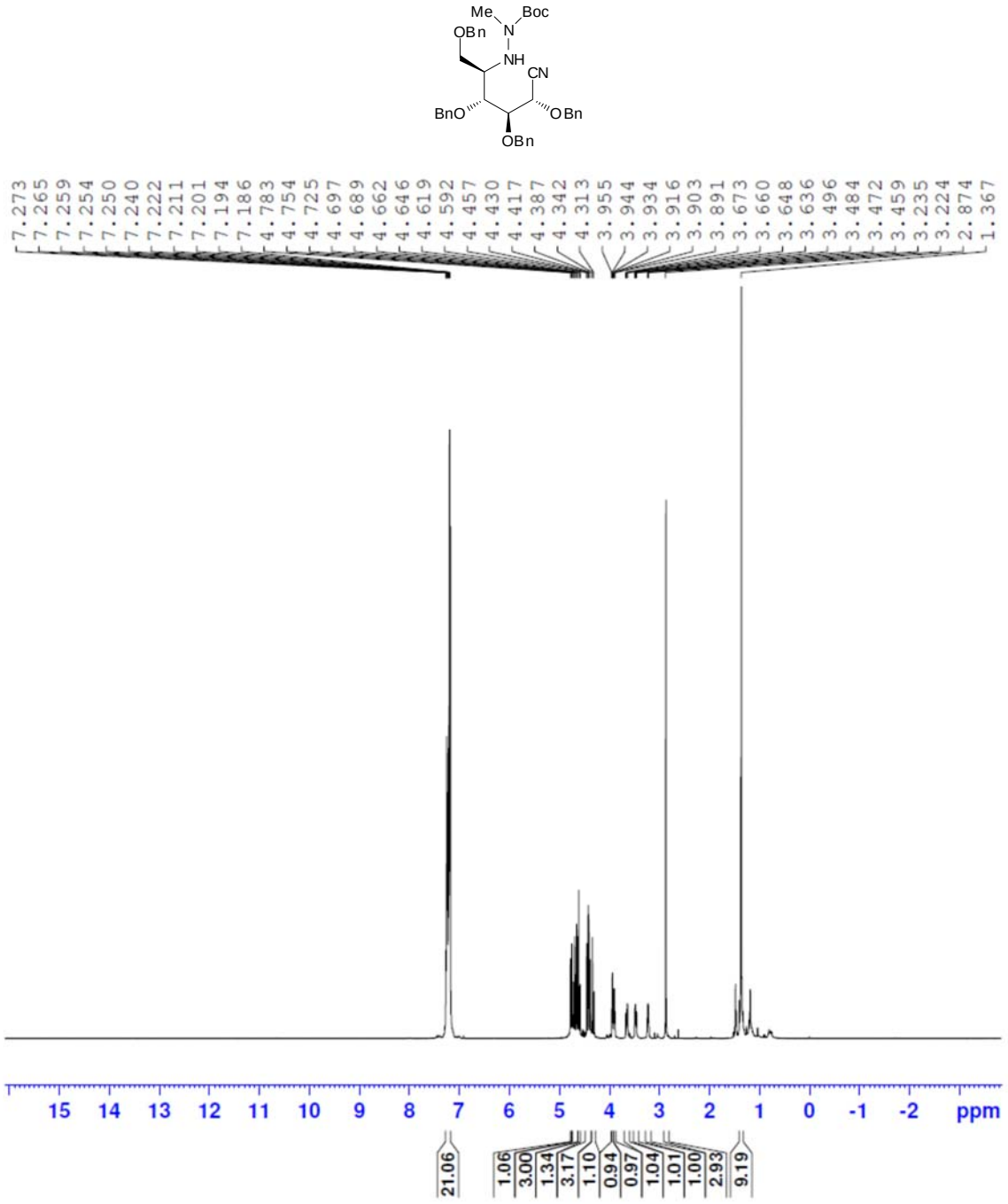
^{1}H-NMR of compound **9** ($CDCl_3$, 400 MHz)



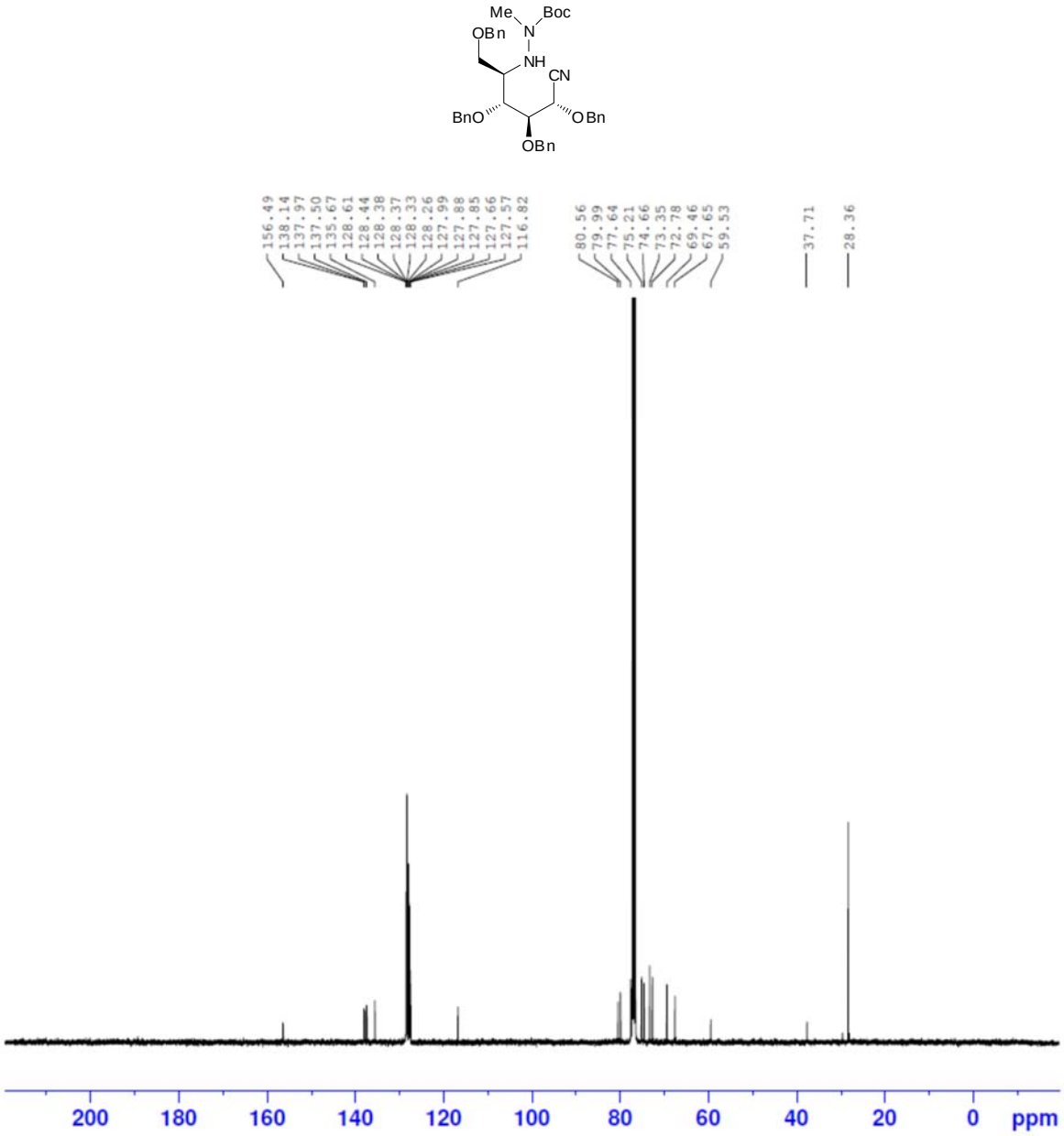
^{13}C-NMR of compound **9** ($CDCl_3$, 100 MHz)



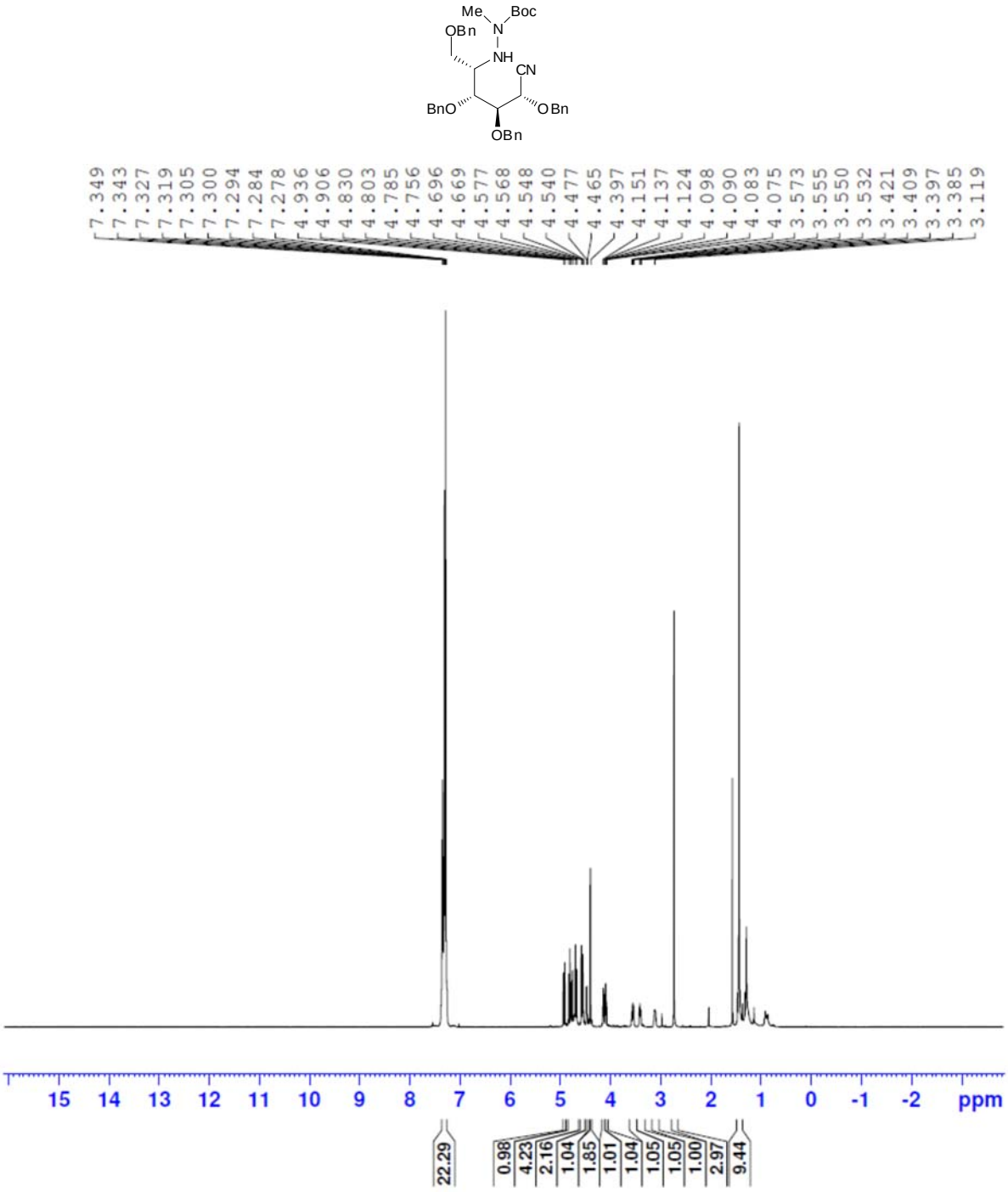
^{1}H-NMR of compound **10** ($CDCl_3$, 400 MHz)



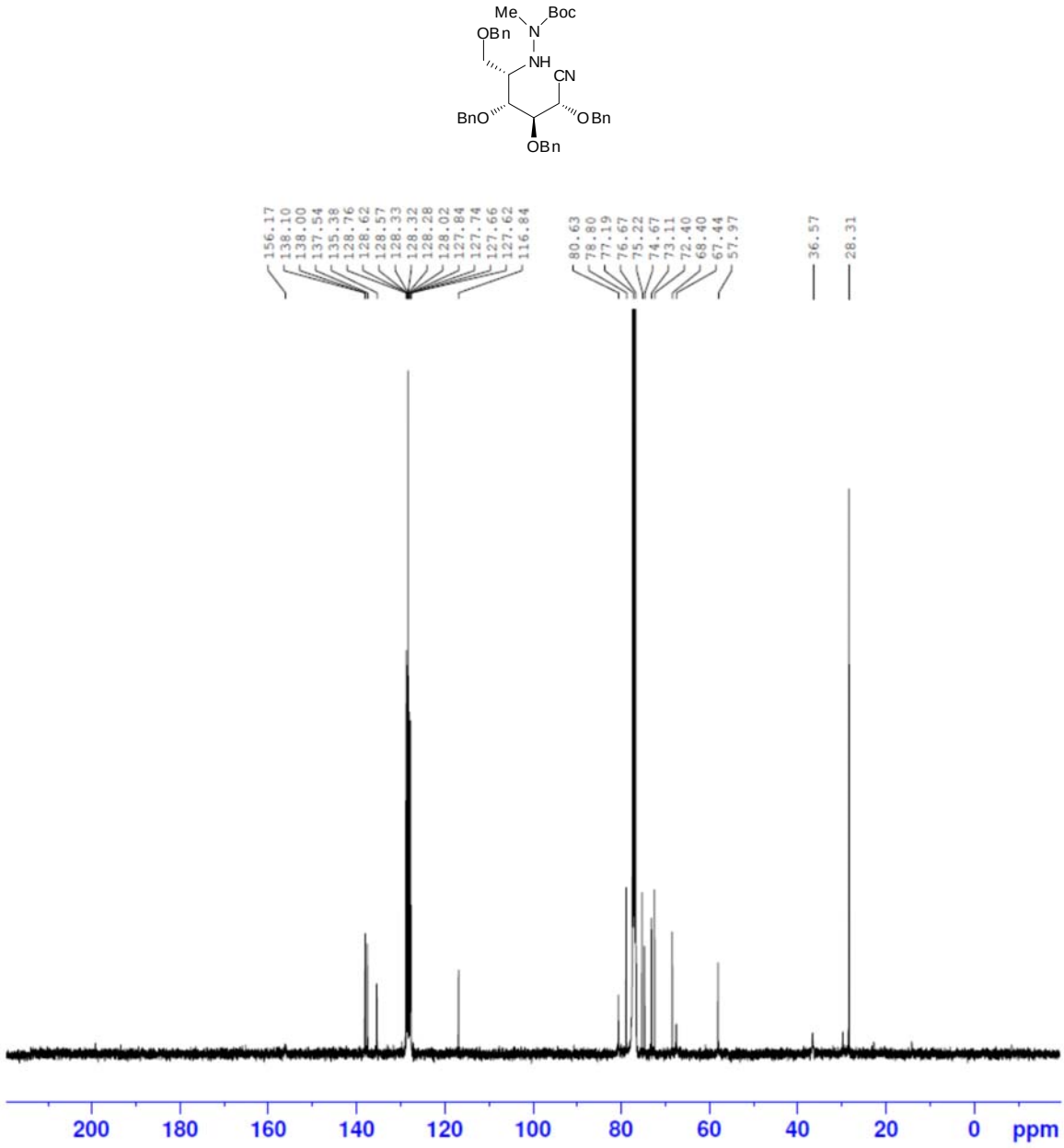
^{13}C-NMR of compound **10** ($CDCl_3$, 100 MHz)



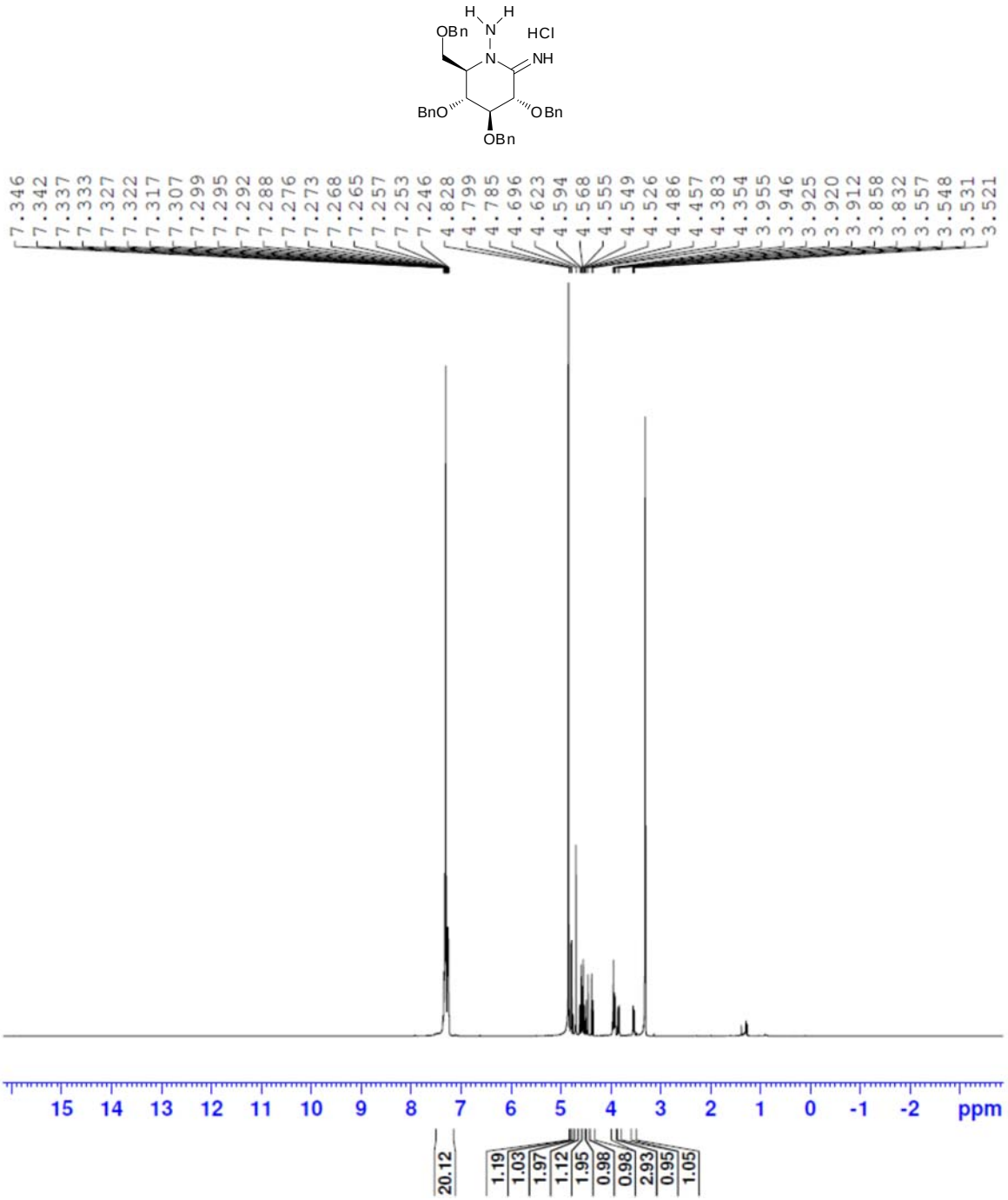
^{1}H-NMR of compound **11** ($CDCl_3$, 400 MHz)



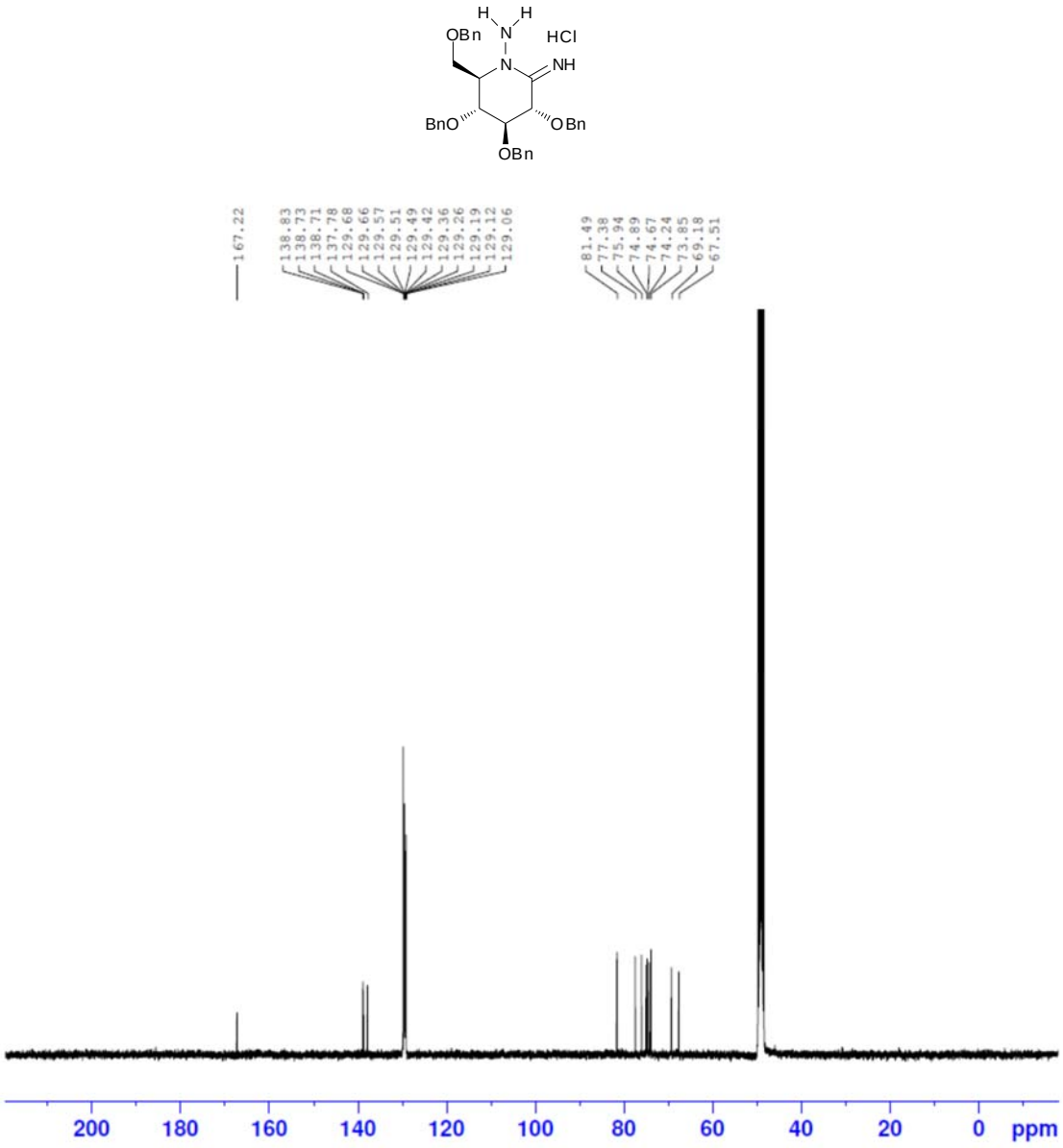
^{13}C-NMR of compound **11** ($CDCl_3$, 100 MHz)



^{1}H-NMR of compound **12** (CD_3OD, 400 MHz)



^{13}C-NMR of compound **12** (CD_3OD, 100 MHz)



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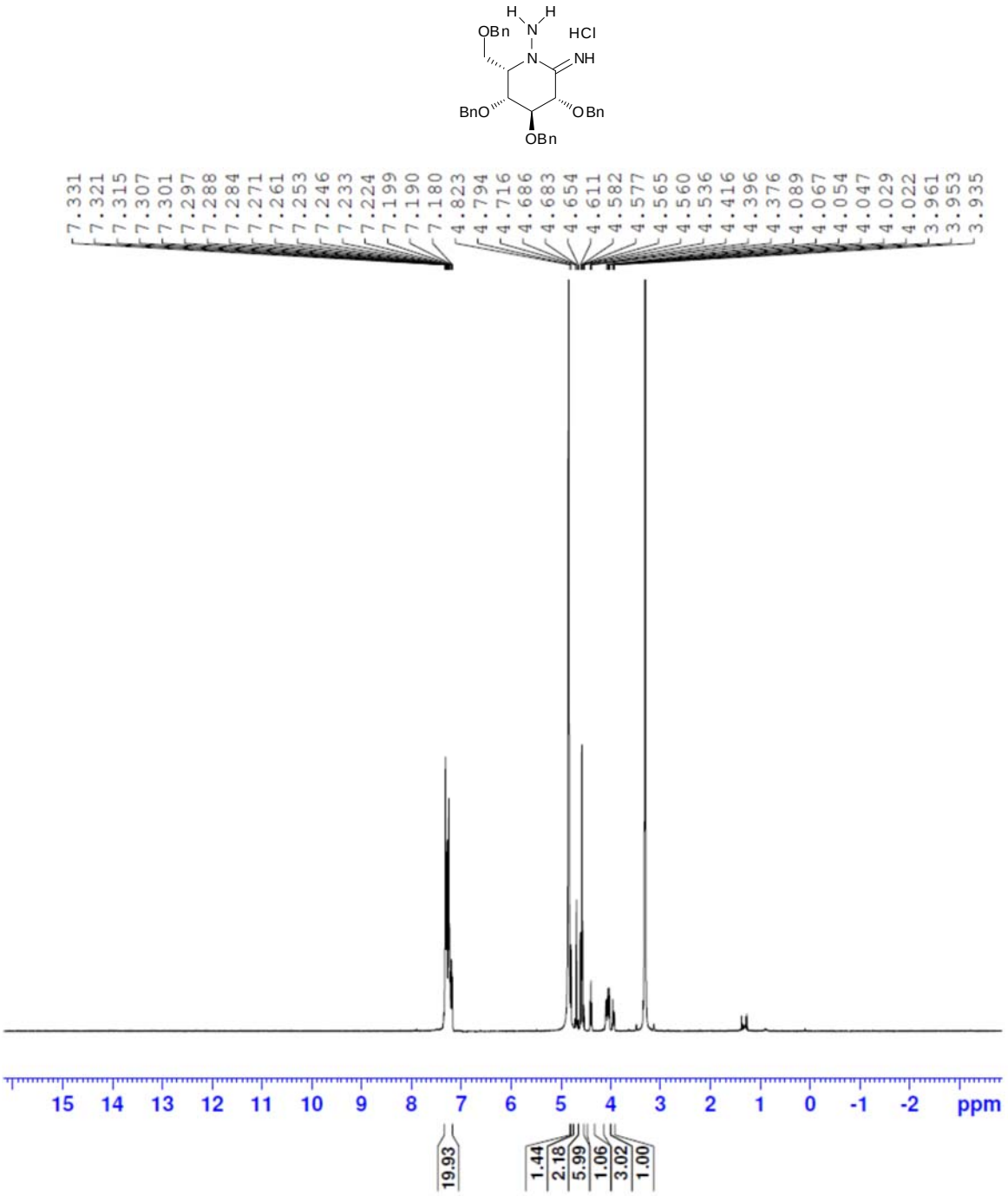

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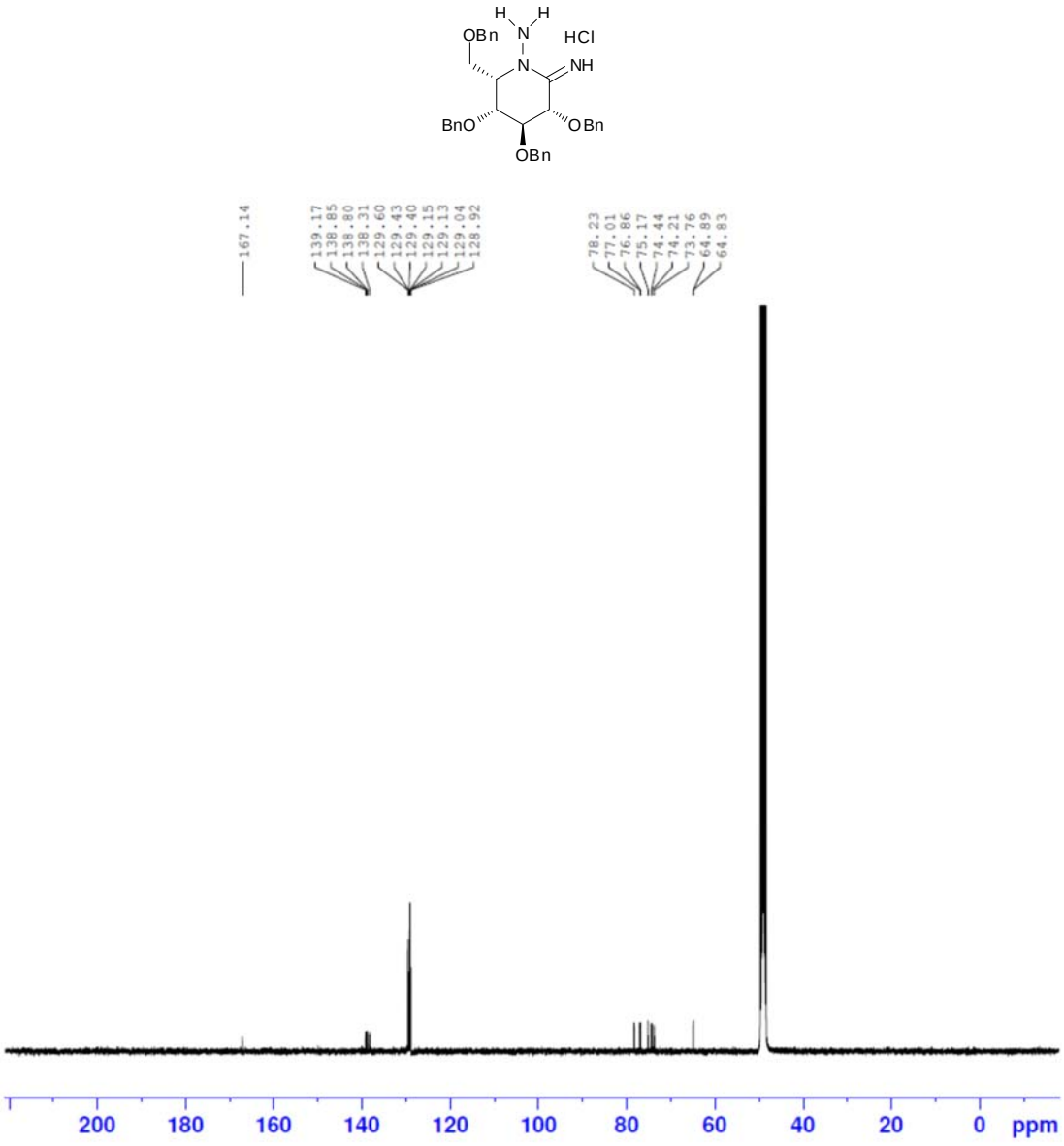
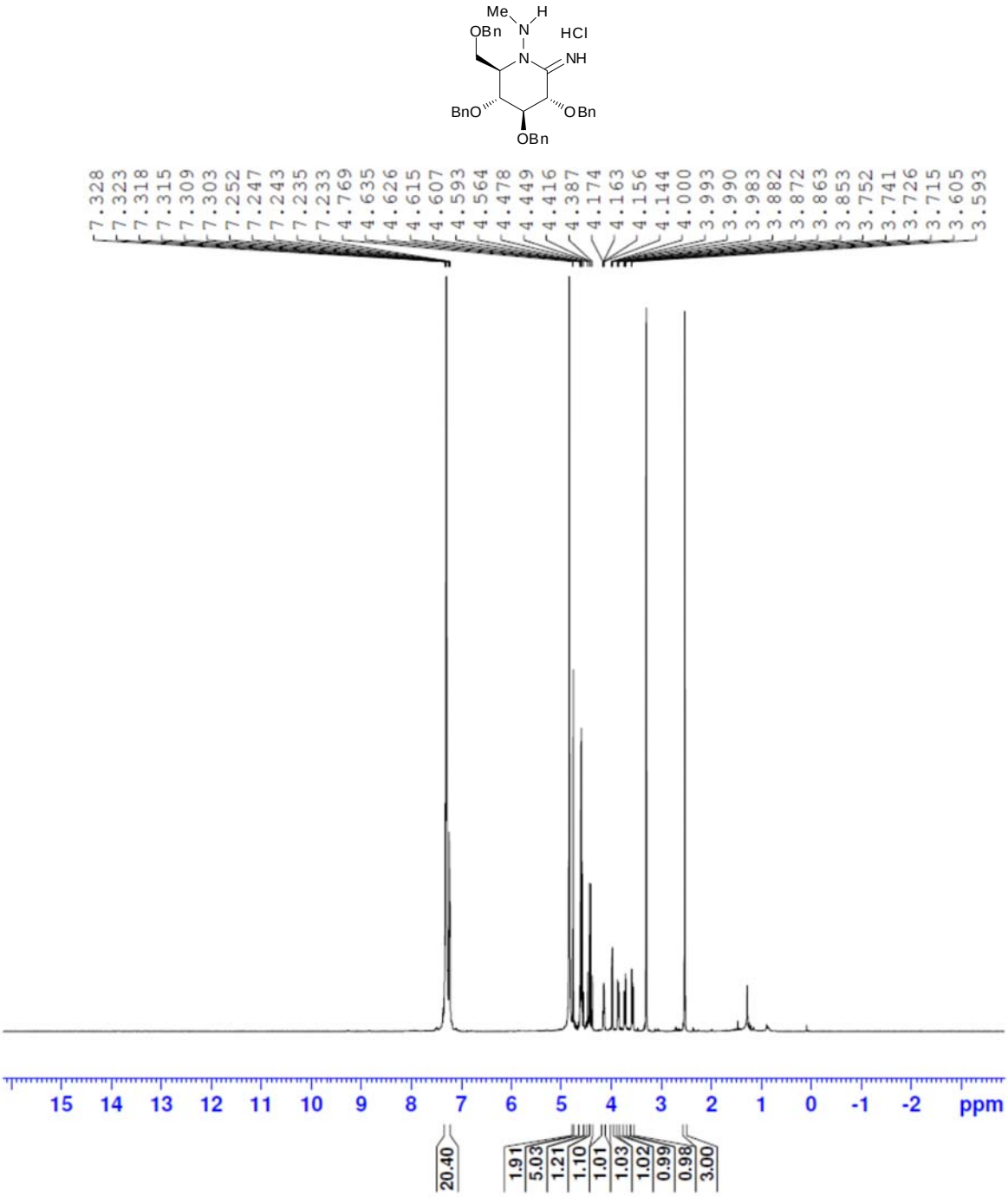
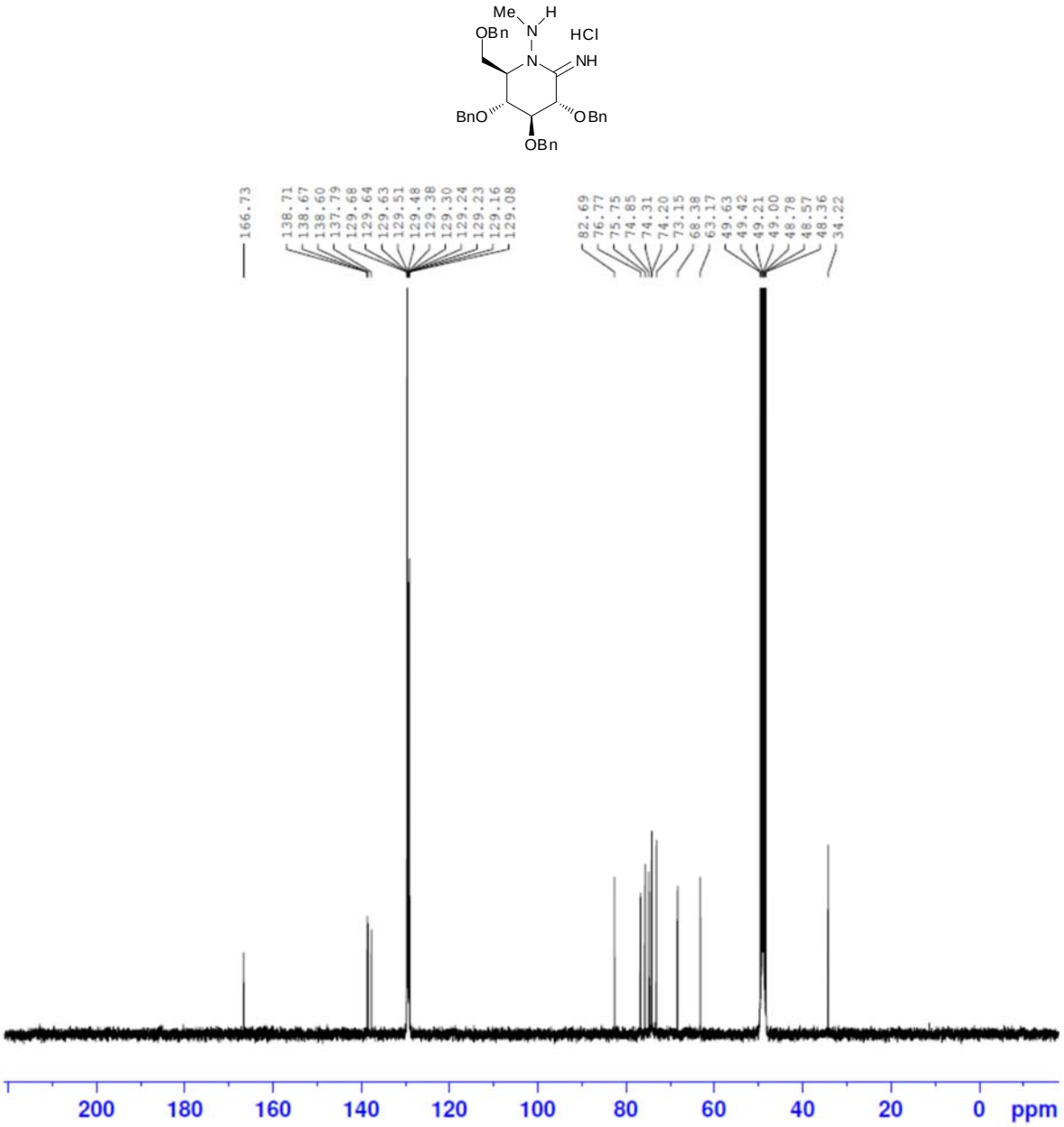


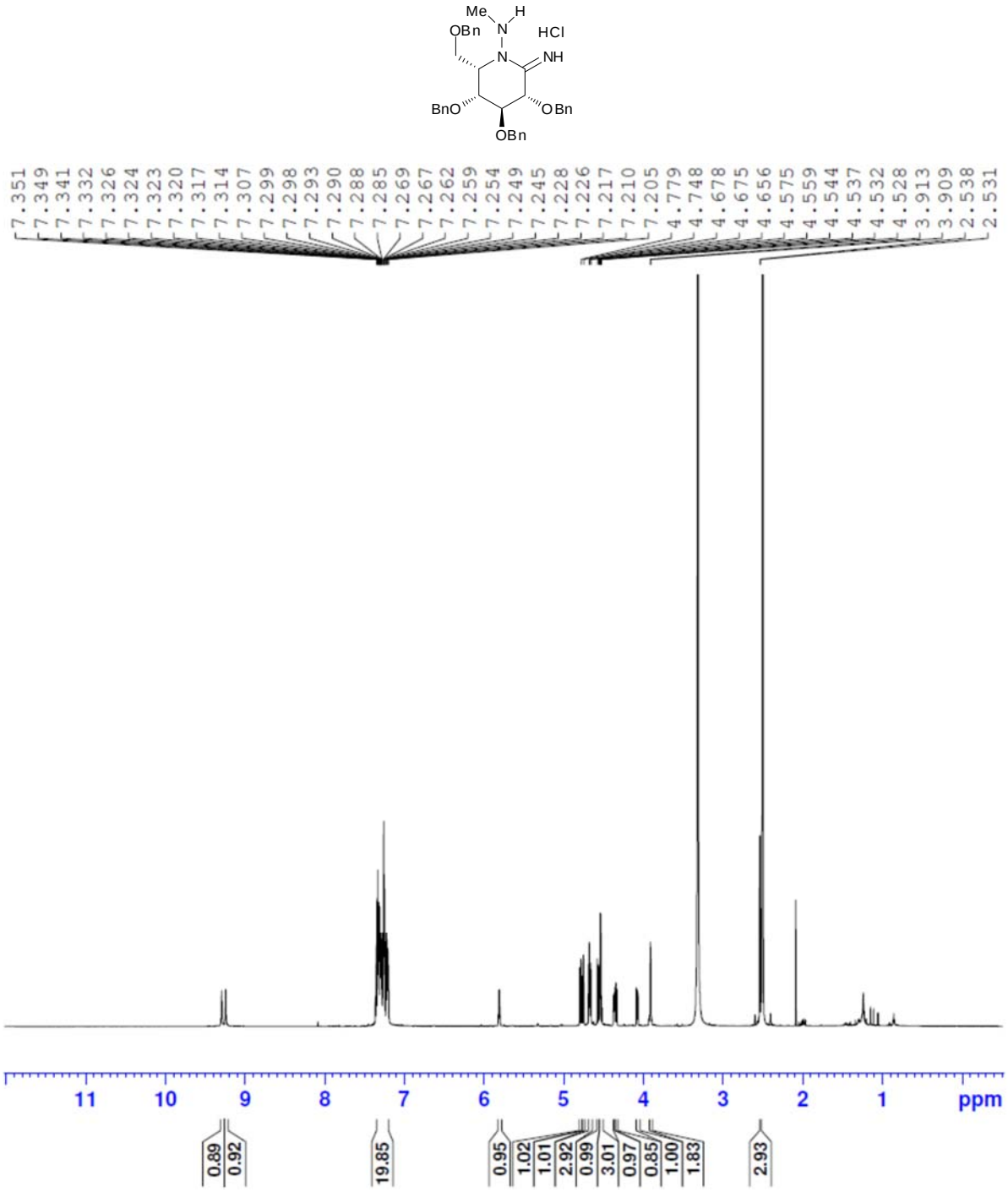
^{1}H-NMR of compound **14** (CD_3OD, 400 MHz)



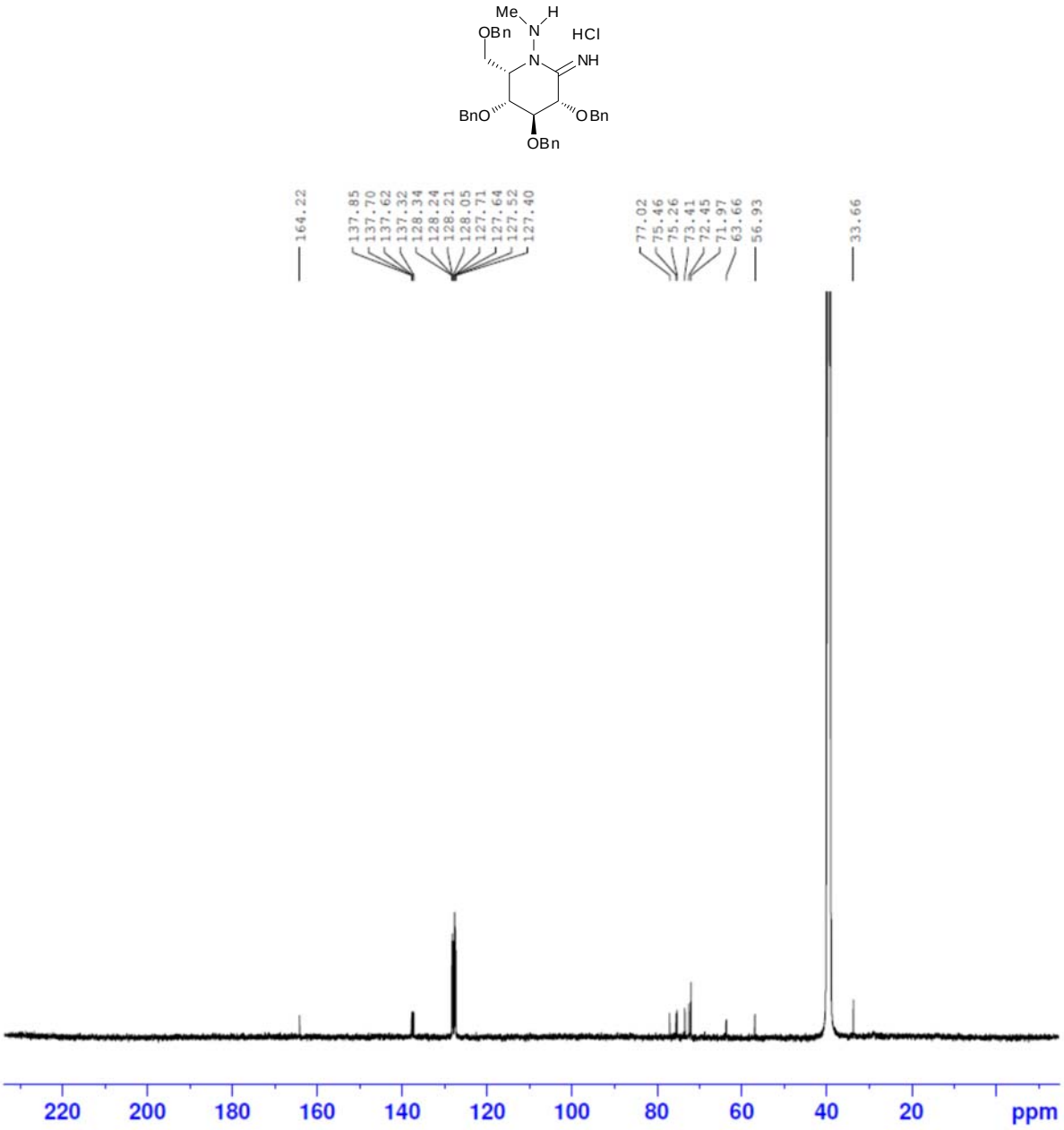
^{13}C-NMR of compound **14** (CD_3OD, 100 MHz)



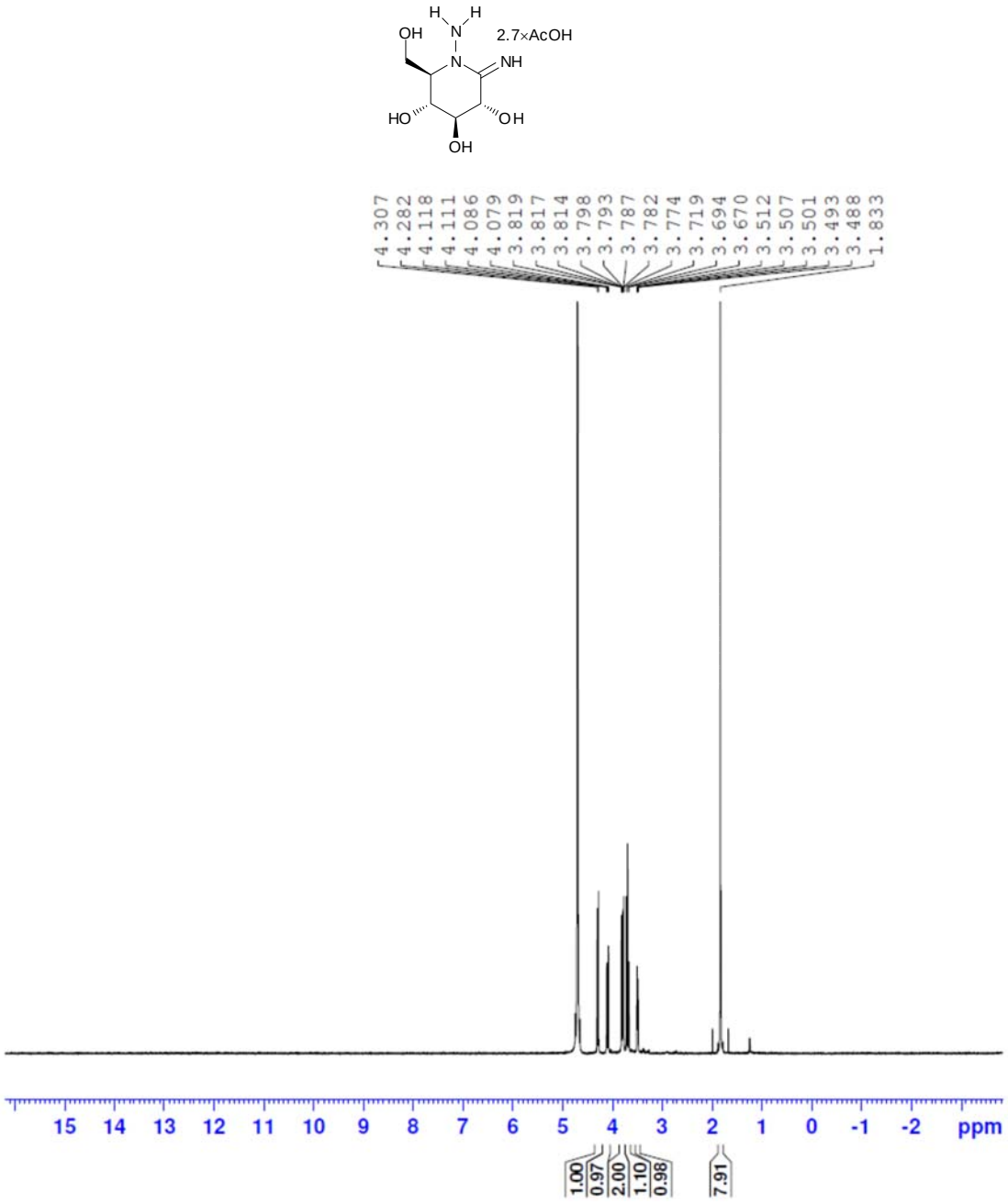
^{1}H-NMR of compound **15** (DMSO-d_6, 700 MHz)



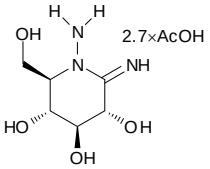
^{13}C-NMR of compound **15** (DMSO-d_6, 125 MHz)

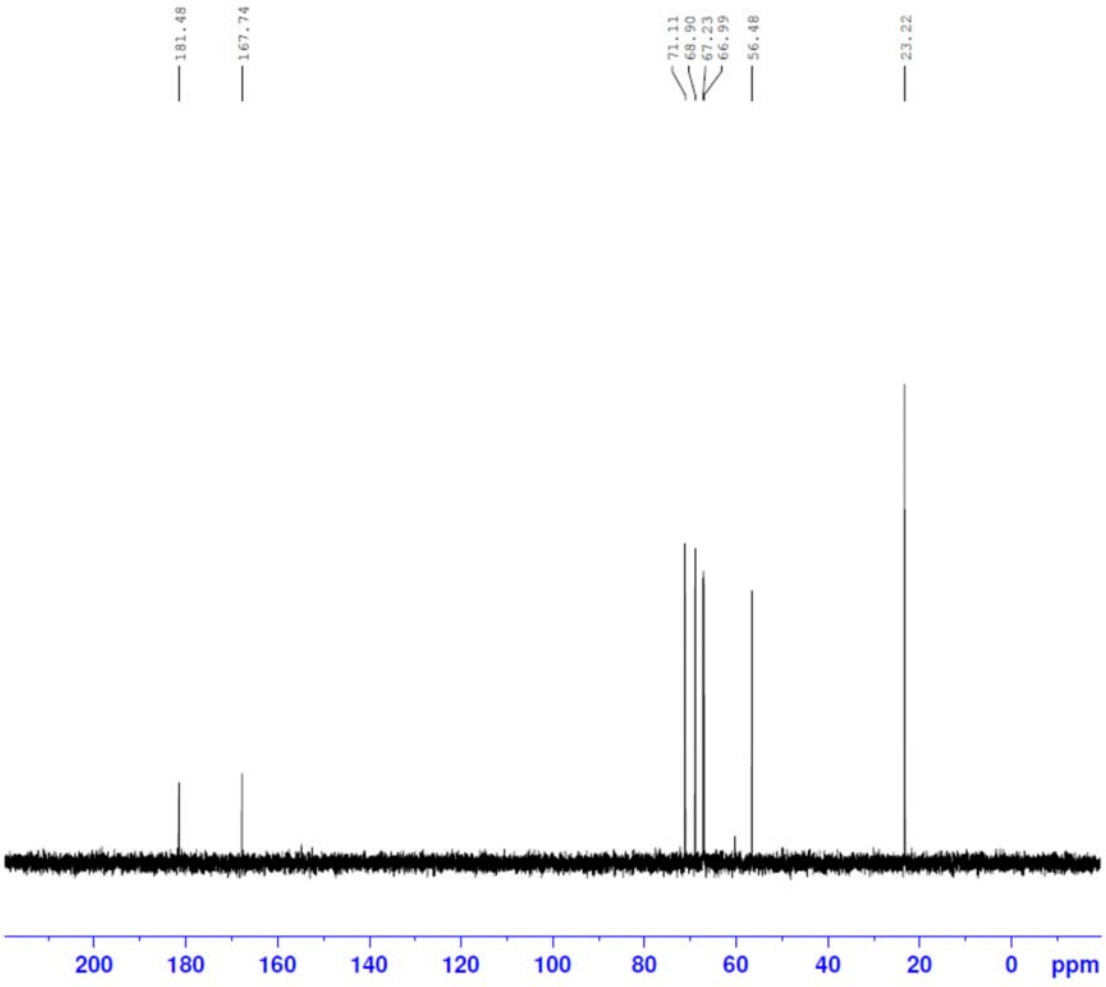


^{1}H-NMR of compound **5** (D_2O, 400 MHz)

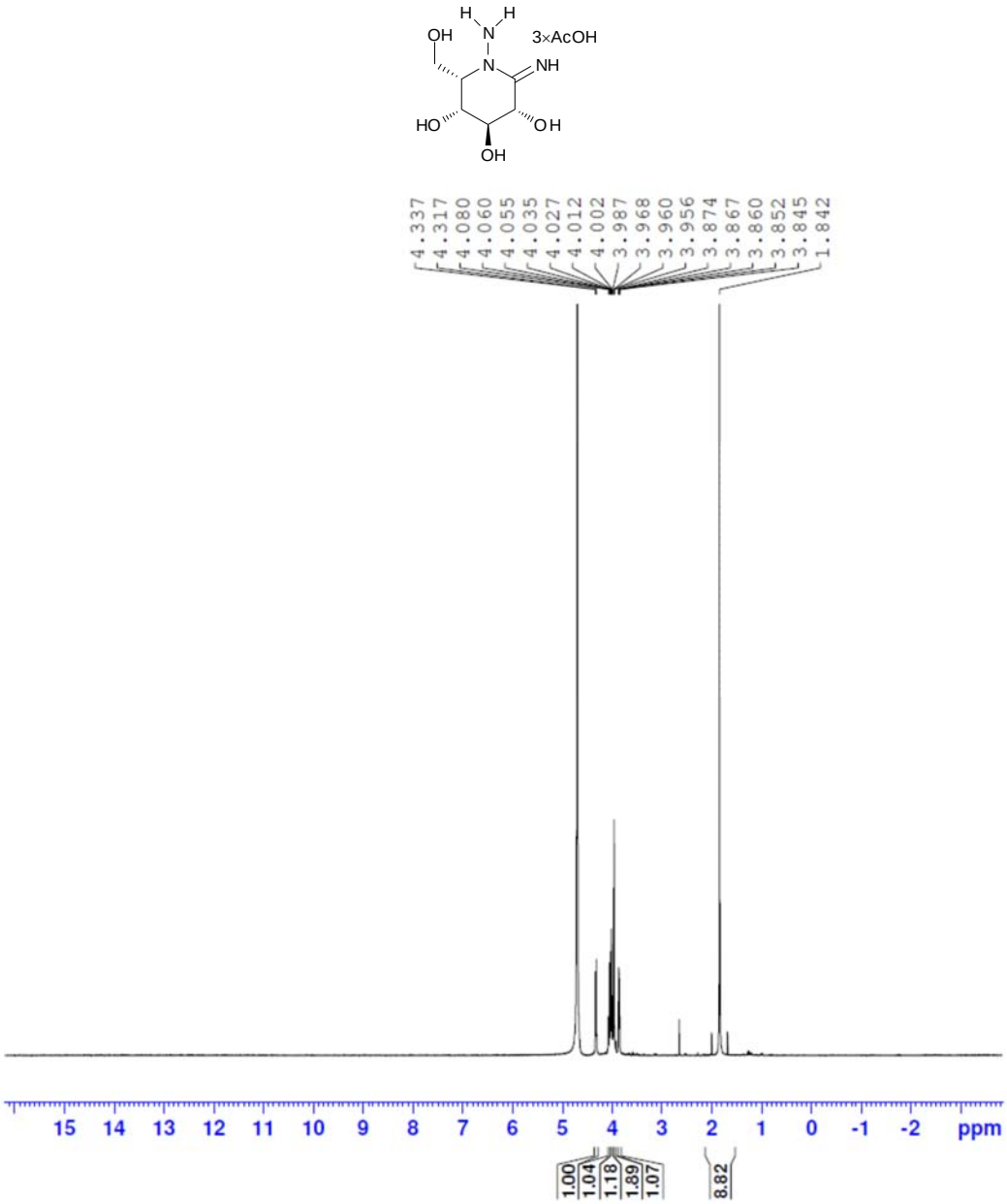


^{13}C-NMR of compound **5** (D_2O, 100 MHz)

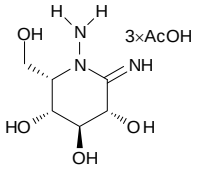


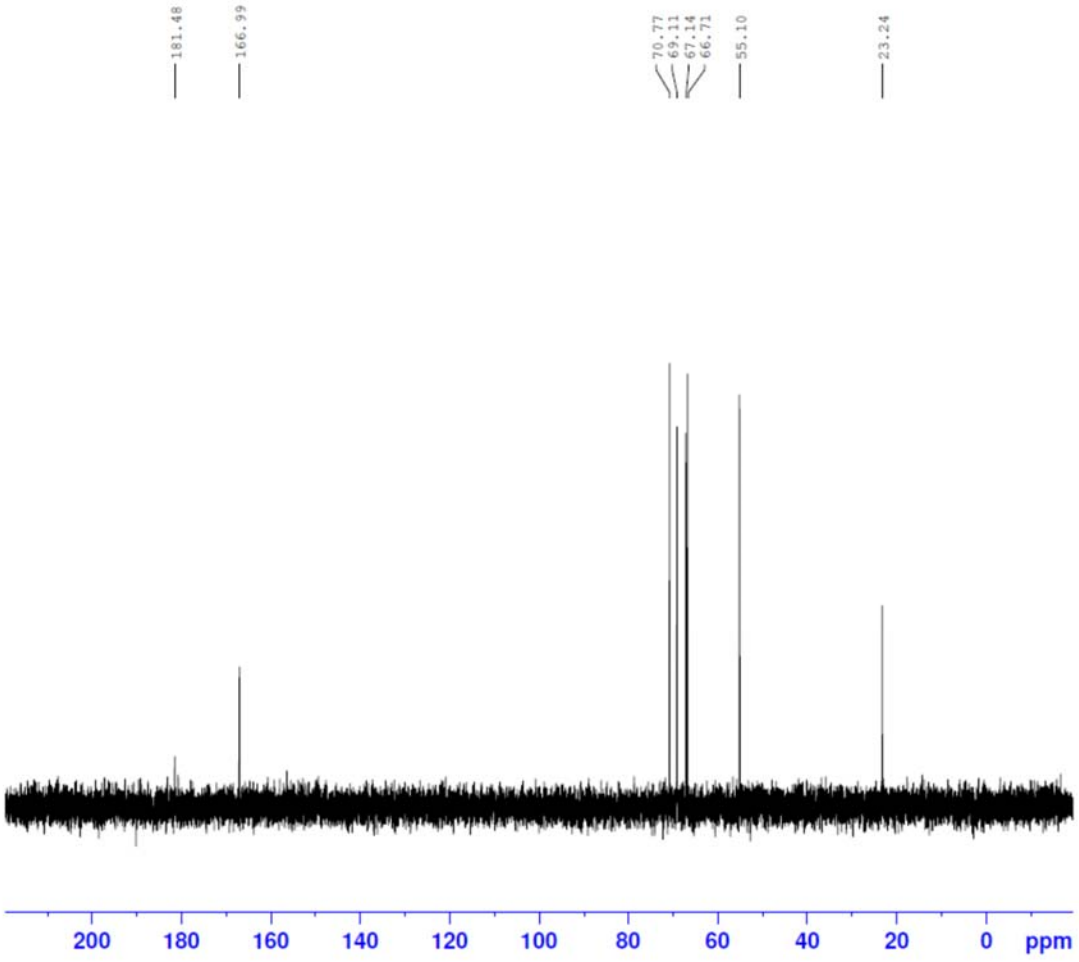


^{1}H-NMR of compound **16** (D_2O, 400 MHz)

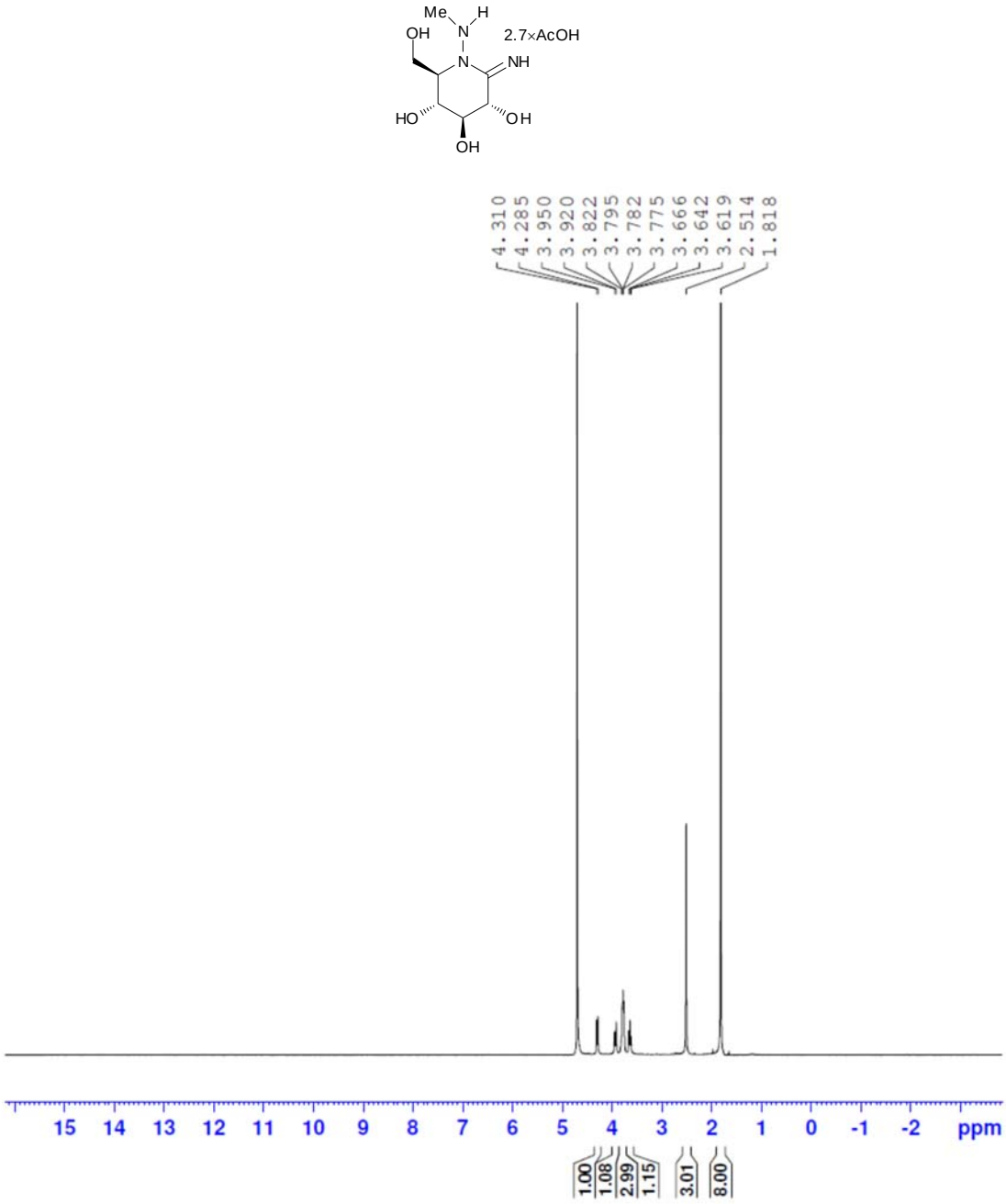


^{13}C-NMR of compound **16** (D_2O, 100 MHz)

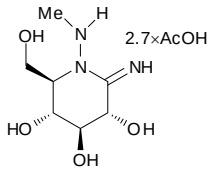


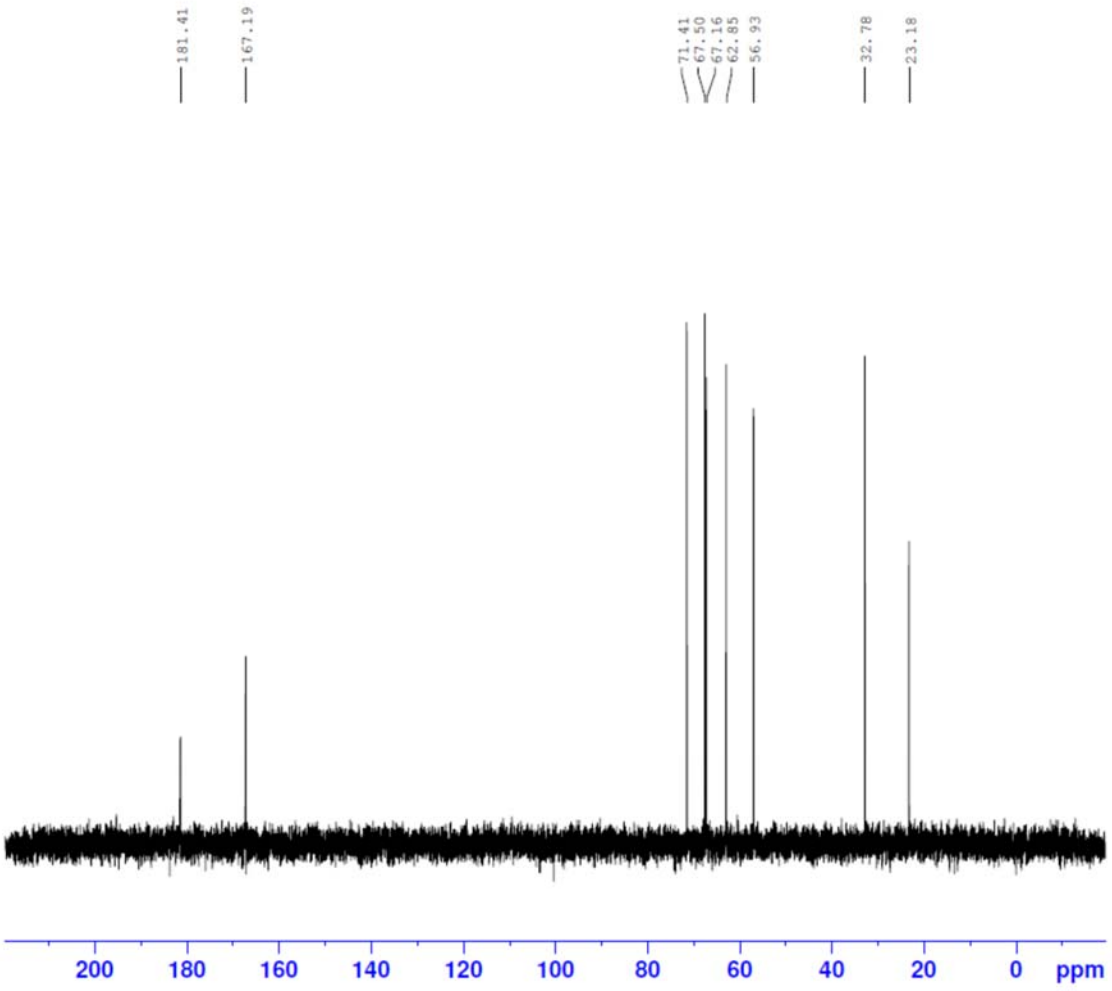


^{1}H-NMR of compound **6** (D_2O, 400 MHz)

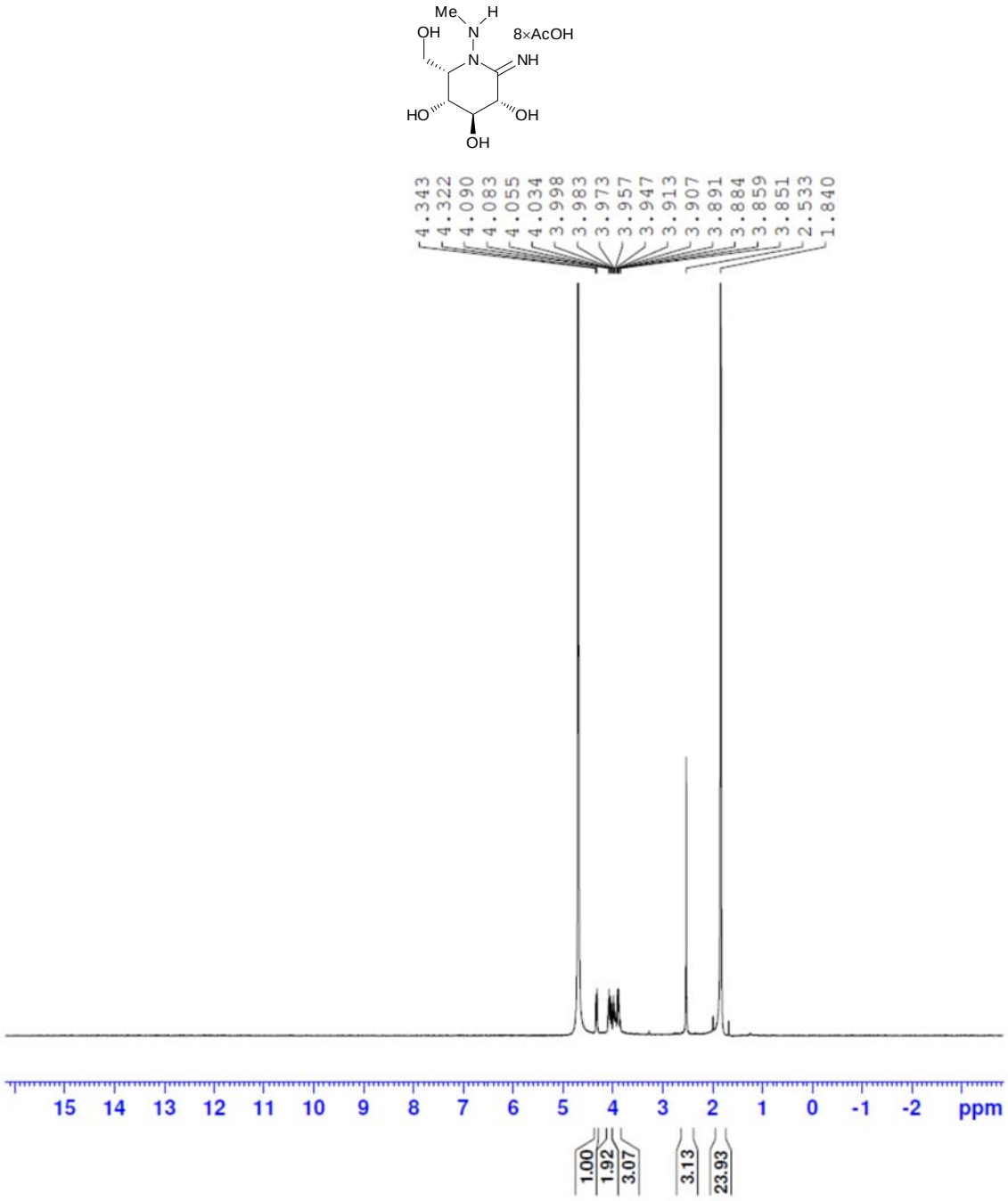


^{13}C-NMR of compound **6** (D_2O, 100 MHz)





^{1}H-NMR of compound **17** (D_2O, 400 MHz)



^{13}C-NMR of compound **17** (D_2O, 100 MHz)