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EXPERIMENTS DIRECTED TOWARD STEREOCONTROLLED SYNTHESIS OF O-LINKED GLYCAN WHICH CONTAINS REPEATING LACTOSAMINE UNIT[1]

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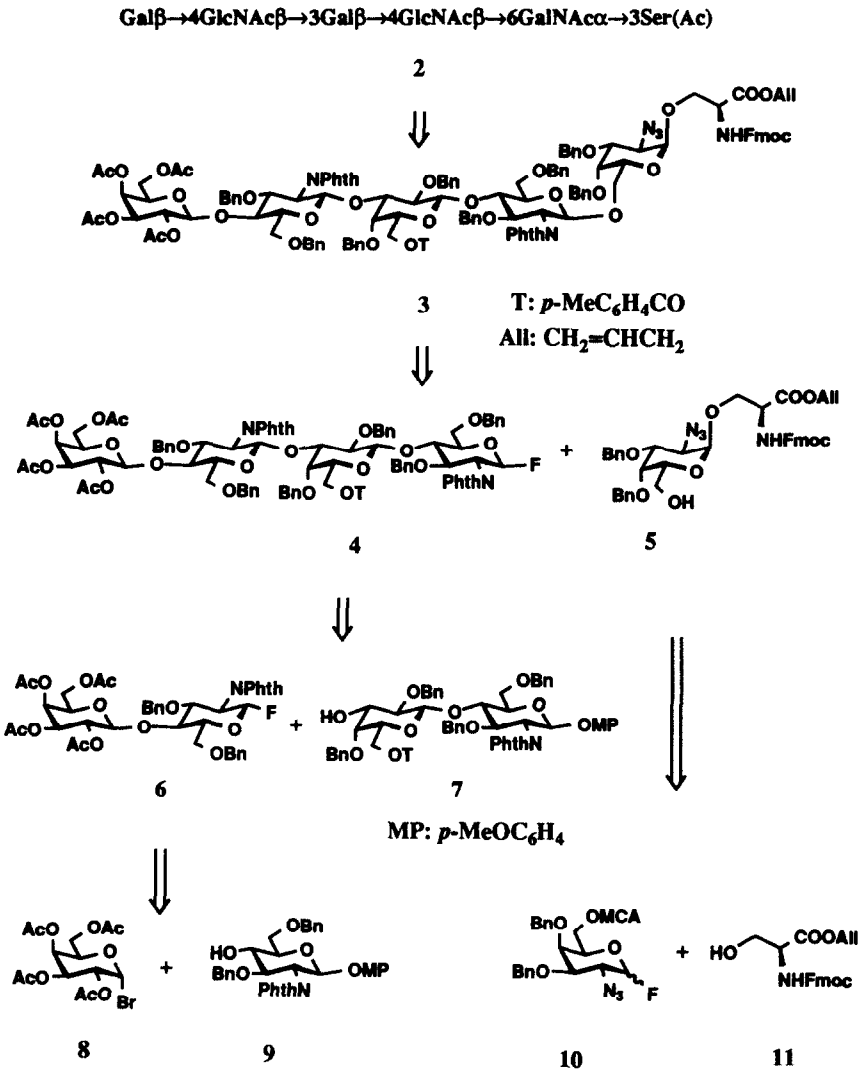
Abstract: Repeating lactosamine containing mucin-type oligosaccharide was synthesized as a serine-linked form in a stereocontrolled manner.

Chemical synthesis of glycoprotein and glycopeptide is a subject of active investigations.[2] Among structurally diverse glycoprotein oligosaccharides, those containing repeating N-acetyllactosamine (LacNAc) structures (polylactosamine) attract much attention in connection with various biological events, most notably as onco-differentiation markers.[3] In addition to widespread occurrence in aspargine-linked glycoproteins and glycosphingolipids, polylactosamine structures have been revealed to exist in various O-linked (mucin type) oligosaccharides.[4] As members of this subgroup, oligosaccharides of structure **1** were found from a variety of sources such as human skim milk mucin, equine chorionic gonadotropin and human secretory immunoglobulin A hinge.[5] A salient character of **1** is that a linearly extended backbone with repeating LacNAc is attached to GalNAc via 6-OH, forming so called core II structure. As part of our project on the synthesis of mucin-type oligosaccharide with biological significance,[6] we report here the synthesis of backbone structure of such oligosaccharides in a serine-linked form (i.e. **2**).

±(NeuAcα→3)Galβ→4GlcNAcβ→3Galβ→4GlcNAcβ→6(Galβ→3)GalNAcα→Ser or Thr

1

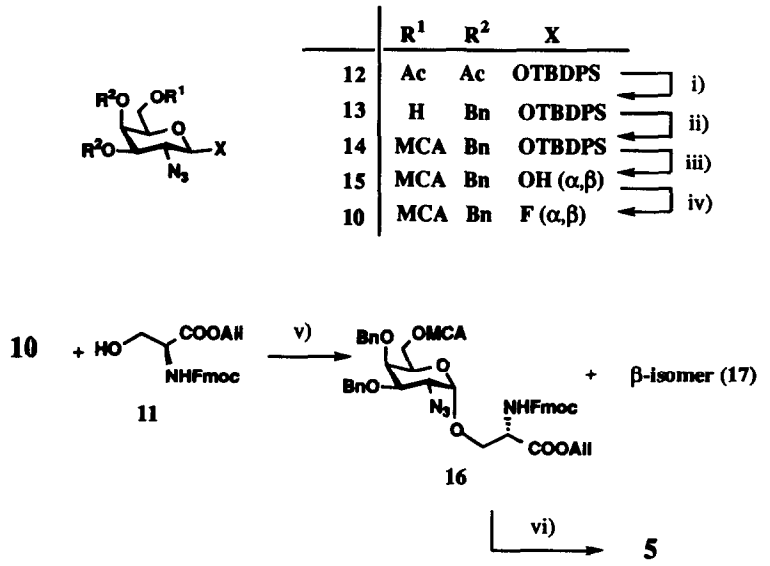
Our synthetic plan is depicted in **Scheme 1**. The GalNAc residue was designed as an azide **10** so that the α-glycosidic linkage can be constructed with exclusion of participation from C-2 substituent. Since it was anticipated that the stereoselective coupling with serine should be most easily achieved at the stage of monosaccharide,[7] LacNAc units were planned to be incorporated onto GalNAc-serine **5**. It is well established in our previous studies on polylactosamine-type glycosphingolipids[8] that the introduction of LacNAc units can be achieved under complete stereochemical control in a high yield by taking advantage of strong 1,2-trans directing nature of a NPhth group[9] and highly efficient activation of anomeric fluoride.[10] Based on such considerations, two LacNAc units were designed as **6** and **7**.



Scheme 1

First, the glycosyl serine **5**[11] was synthesized as shown in **Scheme 2**. Readily available **12** (prepared in 6 steps from D-galactose based on Lemieux's method)[12] was converted into the fluoride **10**[11] (α:β = 1:1), via **13**, **14** and **15**. Glycosylation of **10** with serine derivative **11**[13] in the presence of

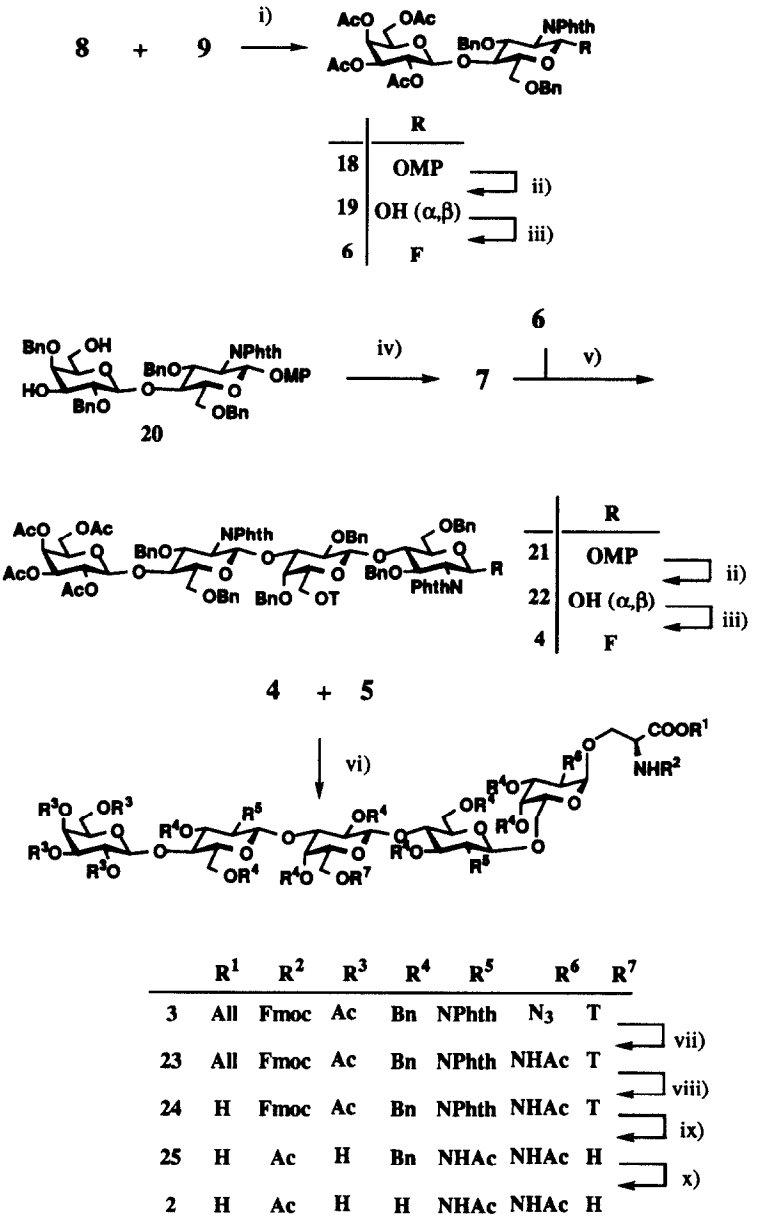
$Cp_2ZrCl_2/AgClO_4$[10a] afforded desired α-glycoside **16**[11] together with corresponding β-isomer **17**[11] in 84 % yield (α/β = 3.33). Further conversion into **5** was achieved by treatment with thiourea[14].



i) a) NaOMe / MeOH-toluene, r.t.; b) DMTr-Cl / pyridine, r.t.; c) BnBr, NaH / THF, 60°C; d) TsOH / MeOH-$CHCl_3$; 80% overall. ii) $(ClCH_2CO)_2O$, DMAP / CH_2Cl_2-Et_3N; 81%. iii) n-Bu_4NF / THF-AcOH, r.t.; 97%. iv) DAST / $(ClCH_2)_2$; 90%. v) $AgClO_4$- Cp_2ZrCl_2 (2:1) / $(ClCH_2)_2$, -23°C; 84%. vi) thiourea / DMF, 90°C; 58%.

Scheme 2

Construction of tetrasaccharide donor **4**[11] and coupling with **5** were performed as follows (**Scheme 3**). Glycosylation of the bromide **8** with **9** afforded **18**[11] in 69% yield. Subsequent removal of 4-methoxyphenyl group[15] gave hemiacetal **19**[11] and fluorination[16] furnished donor **6**[11]. Selective acylation of **20**[17] was cleanly achieved with toluoyl chloride at 0°C. Resulting **7**[11]was glycosylated with **6** by using Cp_2HfCl_2-AgOTf[10b] as a promoter to produce **21**[11]. After conversion into **4**, coupling with **5** was performed again under Suzuki's conditions.[10b] The reaction proceeded smoothly to give the coupling product **3**[11] in good yield, while $SnCl_2$-AgOTf[18] promoted reaction required longer reaction time and gave lower yield (ca. 50%).



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	
3	All	Fmoc	Ac	Bn	NPhth	N_3	T	vii)
23	All	Fmoc	Ac	Bn	NPhth	NHAc	T	viii)
24	H	Fmoc	Ac	Bn	NPhth	NHAc	T	ix)
25	H	Ac	H	Bn	NHAc	NHAc	H	x)
2	H	Ac	H	H	NHAc	NHAc	H	

i) AgOTf / $(CH_2Cl)_2$, -15°C-r.t.; 69%. ii) CAN / toluene-MeCN-H_2O; 85-91%. iii) DAST / $(CH_2Cl)_2$, 0°C; 85-91%. iv) toluoyl chloride / $(CH_2Cl)_2$- pyridine, 0°C; 93%. v) Cp_2HfCl_2-AgOTf (1:2) / CH_2Cl_2, -23°C-0°C; 87%. vi) Cp_2HfCl_2-AgOTf / $(CH_2Cl)_2$, -25°C-r.t.; 79% or AgOTf-$SnCl_2$ / $(CH_2Cl)_2$, r.t.; 50%. vii) MeCOSH / pyridine, r.t.; 89%. viii) $Pd(PPh_3)_4$, MeNHPh / THF; 93%. ix) NH_2NH_2-H_2O / EtOH, 80°C then Ac_2O / aq. dioxane, r.t.; 38%. x) H_2, $Pd(OH)_2$ / MeOH-H_2O-AcOH (40:10:1); 87%.

Scheme 3

Further transformation into **2** was conducted as follows. One-step conversion of azide **3** to acetamide **23**[11] was realized by treatment with AcSH/Pyridine (2:1 volume)[19]. Subsequent deallylation into carboxylic acid **24** was accomplished with $Pd(PPh_3)_4$ and MeNHPh.[20] Exposure of **24** to hydrazine/ethanol (1:2) at 80° C for 3 days followed by N-selective acetylation with 40 equiv. of acetic anhydride in aq. dioxane afforded **25**. Hydrogenolysis with 20% $Pd(OH)_2/C$ proceeded smoothly to produce **2**[11] as a white foam after lyophilization. Spectrum data (NMR, MS) are well in agreement with the structure.

As is described above, we have established a reasonably practical synthetic route to O-linked glycan chain which contains repeated LacNAc units. This strategy, after slight modification, should be applicable to glycan chain with properly functionalized amino acid residue, which can be incorporated into peptide sequences.

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11. Selected physical data for key compounds are given below. Values of $[\alpha]_D$ and ^1H- and ^{13}C-NMR were recorded at 25±3°C for solutions in $CHCl_3$ or $CDCl_3$ respectively, unless otherwise indicated. **2**: Rf 0.33 in 1:1:1:1 nBuOH-EtOH-H_2O-HOAc; $[\alpha]_D$ +19° (c 0.1, H_2O); δ_H (D_2O, 70°C) 4.83(d, J 3.7 Hz, 1H, H-1a), 4.73 and 4.59(2d, J 7.7 Hz, 7.3 Hz, 2H, H-1b, and H-1d), 4.46 and 4.45(2d, J 7.6 Hz and 8.1 Hz, 2H, H-1c and H-1e), 4.15 (dd, J 4.0 and 11 Hz, 1H, H-2a), 4.13(d, J 4.0 Hz, 1H, H-4a), 2.04, 2.03, 2.02, 2.01(4s, 12H, 4Ac); Positive FAB-MS 1081(M), 1104(M+Na); Negative FAB-MS 1080(M-

1). **3**: Rf 0.36 in 2:1 toluene-EtOAc; $[\alpha]_D$ +34° (c 0.4); δ_H 5.82(m, 2H,=CH, NH), 5.36(d, J 8.6Hz, 2H, H-1b and 1c), 5.28(dd, J 1.4 and 15.2 Hz, 1H, $=CH_2$), 5.26(dd, J 2.9 Hz, H-4e), 5.20(dd, J 1.5 and 10 Hz, 1H, $=CH_2$), 5.14 (dd, J 7.9 and 10.3 Hz, H-2e), 2.35(s, 3H, $\underline{CH_3}$-C_6H_4), 2.062, 2.012, 1.989, and 1.977(4s, 12H, 4 Ac); δ_C 102.89, 100.31, 99.84, 99.81, 99.25. **4**: Rf 0.46 in 1:1 hexane-EtOAc; $[\alpha]_D$ +17° (c 0.4); δ_H 5.70(dd, J 7.9 and 54 Hz, 1H, H-1a), 5.40(d, J 8.2Hz, 1H, H-1c), 5.27(d, J 3.3 Hz, 1H, H-4d), 5.18(dd,J 10.6 and 7.9 Hz, 1H, H-2d), 2.065, 2.021, 1.997, and 1.978(4s, 12H, 4Ac). **7**: Rf 0.33 in 1:1 hexane-EtOAc; $[\alpha]_D$ +50.5° (c 0.5) δ_H 5.84(dd, J 54 and 7.3 Hz, 1H, H-1a), 5.27(d, J 2.7 Hz, 1H, H-4b), 5.15(dd, J 7.9 and 10.6 Hz, 1H,H-2b), 4.45(d, J 7.9 Hz, 1H, H-1b), 2.078, 2.063, 2.043, and 2.011(4s, 12H, 4 Ac). **10α**: Rf 0.74 in 2:1 Hexane-EtOAc; $[\alpha]_D$ +48.7° (c 0.67); δ_H 5.62(dd, J 2.4 and 53 Hz, 1H, H-1). **10β**: Rf 0.65 in 2:1 hexane-EtOAc; $[\alpha]_D$ -20.1° (c 1); δ_H 4.96(dd, J 7.6 and 53.5 Hz, 1H, H-1), 3.97(s, 2H, $COCH_2Cl$). **16** Rf 0.66 in 3:1 toluene-EtOAc; $[\alpha]_D$ +56° (c 0.6); δ_H 5.91(m, 1H, CH=), 5.33(dd, J 17.2 and 1.3 Hz, 1H, $=CH_2$), 5.25(dd, J 10.6 and 1 Hz, $=CH_2$), 4.80(d, J 2 Hz, 1H, H-1), 3.95(s, 2H, $COCH_2Cl$); δ_C 99.64 (C-1). **17**: Rf 0.29 in 3:1 toluene-EtOAc; $[\alpha]_D$ -4.8° (c 0.4); δ_H 5.91(m, 1H, CH=), 5.33(dd, J 1.3 and 17.2 Hz, 1H, $=CH_2$), 5.22(dd, J 1.3 and 10.6 Hz, $=CH_2$), 4.12(d, J 7.3 Hz, 1H, H-1), 3.90(s, 2H, $COCH_2Cl$); δ_C 102.6 (C-1). **18**: Rf 0.40 in 2:1 hexane-EtOAc; $[\alpha]_D$ +60° (c 0.35); δ_H 5.62(d, J 8.3 and 10.5 Hz, H-2b), 5.30(d, J 2.9 Hz, 1H, H-4b), 5.18(dd, J 8 and 10.5 Hz, H-2b), 4.50(d, J 9.2 Hz, H-1b), 3.70(s, 3H, OCH_3), 2.056, 2.039, 2.036, and 2.029(4s, 12H, 4Ac) δ_C 100.04(C-1b), 97.5(C-1a). **21**: Rf 0.25 in hexane-EtOAc; $[\alpha]_D$ +30.4° (c 2); δ_H 5.44(d, J 8.3 Hz, 1H, H-1a), 5.22(d, J 2Hz, 1H, H-4d), 5.38(d, J 7.9 Hz, 1H, H-1c), 3.65(s, 3H, OCH_3), 2.39(s, 3H, $\underline{CH_3}$-C_6H_4), 2.06, 2.02, 1.99, and 1.97(4s, 12H, 4Ac). **23**: Rf 0.81 in 9:1 $CHCl_3$-CH_3OH; $[\alpha]_D$ +23.6° (c 0.5); δ_H 5.80(m, 1H, CH=), 5.62(d, J 10 Hz, 1H, NH), 5.37(d, J 8.6 Hz, 2H, H-1b and H-1d), 2.35(s, 3H, $\underline{CH_3}$-C_6H_4), 2.066, 2.013, 1.992, 1.979, and 1.824(5s, 15H, 5Ac). **25**: Rf 0.2 in 3:1 CH_3Cl-CH_3OH; $[\alpha]_D$+28° (c 0.2, CH_3OH); δ_H(CD_3OD) 7.13-7.40(m, aromatic-H), 5.134(d, J10.7 Hz, 1H, O$\underline{CH_2}$Ph), 5.040(d, J 11.7 Hz, 1H, O$\underline{CH_2}$Ph), 5.000(d,J 10.2 Hz, 1H, O$\underline{CH_2}$Ph), 1.990, 1.984, 1.939, and 1.623(4s, 12H, 4 Ac); Positive FAB-MS 1801(M) 1824(M+Na).

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