Electronic Supplementary Material (ESI) for MedChemComm.

Electronic Supplementary Information

for

Exploring Eukaryotic Versus Prokaryotic Ribosomal RNA Recognition with Aminoglycoside Derivatives

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SECTION SI

Simulation Methods

Preparation of systems for simulations

The crystal structure of the yeast 80S ribosome complexed with geneticin (PDB ID: 4U4O[1]) was used as a template to prepare models for simulations. An initial A-site model was built as a sphere containing all residues within 25 Å from the geneticin's center of mass (Figure S1). All of osmium (III) hexamine molecules were removed and all magnesium ions were preserved. Hydrogen and missing heavy atoms of the terminal residues were added in the *leap* program (AmberTools16) (http://ambermd.org/). The parameters of RNA and protein fragments were assigned using ff99OL3[2,3] and ff14SB[4] from the AMBER force field, respectively. The net molecular charge of the free A site was -77e. Three-dimensional structures of compounds **2**, **4**, **8**, and **9** were built in *leap* based on the crystal structure of compound **4** in the prokaryotic ribosomal decoding site (PDB code: 2O3X[5]).

All amine groups in the compounds were protonated. In **8**, the carboxyl group was deprotonated. The molecular total charge was: +4e for **2**, **4**, and **9**, and +3e for **8**. Next, the geometries of the compounds were optimized using the Gaussian 09 suite (http://gaussian.com/) at the RHF/6-31G(d) level of theory in vacuum (in accord with the Amber parameterization procedure). Merz-Kollmann atomic charges were obtained and used for the RESP procedure carried out in *antechamber*[6] (AmberTools16) to obtain the RESP atomic charges[7]. Bonded and non-bonded force field parameters of aminoglycosides were found using *parmchk2* (Ambertools16) and GAFF[8] (General AMBER Force Field). The initial position of the derivatives in the ribosomal A-site was achieved by aligning their rings (I and II) to the conformation that they acquire in geneticin (G418). We did not detect any clashes, i.e., atoms overlapping between the ribosomal decoding site and aminoglycosides.

The free A-site and A-site with bound aminoglycosides were then solvated with a 15 Å layer of OPC explicit water molecules. The OPC water model[9] was used due to its better representation of water charge and bulk properties. The whole system consisted of about 106 000 atoms with the box dimensions of 90Åx90Åx90Å. The negative charge of the solutes was neutralized with sodium ions. To mimic physiological conditions, ionic strength of 100 mM NaCl (45 Na^+ and 45 Cl^- ions) was added. Systems containing only aminoglycosides were prepared in analogous way as the complexes but were

solvated with a 20 Å layer of OPC water, neutralized, and additional 10 Na^+ and 10 Cl^- ions were added to achieve a 100 mM ionic strength.

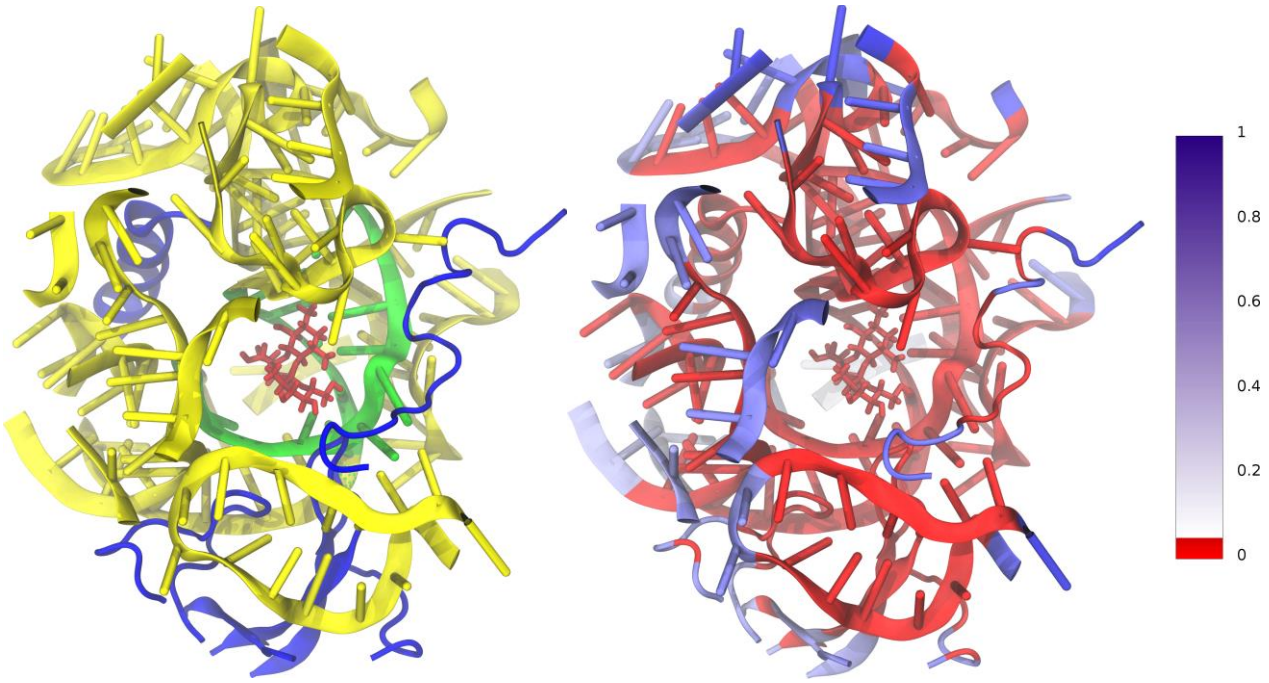

Figure S1. Left: Ribbon model of the A-site containing system in the complex with compound **4** (in red). RNA is in yellow, A-site nucleotides are in green, and protein fragments are in blue. Right: Restraints applied to the system in the production phase to mimic ribosomal surrounding. Force constant values in the legend are in kcal/mol/A^2 for the border residues (group 1), red color means no restraints (group 2). Explicit water and ions are not shown for clarity.

Molecular Dynamics (MD) Simulations

The MD simulation protocol consisted of energy minimization, thermalization, equilibration, and production. In minimization and thermalization harmonic constraints were imposed on heavy atoms of the solute. For border residues (at the 20 Å sphere) centered at geneticin's the center of mass (group 1), the force constants were set equal to their residue-averaged crystallographic beta factors taken from the 4U4O.pdb file[1]. For the remaining solute non-hydrogen atoms (inner residues, group 2), the restraining force constants were set to 10 kcal/mol/$Å^2$.

First, all systems were energy minimized with the above restraints with 5000 steps of steepest descent followed by 4000 steps of conjugate gradient minimization methods using *sander* (Amber 12). The next phases were carried out with NAMD[10]. Second, during thermalization (in the NVT ensemble), each system was heated from 10 to 310 K, increasing the temperature by 20 K every 200 ps, and

keeping the above restraints. Then 800 ps simulations at 310 K were carried out. Third, equilibration was performed in the NpT ensemble with a constant pressure of 1 atm controlled using Langevin Piston method and at constant temperature of 310 K regulated by Langevin dynamics. During equilibration the restraints have been gradually decreased: a scaling factor on the force constant for heavy atoms of group 1 and 2 has been decreased from 1 to 0.025 for 3 ns in 10 time windows. Additionally, 1 ns MD was carried out with the 0.025 scaling factor.

Further, in the production runs, the constraints on heavy atoms of group 2 were completely released while those imposed on atoms of the border group 1 were preserved but scaled by a factor of 0.01. This was to assure that the boundary atoms taken out from the ribosome structure still "feel" the ribosome environment. Group 1 and 2 residues and restraints are schematically shown in Figure S1. The protocol for free aminoglycosides was similar as above but no restraints were applied in the production phase. Periodic boundary conditions and Particle Mesh Ewald method with grid spacing of 1 Å were used. The SHAKE algorithm[11] and the integration time step of 2 fs were applied. For non-bonded interactions a short-range cutoff of 12 Å was used.

The production runs of 500 ns were performed three times for free solute and in the complexes with compounds **2**, **4**, **8**, **9** and of 250 ns for free aminoglycosides, each run started with different velocities. The classical MD production simulations totaled to 3.5 μs. Root mean square deviation (RMSD) analyses confirmed the stability of the solute in classical MD simulations (see Figure S2).

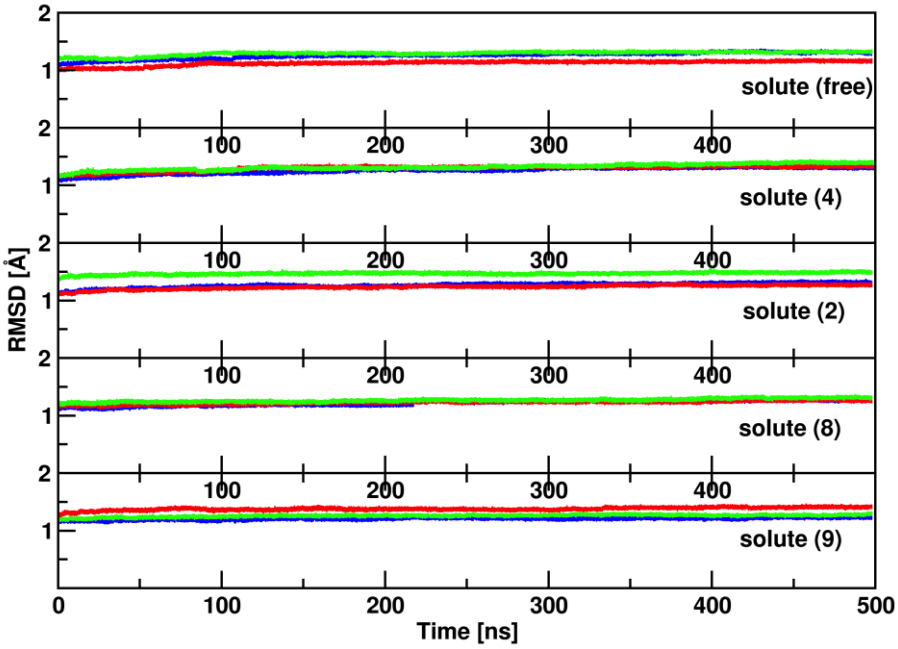

Figure S2. RMSD from the starting structure calculated for the heavy atoms of the solutes (RNA and

protein fragments but excluding solvent and ions) as a function of the simulation time in three independent classical MD simulations of the free solute and in the complexes with different compounds marked in the legend.

Random Acceleration Molecular Dynamics (RAMD) Simulations

To assess kinetic stability of aminoglycosides within the A site, we used random acceleration molecular dynamics (RAMD)[12]. In RAMD, constant force in a random direction is applied to the aminoglycoside to more extensively sample the conformational space and dissociate aminoglycoside from its binding site. The starting structures for RAMD were taken from classical MD production trajectories. Additional force was a randomly generated normalized vector multiplied by an acceleration factor of 35 kcal/mol/Å. The force was imposed on the oxygen atom of the linkage between aminoglycoside ring I and II because this atom is the closest to the center of mass of aminoglycoside non-hydrogen atoms (see Figure 1 in the main text). Every 100 steps, the distance from the starting position of this oxygen to the current one and the distance travelled by this oxygen were calculated. If aminoglycoside oxygen travelled at least 0.0002 Å in 100 steps and the distance from the starting point was larger than in the previous evaluation, the simulation continued with the same force direction. Otherwise, a new random force vector was generated. We assumed that aminoglycoside escaped A site when the distance of the above oxygen atom from the initial position was at least 10 Å. However, if aminoglycoside did not escape for at least 50 ns, we stopped RAMD simulation. For 4 aminoglycoside complexes and 3 starting structures from classical MD production runs, 30 RAMD runs were performed totaling to 360 RAMD simulations and about 6.6 μs.

Trajectory analysis

MD trajectories were analyzed with *cpptraj* of AmberTools16. VMD 1.9.3[13] was used for visualization. Plots were generated with xmgrace and Gnuplot (v4.6). To detect short-range interactions, we used the geometric criteria of 3.2 Å between the donor and acceptor atoms and no less than 150 degrees donor-hydrogen-acceptor angle. To extract dominant conformations clustering analysis on combined trajectories was performed with the kmeans algorithm. The optimal number of clusters (between 2 to 5) was identified by trial and error. The quality of clustering was evaluated by comparing DBI (Davies-Bouldin Index), which is a measure of the separation of clusters, and pSF (pseudo-F statistic), which estimates the tightness of clusters. The lower the DBI and the higher pSF the better.

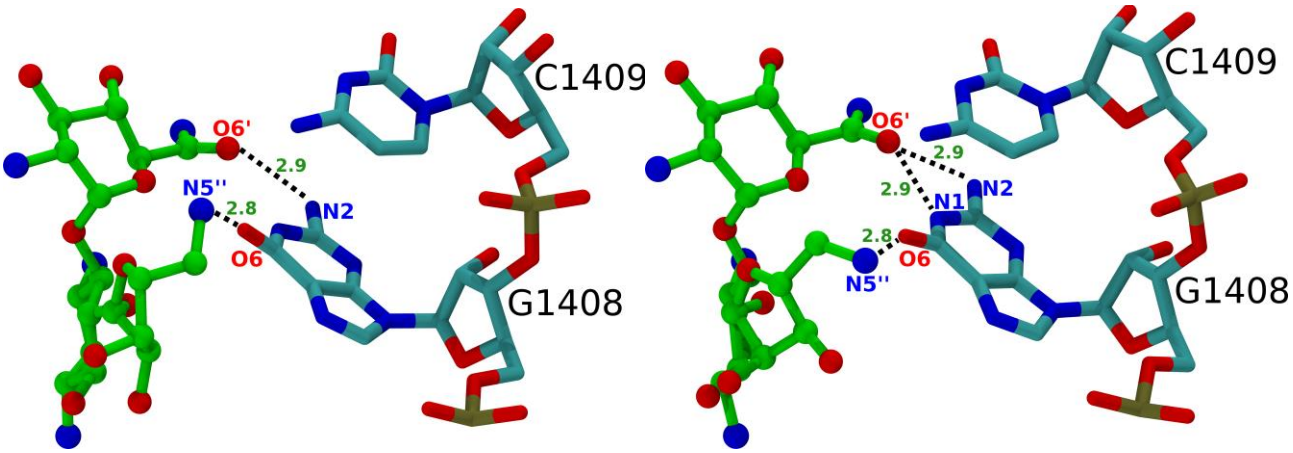

Figure S3. Interactions of compound **9** with G1408 marked with black dashed lines on representative trajectory frames. The distances (in Å) represent averages from frames of the most occupied cluster. The conformations show a representative binding mode from MD simulations.

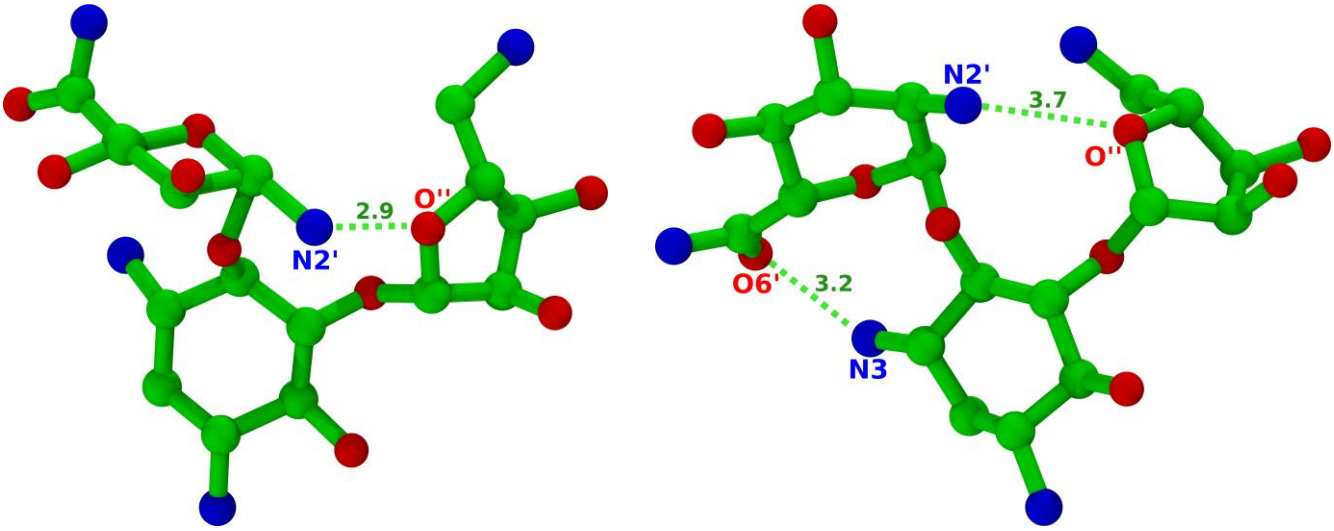

Figure S4. Examples of intra-molecular contacts in compound **9** in solvent from MD simulations. The distances (in Å) represent averages from frames of the most occupied cluster. The conformations show a representative binding mode from MD simulations.

References (Section I)

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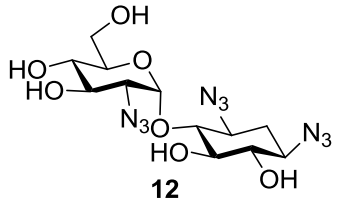
SECTION SII

Chemistry Part

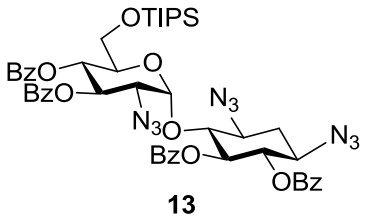
General Techniques: NMR spectra (including ^{1}H, ^{13}C, DEPT, 2D-COSY, 1D TOCSY, HMQC, HMBC) were routinely recorded on a Bruker AvanceTM 500 spectrometer, and chemical shifts reported (in ppm) are relative to internal Me_4Si (δ=0.0) with $CDCl_3$ as the solvent, and to MeOD (δ=3.35) as the solvent. ^{13}C NMR spectra were recorded on a Bruker AvanceTM 500 spectrometer at 125.8 MHz, and the chemical shifts reported (in ppm) relative to the solvent signal for $CDCl_3$ (δ =77.16), or to the solvent signal for MeOD (δ=49.0). Mass spectra analysis were obtained either on a Bruker Daltonix Apex 3 mass spectrometer under electron spray ionization (ESI) or by a TSQ-70B mass spectrometer (Finnigan Mat). Reactions were monitored by TLC on Silica Gel 60 F_{254} (0.25 mm, Merck), and spots were visualized by charring with a yellow solution containing $(NH_4)Mo_7O_{24}$. $4H_2O$ (120 g) and $(NH4)_2Ce(NO_3)_6$ (5 g) in 10% H_2SO_4 (800 mL). Flash column chromatography was performed on Silica Gel 60 (70-230 mesh). All reactions were carried out under an argon atmosphere with anhydrous solvents, unless otherwise noted. All chemicals and biochemicals, unless otherwise stated, were obtained from commercial sources. In all biological tests, all the tested aminoglycosides were in their sulfate salt forms.



12

Compound 12: The title compound was prepared according to previously published (*J. Am. Chem. Soc.* **2002**, *124*, 10773-10778). Briefly, the paromamine (1.0 g, 3.0 mmol), $NaHCO_3$ (3.1 g, 36.9 mmol) and copper (II) sulfate (6 mg, 0.24 mmol) were dissolved in water (5.0 mL). Triflic azide stock solution prepared from Tf_2O (4.6 mL, 27.6 mmol) and NaN_3 (3.6 g, 55.7 mmol) was added followed by the addition of methanol (40 mL) to reach the homogeneous solution. The reaction mixture (blue color) was stirred vigorously at room temperature and the completion of the reaction was indicated by the change of blue color to green. After stirring for 48 h, TLC (EtOAc/MeOH 95:5) analysis finally indicated the completion of the reaction. The solvents were evaporated to dryness and the residue was subjected to column chromatography (EtOAc 100%) to yield compound **12** (650 mg, 52 %). ^{1}H NMR (500 MHz, MeOD): 'Ring I': δ_H 5.69 (d, 1H, *J* = 3.7 Hz, H-1), 3.99 (ddd, 1H, *J* = 9.9, 4.1, 2.6 Hz, H-5),

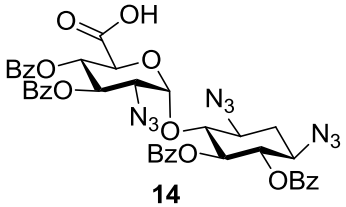
3.94 (dd, 1H, *J* = 10.2, 9.1 Hz, H-3), 3.84 (dd, 1H, *J* = 11.9, 2.3 Hz, H-6), 3.78 (dd, 1H, *J* = 11.8, 4.4 Hz, H-6), 3.46 (dd, 1H, *J* = 9.7, 9.3 Hz, H-4), 3.13 (dd, 1H, *J* = 10.5, 3.7 Hz, H-2); 'Ring II': δ_H 3.80 (t, 1H, *J* = 8.8 Hz, H-5), 3.77 – 3.67 (m, 3H, H-1, H-3, H-4), 3.56 (t, 1H, *J* = 9.6 Hz, H-6), 2.59 – 2.48 (m, 1H), 1.68 (dd, 1H, *J* = 26.3, 12.7 Hz, H-2). ^{13}C NMR (125 MHz, MeOD): δ_C 99.3 (C1'), 80.7, 77.8 (C5), 77.7 (C6), 73.9 (C5'), 72.4 (C3'), 71.6, 64.8 (C2'), 62.1 (C6'), 61.6, 60.9, 33.1 (C2). MALDI TOFMS calculated for $C_{12}H_{19}N_9O_7$ ($[M+K]^+$) m/e 440.3; measured m/e 440.2).



13

Compound 13: Compound **12** (11.6 g, 28.9 mmol) was dissolved in anhydrous DMF (80 mL) and cooled to 0 ^{o}C. Triisopropylsilyl chloride (TIPSCl, 8 mL, 37.3 mmol) was added dropwise, followed by addition of 4-DMAP (10.6 g, 86.7 mmol). The reaction mixture was allowed to attain the room temperature under stirring, and the reaction progress was monitored by TLC (EtOAc/Hexane 7:3), which indicated the completion after 5 h. The reaction mixture was diluted with ethyl acetate (50 mL) and H_2O (20 mL), and the two layers were separated. The aqueous layer was thoroughly washed with ethyl acetate (4 X 30 mL). The combined organic layers were washed with sat. NaCl solution and dried over anhydrous $MgSO_4$. The solvent was evaporated to dryness and the residue was subjected to column chromatography (EtOAc/Hexane 25:75) to yield corresponding silyl ether (13.3 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): **'Ring I'**: δ_H 5.14 (d, 1H, *J* = 4.0 Hz, H-1), 4.09 – 4.02 (m, 2H, H-3, H-6), 3.98 (td, 1H, J_1 = 8.0, J_2 = 4.5 Hz, H-5), 3.82 (dd, 1H, J_1 = 9.5, J_2 = 8.0 Hz, H-6), 3.66 (t, 1H, *J* = 9.0 Hz, H-4), 3.48 (dd, 1H, J_1 = 10.5, J_2 = 4.0 Hz, H-2); **'Ring II'**: δ_H 3.52 (t, 1H, *J* = 8.0 Hz, H-5), 3.47 – 3.37 (m, 2H, H-1, H-6), 3.34 – 3.22 (m, 2H, H-3, H-4), 2.29 (dt, 1H, J_1 = 12.0, J_2 = 4.0 Hz, H-2eq), 1.47 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.0 Hz, H-2ax); *The additional peaks in the spectrum were identified as follow:* δ_H 1.16 – 1.09 (m, 3H, TIPS), 1.07 (s, 12H, TIPS), 1.06 (s, 6H, TIPS). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 99.3 (C1'), 83.4 (C4), 76.1 (C5), 75.5 (C6), 75.1 (C4'), 72.6 (C3'), 69.6 (C5'), 66.0 (C6'), 63.5 (C2'), 59.8 (C1), 58.9 (C3), 32.1 (C2), 17.9 (2C, TIPS), 11.8 (TIPS). MALDI TOFMS calculated for $C_{21}H_{39}N_9O_7Si$ ($[M+Na]^+$) m/e 580.6; measured m/e 580.3).

To a stirred solution of the silyl ether from above step (1.0 g, 1.79 mmol) under argon atmosphere in dry pyridine (15 mL), was added dropwise BzCl (1.5 mL, 12.9 mmol) followed by addition of 4-DMAP (0.150 g, 1.23 mmol) to maintain the pH ~9-10. The reaction was refluxed at 80 ^{o}C. The progress of the reaction was monitored by TLC (EtOAc/Hexane 3:7), which indicated completion after 6 h. The reaction mixture was diluted with EtOAc and extracted with H_2O, HCl (2%), $NaHCO_3$ (sat.), and brine. The combined organic layer was dried over $MgSO_4$, evaporated to dryness, and purified by column chromatography (silica gel, EtOAc/Hexane) to yield compound **13** (1.1g, 80%). ^{1}H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 5.89 (dd, 1H, J_1 = 10.7, J_2 = 9.5 Hz, H-3), 5.52 (t, 1H, J = 9.8 Hz, H-4), 5.33 (d, 1H, J = 3.8 Hz, H-1), 4.48 (ddd, 1H, J_1 = 10.2, J_2 = 3.8, J_3 = 2.4 Hz, H-5), 3.94 – 3.86 (m, 2H, H-6, H-6), 3.27 (dd, 1H, J_1 = 10.7, J_2 = 3.8 Hz, H-2); **'Ring II'**: δ_H 5.72 (t, 1H, J = 9.8 Hz, H-5), 5.40 (t, 1H, J = 10.1 Hz, H-6), 4.04 (t, 1H, J = 9.8 Hz, H-4), 3.87 (td, 1H, J_1 = 12.4, J_2 = 8.7 Hz, H-1), 3.75 (td, 1H, J_1 = 12.5, J_2 = 5.2 Hz, H-3), 2.58 (dt, 1H, J_1 = 13.6, J_2 = 4.6 Hz, H-2eq), 1.79 (ddd, 1H, J_1 = J_2 = J_3 = 13.3 Hz, H-2ax); *The additional peaks in the spectrum were identified as follow:* δ_H 7.92 – 7.85 (m, 8H, Ar), 7.51 – 7.44 (m, 4H, Ar), 7.37 – 7.29 (m, 8H, Ar), 1.10 – 0.97(m, 21H, TIPS). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 165.7 (C=O), 165.6 (C=O), 165.19 (C=O), 165.15 (C=O), 133.5 (Ar), 133.4 (Ar), 133.29 (Ar), 133.27 (Ar), 129.99 (Ar), 129.95 (Ar), 129.86 (Ar), 129.7 (Ar), 129.4 (Ar), 129.2 (Ar), 129.0 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.43 (Ar), 128.4 (Ar), 98.8 (C1'), 77.7 (C4), 74.1 (C6), 73.9 (C5), 71.7 (C5'), 70.8 (C3'), 69.1 (C4'), 62.5 (C6'), 61.2 (C2'), 59.3 (C3), 58.2 (C1), 32.4 (C2), 18.0 (TIPS), 12.0 (TIPS). MALDI TOFMS calculated for $C_{49}H_{55}N_9O_{11}Si$ ($[M+Na]^+$) m/e 996.1; measured m/e 996.2).



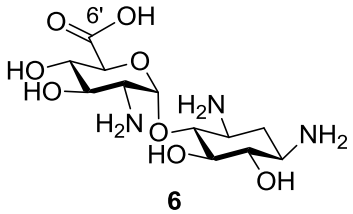
14

Compound 14: A stirred solution of compound **13** (1.1 g, 1.12 mmol) under argon in dry pyridine (10 mL) was cooled to 0-4^{o}C in a polyethylene vessel. At this temperature, the solution of HF in pyridine (5 mL) was added dropwise. The progress of the reaction was monitored by TLC (EtOAc/Hexane 3:7), which indicated completion after 3.5 h. The reaction mixture was diluted with EtOAc and extracted with H_2O, $NaHCO_3$ (sat.). The organic layers again washed with HCl (2%), $NaHCO_3$ (sat.), and brine. The combined organic layer was dried over $MgSO_4$, evaporated to dryness, and purified by column chromatography (silica gel, EtOAc/Hexane) to yield corresponding primary alcohol (0.756 g, 82%). ^{1}H

NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 6.0 (dd, 1H, J_1 = 10.7, J_2 = 9.5 Hz, H-3), 5.39 (d, 1H, J = 3.9 Hz, H-1), 5.34 (dd, 1H, J_1 = 10.1, J_2 = 9.6 Hz, H-4), 4.39 (dt, 1H, J_1 = 10.3, J_2 = 2.3 Hz, H-5), 3.86-3.84 (m, 1H, H-6), 3.76-3.67 (m, 1H, H-6), 3.32 (dd, 1H, J_1 = 10.7, J_2 = 3.9 Hz, H-2); **'Ring II'**: δ_H 5.72 (t, 1H, J = 9.9 Hz, H-5), 5.42 (t, 1H, J = 10.1 Hz, H-6), 4.01 (t, 1H, J = 9.8 Hz, H-4), 3.88 (ddd, 1H, J_1 = 12.2, J_2 = 10.4, J_3 = 4.9 Hz, H-1), 3.74 (ddd, 1H, J_1 = 12.1, J_2 = 10.2, J_3 = 5.1 Hz, H-3), 2.59 (dt, 1H, J_1 = 13.5, J_2 = 4.6 Hz, H-2eq), 1.84 (ddd, 1H, J_1 = J_2 = J_3 = 12.9 Hz, H-2ax); *The additional peaks in the spectrum were identified as follow:* δ_H 7.96 (d, 1H, J = 1.1 Hz, Ar), 7.95 (t, 1H, J = 1.5 Hz, Ar), 7.92 (d, 1H, J = 1.1 Hz, Ar), 7.91 (t, 1H, J = 1.6 Hz, Ar), 7.89 – 7.88 (m, 2H, Ar), 7.87 (dd, 2H, J_1 = 3.4, J_2 = 1.3 Hz, Ar), 7.55 – 7.50 (m, 1H, Ar), 7.50 – 7.44 (m, 3H, Ar), 7.39 – 7.29 (m, 8H, Ar), 2.76 (brs, 1H, 6'-OH). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C166.7 (C=O), 165.6 (C=O), 165.4 (C=O), 165.0 (C=O), 133.9 (Ar), 133.58 (Ar), 133.51 (Ar), 133.4 (Ar), 130.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.5 (Ar), 128.9 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.46 (Ar), 128.4 (Ar), 99.1 (C1'), 78.4 (C4), 74.0 (C6), 73.7 (C5), 71.0 (C5'), 69.9 (C3'), 69.5 (C4'), 61.1 (C2'), 60.6 (C6'), 59.0 (C1), 58.1 (C3), 32.1 (C2). MALDI TOFMS calculated for $C_{40}H_{35}N_9O_{11}$ ($[M+H]^+$) m/e 818.7; measured m/e 818.9).

The primary alcohol product from the previous step (0.1 g, 0.12 mmol) was dissolved in CH_2Cl_2 and cooled to 5 ^oC. Then water (1.0 mL), TEMPO (3 mg, 0.019 mmol), and BIAB (98 mg, 0.30 mmol) were added. The reaction mixture was stirred at 5 ^oC for 40 min and then allowed slowly to warm to room temperature. The progress of the reaction was monitored by TLC which indicated completion after 4.5 h. The reaction mixture was diluted with EtOAc and quenched with $Na_2S_2O_3$ and washed with brine. The combined organic layer was dried over $MgSO_4$, evaporated to dryness, and purified by column chromatography (silica gel, EtOAc/Hexane) to yield acid **14** (0.1 g, ~100%). ^{1}H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 5.91 (t, 1H, J = 9.9 Hz, H-3), 5.52 (dd, 1H, J_1= 13.2, J_2= 5.9 Hz, H-4), 5.4 (d, 1H, J = 4.3 Hz, H-1), 5.1 (d, 1H, J = 10.4 Hz, H-5), 3.39 (dd, 1H, J_1 = 10.6, J_2 = 3.8 Hz, H-2); **'Ring II'**: δ_H 5.71 (t, 1H, J = 9.8 Hz, H-5), 5.44-5.37 (m, 1H, H-6), 4.02 (t, 1H, J = 8.9 Hz, H-4), 3.87 (dd, 1H, J_1 = 11.9, J_2 = 10.2 Hz, H-1), 3.77 (td, 1H, J_1 = 9.8, J_2 = 4.8 Hz, H-3), 2.64-2.53 (m, 1H, H-2eq), 1.84 (ddd, 1H, J_1 = J_2 = J_3 = 12.9 Hz, H-2ax); *The additional peaks in the spectrum were identified as follow:* δ_H 7.93 – 7.82 (m, 8H, Ar), 7.48 (dd, 4H, J_1 = 16.8, J_2 = 9.3 Hz, Ar), 7.33 (dd, 8H, J_1 = 16.9, J_2 = 9.3 Hz, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 171.4 [(OH)C=O], 165.7 (2xC=O), 165.3 (C=O), 165.2 (C=O), 133.59 (Ar), 133.56 (Ar), 133.4 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 128.9 (Ar), 128.79 (Ar), 128.73 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 99.0 (C1'), 79.1 (C4),

74.1 (C6), 73.5 (C5), 70.0 (C4'), 69.9 (C3'), 69.0 (C5'), 60.7 (C2'), 58.5 (C3), 58.2 (C1), 31.8 (C2). MALDI TOFMS calculated for $C_{40}H_{33}N_9O_{12}$ ($[M+Na]^+$) m/e 854.2146; measured m/e 854.2114).

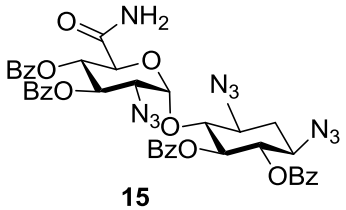


6

Compound 6: A stirred solution of compound **14** (0.35 g, 0.42 mmol) in dry MeOH (10 mL), was cooled to 0 °C and NaOMe (136 mg, 2.51 mmol) was added. The reaction mixture was stirred at 0 °C for 1.0 hr and then allowed slowly to warm to 60 °C. The progress of the reaction was monitored by TLC (EtOAc/MeOH 8:2), which indicated completion after 4 h. The reaction mixture was passed through a silica gel column and product contained fractions were combined and evaporated. The crude product was purified by column chromatography (silica gel, EtOAc/MeOH) to yield corresponding tetraol compound (153 mg, 88%). ^{1}H NMR (600 MHz, MeOD): **'Ring I'**: δ_H 5.92 (s, 1H, H-1), 4.42 (d, 1H, J = 9.5 Hz, H-5), 3.99 (t, 1H, J = 9.5 Hz, H-3), 3.61 (d,1H, J = 5.7 Hz, H-4), 3.25 (d, 1H, J = 8.0 Hz, H-2); **'Ring II'**: δ_H 3.66-3.60 (m, 1H, H-5), 3.54 (dt, 2H, J_1= 15.7, J_2 = 11.0 Hz, H-6, H-1), 3.43 (dd, 1H, J_1= 10.0, J_2 = 3.5 Hz, H-3), 3.37 (dd, 1H, J_1= 12.8, J_2 = 9.4 Hz, H-6), 2.21 (dd, 1H, J_1 = 8.4, J_2 = 4.2 Hz, H-2eq), 1.43 (ddd, 1H, J_1 = J_2 = J_3 = 13.1 Hz, H-2ax); ^{13}C NMR (150 MHz, MeOD): δ_C 178.3 [(OH)C=O], 98.6 (C1'), 79.6 (C4), 77.9, 77.6, 73.7, 72.5, 71.9, 63.6 (C2'), 61.5 (C3), 61.0 (C1), 49.8 (C6), 33.1 (C2).

To a stirred solution of the tetraol acid from the above step (278 mg, 0.669 mmol) in a mixture of THF (3.0 mL) and aqueous NaOH (1 mM, 5.0 mL), PMe_3 (1 M solution in THF, 3.0 mL, 7.8 equiv.) was added. The progress of the reaction was monitored by TLC [CH_2Cl_2/MeOH/H_2O/$MeNH_2$ (33% solution in EtOH), 10:15:6:15], which indicated completion after 4.0 h. The reaction mixture was purified by flash chromatography on a short column of silica gel. The column was washed with the following solvents: THF (100 mL), CH_2Cl_2 (100 mL), EtOH (50 mL), and MeOH (100 mL). The product was then eluted with the mixture of 5% $MeNH_2$ solution (prepared from the 33% stock solution of $MeNH_2$ in EtOH) MeOH. Fractions containing the product were combined and evaporated under vacuum. The pure product was obtained by passing the above product through a short column of Amberlite CG50 (NH_4^+ form). First, the column was washed with water, then the product was eluted

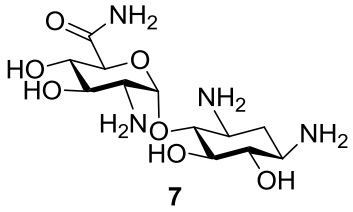
with a mixture of 10% NH_4OH in water to yield the acid **6** (150 mg, 67%). For the storage and biological tests, the compound was converted to its sulfate salt form as follow. The free base form was dissolved in water, the pH was adjusted to 7.0 with H_2SO_4 (0.1 N) and lyophilized to afford the sulfate salt of **6**. ^{1}H NMR (600 MHz, D_2O): **'Ring I'**: δ_H 5.32 (d, 1H, J = 3.9 Hz, H-1), 4.03 (d, 1H, J = 9.9 Hz, H-5), 3.61 (dd, 1H, J_1= 10.2, J_2= 9.2 Hz, H-3), 3.51 (dd, 1H, J_1= 10.4, J_2= 8.5 Hz, H-4), 2.91 (dd, 1H, J_1 = 10.3, J_2 = 3.9 Hz, H-2); **'Ring II'**: δ_H 3.54 (t, 1H, J = 8.5 Hz, H-5), 3.37 (t, 1H, J = 9.4 Hz, H-4), 3.38-3.31 (m, 1H, H-6), 2.96 (ddt, 2H, J_1 = 12.3, J_2 = 9.1, J_3 = 4.5 Hz, H-1, H-3), 2.11 (dt, 1H, J_1 = 12.8, J_2 = 4.2 Hz, H-2eq), 1.39 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.5 Hz, H-2ax); ^{13}C NMR (150 MHz, D_2O): δ_C 176.3 [(OH)C=O], 99.9 (C1'), 85.1 (C4), 75.6 (C4'), 74.8 (C6), 73.3 (C5'), 72.7 (C3'), 72.0 (C5), 54.8 (C2'), 50.5 (C3), 49.2 (C1), 32.9 (C2). MALDI TOFMS calculated for $C_{12}H_{23}N_3O_8$ ($[M+H]^+$) m/e 338.1563; measured m/e 338.1524).



15

Compound 15: To a cooled solution of anhydrous CH_2Cl_2 (10 mL) at -30 °C, oxalyl chloride (1.1 mL, 12.99 mmol) and DMF (1.0 mL, 12.98 mmol) were added successively and then allowed slowly to reach 0 °C in about 1.5 h. The reaction mixture was again cooled to -30 °C and compound **14** (1.1 g, 1.32 mmol) dissolved in anhydrous CH_2Cl_2 (3 mL) was added dropwise. The solution was allowed slowly to reach 0 °C in 1.5 h. Then, the reaction mixture was cooled to -78 °C and the gaseous ammonia (NH_3) was bubbled into the reaction mixture to allow the condensation of the NH_3 gas into the reaction mixture. The excess ammonia was allowed to evaporate from the flask by leaving the reaction slowly to reach to room temperature. The residue was then diluted with ethyl acetate and washed with $NaHCO_3$ solution, brine. The combined organic layers were evaporated to dryness and the residue was purified by column chromatography to yield the amide **15** (0.681g, 62%). ^{1}H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 5.90 (dd, 1H, J_1 = 10.6, J_2 = 9.4 Hz, H-3), 5.56 (dd, 1H, J_1= 10.3, J_2= 9.4 Hz, H-4), 5.32 (d, 1H, J = 3.9 Hz, H-1), 4.92 (d, 1H, J = 10.3 Hz, H-5), 3.40 (dd, 1H, J_1 = 10.7, J_2 = 3.9 Hz, H-2); **'Ring II'**: δ_H 5.76 (t, 1H, J = 9.9 Hz, H-5), 5.41 (t, 1H, J = 10.1 Hz, H-6), 3.95 (t, 1H, J = 9.8 Hz, H-4), 3.87 (ddd, 1H, J_1 = 12.4, J_2 = 10.1, J_3 =4.5 Hz, H-1), 3.78 (ddd, 1H, J_1 = 12.4, J_2 = 9.9, J_3 =4.7 Hz, H-3), 2.57 (dt, 1H, J_1 = 13.4, J_2 = 4.6 Hz, H-2eq), 1.80 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.8 Hz, H-2ax); *The additional peaks in the spectrum were identified as follow:* δ_H 7.96 – 7.95 (m, 1H, Ar), 7.94

(t, 1H, J = 1.6 Hz, Ar), 7.92 – 7.91 (m, 1H, Ar), 7.90 (t, 1H, J = 1.6 Hz, Ar), 7.89 – 7.88 (m, 1H, Ar), 7.87 (dd, 2H, J_1 = 2.8, J_2 = 1.5 Hz, Ar), 7.86 (t, 1H, J = 1.6 Hz, Ar), 7.50 – 7.42 (m, 4H, Ar), 7.36 – 7.28 (m, 8H, Ar), 6.41 (brs, 1H, NH), 5.92 (brs, 1H, NH).^{13}C NMR (150 MHz, $CDCl_3$): δ_C 169.3 [$(NH_2)C=O$], 165.7 (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O), 133.6 (Ar), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.5 (Ar), 129.2 (Ar), 129.0 (Ar), 128.8 (Ar), 128.63 (Ar), 128.61 (Ar), 128.5 (Ar), 128.4 (Ar), 99.1 (C1'), 79.7 (C4), 74.1 (C6), 73.4 (C5), 70.2 (C3'), 70.0 (C4'), 69.3 (C5'), 61.0 (C2'), 58.6 (C3), 58.0 (C1), 32.0 (C2). MALDI TOFMS calculated for $C_{40}H_{34}N_{10}O_{11}$ ($[M+H]^+$) m/e 831.76; measured m/e 831.05).

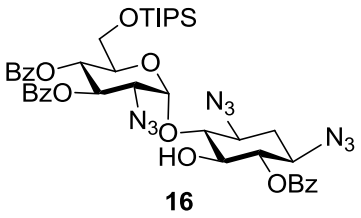


7

Compound 7: To a stirred solution of compound **15** (0.681 g, 0.82 mmol) in dry MeOH (15 mL) was added NaOMe (265 mg, 4.90 mmol) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 1.0 hr and then allowed slowly to warm to 60 ^{o}C. The progress of the reaction was monitored by TLC (EtOAc/MeOH 8:2), which indicated completion after 4 h. The reaction mixture was passed through a silica gel column and product contained fraction were combined, evaporated. The crude product was purified by column chromatography (silica gel, EtOAc/MeOH) to yield corresponding the tetraol amide product (261 mg, 77%). ^{1}H NMR (600 MHz, MeOD): **'Ring I'**: δ_H 5.89 (s, 1H, H-1), 4.40 (d, 1H, J = 10.2 Hz, H-5), 3.97 (t, 1H, J = 9.5 Hz, H-3), 3.61-3.54 (m,1H, H-4), 3.21 (d, 1H, J = 11.6 Hz, H-2); **'Ring II'**: δ_H 3.58 (d, 1H, J = 9.7 Hz, H-5), 3.56 – 3.47 (m, 2H, H-4, H-3), 3.45 – 3.38 (m, 1H, H-1), 3.37 – 3.31 (m, 1H, H-6), 2.21 (dt, 1H, J_1 = 12.1, J_2 = 3.8 Hz, H-2eq), 1.42 (ddd, 1H, J_1 = J_2 = J_3 = 12.1 Hz, H-2ax); ^{13}C NMR (150 MHz, MeOD): δ_C 178.1 [$(NH_2)C=O$], 98.7 (C1'), 79.7 (C4), 78.0 (C5), 77.7 (C6), 73.7 (C4'), 72.4 (C5'), 71.9 (C3'), 63.8 (C2'), 61.6 (C3), 61.1(C1), 49.8 (C6), 33.1 (C2). MALDI TOFMS calculated for $C_{12}H_{18}N_{10}O_7$ ($[M]^+$) m/e 414.33; measured m/e 414.93).

To a stirred solution of the tetraol amide product from the above step (261 mg, 0.63 mmol) in a mixture of THF (3.0 mL) and aqueous NaOH (1 mM, 5.0 mL), PMe_3 (1 M solution in THF, 3.0 mL, 7.8 equiv.) was added. The progress of the reaction was monitored by TLC [CH_2Cl_2/MeOH/H_2O/$MeNH_2$ (33% solution in EtOH), 10:15:6:15], which indicated completion after 3.5 h. The reaction mixture was purified by flash chromatography on a short column of silica gel. The

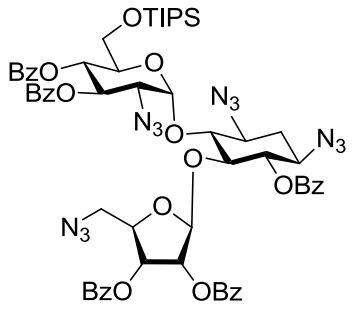
column was washed with the following solvents: THF (100 mL), CH_2Cl_2 (100 mL), EtOH (50 mL), and MeOH (100 mL). The product was then eluted with the mixture of 5% $MeNH_2$ solution (33% solution in EtOH) in 80% MeOH. Fractions containing the product were combined and evaporated under vacuum. The pure product was obtained by passing the above product through a short column of Amberlite CG50 (NH_4^+ form). First, the column was washed with water, then the product was eluted with a mixture of 10% NH_4OH in water to yield compound **7** (120 mg, 57%). For the storage and biological tests, the compound was converted to its sulfate salt form as follow. The free base form was dissolved in water, the pH was adjusted to 7.0 with H_2SO_4 (0.1 N) and lyophilized to afford the sulfate salt of **7**. ^{1}H NMR (600 MHz, D_2O): **'Ring I'**: δ_H 5.30 (d, 1H, J = 3.7 Hz, H-1), 4.01 (d, 1H, J = 9.9 Hz, H-5), 3.60 (t, 1H, J= 9.9 Hz, H-3), 3.52-3.42 (m, 1H, H-4), 2.89 (dd, 1H, J_1 = 10.4, J_2 = 3.7 Hz, H-2); **'Ring II'**: δ_H 3.49 (t, 1H, J = 9.0 Hz, H-5), 3.36 (t, 1H, J = 9.7 Hz, H-4), 3.30 (t, 1H, J = 9.9 Hz, H-6), 2.94 (ddd, 2H, J_1 = 16.0, J_2 = 10.4, J_3 = 5.6 Hz, H-1, H-3), 2.09 (dt, 1H, J_1 = 12.9, J_2 = 3.8 Hz, H-2eq), 1.37 (ddd, 1H, J_1 = J_2 = J_3 = 12.4 Hz, H-2ax); ^{13}C NMR (150 MHz, D_2O): δ_C 176.3 [$(NH_2)C$=O], 99.8 (C1'), 85.0 (C4), 75.5 (C4'), 74.7 (C6), 73.3 (C5'), 72.6 (C3'), 72.0 (C5), 54.8 (C2'), 50.4 (C3), 49.1 (C1), 32.8 (C2). MALDI TOFMS calculated for $C_{12}H_{24}N_4O_7$ ($[M+H]^+$) m/e 337.34; measured m/e 337.00).



16

Compound 16: To a stirred solution of 6'-*O*-triisopropylsilyl perazido paromamine (4.0 g, 7.17 mmol) in dry pyridine (60 mL, - 5 ^{o}C), BzCl (3.72 mL, 32.0 mmol) was added dropwise, followed by addition of 4-DMAP (0.876 g, 7.17 mmol) to maintain the pH ~9-10. The reaction mixture was slowly allowed to attain the room temperature, and stirred at this temperature overnight. The progress of the reaction was monitored by TLC (EtOAc/Hexane 3:7), which indicated completion after 12 h. The reaction mixture was diluted with EtOAc and extracted with H_2O, HCl (2%), $NaHCO_3$ (sat.), and brine. The combined organic layer was dried over $MgSO_4$, evaporated to dryness, and purified by column chromatography (silica gel, EtOAc/Hexane) to yield compound **16** (2.75g, 44%). ^{1}H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 5.97 (t, 1H, J = 10.2 Hz, H-3), 5.65 (t, 1H, J = 9.8 Hz, H-4), 5.56 (d, 1H, J = 3.6 Hz, H-1), 4.41 (dt, 1H, J_1 = 10.1, J_2 = 3.0 Hz, H-5), 3.93 – 3.86 (m, 2H, H-6, H-6), 3.77 (dd, 1H, J_1 = 10.3, J_2 = 4.1 Hz, H-2); **'Ring II'**: δ_H 5.24 (t, 1H, J = 9.9 Hz, H-6), 3.91 (t, 1H, J = 9.4 Hz, H-5), 3.71

(ddd, 2H, J_1 = 12.4, J_2 = 10.3, J_3 = 4.8 Hz, H-1), 3.66 (t, 1H, J = 9.6 Hz, H-4), 3.49 (ddd, 2H, J_1 = 12.3, J_2 = 10.0, J_3 = 4.8 Hz, H-3), 2.47 (dt, 1H, J_1 = 12.7, J_2 = 4.5 Hz, H-2eq), 1.73 (ddd, 1H, J_1 = J_2 = J_3 = 12.8 Hz, H-2ax); *The additional peaks in the spectrum were identified as follow:* δ_H 8.11 (t, 1H, J = 1.5 Hz, Ar), 8.10 (t, 1H, J = 1.6 Hz, Ar), 7.96 (t, 1H, J = 1.4 Hz, Ar), 7.95 (t, 1H, J = 1.6 Hz, Ar), 7.92 (t, 1H, J = 1.4 Hz, Ar), 7.91 (t, 1H, J = 1.6 Hz, Ar), 7.61 – 7.56 (m, 1H, Ar), 7.52 – 7.44 (m, 4H, Ar), 7.38 – 7.33 (m, 4H, Ar), 1.07 – 1.01 (m, 3H, TIPS), 1.01 – 0.98 (m, 18H, TIPS). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 166.2 (C=O), 165.9 (C=O), 165.0 (C=O), 133.6 (Ar), 133.4 (Ar), 133.2 (Ar), 130.2 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.3 (Ar), 129.1 (Ar), 128.9 (Ar), 128.6 (Ar), 128.5 (Ar), 128.48 (Ar), 128.40 (Ar), 98.9 (C1'), 82.6 (C4), 75.6 (C6), 74.8 (C5), 71.9 (C3'), 71.5 (C5'), 68.8 (C4'), 62.4 (C2'), 62.1 (C6'), 58.6 (C3), 58.3 (C1), 32.2 (C2), 17.94 (TIPS), 17.91 (TIPS), 11.9 (TIPS). MALDI TOFMS calculated for $C_{42}H_{51}N_9O_{10}Si$ ($[M+NH_4]^+$) m/e 887.99; measured m/e 887.15).

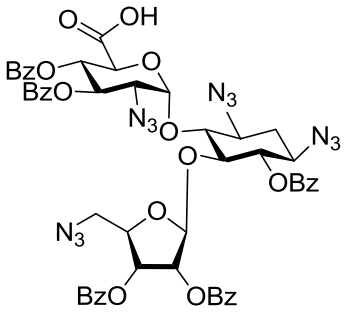


19

Compound 19: Anhydrous CH_2Cl_2 (20 mL) was added to a powdered, flame-dried 4 Å molecular sieves (2.0 g), followed by the addition of acceptor **16** (874 mg, 1.0 mmol) and donor **17** (2.11 g, 4.01 mmol). The reaction mixture was stirred for 10 min at room temperature and was then cooled to -30°C. At this temperature, catalytic amount of BF_3-Et_2O (0.2 mL) was added and the mixture was stirred at -30 °C and the reaction progress was monitored by TLC, which indicated the completion after 60 min. The reaction mixture was diluted with ethyl acetate and washed with saturated $NaHCO_3$ and brine. The combined organic layer was dried over $MgSO_4$, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the titled compound **19**.

^{1}H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 6.18 (d, 1H, J = 3.7, H-1), 6.00 (dd, 1H, J_1 = 10.7, J_2 = 9.5 Hz, H-3), 5.58 (dd, 1H, J_1 = 16.1, J_2 = 6.2 Hz, H-4), 4.53 (ddd, 1H, J_1 =10.2, J_2 =4.4, J_3 = 2.7 Hz, H-5), 3.96-3.88(m, 2H, H-6, H-6), 3.55 (dd, 1H, J_1 = 10.4, J_2 = 4.4 Hz, H-2); **'Ring II':** δ_H 5.32 (t, 1H, J = 9.9 Hz, H-6), 4.20 (t, 1H, J = 9.2 Hz, H-5), 4.00 (t, 1H, J = 9.5 Hz, H-4), 3.71 (ddd, 1H, J_1 =12.3,

J_2 =10.0, J_3 = 4.7 Hz, H-1), 3.65 (ddd, 1H, J_1 =14.7, J_2 = 7.3, J_3 = 3.5 Hz, H-3), 2.46 (dt, 1H, J_1 = 13.3, J_2 = 4.6 Hz, H-2eq), 1.69 (ddd, 1H, J_1 = J_2 = J_3 = 12.9 Hz, H-2ax); **'Ring III'**: δ_H 5.68 (d, 1H, J = 2.2 Hz, H-1), 5.43 (dd, 1H, J_1 = 5.1, J_2 = 2.2 Hz, H-2), 5.32 (dd, 1H, J_1 = 6.4, J_2 = 5.0 Hz, H-3), 4.29 (dd, 1H, J_1 = 6.7, J_2 = 2.6 Hz, H-4), 3.70 (ddd, 1H, J_1 = 7.4, J_2 = 5.7, J_3 = 1.8 Hz, H-5), 3.65 (dt, 1H, J_1 = 13.2, J_2 = 5.8 Hz, H-5); *The additional peaks in the spectrum were identified as follow*: δ_H 8.06 – 8.01 (m, 3H, Ar), 7.98 – 7.96 (m, 2H, Ar), 7.93 – 7.89 (m, 3H, Ar), 7.70 (dd, 2H, J_1 = 8.3, J_2 = 1.2 Hz, Ar), 7.61 (dd, 2H, J_1 = 8.3, J_2 = 1.2 Hz, Ar), 7.60 – 7.56 (m, 5H, Ar), 7.55 – 7.46 (m, 1H, Ar), 7.43 (dd, 1H, J_1 = 10.9, J_2 = 4.7 Hz, Ar), 7.39 – 7.29 (m, 3H, Ar), 7.26 – 7.20 (m, 3H, Ar), 1.12 – 0.98 (m, 21H, TIPS). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 166.0 (C_6H_5-CO), 165.5 (C_6H_5-CO), 165.3 (C_6H_5-CO), 165.2 (C_6H_5-CO), 165.1 (C_6H_5-CO), 133.7 (Ar), 133.6 (Ar), 133.59 (Ar), 133.50 (Ar), 130.04 (Ar), 130.02 (A), 129.97 (Ar), 129.90 (Ar), 129.85 (Ar), 129.81 (Ar), 128.85 (Ar), 128.75 (Ar), 128.75 (Ar), 128.70 (Ar), 128.64 (Ar), 128.60 (Ar), 128.57 (Ar), 128.47 (Ar), 106.7 (C1"), 96.8 (C1'), 80.9 (C5), 80.7 (C4"), 76.1 (C4), 75.6 (C3"), 75.3 (C2"), 75.1 (C6), 71.5 (C5'), 71.1 (C3'), 69.3 (C4'), 62.8 (C6'), 61.7 (C2'), 59.7 (C3), 58.3 (C1), 52.8 (C5"), 32.1 (C2), 18.0 (TIPS), 12.0 (TIPS).



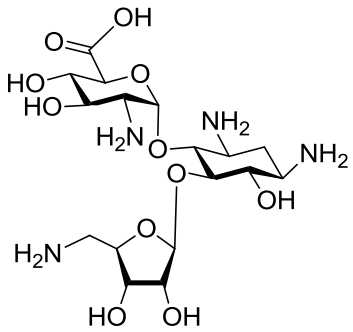
21

<u>Compound 21:</u> A stirred solution of compound **19** (1.71 g, 1.38 mmol) under argon in dry pyridine (10 mL) was cooled to 0-4^{o}C in a polyethylene vessel. To the stirred reaction, the solution of HF in pyridine (4.5 mL) was added dropwise. The progress of the reaction was monitored by TLC (EtOAc/Hexane 3:7), which indicated completion after 3.5 h. The reaction mixture was diluted with EtOAc and extracted with H_2O, $NaHCO_3$ (sat.). The organic layers again washed with HCl (2%), $NaHCO_3$ (sat.), and brine. The combined organic layer was dried over $MgSO_4$, evaporated to dryness, and purified by column chromatography (silica gel, EtOAc/Hexane) to yield corresponding primary alcohol (0.825 g, 76% over 2 steps). ^{1}H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 6.09 (d, 1H, J = 3.8, H-1), 6.01 (dd, 1H, J_1 = 10.7, J_2 = 9.5 Hz, H-3), 5.43 (dd, 1H, J_1 = 10.3, J_2 = 9.3 Hz, H-4), 4.39 (dt, 1H, J_1

=10.5, J_2 =3.0 Hz, H-5), 3.80 (d, 1H, J = 11.2 Hz, H-6), 3.64 (d, 1H, J = 11.6 Hz, H-6), 3.52 (dd, 1H, J_1 = 10.6, J_2 = 4.2 Hz, H-2); **'Ring II':** δ_H 5.28 (t, 1H, J = 9.9 Hz, H-6), 4.11 (t, 1H, J = 9.3 Hz, H-5), 3.87 (t, 1H, J = 9.5 Hz, H-4), 3.60 (ddd, 2H, J_1 = 22.3, J_2 = 11.8, J_3 = 4.8 Hz, H-1, H-3), 2.41 (dt, 1H, J_1 = 12.7, J_2 = 4.5 Hz, H-2eq), 1.66 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.9 Hz, H-2ax); **'Ring III'**: δ_H 5.61 (d, 1H, J = 2.4 Hz, H-1), 5.37 (dd, 1H, J_1 = 5.2, J_2 = 2.4 Hz, H-2), 5.22 (dd, 1H, J_1 = 5.9, J_2 = 5.4 Hz, H-3), 4.22 (td, 1H, J_1 = 5.8, J_2 = 3.1 Hz, H-4), 3.60 (dq, 2H, J_1 = 13.3, J_2 = 4.3Hz, H-5, H-5); *The additional peaks in the spectrum were identified as follow:* δ_H 8.0 – 7.97 (m, 2H, Ar), 7.90 (ddt, 4H, J_1 = 9.7, J_2 = 8.5, J_3 = 1.6 Hz, Ar), 7.69 – 7.66 (m, 2H, Ar), 7.55 – 7.52 (m, 2H, Ar), 7.48-7.39 (m, 4H, Ar), 7.33-7.27 (m, 4H, Ar), 7.25 (dd, 2H, J_1 = 10.9, J_2 = 4.8 Hz, Ar), 7.19-7.14 (m, 5H, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 166.3 (C_6H_5-CO), 165.8 (C_6H_5-CO), 165.07 (2 x C_6H_5-CO), 165.03 (C_6H_5-CO), 133.8 (Ar), 133.6 (Ar), 133.5 (Ar), 133.4 (Ar), 130.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.1 (Ar), 128.8 (Ar), 128.74 (Ar), 128.70 (Ar), 128.6 (Ar), 128.5 (Ar), 128.46 (Ar), 128.44 (Ar), 128.41 (Ar), 106.5 (C1"), 97.2 (C1'), 80.8 (C5), 80.4 (C4"), 76.9 (C4), 75.02 (C2"), 75.01 (C6), 71.5, 71.3 (C3"), 70.9 (C5'), 70.4 (C3'), 69.2 (C4'), 61.7 (C2'), 61.0 (C6'), 59.6 (C3), 58.5 (C1), 52.9 (C5"), 32.0 (C2).

The primary alcohol product from the above step (0.802 g, 0.743 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled to 5 ^oC. Then water (5.0 mL), TEMPO (23 mg, 0.147 mmol), and BIAB (598 mg, 1.85 mmol) were added. The reaction mixture was stirred at 5 ^oC for 40 min and then allowed slowly to warm to room temperature. The progress of the reaction was monitored by TLC which indicated completion after 4.5 h. The reaction mixture was diluted with EtOAc and quenched with $Na_2S_2O_3$ and washed with brine. The combined organic layer was dried over $MgSO_4$, evaporated to dryness, and purified by column chromatography (silica gel, EtOAc/Hexane) to yield acid **21** (0.775 g, 92%). ^{1}H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 6.04 (d, 1H, J = 3.2 Hz, H-1), 5.91(dd, 1H, J_1 = 9.9, J_2 = 9.3 Hz, H-3), 5.62 (t, 1H, J = 9.4 Hz, H-4), 5.15 (d, 1H, J =9.8 Hz, H-5), 3.67 (dd, 1H, J_1 = 10.3, J_2 = 3.5 Hz, H-2); **'Ring II':** δ_H 5.27 (t, 1H, J = 9.8 Hz, H-6), 4.08 (t, 1H, J = 9.1 Hz, H-5), 3.89 (t, 1H, J = 9.2 Hz, H-4), 3.64-3.57 (m, 2H, H-1, H-3), 2.40 (dt, 1H, J_1 = 8.7, J_2 = 4.6 Hz, H-2eq), 1.70 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.6 Hz, H-2ax); **'Ring III'**: δ_H 5.59 (d, 1H, J = 1.9 Hz, H-1), 5.37 (dd, 1H, J_1 = 5.1, J_2 = 1.9 Hz, H-2), 5.19 (dd, 1H, J_1 = 6.5, J_2 = 1.4 Hz, H-3), 4.18 (td, 1H, J_1 = 5.7, J_2 = 3.8 Hz, H-4), 3.56-3.50 (m, 2H, H-5, H-5); *The additional peaks in the spectrum were identified as follow:* δ_H 8.00 – 7.96 (m, 2H, Ar), 7.90 (d, 2H, J = 7.5 Hz, Ar), 7.86 (d, 2H, J = 7.6 Hz, Ar), 7.67 (d, 2H, J = 7.2 Hz, Ar),

7.54 – 7.51 (m, 2H, Ar), 7.46 – 7.36 (m, 4H, Ar), 7.29 – 7.22 (m, 6H, Ar), 7.18 – 7.12 (m, 5H, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C170.54 [(OH)C=O], 165.7 (C_6H_5-CO), 165.4 (C_6H_5-CO), 165.14 (C_6H_5-CO), 165.10 (C_6H_5-CO), 165.01 (C_6H_5-CO), 133.59 (Ar), 133.58 (Ar), 133.52 (Ar), 133.49 (Ar), 133.48 (Ar), 130.1 (Ar), 130.06 (Ar), 130.05 (Ar), 129.8 (Ar), 129.7 (Ar), 129.0 (Ar), 128.9 (Ar), 128.8 (Ar), 128.7 (Ar), 128.6 (Ar), 128.56 (Ar), 128.53 (Ar), 128.48 (Ar), 128.44 (Ar), 128.40 (Ar), 106.7 (C1”), 97.5 (C1’), 80.8 (C4”), 80.1 (C5), 78.0 (C4), 75.1 (C2”), 74.9 (C6), 71.6 (C3”), 70.3 (C3’), 69.6 (C4’, C5’), 61.0 (C2’), 59.0 (C3), 58.5 (C1), 52.9 (C5”), 31.7 (C2).

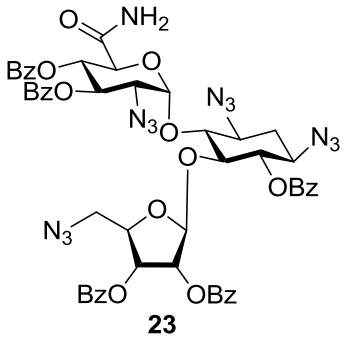


8

<u>Compound 8:</u> To a stirred solution of compound **21** (0.4 g, 0.365 mmol) in dry MeOH (10 mL) was added NaOMe (158 mg, 2.92 mmol) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 1.0 hr and then allowed slowly to warm to 60 ^{o}C temperature. The progress of the reaction was monitored by TLC (EtOAc/MeOH 8:2), which indicated completion after 4 h. The reaction mixture was passed through a silica gel column and product contained fraction were combined, evaporated. The crude product was purified by column chromatography (silica gel, EtOAc/MeOH) to yield corresponding deprotected compound (177 mg, 89%). ^{1}H NMR (600 MHz, MeOD): **‘Ring I’**: δ_H 5.87 (s, 1H, H-1), 4.36 (dd, 1H, J_1 = 7.4, J_2 = 3.3 Hz, H-5), 3.97 (t, 1H, J = 9.9 Hz, H-3), 3.58-3.52(m, 1H, H-4), 3.21 (dd, 1H, J = 8.8 Hz, H-2); **‘Ring II’:** δ_H 3.74 - 3.63 (m, 2H, H-5, H-6), 3.69 (dd, 2H, J_1 = 9.1, J_2 = Hz, H-4, H-3), 3.48-3.41 (m, 1H, H-1), 3.34 – 3.31 (m, 1H, H-2eq), 2.24 - 2.16(m, 1H, H-2ax); **‘Ring III’**: δ_H 5.41 (s, 1H, H-1), 4.20 (d, 1H, J = 5.4 Hz, H-2), 4.12 (t, 1H, J = 7.4 Hz, H-3), 4.06 (s, 1H, H-4), 3.58-3.52 (m, 2H, H-5, H-5). ^{13}C NMR (150 MHz, MeOD): δ_C 178.22 [(OH)C=O], 109.4 (C1”), 97.8 (C1’), 84.6 (C6), 82.3 (C4”), 76.8, 76.5 (C2”), 73.8 (C4’), 72.3 (C3’), 72.3 (C3”), 72.1 (C5’), 64.2 (C2’), 61.6, 61.2, 59.0, 58.5, 53.9 (C5”), 32.9 (C2).

To a stirred solution of the product from the above step (325 mg, 0.595 mmol) in a mixture of

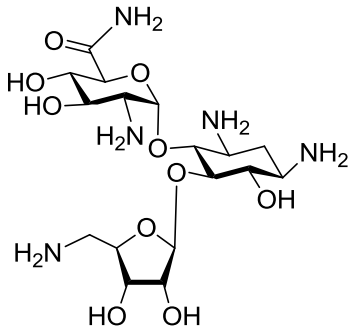
THF (3.0 mL) and aqueous NaOH (1 mM, 5.0 mL), PMe_3 (1 M solution in THF, 5.0 mL, 7.8 equiv.) was added. The progress of the reaction was monitored by TLC [CH_2Cl_2/MeOH/H_2O/$MeNH_2$(33% solution in EtOH), 10:15:6:15], which indicated completion after 4.0 h. The reaction mixture was purified by flash chromatography on a short column of silica gel. The column was washed with the following solvents: THF (100 mL), CH_2Cl_2 (100 mL), EtOH (50 mL), and MeOH (100 mL). The product was then eluted with the mixture of 5% $MeNH_2$ solution (33% solution in EtOH) in 80% MeOH. Fractions containing the product were combined and evaporated under vacuum. The pure product was obtained by passing the above product through a short column of Amberlite CG50 (NH_4^+ form). First, the column was washed with water, then the product was eluted with a mixture of 10% NH_4OH in water to yield the 6'-acid **8** (161 mg, 58%). For the storage and biological tests, compound was converted to its sulfate salt form as follow. The free base form was dissolved in water, the pH was adjusted to 7.0 with H_2SO_4 (0.1 N) and lyophilized to afford the sulfate salt of the acid **8**. ^{1}H NMR (600 MHz, D_2O): **'Ring I'**: δ_H 5.38 (s, 1H, H-1), 4.09 (d, 1H, J = 9.4 Hz, H-5), 3.64 (dd, 1H, J_1 = 13.2, J_2 =6.1 Hz, H-3), 3.54 (t, 1H, J =8.8 Hz, H-4), 2.85 (d, 1H, J = 5.3Hz, H-2); **'Ring II':** δ_H 3.71 (t, 1H, J = 8.9 Hz, H-5), 3.54 (t, 1H, J = 9.6 Hz, H-4), 3.36 (t, 1H, J = 10.6 Hz, H-6), 2.98 (d, 1H, J = 10.2 Hz, H-3), 2.87 (d, 1H, J = 9.8 Hz, H-1), 2.04 (dd, 1H, J_1 = 12.6, J_2 = 2.9 Hz, H-2eq), 1.31 (ddd, 1H, J_1 = J_2 = J_3 = 12.0 Hz, H-2ax); **'Ring III'**: δ_H 5.31 (s, 1H, H-1), 4.23-4.17 (m, 1H, H-2), 4.14 (dd, 1H, J_1 = 3.9, J_2 = 2.5 Hz, H-3), 4.05-4.00 (m, 1H, H-4), 3.21 (d, 1H, J = 13.3 Hz, H-5), 3.02 (t, 1H, J = 11.8 Hz, H-5). ^{13}C NMR (150 MHz, D_2O): δ_C 176.4 [(OH)C=O], 109.1 (C1"), 98.7 (C1'), 83.7 (C5), 82.4 (C4), 80.1 (C4"), 76.0 (C6), 74.7 (C2"), 73.7 (C5'), 72.9 (C3'), 72.1 (C4'), 71.1(C3"), 54.39 (C2'), 50.4 (C3), 49.9 (C1), 42.4 (C5"), 34.0 (C2).



23

Compound 23: To a cooled solution of anhydrous CH_2Cl_2 (10 mL) at -30 °C, oxalyl chloride (0.878 mL, 10.37 mmol) and DMF (0.795 mL, 10.32 mmol) were added successively and then allowed slowly to reach 0 °C in 1.5 h. The reaction mixture was again cooled to -30 °C and compound **21** (0.896, 0.819 mmol) dissolved in anhydrous CH_2Cl_2 (3 mL) was added dropwise. The reaction was allowed slowly to

reach 0 °C in about 1.5 h. Then, the mixture was cooled to -78 °C and the gaseous ammonia (NH_3) was bubbled carefully into the reaction vessel. The excess ammonia was evaporated from the flask by allowing the reaction to reach to room temperature. The residue was then diluted with ethyl acetate and washed with $NaHCO_3$ solution, brine. The organic layer was evaporated to dryness, and purified by column chromatography to yield the corresponding amide derivative **23** (0.615g, 69%). ^{1}H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 6.09 (d, 1H, *J* = 3.4 Hz, H-1), 5.92 (dd, 1H, J_1 = 10.6, J_2 = 9.4 Hz, H-3), 5.62-5.58 (m, 1H, H-4), 4.94 (d, 1H, *J* =10.3 Hz, H-5), 3.54 (dd, 1H, J_1 = 13.3, J_2 = 5.6 Hz, H-2); **'Ring II':** δ_H 5.28 (t, 1H, *J* = 9.9 Hz, H-6), 4.10 (t, 1H, *J* = 9.2 Hz, H-5), 3.86 (t, 1H, *J* = 9.4 Hz, H-4), 3.60 (ddd, 1H, J_1 = 16.4, J_2 = 13.8, J_3 = 5.1 Hz, H-1, H-3), 2.39 (dt, 1H, J_1 = 13.3, J_2 = 4.6 Hz, H-2eq), 1.67 (ddd, 1H, $J_1 = J_2 = J_3$ = 13.1 Hz, H-2ax); **'Ring III'**: δ_H 5.60 (s, 1H, H-1), 5.39 (dd, 1H, J_1 = 5.1, J_2 = 2.0 Hz, H-2), 5.21 (dd, 1H, J_1 = 6.3, J_2 = 5.2 Hz, H-3), 4.22 (td, 1H, J_1 = 5.9, J_2 = 2.9 Hz, H-4), 3.61 (d, 1H, *J* = 12.8 Hz, H-5), 3.54 (dd, 1H, J_1 = 13.6, J_2 = 4.4 Hz, H-5); *The additional peaks in the spectrum were identified as follow:* δ_H 8.00 – 7.95 (m, 2H, Ar), 7.91-7.88 (m, 4H, Ar), 7.70-7.66 (m, 2H, Ar), 7.54 (dt, 2H, J_1 = 8.4, J_2 = 1.5 Hz, Ar), 7.47-7.37 (m, 4H, Ar), 7.31 – 7.23 (m, 6H, Ar), 7.19 – 7.16 (m, 2H, Ar), 7.16 – 7.12 (m, 3H, Ar), 6.51(d, 1H, *J* = 2.7 Hz, -NH), 6.51(d, 1H, *J* = 2.6 Hz, -NH). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 169.5 [$(NH_2)C$=O], 165.7 (C_6H_5-CO), 165.5 (C_6H_5-CO), 165.1 (C_6H_5-CO), 165.09 (C_6H_5-CO), 165.04 (C_6H_5-CO), 133.6 (Ar), 133.55 (Ar), 133.53 (Ar), 133.5 (Ar), 133.2 (Ar), 130.06 (Ar), 130.0 (Ar), 129.8 (Ar), 129.7 (Ar), 129.3 (Ar), 129.0 (Ar), 128.7 (Ar), 128.65 (Ar), 128.62 (Ar), 128.5 (Ar), 128.45 (Ar), 128.41 (Ar), 128.35 (Ar), 106.8 (C1"), 97.2 (C1'), 80.7 (C4"), 80.4 (C5), 77.6 (C4), 75.0 (C2"), 74.9 (C6), 71.4 (C3"), 70.3 (C3'), 69.8 (C4'), 69.3(C5'), 61.4 (C2'), 59.1 (C3), 58.4 (C1), 52.7 (C5"), 31.8 (C2).



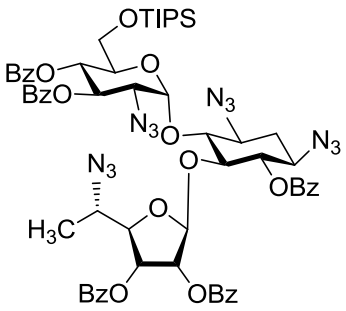
9

Compound 9: To a stirred solution of compound **23** (0.541 g, 0.495 mmol) in dry MeOH (10 mL, 0 °C), NaOMe (214 mg, 3.96 mmol) was added. The reaction mixture was stirred at 0 °C for 1.0 hr and then allowed slowly to warm to 60 °C. The progress of the reaction was monitored by TLC

(EtOAc/MeOH 8:2), which indicated completion after 4 h. The reaction mixture was passed through a silica gel column and product contained fraction were combined, evaporated. The crude product was purified by column chromatography (silica gel, EtOAc/MeOH) to yield the corresponding amide alcohol (283 mg, ~100%). ^{1}H NMR (600 MHz, MeOD): **'Ring I'**: δ_H 5.91 (d, 1H, *J* = 3.6 Hz, H-1), 4.38 (d, 1H, *J* = 10.1Hz, H-5), 3.96 (t, 1H, *J* = 9.7 Hz, H-3), 3.57-3.50 (m, 1H, H-4), 3.19 (dd, 1H, J_1 = 10.5, J_2 = 3.7 Hz, H-2); **'Ring II':** δ_H 3.72 (t, 1H, *J* = 9.2 Hz, H-6), 3.66 (t, 1H, *J* = 8.9 Hz, H-5), 3.53 (dd, 1H, J_1 = 17.8, J_2 = 7.2 Hz, H-4, H-1), 3.46 – 3.39 (m, 1H, H-3), 2.22 – 2.16 (m, 1H, H-2eq), 1.38 (ddd, 1H, J_1 = J_2 = J_3 = 12.9 Hz, H-2ax); **'Ring III'**: δ_H 5.39 (s, 1H, H-1), 4.18 (d, 1H, *J* = 4.7 Hz, H-2), 4.08 (dd, 1H, J_1 = 7.0, J_2 = 4.9 Hz, H-3), 4.03 (td, 1H, J_1 = 6.8, J_2 = 3.2 Hz, H-4), 3.58 (d, 1H, *J* = 10.9 Hz, H-5), 3.51 (dd, 1H, J_1 = 13.1, J_2 = 6.5 Hz, H-5). ^{13}C NMR (150 MHz, MeOD): δ_C 178.0 [(NH_2) C=O], 110.1 (C1"), 97.8 (C1'), 84.8 (C5), 82.3 (C4"), 77.1, 76.9 (C6), 76.4 (C2"), 73.9 (C4') 72.5 (C3"), 72.2 (C3'), 72.0 (C5'), 64.4 (C2'), 61.7 (C3), 61.2 (C1), 54.3 (C5"), 33.0 (C2).

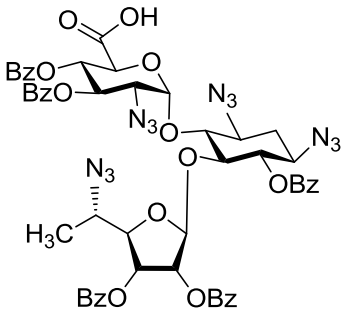
To a stirred solution of the amide alcohol product from the above step (281 mg, 0.491 mmol), in a mixture of THF (3.0 mL) and aqueous NaOH (1 mM, 5.0 mL), PMe_3 (1 M solution in THF, 4.5 mL, 7.8 equiv.) was added. The progress of the reaction was monitored by TLC [CH_2Cl_2/MeOH/H_2O/$MeNH_2$ (33% solution in EtOH), 10:15:6:15], which indicated completion after 4.0 h. The reaction mixture was purified by flash chromatography on a short column of silica gel. The column was washed with the following solvents: THF (100 mL), CH_2Cl_2 (100 mL), EtOH (50 mL), and MeOH (100 mL). The product was then eluted with the mixture of 5% $MeNH_2$ solution (33% solution in EtOH) in 80% MeOH. Fractions containing the product were combined and evaporated under vacuum. The pure product was obtained by passing the above product through a short column of Amberlite CG50 (NH_4^+ form). First, the column was washed with water, then the product was eluted with a mixture of 10% NH_4OH in water to yield the 6'-amide **9** (106 mg, 47%). For the storage and biological tests, compound was converted to its sulfate salt form as follow. The free base form was dissolved in water, the pH was adjusted to 7.0 with H_2SO_4 (0.1 N) and lyophilized to afford the sulfate salt of the compound **9**. ^{1}H NMR (600 MHz, D_2O): **'Ring I'**: δ_H 5.35 (d, 1H, *J* = 3.6 Hz, H-1), 4.07 (d, 1H, *J* = 9.7 Hz, H-5), 3.62 (t, 1H, *J* = 9.5 Hz, H-3), 3.55-3.49 (m, 1H, H-4), 2.82 (dd, 1H, J_1 = 9.9, J_2 = 3.6 Hz, H-2); **'Ring II':** δ_H 3.69 (t, 1H, *J* = 9.1 Hz, H-5), 3.52 (t, 1H, *J* = 9.7 Hz, H-4), 3.34 (t, 1H, *J* = 10.0 Hz, H-6), 2.96 (t, 1H, *J* = 8.4 Hz, H-3), 2.85 (t, 1H, *J* = 8.3 Hz, H-1),2.02 (dt, 1H, J_1 = 8.2, J_2 = 3.9 Hz, H-2eq), 1.29 (ddd, 1H, J_1 = J_2 = J_3 = 12.4 Hz, H-2ax); **'Ring III'**: δ_H 5.29 (d, 1H, *J* = 1.7 Hz, H-1), 4.18 (dd, 1H, J_1 = 4.9, J_2 = 1.9 Hz, H-2), 4.12 (dd, 1H, J_1 = 6.6, J_2 = 5.2 Hz, H-3), 4.01 (td, 1H, J_1 = 7.4, J_2 = 3.5 Hz, H-4), 3.18 (dd, 1H, J_1 = 13.2, J_2 = 3.8 Hz, H-5), 3.00 (dd, 1H, J_1 = 13.2, J_2 = 8.6

Hz, H-5). ^{13}C NMR (150 MHz, D_2O): δ_C 176.4 [(NH_2) C=O], 109.0 (C1”), 98.8 (C1’), 83.7 (C5), 82.6 (C4’), 80.2 (C4”), 76.0 (C6), 74.7 (C2”), 73.7 (C5’), 73.0 (C3’), 72.1 (C4), 71.1 (C3”), 55.0 (C2’), 50.4 (C1), 50.0 (C3), 42.5 (C5”), 34.1 (C2).



20

Compound 20: Anhydrous CH_2Cl_2 (20 mL) was added to a powdered, flame-dried 4 Å molecular sieves (2.0 g), followed by the addition of acceptor (**16**) (1.6 g, 1.84 mmol) and donor (**18**) (3.26 g, 6.01 mmol). The reaction mixture was stirred for 10 min at room temperature and was then cooled to -30°C. At this temperature, catalytic amount of BF_3-Et_2O (0.2 mL) was added and the mixture was stirred at -30 °C and the reaction progress was monitored by TLC, which indicated the completion after 60 min. The reaction mixture was diluted with ethyl acetate and washed with saturated $NaHCO_3$ and brine. The combined organic layer was dried over $MgSO_4$, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the glycosylation product **20**.



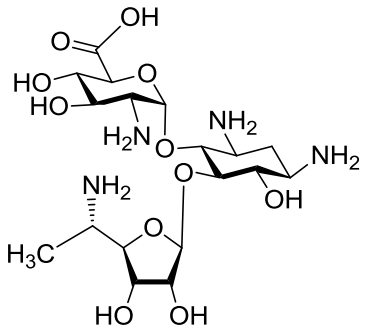
22

Compound 22: A stirred solution of the glycosylation product **20** (2.225 g, 1.78 mmol) in dry pyridine (10 mL) was cooled to 0-4^{o}C in a polyethylene vessel. The solution of HF in pyridine (10 mL) was added dropwise to the cooled reaction mixture. The progress of the reaction was monitored by TLC (EtOAc/Hexane 3:7), which indicated completion after 6.0 h. The reaction mixture was diluted with EtOAc and extracted with H_2O, $NaHCO_3$ (sat.). The organic layers again washed with HCl (2%), $NaHCO_3$ (sat.), and brine. The combined organic layer was dried over $MgSO_4$, evaporated to dryness,

and purified by column chromatography (silica gel, EtOAc/Hexane) to yield corresponding primary alcohol (1.34 g, 67% over 2 steps). ^{1}H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 6.18 (d, 1H, *J* = 3.8, H-1), 6.08 (dd, 1H, J_1 = 10.6, J_2 = 9.6 Hz, H-3), 5.51 (t, 1H, *J* = 9.9 Hz, H-4), 4.45 (dt, 1H, J_1 =10.3, J_2 =2.7 Hz, H-5), 3.87 (d, 1H, *J* = 12.7 Hz, H-6), 3.72 (d, 1H, *J* = 12.6 Hz, H-6), 3.66 (dd, 1H, J_1 = 10.4, J_2 = 4.5 Hz, H-2); **'Ring II':** δ_H 5.34 (t, 1H, *J* = 9.7 Hz, H-6), 4.22 (t, 1H, *J* = 9.1 Hz, H-5), 3.95 (dd, 1H, J_1 = 9.7, J_2 =8.8 Hz, H-4), 3.73 – 3.63 (m, 2H, H-1, H-3), 2.48 (dt, 1H, J_1 = 13.3, J_2 = 4.6 Hz, H-2eq), 1.75 (ddd, 1H, J_1 = J_2 = J_3 = 12.7 Hz, H-2ax); **'Ring III'**: δ_H 5.66 (s, 1H, H-1), 5.45 – 5.41 (m, 2H, H-2, H-3), 4.11 (t, 1H, *J* = 5.4 Hz, H-4), 3.81 – 3.72 (m, 1H, H-5), 1.31 (d, 3H, *J* = 6.8Hz, 6-CH_3); *The additional peaks in the spectrum were identified as follow:* δ_H 8.08 (dt, 2H J_1 = 3.9, J_2 = 2.3 Hz, Ar), 7.99 – 7.94 (m, 4H, Ar), 7.75 – 7.72 (m, 2H, Ar), 7.66 – 7.63 (m, 2H, Ar), 7.54 – 7.45 (m, 4H, Ar), 7.37 (ddd, 4H, J_1 = 9.5, 2_1 = 8.7, J_3 = 4.8 Hz, Ar), 7.32 (t, 2H, *J* = 7.8 Hz, Ar), 7.27 – 7.22 (m, 5H, Ar), 2.58 (brs, 1H, 6'-OH). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 166.3 (C_6H_5-CO), 165.7 (C_6H_5-CO), 165.1 (C_6H_5-CO), 164.9 (C_6H_5-CO), 164.7 (C_6H_5-CO), 133.7 (Ar), 133.5 (Ar), 133.47 (Ar), 133.43 (Ar), 130.17 (Ar), 130.12 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.1 (Ar), 128.86 (Ar), 128.80 (Ar), 128.7 (Ar), 128.6 (Ar), 128.56 (Ar), 128.52 (Ar), 128.3 (Ar), 106.7 (C1"), 97.0 (C1'), 83.8 (C4"), 80.3 (C5), 77.1 (C4), 75.3 (C2"), 75.0 (C6), 71.1 (C5'), 70.9 (C3"), 70.6 (C3'), 69.3 (C4'), 61.8 (C2'), 61.0 (C6'), 59.6 (C3), 58.6 (C1), 58.3 (C5"), 31.9 (C2), 15.65 (6"-CH_3).

The primary alcohol product from the above step (1.3 g, 1.19 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled to 5 ^{o}C. To the stirred solution, the water (5.0 mL), TEMPO (37 mg, 0.236 mmol), and BIAB (958 mg, 2.97 mmol) were added. The reaction mixture was stirred at 5 ^{o}C for 40 min and then allowed slowly to warm to room temperature. The progress of the reaction was monitored by TLC which indicated completion after 4.5 h. The reaction mixture was diluted with EtOAc and quenched with $Na_2S_2O_3$ and washed with brine. The combined organic layer was dried over $MgSO_4$, evaporated to dryness, and purified by column chromatography (silica gel, EtOAc/Hexane) to yield the acid **22** (1.23g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$): **'Ring I'**: δ_H 6.12 (d, 1H, *J* = 3.7 Hz, H-1), 5.97 (t, 1H, *J* = 9.6 Hz, H-3), 5.69 (t, 1H, *J* = 9.4 Hz, H-4), 5.20 (d, 1H, *J* =9.8 Hz, H-5), 3.83 (dd, 1H, J_1 = 10.1, J_2 = 3.7 Hz, H-2); **'Ring II':** δ_H 5.34 (t, 1H, *J* = 9.8 Hz, H-6), 4.20 (t, 1H, *J* = 9.2 Hz, H-5), 3.97 (t, 1H, *J* = 9.3 Hz, H-4), 3.70 (dt, 2H, J_1 = 17.7, J_1 = 7.8 Hz, H-1, H-3), 2.49 (dt, 1H, J_1 = 13.2, J_2 = 4.4 Hz, H-2eq), 1.78 (ddd, 1H, J_1 = J_2 = J_3 = 12.7 Hz, H-2ax); **'Ring III'**: δ_H 5.64 (s, 1H, H-1), 5.43 (d, 1H, *J* = 5.2 Hz, H-2), 5.38 (dd, 1H, J_1 = 7.7, J_2 = 5.2 Hz, H-3), 4.11 (dt, 1H, J_1 = 12.4, J_2 = 6.7 Hz, H-4), 3.71(p, 1H, *J* = 6.8 Hz, H-5), 1.25 (d, 3H, *J* = 6.7 Hz, 6-CH_3*); The additional peaks in the spectrum were identified as follow:* δ_H 8.12 – 8.06 (m, 2H, Ar), 7.96 (dd, 4H, J_1 = 12.5, J_2 = 7.6 Hz, Ar), 7.74 (d,

2H, *J* = 7.5 Hz, Ar), 7.63 (d, 2H, *J* = 7.6 Hz, Ar), 7.53 – 7.43 (m, 4H, Ar), 7.33 (dt, 6H, J_1 = 15.6, J_2 = 7.5 Hz, Ar), 7.25 (q, 5H, *J* = 8.2 Hz, Ar). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 170.5 [(OH)C=O], 165.6 (C_6H_5-CO), 165.4 (C_6H_5-CO), 165.2 (C_6H_5-CO), 165.0 (C_6H_5-CO), 164.6 (C_6H_5-CO), 133.58 (Ar), 133.56 (Ar), 133.49 (Ar), 133.42 (Ar), 130.1 (Ar), 130.06 (Ar), 130.02 (Ar), 129.8 (Ar), 129.6 (Ar), 128.96 (Ar), 128.91 (Ar), 128.8 (Ar), 128.7 (Ar), 128.55 (Ar), 128.54 (Ar), 128.51 (Ar), 128.39 (Ar), 128.37 (Ar), 106.7 (C1”), 97.3 (C1’), 84.0 (C4”), 79.9 (C5), 78.3 (C4), 75.3 (C2”), 74.9 (C6), 71.1 (C3”), 70.4 (C3’), 69.6 (C4’), 69.1 (C5’), 61.2 (C2’), 59.0 (C5”), 58.6 (C1), 58.4 (C3), 31.6 (C2), 15.5 (6”-CH_3).

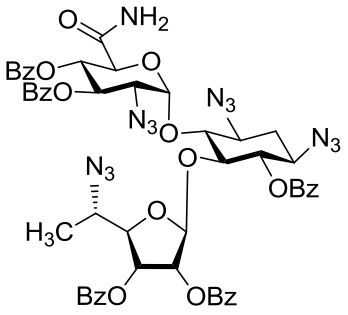


10

<u>Compound 10:</u> To a stirred solution of compound **22** (0.6 g, 0.542 mmol) in dry MeOH (15 mL, 0 ^oC), NaOMe (234 mg, 4.33 mmol) was added. The reaction mixture was stirred at 0 ^oC for 1.0 hr and then allowed slowly to warm to 60 ^oC. The progress of the reaction was monitored by TLC (EtOAc/MeOH 8:2), which indicated completion after 4 h. The reaction mixture was passed through a silica gel column and product contained fraction were combined, evaporated. The crude product was purified by column chromatography (silica gel, EtOAc/MeOH) to yield corresponding perazido acid (318 mg, 100%). ^{1}H NMR (600 MHz, MeOD): **‘Ring I’**: δ_H 5.88 (s, 1H, H-1), 4.36 (s, 1H, H-5), 4.00 (t, 1H, *J* = 9.2 Hz, H-3), 3.62-3.52 (m, 1H, H-4), 3.22 (d, 1H, *J* = 7.5 Hz, H-2); **‘Ring II’:** δ_H 3.77-7.71 (m, 1H, H-6), 3.68 (t, 1H, *J* = 8.3 Hz, H-5), 3.56 (dd, 1H, J_1 = 13.4, J_2 = 6.5 Hz, H-4, H-1), 3.46 (dd, 1H, J_1 = 19.3, J_2 = 7.1 Hz, H-3), 2.20 (dd, 1H, J_1 = 8.8, J_2 = 2.6 Hz, H-2eq), 1.37 (m, 1H, H-2ax); **‘Ring III’**: δ_H 5.41 (s, 1H, H-1), 4.20 (d, 1H, *J* = 11.4 Hz, H-2, H-3), 3.84 (s, 1H, H-4), 3.72 (d, 1H, *J* = 13.5 Hz, H-5), 1.37 (d, 3H, *J* = 6.5 Hz, 6-CH_3). ^{13}C NMR (150 MHz, MeOD): δ_C 178.2 [(OH) C=O], 108.8 (C1”), 97.7 (C1’), 86.0 (C4”), 84.5 (C6), 76.6 (C2”), 73.8 (C4’), 72.4 (C3”), 72.1 (C3’), 71.9 (C5’), 64.1 (C2’), 61.4 (C3), 61.2 (C1), 60.5 (C5”), 33.0 (C2), 16.3 (6”-CH_3).

To a stirred solution of the perazido acid product from the above step (318 mg, 0.542 mmol) in a mixture of THF (3.0 mL) and aqueous NaOH (1 mM, 5.0 mL), PMe_3 (1 M solution in THF, 5.0 mL,

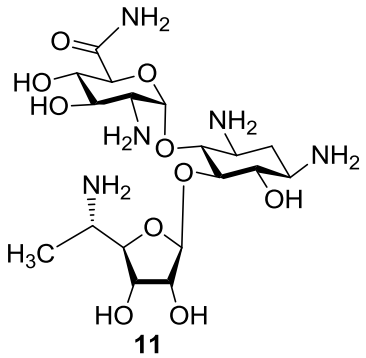
7.8 equiv.) was added. The progress of the reaction was monitored by TLC [CH_2Cl_2/MeOH/H_2O/$MeNH_2$ (33% solution in EtOH), 10:15:6:15], which indicated completion after 4.0 h. The reaction mixture was purified by flash chromatography on a short column of silica gel. The column was washed with the following solvents: THF (100 mL), CH_2Cl_2 (100 mL), EtOH (50 mL), and MeOH (100 mL). The product was then eluted with the mixture of 5% $MeNH_2$ solution (33% solution in EtOH) in 80% MeOH. Fractions containing the product were combined and evaporated under vacuum. The pure product was obtained by passing the above product through a short column of Amberlite CG50 (NH_4^+ form). First, the column was washed with water, then the product was eluted with a mixture of 10% NH_4OH in water to yield the 6'-acid **10** (190 mg, 72%). For the storage and biological tests, compound was converted to its sulfate salt form as follow. The free base form was dissolved in water, the pH was adjusted to 7.0 with H_2SO_4 (0.1 N) and lyophilized to afford the sulfate salt of the acid **10**. ^{1}H NMR (600 MHz, D_2O): **'Ring I'**: δ_H 5.41 (d, 1H, *J* =3.5 Hz, H-1), 4.10 (d, 1H, *J* = 9.6 Hz, H-5), 3.66 (t, 1H, *J* = 9.5 Hz, H-3), 3.60 – 3.53 (m, 1H, H-4), 2.83 (dd, 1H, J_1 = 10.0, J_2 = 3.6 Hz, H-2); **'Ring II':** δ_H 3.73 (t, 1H, *J* = 9.1 Hz, H-5), 3.57 (t, 1H, *J* = 9.4 Hz, H-4), 3.37 (t, 1H, *J* = 9.7 Hz, H-6), 2.98 (ddd, 1H, J_1 = 9.2, J_2 = 7.8, J_3 = 4.1Hz, H-3), 2.87 (ddd, 1H, J_1 = 10.0, J_2 = 8.2, J_3 = 4.5 Hz, H-1), 2.06 (dt, 1H, J_1 = 12.6, J_2 = 3.7 Hz, H-2eq), 1.33 (ddd, 1H, J_1 = J_2 = J_3 = 12.7 Hz, H-2ax); **'Ring III'**: δ_H 5.33 (d, 1H, *J* =1.1 Hz, H-1), 4.25-4.20 (m, 2H, H-2, H-3), 3.77 (dd, 1H, J_1 = 8.2, J_2 = 6.0 Hz, H-4), 3.31 (dq, 1H, J_1 = 13.5, J_2 = 6.6 Hz, H-5), 1.31 (d, 3H, *J* = 6.6 Hz, 6-CH_3). ^{13}C NMR (150 MHz, D_2O): δ_C 176.4 [(OH)C=O], 108.5 (C1"), 98.6 (C1'), 84.0 (C4"), 83.5 (C5), 82.3 (C4), 76.1 (C6), 75.0 (C3"), 73.8 (C5'), 73.1 (C3'), 72.2 (C4'), 71.4(C2"), 54.9 (C2'), 50.5 (C1), 50.2 (C3), 50.0 (C5"), 34.3 (C2), 15.8 (6"-CH_3).



24

Compound 24: To a cooled solution of anhydrous CH_2Cl_2 (10 mL) at -30 °C, oxalyl chloride (0.5 mL, 5.9 mmol) and DMF (0.5 mL, 6.49 mmol) were added successively and then allowed slowly to reach 0 °C in 1.5 h. The reaction mixture was cooled to -30 °C and the acid compound **22** (0.62, 0.56 mmol)

dissolved in anhydrous CH_2Cl_2 (3 mL) was added dropwise. The reaction was allowed slowly to reach 0 ^oC in 1.5 h. The mixture was then cooled to -78 ^oC and gaseous ammonia (NH_3) was bubbled carefully into the reaction vessel. The excess ammonia was allowed to evaporate from the flask by letting the reaction to warm to room temperature. The residue was then diluted with ethyl acetate and washed with $NaHCO_3$ solution, brine. The combined organic layers were evaporated to dryness and purified by column chromatography to yield the corresponding amide derivative **24** (0.44g, 71%). ^1H NMR (600 MHz, $CDCl_3$): **'Ring I'**: δ_H 6.13 (d, 1H, J = 3.8 Hz, H-1), 5.91 (dd, 1H, J_1 = 10.4, J_2 = 9.5 Hz, H-3), 5.61-5.58 (m, 1H, H-4), 4.91 (d, 1H, J =10.2 Hz, H-5), 3.65 (dd, 1H, J_1 = 10.0, J_2 = 4.7 Hz, H-2); **'Ring II':** δ_H 5.27 (t, 1H, J = 9.8 Hz, H-6), 4.13 (t, 1H, J = 9.2 Hz, H-5), 3.86 (t, 1H, J = 9.5 Hz, H-4), 3.61 (ddd, 1H, J_1 = 17.5, J_2 = 14.5, J_3 = 5.3 Hz, H-1, H-3), 2.39 (dt, 1H, J_1 = 9.0, J_2 = 4.2 Hz, H-2eq), 1.68 (ddd, 1H, J_1 = J_2 = J_3 = 13.1 Hz, H-2ax); **'Ring III'**: δ_H 5.58 (d, 1H, J = 0.6 Hz, H-1), 5.37 (dd, 1H, J_1 = 5.1, J_2 = 1.0 Hz, H-2), 5.34 (dd, 1H, J_1 = 7.1, J_2 = 5.1 Hz, H-3), 4.03 (dd, 1H, J_1 = 7.1, J_2 = 4.9 Hz, H-4), 3.64 (dt, 1H, J_1 = 13.4, J_1 = 6.7 Hz, H-5), 1.23 (d, 3H, J = 6.8 Hz, 6-CH_3); *The additional peaks in the spectrum were identified as follow:* δ_H 8.03 – 7.99 (m, 2H, Ar), 7.89 (dd, 4H, J_1 = 8.3, J_2 = 1.2 Hz, Ar), 7.67 (dd, 2H, J_1 = 8.3, J_2 = 1.2 Hz, Ar), 7.57 (dd, 2H, J_1 = 8.3, J_2 = 1.2 Hz, Ar), 7.45 – 7.36 (m, 4H, Ar), 7.25 (ddd, 6H, J_1 = 11.7, J_2 = 8.1, J_3 = 4.8 Hz, Ar), 7.18 – 7.14 (m, 5H, Ar), 6.46 (brs, 1H, -NH), 5.57 (brs, 1H, -NH). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 169.4 [$(NH_2)C=O$], 165.69 (C_6H_5-CO), 165.60 (C_6H_5-CO), 165.2 (C_6H_5-CO), 164.9 (C_6H_5-CO), 164.6 (C_6H_5-CO), 133.59 (Ar), 133.52 (Ar), 133.4 (Ar), 133.2 (Ar), 130.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 129.3 (Ar), 129.0 (Ar), 128.8 (Ar), 128.7 (Ar), 128.55 (Ar), 128.50 (Ar), 128.43 (Ar), 128.40 (Ar), 107.0 (C1"), 96.9 (C1'), 84.0 (C4"), 80.4 (C5), 77.9 (C4), 75.3 (C2"), 74.8 (C6), 71.1 (C3"), 70.5 (C3'), 69.9 (C4'), 69.3 (C5'), 61.6 (C2'), 59.2 (C3), 58.5 (C1), 58.2 (C5"), 31.7 (C2), 15.7 (6"-CH_3).



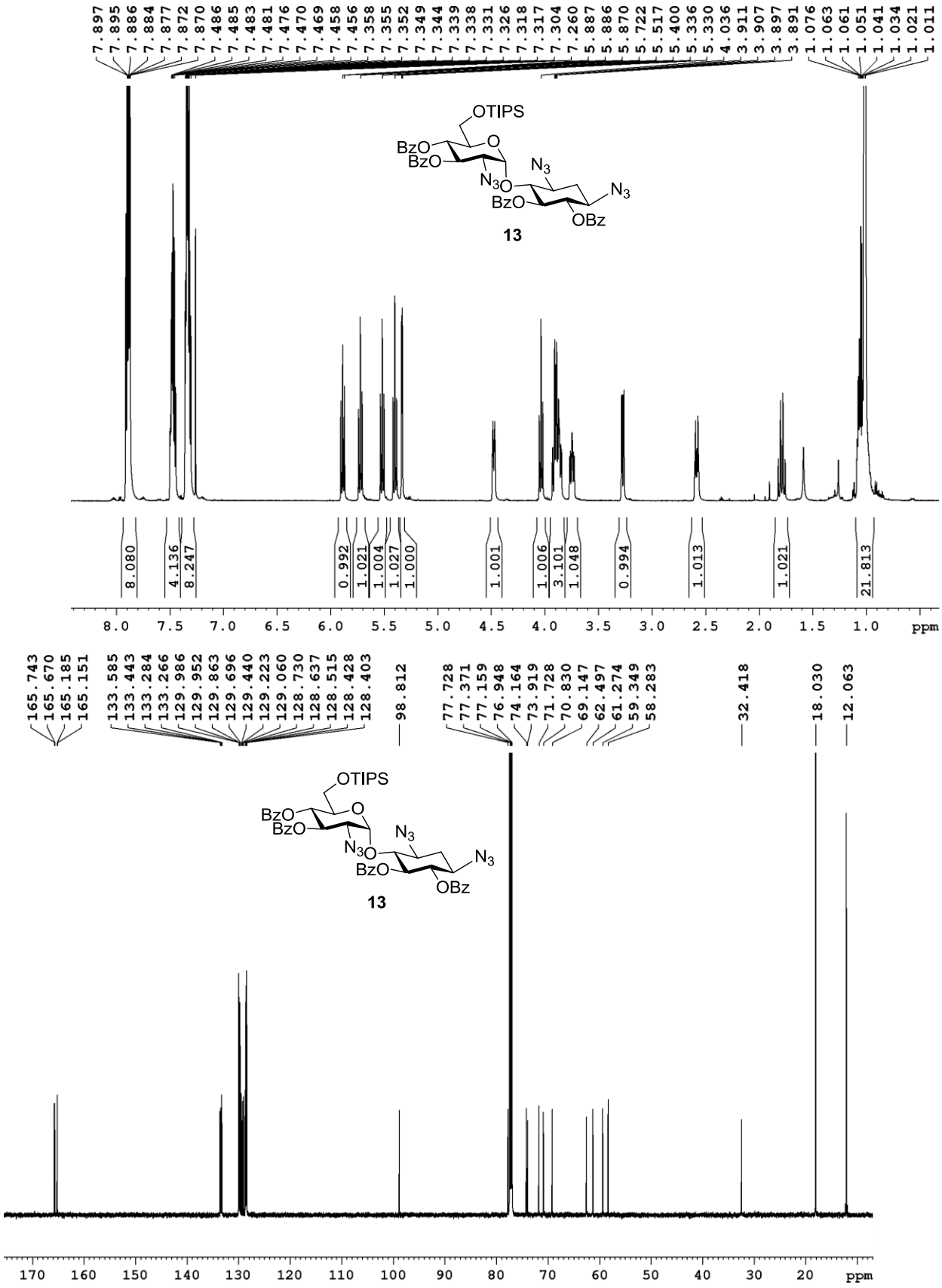
11

<u>Compound 11:</u> To a stirred solution of compound **24** (0.44 g, 0.398 mmol) in dry MeOH (15 mL, 0 ^oC), NaOMe (171 mg, 3.16 mmol) was added. The reaction mixture was stirred at 0 ^oC for 1.0 hr and

then allowed slowly to warm to 60 ºC. The progress of the reaction was monitored by TLC (EtOAc/MeOH 8:2), which indicated completion after 4 h. The reaction mixture was passed through a silica gel column and the fractions containing the product (TLC) were combined and evaporated to dryness. The crude product was purified by column chromatography (silica gel, EtOAc/MeOH) to yield corresponding perazido intermediate product (244 mg, ~ 100%). ^{1}H NMR (600 MHz, MeOD): **'Ring I'**: δ_H 5.88 (s, 1H, H-1), 4.39 (d, 1H, *J* = 9.4 Hz, H-5), 4.00 (t, 1H, *J* = 9.6 Hz, H-3), 3.60 -3.55 (m, 1H, H-4), 3.23 (d, 1H, *J* = 6.3 Hz, H-2); **'Ring II':** δ_H 3.76 (t, 1H, *J* = 6.4 Hz, H-6), 3.69 (dd, 1H, J_1 = 9.7, J_2 = 6.5 Hz, H-5), 3.58 - 3.53 (m, 2H, H-4, H-1), 3.52 – 3.43 (m, 1H, H-3), 2.22 (dd, 1H, J_1 = 8.6, J_2 = 3.5 Hz, H-2eq), 1.39 (ddd, 1H, $J_1 = J_2 = J_3$ = 11.8 Hz, H-2ax); **'Ring III'**: δ_H 5.42 (s, 1H, H-1), 4.25 – 4.13 (m, 2H, H-2, H-3), 3.86 – 3.81 (m, 1H, H-4), 3.76 – 3.69 (m, 1H, H-5), 1.38 (d, 3H, *J* = 6.7 Hz, 6-CH_3).^{13}C NMR (150 MHz, MeOD): δ_C 178.0 [(NH_2) C=O], 108.9 (C1"), 97.8 (C1'), 86.1 (C4"), 84.5 (C5), 76.68 (C6), 76.61 (C2"), 73.8 (C4'), 72.4 (C3'), 72.2 (C3"), 71.9 (C5'), 64.2 (C2'), 61.5 (C3), 61.2 (C1), 60.6 (C5"), 32.9 (C2), 16.2 (6"-CH_3).

To a stirred solution of the perazido product from the above step (244 mg, 0.416 mmol) in a mixture of THF (3.0 mL) and aqueous NaOH (1 mM, 5.0 mL), PMe_3 (1 M solution in THF, 5.0 mL, 7.8 equiv.) was added. The progress of the reaction was monitored by TLC [CH_2Cl_2/MeOH/H_2O/$MeNH_2$ (33% solution in EtOH), 10:15:6:15], which indicated completion after 4.0 h. The reaction mixture was purified by flash chromatography on a short column of silica gel. The column was washed with the following solvents: THF (100 mL), CH_2Cl_2 (100 mL), EtOH (50 mL), and MeOH (100 mL). The product was then eluted with the mixture of 5% $MeNH_2$ solution (33% solution in EtOH) in 80% MeOH. Fractions containing the product were combined and evaporated under vacuum. The pure product was obtained by passing the above product through a short column of Amberlite CG50 (NH_4^+ form). First, the column was washed with water, then the product was eluted with a mixture of 10% NH_4OH in water to yield the 6'-amide **11** (143 mg, 71%). For the storage and biological tests, compound was converted to its sulfate salt form as follow. The free base form was dissolved in water, the pH was adjusted to 7.0 with H_2SO_4 (0.1 N) and lyophilized to afford the sulfate salt of **11**. ^{1}H NMR (500 MHz, D_2O): **'Ring I'**: δ_H 5.37 (d, 1H, *J* = 3.4 Hz, H-1), 4.06 (d, 1H, *J* = 9.6 Hz, H-5), 3.61 (t, 1H, *J* = 9.5 Hz, H-3), 3.54-3.48 (m, 1H, H-4), 2.79 (dd, 1H, J_1 = 10.5, J_2 = 6.2 Hz, H-2); **'Ring II':** δ_H 3.68 (t, 1H, *J* = 9.3 Hz, H-5), 3.52 (t, 1H, *J* = 9.3 Hz, H-6), 3.32 (t, 1H, *J* = 9.6 Hz, H-4), 2.94 (td, 1H, J_1 = 9.3, J_1 = 4.7 Hz, H-1), 2.81 (td, 1H, J_1 = 9.9, J_1 = 4.3 Hz, H-3), 2.00 (dt, 1H, J_1 =12.8, J_2 = 4.1 Hz, H-2eq), 1.27 (ddd, 1H, $J_1 = J_2 = J_3$ = 13.2 Hz, H-2ax); **'Ring III'**: δ_H 5.28 (d, 1H, *J* = 1.0 Hz, H-1), 4.21- 4.16 (m, 2H, H-2, H-3), 3.73 (dd, 1H, J_1 = 8.2, J_2 = 6.0 Hz, H-4), 3.26 (dd, 1H, J_1

= 14.2, J_2 = 7.3 Hz, H-5), 1.26 (d, 3H, J = 6.6 Hz, 6-CH_3). ^{13}C NMR (125 MHz, D_2O): δ_C 176.3 [(NH_2) C=O], 108.3 (C1”), 98.4 (C1’), 83.7 (C4”), 83.3 (C5), 82.0 (C6), 76.0 (C4), 74.8 (C3”), 73.6 (C5’), 72.9 (C3’), 71.9 (C4’), 71.2 (C2”), 54.7 (C2’), 50.3 (C3), 50.0 (C5”), 49.7 (C1), 34.1 (C2), 15.5 (6”-CH_3).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N3
N3O
N3
BzO
OBz
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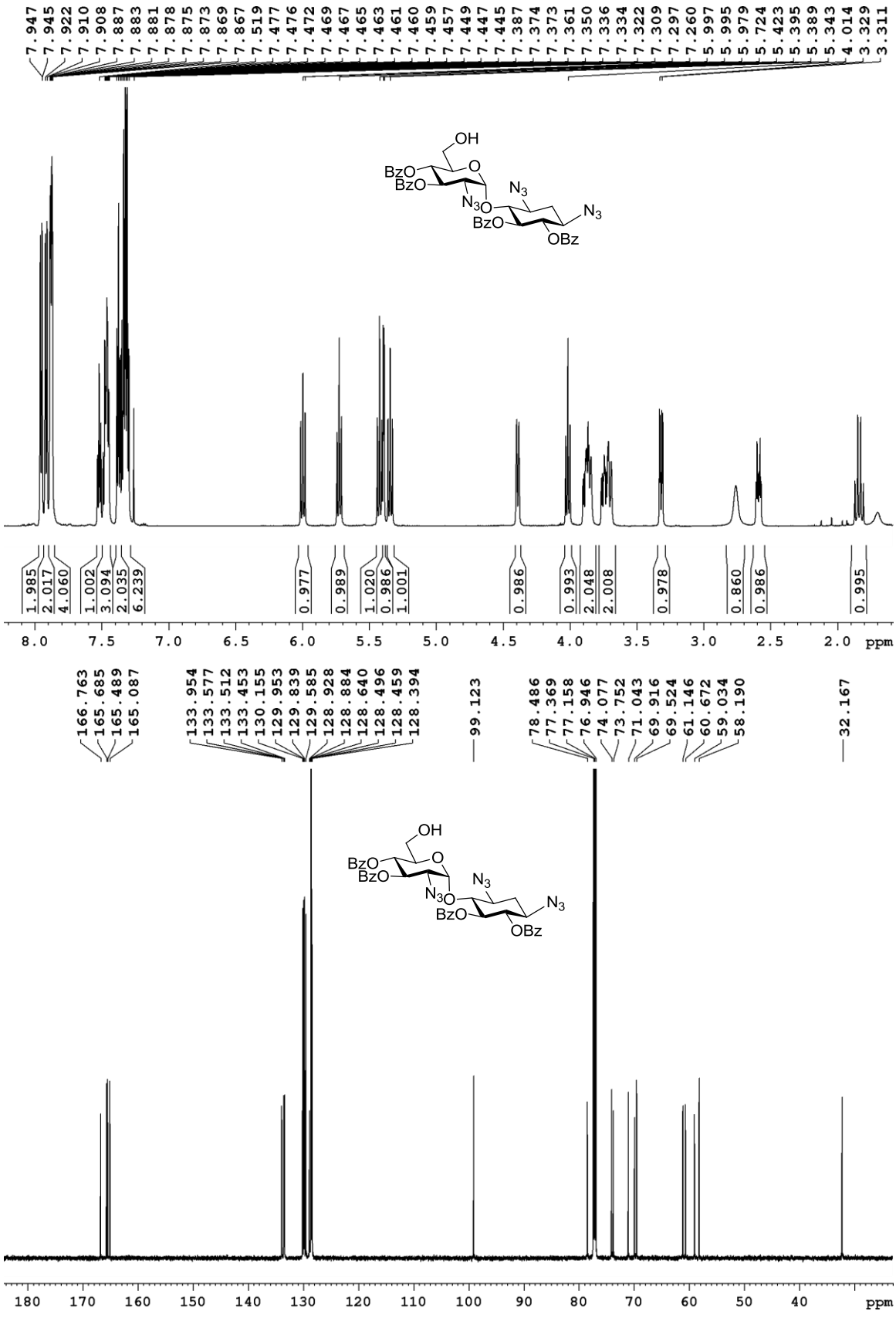
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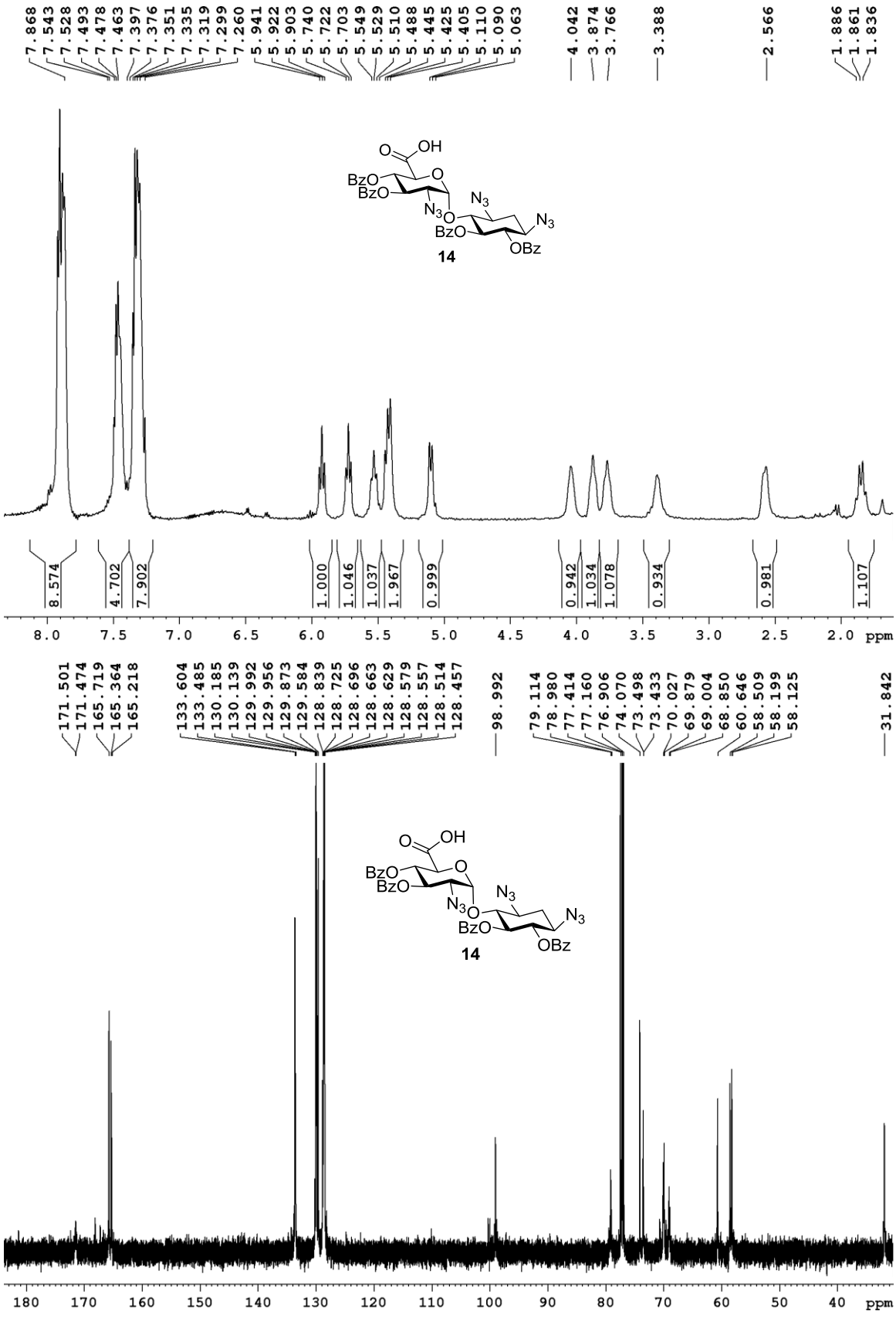
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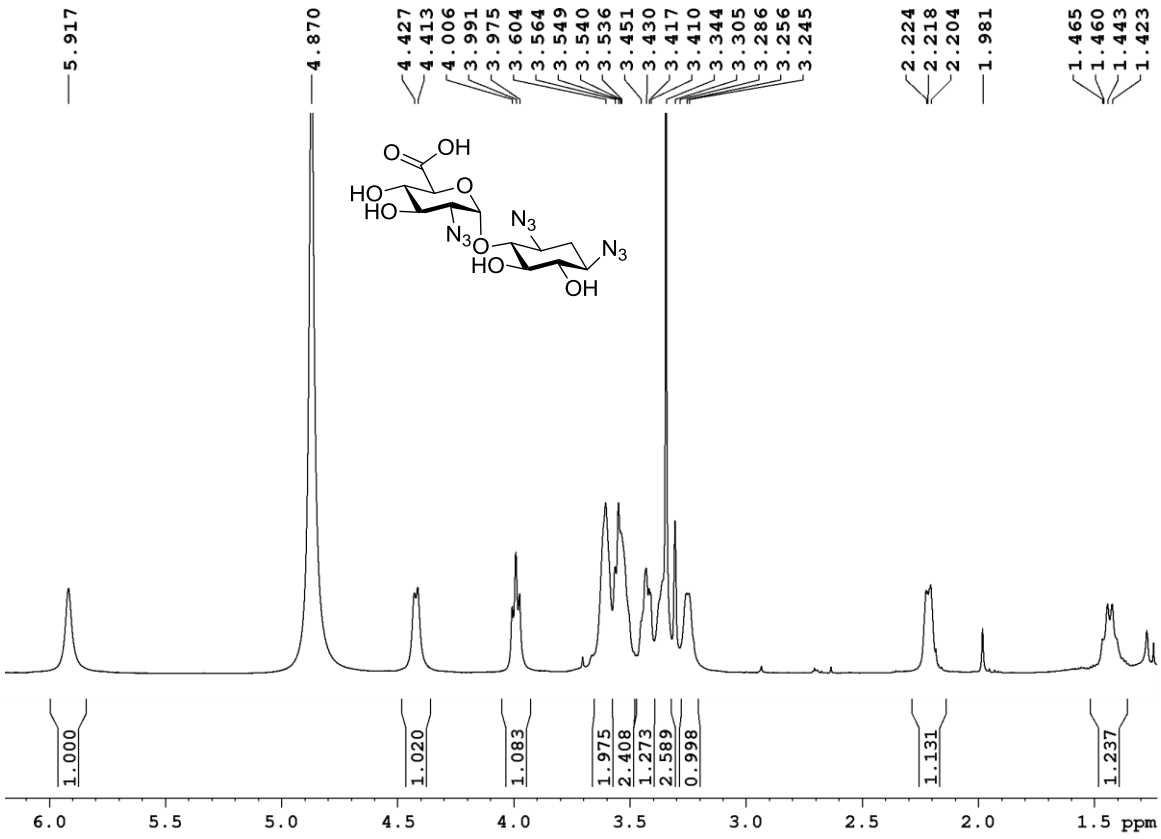
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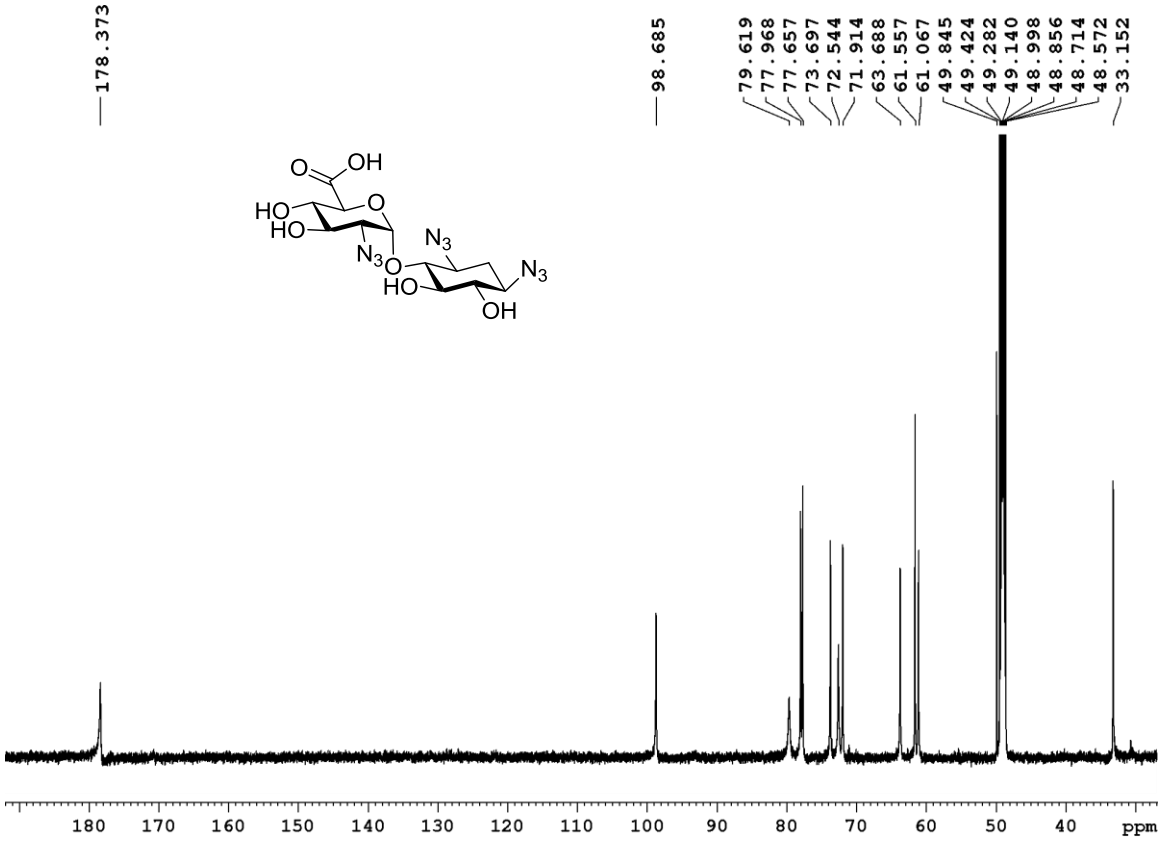
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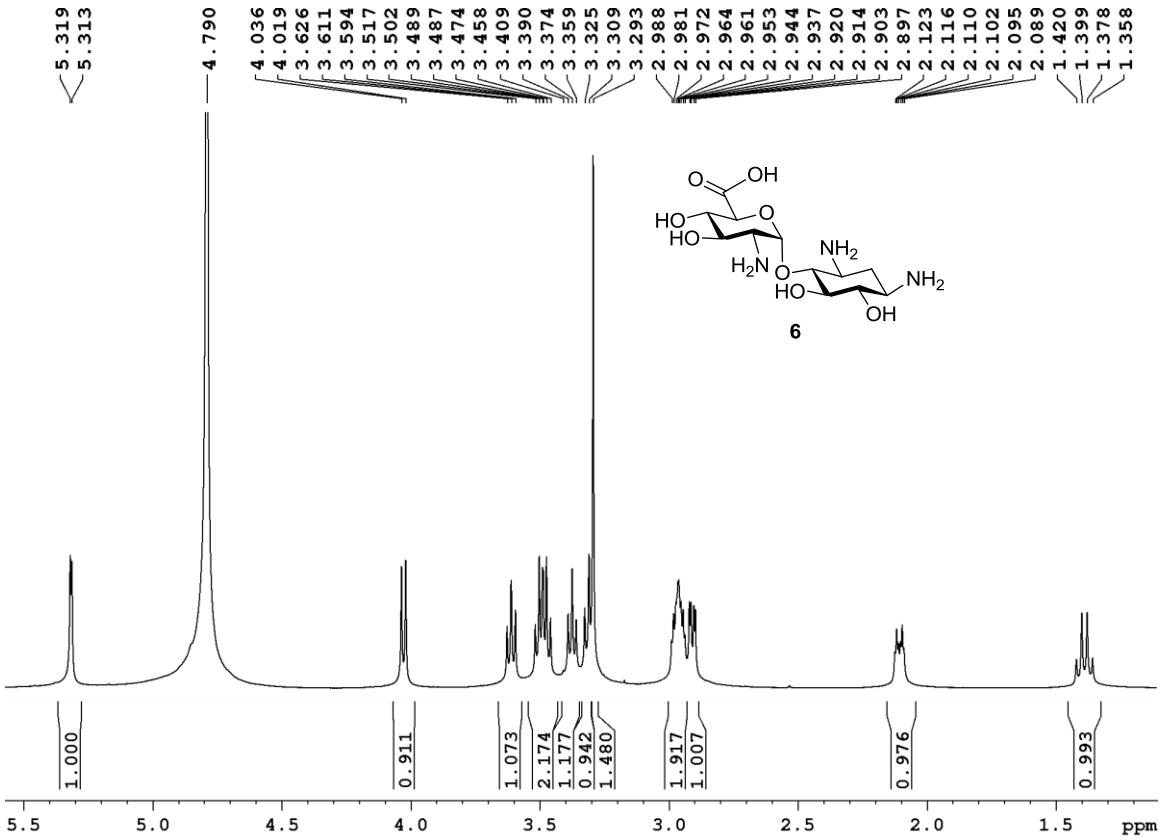
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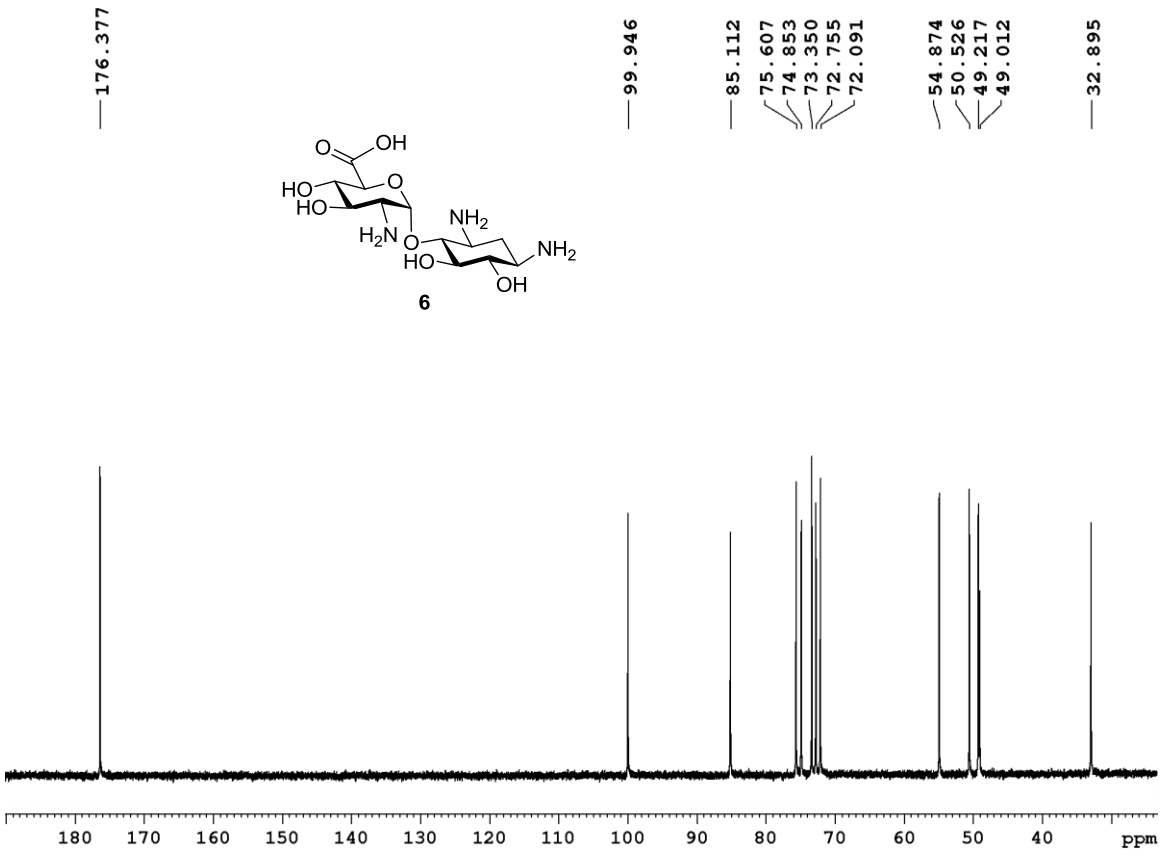
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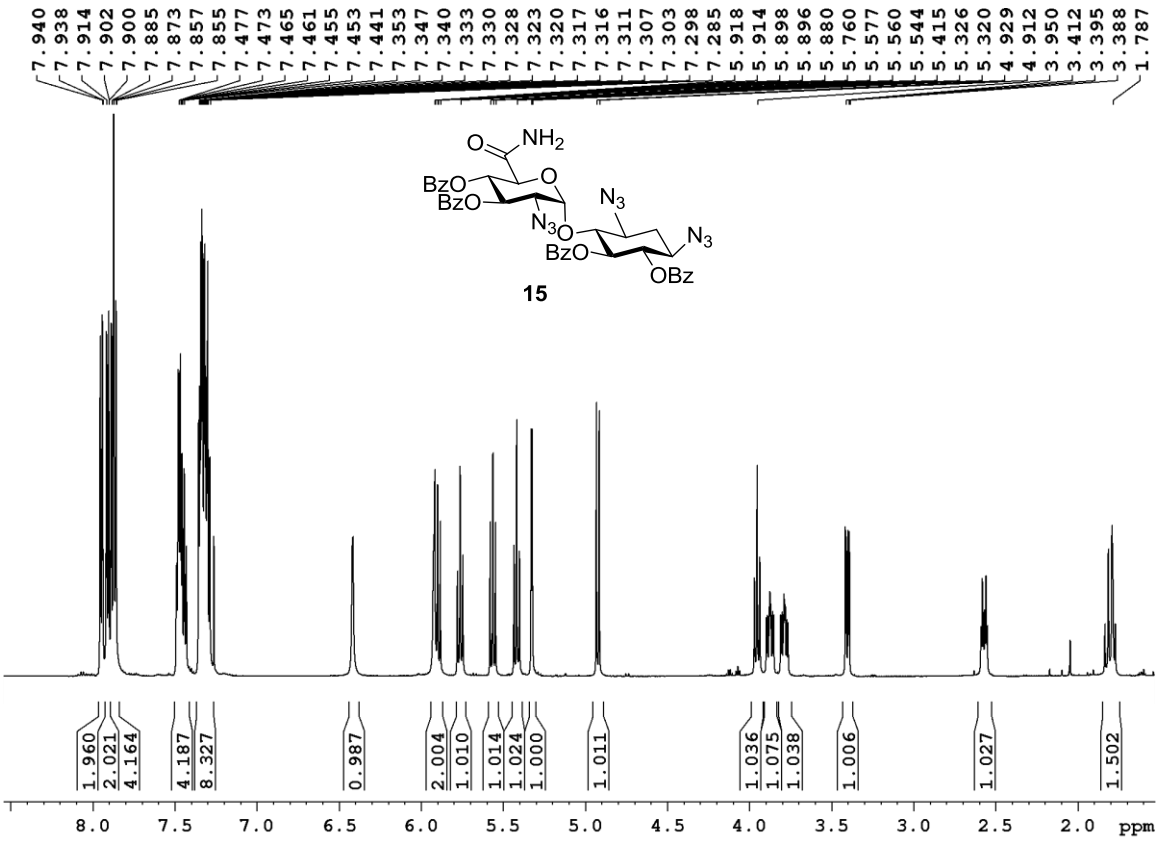
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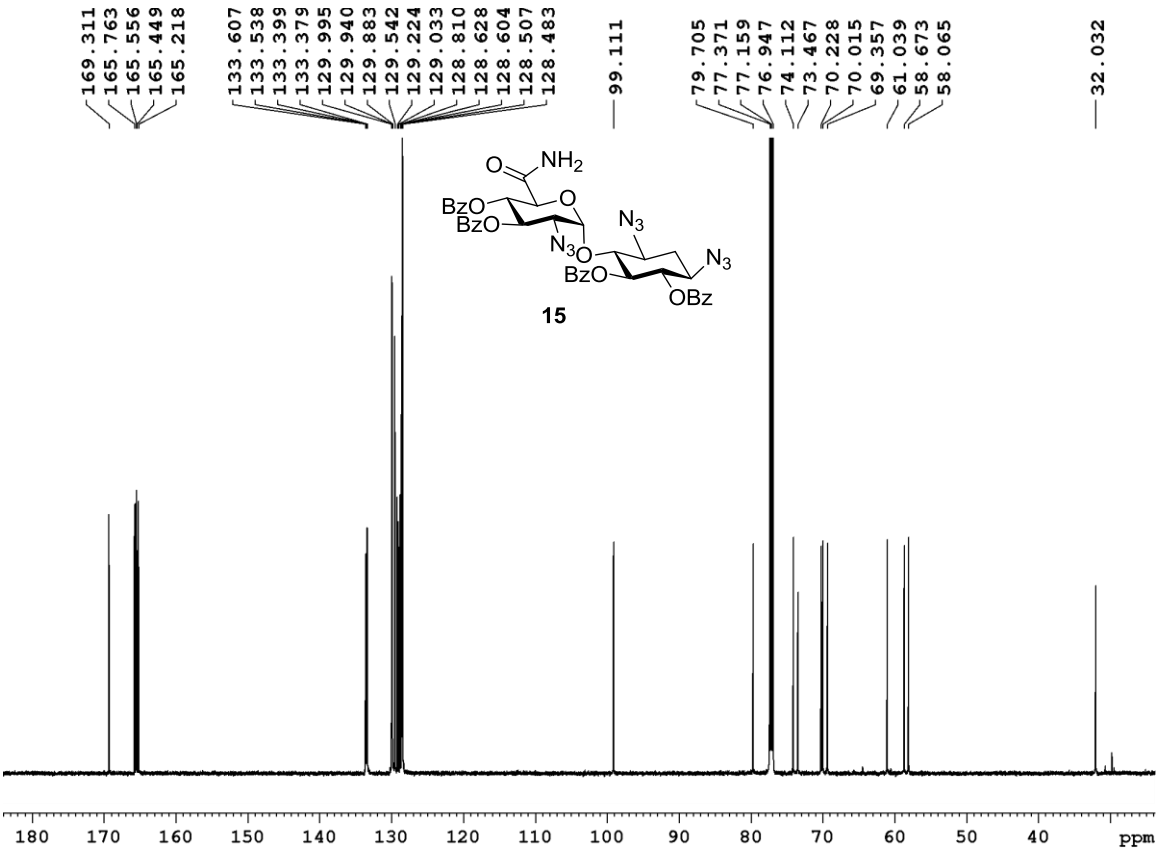
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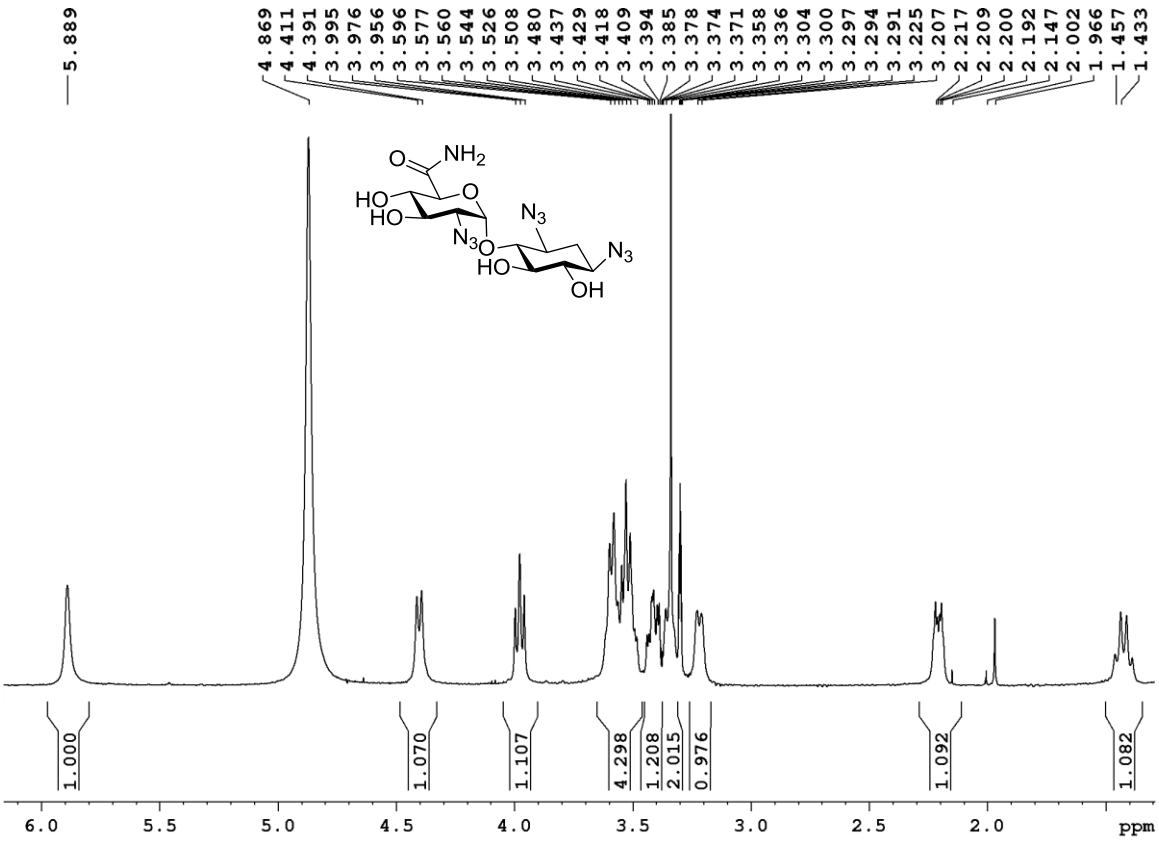
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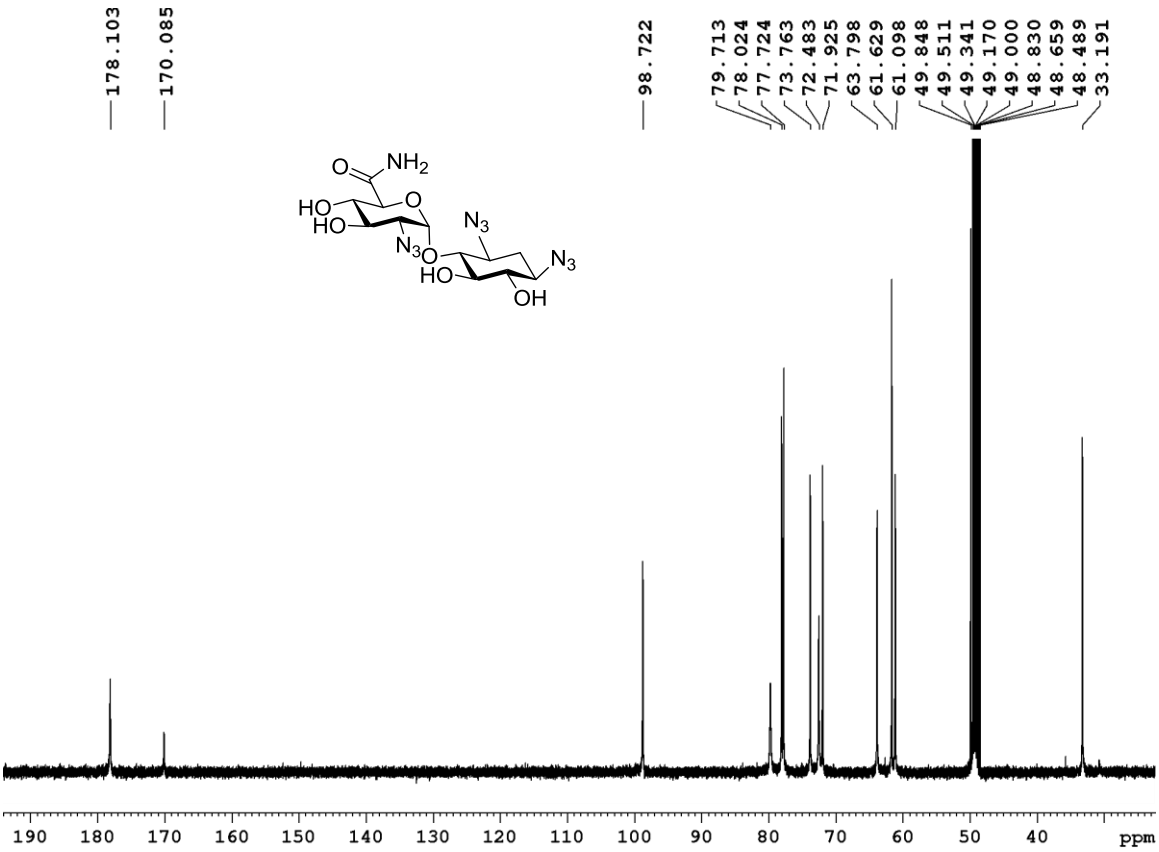
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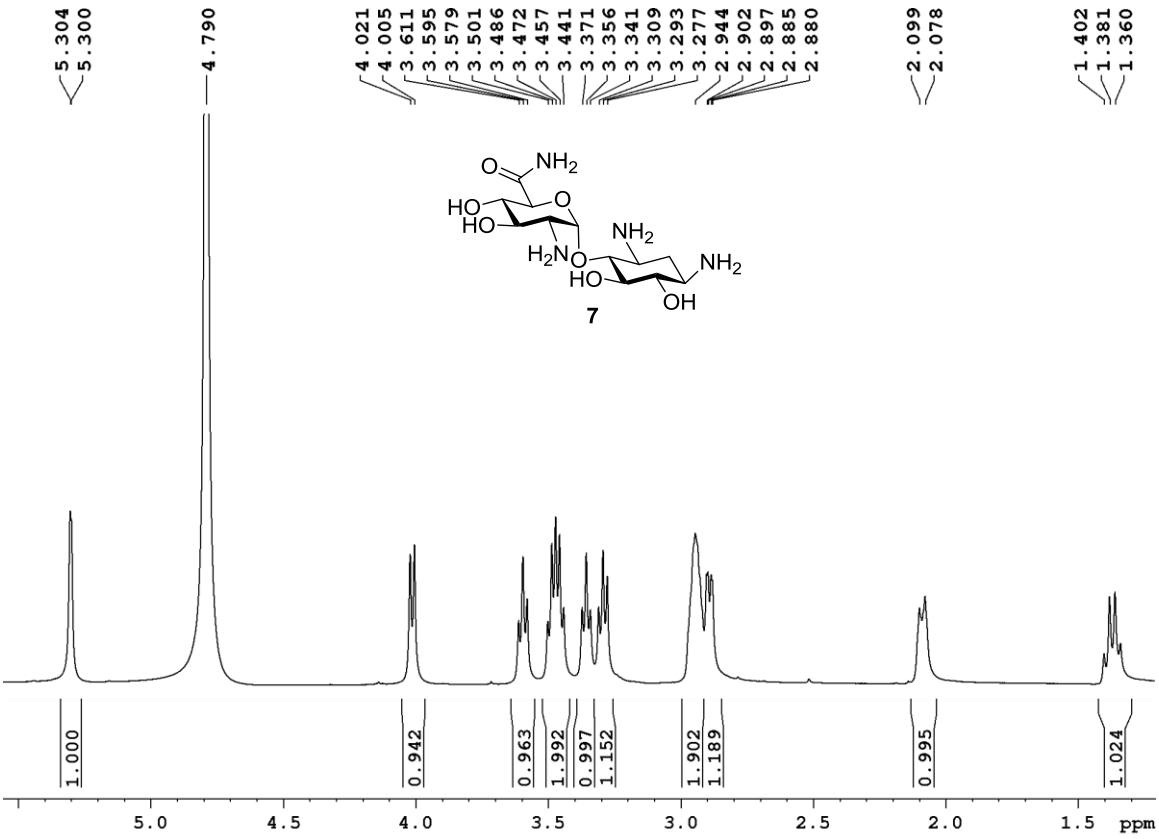
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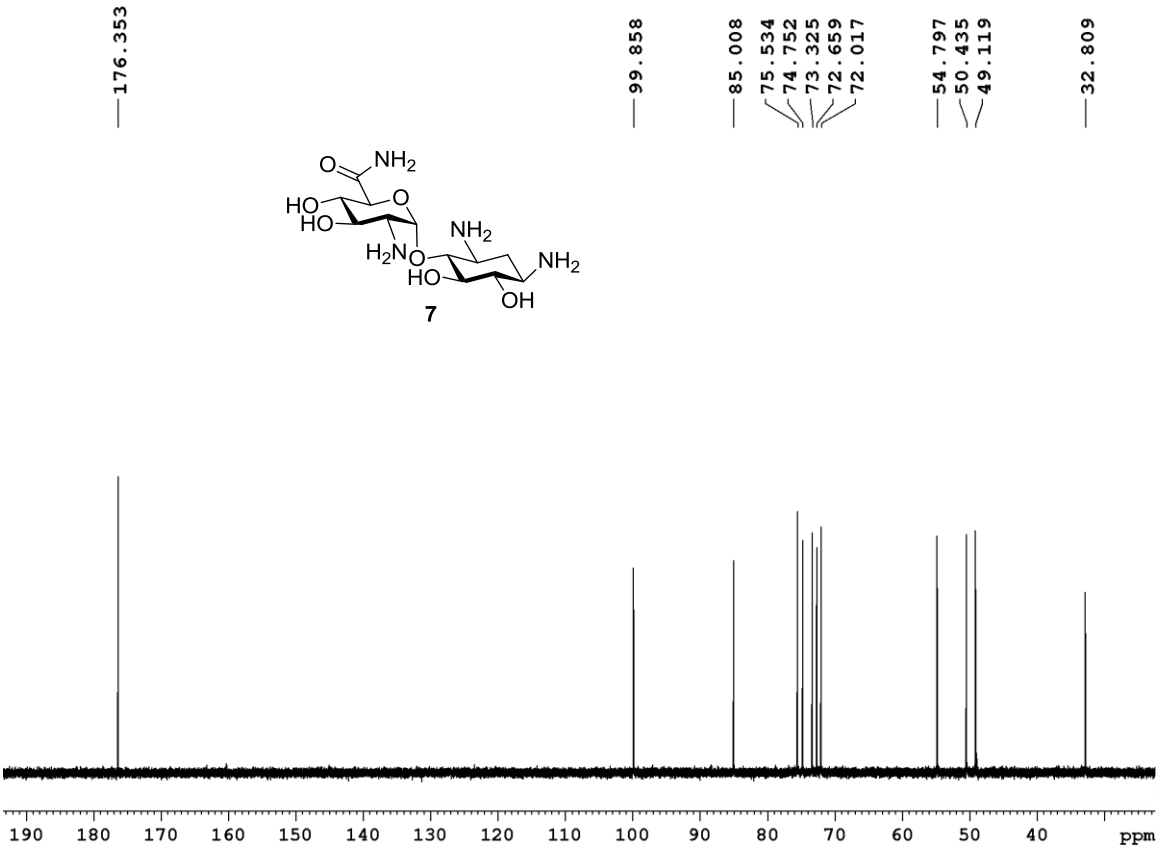
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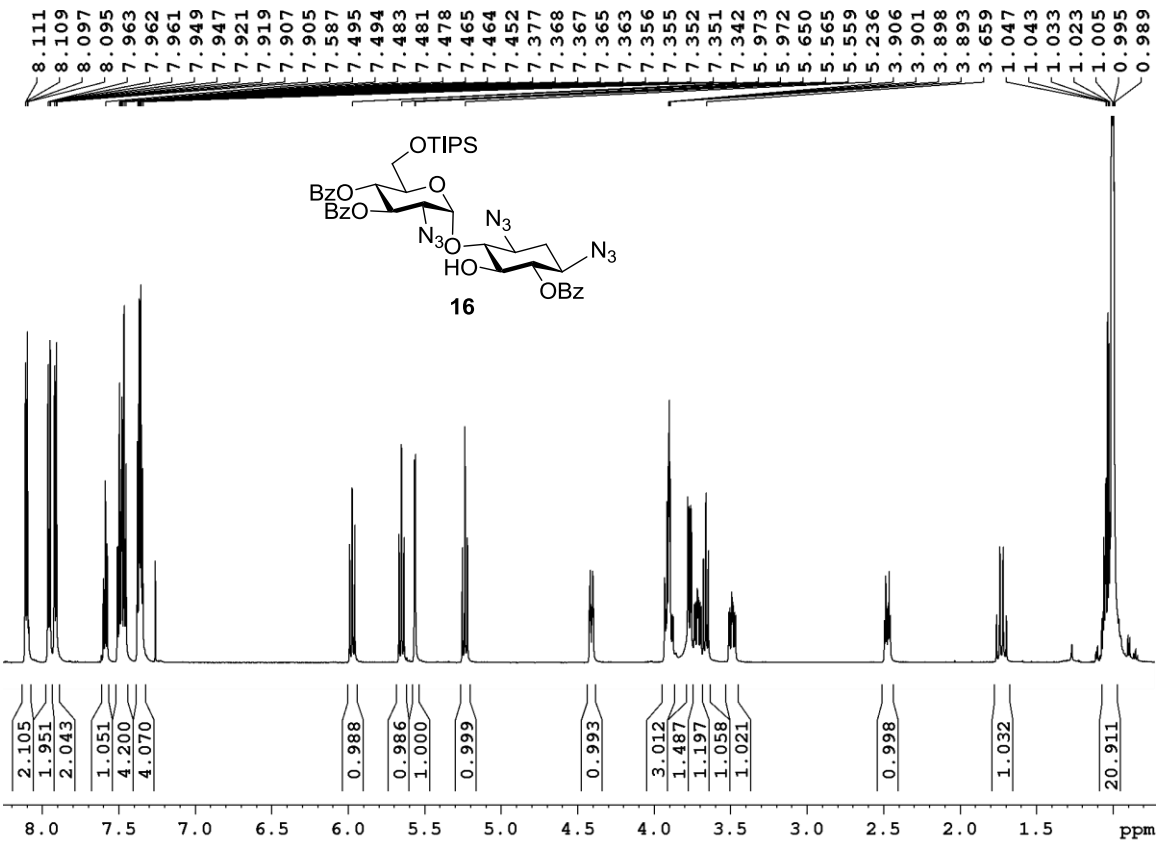
ppm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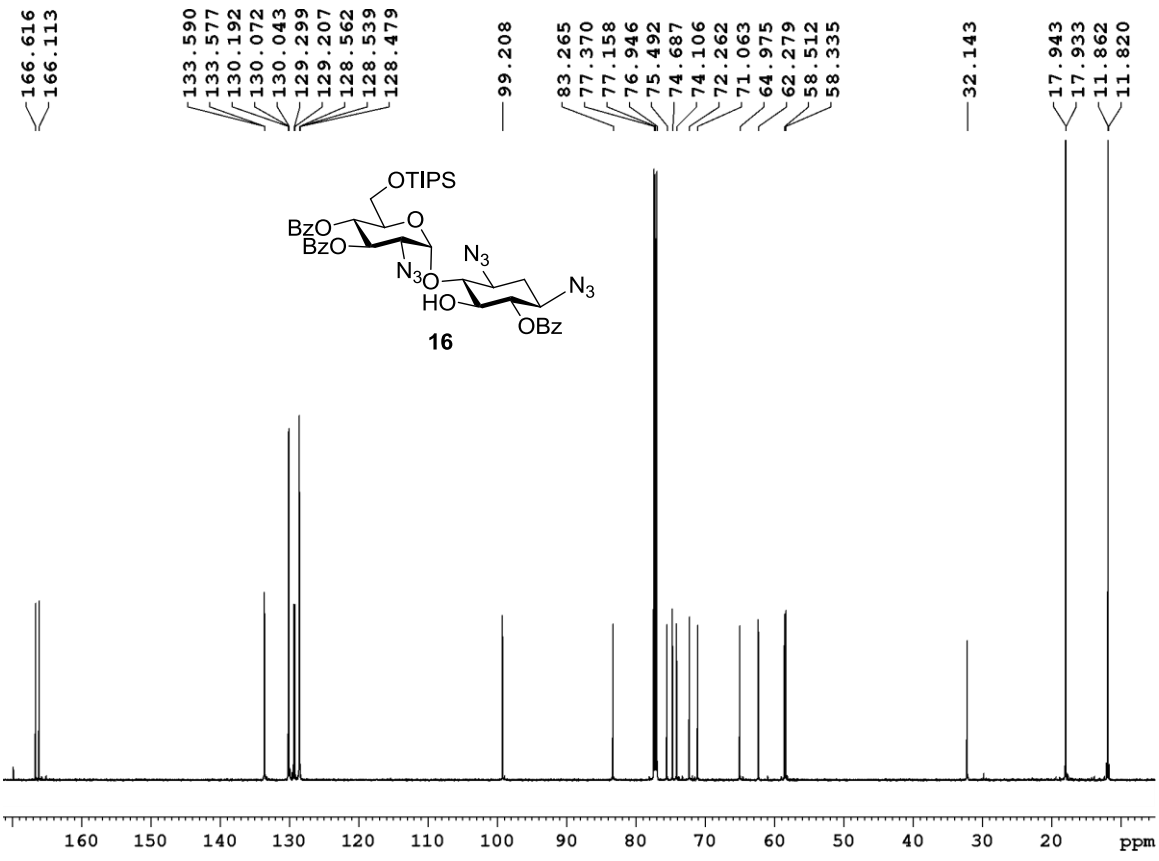
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BzO
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HO
N3
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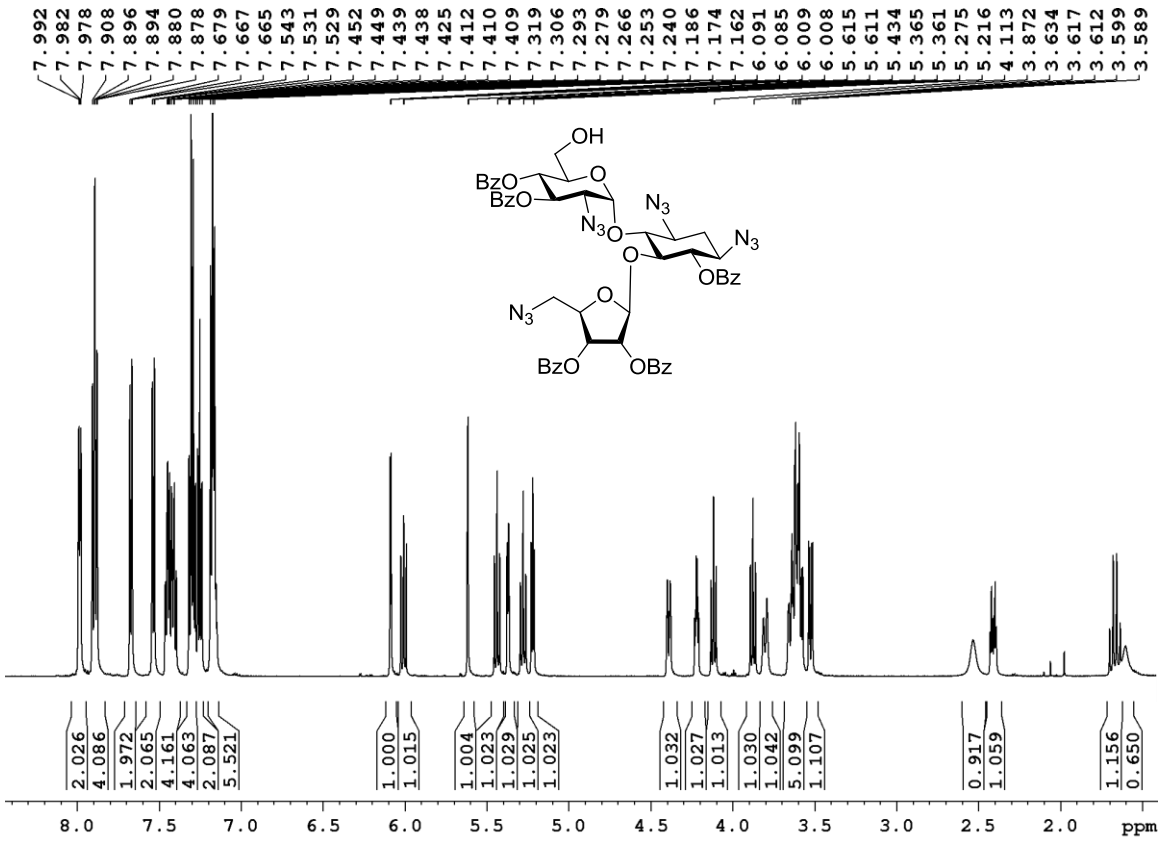
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7.529
7.452
7.449
7.439
7.438
7.425
7.412
7.410
7.409
7.319
7.306
7.293
7.279
7.266
7.253
7.240
7.186
7.174
7.162
6.091
6.085
6.009
6.008
5.615
5.611
5.434
5.365
5.361
5.275
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4.113
3.872
3.634
3.617
3.612
3.599
3.589
OH
BzO
BzO
O
N3
N3
O
N3
N3
O
OBz
O
N3
BzO
OBz
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2.065
4.161
4.063
2.087
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1.000
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1.004
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1.029
1.025
1.023
1.032
1.027
1.013
1.030
1.042
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1.107
0.917
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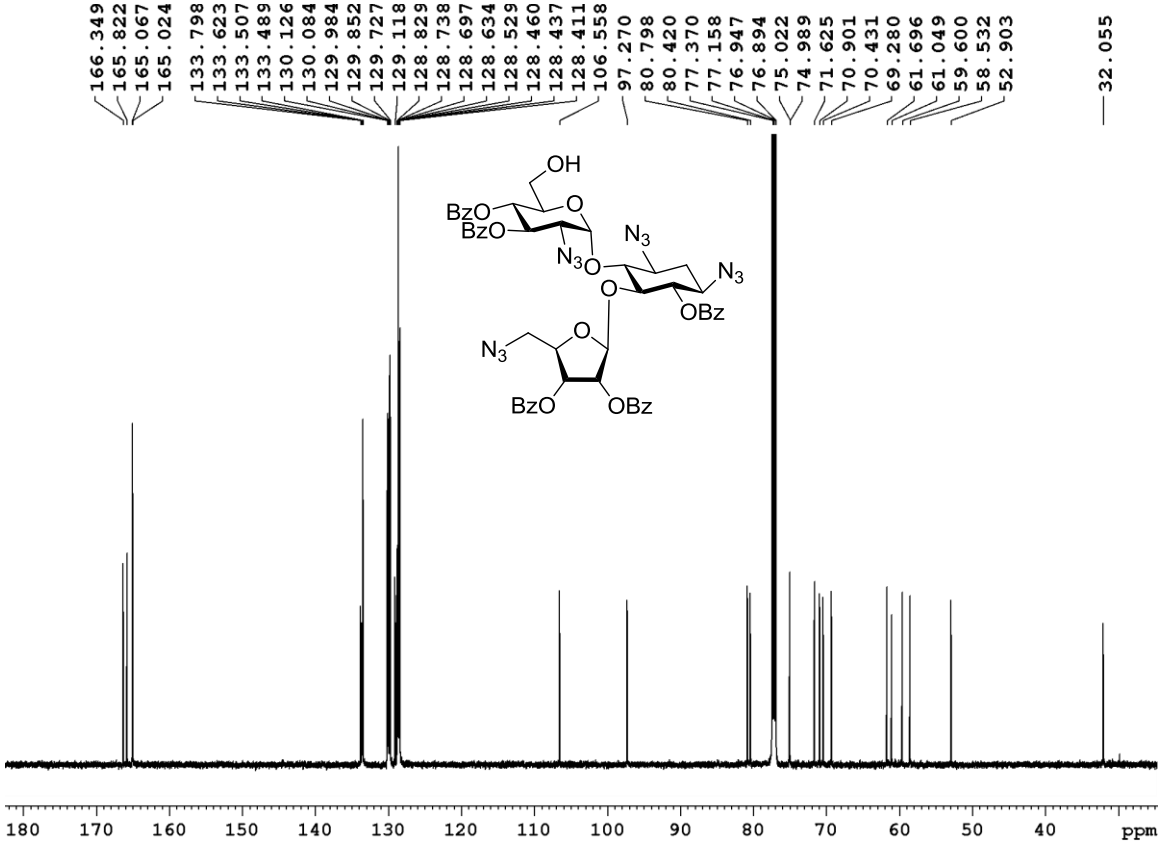
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77.158
76.947
76.894
75.022
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71.625
70.901
70.431
69.280
61.696
61.049
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58.532
52.903
32.055
OH
BzO
BzO
O
N3
N3
O
N3
N3
O
OBz
O
N3
BzO
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50
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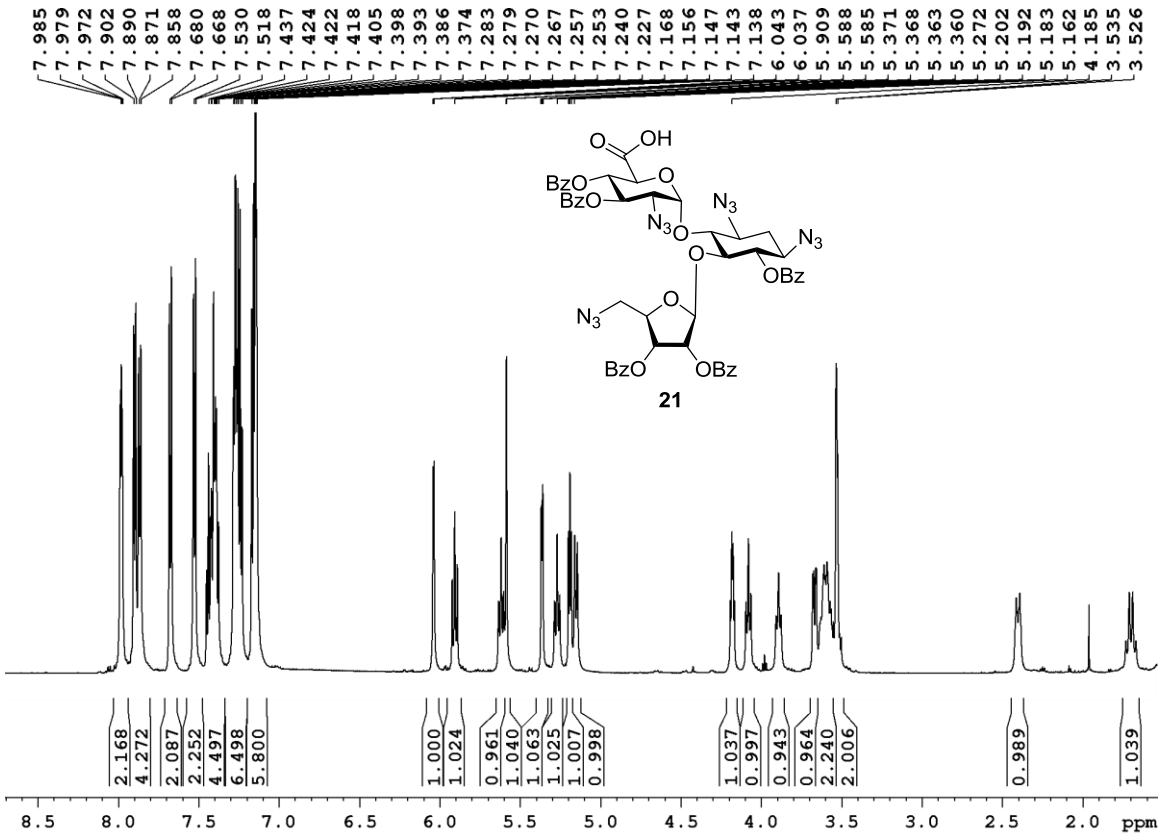
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7.422
7.418
7.405
7.398
7.393
7.386
7.374
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7.279
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7.257
7.253
7.240
7.227
7.168
7.156
7.147
7.143
7.138
6.043
6.037
5.909
5.588
5.585
5.371
5.368
5.363
5.360
5.272
5.202
5.192
5.183
5.162
4.185
3.535
3.526
21
2.168
4.272
2.087
2.252
4.497
6.498
5.800
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1.024
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1.040
1.063
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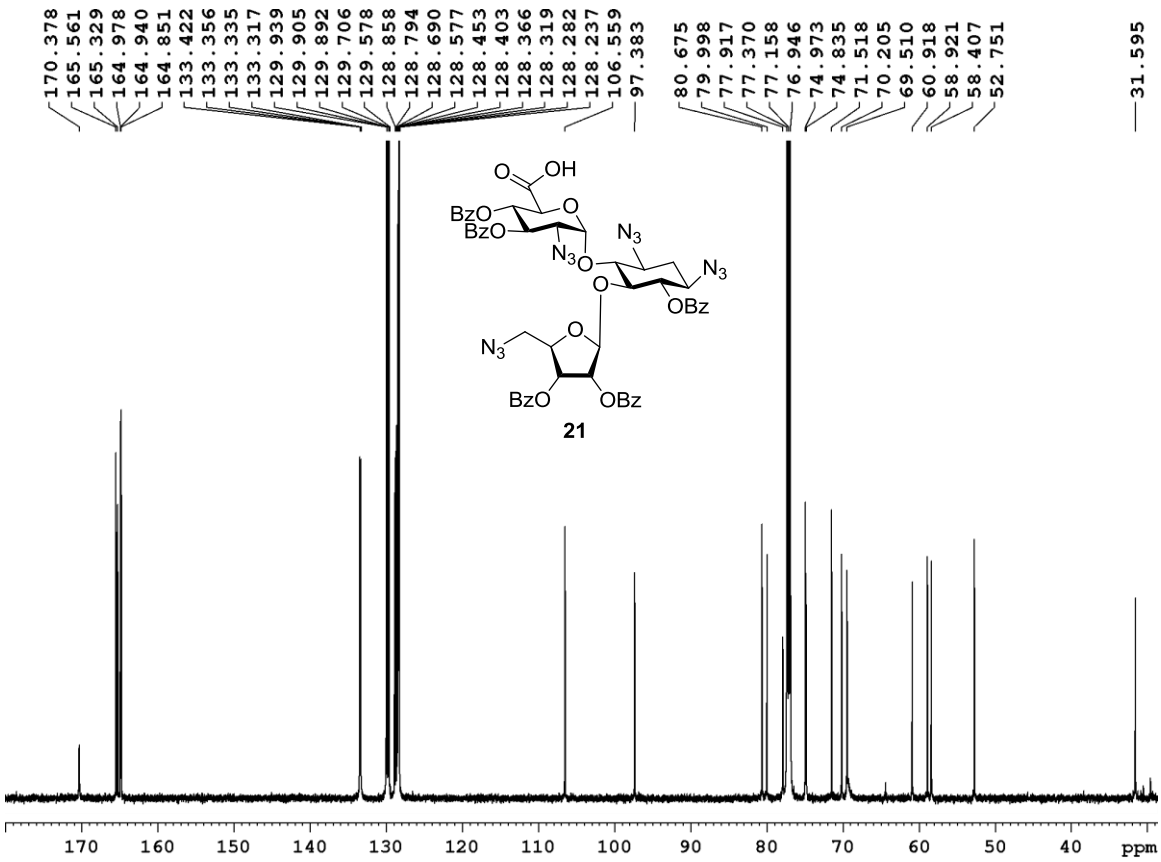
ppm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129.706
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128.858
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128.577
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128.403
128.366
128.319
128.282
128.237
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97.383
80.675
79.998
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77.158
76.946
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21
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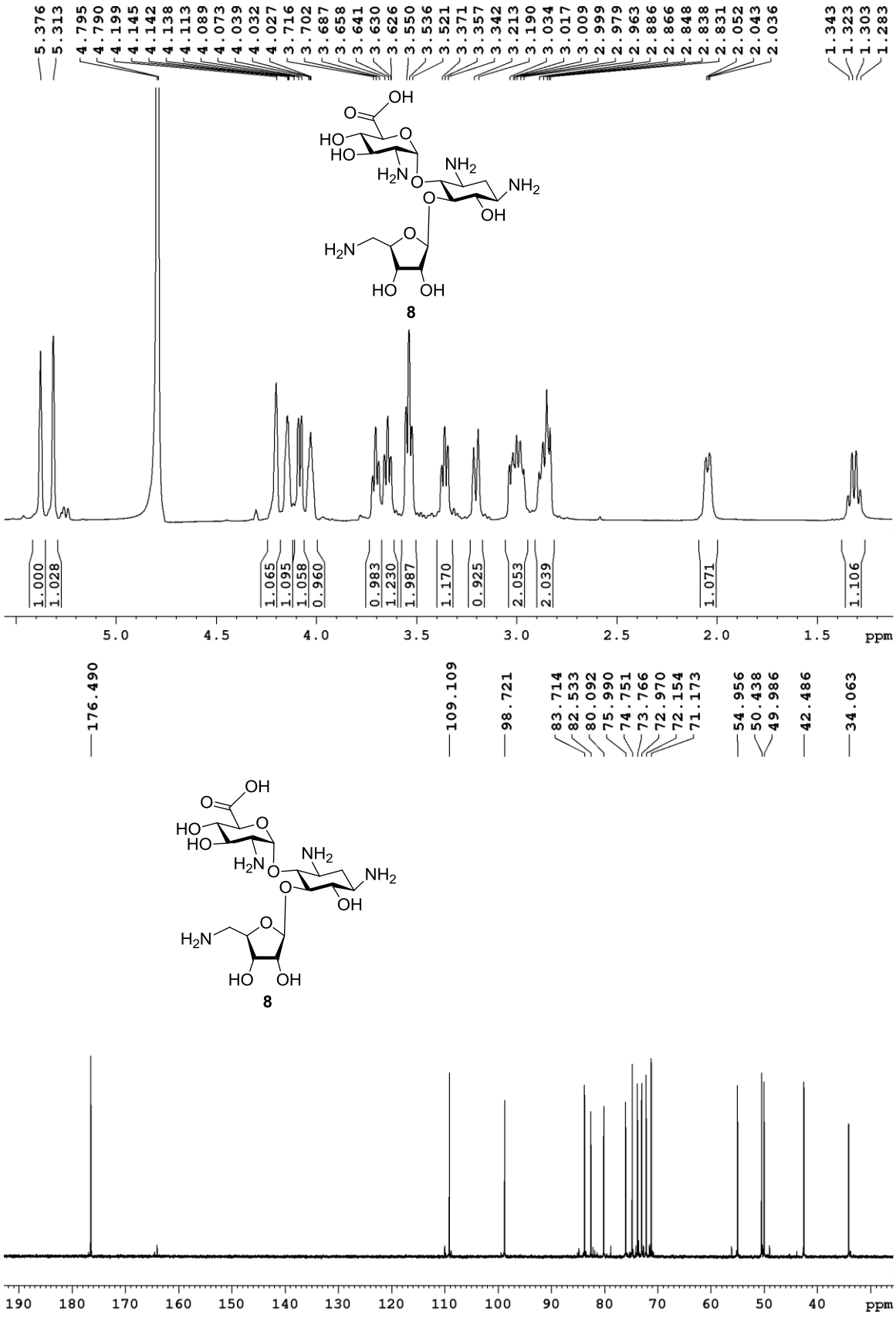
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4.027
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3.702
3.687
3.658
3.641
3.630
3.626
3.550
3.536
3.521
3.371
3.357
3.342
3.213
3.190
3.034
3.017
3.009
2.999
2.979
2.963
2.886
2.866
2.848
2.838
2.831
2.052
2.043
2.036
1.343
1.323
1.303
1.283
8
1.000
1.028
1.065
1.095
1.058
0.960
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1.230
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1.170
0.925
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4.0
3.5
3.0
2.5
2.0
1.5
ppm
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75.990
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71.173
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42.486
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180
170
160
150
140
130
120
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100
90
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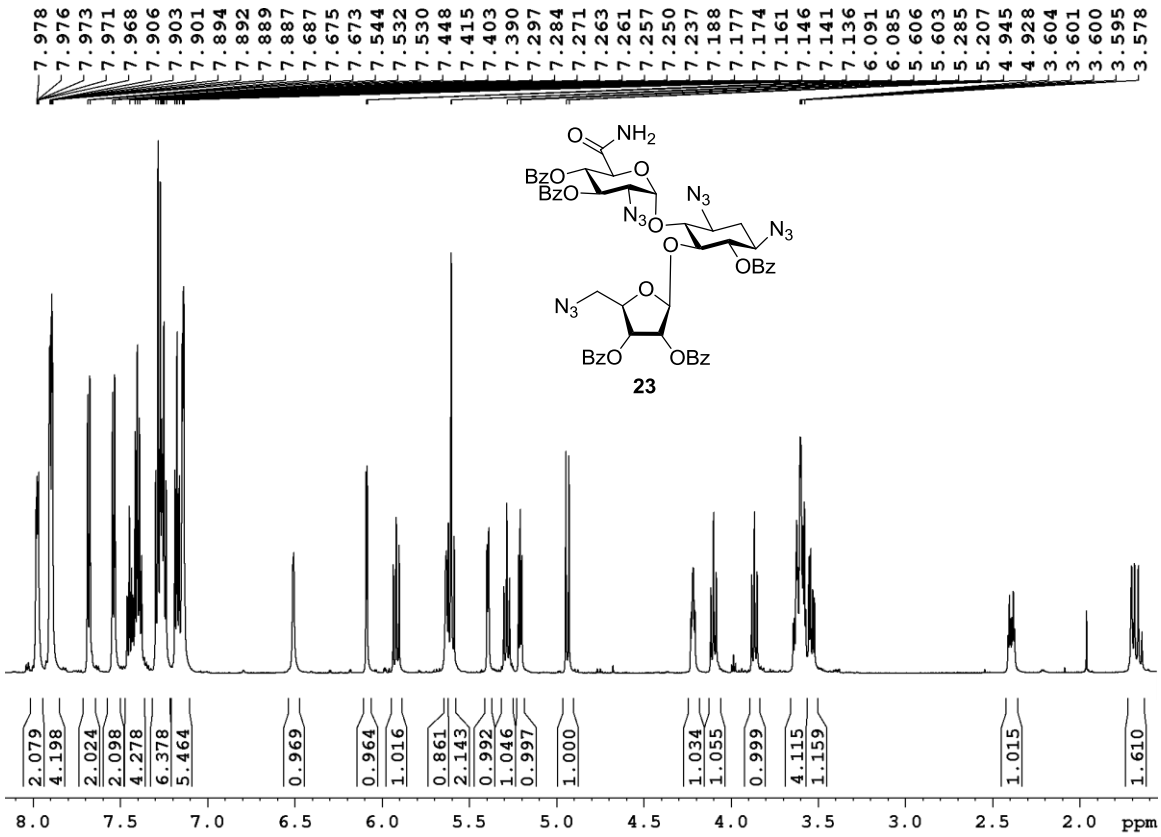
70
60
50
40
ppm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7.889
7.887
7.687
7.675
7.673
7.544
7.532
7.530
7.448
7.415
7.403
7.390
7.297
7.284
7.271
7.263
7.261
7.257
7.250
7.237
7.188
7.177
7.174
7.161
7.146
7.141
7.136
6.091
6.085
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5.603
5.285
5.207
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4.928
3.604
3.601
3.600
3.595
3.578
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BzO
BzO
N3
N3
N3
OBz
BzO
OBz
23
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2.024
2.098
4.278
6.378
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0.861
2.143
0.992
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1.000
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1.055
0.999
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1.159
1.015
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6.0
5.5
5.0
4.5
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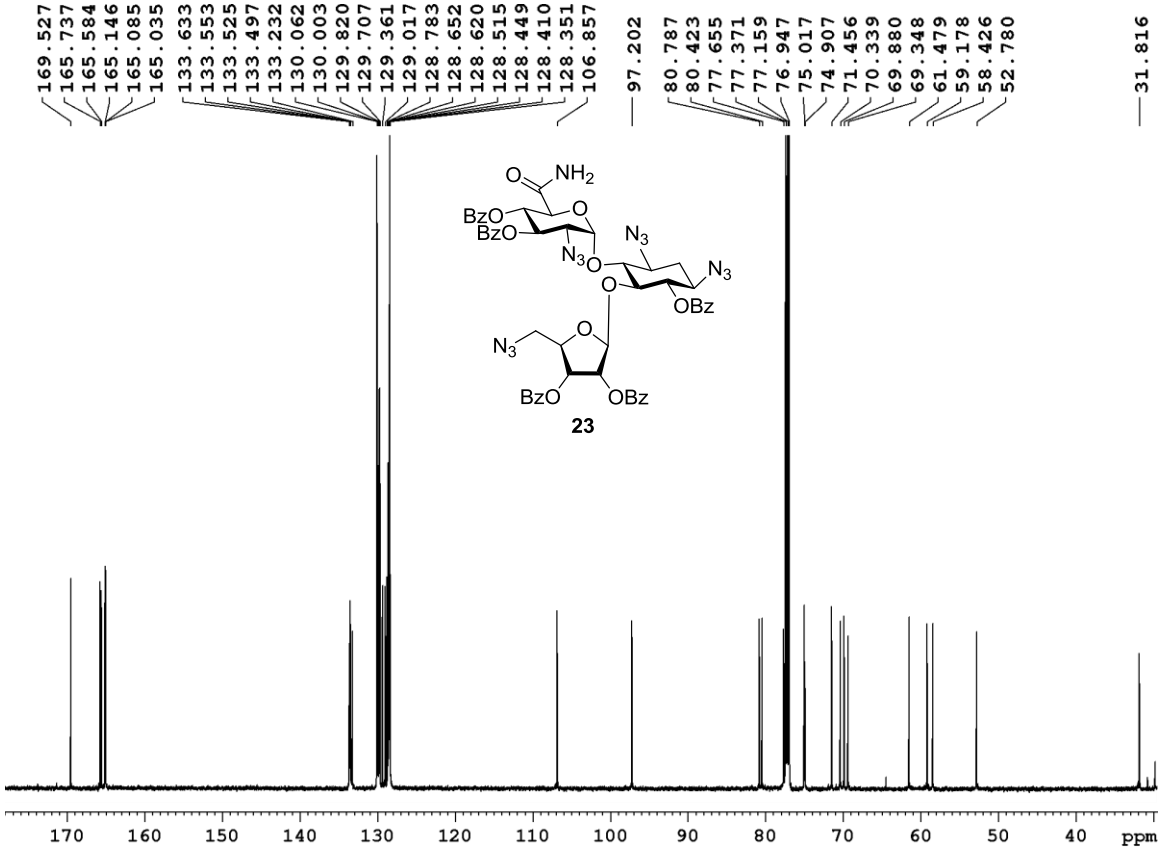
3.5
3.0
2.5
2.0
ppm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130.003
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129.361
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128.783
128.652
128.620
128.515
128.449
128.410
128.351
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97.202
80.787
80.423
77.655
77.371
77.159
76.947
75.017
74.907
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52.780
31.816
NH2
BzO
BzO
N3
N3
N3
OBz
BzO
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23
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150
140
130
120
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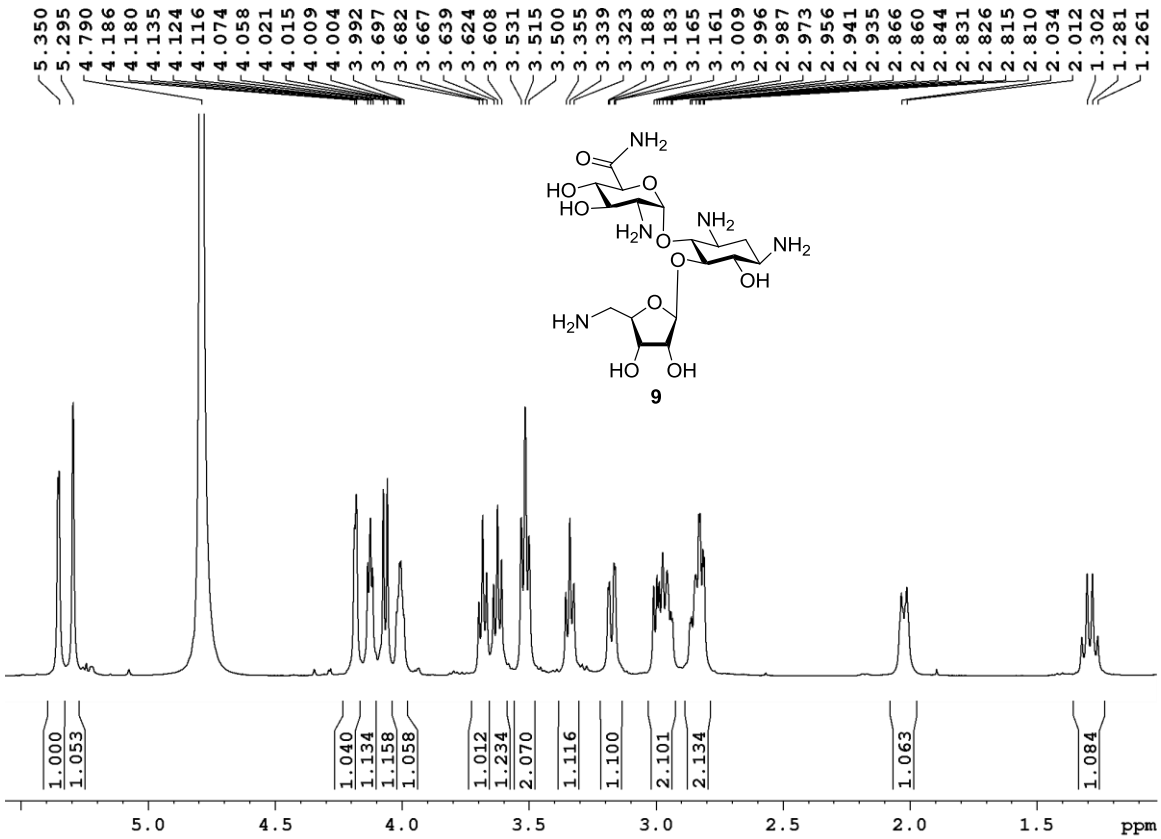
100
90
80
70
60
50
40
ppm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4.021
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4.009
4.004
3.992
3.697
3.682
3.667
3.639
3.624
3.608
3.531
3.515
3.500
3.355
3.339
3.323
3.188
3.183
3.165
3.161
3.009
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2.987
2.973
2.956
2.941
2.935
2.866
2.860
2.844
2.831
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2.815
2.810
2.034
2.012
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1.281
1.261
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HO
HO
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H2N
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HO
OH
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4.0
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3.0
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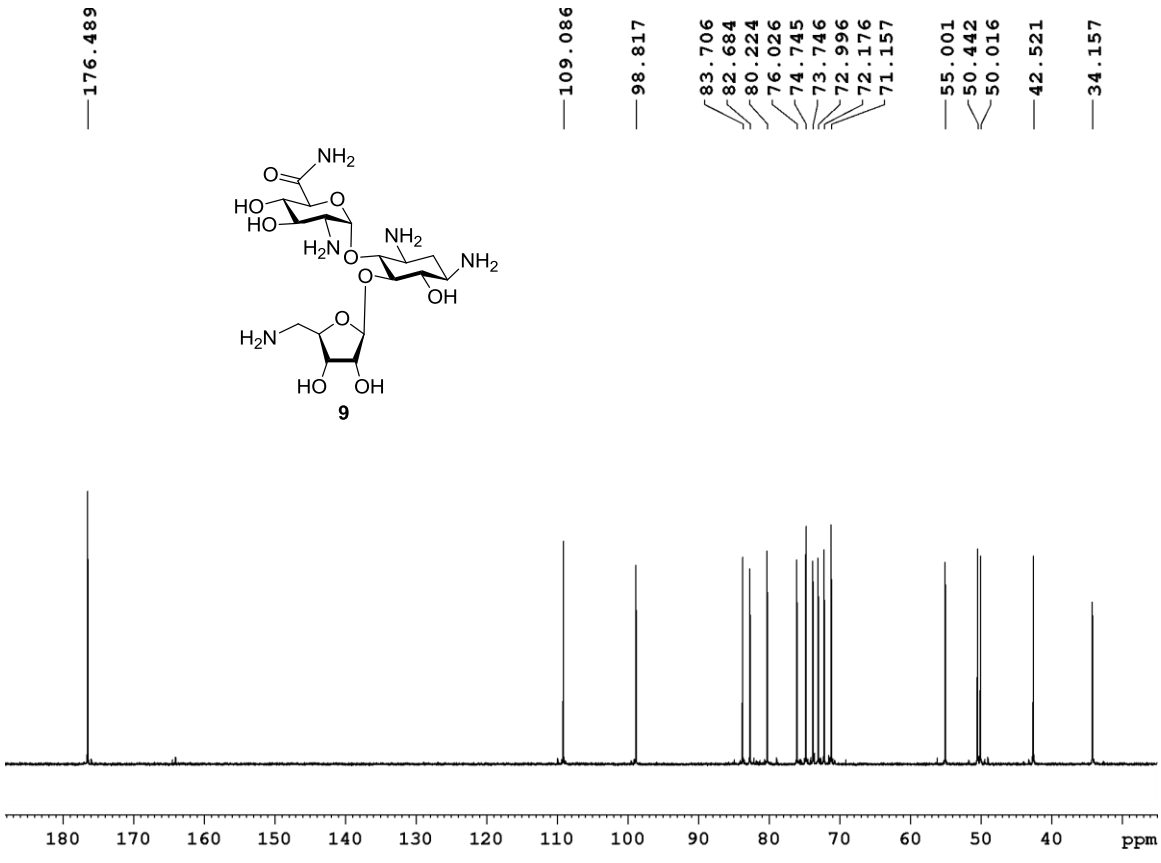
2.0
1.5
ppm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55.001
50.442
50.016
42.521
34.157
NH2
O
HO
HO
O
H2N
O
NH2
NH2
O
OH
H2N
O
HO
OH
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150
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70
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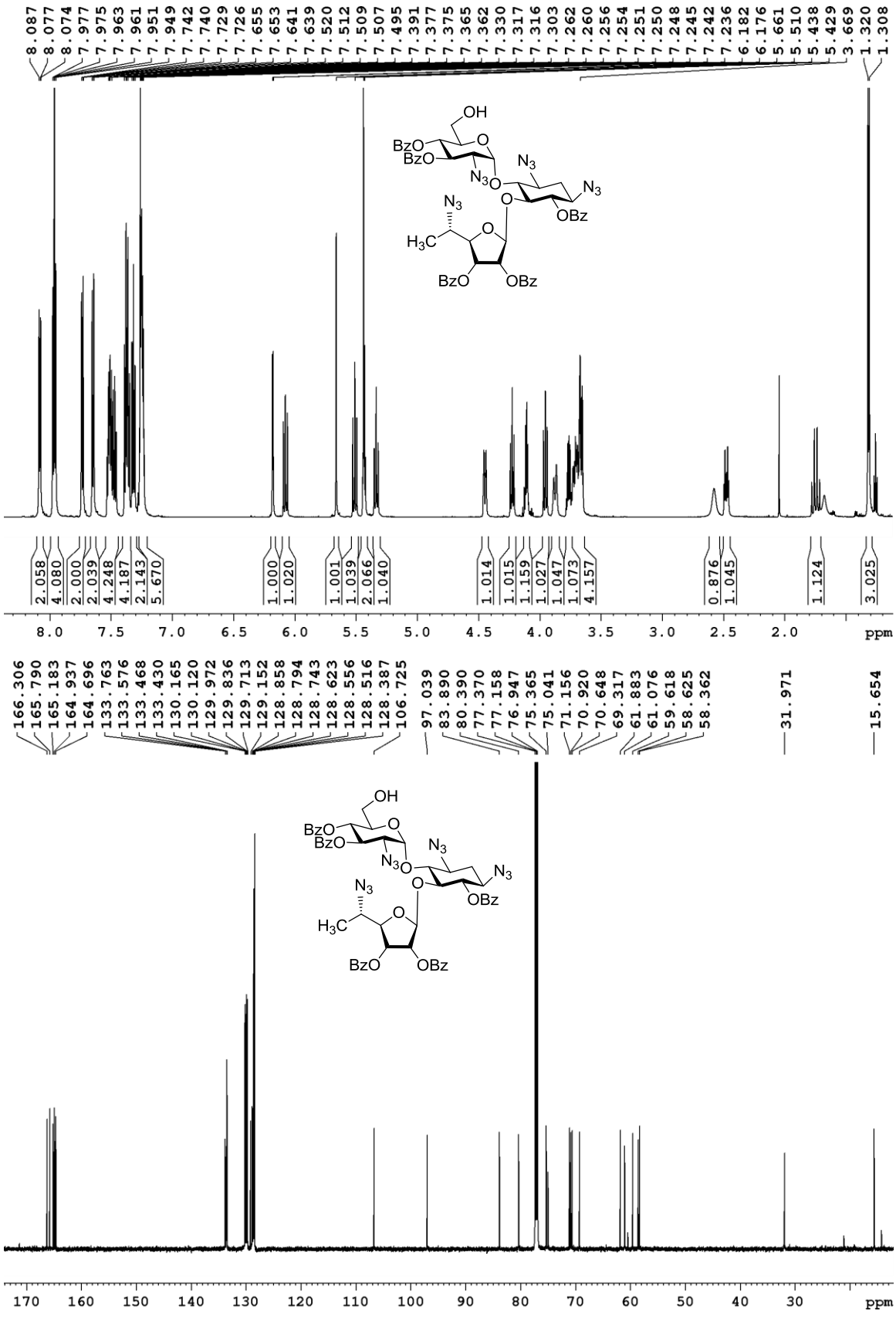
50
40
ppm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7.509
7.507
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7.375
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7.316
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7.251
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7.245
7.242
7.236
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6.176
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5.429
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1.308
OH
BzO
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N3
N3
O
N3
N3
OBz
N3
H3C
O
BzO
OBz
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6.0
5.5
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3.5
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2.5
2.0
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128.516
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76.947
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75.041
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61.076
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58.625
58.362
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OH
BzO
BzO
O
N3
N3
O
N3
N3
OBz
N3
H3C
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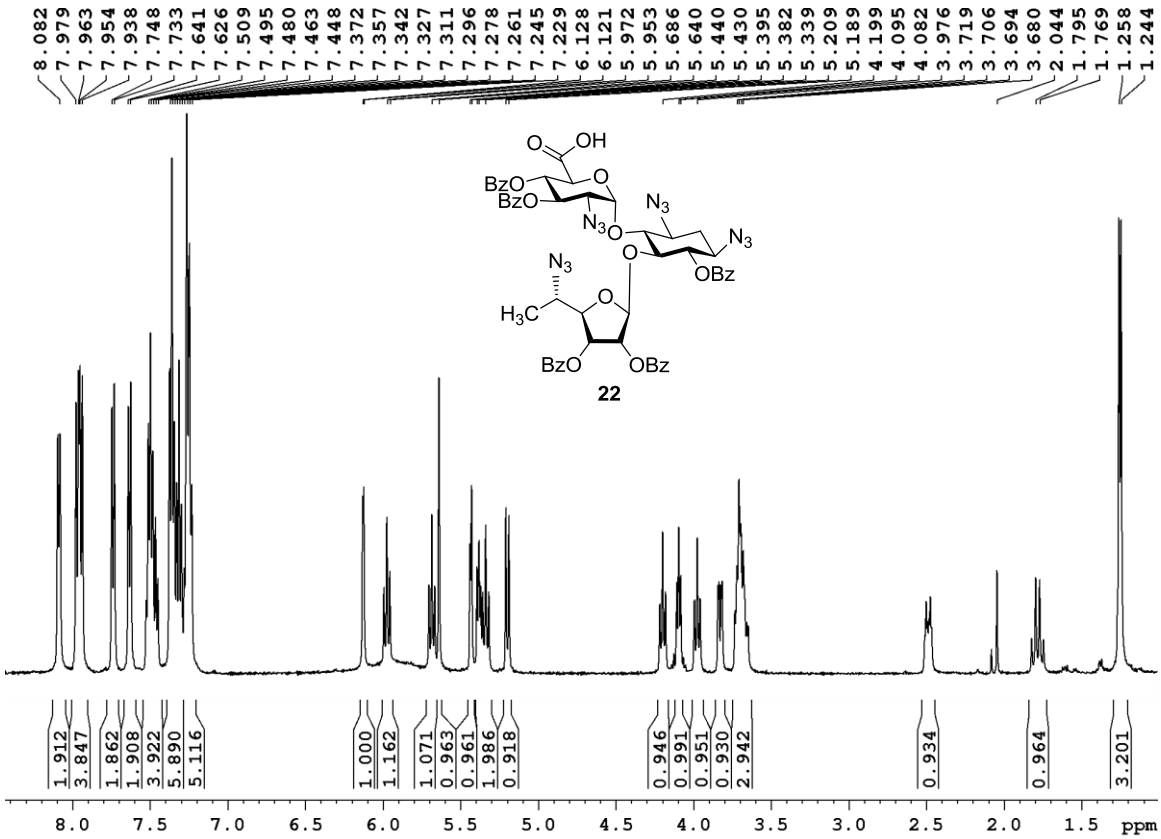
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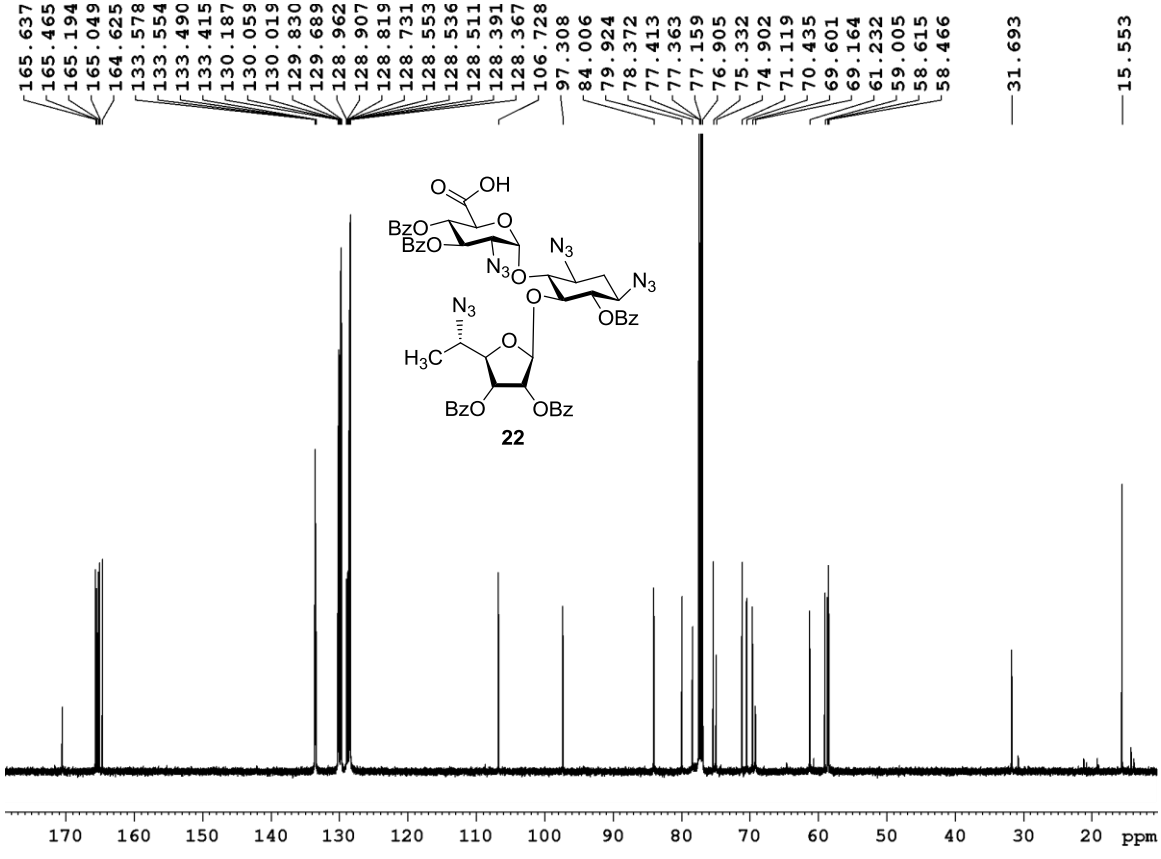
ppm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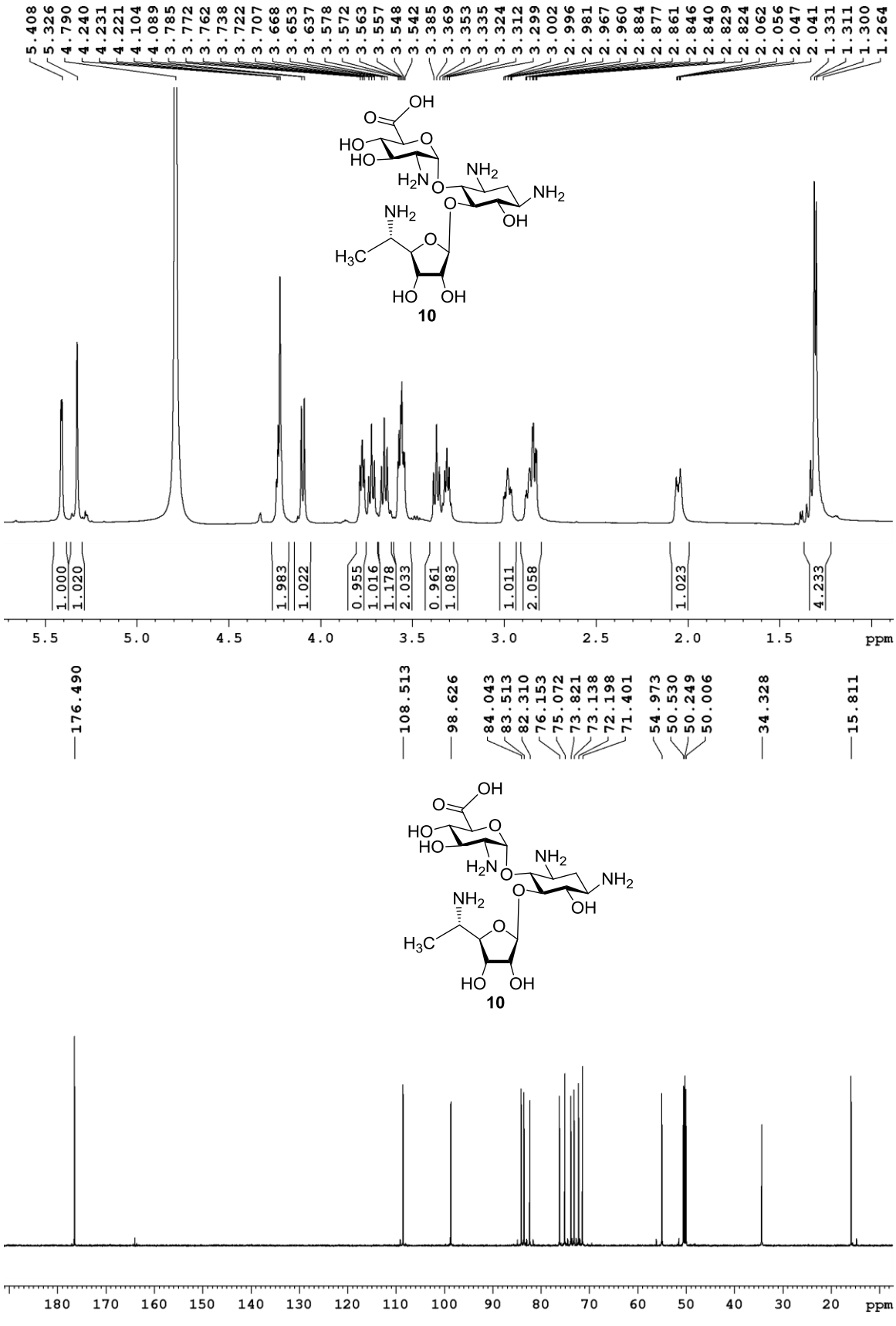
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4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
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82.310
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72.198
71.401
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80
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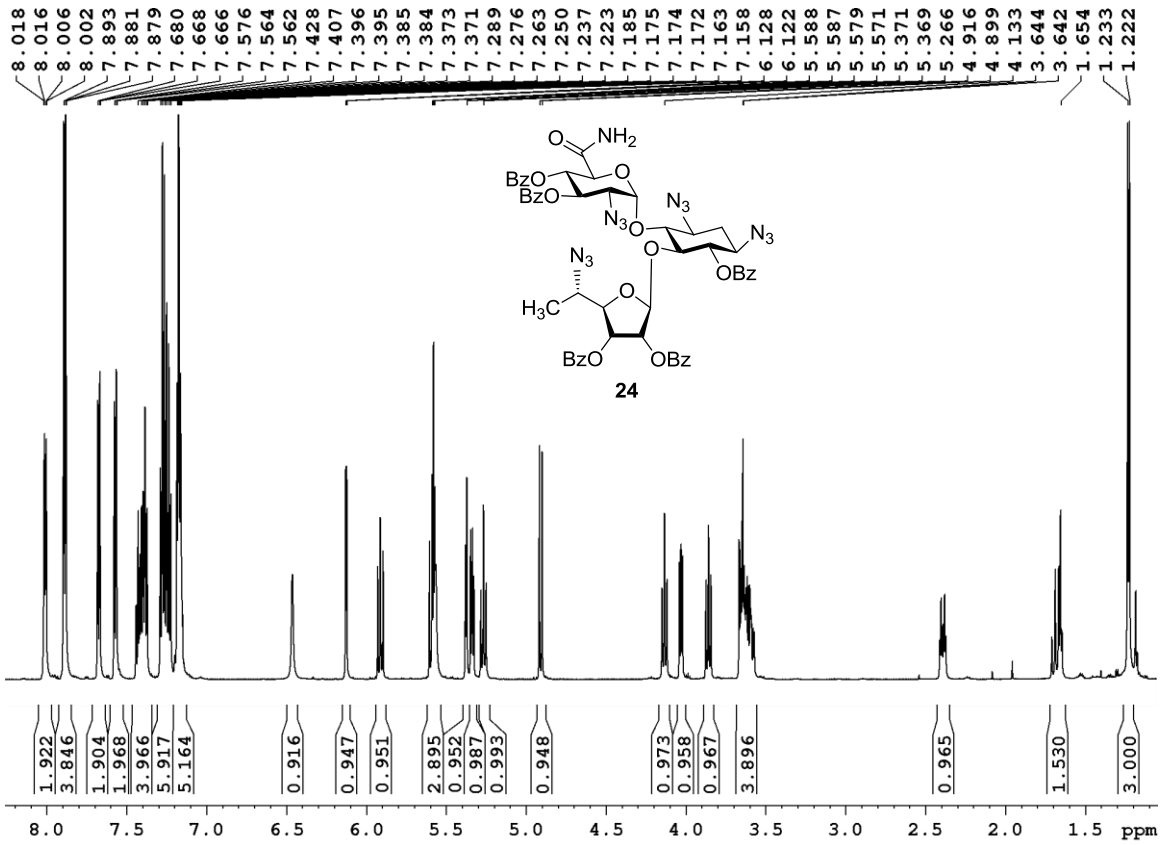
ppm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1.922 3.846 1.904 1.968 3.966 5.917 5.164 0.916 0.947 0.951 2.895 0.952 0.987 0.993 0.948 0.973 0.958 0.967 3.896 0.965 1.530 3.000
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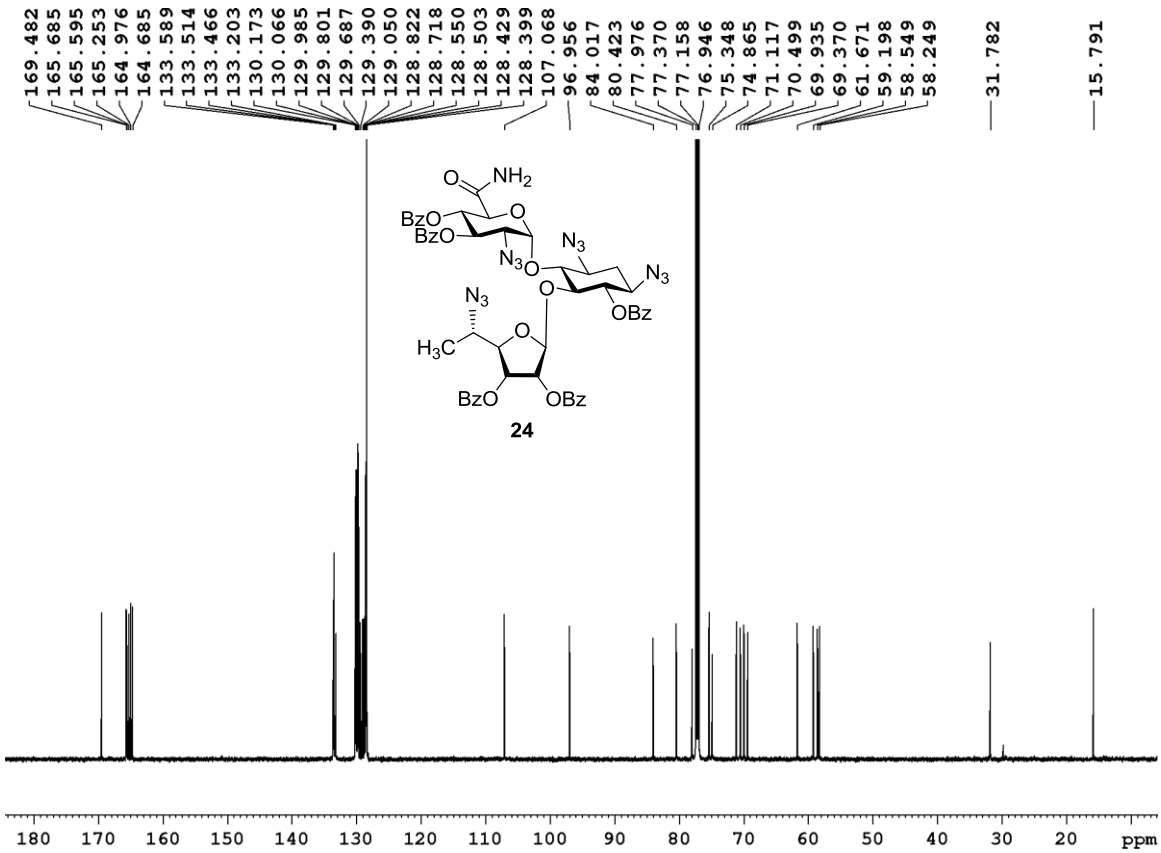
24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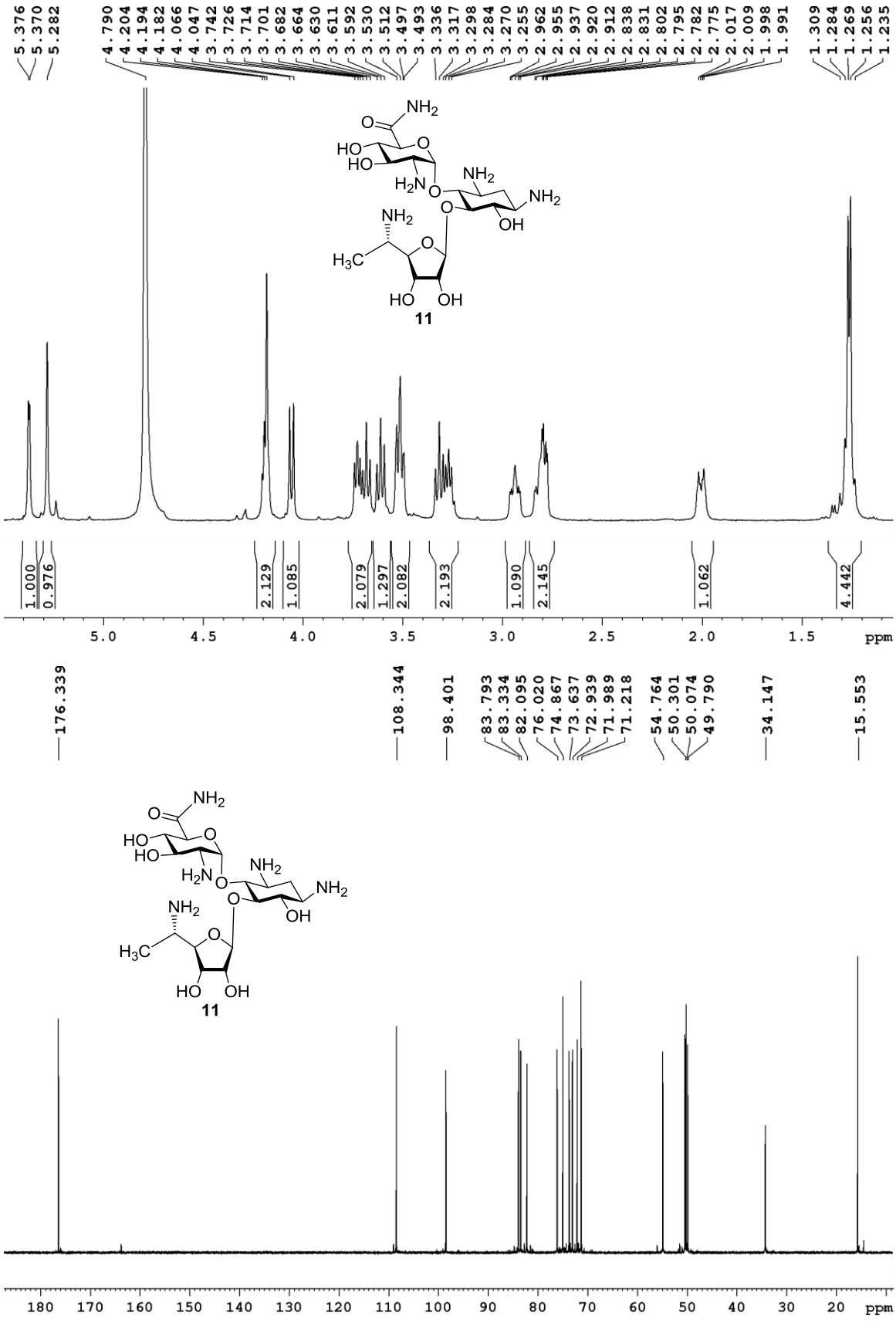
24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2.079
1.297
2.082
2.193
1.090
2.145
1.062
4.442
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
176.339
108.344
98.401
83.793
83.334
82.095
76.020
74.867
73.637
72.939
71.989
71.218
54.764
50.301
50.074
49.790
34.147
15.553
11
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm