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# Synthesis of a Versatile Tetrasaccharide of Sialyl $Le^x$ and $Le^x$ Antigens

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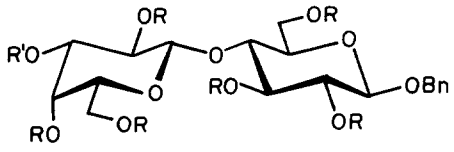
**Abstract** : Benzyl 4-O-[2,4,6-tri-O-benzyl-3-O-(4-methoxybenzyl)-β-D-galactopyranosyl]-2,3,6-tri-O-benzyl-β-D-glucopyranoside (4) was synthesised from benzyl lactoside (2) via regioselective protection of 3'-OH with 4-methoxybenzyl group. Oxidative deprotection of 4 afforded the valuable acceptor 5. The trisaccharide 7 was obtained through coupling of 5 and phenyl 2-deoxy-3,4,6-tri-O-acetyl-2-phthalimido-1-thio-β-D-glucopyranoside (6). The desired tetrasaccharide 1 was built up through fucosylation of the benzylidene derivative 11 obtained from 7. 

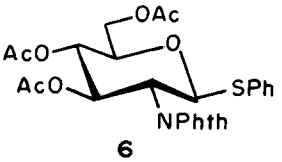
In recent years, attention has been directed towards developing a new class of sugar derived antiinflammatory and antitumor drugs[1] based on the discovery of involvement[2] of Sialyl Lewis x[2] in the cell adhesion process through binding to the glycoprotein E-selectin.[3] Sulphofucooligosaccharides[4] have also been reported as ligands for E-selectin due to their surprising binding activity.

In order to synthesise glycosphingolipids[5] and sulphated oligosaccharides[4] for evaluation of their therapeutic activity it is necessary to develop a practical and inexpensive synthesis of the common key tetrasaccharide 1. Herein the construction of 1 is reported.

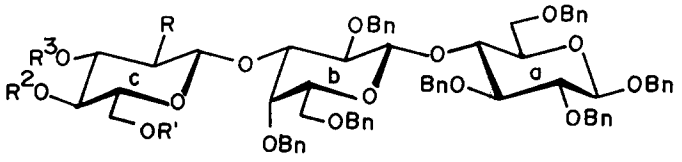
The presence of a lactose unit at the reducing end is a common feature of glycosphingolipids[5] (lacto, neolacto etc.). In order to synthesise 1, the task was to prepare the lactose unit unprotected at 3'-OH. The key compound benzyl 4-O-[3-O-(4-methoxybenzyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (3) was obtained from the well-known benzyl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside[6] through de-acetylation and subsequent regioselective 4-methoxy benzylation at 3'-OH via, the stannylene complex of 2. The structure of compound 3 was established on the basis of spectral data. The $^1H$ NMR analysis of the compound exhibited a singlet at $\delta$3.84 indicating the presence of one methoxy and the mass spectrum showed m/z $(M+Na)^+$ 575. Perbenzylation and oxidation[7] of the



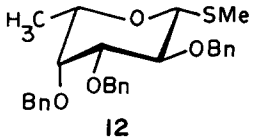
**2** R = R' = H
**3** R = H, R' = pMeOBn
**4** R = Bn, R' = pMeOBn
**5** R = Bn, R' = OH



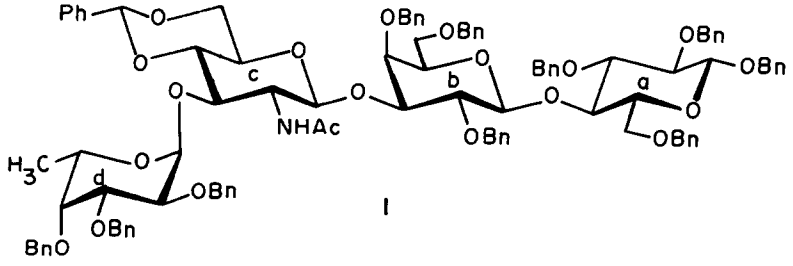
**6**



**7** R = NPhth, $R^1 = R^2 = R^3 = Ac$
**8** R = NPhth, $R^1 = R^2 = R^3 = H$
**9** $R = NH_2$, $R^1 = R^2 = R^3 = H$
**10** R = NHAc, $R^1 = R^2 = R^3 = H$
**11** R = NHAc, $R^1$, $R^2$ = benzylidene, $R^3 = H$



**12**



**1**

compound with 2,3-dichloro-5,6-dicyanobenzoquinone afforded 5, the physical data of which are in agreement with the literature.[6] In an earlier preparation[6] of compound 5, the allyl group has been used for special protection of 3'-OH but this necessitates use of $Rh(Ph_3P)_3Cl$ for deprotection which is expensive. The thioglycoside 6 was prepared[8] from 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose with thiophenol using boron trifluoride diethyl etherate in 89% yield. The trisaccharide moiety 7 was prepared in 71% yield by coupling of the acceptor 5 with 6 using N-iodosuccinimide/trifluoromethanesulfonic acid.[9] The new glycosidic bond was established as β from the coupling constant of 8Hz of the characteristic proton ($H\text{-}1^C$) due to the β-directing power of the phthalimido group.

The trisaccharide 7 was transformed into 10 through Zemplen de-acetylation followed by dephthalimidation using hydrazine hydrate and selective N-acetylation with acetic anhydride-methanol. Benzylidenation of compound 10 with benzaldehyde dimethyl acetal in the presence of 4-toluene sulphonic acid in DMF afforded 11 in good yield. The presence of the three proton singlet at δ1.47 and also a singlet at δ5.59 confirmed the presence of N-acetyl and the benzylidene group in the glucosamine unit. The methyl thioglycoside[10] 12 was coupled with 11 stereoselectively in the presence of $CuBr_2$ and tetrabutylammonium bromide[11] in ethylene chloride and DMF (5:1) mixture. This resulted in an efficient fucosylation at $3\text{-}OH^C$. Compound 1 was characterised from its NMR data. The presence of a doublet of $H\text{-}1^d$ at δ5.26 with J=3.3 Hz confirmed the presence of the L-fucosyl moiety with α-stereoselectivity.

Compound 1 is the core unit of Sialyl Lewis X and Lewis X family of compounds and certainly would be of potential use in related glycosphingolipid synthesis.

## EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on a JASCO 700 spectromter. $^1H$ NMR spectra were run on a JEOL FX-100 FT spectrometer using TMS as internal standard. High field NMR spectra were recorded on a Brucker AM-300L and Bruker AC-200. FAB-MS spectra were recorded on a JEOL AX-500 spectrometer. TLC was performed on silica gel, $F_{254}$ Merck, Darmstadt, Germany. Petroleum ether of boiling range 60-80°C was used. Optical rotations were measured at 25°C on a JASCO, DIP-360 polarimeter.

**Benzyl 4-O-[3-O-(4-methoxybenzyl-β-D-galactopyranosyl]-β-D-glucopyranoside (3) :**

A mixture of 2 (6.4 g, 14 mmol) and dibutyltin oxide (4.3 g) in dry methanol

(220 ml) was refluxed with stirring for 13h. After removal of the solvent the residue was dried in vacuum. To the residue in dry benzene (80 ml), MS 4Å (6.4 g), 4-methoxybenzyl chloride (3.84 ml) and tetrabutylammonium bromide (2.27 g) were added and the mixture refluxed for 6 h. After removal of the solvent the residual mass was column chromatographed on silica gel. Successive elution with dichloromethane and dichloromethane-methanol (19:1, 18:2) mixture afforded **3** (4.2 g, 54%) as amorphous material $[\alpha]_D$-6.4° (c 0.73, methanol); Rf 0.6 (methanol-chloroform,1:9); IR (Neat) : 720, 692 $cm^{-1}$ (aromatic); $^1H$ NMR (100 MHz, $CDCl_3$) : $\delta$3.84 (s,3H,OMe), 6.92 (2H,d,J=8Hz, p-substituted aromatic), 7.2-7.5 (brd.7H, aromatic); FAB-MS : m/z $(M+Na)^+$ 575, $(M-MeOH)^+$ 520. Anal. Calcd, for $C_{27}H_{36}O_{12}$ : (552.5) C, 58.68 ; H, 6.56. Found C, 58.70 ; H, 6.58.

**Benzyl 4-O-[2,4,6-tri-O-benzyl-3-O-(4-methoxybenzyl)-β-D-galactopyranosyl]-2,3,6-tri-O-benzyl-β-D-glucopyranoside (4) :**

A mixture of sodium hydride (2.2 g), **3** (2.6 g, 4.7 mmol) and DMF (25 ml) was stirred for half an hour at 0°C followed by dropwise addition of benzyl chloride (6.46 ml, 46 mmol). The stirring was continued overnight at room temperature, and the reaction monitored by TLC. The reaction mixture was quenched with methanol and then extracted with dichloromethane. The organic layer was dried ($Na_2SO_4$) and then concentrated. Purification of the residue by column chromatography on silica gel with successive elution using ethylacetate-petroleum ether (1:5;1:4) yielded (76%) a syrup **4** (4.0 g). $[\alpha]_D$+25.92° (c 0.27 chloroform) ; Rf 0.65, ethylacetate-petroleum ether (1:1); IR (Neat) : 742, 688, 657 $cm^{-1}$ (aromatic); $^1H$ NMR (100 MHz, $CDCl_3$) :$\delta$3.82 (s,3H,OMe), 6.86 (d,2H, J=8Hz, p-substituted aromatic), 7.00-7.64 (m,37H, aromatic).

**Benzyl 2,3,6-tri-O-benzyl-4-O-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyrasnoside (5) :**

To a solution of **4** (2.6 g, 2,3 mmol) in dichloromethane (46.8 ml), 2,3-dichloro-5,6-dicyanobenzoquinone (738 mg, 3.25 mmol) and water (2.6 ml) were added. The mixture was stirred for 1h at room temperature. It was then filtered, and washed repeatedly with dichloromethane. The filtrate was washed with saturated $NaHCO_3$ solution, dried ($Na_2SO_4$) and then concentrated. Column chromatography using ethylacetate-petroleum ether (1:5.5) of the residue afforded a thick syrup characterised as **5** (1.6 g, 74%); $[\alpha]_D$-4.76° (c 2.17, chloroform) [ref.[6b] $[\alpha]_D$-5.7°(c 1.5, chloroform)]; IR (Neat): 724, 688 $cm^{-1}$ (aromatic); $^1H$ NMR (200 MHz, $CDCl_3$):$\delta$ 3.80-4.01 (m,12H), 4.26-5.01 (m,16H,7$CH_2$,1-H,1'-H), 7.16-7.33 (m,35H).

**Phenyl 2-deoxy-3,4,6-tri-O-acetyl-2-phthalimido-1-thio-β-D-glucopyranoside (6) :**

To a stirred solution of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glycopyranose (4 g, 8.3 mmol) in dichloromethane (40 ml) at 0°C, thiophenol (0.94 ml, 9.13 mmol) and then boron trifluoride etherate (2.5 ml) were added dropwise in succession. The reaction mixture was allowed to come to room temperature. At the end of the reaction (monitored by TLC), the ice cooled water was added to the mixture and it was washed with saturated $NaHCO_3$ solution. The organic layer was then dried ($Na_2SO_4$) and concentrated. Column chromatography of the residue yielded a solid which was recrystallised from dichlormethane-petroleum ether to afford 6 as needles (3.54 g); m.p. 152°C; $[\alpha]_D$+62.2° (c 1.8, chloroform); IR (KBr) : 1718 (ester), 717 and 636 $cm^{-1}$ (aromatic); $^1H$ NMR (100 MHz, $CDCl_3$) : δ1.84, 2.02, 2.1 (3s,9H,3Ac), 3.80-4.04 (H-5), 5.16 (t,$J_{2,3}=J_{3,4}$=9Hz,H-3), 5.74 (d,1H,J=9Hz,H-1), 5.82 (t,1H, $J_{3,4}=J_{4,5}$=10Hz,H-4), 7.20-7.60 (m,5H,Ph), 7.72-7.96 (m,4H,Phthaloyl-H); FAB-MS : m/z $(M+Na)^+$ 550.

**Benzyl O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (7) :**

To a solution of 5 (876 mg, 0.9 mmol) in dichloromethane (15 ml), compound 6 (664 mg, 1.39 mmol) and freshly activated MS 3A (1.54 g) were added under nitrogen. The mixture was stirred for 6h at room temperature and then cooled to -35°C. N-Iodosuccinimide (438 mg) and a solution of trifluoromethanesulphonic acid (23.7 μl) in dichloromethane were added dropwise to the reaction mixture under stirring. After 45 minutes it was quenched with triethyl amine. The mixture was filtered, and washed with dichloromethane. The organic layer was washed with saturated $NaHCO_3$ solution followed by 10% sodium thiosulphate solution. It was then dried ($Na_2SO_4$), concentrated and the residue was subjected to column chromatography using ethylacetate-petroleum ether (2:3) on silica gel. This gave an amorphous material 7 (900 mg); Rf 0.52, ethylacetate-petroleum ether (4:1); $[\alpha]_D$-4.68° (c 0.47, chloroform); IR ($CHCl_3$) : 1751, 1222 (ester), 1720 (imide), 743 $cm^{-1}$ (aromatic); $^1H$ NMR (200 MHz, $CDCl_3$) : δ1.83, 1.93, 2.01 (3s,9H,3Ac), 3.25-3.54 (m,10H), 5.01 (t, $J_{2c,3c}=J_{3c,4c}$=10Hz,H-3c), 5.62 (d,1H,$J_{1c,2c}$=8Hz,H-1c), 5.86 (dd, 1H,$J_{4c,5c}$=8Hz,H-4c) and 6.87-7.47 (m,39H,7Ph,phthaloiyl-H); FAB-MS : m/z $(M+H)^+$ 1390.

**Benzyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (10) :**

Sodium methoxide (26 mg) was added to a solution of 7 (700 mg, 0.5 mmol) in

methanol (4 ml) and the reaction mixture allowed to stand for 2h at room temperature. It was then treated with Amberlite IR-120($H^+$) resin, filtered, washed and the filtrate concentrated. The residue was refluxed for 2h in a mixture of alcohol (7 ml), water (0.5 ml) and hydrazine hydrate (127 µl). The mixture was filtered, washed with a little alcohol and the filtrate concentrated. The residue was treated with a mixture of acetic anhydride (0.28 ml), methanol (3.7 ml) and pyridine (0.44 ml) and kept at room temperature for 3h. After this, the reagents were removed under reduced pressure and the residue was dissolved in dichloromethane. The organic layer was thoroughly washed with water, dried ($Na_2SO_4$) and concentrated. The thick residual mass was subjected to column chromatography using methanol-dichloromethane (3:97) to afford **10** (510 mg, 86.6%) as amorphous powder; $[\alpha]_D$-18.75° (c 1.12, chloroform-methanol, 1:1); IR ($CHCl_3$) : 3314(OH), 1660 and 1560 (amide), 727 and 688 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 1.85 (s,3H,NAc), 7.20-7.40 (m,35H,7Ph).

**Benzyl O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (11):**

To a solution of compound **10** (338 mg, 0.29 mmol) in DMF (2 ml), benzaldehyde dimethyl acetal (87.4 µl, 0.57 mmol), 4-toluene sulphonic acid monohydrate (catalytic amount) and anhydrous calcium sulfate (338 mg) were added. The mixture was stirred for 48h at room temperature and then neutralised with triethylamine. It was then filtered and washed with dichloromethane. The organic layer was washed with water, dried ($Na_2SO_4$) and concentrated. The residue on column chromatography over silica gel using methanol-dichloromethane (1:19) afforded a syrup, characterisd as **11**, $[\alpha]_D$-16.6° (c 0.9, chloroform); IR ($CHCl_3$) : 3296(OH), 1658 and 1553 (amide), 698 and 667 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$) : δ1.47 (s,3H,NAc), 5.36 (d,br,1H,NH), 5.59 (s,1H,PhCH) and 7.10-7.50 (m,40H,8Ph); $^{13}C$ NMR ($CDCl_3$) : 172.37 (-NHCO-), 125.94-138.94 (Ar), 102.35, 102.45 (C-1,a,b), 101.86, 82.73, 81.68, 81.54, 81.42, 79.93-72.67, 68.53, 68.02, 66.42, 59.02,(C-2,c), 22.59 ($-NCOCH_3$).

**Benzyl O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-2,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (1) :**

A soluction of **11** (52 mg, 0.04 mmol) and **12** (38 mg, 0.08 mmol) in 1 ml of $ClCH_2CH_2Cl$-DMF (5:1) were added to stirred mixture of $CuBr_2$ (22.95 mg, 0.1 mmol) tetrabutylammonium bromide (0.33 mg, 0.1 mmol) and freshly activated powdered MS 4Å (110 mg) under nitrogen. The stirring was continued for 50h at room temperature. The reaction mixture was filtered and washed with dichloromethane. The combined

filtrate and washings were washed with saturated $NaHCO_3$ solution and then with water.It was then dried ($Na_2SO_4$) and concentrated. Column chromatography of the residue with ethyl acetate-petroleum ether (3:7) yielded the syrupy product **1** (50 mg, 70%); Rf 0.52 in chloroform-methanol (40:1); $[\alpha]_D$-39.06° (c 1.29, chloroform); IR ($CHCl_3$) : 1673,1605 (amide), 695, 669 $cm^{-1}$ (aromatic); $^1H$ NMR (300 MHz, $CDCl_3$) : $\delta$1.17 (d,3H,$J_{5,6}$=7.8Hz, Fuc), 1.42 (s,3H,NAc), 5.26 (d,1H,J=3.3Hz,H-1, Fuc), 5.57 (s,1H,PhCH) and 7.10-7.46 (m,55H,11Ph); FAB-MS : m/z $(M+Na)^+$ 1702.

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