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A Novel Mimic of the Sialyl Lewis x Determinant

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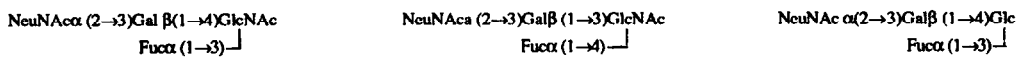
Abstract: The syntheses of two novel disaccharides are described which block the interaction of the lectin domain of E-selectin with its glycosylated ligands on neutrophils.

The interaction of tumour cells and neutrophils with the activated endothelium of blood vessels is the initial event which allows them to pass from the circulation into the tissue. The process is mediated by several factors, one of which is the interaction of the lectin domain in E-selectin with its glycosylated ligands on the circulating cells[1]. Blockade of this interaction might represent a possible therapy for; (i) reperfusion injury, ARDS and Crohn's disease in which excessive accumulations of neutrophils are found in the inflamed tissue, (ii) prevention of metastasis of certain types of tumour.

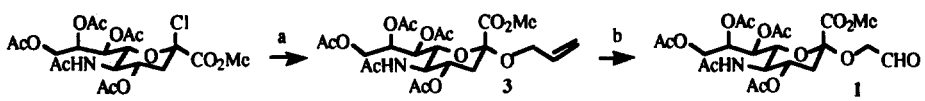
Figure 1



sialyl Lewis x *sialyl Lewis A* *3'sialyl-3-fucosyl lactose*

sLex[2], sLea[3] and 3'-sialyl-3-fucosyl lactose[4] (Figure 1) all have been shown to bind to the E-selectin. It would appear that they possess a common epitope[4,5], formed by the fucose, sialic acid and by parts of the galactose residue which interacts with the receptor. In order to interfere with this process using simpler molecules, we decided to link the C-4 position of fucose to the C-2 position of sialic acid by a 6 atom chain, hoping to create a mimic of this common domain. The chain would serve not only to maintain the relative distance and orientation of these residues but would also result in the introduction of functionality with which we could represent the galactose.

Figure 2

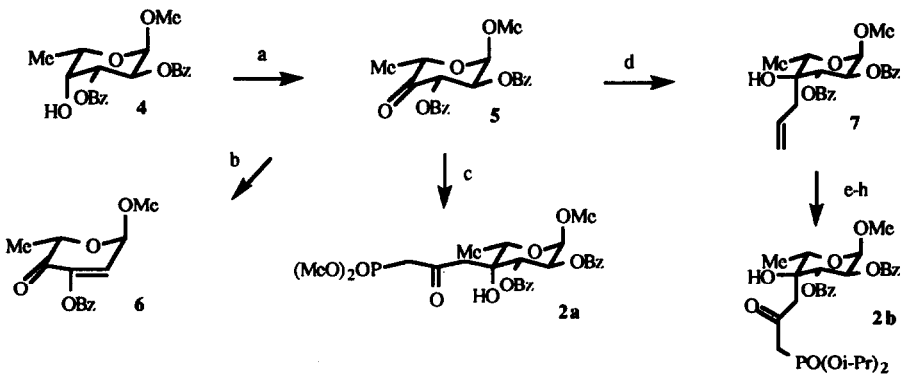


a) Silver salicylate/ CH_2=$CHCH_2OH$/ 4A sieves 95% b) O_3/ MeOH 0°C then Me_2S 60%.

The linker was constructed by coupling a sialic acid aldehyde **1** to a fucosyl ketophosphonate **2a**. The sialic acid aldehyde was prepared in two steps from methyl tetra-O-acetyl-α2-chloro-N-acetylneuraminidate[6] (Figure 2). Reaction with allyl alcohol in the presence of silver salicylate[6] and powdered 4A sieves gave the α-allyl glycoside **3** in 95% yield. Ozonolysis in methanol at 0°C followed by a dimethyl sulphide workup gave the sialic acid aldehyde **1** in 55% yield after chromatography on silica.

The starting material for the fucose ketophosphonate **2a** was methyl 2,3-di-O-benzoyl-α-L-fucopyranoside **4**[7] (Figure 3). Oxidation with pyridinium chlorochromate and 4A sieves gave the fucosyl ketone **5** in 80% yield. Elaboration of **5** to the desired ketophosphonate proved difficult because of its sensitivity to base, namely the elimination of the 2-O benzoyl group to the enone **6**. It was therefore decided to add a 3-carbon nucleophile to the *Si* face of **5** under Lewis acid conditions and then to develop this into an equatorial ketophosphonate group. Although addition of allyl silane using $TiCl_4$[8] or $SnCl_4$ in CH_2Cl_2 at -78°C gave excellent yields of a homoallylic alcohol **7**, NOE studies[9] showed that attack had occurred exclusively from the opposite face. Although the undesired isomer had been obtained, it was decided to take this through to the ketophosphonate **2b**. After much experimentation we found this could be achieved via epoxidation with MCPBA (92%), formation of a pair of bromohydrins with Li_2NiBr_4[10](98%), oxidation with PCC and 4A sieves (78%), and an Abusov reaction with triisopropylphosphite[11] at 80°C (39%) which yielded **2b**[12]. However, attempts to invert the 4-hydroxyl on either **7** or **2b** were unsuccessful. This forced us to re-examine basic nucleophilic additions to compound **5**.

Figure 3

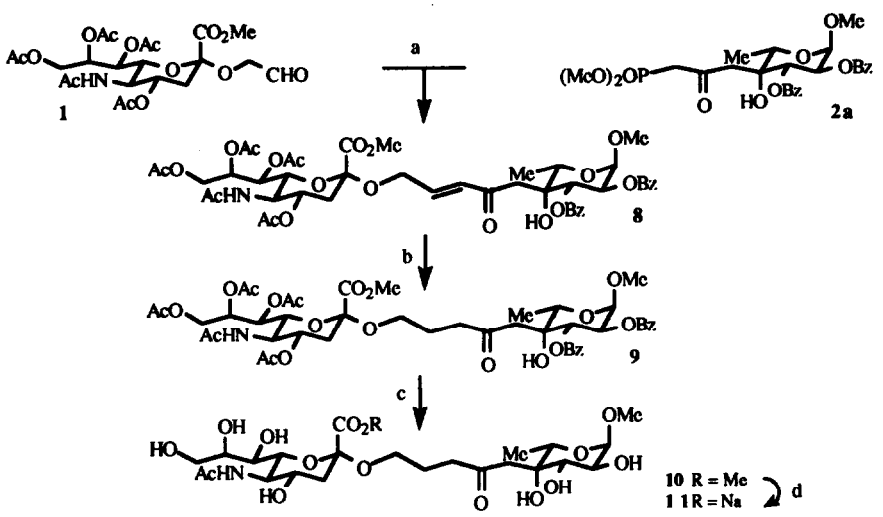


a. PCC CH_2Cl_2 4A sieves 12hr 80% b. Ph_3PCHCO_2Et PhH 20% c. (1) $CH_3COCH_2PO(OMe)_2$, 2nBuLi THF 0°C (2) $ClTi(Oi\text{-}Pr)_3$ -78°C (3) **5** -78°C 2hr 43% d. $CH_2{=}CHCH_2Si(Me)_3$ $TiCl_4$ CH_2Cl_2 -78°C 2hr 95% e. MCPBA CH_2Cl_2 48hr 92% f. Li_2NiBr_4 THF 2hr 98% g. PCC CH_2Cl_2 4A sieves 12hr 78% h. $P(Oi\text{-}Pr)_3$ 80°C 2hr 39%.

Addition of the lithium dianion of dimethyl-2-oxopropyl phosphonate[13] gave a 15% yield of the desired equatorial fucose ketophosphonate **2a** with no contaminating axial byproduct. The stereochemistry at C-4 was assigned from the NOE enhancements of 3% and 5% observed from the axial H-3 and H-5 protons of the fucose ring to the CH_2 protons between the ketone and C-4. No enhancement was observed between the CH_2 group and H-2. Encouraged by this result, we examined the effects of metal additives to modify the basicity of the nucleophile. Conducting the addition in the presence of $CeCl_3$[14]gave a 29% yield of **2a**, however the best yield was obtained using $TiCl(Oi\text{-}Pr)_3$[15]. Thus addition of one equivalent of **5** to two equivalents of the lithium dianion of dimethyl-2-oxopropyl phosphonate with 1.2 equivalents of chlorotriisopropoxy titanium in THF at -78°C gave only the desired equatorial fucose ketophosphonate **2a** in 43% yield.

The Wadsworth-Emmons reaction of sialic acid-aldehyde **1** with the fucosyl ketophosphonate **2a** (Figure 4) proved unexpectedly demanding owing to the base sensitivity of the aldehyde component and of the disaccharide enone **8**. Caesium carbonate in t-butanol[16] was much superior to any of fifteen other conditions tried for this reaction. The reaction of **1** with **2a** gave an optimal 58% yield of **8** after 2hr. The enone was quantitatively converted to the ketone **9** by reduction with hydrogen over a 10% palladium on charcoal catalyst. The alcohol protecting groups were removed with a catalytic amount of sodium in methanol to give the methyl ester **10** in 65% yield. **10** was quantitatively saponified to the sodium carboxylate salt **11** with sodium hydroxide in water.

Figure 4



a. Cs_2CO_3 t-BuOH 2hr 57% b. H_2 10% Pd-C MeOH 100% c. NaOMe (cat) MeOH 12hr 65% d. NaOH,H_2O 100%.

Both methyl ester **10** and the sodium carboxylate salt **11** blocked the adhesion of an sLex expressing myeloid cell line U937 to activated endothelial cell cultures at twenty-five and thirty-fold higher concentrations than sLex. Full details of the biological results will be published elsewhere.

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12. In the presence of Cs_2CO_3 and t-butanol **2b** also reacted with aldehyde **1** to give a 38% yield of the 4-fucosyl hydroxy epimer of disaccharide **8**.

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Selected analytical data on compounds **2b** and **10**. The following abbreviations are used: S for sialic acid, F for fucose and L for the linker. The numbering system in the linker runs away from the fucosyl ring.

Compound **2b:** ^{1}H nmr: ($CDCl_3$) δ 7.99-7.87 (4H, 2d, 2 x OBz); 7.52-7.26 (6H, m, 2 x OBz); 5.82(H, d, J = 10.2 Hz, H-3); 5.49 (H, dd, J = 3.8 and 10.2 Hz, H-2); 5.14 (H, d, J = 3.8 Hz, H-1); 4.24 (H, q, J = 6.4 Hz, H-5); 4.10 (H, br s, OH); 3.71 (3H, d, J = 11.3 Hz, MeOP); 3.64 (3H, d, J = 11.3 Hz, MeOP); 3.41 (3H, s, F1-OMe); 3.10-2.99 (2H, dd, J = 5.5 and 22.6 Hz, $(MeO)_2OPCH_2CO$-); 2.86 (2H, s, $COCH_2$C-4); 1.30 (3H, d, J = 6.4 Hz). ^{13}C nmr: ($CDCl_3$) δ 200.6 (-CO-), 166.2, 166.0 (2 x OCOPh), 133.3, 133.3, 129.8, 129.7, 129.3, 129.2, 128.4, 128.2 (2 x Ph); 97.0 (C-1), 75.1 (C-4), 72.9, 70.6, 67.8, 60.3 C1-OCH_3), 53.0, 52.9 ($P(OCH_3)_2$), 46.8 ($COCH_2$C-4), 43.58 and 41.6 (PCH_2CO), 13.5 (C-6).
High resolution mass spectrum: $C_{26}H_{31}O_{11}P$ [M+H]+ calc.551.16823; found 551.16769.

Compound **10**: ^{1}H nmr: (D_2O) δ 4.84 (H, d, F-1); 4.07 (H, q, J = 6.4 Hz, F-5); 3.83 (3H, s, S-OMe); 3.82-3.39 (11H, m, F-2, F-3, S-4, S-5, S-6, S-7, S-8, 2 x S-9, 2 x L-5); 3.36 (3H, s, F-OMe); 2.87 (H, d, J = 16.4 Hz, L-1); 2.66-2.59 (4H, m, L-1', 2 x L-3, S-3e); 1.99 (3H, s, NHAc); 1.87-1.70 (3H, m, 2 x L-4, S-3a); 1.12 (3H, d, J = 6.4 Hz, F-6). ^{13}C nmr: (D_2O) δ 213.5 (L-2), 175.2 (S-1), 178.4 (NHAc), 99.5 (S-2), 99.4 (F-1), 76.0 (F-4), 73.1 (S-8), 71.3 (F-2), 70.9 (F-3), 69.2 (S-7), 68.5 (S-6), 68.1 (F-5), 67.4 (S-4), 64.1 (L-5), 63.3 (S-9), 55.3 (F-OMe), 53.6 (S-OMe), 52.0 (S-5), 45.5 (L-1), 40.6 (L-3), 39.4 (S-3), 23.2 (L-4), 22.3 (NHAc), 13.0 (F-6).
High resolution mass spectrum: $C_{24}H_{41}NO_{15}$ [M+Na]+ calc.606.23739; found 606.23763.

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