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The First Synthesis of Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-Ser — a Newly Discovered Component of α-dystroglycan

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***Abstract*:** Glycopeptide (1), Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-Ser, was synthesized using a chemoenzymatic strategy. Galβ1-4GlcNAcβ1-2Man trisaccharide was prepared using glycosidase assisted oligosaccharide synthesis. After coupling of this trisaccharide with a serine derivative by chemical glycosylation, sialic acid was introduced using sialyltransferase to produce a tetrasaccharide serine derivative. Removal of protecting group afforded glycopeptide (1). Use of a chemoenzymatic strategy allowed for the elimination of numerous synthetic steps and efficient preparation of the target compound.

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Neu5Acα2-3Galβ1-4GlcNAcβ1-2Man-Ser/Thr which binds to a laminin molecule in the extracellular matrix was discovered between the 317th - 488th amino acid residues of α-dystroglycan[1] by Endo *et. al.*.[2] *O*-linked sugar chains which are bound via a Man-Ser/Thr linkage occur frequently in yeast but are extremely rare in mammals. The carbohydrate moieties of α-dystroglycan are thought to be essential to its biological functions.[3] For the purpose of elucidating the biological role of Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα-Ser (**1**), we have developed a short and direct synthesis of **1**.

The synthetic plan for the tetrasaccharide derivative bound to a serine residue (**1**) is shown in Figure 1. Trisaccharide (Galβ1-4GlcNAcβ1-2Man) was obtained by glycosidase assisted synthesis and was then converted into glycosyl donor **3**. Coupling of **3** and serine derivative **4** was performed by chemical glycosylation. Finally, the sialic acid residue was introduced using sialyl transferase.

The synthesis of thioglycoside **3** by use of glycosidase was performed as follows: disaccharide **5** was prepared according to our previous report.[4] Reverse hydrolysis of mannose and *N*-acetylglucosamine in the presence of *β-N*-acetylglucosaminidase from *Bacillus circulans* gave GlcNAcβ1-2Man (**5**) and GlcNAcβ1-6Man in 0.5% and 2.4% yield, respectively. Thiophenyl group was introduced to the anomeric position of the mannose residue in **5** to give **6**[6] in 2 steps in 92 % overall yield. Subsequent removal of the acetyl groups using NaOMe/MeOH at room temperature gave **7**[5] in quantitative yield. Galactosylation of **7** was performed by

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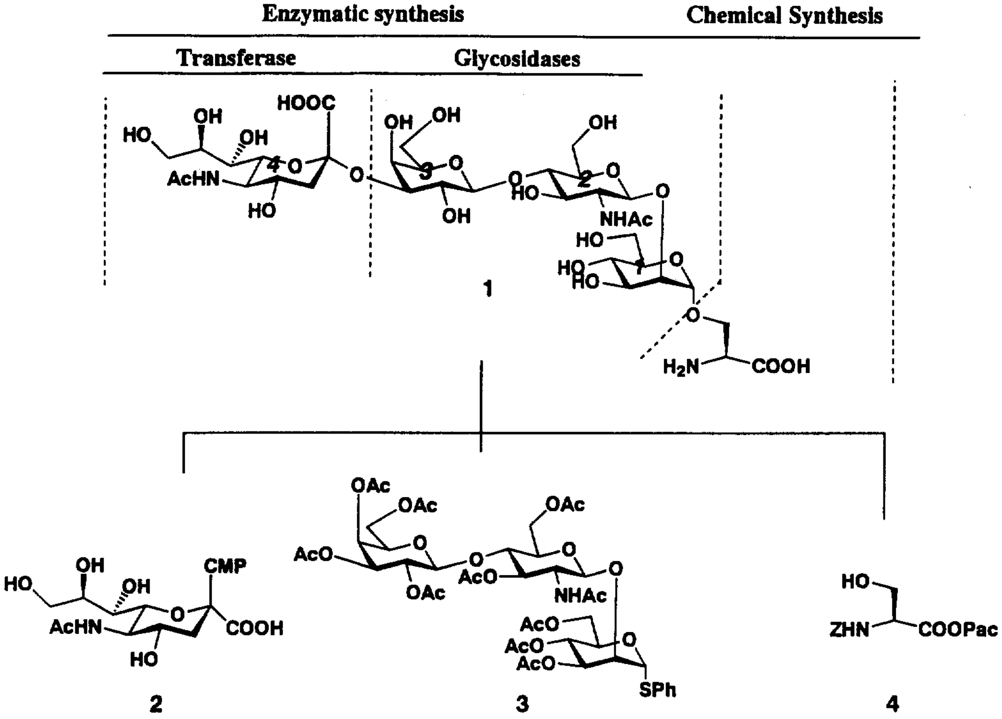
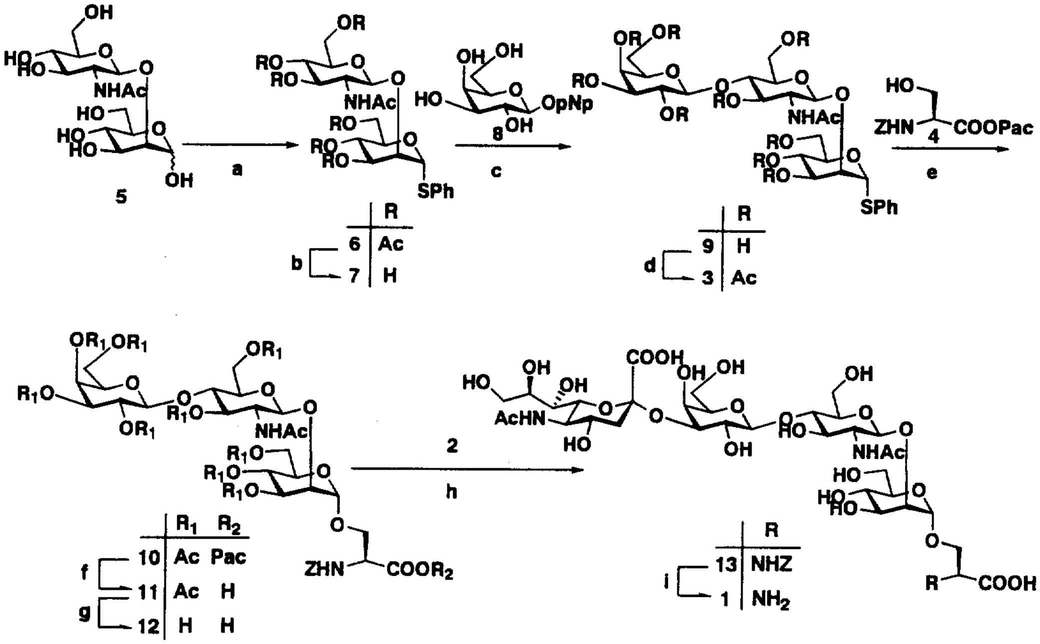


Figure 1

transglycosylation using β-galactosidase. When the disaccharide 7 (100 mg, 0.21 mmol) and *p*-nitrophenyl (pNp)-galactoside **8** (64 mg, 0.21 mmol) in 0.1 M phosphate buffer (pH 6.0) containing 10 % DMSO were incubated in the presence of β-galactosidase from ***bifidobacterium bifidum*** (12 U), galactose residue was introduced to the C-4 position of *N*-acetylglucosamine in the disaccharide 7 regioselectively to give **9**[5] (20 mg, 0.03 mmol) in 15 % yield (47 % of 7 was recovered). Treatment of **9** with acetic anhydride and pyridine gave the trisaccharide **3**[5] in 83 % yield. Coupling of **3** and the serine derivative **4** whose α-carboxyl group is protected with a Phenacyl (Pac) group was performed by using 5eq. of NIS and 1eq. of TfOH[6] in CH_2Cl_2 at -78 °C to give the glycopeptide **10**[5] in 98 % yield. The α-configuration of the newly formed glycosidic bond was confirmed by the $^1J_{C\text{-}H}$ and $^3J_{H1\text{-}H2}$ coupling constants of the NMR spectrum (172 Hz, and ~0 Hz, respectively). Removal of all protecting groups except the Z group were performed as follows: the Pac group of the serine residue in **10** was removed by using Zn/AcOH[7] at room temperature, and deacetylation of **11** was performed by using NaOMe/MeOH at 0 °C to give compound **12**[5] (overall yield 53 %). Epimerization of the serine residue of **12** under these conditions was not observed by HPLC or ^1H NMR spectroscopy. Although the enzymatic sialylation might be performed by either sialidase[8] or sialyltransferase[9], we chose the latter since high reaction yields and regioselectivities were expected.[9] Sialylation of **12** was performed as follows: a mixture of **12** (11 mg, 14.3 μmol), CMP-NeuAc **2** (22 mg, 35.8 μmol), and α-2,3-sialyltransferase[10] (300 mU) in 0.05 M cacodylate buffer (pH 6, 2 mL) containing 0.05 M NaCl and bovine serum albumin (1.9 mg) was incubated at 37 °C for 2 days. The reaction was monitored by an HPLC fitted with an ODS column and a UV monitor. The mixture was purified by an HPLC fitted with an ODS column (20 % aq. CH_3CN containing 0.1% TFA) to give compound 13[4] (13 mg, 12.3 μmol) in 86 % yield. The structure of the sialylated compound 13 was determined by ^1H and ^{13}C NMR spectroscopy to be on α-2,3-linkage (sialic acid residue of H-3^e: 2.69 ppm, H-3^a: 1.76 ppm, and C-3: 41.03 ppm). Removal of the Z group was accomplished by hydrgenolysis using H_2 / Pd-black in water to give target compound 1[4] in quantitative yield.



Scheme 1: conditions; a) 1) Ac_2O, Pyridine. 2) PhSH, $SnCl_4$, CH_2Cl_2. b) NaOMe, MeOH. c) β-galactosidase from *b. bifidum*, aq. DMSO. d) Ac_2O, Pyridine. e) NIS, TfOH, CH_2Cl_2. f) Zn, aq. AcOH. g) NaOMe, MeOH. h) α-2,3-(N)-sialyltransferase from rat, recombinant, cacodylate buffer pH 6. i) Pd-black, H_2, H_2O.

In summary, Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα-Ser (1) was synthesized in only 9 steps from the disaccharide 5 using a chemoenzymatic strategy. Use of both a glycosidase and a transferase in the synthesis of the oligosaccharide portion ensured formation of glycosidic bonds in high stereo- and regio-selectivity and eliminated many complicated synthetic steps as necessary in traditional chemical oligosaccharide synthesis. Moreover, by use of the aromatic protecting groups, the products were made easy to separate by HPLC using an ODS column due to the resulting hydrophobicity and their ability to be monitored using a UV detector.

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REFERENCES AND NOTES

1 (a) Spear, P. G. *Science*, **1998**, *282*, 1999-2000. (b) Henry, M. D.; Campbell, K. P. *Cell*, **1998**, *95*, 859-870.

2 Chiba, A.; Matsumura, K.; Yamada, H.; Inazu, T.; Shimizu, T.; Kusunoki, S.; Kanazawa, I.; Kobata, A.; Endo, T. *J. Biol. Chem.* **1997**, *272*, 2156-2162.

3 Sasaki, T.; Yamada, H.; Matsumura, K.; Shimizu, T.; Kobata, A.; Endo, T. *Biochem. Biophys. Acta*, **1998**, *24707*, 1-8.

4 Fujimoto. H.; Isomura, M.; Miyazaki, T.; Matsuo, I.; Walton, R.; Sakakibara, T.; Ajisaka, K. *Glycoconjugate J.* **1997**, *14*, 75.

5 ^{1}H and ^{13}C NMR data for key compounds are described below. NMR spectra were measured on a UNITY INOVA 500 spectrometer in CDCl$_3$ (Me$_4$Si as internal standard) or D$_2$O (CH$_3$CN as internal standard). Signal assignments such as 1^3 stand for a proton or carbon at C-1 of sugar redidue 3. **6**; ^{1}H (CDCl$_3$) δ 5.43 (bs, 1^1), 5.00 (d, 8.3 Hz, 1^2); ^{13}C: δ 98.36 (1^2), 85.06 (1^1). **7**; ^{1}H (D$_2$O) δ 5.39 (d, 1.5 Hz, 1^1), 4.45 (d, 8.4 Hz, 1^2); ^{13}C δ 99.41 (1^2), 85.59 (1^1), 70.30 (4^2). **9**; ^{1}H (D$_2$O) δ 5.46 (bs, 1^1), 4.53 (d, 8.1 Hz, 1^2), 4.40 (d, 7.6 Hz, 1^3), 1.91 (s, Me); ^{13}C δ 103.33 (1^3), 99.19 (1^2), 85.49 (1^1), 78.85 (4^2). **3**; ^{1}H (CDCl$_3$) δ 5.47 (bs, 1^1), 4.67 (d, 7.6 Hz, 1^2), 4.48 (d, 7.6 Hz, 1^3); ^{13}C δ 100.81 (1^2), 98.91 (1^2), 84.77 (1^1). **10**; ^{1}H (CDCl$_3$) δ 5.21 (bs, 1^1), 4.67 (m, Ser$^{\alpha}$), 4.53 (d, 8.5 Hz, 1^2), 4.46 (d, 8.0 Hz, 1^3); ^{13}C δ 100.92 (1^3), 100.76 (1^2), 98.55 (1^1), 54.12 (Ser$^{\alpha}$). **12**; ^{1}H (D$_2$O) δ 4.80 (bs, 1^1), 4.49 (d, 7.3 Hz, 1^2), 4.47 (m, Ser$^{\alpha}$), 4.40 (d, 7.9 Hz, 1^3); ^{13}C δ 103.39 (1^3), 99.91 (1^2), 97.88 (1^1), 54.69 (Ser$^{\alpha}$). **13**; ^{1}H (D$_2$O) δ 4.79 (bs, 1^1), 4.47(d, 7.7 Hz, 2H, 1^2 and 1^3), 4.44 (m, Ser$^{\alpha}$), 2.69 (dd, 4.8 Hz, 3^{4e}), 1.96 (s, 6H, Me), 1.76 (t, 12.4 Hz, 3^{4a}); ^{13}C (^{t}BuOH as internal standard) δ 104.18 (1), 101.07 (1), 98.99 (1^1), 56.03 (Ser$^{\alpha}$), 41.03 (3^4), 23.92, 23.66 (Me). **1**; ^{1}H (D$_2$O) δ 4.80 (bs, 1^1), 4.51 (d, 7.4 Hz, 1), 4.48 (d, 7.9 Hz, 1), 2.69 (dd, 4.5 Hz, 3^{4e}), 1.98, 1.96 (s, Me), 1.73 (t, 12.3 Hz, 3^{4a}).

6 (a)Konradsson, P.; Mootoo, D.R.; Mcdevitt, R. E.; Fraser-Reid, B. *J. Chem. Soc.,Chem. Commun.* **1990**, 270-272. (b) Veeneman, G. H.; Van Leeuwen, S. H.; Van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331-1332. (c) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B.*Tetrahedron Lett.* **1990**, *31*, 4313-4314.

7 Hendrickson, J. B.; Kandall, C. *Tetrahedron Lett.* **1979**, 343-344.

8 (a) Ajisaka, K.; Fujimoto, H.; Isomura, M. *Carbohydr. Res.* **1994**, *259*. 103-115. (b) Makimura, Y.; Ishida, H.; Kondo, A.; Hasegawa, A.; Kiso, M. *J. Carbohydr. Chem.* **1998**, *17*, 975-979.

9 (a) Ichikawa, Y.; Look, G.-C.; Wong, C.-H. *Anal. Biochem.* **1992**, *202*, 215-238. (b) Theim, J.; Treder, W. *Angew. Chem., Int. Ed. Engl.* **1986**, *12*, 1096-1097. (c) Auge, C.; Gautheron, C.; Pora, H. *Carbohydr. Res.* **1989**, *288*. 288-292. (d) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G. -J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C. -H. *J. Am. Chem.Soc.* **1992**, *114*, 9283-9298 (e) Ito, Y.; Paulson, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 7862-7863.

10 Williams, M. A.; Kitagawa, H.; Datta, A. K.; Paulson, J. C.; Jamieson, J. C. *Glycoconjugate J.* **1995**, *12*, 755-761.