

## Supporting Information

### A neoglycoprotein-immobilized fluorescent magnetic bead suspension multiplex array for galectin-binding studies

Libo Zhang<sup>1</sup>, Hai Yu<sup>1</sup>, Yuanyuan Bai<sup>1</sup>, Bijoyananda Mishra<sup>1</sup>, Xiaoxiao Yang<sup>1</sup>, Jing Wang<sup>1,2</sup>, Evan B. Yu<sup>1</sup>, Riyao Li<sup>1</sup>, and Xi Chen<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, University of California, Davis, California, United States

<sup>2</sup>Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

\*Correspondence: xiichen@ucdavis.edu; Tel.: 1-530-754-6037

#### Table of content

<b>Table S1.</b> The list of glycans, glycan-BSA conjugates, and bead region numbers.....	S2–S3
<b>Reference</b> .....	S3
<b>Table S2.</b> Glycan valency dependence on the ratio of lactosyl squarate monoamide and BSA.....	S4
<b>Figure S1.</b> RCA-I binding results using MagPlex beads immobilized with different amounts of Lac $\beta$ -BSA.....	S4
<b>Figure S2.</b> MALDI-TOF analysis results of glycan-BSA conjugates.....	S5–S7
<b><sup>1</sup>H and <sup>13</sup>C{<sup>1</sup>H} NMR spectra of compounds 1–3, 15–18, 20, and 22.....</b>	<b>S8–S16</b>

**Table S1.** The list of glycans, glycan-BSA conjugates, and bead region numbers.

Compound number	Glycan	Ref.	HRMS of glycan squarate	Average glycan number per BSA	Beads Region number
1	Glc $\alpha$ PropN <sub>3</sub>	This study	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>15</sub> H <sub>23</sub> NO <sub>9</sub> Na 384.1271; found 384.1232	12	64
				35	65
2	Glc $\beta$ PropN <sub>3</sub>	This study	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>15</sub> H <sub>23</sub> NO <sub>9</sub> Na 384.1271; found 384.1256	6	18
				19	15
3	Gal $\alpha$ PropN <sub>3</sub>	This study	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>15</sub> H <sub>23</sub> NO <sub>9</sub> Na 384.1271; found 384.1234	10	58
				28	61
4	Gal $\beta$ PropN <sub>3</sub>	[1]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>15</sub> H <sub>23</sub> NO <sub>9</sub> Na 384.1271; found 384.1257	15	20
				24	19
5	GlcNAc $\alpha$ PropN <sub>3</sub>	[2]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>17</sub> H <sub>26</sub> N <sub>2</sub> O <sub>9</sub> Na 425.1536; found 425.1517	20	51
				27	47
6	GlcNAc $\beta$ PropN <sub>3</sub>	[2]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>17</sub> H <sub>26</sub> N <sub>2</sub> O <sub>9</sub> Na 425.1536; found 425.1496	11	30
				30	29
7	GalNAc $\alpha$ PropN <sub>3</sub>	[3]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>17</sub> H <sub>26</sub> N <sub>2</sub> O <sub>9</sub> Na 425.1536; found 425.1524	18	46
				26	45
8	GalNAc $\beta$ PropN <sub>3</sub>	[3]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>17</sub> H <sub>26</sub> N <sub>2</sub> O <sub>9</sub> Na 425.1536; found 425.1518	17	22
				28	21
9	Gal $\beta$ 3GlcNAc $\alpha$ PropN <sub>3</sub>	[4]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>23</sub> H <sub>36</sub> N <sub>2</sub> O <sub>14</sub> Na 587.2064; found 587.2053	21	35
				42	34
10	Gal $\beta$ 3GlcNAc $\beta$ PropN <sub>3</sub>	[4]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>23</sub> H <sub>36</sub> N <sub>2</sub> O <sub>14</sub> Na 587.2064; found 587.2057	16	54
				30	52
11	Gal $\beta$ 3GalNAc $\alpha$ PropN <sub>3</sub>	[4]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>23</sub> H <sub>36</sub> N <sub>2</sub> O <sub>14</sub> Na 587.2064; found 587.2060	14	77
				29	78
12	Gal $\beta$ 3GalNAc $\beta$ PropN <sub>3</sub>	[4]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>23</sub> H <sub>36</sub> N <sub>2</sub> O <sub>14</sub> Na 587.2064; found 587.2057	16	42
				24	39
13	Gal $\beta$ 4Glc $\beta$ PropN <sub>3</sub> (Lac $\beta$ ProN <sub>3</sub> )	[1, 3]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>21</sub> H <sub>33</sub> NO <sub>14</sub> Na 546.1799; found 546.1753	12	73
				36	74
14	Gal $\beta$ 4GlcNAc $\beta$ PropN <sub>3</sub> (LacNAc $\beta$ ProN <sub>3</sub> )	[2]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>23</sub> H <sub>36</sub> N <sub>2</sub> O <sub>14</sub> Na 587.2064; found 587.2044	6	75
				14	76
				18	7
				37	36
15	Gal $\alpha$ 3Lac $\beta$ PropN <sub>3</sub> (iGb3)	This study	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>27</sub> H <sub>43</sub> NO <sub>19</sub> Na 708.2327; found 708.2319	12	44
				19	43
16	Gal $\alpha$ 3LacNAc $\beta$ PropN <sub>3</sub>	This study	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>29</sub> H <sub>46</sub> N <sub>2</sub> O <sub>19</sub> Na 749.2592; found 749.2559	8	53
				11	55
17	Gal $\alpha$ 4Lac $\beta$ PropN <sub>3</sub> (Gb3)	This study	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>27</sub> H <sub>43</sub> NO <sub>19</sub> Na 708.2327; found 708.2338	9	67
				26	72
18	Gal $\alpha$ 4LacNAc $\beta$ PropN <sub>3</sub>	This study	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>29</sub> H <sub>46</sub> N <sub>2</sub> O <sub>19</sub> Na 749.2592; found 749.2594	11	38
				15	37

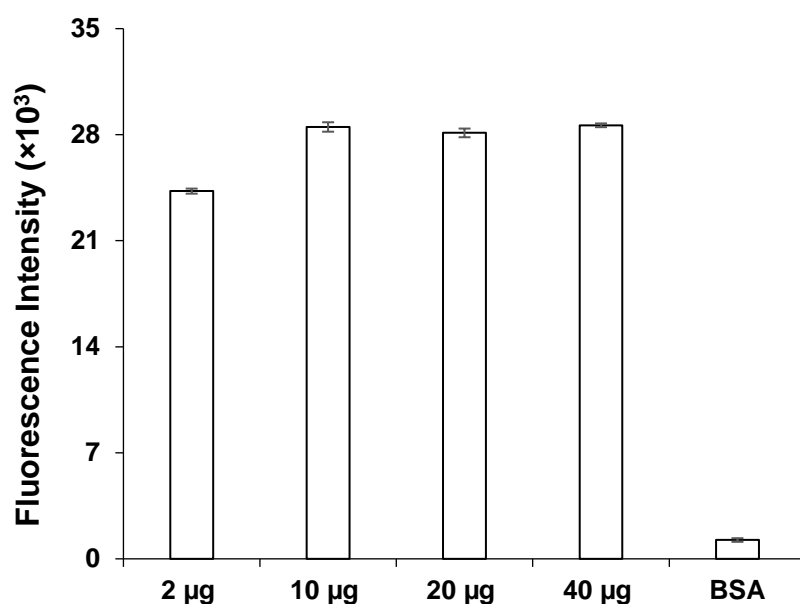
19	GlcNAc $\beta$ 3Lac $\beta$ PropN <sub>3</sub>	[5]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>29</sub> H <sub>46</sub> N <sub>2</sub> O <sub>19</sub> Na 749.2592; found 749.2588	9	26
				16	25
20	GalNAc $\beta$ 3Lac $\beta$ PropN <sub>3</sub>	This study	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>29</sub> H <sub>46</sub> N <sub>2</sub> O <sub>19</sub> Na 749.2592; found 749.2586	9	67
				26	72
21	GalNAc $\beta$ 4Lac $\beta$ PropN <sub>3</sub> (GA2)	[6]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>29</sub> H <sub>46</sub> N <sub>2</sub> O <sub>19</sub> Na 749.2592; found 749.2559	8	9
				14	8
22	Gal $\beta$ 3GlcNAc $\beta$ 3Lac $\beta$ PropN <sub>3</sub> (LNT)	This study	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>35</sub> H <sub>56</sub> N <sub>2</sub> O <sub>24</sub> Na 911.3121; found 911.3041	6	63
				17	62
23	Gal $\beta$ 3GalNAc $\beta$ 4Lac $\beta$ PropN <sub>3</sub> (GA1)	[6]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>35</sub> H <sub>56</sub> N <sub>2</sub> O <sub>24</sub> Na 911.3121; found 911.3101	7	13
				22	12
24	Gal $\beta$ 4GlcNAc $\beta$ 3Lac $\beta$ PropN <sub>3</sub> (LNnT)	[5]	m/z: [M+Na] <sup>+</sup> Calcd for C <sub>35</sub> H <sub>56</sub> N <sub>2</sub> O <sub>24</sub> Na 911.3121; found 911.3103	9	28
				20	27

## References:

1. Yu, H.; Chokhawala, H.; Karpel, R.; Yu, H.; Wu, B.; Zhang, J.; Zhang, Y.; Jia, Q.; Chen, X. A multifunctional *Pasteurella multocida* sialyltransferase: a powerful tool for the synthesis of sialoside libraries. *J. Am. Chem. Soc.* **2005**, *127*, 17618-17619, doi:10.1021/ja0561690.
2. Lau, K.; Thon, V.; Yu, H.; Ding, L.; Chen, Y.; Muthana, M. M.; Wong, D.; Huang, R.; Chen, X. Highly efficient chemoenzymatic synthesis of beta1-4-linked galactosides with promiscuous bacterial beta1-4-galactosyltransferases. *Chem. Commun.* **2010**, *46*, 6066-6068, doi:10.1039/c0cc01381a.
3. Yu, H.; Huang, S.; Chokhawala, H.; Sun, M.; Zheng, H.; Chen, X. Highly efficient chemoenzymatic synthesis of naturally occurring and non-natural alpha-2,6-linked sialosides: a *P. damsela* alpha-2,6-sialyltransferase with extremely flexible donor-substrate specificity. *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 3938-3944, doi:10.1002/anie.200600572.
4. Yu, H.; Thon, V.; Lau, K.; Cai, L.; Chen, Y.; Mu, S.; Li, Y.; Wang, P. G.; Chen, X. Highly efficient chemoenzymatic synthesis of beta1-3-linked galactosides. *Chem. Commun.* **2010**, *46*, 7507-7509, doi:10.1039/c0cc02850a.
5. Yu, H.; Zeng, J.; Li, Y.; Thon, V.; Shi, B.; Chen, X. Effective one-pot multienzyme (OPME) synthesis of monotreme milk oligosaccharides and other sialosides containing 4-O-acetyl sialic acid. *Org. Biomol. Chem.* **2016**, *14*, 8586-8597, doi:10.1039/c6ob01706a.
6. Yang, X.; Yu, H.; Yang, X.; Kooner, A. S.; Luu, B.; Chen, X. One-pot multienzyme (OPME) chemoenzymatic synthesis of brain ganglioside glycans with human ST3GAL II expressed in *E. coli*. Submitted.

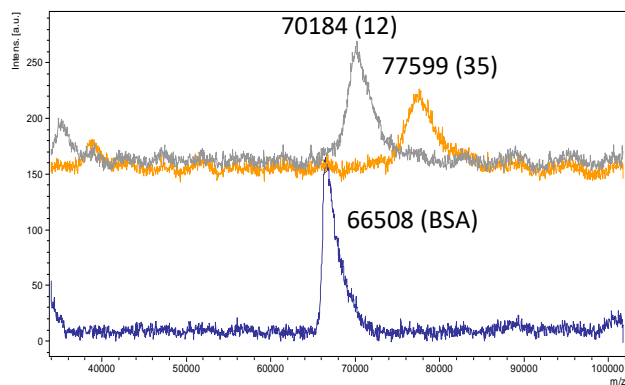
**Table S2.** Glycan valency (average number of glycans per BSA molecule) dependence on the ratio of lactosyl squarate monoamide and BSA. Conditions used: 2 mg/mL BSA was incubated with different ratios (20:1 to 100:1) of lactosyl squarate monoamide at room temperature for 20 h with shaking (850 rpm).

Lactoside:BSA ratio	Glycan valency
20:1	12
40:1	20
60:1	28
80:1	32
100:1	36

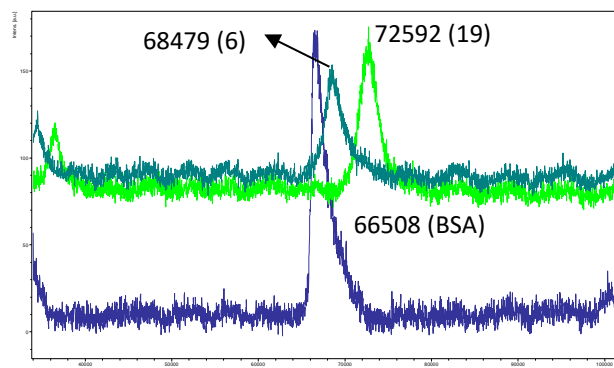


**Figure S1.** RCA-I binding results using MagPlex beads immobilized with different amounts of Lacβ-BSA. Different amounts of Lacβ-BSA with a valency of 36 glycan per BSA molecule were incubated with 25 μL ( $0.3 \times 10^6$  beads) of EDC/NHS-activated MagPlex beads (Region numbers 7, 8, 12, 13 for 2, 10, 20, 40 μg Lacβ-BSA respectively) at room temperature for 2 h. BSA (10 μg) immobilized (Region number 44) under the same condition was used as a control. RCA-I (15 μg/mL) was used in PBS+0.2% BSA as the binding buffer and PBS+0.1% Tween 20 was used as the washing buffer in the assay.

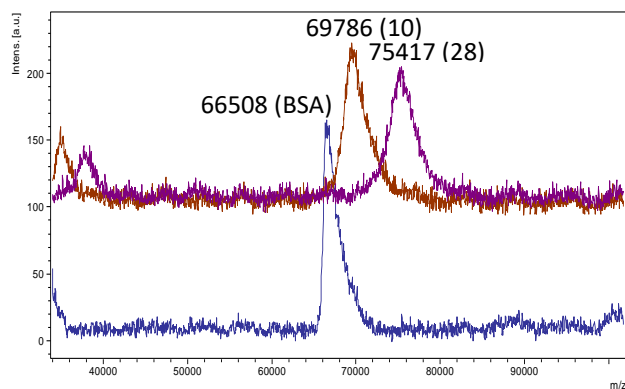
**Glc $\alpha$ -BSA**



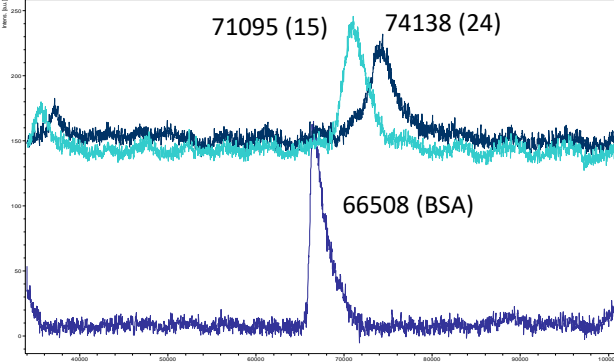
**Glc $\beta$ -BSA**



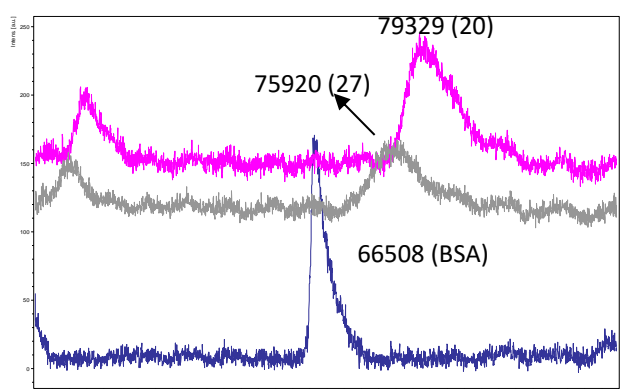
**Gal $\alpha$ -BSA**



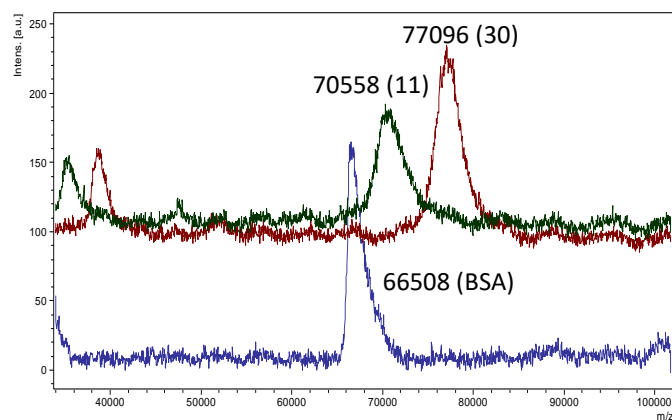
**Gal $\beta$ -BSA**



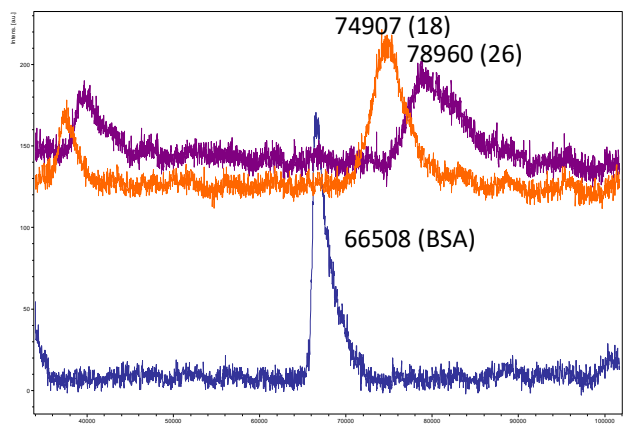
**GlcNAc $\alpha$ -BSA**



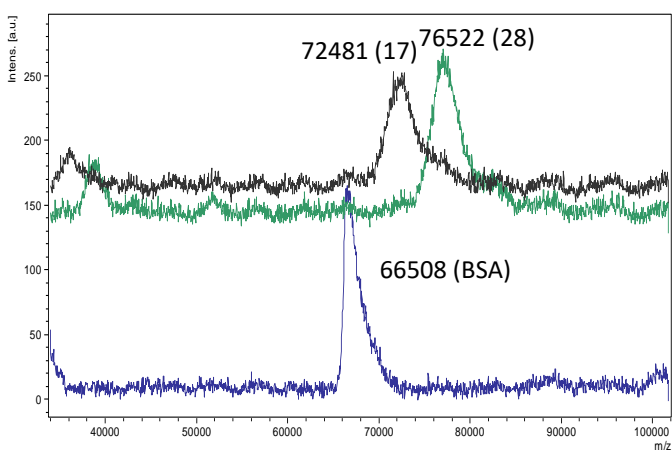
**GlcNAc $\beta$ -BSA**



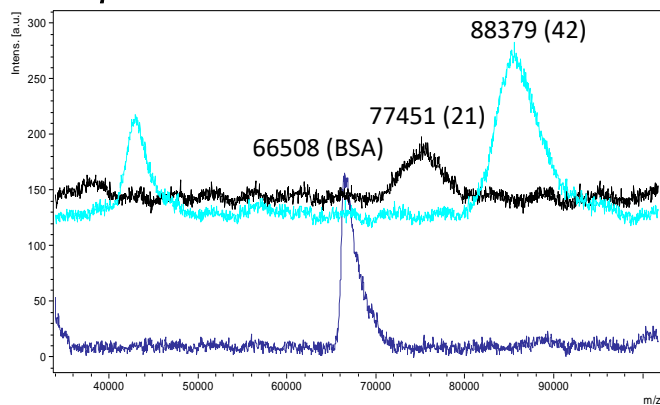
**GalNAc $\alpha$ -BSA**



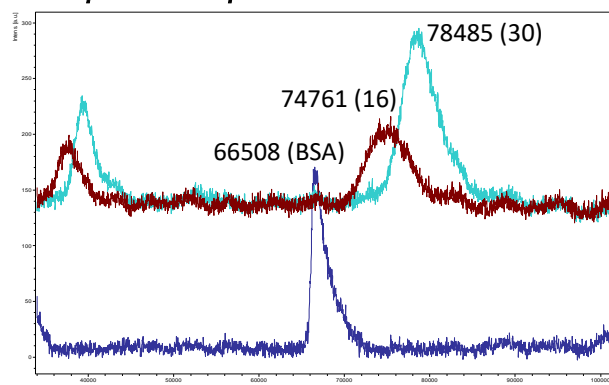
**GalNAc $\beta$ -BSA**



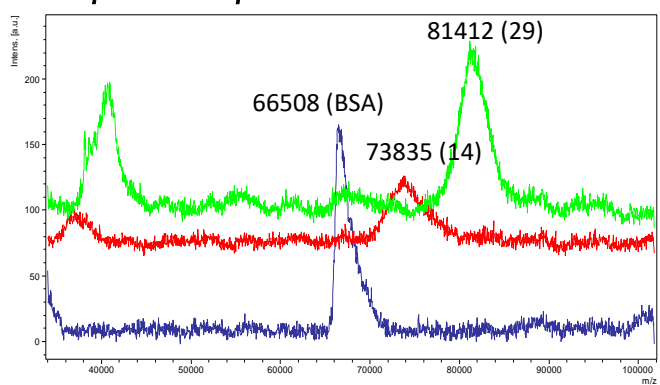
**Gal $\beta$ 3GlcNAc $\alpha$ -BSA**



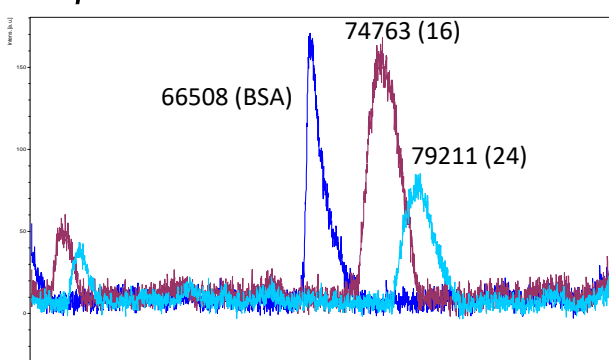
**Gal $\beta$ 3GlcNAc $\beta$ -BSA**



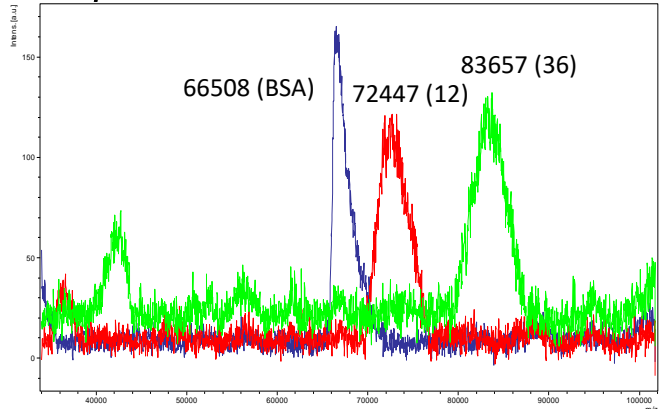
**Gal $\beta$ 3GalNAc $\beta$ -BSA**



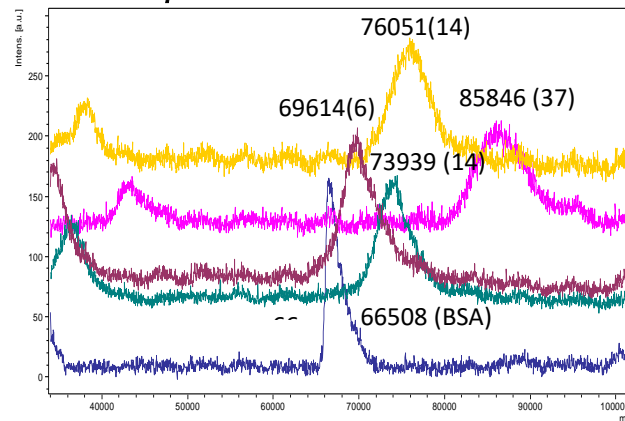
**Gal $\beta$ 3GalNAc $\alpha$ -BSA**



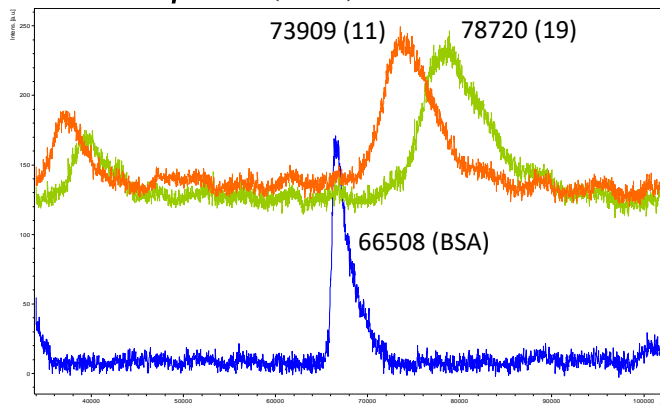
**Lac $\beta$ -BSA**



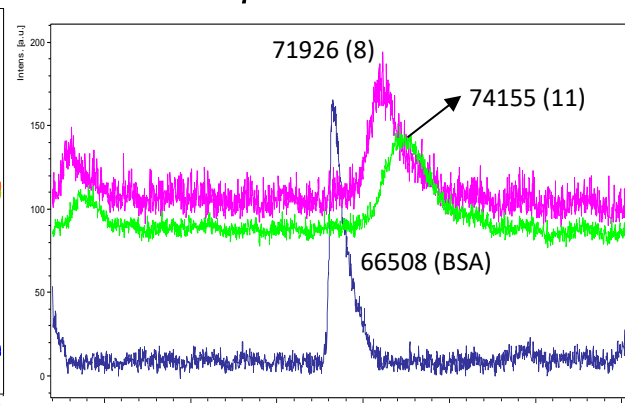
**LacNAc $\beta$ -BSA**

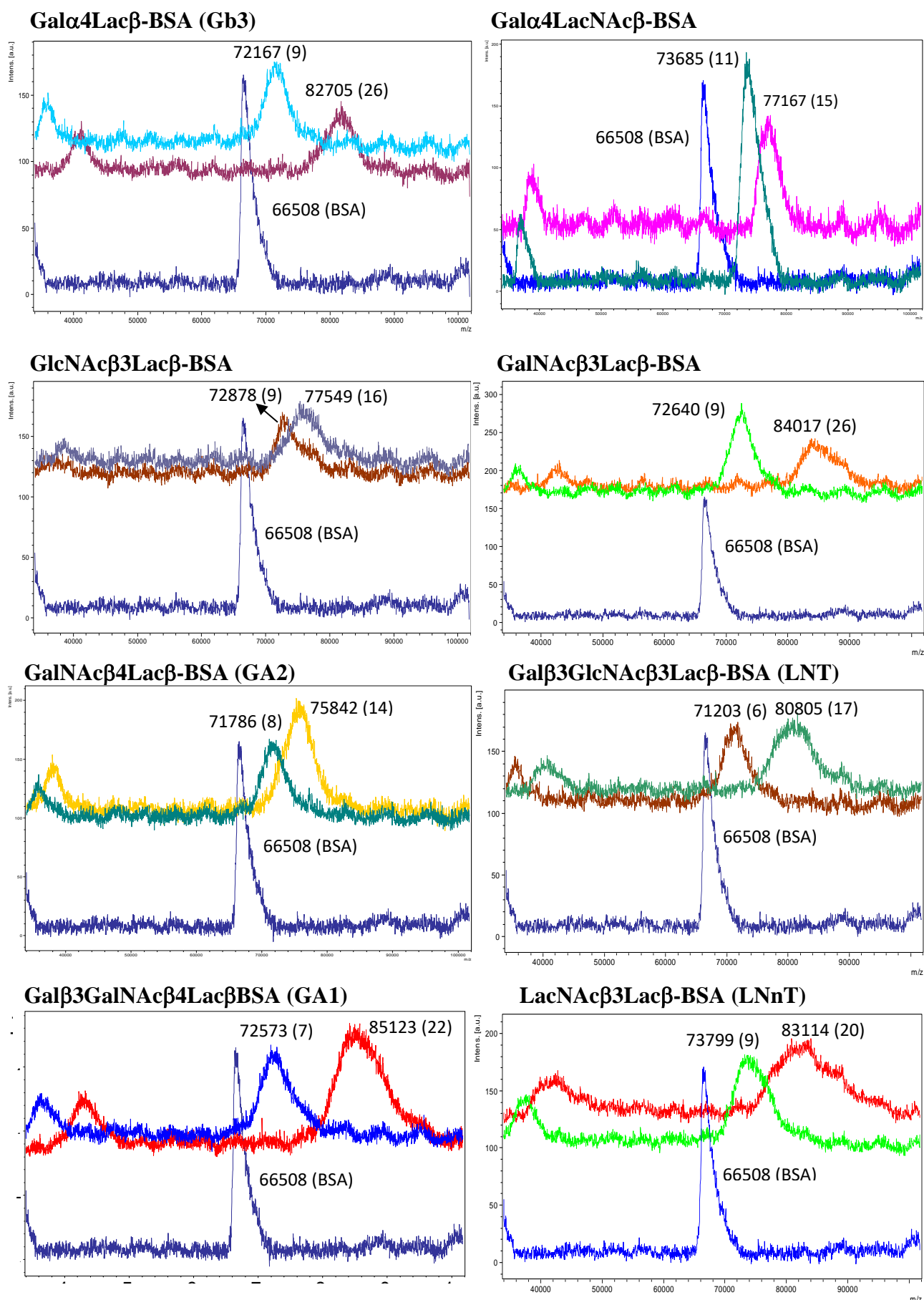


**Gal $\alpha$ 3Lac $\beta$ -BSA (iGb3)**



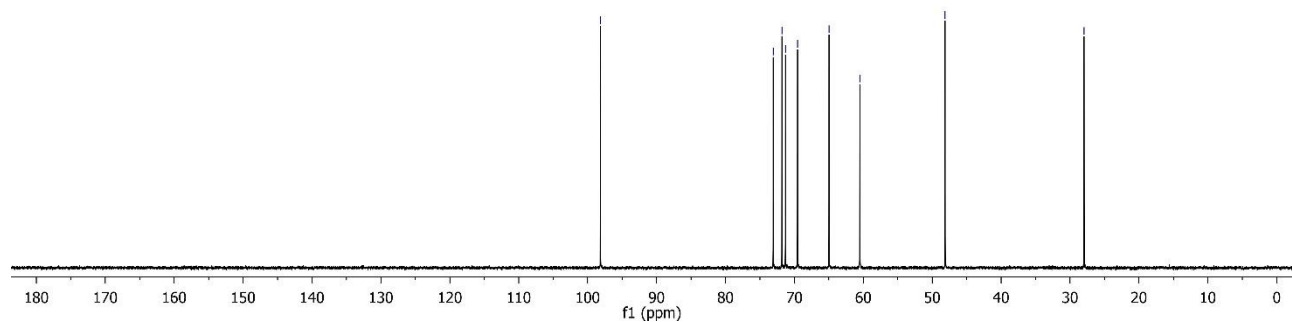
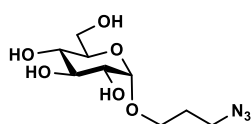
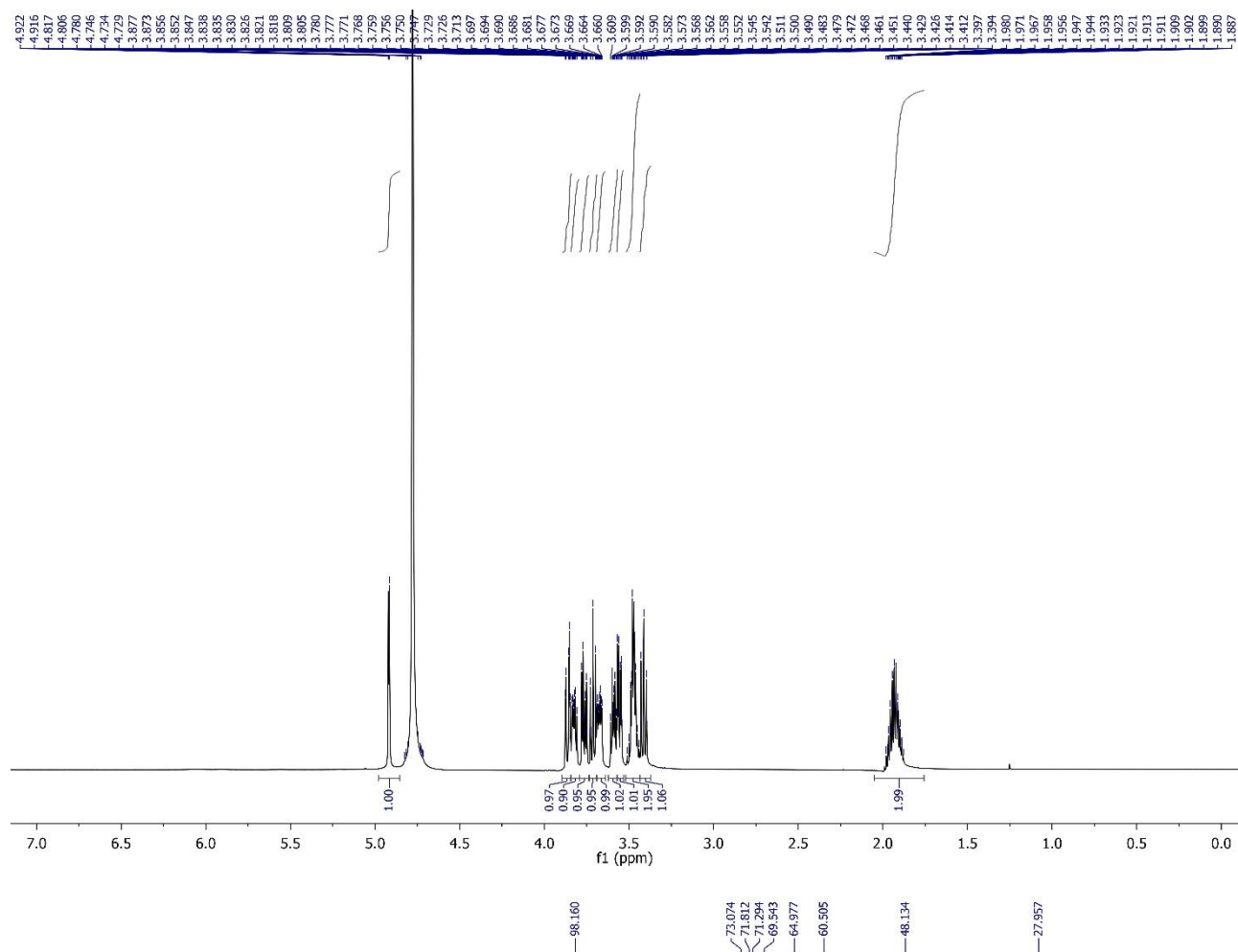
**Gal $\alpha$ 3LacNAc $\beta$ -BSA**





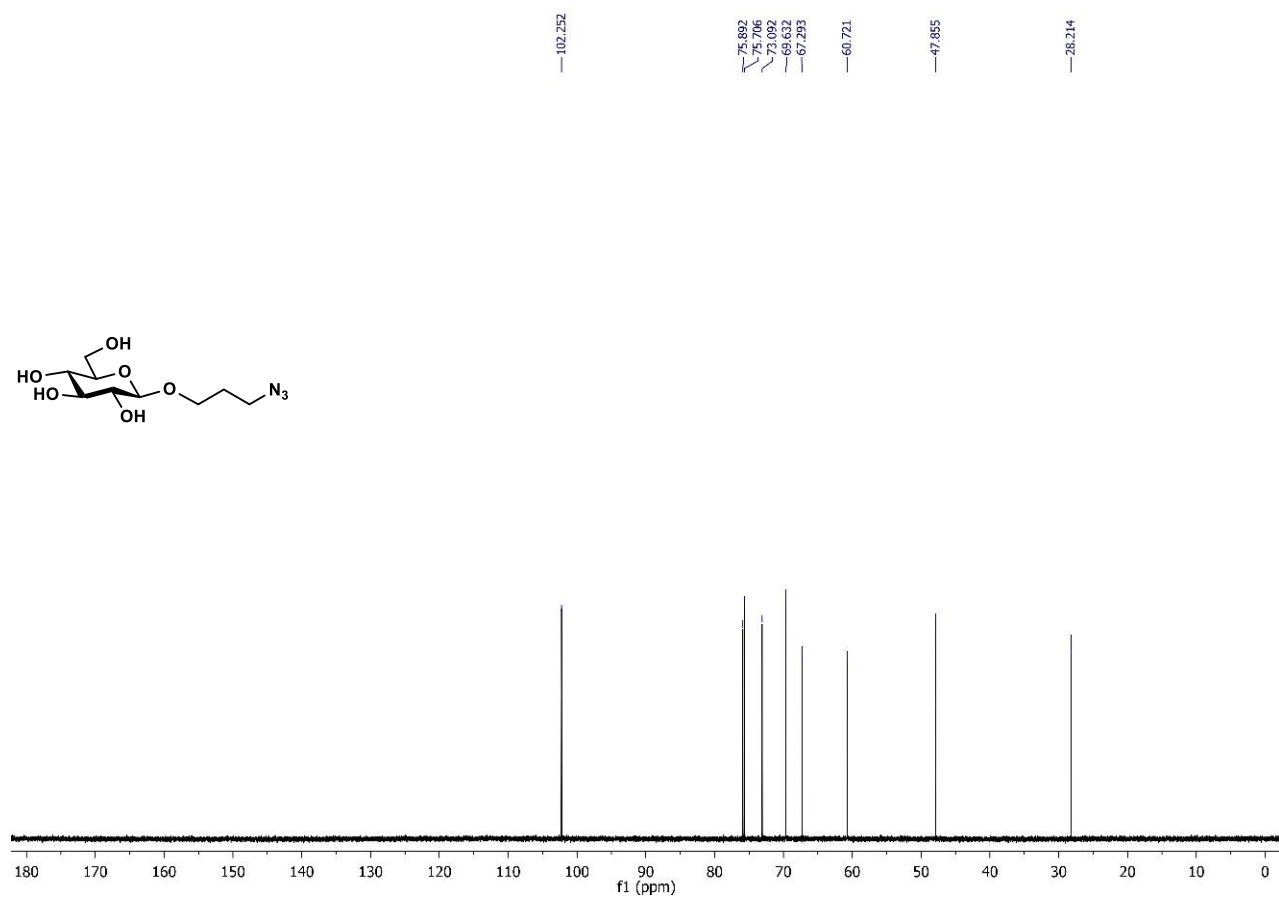
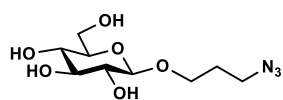
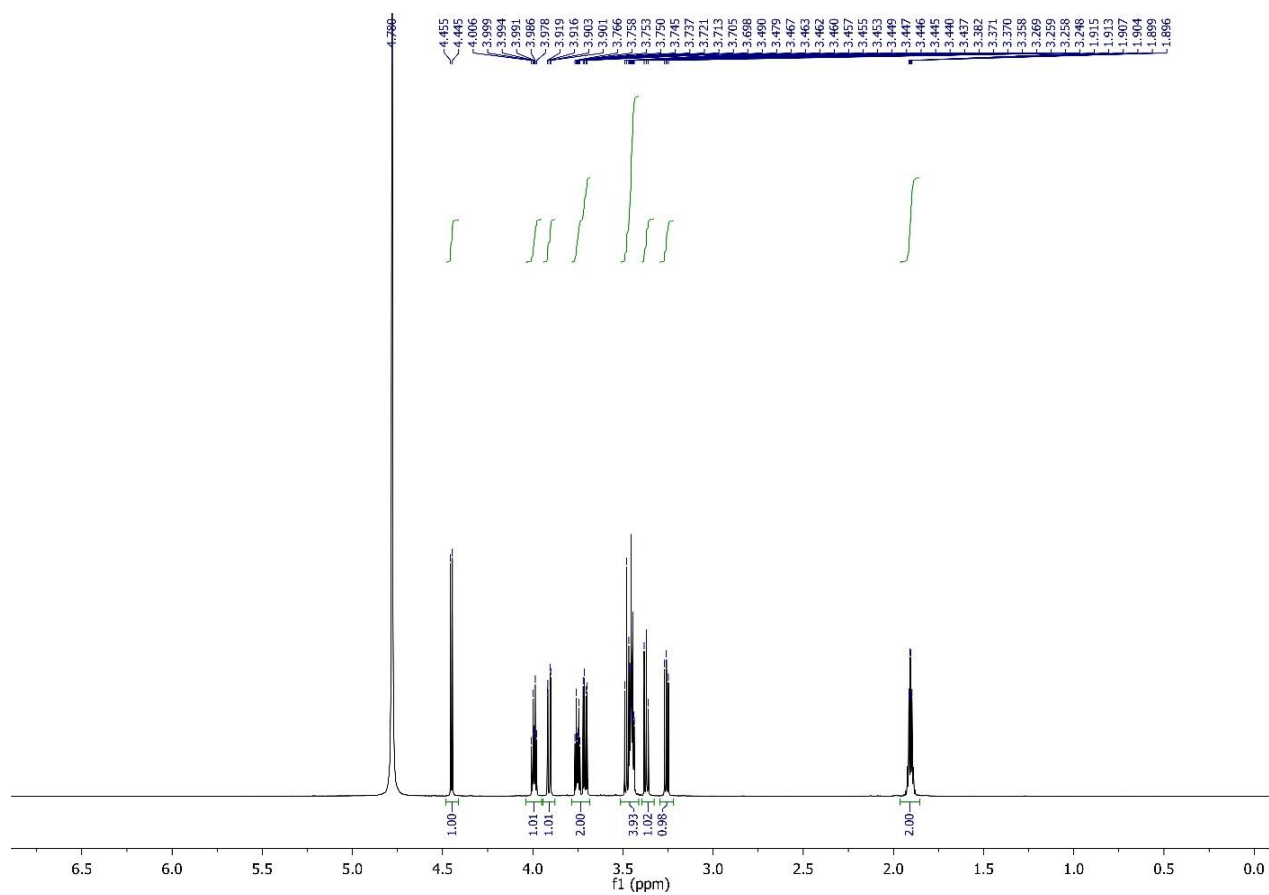
**Figure S2.** MALDI-TOF analysis results of glycan-BSA conjugates which were used to calculate the average numbers of glycans per BSA molecule (glycan valencies, the numbers shown in parentheses).

600 MHz  $^1\text{H}$  and 150 MHz  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of Glc $\alpha$ PropN $_3$  (**1**) in D $_2$ O

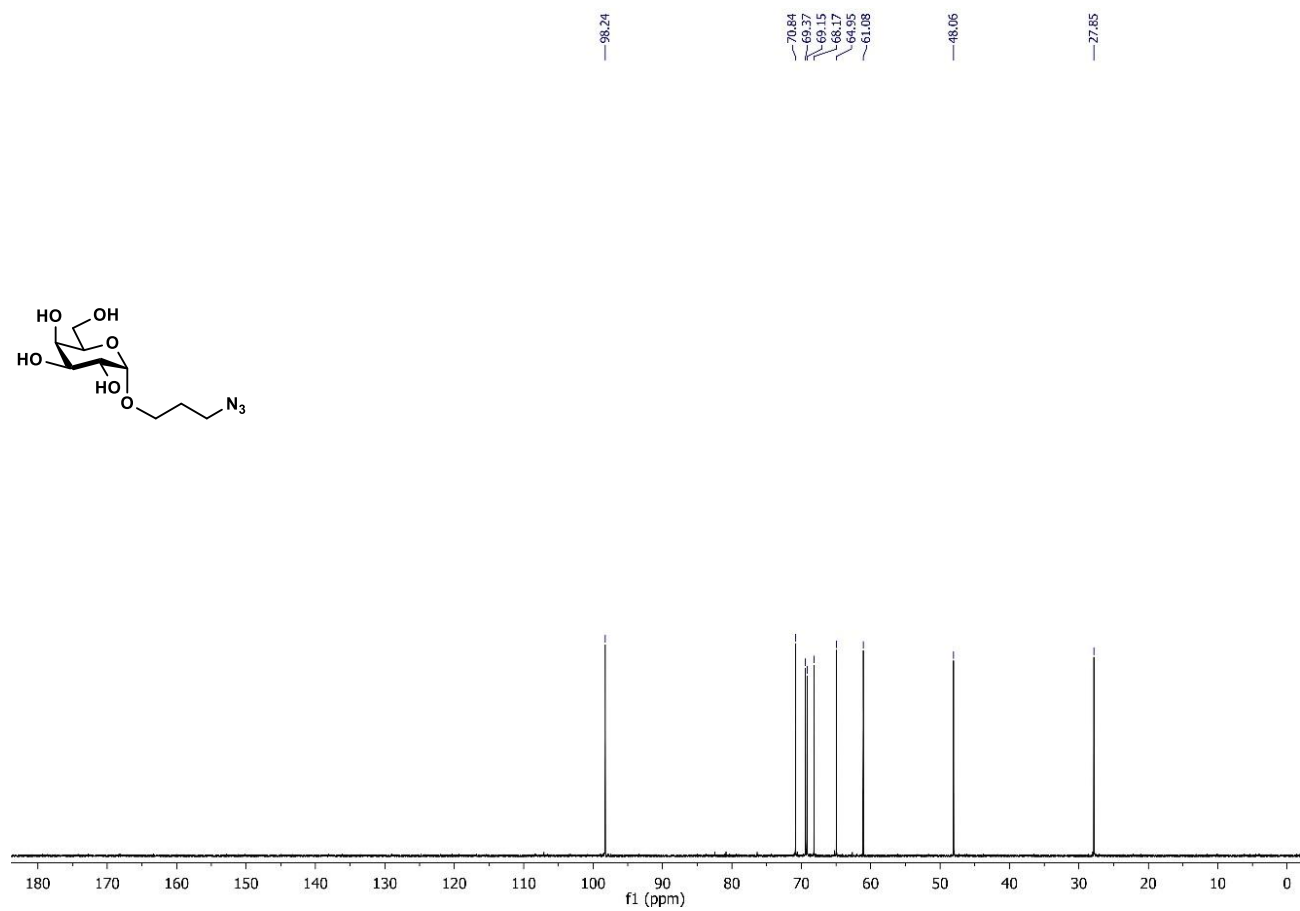




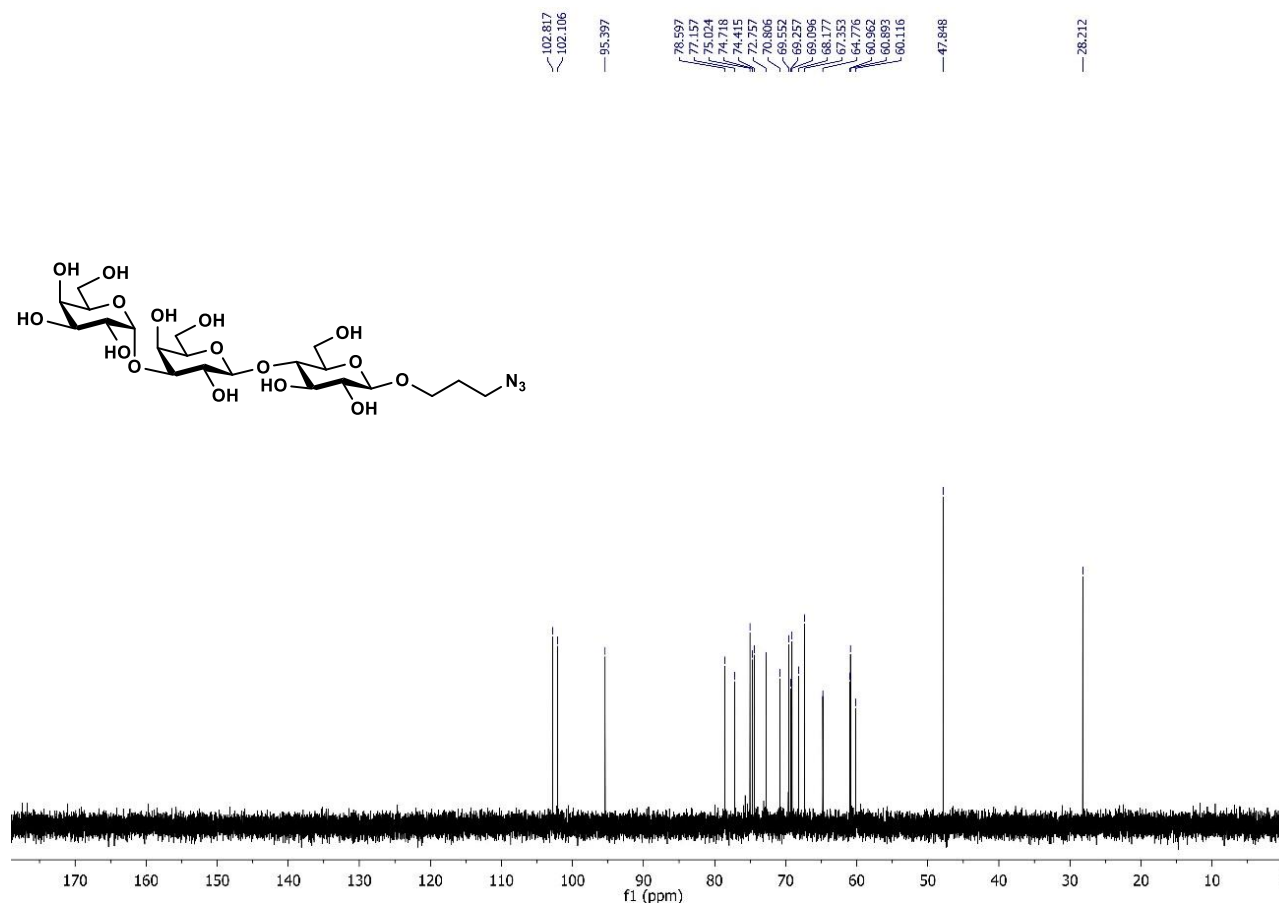
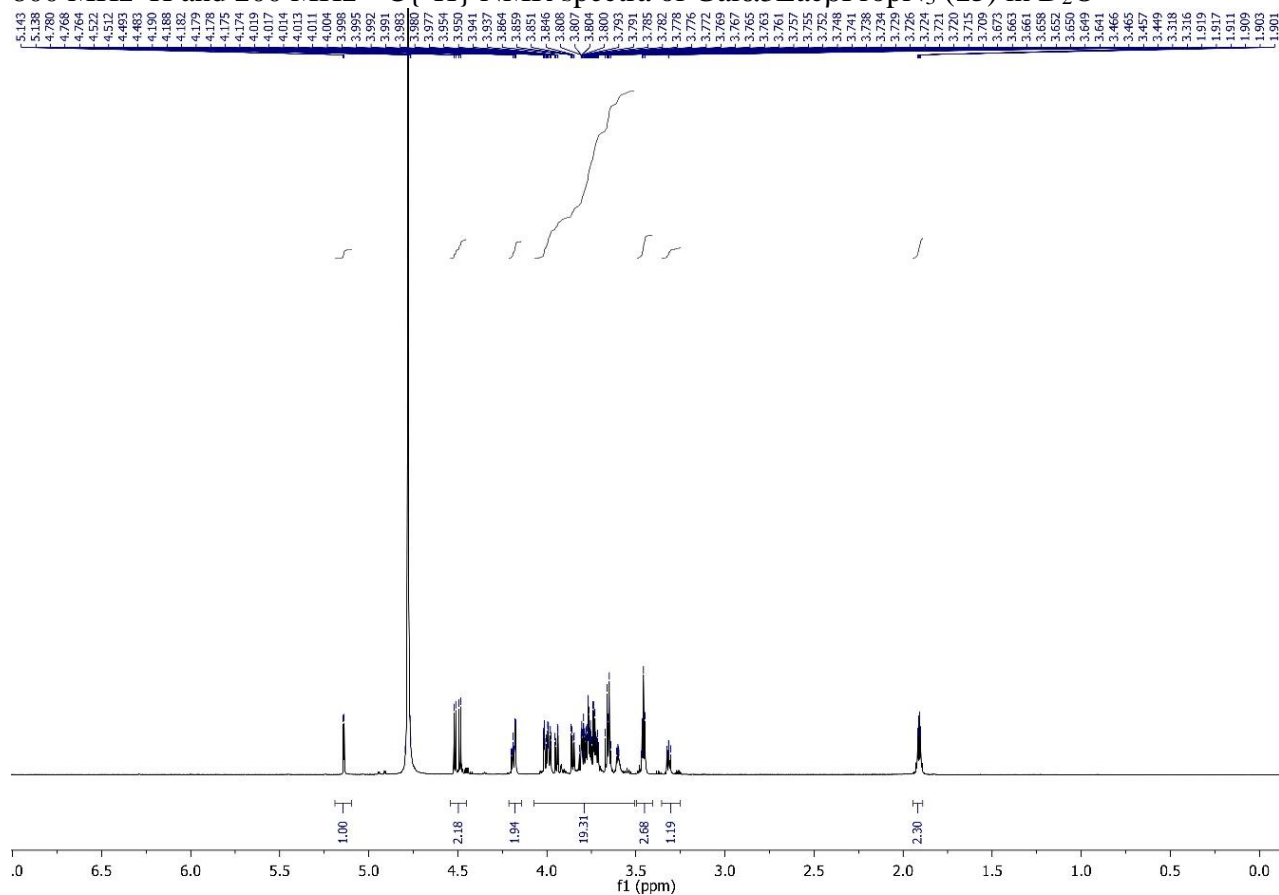
800 MHz  $^1\text{H}$  and 200 MHz  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of Glc $\beta$ PropN $_3$  (**2**) in D $_2$ O



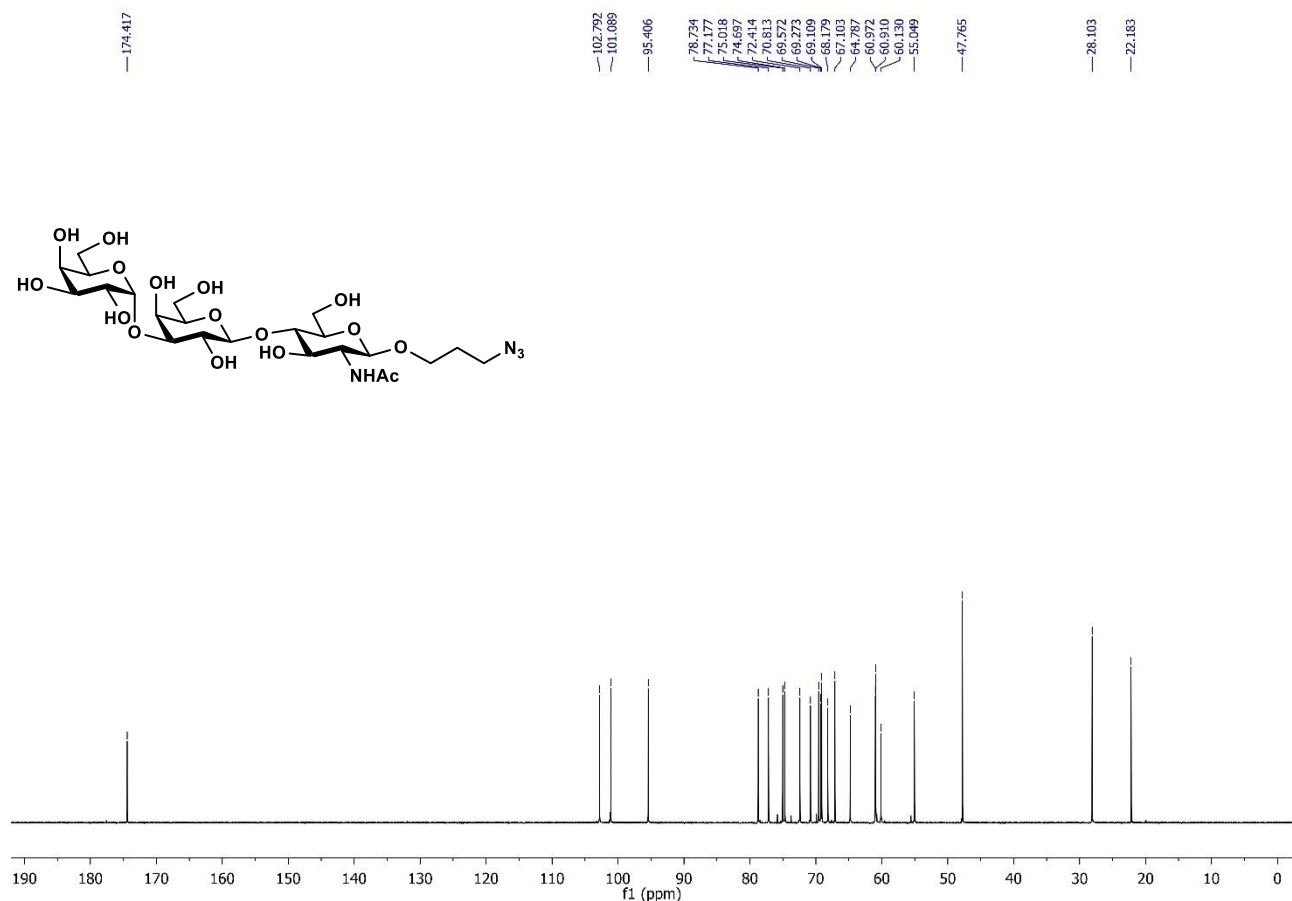
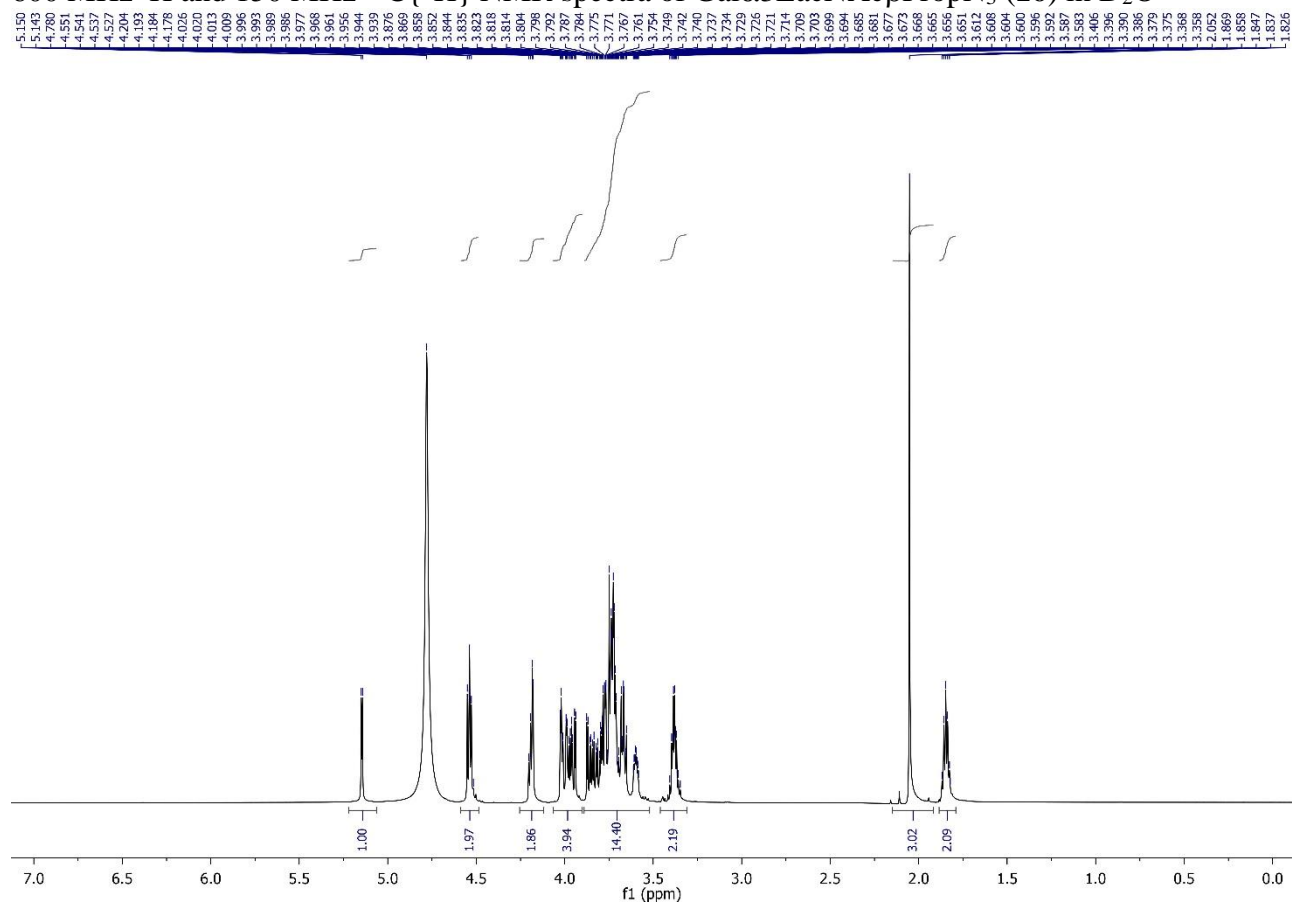
<sup>1</sup>H NMR spectrum of compound 10b in CDCl<sub>3</sub>. The spectrum shows peaks at 4.937, 4.932, 4.816, 4.763, 4.751, 3.971, 3.966, 3.931, 3.924, 3.916, 3.858, 3.854, 3.845, 3.841, 3.826, 3.817, 3.819, 3.814, 3.806, 3.801, 3.797, 3.737, 3.735, 3.728, 3.593, 3.585, 3.580, 3.578, 3.572, 3.572, 3.565, 3.467, 3.454, 3.446, 3.439, 1.964, 1.945, 1.936, 1.925, 1.927, 1.925, 1.920, 1.916, 1.908, 1.899, 1.893, 1.892, and 1.890 ppm. Integration values are 1.00, 0.94, 0.92, 0.92, 1.01, 1.96, and 2.20.



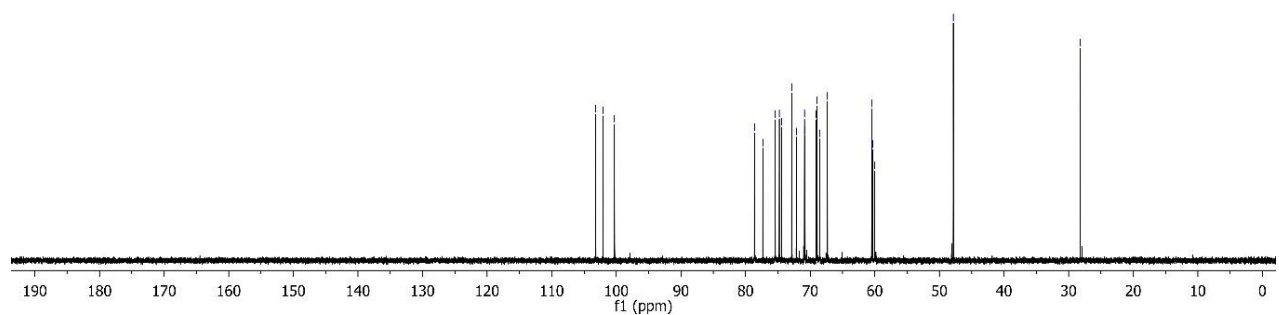
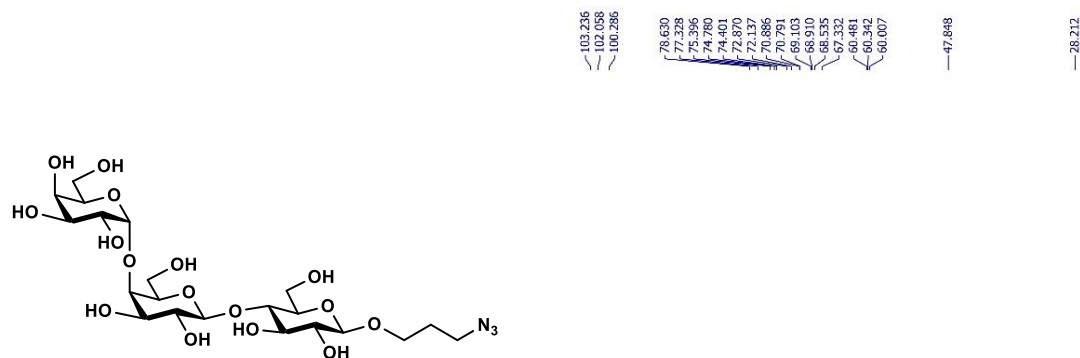
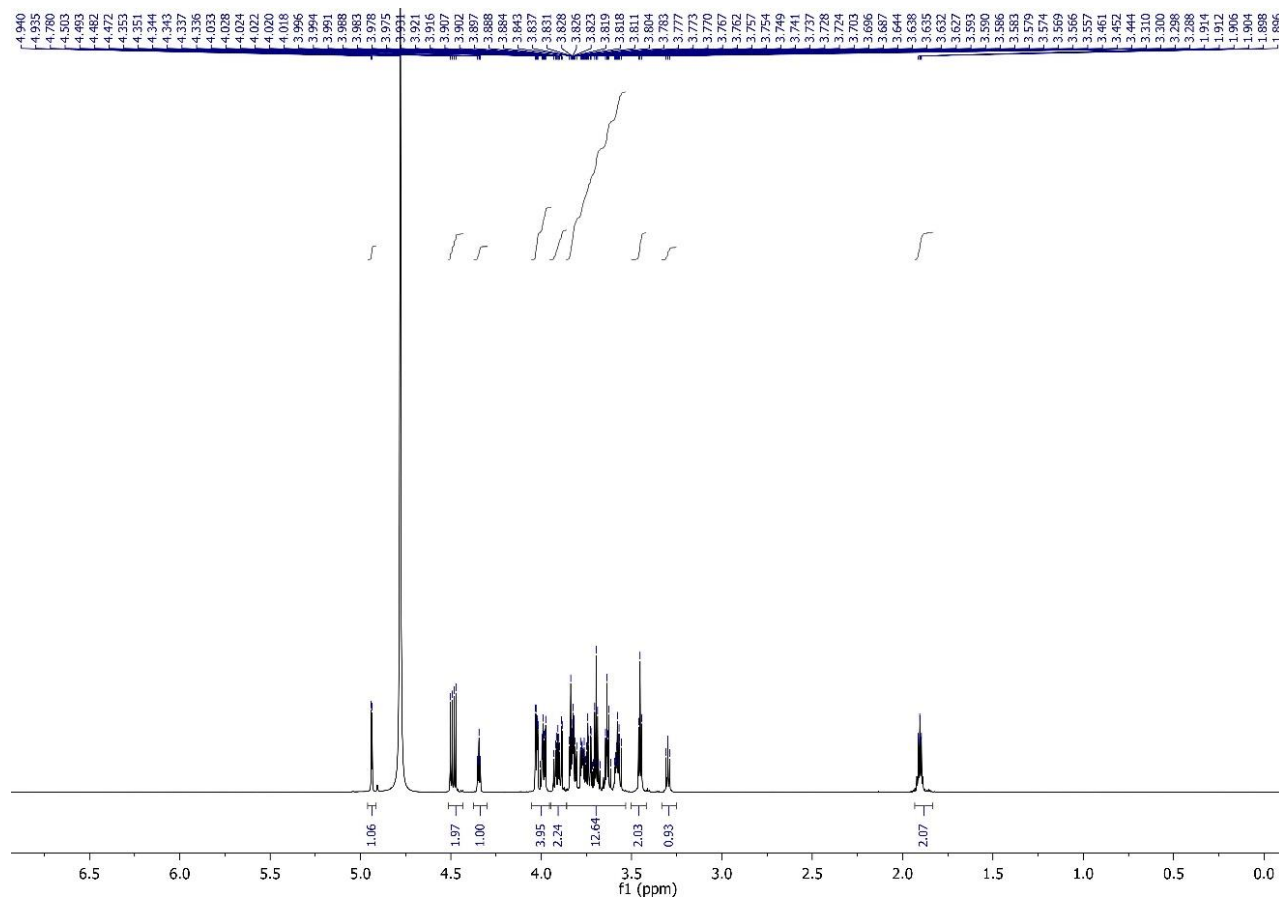
800 MHz  $^1\text{H}$  and 200 MHz  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of Gal $\alpha$ 3Lac $\beta$ PropN $_3$  (**15**) in D $_2$ O



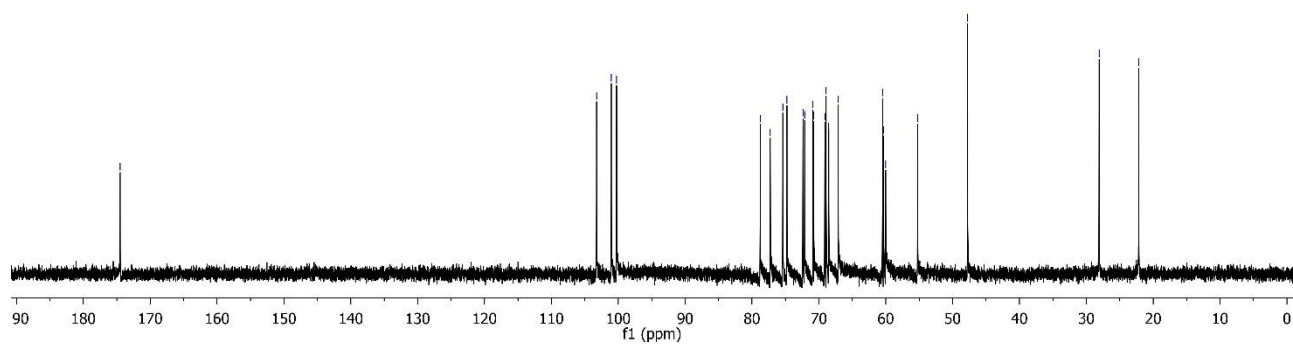
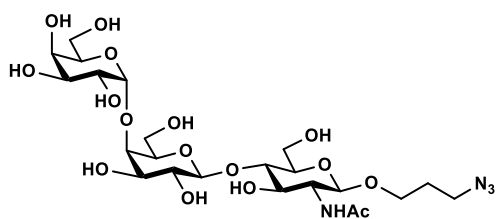
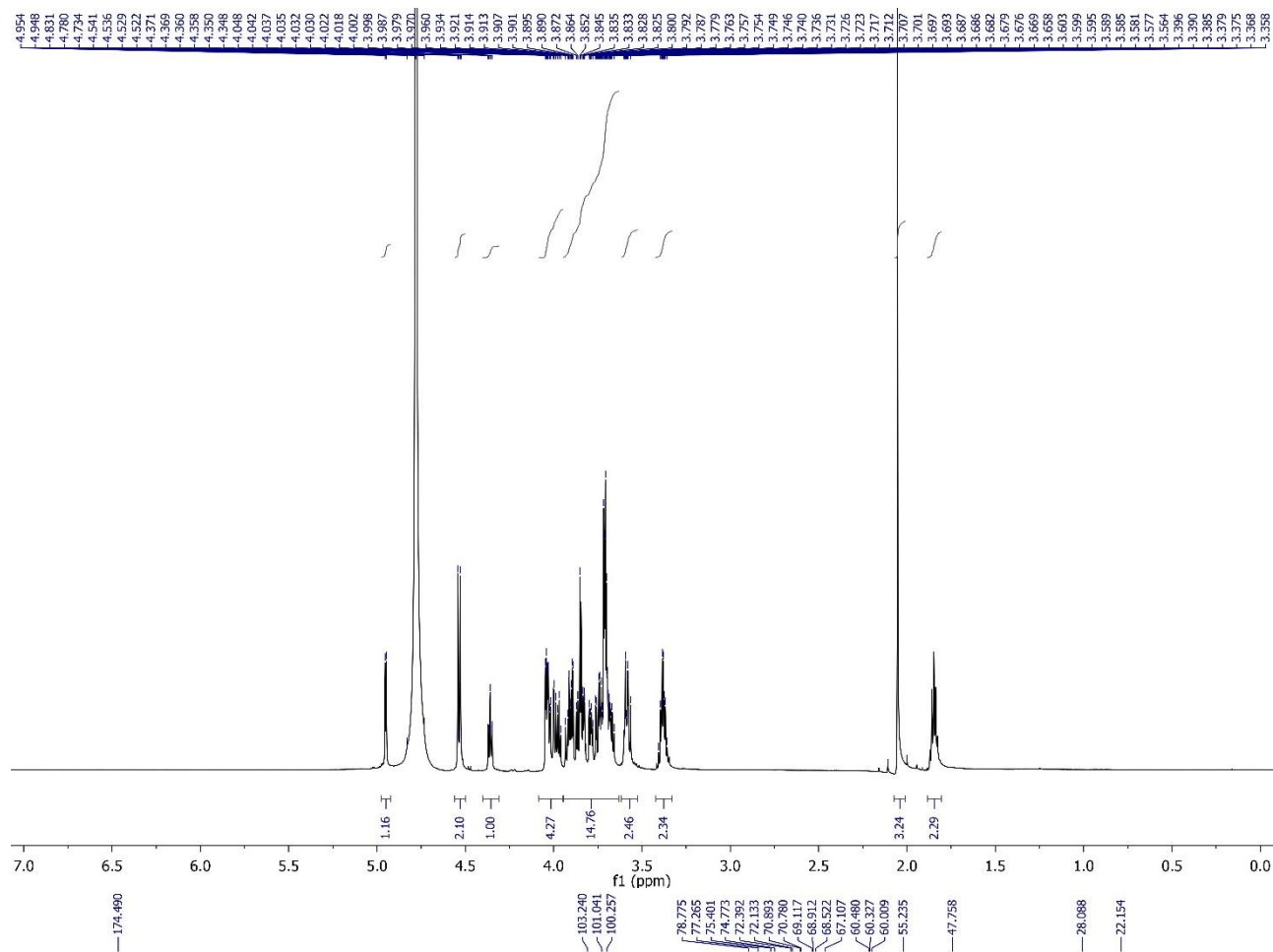
600 MHz  $^1\text{H}$  and 150 MHz  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of Gal $\alpha$ 3LacNAc $\beta$ PropN $_3$  (**16**) in D $_2$ O



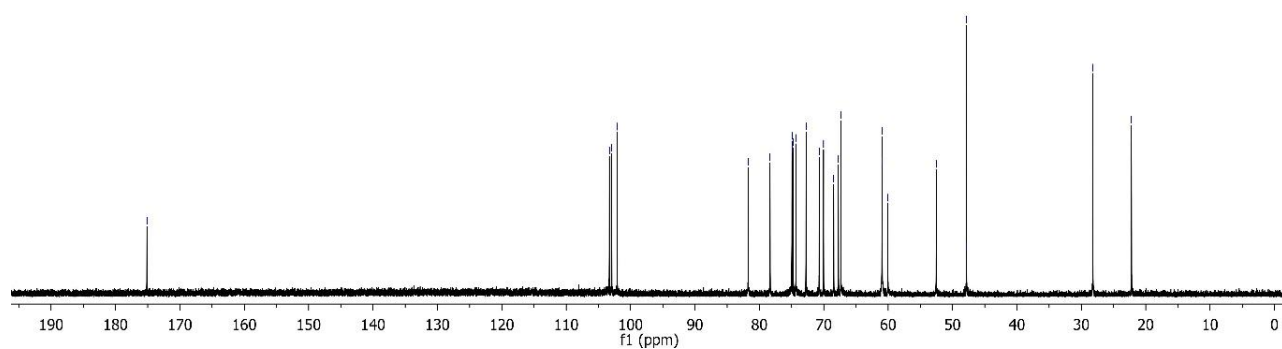
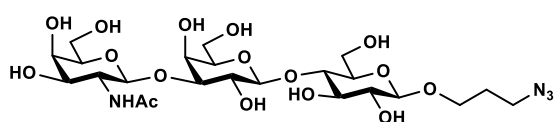
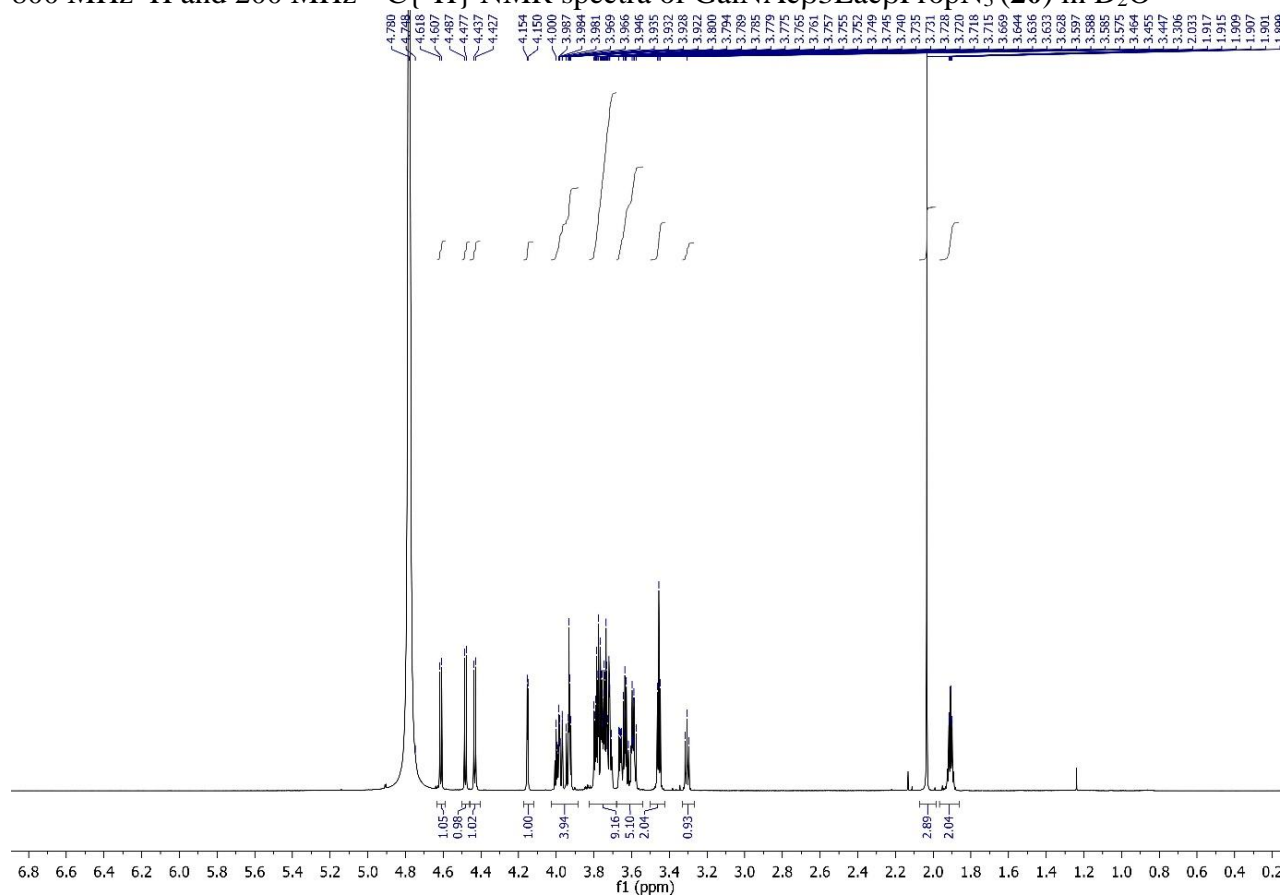
800 MHz  $^1\text{H}$  and 200 MHz  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of Gal $\alpha$ 4Lac $\beta$ PropN $_3$  (**17**) in D $_2$ O



600 MHz  $^1\text{H}$  and 150 MHz  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of Gal $\alpha$ 4LacNAc $\beta$ PropN $_3$  (**18**) in D $_2$ O



800 MHz  $^1\text{H}$  and 200 MHz  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of GalNAc $\beta$ 3Lac $\beta$ PropN $_3$  (**20**) in  $\text{D}_2\text{O}$



800 MHz  $^1\text{H}$  and 200 MHz  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of Gal $\beta$ 3GlcNAc $\beta$ 3Lac $\beta$ PropN $_3$  (LNT, **22**) in  $\text{D}_2\text{O}$

