

Supporting Information for

The Hexosamine Template – A Platform for Modulating Gene Expression and for Sugar-based Drug Discovery

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1. Synthesis and Characterization of SCFA-Hexosamine Analogs

1.1 Materials and Methods

The starting materials *N*-acetyl-D-glucosamine (GlcNAc) and *N*-acetyl-D-galactosamine (GalNAc) were purchased from Sigma-Aldrich and *N*-acetyl-D-mannosamine (ManNAc) was purchased from New Zealand Pharmaceuticals, and the corresponding fully acylated derivatives, namely, 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-butanoyl- α,β -D-mannopyranose (**1a**), 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl- α,β -D-mannopyranose (**1d**), 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl- α,β -D-mannopyranose (**1f**), 2-acetamido-2-deoxy-3,4,6-tri-*O*-butanoyl- α,β -D-mannopyranose (**1b**), 2-acetamido-2-deoxy-1,3,4-tri-*O*-butanoyl- α,β -D-mannopyranose (**1c**), 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(4-oxopentanoyl)amino- α,β -D-mannopyranose (**1e**) and 2-acetamido-2-deoxy-3,4,6-tri-*O*-

butanoyl- α,β -D-glucopyranose (**2a**) were synthesized following reported procedures (1-3). The synthesis and characterization of the novel compounds **1g**, **1h**, **3** and **3a** are reported below.

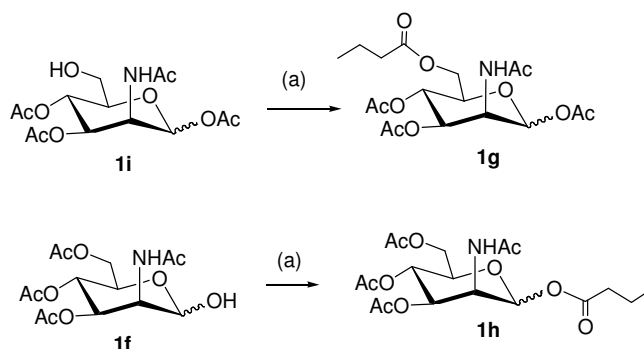
Commercial reagents were used without further purification. Thin layer chromatography (TLC) was performed on silica gel coated glass plates (Cat. No. 21521, ANALTECH). Column chromatography was performed using silica gel 60 Å. ^1H and ^{13}C NMR spectra were obtained using a 400 MHz Bruker instrument at 22 °C; the chemical shifts values are reported in 'δ' and coupling constants (J) in Hz. Mass spectrometry was performed using either ESI-MS, high resolution FAB-MS, or MALDI-TOF (Voyager DE-STR, Applied Biosystems). Elemental analysis of unreported compounds was obtained from Atlantic Microlab, Inc (www.atlanticmicrolab.com). Molecular sieves (4 Å, Sigma-Aldrich) was activated at 150 °C overnight, cooled in a desiccator and powdered freshly before use. Solvent evaporations were performed on a rotary evaporator under reduced pressure at 30-35 °C.

1.2 General Notes on Analog Solubility, Stability, and Anomers

Stock solutions of analogs were made at 10 to 50 mM in ethanol or DMSO to maintain sterility and also because the SCFA-derivatized sugars typically were not soluble in aqueous solutions (e.g., in tissue culture media) above ~500 to 700 μM . When stored at either 4 °C or -20 °C the analogs were stable in for solution for several months (i.e., migration of SCFA groups to the free hydroxyl of triacetylated or tributanoylated analogs was not observed). Analogues were used as the α,β mixtures obtained from column chromatography.

2. General Procedure for the 'Mix & Match' Synthesis of Peracyl ManNAc Analogs with SCFAs of Different Acyl Chain Lengths

Compounds **1g** and **1h** were synthesized from **1i** and **1f**, respectively, by following the procedure shown in **Scheme 1**:



Scheme 1: Scheme for the synthesis of peracyl ManNAc analogs with different acyl chain lengths; conditions: (a) Butyric anhydride, pyridine, DMAP, 22 °C

2.1 Synthesis of 2-Acetamido-1,3,4-tri-O-acetyl-6-O-butanoyl-2-deoxy- α,β -D-manno-pyranose (1g)

To a stirred mixture of **1i** (1.0 g, 2.88 mmol) (**2**) and butyric anhydride (2.0 ml, 12.22 mmol) in pyridine (1.44 ml, 18.32 mmol) at 0 °C (ice-water bath) was added DMAP (cat.) and allowed to warm up to 22 °C. After ~ 24 h, the reaction mixture was concentrated with toluene (3 x 20 ml) and extracted with a mixture of dichloromethane and water. The combined organic layers were dried over anhydrous Na_2SO_4 , filtered and concentrated. Column chromatography of the residue (hexanes: AcOEt 2:3) gave a mixture of anomers of **1g** with 85 % yield and greater than 98 % purity based on the basis of NMR spectra, FAB-MS and elemental analysis (CHN).

2.2 Characterization of 2-Acetamido-1,3,4-tri-O-acetyl-6-O-butanoyl-2-deoxy- α,β -D-manno-pyranose (1g)

Syrup, (Mixture of anomer, Major : Minor = 68 : 32): $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 6.03 (d, 0.32H, $J=2.0$ Hz, H-1 minor), 5.88 (d, 0.68H, $J=2.0$ Hz, H-1 major), 5.87 (d, 0.32 H, NH), 5.84 (m, 0.68H, NH), 5.34 (dd, 0.32 H, $J=4.4$ & 10.0 Hz), 5.17 (t, 0.32H, $J=10.0$ Hz), 5.13 (t, 0.68H, $J=10.0$ Hz), 5.04 (dd, 0.68H, $J=4.0$ & 10.0 Hz), 4.79 (m, 0.68H, H-2), 4.64 (m, 0.32H, H-2), 4.31-4.24 (m, 1H), 4.17-4.00 (m, 1.32H, H-6b & H-5), 3.80 (m, 0.68H), 2.37-2.32 (t, 2H, CH_2), 2.18, 2.16, 2.14, 2.12, 2.12, 2.06, 2.02 (s, 2.04H, NHAc), 1.99 (s, 0.96H, NHAc), 1.71-1.67 (q, 2H), 1.00-0.96 (t, 3H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) : δ 173.1, 173.1, 170.6, 170.1, 170.1, 170.0, 169.7, 169.7, 168.3 (NHCO), 168.1 (NHCO), 91.7 (C-1minor), 90.6 (C-1 major), 73.5, 71.4, 70.2, 68.8, 65.6, 65.3, 61.9 (C-6), 61.7 (C-6), 49.5 (C-2), 49.3 (C-2), 35.9, 35.8, 23.3, 23.3, 20.8, 20.7, 20.7, 20.7, 20.7, 20.6, 20.6, 20.6, 18.4, 18.2, 13.6, 13.6; FAB-MS : Calcd for $\text{C}_{18}\text{H}_{28}\text{NO}_{10}$ ($[\text{M}+\text{H}]^+$): 418.1716, found: 418.1711; Anal. Calcd. for $\text{C}_{18}\text{H}_{28}\text{NO}_{10}$: C, 51.79; H, 6.52; N, 3.36. Found: C, 51.78; H, 6.66; N, 3.20.

2.3 Synthesis of 2-Acetamido-3,4,6-tri-O-acetyl-1-O-butanoyl-2-deoxy- α,β -D-manno-pyranose (1h)

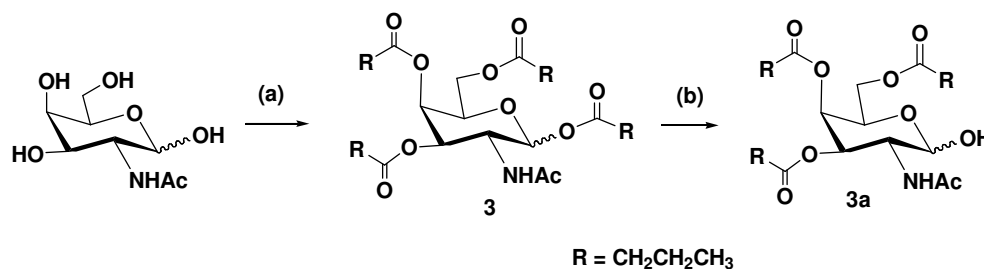
To a stirred solution of **1f** (1.0 g, 2.88 mmol) (**2**) and butyric anhydride (2.0 ml, 12.22 mmol) in pyridine (1.44 ml, 18.32 mmol) at 0 °C (ice water bath) was added DMAP (cat.) and allowed to warm up to 22 °C. After ~ 24 h, the reaction mixture was concentrated, co-concentrated with toluene (3 x 10 ml) and the residue was extracted with a mixture of dichloromethane (100 ml) and water (50 ml). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered and concentrated. Column chromatography of the residue (hexanes: AcOEt) gave **1h** (75 %) as a mixture of anomers with greater than 99 % purity based on NMR spectra, FAB-MS and elemental analysis (CHN).

2.4 Characterization of 2-Acetamido-3,4,6-tri-O-acetyl-1-O-butanoyl-2-deoxy- α,β -D-mannopyranose (1h)

Syrup, (Mixture of anomers; Major : Minor = 90:10): $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 6.07 (d, 0.9H, $J = 1.6$ Hz, H-1), δ 5.89 (d, 0.1H, $J = 1.6$ Hz, H-1), 5.76 (d, 0.1H, $J = 9.2$ Hz, NH), 5.74 (d, 0.9H, $J = 9.2$ Hz, NH), 5.35 (dd, 0.9H, $J = 4.4$ & 10.4 Hz), 5.18 (t, 0.9H, $J = 10.4$ Hz), 5.10 (t, 0.1H, $J = 9.6$ Hz), 5.08 (dd, 0.1H, $J = 3.6$ & 9.6 Hz), 4.78 (m, 0.1H, H-2), 4.66 (m, 0.9H, H-2), 4.33 (dd, 0.1H), 4.30 (dd, 0.9H, $J = 5.4$ & 12.3 Hz), 4.10-4.00 (m, 1.9H), 3.82 (m, 0.1H, H-5), 2.40 (t, 1.8H, CH_2), 2.36 (t, 0.2H, CH_2), 2.16, 2.11, 2.10, 2.09, 2.08, 2.08 (6s, 9H), 2.04 (s, 0.3H, NHAc), 2.02 (s, 2.7H, NHAc), 1.74 (q, 1.8H, CH_2), 1.67 (q, 0.2H, CH_2), 1.00 (t, 2.7H, CH_3), 0.80 (t, 0.3H, CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ 171.0, 170.7, 170.6, 170.5, 170.1, 170.1, 170.0, 170.0, 169.7 (NHCO), 169.6 (NHCO), 91.5 (C-1), 90.6 (C-1), 73.4, 71.3, 70.2, 68.9, 65.6, 65.4, 62.1 (C-6), 62.0 (C-6), 49.6 (C-2), 49.4 (C-2), 35.9, 35.7, 23.3, 23.3, 20.7, 20.6, 20.6, 18.3, 17.9, 13.5, 13.4; FAB-MS : Calcd for $\text{C}_{18}\text{H}_{28}\text{NO}_{10}$ ($[\text{M}+\text{H}]^+$): 418.1716, found: 418.1713. Anal. Calcd. for $\text{C}_{18}\text{H}_{28}\text{NO}_{10}$: C, 51.79; H, 6.52; N, 3.36. Found: C, 51.81; H, 6.58; N, 3.34.

3. Synthesis of 2-Acetamido-3,4,6-tri-O-butanoyl-2-deoxy- α,β -D-galactopyranose

The titled compounds were synthesized by following the reported procedure (2) starting from D-galactopyranose in two steps as shown in **Scheme 2**:



Scheme 2: Synthesis of 2-acetamido-1,3,4,6-tetra-O-butanoyl-2-deoxy- α,β -D-galactopyranose (**3**), Synthesis of 2-acetamido-3,4,6-tri-O-butanoyl-2-deoxy- α,β -D-galactopyranose (**3a**), Conditions: (a) $(\text{RCO})_2\text{O}$, pyridine, DMAP, 22 °C, 24 h; (b) Molecular sieves 4 Å, MeOH, 22 °C, 7–12h (5)

3.1 Synthesis of 2-Acetamido-1,3,4,6-tetra-O-butanoyl-2-deoxy- α -D-galactopyranose (**3**)

To a stirred solution of 2-acetamido-2-deoxy- α,β -D-galactopyranose (2.16 mmol) in pyridine (1.46 ml, 18 mmol) at 0 °C (ice-water bath), butyric anhydride (12 mmol) was added. The reaction mixture was allowed to warm up to 22 °C and monitored by TLC (hexanes: AcOEt 1:1). After 24 h, the mixture was concentrated with toluene (3 x 10 ml), and extracted using a mixture of dichloromethane (100 ml) and water (50 ml). The organic layers were collected, dried over anhydrous Na_2SO_4 filtered and concentrated. Column chromatography of the residue (hexanes: AcOEt) gave the titled compound with more than 96 % purity.

3.2 Characterization of 2-Acetamido-1,3,4,6-tetra-O-butanoyl-2-deoxy- α -D-galactopyranose (3)

Syrup, Yield: 79 % : $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 6.20 (d, 0.16H, $J = 3.6$ Hz, H-1), 5.50-5.30 (m, 2H, H-4, NH), 5.23 (m, 1H, H-3), 4.70 (m, 1H, H-2), 4.21 (m, 1H, H-5), 4.15-3.90 (m, 2H, H-6a & H-6b), 2.50-2.15 (m, 8H), 1.92 (s, 3H, NHAc), 1.70-1.1.40 (m, 8H), 1.10-0.78 (m, 12H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) : δ 173.8, 172.9, 172.7, 171.4, 169.8 (NHCO), 91.1 (C-1), 68.8, 67.7, 66.6, 61.3 (C-6), 47.2 (C-2), 36.0, 36.0, 35.9, 35.8, 23.1, 18.5, 18.4, 18.4, 18.3, 13.7, 13.6, 13.6, 13.5. MALDI-MS: Calcd for $\text{C}_{24}\text{H}_{39}\text{N O}_{10}\text{Na}$ ($[\text{M}+\text{Na}]^+$): 524.2472, found: 524.2490.

3.3 Synthesis of 2-Acetamido-3,4,6-tri-O-butanoyl-2-deoxy- α -D-galactopyranose (3a)

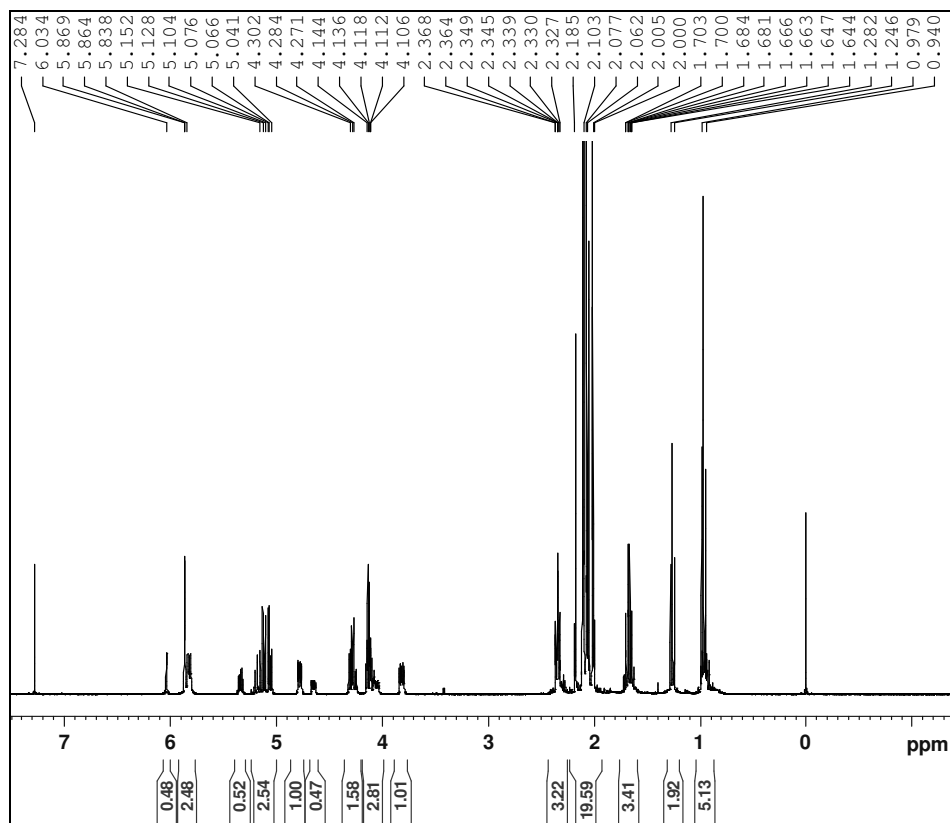
The 2-acetamido-1,3,4,6-tetra-O-butanoyl-2-deoxy- α -D-galactopyranose **3** (2.0 mmol) was mixed with activated and powdered molecular sieves 4\AA (4.0 g) in methanol (100 ml) and stirred at $22\text{ }^\circ\text{C}$. The reaction mixture was monitored by TLC {hexanes: ethyl acetate (AcOEt)} to maximize conversion to the hemi-acetal while minimizing de-acylation at positions other than C1. After $\sim 7 - 12$ h, the reaction mixture was filtered through a pad of celite, washed twice with methanol (10 ml) and the combined filtrate was concentrated. Column chromatography of the residue (hexanes: ethyl acetate (AcOEt)) was done to separate unreacted starting material, respectively from the hemiacetals (**3a**) with more than 97 % purity NMR spectra, MALDI-MS and elemental analysis (CHN).

3.4 Characterization of 2-Acetamido-3,4,6-tri-O-butanoyl-2-deoxy- α -D-galactopyranose (3a)

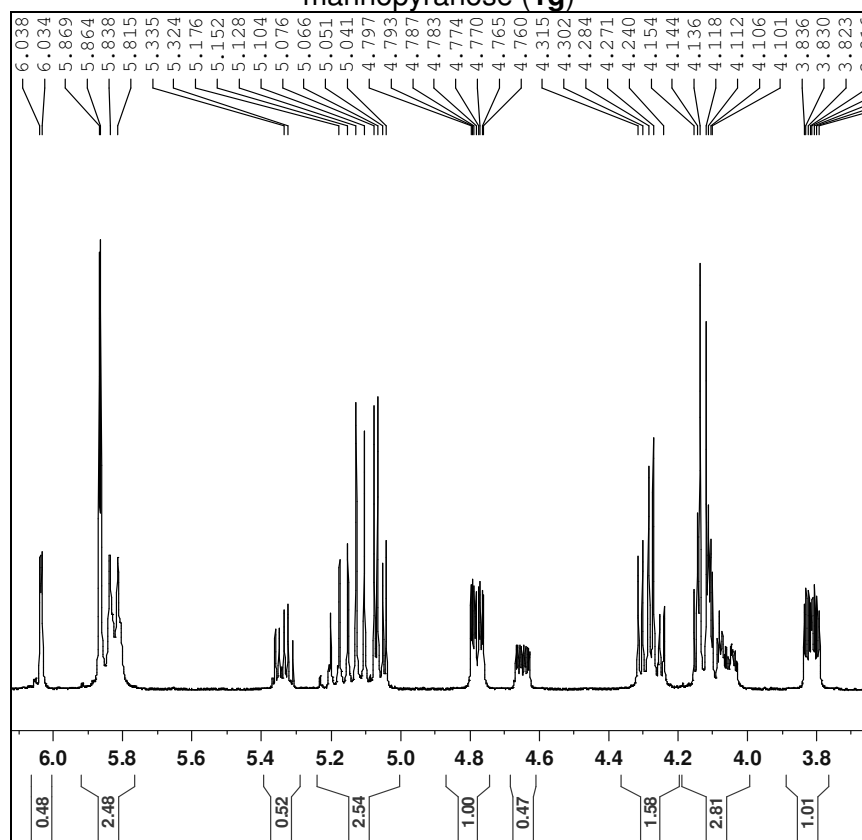
Syrup, Yield: 82 %: δ $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 5.82 (d, 1H, $J = 9.2$ Hz, NH), 5.42-5.38 (m, 1H, H-4 major), 5.30 (t, 1H, $J = 3.6$ Hz, H-1), 5.26 (dd, 1H, $J = 3.6$ & 10.8 Hz, H-3), 4.55 (m, 1H, H-2), 4.41 (m, 1H, H-5), 4.20-4.00 (m, 2H), 3.40 (m, 1H, C-1OH), 2.43-2.13 (m, 6H), 1.96 (s, 3H, NHAc), 1.74-1.50 (m, 6H), 1.10-0.86 (m, 9H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ 173.5, 173.2, 170.8, 170.0, 92.4, 67.8, 67.3, 66.8, 61.9, 48.2, 36.1, 36.0, 35.9, 23.3, 18.6, 18.3, 18.2, 13.5, 13.5, 13.5. MALDI-MS: Calcd for $\text{C}_{20}\text{H}_{33}\text{NO}_9\text{Na}$ ($[\text{M}+\text{Na}]^+$): 454.2053, found: 454.2043; Anal. Calcd. for $\text{C}_{20}\text{H}_{33}\text{NO}_9$: C, 55.67; H, 7.71; N, 3.25. Found: C, 55.91; H, 7.67; N, 3.26.

4. Spectra of Selected Compounds

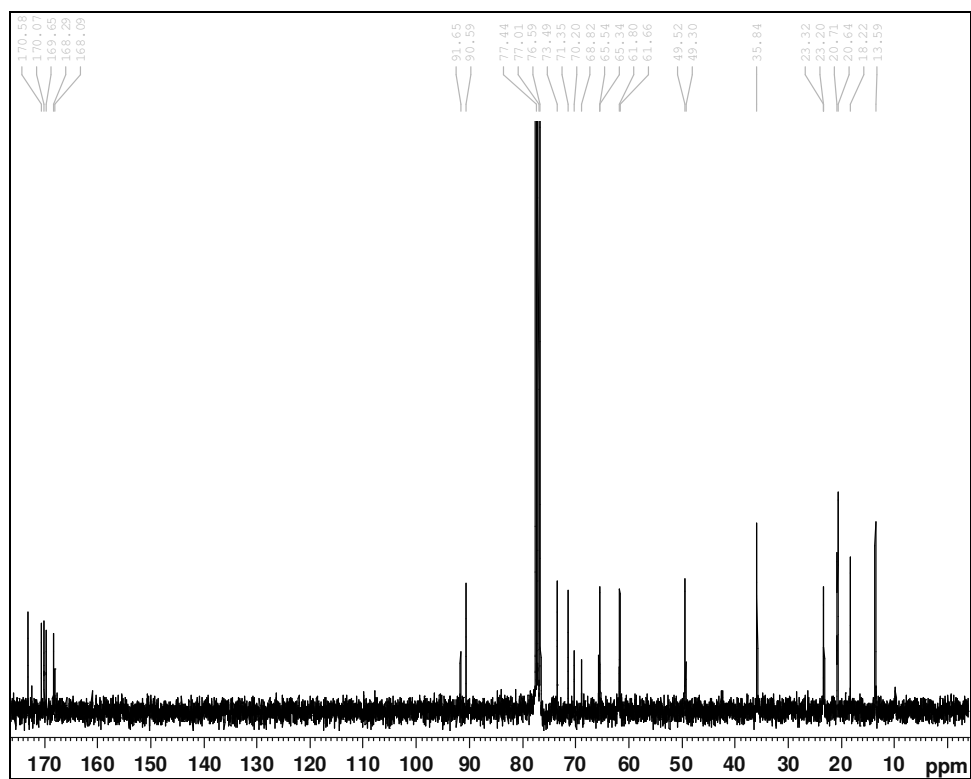
Proton (^1H) and ^{13}C NMR spectra are presented on the following pages for compounds **1g**, **1h**, and **3a** and a ^1H spectrum is given for the intermediate compound **3**.



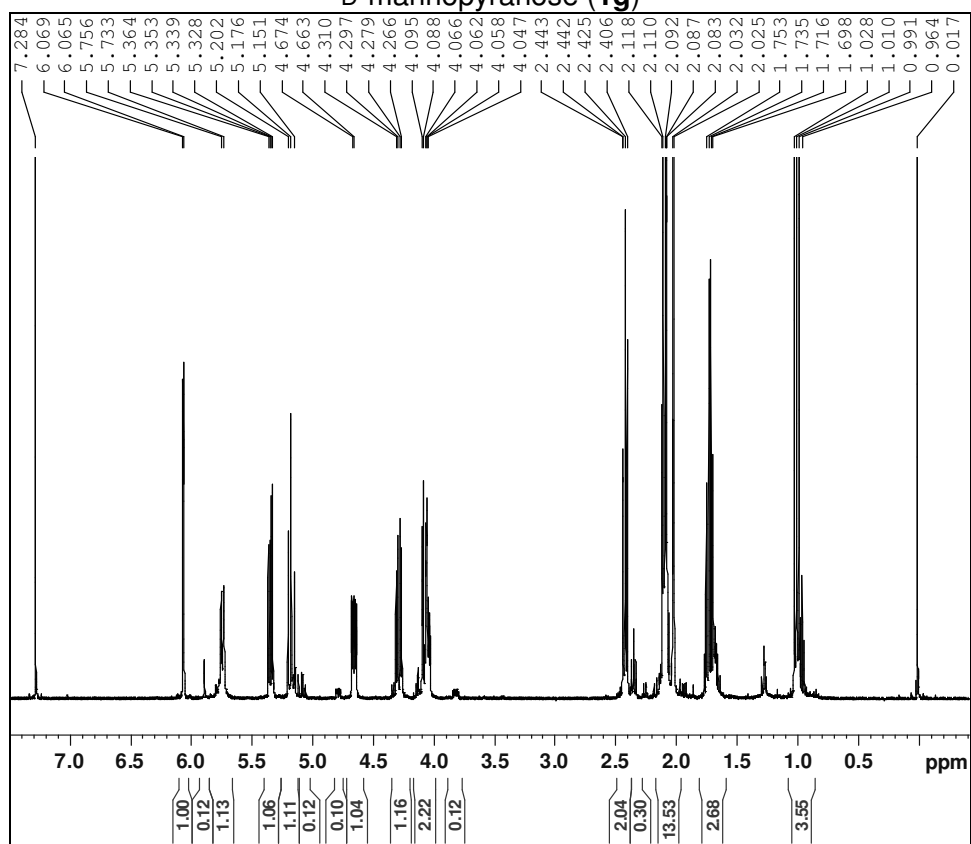
$^1\text{H-NMR}$ (400 MHz, CDCl_3) spectrum of 2-acetamido-1,3,4-tri-*O*-acetyl-6-*O*-butanoyl-2-deoxy- α,β -D-mannopyranose (**1g**)



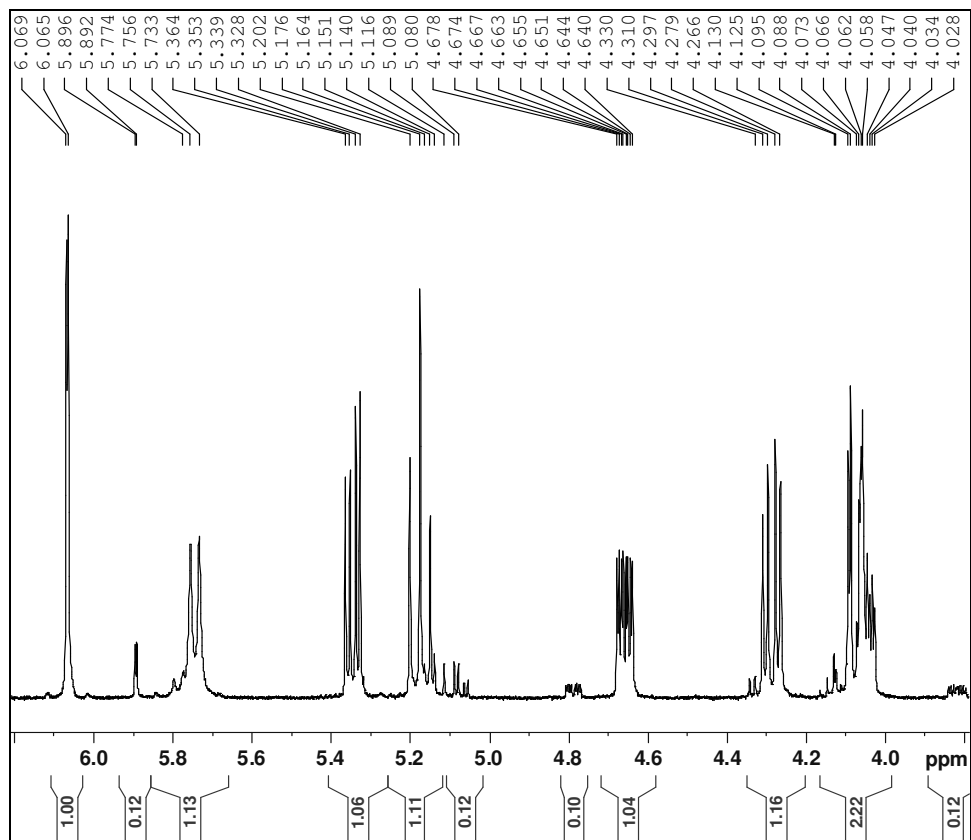
Expanded $^1\text{H-NMR}$ (400 MHz, CDCl_3) spectrum of 2-acetamido-1,3,4-tri-*O*-acetyl-6-*O*-butanoyl-2-deoxy- α,β -D-mannopyranose (**1g**)



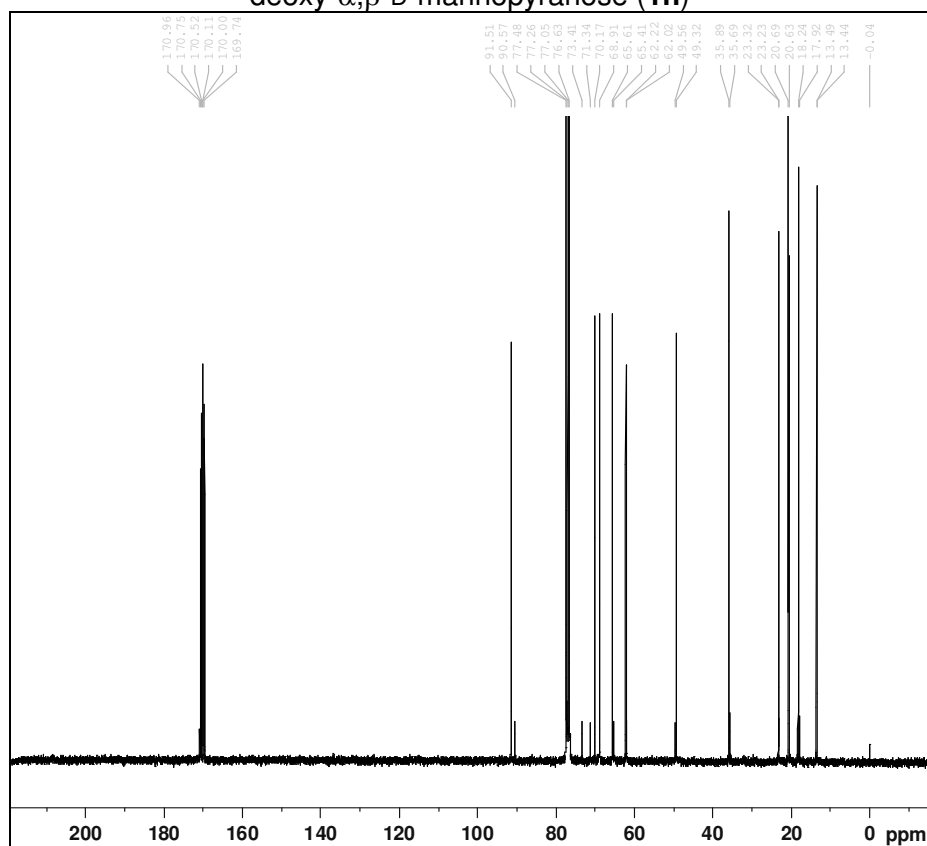
^{13}C -NMR (100 MHz, CDCl_3) spectrum of 2-acetamido-1,3,4-tri-*O*-acetyl-6-*O*-butanoyl-2-deoxy- α,β -D-mannopyranose (**1g**)



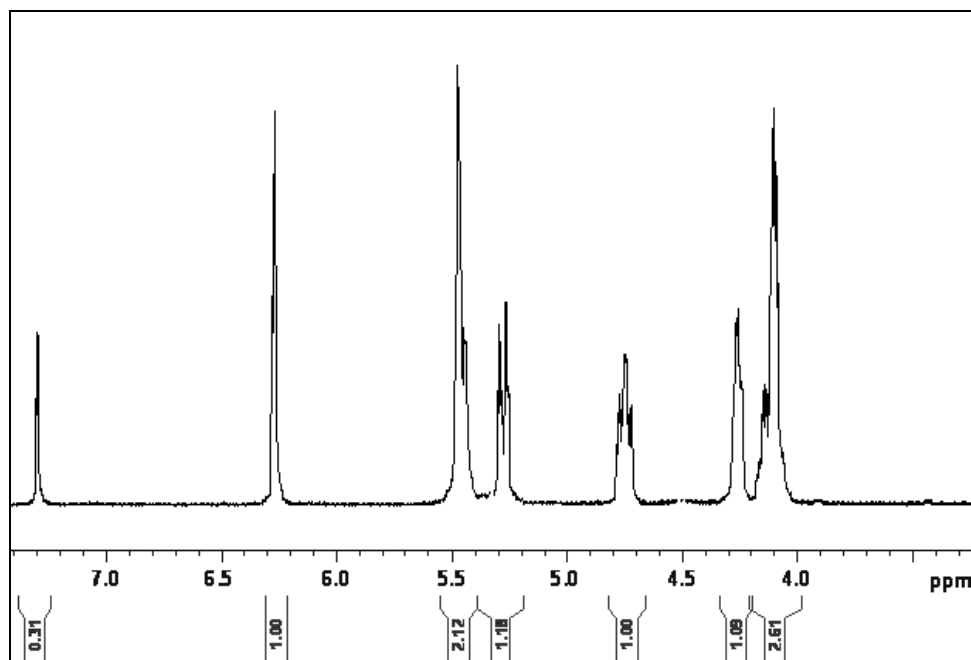
^1H -NMR (400 MHz, CDCl_3) spectrum of 2-Acetamido-3,4,6-tri-*O*-acetyl-1-*O*-butanoyl-2-deoxy- α,β -D-mannopyranose (**1h**)



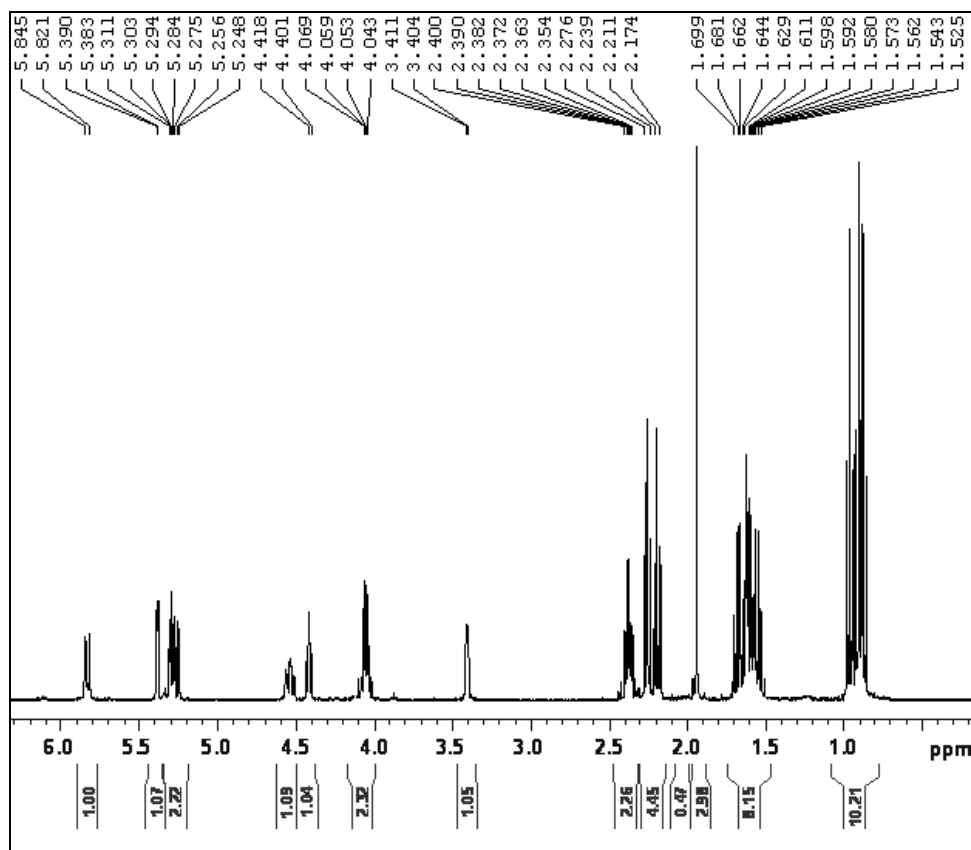
Expanded $^1\text{H-NMR}$ (400 MHz, CDCl_3) spectrum of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*O*-butanoyl-2-deoxy- α,β -D-mannopyranose (**1h**)



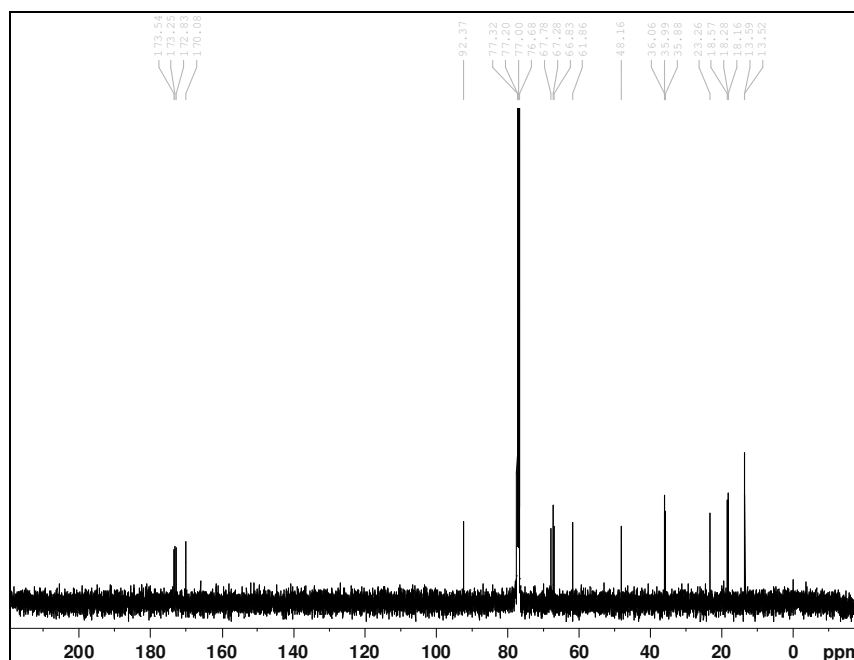
$^{13}\text{C-NMR}$ (100 MHz, CDCl_3) spectrum of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*O*-butanoyl-2-deoxy- α,β -D-mannopyranose (**1h**)



¹H-NMR (400 MHz, CDCl₃) spectrum of 2-acetamido-1,3,4,6-tetra-*O*-butanoyl-2-deoxy- α -D-galactopyranose (**3**)



¹H-NMR (400 MHz, CDCl₃) spectrum of 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy- α -D-galactopyranose (**3a**)



^{13}C -NMR (100 MHz, CDCl_3) spectrum of 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy- α -D-galactopyranose (**3a**)

5. Protocol for Ligand and Protein Binding Analysis by AutoDock

Binding analysis was done using the software packages: AutoDock Tools v1.5.2r2 (Molecular Graphics Laboratory, The Scripps Research Institute, La Jolla, CA) and ChemDraw Pro v8.0.3 (CambridgeSoft Corporation, Cambridge, MA)

5.1 Generation of 3D Coordinates for the Ligand and Protein

The ligand was drawn in ChemDraw and copied by opening the 'Edit' menu and selecting 'Copy As'-'SMILES'. The website http://www.molecular-networks.com/online_demos/corina_demo.html was opened in a web browser. The SMILES code for the ligand was pasted in the form on the website and the 'Submit' button was clicked. The option to 'Download 3D structure as PDB file' was selected and the PDB file was saved. AutoDock was opened, and in the 'Ligand' menu, the 'Input'-'Open' option was selected. Taking care to ensure the 'Files of Type' drop-down menu read 'PDB' and not 'PDBQT', the recently saved PDB file was located and opened. In the 'Ligand' menu, the 'Output'-'Save as PDBQT' option was selected and the PDBQT file of the ligand was saved. The ligand was closed by selecting its name and pressing the Delete key on the keyboard.

In the 'File' menu, the 'Read'-'Molecule From Web' option was selected. The PDB code for the protein was entered and the protein was downloaded from the internet. From the 'Select' menu, the 'Select From String' option was selected. 'HOH*' was entered in the box for residues and the 'Add'

button was clicked. The dialog that appeared was dismissed. The Delete key on the keyboard was pressed and the 'Continue' option selected on the dialog that appeared to delete any water molecules downloaded with the protein. From the 'Edit' menu, the 'Hydrogens'-'Add'-'Add All' option was selected and the dialog box was dismissed by clicking the 'Ok' button. From the 'Flexible Residues' menu, the 'Input'-'Choose Macromolecule'-'Choose Your Protein' option was selected. In the resulting dialog asking to merge non-polar hydrogens, the 'Yes' option was selected and the dialog box was dismissed by clicking the 'Ok' button. From the 'File' menu, the 'Save'-'Write PDBQT' option was selected and the protein was saved. AutoDock was closed.

5.2 Running Ligand-Protein Analysis after Generation of Ligand and Protein Files

AutoDock was opened. From the 'Grid' menu, the 'Macromolecule'-'Open' option was selected. The PDBQT file of the protein of interest was located and opened. When asked to preserve charges, the 'Yes' option was selected. The dialog box that appeared was dismissed. From the 'Grid' menu, the 'Grid Box' option was selected. The wheels were adjusted so that the entire protein was covered by the grid box. To spin the protein to confirm that all dimensions were properly covered, from the '3D Graphics' menu, the 'Spin-Bounce-Oscillate' option was selected and the 'Spin' option clicked. The 'Off' button was then clicked and the spin dialog box closed. In the Grid Box dialog, the 'File' menu was opened and the 'Close Saving Current' option was selected. In the 'Grid' menu, the 'Set Map Type'-'Open Ligand' option was selected and the PDBQT file of the ligand of interest was located and opened. In the 'Grid' menu, the 'Output'-'Save GPF' was selected. An appropriately named GPF file was saved. In the 'Run' menu, the 'Run AutoGrid' option was selected. The appropriate path to the program 'autogrid4.exe' was entered in the box marked 'Program Pathname' and the correct GPF file was confirmed to be in the box marked 'Parameter Filename.' The 'Launch' button was clicked and AutoGrid executed. When execution was complete (approximately two minutes), the log dialog box was dismissed.

In the 'Docking' menu, the 'Macromolecule'-'Set Rigid Filename' option was selected. The protein PDBQT file was located and selected. In the 'Docking' menu, the 'Ligand'-'Choose' option was selected. The ligand was selected and the 'Select Ligand' button was clicked. In the 'Docking' menu, the 'Search Parameters'-'Genetic Algorithm' command was selected. The 'Number of GA Runs' was set to 50, and the drop-down menu under 'maximum number of evals' was set to 'Long'. The box was dismissed by clicking 'Accept.' In the 'Docking' menu, the 'Output'-'Lamarckian GA' command was selected. The DPF file was assigned a name identical to the corresponding GPF file (except for the file extension) and placed in the same folder as the GPF file. In the 'Run' menu, the 'Web Services' command was selected. In the 'Web Services Location' box, the following URL was entered: 'http://ws.nbc.net/opal/services'. In the section of the dialog box noted as 'AutoDock', the path to the DPF file was entered. The 'Run AutoDock' button was clicked. After approximately one

minute, the 'Web Services Status' read 'ACTIVE' The program was left open for the approximately two days until 'Web Services Status' indicated that the execution had completed.

5.3 Analysis of Docking Results

AutoDock was restarted. In the 'Analyze' menu, the 'Dockings'-'Open' command was selected. The docking log file (DLG) that was generated had the same name as the input DPF file (except for the file extension). That file was opened, and the dialog box that appeared was dismissed. In the 'Analyze' menu, the 'Macromolecule'-'Open' command was selected. In the 'Analyze' menu, the 'Conformations'-'Play, ranked by energy' command was selected. The r, c, and n keys on the keyboard were pressed simultaneously to center the display of the protein and ligand in the viewing area. In the control dialog that opened when the 'Play, ranked by energy' command was selected, the arrows were pressed to cycle through the various conformations. In the '3D Graphics' menu, the 'SetBackgroundColor' command was selected. The color white was selected and the dialog box was dismissed. The ligand was selected by clicking the square in the 'Sel.' column next to the name of the ligand in the molecule list. In the 'Display' menu, the 'Sticks and Balls' command was selected. In the dialog box that appeared, the 'Sticks and Balls' radio button was selected. 'Stick Radius' was set to 0.10 and the 'Ok' button was clicked. The ligand was deselected by the same process it was selected. The ligand was colored by atom by clicking on the diamond in the 'Atom' column next to the name of the ligand in the molecule list. The protein was selected by the same process the ligand was selected. In the 'Display' menu, the 'Secondary Structure' command was selected. In the dialog box that appeared, the 'display only' radio button was checked and the 'Ok' button was pressed. The protein was colored by secondary structure by clicking on the diamond in the 'Sec. Str.' column next to the name of the protein in the molecule list. With the protein still selected, in the 'Color' menu, the 'by Atom Type' command was selected. The checkbox named 'lines' was checked and 'Ok' as pressed.

In the dialog that opened when the 'Play, ranked by energy' command was selected, the '&' button was clicked. Clicking the checkbox 'Show Info' showed relevant parameters such as binding energy and inhibition constant. The 'Build H-Bonds' checkbox was clicked. In the hydrogen bonding dialog box that appeared, 'Show All' and 'Show Distances' were checked. In the 'Hydrogen Bonds' menu, the 'Display'-'As Spheres' command was selected. The 'Choose Color' button was pressed on the dialog box that appeared. The color black was selected and the color dialog box was closed. The spacing of the spheres was set to 0.20 and the Enter key was pressed on the keyboard. The docking was rotated and zoomed until a good picture could be captured. The open dialog boxes were moved out of the way so that the picture could be seen fully. In the 'File' menu, the 'Save'-'Save Image As' command was selected. The path where the image was to be stored was entered in the box that appeared and the image was saved.

6. References

1. Sampathkumar, S.-G., Jones, M. B., Meledeo, M. A., Campbell, C. T., Choi, S. S., Hida, K., Gomutputra, P., Sheh, A., Gilmartin, T., Head, S. R., and Yarema, K. J. (2006) Targeting glycosylation pathways and the cell cycle: sugar- dependent activity of butyrate-carbohydrate cancer prodrugs, *Chem. Biol.* *13*, 1265-1275.
2. Aich, U., Campbell, C. T., Elmouelhi, N., Weier, C. A., Sampathkumar, S.-G., Choi, S. S., and Yarema, K. J. (2008) Regioisomeric SCFA attachment to hexosamines separates metabolic flux from cytotoxicity and MUC1 suppression, *ACS Chem. Biol.* *3*, 230-240.
3. Campbell, C. T., Aich, U., Weier, C. A., Wang, J. J., Choi, S. S., Wen, M. M., Maisel, K., Sampathkumar, S.-G., and Yarema, K. J. (2008) Targeting pro-invasive oncogenes with short chain fatty acid-hexosamine analogs inhibits the mobility of metastatic MDA-MB-231 breast cancer cell, *J. Med. Chem.* *51*, 8135–8147.