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SYNTHESIS OF A GLYCOTETRAOSYL SERINE, A PARTIAL STRUCTURE OF AN OVARIAN CYST MUCIN GLYCOPROTEIN OF BLOOD GROUP A ACTIVITY[1]

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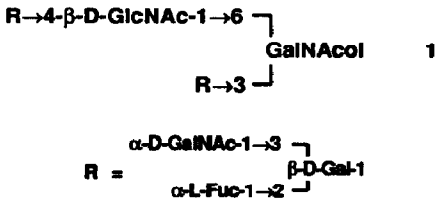
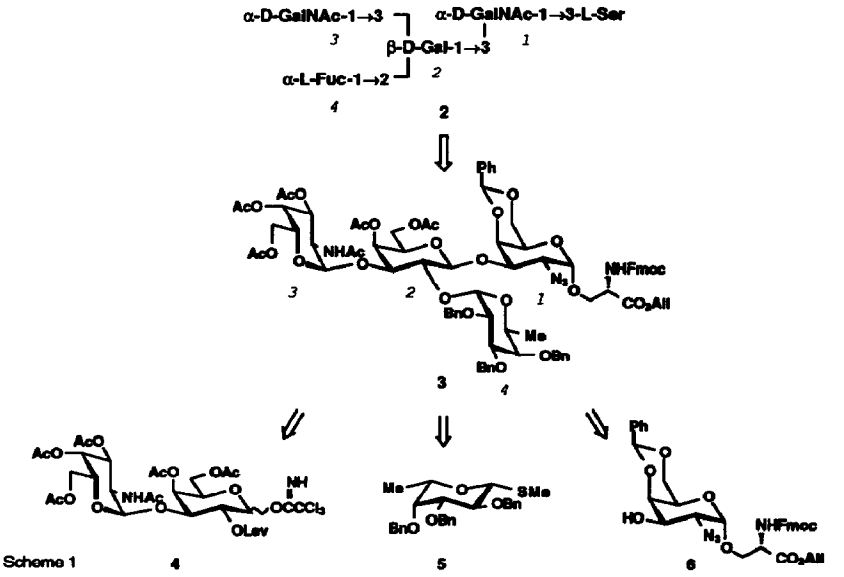
Abstract: A glycotetraosyl serine containing blood group A determinant tetrasaccharide and its properly protected equivalent were synthesized in a regio- and stereocontrolled manner.

In 1986, Bush and his co-workers[2] reported the isolation of complex oligosaccharide alditols such as **1** after alkaline borohydride degradation of ovarian cyst mucin glycoproteins with blood group A activity and their characterization through a combined use of high pressure liquid chromatography and ^{1}H NMR spectroscopy. It is to be noted that structure **1** bears two blood group A determinant trisaccharide composite units α-D-GalNAc-(1→3)-{α-L-Fuc-(1→2)-}-β-D-Gal and belong to the core II type structure[3].



In connection with our on-going project on the synthesis of glycopeptide fragments of glycophorin, we have been interested in the development of general synthetic approaches toward O-linked glycan of glycoproteins with blood group activity. In order to study a plausible synthetic strategy directed towards highly branched structures such as **1**, a partial structure of **1** was extracted and designed as a target molecule **2** that contains L-serine as a possible linking residue of the carbohydrate periphery to the peptide backbone. Hitherto synthetic studies towards blood group A active oligosaccharides that carry no amino acid have been reported from

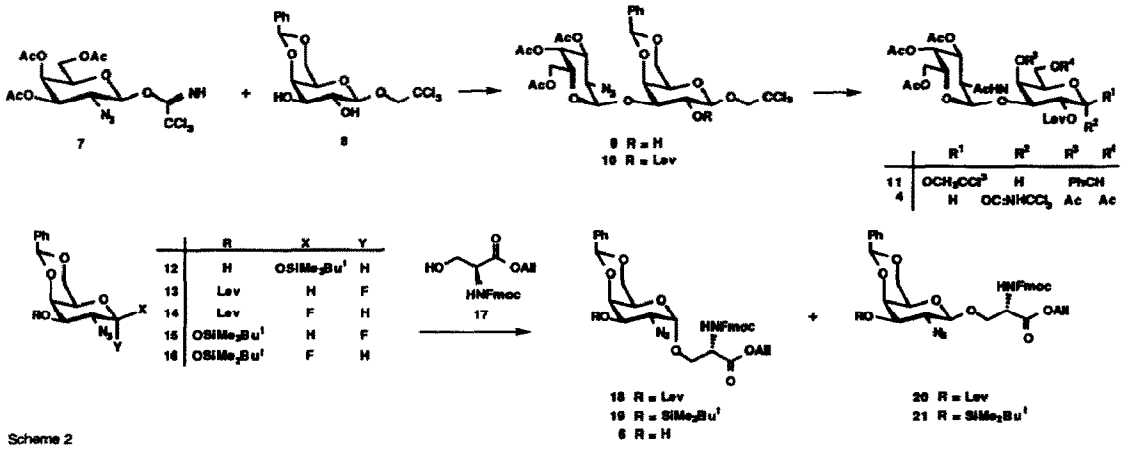


Scheme 1

several groups[4]. Herein we report on the stereoselective synthesis of compounds **2**.

Retrosynthetic analysis of **2**, which belongs to the core I type structure, was carried out by first designing a completely protected glycotetraosyl serine **3** which should be suitable for further elaboration at O-6^{I} of **3** in order to approach a glycosyl serine with core II type structure such as **1**. Disconnection of **3** led us to design two glycosyl donors **4** and **5**, and a glycosyl acceptor **6**.

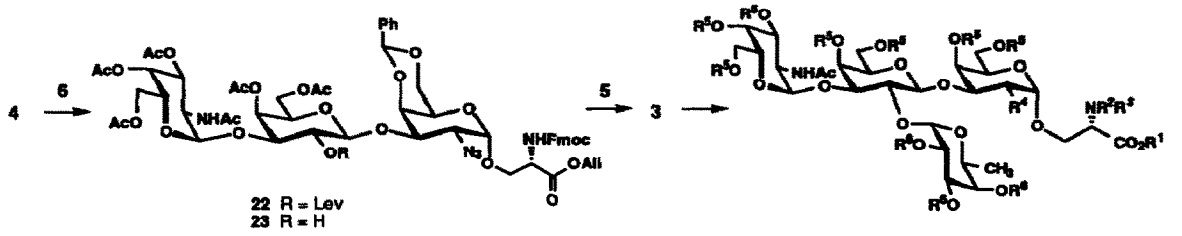
The glycosyl donor **4** was synthesized in a following way. Glycosylation of diol **8**[5] with 1.2 equivalents of β-trichloroacetimidate **7**[6] in the presence of powdered molecular sieves 4A (MS4A) and TMSOTf[7] in CH_2Cl_2 at -20° proceeded smoothly to give a regioselectively glycosylated product **9**[8] in 77% which was treated with levulinic anhydride[9] and DMAP in pyridine to give 86% of **10**[8]. Conversion of **10** into **4**[8] was performed in 4 steps; *1*) AcSH[10] in CH_2Cl_2, 4 days at 20°, *2*) 8:2:1 CF_3COOH-H_2O-CH_2Cl_2, overnight at 20°, *3*) Ac_2O, DMAP in pyridine, *4*) Zn, 2:5 AcOH-THF, *5*) CCl_3CN[11], DBU in CH_2Cl_2, 83% overall.



Scheme 2

The glycosyl acceptor **6** was prepared as follows. A readily available **12**[12] was converted into α- and β-fluorides **13**[8] and **14**[8] in 40% and 35% overall yield in three steps; *1*) $(Lev)_2O$, DMAP in pyridine, *2*) nBu_4NF, AcOH in THF[13], *3*) DAST[14] in CH_2Cl_2. Glycosylation of the serine derivative **17**[15,20] with 0.4 equivalents of **13** in the presence of $Cp_2Zr(ClO_4)_2$[16] and MS4A in CH_2Cl_2 afforded 46% of desired **18**[8] and 28% of crude β-isomer **20**. A similar observation was made under the same reaction condition by use of β-fluoride **14** and 52% of α-isomer **18** was obtained along with 21% of crude β-isomer **20**. Compounds **13** and **14** was then converted into another pair of glycosyl donors **15**[8] and **16**[8] in 90 and 89% yield, respectively, in two steps: *1*) NH_2NH_2AcOH in 1:5 PhMe-EtOH[17], *2*) $^{t}BuMe_2SiCl$, imidazole, DMAP in DMF. Now glycosylation of **17** with 0.96 equivalents of **15** according to the Suzuki procedure as described above gave an improved result and thus a 93% yield of mixture **19**[8] and **21**[8] in a ratio of 10:1 was obtained. Use of the β-fluoride **16** gave virtually the same result. Treatment of **19** with NH_4F[18] in MeOH afforded the designed glycosyl acceptor **6** in 72% yield. TMSOTf catalyzed coupling of **4** with 1.3 equivalents of **6** in the presence of MS4A in CH_2Cl_2 for 12h at -20° gave 58% of **22**[8]. Removal of levulinoyl group of **22** was carried out as described above to give 98% of **23**[8]. Crucial glycosylation of **23** with 10 equivalents of a fucosyl donor **5** was achieved in the presence of

nBu_4NBr-$CuBr_2$-AgOTf[19] in 5:1 $(CH_2Cl)_2$-PhMe to give 58% of the designed key intermediate **3**. The β-isomer[8] of **3** was also formed and was isolated in 14% yield.



	R^1	R^2	R^3	R^4	R^5	R^6
25	All	H	Fmoc	N_3	Ac	Bn
26	All	H	Fmoc	NHAc	Ac	Bn
27	H	H	H	NHAc	H	Bn
28	H	Me	Me	NHAc	H	H

Scheme 3

Finally, in order to confirm the structure, completely protected **3** was converted into free glycotetraosyl serine derivative **28**[8] via **26**[8] and **27**[8] in 5 steps: *1*) 80% aq.AcOH, 6h at 60°, then Ac_2O, DMAP in pyridine, 87%; *2*) AcSH and pyridine, 94%; *3*) $Pd(Ph_3P)_4$, PhNHMe in THF[20], 74%; *4*) aq. NaOH-MeOH, 95%; *5*) 20% $Pd(OH)_2$-C, H_2, 80% aq.MeOH, 89%. Hydrogenolysis of three benzyl groups on fucosyl residues of compound **27** in step *5* did afford N,N-dimethylated serine derivative **28** instead of the expected **2**, most probably due to the presence of trace amount of HCHO in the reaction medium. Since structure of **28** was firmly confirmed by ^{1}H-NMR and FAB-MS data, we are now in a position to extend our synthetic strategy directed further toward glycosyl serines with core II type structure.

In summary, by employing two glycosyl donors **4** and **5** as well as a glycosyl acceptor **6**, a rational synthetic design to core I type glycosyl serine with blood group A activity was developed.

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1. Part 96 in the series "Synthetic Studies on Cell-Surface Glycans". For part 95, see F. Goto and T. Ogawa, *Tetrahedron Lett.*, submitted.
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8. Physical data for novel compounds are presented below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were recorded at 25°±3° for solutions in $CHCl_3$ and $CDCl_3$, respectively, unless otherwise indicated. Signal assignment such as H-3^2 stands for a proton at C-3 of sugar residue *2*. **3**: $[\alpha]_D$ +51.6° (c 0.5); δ_H 0.992 (d, J 6.6 Hz, Fuc-CH_3), 1.917, 1.935, 1.965, 1.997, 2.063, 2.096 (6s, 6Ac), 5.158 (d, J 3.7 Hz, H-1^3), 5.438 (d, J 3.7 Hz, H-1^4), 5.557 (s, PhC*H*), 5.645 (d, J 9.2 Hz, SerN*H* or N*H*Ac); δ_C 94.5 (C-1^3), 98.1 (C-1^4), 100.6 (C-1^1), 101.5 (Ph*C*H). FAB-MS $(M+1)^+$ 1634. β-isomer of **3**: $[\alpha]_D$ +4.6° (c 0.2); δ_H 1.010 (d, J 6.2 Hz, Fuc-CH_3), 1.811, 1.923, 1.988, 2.000, 2.139, and 2.147 (6s, 6Ac), 4.773 (d, J 7.7 Hz, H-1^4), 5.401 (s, PhC*H*); FAB-MS$(M+Na)^+$ 1656. **4**: $[\alpha]_D$ +105° (c 1.9); δ_H 1.974, 1.980, 2.014, 2.020, 2.157, 2.162, 2.204 (7s, 6Ac and Lev), 5.122 (d, J 3.4 Hz, H-1^2), 5.339 (dd, J 3.7 and 10.4 Hz, H-2^1), 5.538 and 5.419 (2d, J 2.4 and 1.9 Hz, H-$4^{1,2}$), 5.886 (d, J 9.8 Hz, N*H*Ac), 6.585 (d, J 3.7 Hz, H-1^1), 8.683 (s, CNH). **6**: $[\alpha]_D$ +97° (c 0.4); δ_H 3.556 (dd, J 3.3 and 10.6 Hz, H-2), 4.036 (dd, J 2.2 and 10.3 Hz, SerβH), 4.573 (m, SerαH), 4.977 (d, J 2.6 Hz, H-1), 5.540 (s, PhC*H*). **9**: M.p. 108-109°C; $[\alpha]_D$ +73° (c 1.2); δ_H 2.040, 2.050, 2.134 (3s, 3Ac), 4.008 (dd, J 11.2 and 7.6 Hz, H-2^1), 4.500 and 4.185 (2d, J 11.9 Hz, CH_2CCl$_3$), 4.633 (d, J 7.6 Hz, H-1^1), 5.243 (d, J 3.4 Hz, H-1^2), 5.476 (d, J 3.1 Hz, H-4^2), 5.587 (s, PhC*H*). **10**: M.p. 182-183°C; $[\alpha]_D$ +72° (c 0.7); δ_H 2.007, 2.039, 2.132 and 2.196 (4s, 3Ac and Lev), 4.842 (d, J 8.1 Hz, H-1^1), 5.455 (dd, J 8.1 and 9.9 Hz, H-2^1), 5.488 (s, PhC*H*), 5.101 (d, J 4.0 Hz, H-1^2), 5.741 (d, J 9.5 Hz, N*H*Ac). **13**: $[\alpha]_D$ -136.4° (c 0.3); δ_H 2.123 (s, Lev), 5.558 (s, PhC*H*), 5.804 (dd, J 2.4 and 52.8 Hz, H-1). **14**: $[\alpha]_D$ +133° (c 0.3); δ_H 2.110 (s, Lev), 5.127 (dd, J 7.6 and 52.5 Hz, H-1), 5.535 (s, PhC*H*). **15**: $[\alpha]_D$ +99° (c 2.6); δ_H 0.148 and 0.184 (2s, 2CH$_3$), 0.926 (s, ^{t}Bu), 5.543 (s, PhC*H*), 5.730 (dd, J 2.4 and 53.1 Hz, H-1). **16**: $[\alpha]_D$ +23.5° (c 0.5); δ_H 0.167, 0.130 (2s, 2CH$_3$), 0.926 (s, ^{t}Bu), 4.022 (t, J 2.4 Hz, H-4), 5.058 (dd, J 7.6, 52.8 Hz, H-1), 5.541 (s, PhC*H*). **18**: $[\alpha]_D$ +168° (c 0.3); δ_H 2.116 (s, Lev), 5.033 (d, J 3.1 Hz, H-1), 5.506 (s, PhC*H*); δ_C 99.9 (C-1), 100.7 (Ph*C*H). **19**: $[\alpha]_D$ +72° (c 1.5); δ_H 0.145 and 0.179 (2s, CH$_3$), 0.934 (s, ^{t}Bu), 3.732 (dd, J 3.4 and 10.0 Hz, H-2), 4.470 (dd, J 7.3 and 10.7 Hz, H-3), 4.964 (d, J 3.4 Hz, H-1), 5.509 (s, PhC*H*). **20**: $[\alpha]_D$ +29° (c 0.7); δ_H 2.105 (s, Lev), 5.491 (s, PhC*H*); δ_C 102.3 (C-1), 100.9 (Ph*C*H). **21**: $[\alpha]_D$ +22° (c 0.5); δ_H 0.117, 0.162 (2s, 2CH$_3$), 0.926 (s, ^{t}Bu), 5.522 (s, PhC*H*). **22**: $[\alpha]_D$ +101° (c 0.3); δ_H 1.949, 1.959, 2.010, 2.034, 2.123, 2.149, 2.172 (7s, 6Ac and Lev), 4.699 (d, J 7.9 Hz, H-1^2), 5.078 (d, J 3.7 Hz, H-1^3), 5.209 (dd, J 7.6 and 10.1 Hz, H-2^2), 5.514 (d, J 3.4 Hz, H-4^2), 5.522 (s, PhC*H*); δ_C 96.3 (170.9 Hz, C-1^3), 100.1 (172.1 H, C-1^1), 100.5 (162.3 Hz, Ph*C*H), 102.1 (159.9 Hz, C-1^2). **23**: $[\alpha]_D$ +53° (c 0.1); δ_H 1.991, 2.012, 2.018, 2.029, 2.159, 2.172 (6s, 6Ac), 4.982 (d, J 3.7 Hz, H-1^1), 5.029 (dd, J 3.0 and 11.3 Hz, H-3^3), 5.040 (d, J 3.1 Hz, H-1^3), 5.536 (s, PhC*H*). **25**: $[\alpha]_D$ +42° (c 0.1); δ_H 1.940, 1.972, 2.011, 2.022, 2.025, 2.076, 2.090, 2.113 (8s, 8Ac), 5.175 (d, J 3.7 Hz, H-1^3), 5.440 (d, J 3.7 Hz, H-1^4), 5.724 (d, J 9.5 Hz, SerN*H* or N*H*Ac), 5.914 (d, J 8.6 Hz, SerN*H* or N*H*Ac). FAB-MS $(M+1)^+$ 1630. **26**: $[\alpha]_D$ +82° (c 0.1); δ_H 1.887, 1.901, 2.095, 2.033, 2.095, 2.121, 2.139 (7s, 7Ac), 2.158 (s, 2Ac), 5.192 (d, J 3.7 Hz, H-1^3), 5.347 (d, J 3.1 Hz, H-1^4). FAB-MS $(M+1)^+$ 1648, $(M+Na)^+$ 1670. **27**: $[\alpha]_D$ +41.2° (c 0.2, CH_3OH); δ_H(CD_3OD) 1.889, 2.002 (2s, 2Ac). FAB-MS $(M+1)^+$ 1090, $(M+Na)^+$ 1112. **28**: $[\alpha]_D$ +55.7° (c 0.1, H_2O); δ_H(D_2O at 50°) 1.222 (d, J 6.7 Hz, Fuc-CH_3), 2.068, 2.085 (2s, 2Ac), 2.710 (s, N(CH_3)$_2$), 4.713 (d, J 7.3 Hz, H-1^2), 4.926 (d, J 3.7 Hz, H-1^1), 5.207 (d, J 4.0 Hz, H-1^3), 5.295 (d, J 4.0 Hz, H-1^4). FAB MS $(M+1)^+$ 848, $(M+Na)^+$ 870.

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