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SYNTHESIS OF Galß1→3(Fucα1→4)GlcNAcß-OR AS POTENTIAL ACCEPTORS FOR A NEW MEMBER OF THE α-1,2-L-FUCOSYLTRANSFERASE FAMILY

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Abstract: A stereoselective synthesis of Galß1→3(Fucα1→4)GlcNAcß-OR (Lewis a) structures containing an anomeric p-nitrophenyl or benzyl group was accomplished through the use of methyl 3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-1-thio-ß-L-fucopyranoside. The compounds were used as acceptors to show in human ovarian tumors and a colon carcinoma cell line (Colo 205), the presence of a new type of α1,2-L-fucosyltransferase which converts the blood group determinant Lewis^a to Lewis^b.

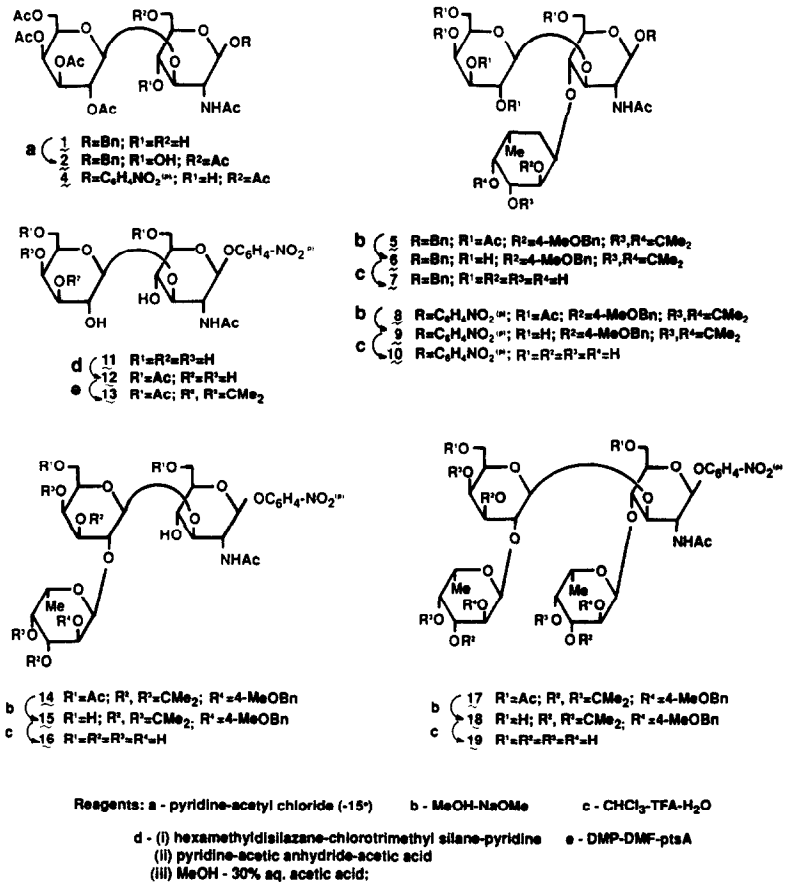
Blood group H-type 1 [Fucα1→2Galß1→3GlcNAcß→] and H-type 2 [Fucα1→2Galß1→4GlcNAcß→] are respectively known as the precursor structures of the blood group determinants, Lewis b [Fucα1→2Galß1→3(Fucα1→4)GlcNAcß→] and Lewis y [Fucα1→2Galß1→4(Fucα1→3)GlcNAcß→][1-4]. Blaszczyk-Thurin et al.[5] identified an α-1,2-L-fucosyltransferase in gastric carcinoma cells that can fucosylate Galß1→3(Fucα1→4)GlcNAcß→ (Le^a sequence) to give Fucα1→2Galß1→3(Fucα1→4)GlcNAcß→ (Le^b sequence). Sarnesto et al.[6] have shown the existence of two ß-galactoside α1→2 fucosyltransferases in human plasma encoded separately by the H and Se genes. It has been demonstrated that sialylation is followed by fucosylation during the biosynthesis of the sialylated Le^a structure, NeuAcα2→3Galß1→3(Fucα1→4)GlcNAc). Questions arise as to whether this new type fucosyltransferase is present in certain cell lines and tissues and whether a new type α-2,3-sialyltransferase exists which is capable of sialylating the Le^a structure. In order to investigate new and unusual types of fucosyltransferase and sialyltransferase activities we targeted the synthesis of Le^a structures[7] containing anomeric benzyl and p-nitrophenyl functional groups. We demonstrated the existence of this new type α-1,2-L-fucosyltransferase in Colo 205 cells and ovarian tumors.

Recently, we developed the synthesis of methyl 3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-1-thio-ß-L-fucopyranoside[8] which was efficiently used for the synthesis of various glycosides containing a benzyl or p-nitrophenyl

functional group. Benzyl 2-acetamido-6-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-ß-D-galactopyranosyl)-2-deoxy-ß-D-glucopyranoside (**2**) and p-nitrophenyl 2-acetamido-6-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-ß-D-galactopyranosyl)-2-deoxy-ß-D-glucopyranoside (**4**) were utilized for the synthesis of our target Lewis a containing oliogosaccharides. Compound **2** was prepared in 55% yield by the selective acetylation of benzyl 2-acetamido-3-O-(2,3,4,6-tetra-O-acetyl-ß-D-galactopyranosyl)-2-deoxy-ß-D-glucopyranoside[9] (**1**) with pyridine-acetyl chloride at -15°. Complete silylation of p-nitrophenyl 2-acetamido-2-deoxy-ß-D-glucopyranoside with hexamethyldisilazane followed by selective substitution of the primary hydroxy with an acetyl group[10-12] produced p-nitrophenyl 2-acetamido-6-O-acetyl-2-deoxy-ß-D-glucopyranoside (**3**) in 59% yield, $[\alpha]_D$ -38 (c 1.1, b); ^{1}H n.m.r. (CD_3OD): δ 1.98 (s, 3 H, NAc), 2.06 (s, 3 H, OAc), 5.24 (d, J = 8.3 Hz, 1 H, H-1), 7.15 (d, J = 9.3 Hz, 2 H, arom.), 8.20 (d, J = 9.2 Hz, 2 H, arom.); ^{13}C n.m.r.: δ 99.74 (C-1), 75.63 (C-5), 75.41 (C-3), 71.88 (C-4), 64.56 (C-6), 57.12 (C-2). Condensation of compound **3** with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide under $Hg(CN)_2$ catalysis afforded compound **4** in 55% yield.

Methyl 3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-1-thio-ß-L-fucopyranoside (0.88 g, 2.5 mmol) on separate reaction with compounds **2** (0.68 g, 1 mmol) and **4** (0.7 g, 1 mmol) in 5:1 (V/V) dichloroethane-N,N-dimethylformamide (48 ml) in the presence of $CuBr_2$ (0.93 g, 4 mmol), tetrabutylammonium bromide[13] (1.28 g, 4 mmol) and 4°A molecular sieves (5 g) for 2 days, afforded crude products **5** and **8** which after de-O-acetylation with methanolic sodium methoxide gave compounds **6** (0.4 g, 52%) and **9** (0.45 g, 57%), respectively, after silica gel column chromatography using 10% methanol in chloroform as the eluent. The removal of both protecting groups from compounds **6** (0.2 g) and **9** (0.23 g) was achieved in one step ($CHCl_3$-TFA- H_2O) to furnish the final trisaccharides, Galß1→3(Fucα1→4)GlcNAcß-OBn (**7**, 0.11 g) and Galß1→3(Fucα1→4) GlcNAcß-OC_6H_4-p-NO_2 (**10**, 0.1 g).

We next became interested in the synthesis of the Le^b structure[14], Fucα1→2Galß1→3(Fucα1→4)GlcNAcß-OR, which will be the enzymatic product resulting from the action of α(1,2)-L-fucosyltransferase upon our Le^a acceptor, Galß1→3(Fucα1→4)GlcNAcß-OR. p-Nitrophenyl 2-acetamido-6-O-acetyl-3-O-(6-O-acetyl-3,4-O-isopropylidene-ß-D-galactopyranosyl)-2-deoxy-ß-D-glucopyranoside (**13**) was a key intermediate acceptor moiety in the synthesis



Reagents: a - pyridine-acetyl chloride (-15°) b - MeOH-NaOMe c - $CHCl_3$-TFA-H_2O

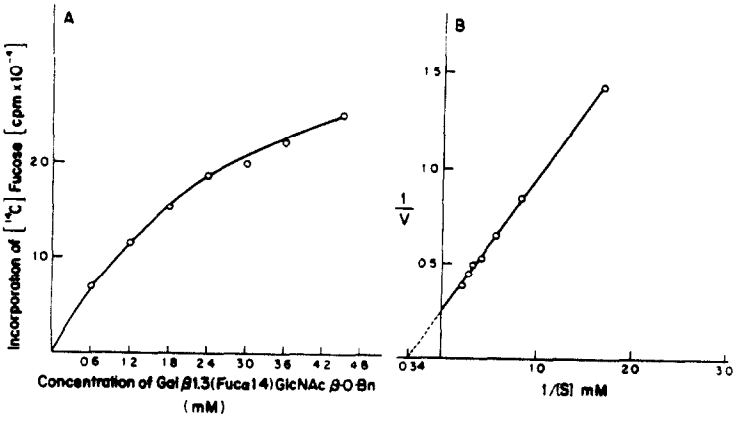
d - (i) hexamethyldisilazane-chlorotrimethyl silane-pyridine e - DMP-DMF-ptsA
(ii) pyridine-acetic anhydride-acetic acid
(iii) MeOH - 30% aq. acetic acid;

of the Le^b structure. It was prepared from p-nitrophenyl 3-O-β-D-galactopyranosyl-2-acetamido-2-deoxy-β-D-glucopyranoside (**11**)[15] in 4 steps. Compound **11** (4.5 g) in pyridine was treated with hexamethyldisilazane-chlorotrimethylsilane to give a fully silylated intermediate which on treatment with pyridine-acetic anhydride-acetic acid followed by the reaction with methanol-30% Aq acetic acid[10-12] provided p-nitrophenyl 2-acetamido-6-O-acetyl-3-O-(6-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (**12**, 3.8 g) in 81% yield. Compound **12** (3.0 g) was treated with acetone-2,2-dimethoxypropane (1:1 V/V) in the presence of p-toluenesulfonic acid to furnish the key glycosyl acceptor **13** (2.25 g) in 71% yield. When diol **13** (0.94 g) was reacted with methyl 3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside (2.6 g) under $CuBr_2$-Bu_4NBr condition it provided monofucosylated trisaccharide **14** (0.8 g) and the difucosylated

tetrasaccharide **17** (0.25 g) in 57.2% and 13.5% yield, respectively. However, the glycosylation of trisaccharide **14** (0.4 g) with the same glycosyl donor under similar reaction condition furnished the tetrasaccharide **17** (0.48 g) in 90.4% yield. Compounds **14** (0.12 g) and **17** (0.15 g) after de-O-acetylation followed by treatment with $CHCl_3$-TFA-H_2O afforded known Fucα1→2Galß1→3GlcNAcß-OC_6H_4-p-NO_2[16] (**16**, 0.04 g, 53%) and Fucα1→2Galß1→3(Fucα1→4)GlcNAcß-OC_6H_4-p-NO_2 (**19**, 0.05 g, 49%) respectively. Structural confirmation of the final products was accomplished through ^{13}C n.m.r. and f.a.b. mass spectroscopy[17].

The acceptor activity of Galß1→3(Fucα1→4)GlcNAcß-O-Bn at various concentrations using Triton X-100 solubilized extract (200 μg protein) of human ovarian tumor as the source of α1,2-L-fucosyltransferase was determined by SEP-PAK C_{18} methodology[18] (Fig. A). The Km was determined by Lineweaver-Burke plot (Fig. B), as 2.94mM. The linkage of [^{14}C]Fuc in the product was identified as α1→2 from its complete release by α-L-fucosidase, which is not active towards Fucα1→3Gal and Fucα1→6Gal. Colo 205 enzyme showed very high activity with the above acceptor and was almost inactive with Galß-O-Bn, whereas human serum enzyme was seven-fold active with Galß-O-Bn as compared to Galß1→3(Fucα1→4)GlcNAcß-O-Bn.

	Incorporation of [^{14}C] Fuc (CPM) into acceptor (3.0 mM)	
Source	Galß1,3(Fucα1,4)GlcNAcßOBn	Gal-ß-O-Bn
Colo 205	13663	71
Serum	1098	7016



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17. All products listed gave satisfactory elemental analysis. Data for compounds are given below as follows: Compound no., yield (%), values of $[\alpha]_D$ measured at 25° ± 3° for solutions in (a) $CHCl_3$ (b) MeOH (c) $CHCl_3$:MeOH (1:1, V/V) (d) H_2O. Compound **2** (55), -15 (c 1.0, a), ^1H n.m.r. (CD_3OD): δ 1.93 (s, 3 H, NAc), 1.94, 2.01, 2.04, 2.08, 2.13 (each s, 3 H, 5 x OAc), 4.46 (d, J = 8.0 Hz, 1 H, H-1'), 7.26-7.33 (m, 5 H, arom.); ^{13}C n.m.r.: δ 102.32 (C-1'), 101.39 (C-1), 84.39 (C-3), 70.27 (C-4), 64.64 (C-6), 62.55 (C-6'), 55.98 (C-2); Compound **4** (51), -18 (c 1.3, a), ^1H n.m.r. (CD_3OD): δ 1.94 (s, 3 H, NAc), 1.99, 2.03, 2.04, 2.08, 2.14 (each s, 3 H, 5 x OAc), 5.23 (d, J = 8.2 Hz, 1 H, H-1), 7.14 (d, J = 9.3 Hz, 2 H, arom.), 8.19 (d, J = 9.3 Hz, 2 H, arom.); ^{13}C n.m.r.: δ 102.29 (C-1'), 99.52 (C-1), 83.84 (C-3), 70.31 (C-4), 64.62

(C-6), 62.58 (C-6'), 55.75 (C-2). Compound **6** (52), -79 (c 0.6, b), ^{1}H n.m.r. ($CDCl_3$): 1.07-1.32 (cluster of s, 9 H, 3 x CMe), 1.88 (s, 3 H, NAc), 3.78 (s, 3 H, OMe), 5.10 (d, J = 3 Hz, 1 H, H-1"), 6.83 (d, J = 9 Hz, 2 H, arom.), 7.10-7.37 (m, 7 H, arom.); Compound **9** (57%), -69 (c 1.1, b), ^{1}H n.m.r. ($CDCl_3$): δ 1.19-1.34 (cluster of s, 9 H, 3 x CMe), 1.91 (s, 3 H, NAc), 3.76 (s, 3 H, OMe), 5.11 (d, J = 3 Hz, 1 H, H-1"), 6.73-7.33 (m, 6 H, arom.), 8.10 (d, J = 9 Hz, 2 H, arom.); Compound **7** (69), -81.5 (c 0.7, d); ^{13}C n.m.r. (D_2O): δ 101.49, 104.85, 99.63 (C-1, C-1', C-1"), 78.45 (C-3), 77.54 (C-4); m/z: 620.4 $(M+1)^+$, 642.4 $(M+Na^+)^+$, 618.2 $(M-1)^-$; Compound **10** (55), -65 (c 0.5, d); ^{13}C n.m.r. (D_2O): δ 105.01, 99.60, 99.69 (C-1', C-1, C-1"), 78.17 (C-3), 77.95 (C-4); m/z: 651.4 $(M+1)^+$, 649.3 $(M-1)^-$; Compound **12** (81), -18 (c 0.5, c), ^{1}H n.m.r. [$(CD_3)_2SO$]: 1.82 (s, 3 H, NAc), 2.03 (s, 6 H, 2 x OAc), 5.46 (d, J = 7 Hz, 1 H, H-1), 7.20 (d, J = 9.2 Hz, 2 H, arom.), 7.87 (d, J = 6.8 Hz, 1 H, NH), 8.22 (d, J = 9.1 Hz, 2 H, arom.); Compound **13** (71), +9 (c 0.4, a), ^{1}H n.m.r. ($CDCl_3$): δ 1.32 and 1.46 (each s, 3 H, 2 x CMe), 2.03 (s, 3 H, NAc), 2.07 and 2.10 (each s, 3 H, 2 x OAc), 5.54 (d, J = 8.3 Hz, 1 H, H-1), 6.18 (d, J = 7.5 Hz, 1 H, NH), 7.07 (d, J = 9.1 Hz, 2 H, arom.), 8.16 (d, J = 9.2 Hz, 2 H, arom.); Compound **14** (57.2), -31.5 (c 0.7, a), ^{1}H n.m.r. ($CDCl_3$): δ 1.26-1.52 (cluster of s, 15 H, 5 x CMe), 1.94 (s, 3 H, NAc), 2.07 and 2.10 (each s, 3 H, 2 x OAc), 3.79 (s, 3 H, OMe), 5.32 (d, J = 3 Hz, 1 H, H-1"), 5.94 (d, J = 8.2 Hz, 1 H, NH), 6.85 (d, J = 8.5 Hz, 2 H, arom.), 7.07 (d, J = 9.1 Hz, 2 H, arom.), 7.27 (d, J = 8.3 Hz, 2 H, arom.), 8.18 (d, J = 9.1 Hz, 2 H, arom.); Compound **16** (53%), -50.5 (c 0.7, Me_2SO); Compound **17** (13.5 from compound **13**, 90.4 from compound **14**), -85 (c 1.2, a), ^{1}H n.m.r. ($CDCl_3$): δ 1.27-1.67 (cluster of s, 27 H, 8 x CMe and NAc), 1.97 and 2.12 (each s, 3 H, 2 x OAc), 3.78 and 3.79 (each s, 3 H, 2 x OMe), 5.42 (d, J = 3 Hz, 2 H, H-1' and H-1"), 6.83 (d, J = 8.1 Hz, 4 H, arom.), 7.09 (d, J = 9.0 Hz, 2 H, arom.), 7.25-7.32 (m, 4 H, arom.), 8.17 (d, J = 9.1 Hz, 2 H, arom.); Compound **19** (47), -87 (c 0.7, d); ^{13}C n.m.r. (D_2O): δ 102.53, 101.59, 100.66, 99.63 (C-1', C-1"', C-1", C-1), 78.39, 78.05, 76.80 (C-3, C-4, C-2').

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19. This publication is part 88 of Synthetic Studies in Carbohydrates, for part 87, see Sarkar, A., Matta, K.L. Carbohydr. Res. (1992) Submitted DCB-92091.