

## **ONLINE RESOURCE 1: COMPOUND CHARACTERIZATION**

**Article Title: Cellular Metabolism of Unnatural Sialic Acid Precursors**

Journal: Glycoconjugate Journal

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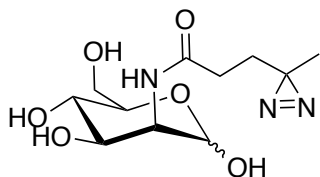
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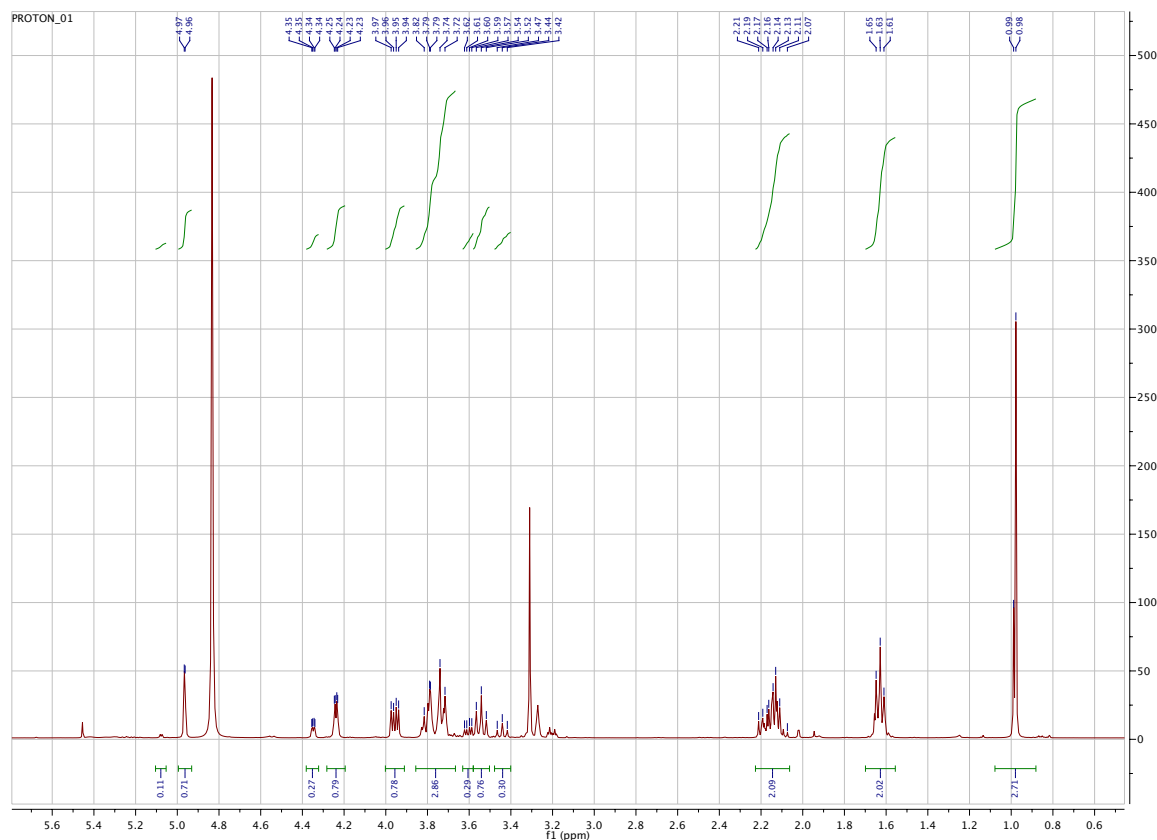
## I. Synthesis of ManNDaz

### 2-Acetyl-*N*-4,4-azo-pentamido-2-deoxy- $\alpha,\beta$ -D-mannopyranose (ManNDaz)

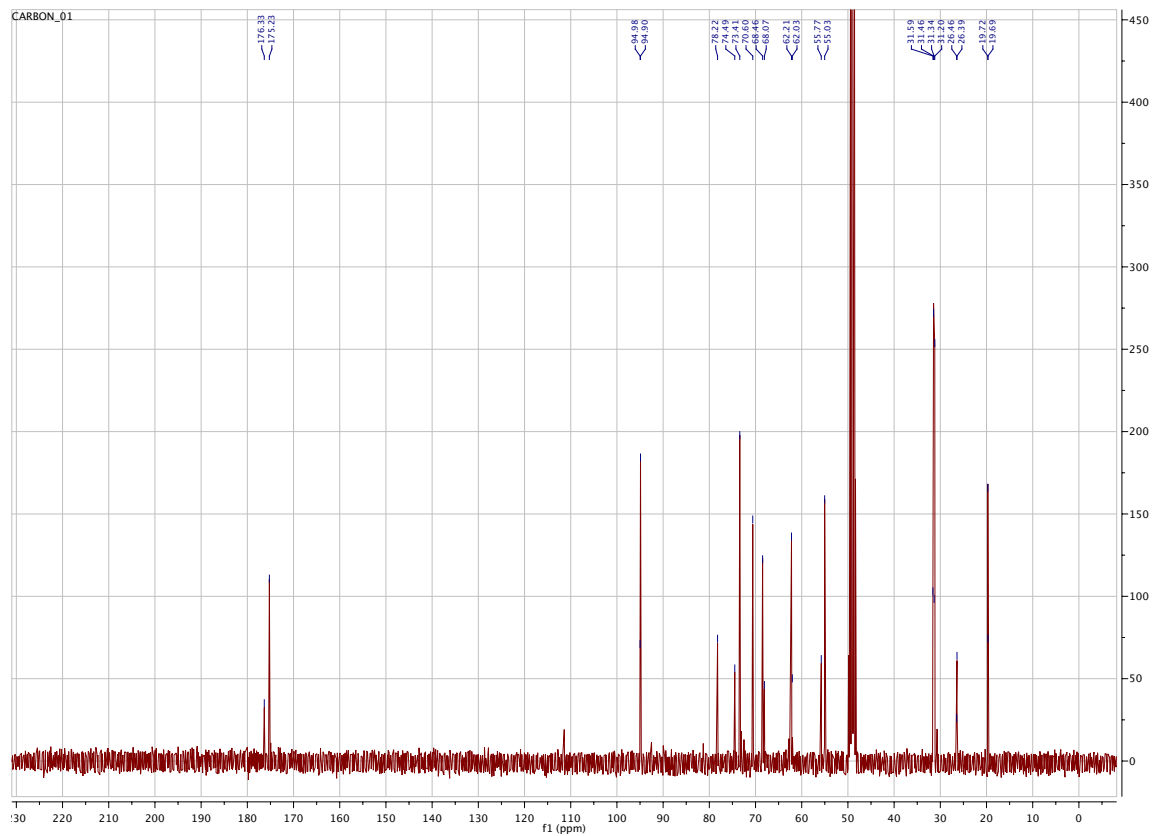


92 mg of Ac<sub>4</sub>ManNDaz (0.20 mmol) were dissolved in 5 mL dry methanol, followed by the addition of 0.2 mL of 0.5 M NaOMe in methanol (0.10 mmol; 0.12 eq.). After stirring at room temperature for 45 min, the solution was run over a Dowex 50WX8-100 resin for neutralization and removal of salt, followed by purification over silica using 12 % methanol in methylene chloride (RF 0.35 % with 20 % methanol in methylene chloride) which afforded 30 mg of ManNDaz (0.10 mmol; 52 % yield) in good purity as a colorless film.

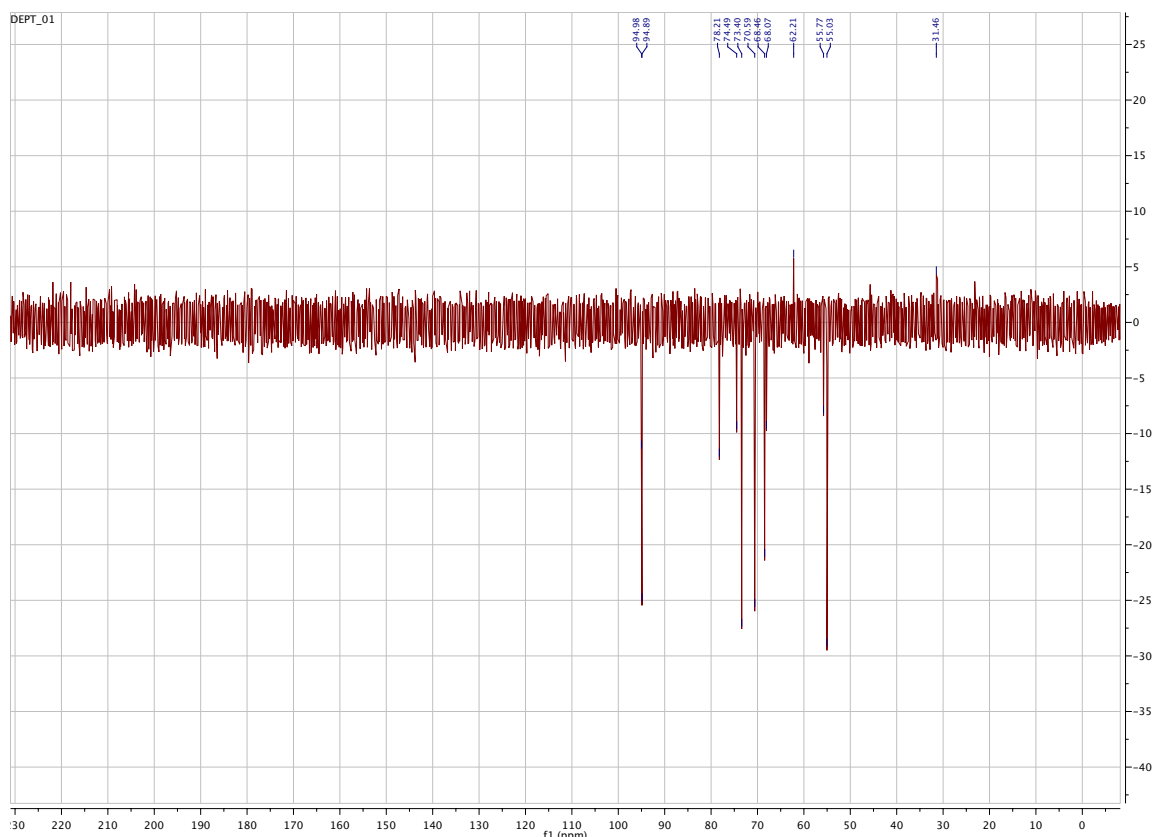
**<sup>1</sup>H-NMR** (400 MHz, CD<sub>3</sub>OD, major anomer):  $\delta$  = 0.98 (3H, s), 1.58–1.67 (2H, m), 2.07–2.23 (2H, m), 3.54 (1H, t,  $J$ = 9.6 Hz), 3.70–3.84 (3H, m), 3.95 (1H, dd,  $J$ = 4.7, 9.7 Hz), 4.24 (1H, dd,  $J$ = 1.7, 4.7 Hz), 4.97 (1H, d,  $J$ = 1.7 Hz). **<sup>13</sup>C-NMR** (400 MHz, CD<sub>3</sub>OD, major anomer):  $\delta$  = 19.7, 26.4, 31.2, 31.5, 55.0 (CH), 62.2, 68.5 (CH), 70.6 (CH), 73.4 (CH), 94.9 (CH), 175.2. **HRMS (ESI-TOF)** for C<sub>11</sub>H<sub>19</sub>N<sub>3</sub>O<sub>6</sub>Na [M+Na]<sup>+</sup> calcd.: 312.1166, found: 312.1155,  $\Delta$  = 3.5 ppm.



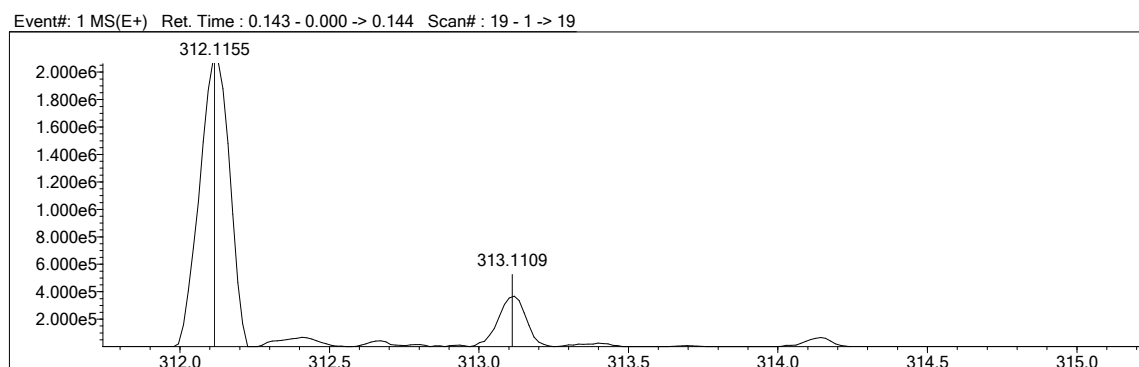
**Figure S1.** <sup>1</sup>H-NMR spectrum of ManNDaz.



**Figure S2.**  $^{13}\text{C}$ -NMR spectrum of ManNDaz.



**Figure S3.** DEPT-90-spectrum of ManNDAz.

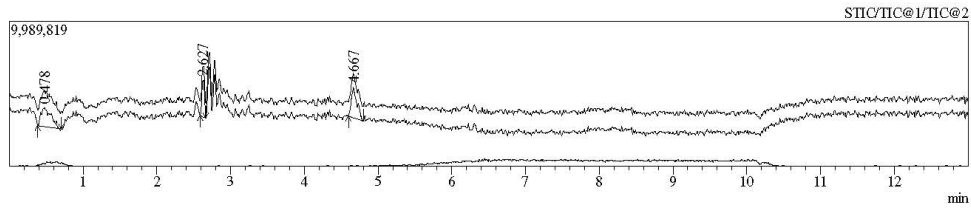


**Figure S4.** ESI-MS of ManNDAz.

## II. Mass spectrometry detection of Neu5Ac-9-P

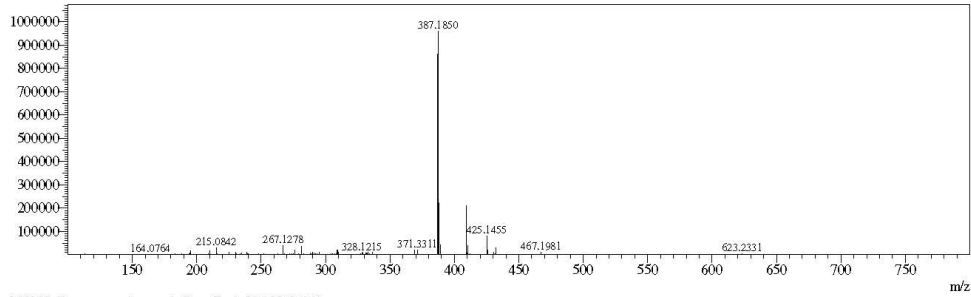
The sample was dissolved in 80% MeOH/20% water and injected onto a high resolution mass spectrometer following liquid chromatography (LCMS IT-TOF (Shimadzu)) with a Gemini 50 x 2.00 mm 5 microns (Phenomex) column with a flow rate of 0.4 mL/min. The solvent system consisted of 95/5/0.1 % H<sub>2</sub>O/ACN/formic acid (solvent A) and 95/5/0.1 % ACN/H<sub>2</sub>O/formic acid (solvent B) with the following gradient of solvent B: 0-0.3 min, 0 %; 0.3-3 min, 50 %; 3-5 min, 95 %; 5-9 min, 95 %; 9-9.1 min, 0 %; 9.1-13.0 min, 0 %.

<Chromatogram>



<Spectrum>

MSMS: Precursor  $m/z$  ----- /+ Base Peak 387.19(959280)



MSMS: Precursor  $m/z$  ----- /- Base Peak 394.89(2400)

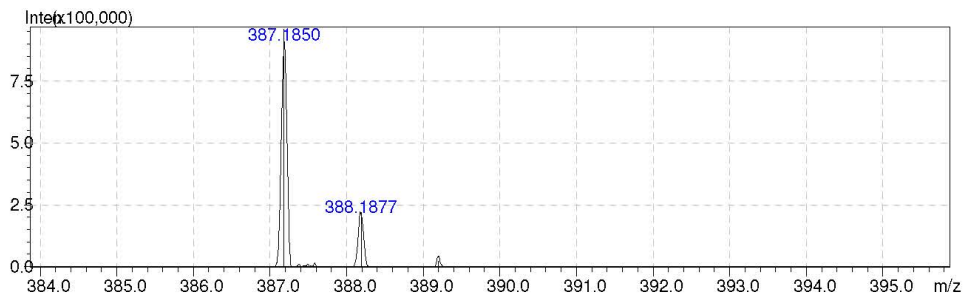
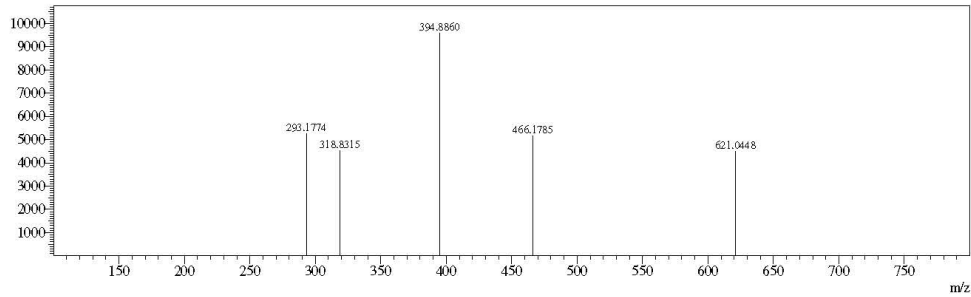


Figure S5. LC-MS identification of Neu5Ac-9-P ( $m/z$  predicted: 387.07; observed: 387.19).