Stereocontrolled Synthesis of Sialyl Le^{x}, the Oligosaccharide Binding Ligand to ELAM-1 (Sialyl = *N*-acetylneuramin)

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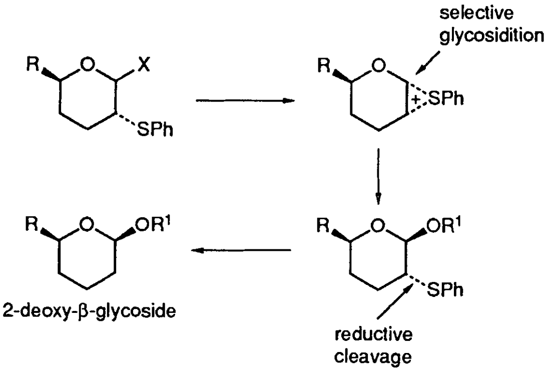
Sialyl Le^{x} **1** the oligosaccharide binding ligand to ELAM-1 is synthesized from building blocks **2**–**5** *via* a short route featuring the principle of a neighbouring PhS group as an auxiliary to facilitate and stereochemically control the formation of the desired glycoside bond in the target molecule.

Endothelial leukocyte adhesion molecule-1 (ELAM-1), found on blood vessel walls, serves an important role in the recruitment of leukocytes to inflammation sites.[1] Recent disclosures[2–4] identified sialyl Le^{x} **1** (Scheme 2), a terminal oligosaccharide fragment of membrane glycoproteins and glycolipids, as the ligand recognized by ELAM-1. These reports dramatically added weight to the notion that carbohydrates play important roles in cellular recognition and physiological functions.[5] Furthermore, this carbohydrate fragment is highly expressed on the surface of tumour and embryonic cells.[6] The demonstration of sialyl Le^{x} **1** as the guiding moiety of leukocytes to sites of injury and its identification as a tumour cell marker coupled with difficulties associated with its isolation from natural sources, prompted us to undertake its chemical synthesis. Reported herein is a stereocontrolled and biologically efficient synthesis of the parent sialyl-Le^{x} **1**.[7]

Previous reports from these laboratories demonstrated the

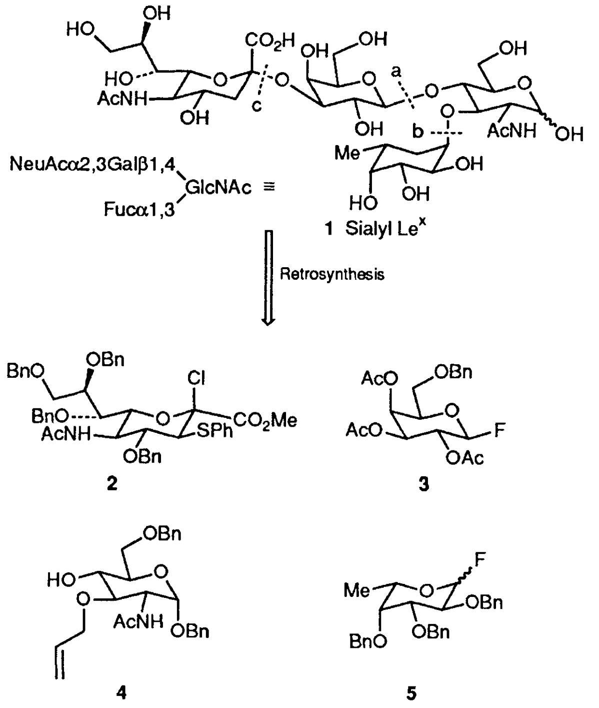


Scheme 1 Stereocontrolled construction of 2-deoxy glycosides

utilization of the PhS group as an auxiliary to facilitate and stereochemically control the formation of α- and β-2-deoxyglycosides[8] (see Scheme 1) such as those present in gangliosides and other sialyl derivatives.[9] Scheme 2 outlines a retrosynthetic analysis of sialyl Le^x **1** based on this principle, and which defines compounds **2–5** as the key intermediates required for its synthesis. The order of bond formation in the synthetic direction was chosen to be a→b→c for optimum efficiency and protecting group manipulation.

The requisite fragments **2**,† **3**,‡ **4**,§ and **5**[10] were synthesized from sialic acid,[11] D-galactose, *N*-acetyl-D-glucosamine, and L-fucose, respectively. Noteworthy is the new, four step procedure, for the synthesis of sialic acid derivative **2** from sialic acid methyl ester.

Scheme 3 summarizes the construction of sialyl Le^x **1** starting with the coupling of intermediates **3** and **4**. Thus, reaction of glycosyl fluoride **3** with glucosamine derivative **4** under the influence of $AgClO_4$–$SnCl_2$[12,13] resulted in the stereospecific formation of β-glycoside **6** in 63% yield. Selective removal of the allyl protecting group from **6** with $RuH_2(PPh_3)_4$–H^+ led to disaccharide **7** (85%) which reacted with glycosyl fluoride **5** under the above mentioned conditions affording trisaccharide **8** as the only detectable product and in 85% yield. Deacetylation of **8** under basic conditions gave triol **9** (95% yield) which reacted with the sialic acid derivative **2** in the presence of $Hg(CN)_2$–$HgBr_2$ in a remarkably regio- and stereo-specific manner, furnishing tetrasaccharide **10** in 63% yield (based on consumed triol **9**).[14,15] Exposure of **10** to Ph_3SnH–AIBN in toluene at 130 °C led to reductive desulphurization and formation of δ-lactone **11**¶ as the major product together with its 4′-regioisomer (**11′**) (77% total yield, *ca*, 3.5 : 1 by ^1H NMR).|| Alkaline hydrolysis of the mixture **11** + **11′** (LiOH, aqueous dioxane) led, in essentially quantitative yield, to hydroxy acid **12**. Finally, catalytic



Scheme 2 Retrosynthetic analysis of sialyl Le^x **1**. Order of bond formation: a→b→c (see structure 1).

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† This compound was prepared from the methyl ester of sialic acid in *ca*. 32% overall yield by the following sequence: (*i*) excess Ac_2O, H_2SO_4 cat., 25 °C, 20 h; (*ii*) 4 equiv. 1 mol dm^{-3} NaOH, H_2O, 25 °C, 2 h; (*iii*) 9 equiv. NaOH, 9 equiv. $PhCH_2Br$, $Bu^n{}_4NI$ cat., dimethylformamide (DMF), 60 °C, 3 h, quench with MeOH at 25 °C and acidify to pH 2 with 1 mol dm^{-3} aq HCl; (*iv*) 2.5 equiv. PhSCl, CH_2Cl_2, 25 °C, 16 h. [See ref. 14(*a*)].

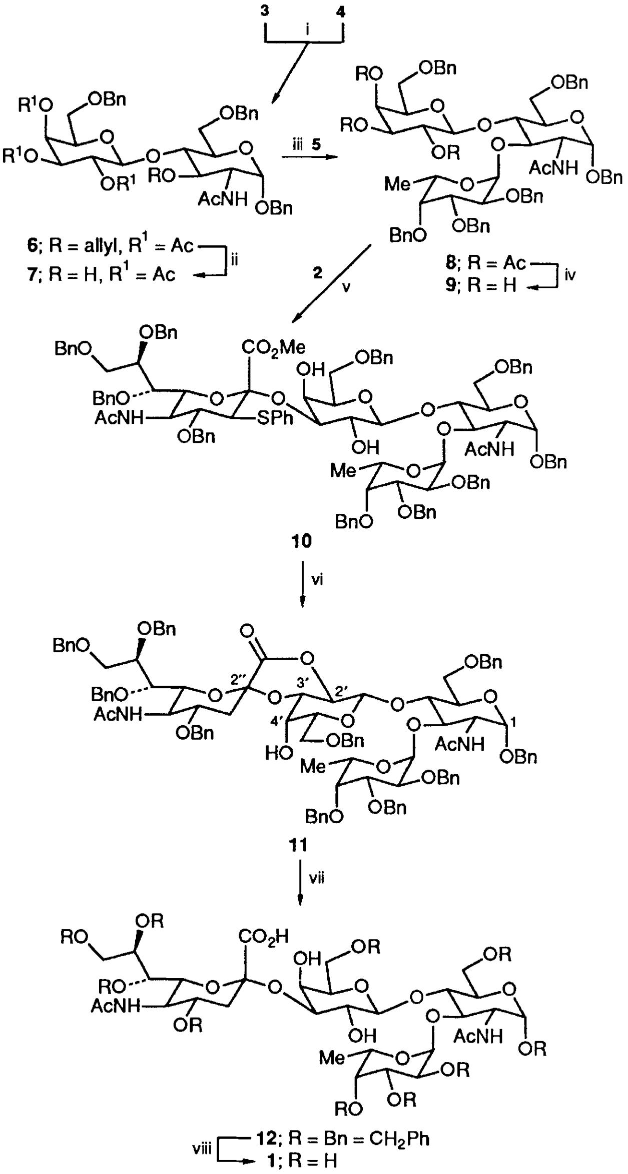
‡ This compound was prepared from the phenylthio β-galactose in *ca*. 37% overall yield by the following sequence: (i) 3 equiv. $PhCH(OMe)_2$, camphosulphonic acid cat., tetrahydrofuran (THF), 50 °C, 15 h; (*ii*) 2 equiv. Ac_2O, 1.4 equiv. Et_3N, 4-dimethylaminopyridine (DMAP) cat., CH_2Cl_2, 0→25 °C, 1 h; (*iii*) 10 equiv. $NaCNBH_3$, ethereal HCl, 3 Å molecular sieves (MS), THF, 8 h, 25 °C; (*iv*) 2 equiv. Ac_2O, 1.4 equiv. Et_3N, DMAP cat., CH_2Cl_2, 0→25 °C, 1 h; (*v*) excess HF–pyridine, 1.5 equiv. *N*-bromosuccinimide (NBS), CH_2Cl_2, −78 to 25 °C, 5 h.

§ This compound was prepared from the *N*-acetylglucosamine in *ca*. 34% overall yield by the following sequence: (*i*) HCl satd. $PhCH_2OH$, 100 °C, 1 h; (*ii*) 3 equiv. $PhCH(OMe)_2$, camphorsulphonic acid cat., THF, 50 °C, 1 h; (*iii*) 1.5 equiv. NaH, 2 equiv. allyl bromide, $Bu^n{}_4NI$ cat., DMF, 0 to 25 °C, 3 h; (*iv*) 15 equiv. $NaCNBH_3$, ethereal HCl, 3 Å MS, CH_2Cl_2, 0.5 h, 25 °C.

¶ *Selected physical properties* for **11**: R_f = 0.19 (silica, 30% ethyl acetate in benzene); $[\alpha]^{25}{}_D$ +0.22° (*c* 0.65, $CHCl_3$); IR ($CHCl_3$) ν_{max}/cm^{-1} 3423m, 3344br, 3009s, 2930s, 1758s, 1718s, 1683s, 1455s and 1094s; ^1H NMR (500 MHz, C_6D_6) δ 7.69–6.93 (m, 50 H, aromatic), 5.61 (d, *J* 3.4 Hz, 1 H, H-1 fuc), 5.35 (dd, *J* 7.8, 10.5 Hz, 1 H, H-2′), 5.28 (d, *J* 10.5 Hz, 1 H, NH), 5.06 (d, *J* 3.1 Hz, 1 H, H-1), 4.96–4.84 (m, 5 H, H-1′, CH_2Ph), 4.78–4.66 (m, 2 H, CHO, CH_2Ph), 4.56–4.27 (m, 13 H, H-2 fuc, CHO, CHN, CH_2Ph), 4.24–3.81 (m, 20 H, H-2, 2 H-6′, H-4″, H-5 fuc, CHO, CH_2Ph), 3.75 (d, *J* 1.9 Hz, 1 H, H-4′), 3.62 (d, *J* 10.7 Hz, 1 H, NH), 3.52 (t, *J* 6.7 and 6.6 Hz, 1 H, H-5′), 3.43 (d, *J* 1.0 Hz, 1 H, CHO), 3.05 (dd, *J* 10.5 and 2.7 Hz, 1 H, H-3′), 2.73 (dd, *J* 13.4 and 5.1 Hz, 1 H, H-3″eq), 1.93 (dd, *J* 13.4 and 11.3 Hz, 1 H, H-3″ax), 1.49 (d, *J* 6.4 Hz, 3 H, H-6 fuc), 1.42 (s, 3 H, NAc) and 1.41 (s, 3 H, NAc); ^{13}C NMR (125 MHz, C_6D_6) δ 170.6, 169.4, 165.5, 139.5, 139.4, 139.3, 139.1, 138.9, 138.8, 138.5, 138.1, 137.6, 136.4, 130.6, 130.4, 129.2, 129.1, 128.9, 128.8, 128.65, 128.61, 128.55, 128.51, 128.48, 128.3, 128.2, 127.8, 127.3, 117.7, 107.6, 101.1, 99.3, 98.0, 97.3, 96.7, 79.5, 79.4, 78.9, 78.1, 76.7, 76.5, 75.7, 75.6, 74.8, 74.7, 74.6, 74.3, 74.0, 73.9, 73.2, 72.9, 72.4, 72.0, 71.4, 69.8, 68.4, 66.9, 66.6, 61.2, 59.5, 59.2, 55.4, 54.1, 49.9, 43.2, 33.9, 30.1, 27.0, 23.2, 20.5, 19.5 and 16.8; HRMS (FAB) Calcd. for $C_{101}H_{110}O_{22}N_2Cs$ (M+Cs): 1835.6605, found: 1835.6691.

For **1**: (mixture of C-1 anomers, *ca*. 3 : 2, α : β): R_f = 0.32 (silica, butan-1-ol : ethanol : water, 2 : 1 : 1); $[\alpha]^{25}{}_D$ +5.8° (*c* 0.24, MeOH); IR (KBr disc) ν_{max}/cm^{-1} 3400vb, 2955s, 1561s, 1413s, 1248s, 1145m, 1040m, 841s; ^1H NMR (500 MHz, D_2O) δ 5.10 (2 d, *J* 3.5 Hz each, H-1 fuc and H-1 α-anomer), 4.73 (d, *J* 8.0 Hz, H-1 β-anomer), 4.54 (dd, *J* 4.5 and 8.0 Hz, 1 H, CHO), 4.1 (dd, *J* 3.5 and 9.44 Hz, 1 H, C HO), 4.09 (dt, *J* 9.9 and 3.2 Hz, 1 H, CHO), 4.02–3.54 (m, 21 H, remaining CHO, CHN), 2.77 (dd, *J* 4.3 and 12.3 Hz, 1 H, H-3″ eq), 2.04 (s, 6 H, acetates), 1.80 (dd, *J* 12.3 and 12.3 Hz, 1 H, H-3″ax) and 1.18 (d, *J* 6.1 Hz, 3 H, H-6 fuc); HRMS (FAB) Calcd. for $C_{31}H_{52}O_{23}N_2Cs$ (M+Cs): 953.2015, found: 953.2079.

|| In addition to products **11** and **11′**, a mixture of δ-lactones (23%) containing the PhS group was also obtained and was converted to **11** and **11′** under the same Ph_3SnH–AIBN desulphurization conditions.



Scheme 3 Synthesis of sialyl Le^x **1**. *Reagents and conditions*: (i) 2.5 equiv. $AgClO_4$, 2.5 equiv. $SnCl_2$, 1.5 equiv. of **4**, 4 Å MS, CH_2Cl_2, 0 °C, 4 h, 63%; (ii) $H_2Ru(PPh_3)_4$ cat., EtOH, 95 °C, 1 h; then TsOH cat., MeOH, 25 °C, 2 h, 85%; (iii) 3 equiv. $AgClO_4$, 3 equiv. $SnCl_2$, 1.6 equiv. of **5**, 4 Å MS, Et_2O, 25 °C, 3 h, 85%; (iv) NaOMe cat., MeOH, 0 °C, 2 h, 95%; (v) 3 equiv. $Hg(CN)_2$, 1 equiv. $HgBr_2$, 1.7 equiv. of **9**, 4 Å MS, CCl_4, 40 °C, 48 h, 63% based on consumed **9**; (vi) 5 equiv. Ph_3SnH, AIBN cat., toluene, 130 °C, 4 h, 77% plus 23% of δ-lactones containing a PhS group; (vii) LiOH, H_2O, dioxane, 25 °C, 24 h, 100%; (viii) H_2, $Pd(OH)_2$ cat., MeOH, 25 °C, 48 h, 95%.

hydrogenolysis of the benzyl groups from **12** gave the desired product, sialyl Le^x **1**‖ which was purified by filtration through Sephadex (95% yield). The alternative finishing sequence involving hydrogenolysis of the benzyl groups from **11**, followed by hydrolysis of the lactone leading to **1**, was also successful (90% overall yield).

The described sequence renders sialyl Le^x **1**, the oligosaccharide binding ligand of ELAM-1, readily available in pure form for extensive biological investigations. Further chemical studies utilizing the present strategy, or modifications of it, may provide powerful biological tools and potential therapeutic agents in the area of inflammation and related disorders.[17]

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