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SYNTHESIS OF THE KEY DISACCHARIDE OF 3'-SULFO LEWIS X AND LEWIS A :THE NOVEL E-SELECTIN LIGAND

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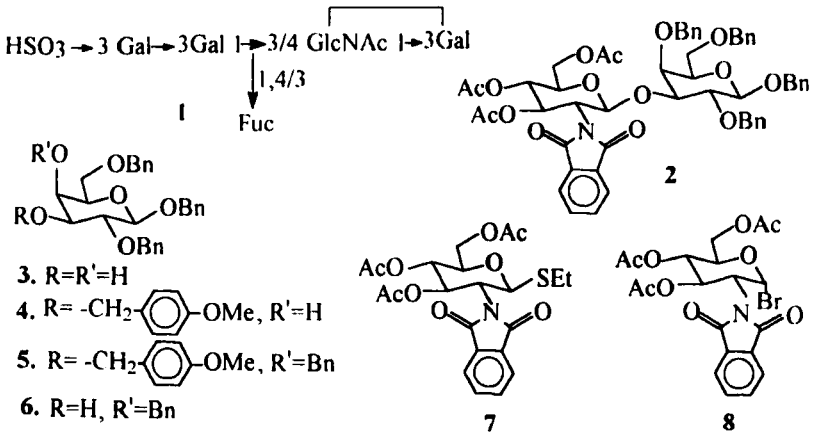
Abstract: Benzyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**6**) was synthesised from regioselective protection of stannylated benzyl 2,6-di-*O*-benzyl -β-D-galactopyranoside (**3**) with 4-methoxybenzyl chloride, followed by benzylation and oxidative removal of the temporary protecting group. The disaccharide (**2**) was prepared by condensation of the common acceptor **6** with **7** through thial activation as well as with **8** following Konigs Knorr procedure.

Since the discovery of Sialyl Lewis X[1] as a ligand[2] of E-selectin[3] in the cell adhesion process leading to inflammation[4], efforts continue to obtain a high avid inhibitor of selectins to cure inflammatory diseases. Feizi *et al*[5] have isolated and identified the novel sulfated ligands of E-selectin. Few syntheses[6] of the ligands are reported. Still development of the methodology directed towards the synthesis of the novel ligand is demanding particularly for studying selectin- carbohydrate interaction[7] in relation to selectin-mediated human diseases. In our endeavour to synthesise the novel sulfated ligands (3'-sulfo Lewis X and Lewis A) the key disaccharide **2** was synthesised as described below.

The presence of galactose unit at the reducing end is a unique feature of biologically important oligosaccharides like glycosphingolipids[7], sulfated oligosaccharides etc. Chain extension towards the non-reducing end usually occur through 3-OH of the galactose ring. So the preparation of the crucial building block **6** is an important task in arriving at the total synthesis of the target molecule **1**. Usually an allyl group[8] is used to protect 3-OH of the stannylated galactoside.



But deallylation[9] with $Rh(Ph_3P)_3Cl$ is expensive particularly for the large scale preparation of **6**. As a consequence the 4-methoxy benzyl group is used here for the regioselective protection of the stannylated intermediate **3** and compound **4** was obtained in excellent yield. Compound **3** was obtained sequentially through reaction of acetobromogalactose with benzyl alcohol in presence of $HgBr_2/HgO$ followed by deacetylation and isopropylidenation. Benzylation of 2 and 6-hydroxyl groups followed by de-isopropylidenation afforded the above compound. Compound **6** was prepared through oxidative cleavage[9] of 4-methoxybenzyl group

in **5** in excellent yield.

To prepare the disaccharide (**2**), thioglycoside[10] **7** was used as glycoyl donor using NIS/TfOH[11] but no appreciable yield was obtained. On the other hand through Konigs Knorr procedure it was obtained in 89% yield The interglycosidic bond was characterised as β from the coupling constant of 7 2 Hz for the anomeric proton

The disaccharide **2** is indeed the key unit of 3'-sulfo Le^x/Le^a ligand (Le^x =Lewis X; Le^a =Lewis A) and biologically active hot molecules like Sialyl Le^x and other important oligosaccharides[11].

Experimental

Benzyl-3-*O*-(4-methoxybenzyl)-2,6-di-*O*-benzyl-β-D-galactopyranoside (4) : A mixture of **3** (5.5 g, 12 mmol) and dibutyltin oxide (3.88 g) in methanol (60 mL) was refluxed for 10 h with stirring. The solvent was removed and dried in vacuum. To the residue in dry benzene (60 mL) was added 4-methoxy benzyl-chloride (3.26 mL), MS4Å (9.5 g) and tetrabutyl ammonium bromide (1.93 g). The mixture was refluxed for 6h. Benzene was evaporated and the residue was chromatographed over silica gel. Elution with dichloromethane-methanol (19:1) afforded **5** (6.8 g, 91%) as an amorphous material. $[\alpha]_D^{25}$-16.88° (c=0.77, $CHCl_3$); IR (Neat): 3466 (OH), 1513, 1453, 733, 693 cm^{-1} (aromatic); ^1H NMR (100 MHz, $CDCl_3$): δ 3.80 (3H, s, OMe), 5.00 (1H, d, J=7 Hz, anomeric), 6.80-6.92 (m, aromatic proton), 7.20-7.50 (aromatic proton). ^{13}C NMR (300MHz,$CDCl_3$) δ 55.12 (OMe),

64.19 (CH_2), 66.82 (CH-OH), 69.19 (CH_2), 70.76, 71.97 (CH_2), 73.58 (CH_2), 78.87, 80.26, 102.45 (C-1), 113.68, 113.75, 126.80, 127.38, 127.44, 127.56, 127.64, 127.8, 127.98, 128.14, 128.22, 128.32, 129.24, 129.38, 129.68, 129.88, 131.87, 137.42, 137.95, 138.51, 159.26. Anal. Calcd. for $C_{35}H_{38}O_7$: C, 84.17; H, 6.71. Found: C, 76.37; H, 6.60. FAB-MS: m/z (M-H)$^+$ 569

Benzyl-3-*O*-(4-methoxybenzyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (5) : Compound **4** (6.8 g , 11 mmol) was taken in DMF (30 mL). Sodium hydride (0.57 g) was added to it and stirred for half an hour. Benzyl chloride (2.3 mL, 0.02 mol) was added dropwise with stirring at 0°C. At room temperature it was stirred overnight. The reaction mixture was quenched with methanol and concentrated in vacuum. The mass was taken in dichloromethane, ice was added to it. The mixture was washed with 2N HCl and then with saturated $NaHCO_3$ solution, dried (Na_2SO_4) and then concentrated. Column chromatography using ethyl acetate - petroleum ether (17 : 3) afforded **5** (81%) as a syrupy mass. $[\alpha]_D^{25}$ -20.11^0 (c=1.56, $CHCl_3$); IR (Neat): 1612, 1585, 1512, 812, 729 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 3.86 (3H, s, OMe), 6.80-7.00 and 7.20-7.60 (aromatic protons). ^{13}C NMR($CDCl_3$,300 MHz) δ 55.12, (OMe), 68.46 (-CH_2), 70.79 (-CH_2), 72.58 (-CH_2), 73.03 (-CH_2), 73.86,74.38 (-CH_2), 74.76, 75.08, 79.19, 81.19, 102.74 (C-1), 113.46, 113.54, 113.65, 113.72, 113.78, 127.38, 127.46, 127.74, 127.86, 127.91, 128.04, 128.10, 128.19, 128.36, 129.03, 129.42, 129.71, 129.92, 130.59, 130.87, 137.64, 136.62, 159.02.Anal. Calcd for $C_{42}H_{44}O_7$: C, 76.34; H, 6.7.Found: 76.42, H, 6.62. (FAB-MS: m/z (M-H)$^+$ 659.

Benzyl 2,4,6-tri-O-benzyl-β-D-galactopyranoside (6):

Water (3 mL) was added to compound **5** (5.6 g, 8.4 mmol) in dichloromethane (54 mL) followed by addition of 2,3-dichloro-5,6-dicyanobenzoquinone (2.86 g) under stirring. The mixture was stirred for 1h at room temperature and monitored by TLC. Dichloromethane was filtered and the mass was repeatedly washed with dichloromethane. Filtrates were collected, washed with $NaHCO_3$ solution, dried (Na_2SO_4) and then concentrated. The residue was subjected to column chromatography and successive elution with dichloromethane-methanol (99:1, 96.5:3.5) yielded **6** (95%) as a syrupy mass. $[\alpha]_D$ -14.22° (c=0.9, $CHCl_3$), lit[12] $[\alpha]_D$ -16°; IR (Neat) 3468 (OH) 1454, 829, 733, 693 cm^{-1} (aromatic); ^1H NMR (100 MHz, $CDCl_3$): δ 7.2-7.6 (aromatic protons).^{13}C NMR($CDCl_3$, 300MHz) 68.78, 70.83, 73.68, 74.06, 74.65, 74.94, 75.16, 75.57, 79.62, 102.54 (C-1), 114.27,127.63, 127.72, 127.88, 128.12, 128.27, 128.29, 128.37, 131.92, 137.44, 137.91, 138.40.

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-galactopyranoside (2):

A mixture of **7** (323 mg, 0.67 mmol), **6** (150 mg, 0.27 mmol) and MS4A (473 mg) in dichloromethane (7 mL) was stirred at room temperature under nitrogen for 24 h. It was cooled to -35 °C and *N*-iodosuccinimide (301.3 mg) was added followed by dropwise addition of trifluoromethane solphonic acid (23.9 μL) in dichloromethane to the reaction mixture under stirring. The progress of the reaction was monitored by TLC and after 40 minutes it was quenched with triethylamine. The reaction mixture was filtered and washed with dichloromethane. The organic layer was washed with saturated $NaHCO_3$ solution followed by

treatment with 10% sodium thiosulfate solution. It was dried (Na_2SO_4) and concentrated. Chromatographic purification over silica gel column with eluent dichloromethane : methanol (99.4 : 0.6) afforded **2** (150 mg, 58.9%) as a syrupy material. $[\alpha]_D^{30}$-5.2° (c=1, $CHCl_3$); IR (Neat): 1715 (ester), 1728 (imide), 1499, 1451, 654, 722 cm^{-1} (aromatic); ^1H NMR (300 MHz, $CDCl_3$): δ 1.84, 2.00, 2.03 (9H,3s, 3 Ac), 5.09 (t, $J_{2c,3b}=J_{3b,4b}=10.95$ 5 Hz, H-3b), 5.72 (d, 1H, $J_{1b,2b}=7.2$ Hz, H-1b), 5.878 (t, 1H, $J_{4c,5c}=8.9$ Hz, H-4b and 6.97 - 7.58, 24H (m, 4Ph, phthaloyl-H); ^{13} C NMR($CDCl_3$, 300MHz) 20.37, 20.58, 22.94, 55.09(C-2, GlcN), 68.14, 68.91, 69.11, 70.54, 70.67, 73.44, 73.73, 74.69, 75.94, 78.28, 81.62, 99.07, 102.5,123.40, 126.82, 127.42, 127.49, 127.74, 127.80, 127.93, 128.08, 128.11, 128.22, 128.37, 128.42, 128.56, 128.77, 131.07, 133.99, 137.18, 138.55, 138.75, 167.44, 169.52, 169.98, 170.53. Anal. Calcd. for $C_{54}H_{55}O_{15}N$: C, 67.7, H, 5.79; N, 1.46. Found: C, 67.5; H, 5.77; N, 1.43. FAB-MS (Electron spray) : m/z (M+ Na) 981.

Synthesis of 2 by Konigs Knorr Procedure :

A mixture of compound **6** (972 mg, 1.8 mmol), silver perchlorate (368 mg), silver carbonate (992 mg) and MS4A (1.8g) in dichloromethane (10 ml) was stirred under nitrogen for 24 h in the dark. Compound **8** (1.58 g) and MS 4A (1.7 g) were taken in dichloromethane (10 mL) and stirred for 24 h. The mixture was added to the flask containing the acceptor at 0°C and stirred for 24 h in the dark at room temp. The reaction was monitored by TLC. The reaction mixture was filtered, the precipitate was washed several times with dichloromethane and washings were pooled, washed with water and dried (Na_2SO_4) and then concentrated. It was

subjected to flash chromatography. Elution with ethylacetate : petroleum ether (1:3) yielded **2** as a syrupy material (1.7 g, 88.8%). IR and NMR data is in good agreement with those obtained for the same compound prepared by thiol activation.

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