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Orthogonal Glycosylation Strategy in Synthesis of Extended Blood Group B Determinant

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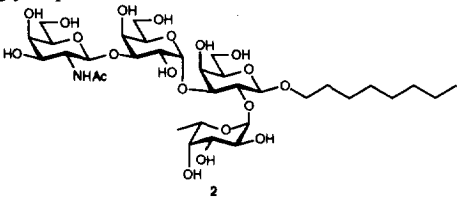
Abstract: The orthogonal glycosylation strategy was applied for the synthesis of extended blood type B determinant (**2**) of a novel glycolipid **1**. Key features in the synthesis are 1) four monosaccharide units were synthesized as either glycosyl fluoride or thioglycoside to be engaged to the orthogonal glycosylation strategy and 2) all necessary manipulations were completed at the monosaccharide level, therefore, manipulations during the elongation of sugar chain were minimized. Copyright © 1996 Elsevier Science Ltd

Natural and non-natural oligosaccharides are useful probes to investigate detailed biological functions of glycoconjugates such as receptor-ligand interactions. Chemical, enzymatic, and combined methods are employed for the synthesis of such oligosaccharides.[1] Therefore, isolation, characterization, and cloning of glycosyltransferases are of great interest for the progress of glycotechnology. The recent discovery[2] of a novel glycolipid, namely, β-D-Gal*N*Ac-(1→3)-α-D-Gal-(1→3)-β-D-[α-L-Fuc-(1→2)]-Gal-(1→3)-β-D-Gal*N*Ac-(1→4)-β-D-Gal-(1→4)-β-D-Glc-(1→1)-Cer (**1**), which carries the extended blood group B structure, is of particular interest in this respect because it suggests the presence of a new *N*-acetylgalactosaminyltranferase in rat species. To investigate the possible precursor(s) and the enzyme(s) responsible for the biosynthesis of structure **1**, we decided to synthesize a tetrasaccharide (**2**) which corresponds to the terminal branched portion of this glycolipid.



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Execution of the synthesis used an orthogonal set of glycosylation reactions,[3] namely activation of thioglycosides and glycosyl fluorides with conditions *a* (NIS-AgOTf[4] or DMTST[5]) and conditions *b* (Cp_2HfCl_2-$AgClO_4$[6]), respectively. Thus, four monosaccharide units **3**, **4**, **6**, and **10** were synthesized as either glycosyl fluoride or thioglycoside to fulfill the criteria of this concept. All necessary selective protections as well as anomeric transformations were completed at the stage of monosaccharide, thereby minimizing manipulations during elongation of the sugar chain (Fig. 1).

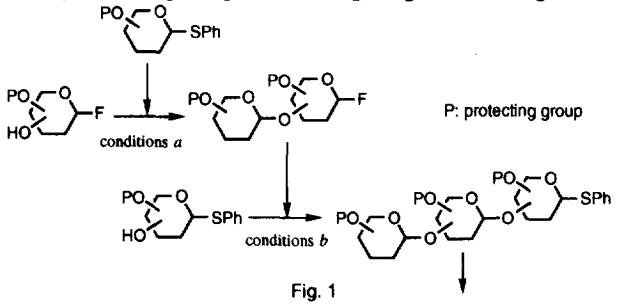
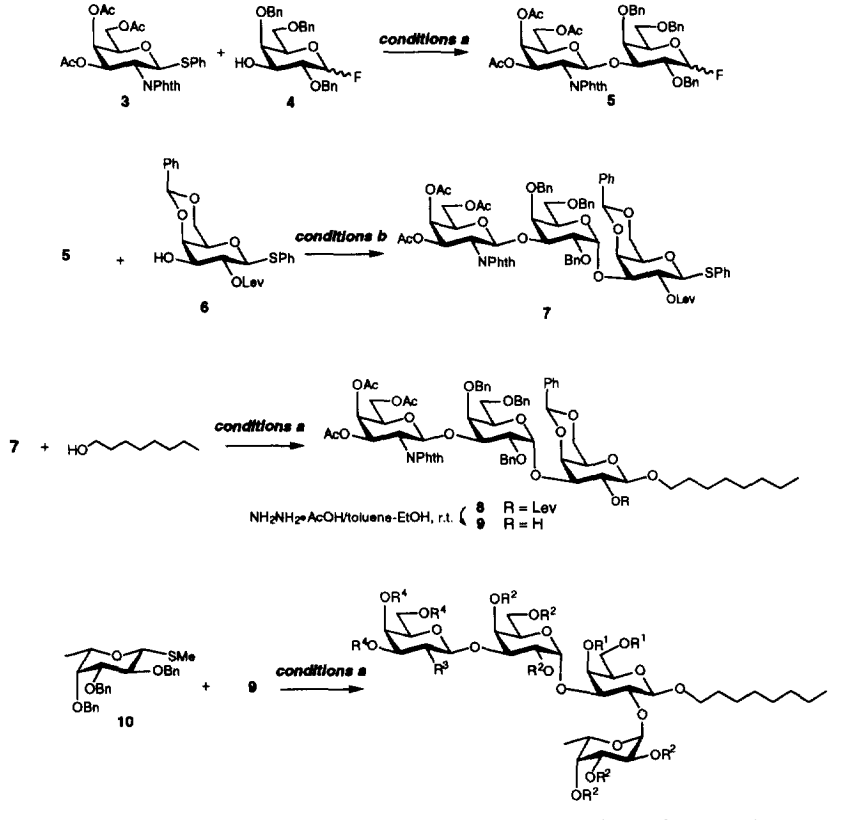


Fig. 1

The synthesis was started from suitably protected galactosyl fluoride **4**[7] ($\alpha:\beta$ = 1.7:1; prepared from **13**[8]) which was glycosylated with Gal*N*Ac precursor **3**[9] under conditions *a* (NIS-AgOTf) in CH_2Cl_2 (-20 → 0 °C) to afford **5**[10] (90%). The stereochemical assignment was confirmed by ^1H NMR, where the $J_{1',2'}$ value of *ca.* 8.5Hz for H-1' indicated the β-D-configuration for Gal*N*Ac residue. No anomerization was observed during the glycosylation reaction which eliminated the potential ambiguity of this strategy. The disaccharide **5** was directly used as a donor for the next glycosylation reaction with the phenylthio glycoside **6**.[11] The glycosylation under conditions *b* in CH_2Cl_2-Et_2O (-78 °C → r.t.) in the presence of $CaSO_4$[12] afforded desired trisaccharide **7**[10] (73.4%). The α-configuration of the newly formed glycosidic linkage was evident from the coupling constant of the anomeric proton at δ 4.77 (J = 3.6Hz). Glycosylation of **7** with octyl alcohol was then carried out under conditions *a* (NIS-AgOTf) in CH_2Cl_2 (-20 °C → r.t.) to yield octyl glycoside **8**[10] (89.7%). Deprotection of the levulinoyl group on trisaccharide **8** using H_2NNH_2•AcOH[13] afforded **9** (85.4%) which was then used as acceptor for the α-fucosylation reaction. Thus, the levulinoyl group was used not only as a participating group to yield β-glycoside but also as a selectively removable protecting group. The coupling reaction of **9** using methylthio glycoside **10**[14] as donor took place smoothly under conditions *a* (DMTST) in benzene-CH_2Cl_2 (0 → 10 °C) to give the desired glycoside **11**[10] (88%). Ethylenediamine treatment[15] of tetrasaccharide **11** followed by selective acetylation of the resulting amine yielded **12**[10] (92%). Hydrogenolysis of **12** under acidic conditions provided the target compound **2**[10] (92.5%).



We have successfully demonstrated the applicability of the orthogonal glycosylation strategy to the synthesis of a branched oligosaccharide with four different types of glycosidic linkages. The synthesis of the target tetrasaccharide was thus achieved in seven steps (38% overall yield) from protected monosaccharide units. Application of this strategy to the polymer supported oligosaccharide synthesis is now under way in our laboratory and will be published elsewhere.

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Supplementary Material Available: Experimental procedures including analyses, ^{1}H and ^{13}C NMR data are available from WWW site at URL: http://carbwww.riken.go.jp/orthogonal2.html or on request to one of the authors (O.K: kokanee@postman.riken.go.jp).

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Special Researcher for Basic Science Program

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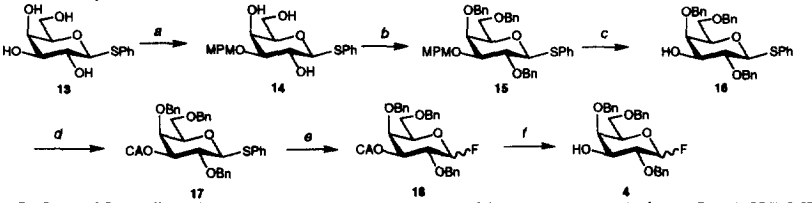
7. Compound **4** was synthesized from **13**[8] as follows;



a) (i) Bu_2SnO/MeOH, reflux, 2 h, (ii) MPMCl-CsF/DMF, 50 °C, 2 days, 45.3%; *b*) BnBr-NaH/DMF, 0 °C, 20 min., 94%; *c*) CAN/CH_3CN-H_2O, 5 °C, 40 min., 87%; *d*) chloroacetyl chloride (CACl)/CH_2Cl_2-Pyr., 0 °C, 30 min., quant.; *e*) DAST-NBS/CH_2Cl_2, -20 °C, 1h, 73%; *f*) Et_3N-MeOH, r.t. over night, quant.

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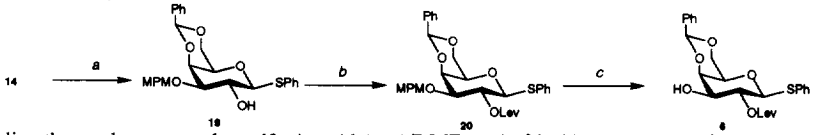
9. Compound **3** was synthesized from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-α,β-D-galactopyranose (Paulsen, H.; Bünsch, A. *Carbohydr. Res.*, **1982**, *100*, 143-167) on treatment with thiophenol and $SnCl_4$ (~quant.).

10. Selected physical data for key compounds are given below. Optical rotations were measured with a JASCO DIP-310 polarimeter at 22°± 2°. ^{1}H-NMR spectra were recorded at 270 MHz (JEOL EX-270) on solution in $CDCl_3$ (internal Me_4Si, δ 0 ppm) or D_2O (internal dioxane, δ 3.55 ppm). **4**: R_f 0.36 and 0.31 (3:1 toluene-EtOAc); NMR δ 5.63 (0.63H, *J* = 2.7, 53.8 Hz, H-1-α) and 5.16 (dd, 0.37H, *J* = 6.6, 52.8 Hz, H-1-β). **5**: R_f 0.33 and 0.30 (5:1 toluene-EtOAc); NMR δ 5.91 (dd, 1H, 0.63H, *J* = 9.6, 3.6 Hz, H-3'-α), 5.85 (dd, 0.37H, *J* = 11.5, 3.3 Hz, H-3'-β), 5.64 (d, 0.63H, *J* = 8.3 Hz, H-1'-α), 5.63 (d, 0.37H, *J* = 8.6 Hz, H-1'-β), 5.52 (broad d, 0.63H, H-4'-α), 5.49 (broad d, 0.37H, H-4'-β), 5.21 (dd, 0.63H, *J* = 3.6, 53.5 Hz, H-1-α), 5.06 (dd, 0.37H, *J* = 6.6, 46.2 Hz, H-1-β), 2.17 and 2.01 (2s, 6H, CH_3CO), 1.86 (s, 1.89H, CH_3CO), and 1.84 (s, 1.11H, CH_3CO). **6**: $[\alpha]_D$ -85.3 (*c* 0.7, $CHCl_3$); R_f 0.10 (2:1 hexane-acetone); NMR δ 5.51 (s, 1H, benzylidene methyne), 5.04 (t, 1H, *J* = 9.6 Hz, H-2), 4.65 (d, 1H, H-1), 4.39 (dd, 1H, *J* = 1.2, 12.5 Hz, H-6a), 4.23 (dd, 1H, *J* = 3.6, 1.0 Hz, H-4), 4.04 (dd, 1H, *J* = 1.7 Hz, H-6b), 3.76 (ddd, 1H, *J* = 10.6 Hz, H-3), 3.55 (broad d, 1H, H-5), 2.54 (d, 1H, OH), and 2.19 (s, 3H, CH_3CO). **7**: $[\alpha]_D$ +8.37 (*c* 1.0, $CHCl_3$); R_f 0.11(5:1 toluene-EtOAc); NMR δ 5.88 (dd, 1H, *J* =11.6, 3.3 Hz, H-3"), 5.62 (d, 1H, *J* = 8.6 Hz, H-1"), 5.48 (broad d, 1H, H-4"), 5.39 (s, 1H, benzylidene methyne), 5.30 (dd, 1H, *J* = 8.9, 9.9 Hz, H-2), 4.77 (d, 1H, *J* = 3.6 Hz, H-1'), 4.61 (dd, 1H, H-2"), 4.49 (d, 1H, *J* = 9.6 Hz, H-1), and 2.19, 2.11, 1.96, and 1.84 (4s, 12H, CH_3CO). **8**: $[\alpha]_D$ +10.6 (*c* 0.8, $CHCl_3$); R_f 0.24 (3:1 toluene-EtOAc); NMR δ 5.88 (dd, 1H, *J* = 11.5, 3.3 Hz, H-3"), 5.67 (d, 1H, *J* = 8.6 Hz, H-1"), 5.49 (d, 1H, H-4"), 5.42 (s, 1H, benzylidene methyne), 5.32 (dd, 1H, *J* = 7.9, 10.2 Hz, H-2), 4.82 (d, 1H, *J* = 4.6 Hz, H-1'), 4.62 (dd, 1H, H-2"), 4.27 (d, 1H, H-1), 2.18, 2.12, 1.98, and 1.84 (4s, 12H, CH_3CO), and 0.88 (t, 3H, CH_3). **11**: $[\alpha]_D$ -9.2 (*c* 0.77, $CHCl_3$); R_f 0.52 (1:1 hexane-EtOAc); NMR δ 5.65 (d, 1H, *J* = 3.6 Hz, H-1'"), 5.09 (dd, 1H, *J* = 11.6, 3.3 Hz, H-3"), 5.41 (d, 1H, H-1"), 5.39 (s, 1H, benzylidene methyne), 5.33 (broad d, 1H, H-4"), 5.24 (d, 1H, *J* = 3.6 Hz, H-1'), 4.32 (d, 1H, *J* = 7.6 Hz, H-1), 2.13, 1.95, and 1.83 (3s, 9H, CH_3CO), 1.22 (d, 3H, *J* = 6.3 Hz, H-6"'), and 0.89 (t, 3H, CH_3). **2**: R_f 0.31 (30:17:2 CH_2Cl_2-MeOH-H_2O); NMR δ 5.11 (d, 1H, *J* = 2.3 Hz, H-1'), 5.02 (d, 1H, *J* = 3.0 Hz, H-1"'), 4.33 (d, 1H, *J* = 8.6 Hz, H-1"), 4.34 (d, 1H, *J* = 7.6 Hz, H-1), 4.27 (q, 1H, *J* = 6.3 Hz, H-5"') 1.83 (s, 3H, CH_3CO), 1.41 (m, 2H, CH_2), 1.08 and 1.01 (broad signal and d, octyl methylene and H-6"'), and 0.66 (t, 3H, octyl methyl).

11. Compound **6** was synthesized from **14**;



a) α,α-dimethoxytoluene-camphorsulfonic acid (cat.)/DMF, at 45 °C, 30 min., 92.4%; *b*) levulinic acid-DCC-DMAP, r.t., 1 h, 87%; *c*) CAN/CH_3CN-H_2O, r.t., 20 min., 89%.

12. During the activation of fluoride **5** under Suzuki's conditions, partial adsorption of acceptor molecule carrying phenylthio group was observed. It seemed that this happened in the case that reaction was carried out in the presence of molecular sieves 4Å. No further activation of fluoride was observed after adsorption. In the case of glycosylation of phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside with corresponding fluoride donor (data not shown), adsorption of the product was observed. The thioglycosides adsorbed on MS 4Å was released during aqueous work-up and were recovered quantitatively.

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