Synthesis of Oligosaccharides Structurally Related to E-Selectin Ligands

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A tri- and a tetra-saccharide derived from methyl 3-fucosyl-β-lactoside with sulfate and *N*-acetylneuraminyl groups at the C-3 position of the galactose unit, analogues of sulfated Lewis X trisaccharide and sialyl Lewis X respectively, have been efficiently prepared from methyl β-lactoside in short syntheses.

Acute inflammatory response requires the recruitment and extravasation of circulating leukocytes at the site of injury or infection. It has been recently shown that the adhesion process involves the interaction between the cell-adhesion protein E-selectin, expressed on cytokine-stimulated endothelial cells, and a ligand displayed on the surface of leukocytes.[1] The ligand recognized by E-selectin was identified to be the tetrasaccharide sialyl Lewis X (**1**).[2] Since then several syntheses of sialyl Lewis X compounds have been reported.[3] More recently other analogues, including **2**,[4] derived from the substitution of the *N*-acetylglucosamine unit for a glucose at the reducing end of **1**, and the sulfated tetrasaccharide **3**,[5] have been shown to be recognized by E-selectin with similar affinity. It has been postulated[6] that the binding domain of **1** in the interaction with E-selectin comes from the carboxylate of the *N*-acetylneuraminic residue and the galactose and fucose residues, while the *N*-acetylglucosamine moiety does not seem to play a relevant role. Owing to the interest of developing a large-scale synthesis of active compounds which can be potentially useful as new antiinflammatory agents, we now report a practical synthesis of the tetrasaccharide **4** (namely, the methyl glycoside of **2**[7]) and the terminal sulfated trisaccharide **5**, as potential ligands in which the less important *N*-acetylglucosamine unit is replaced by glucose. Whereas the synthesis of **1**[3] and **3**[8] requires cumbersome multistep preparations of *N*-acetyllactosaminyl fragments, **4** and **5** can be obtained from the readily available disaccharide, lactose.

The synthesis of **4** (Scheme 1) was carried out in six steps starting from methyl 3′,4′-*O*-isopropylidene-β-lactoside[9] **6**. Partial benzylation[10] of **6** gave as main product, the expected tetrabenzyl derivative **7**† having free the less reactive hydroxy at C-3. α-L-Fucosylation with **8**[11] promoted by mercury(II) bromide gave the protected trisaccharide **9** (82%, δ 5.63, $J_{1',2'}$ 4.0 Hz, H-1′), which was treated with 80% acetic acid to afford diol **10**. The sialylation on **10** was performed with the phosphite donor **11**[12] using trimethylsilyl triflate as catalyst to give regio- and stereo-specifically the tetrasaccharide derivative **13** in 36% yield (80% based upon consumed **10**). The sialylation with donor **12**[13] under similar conditions gave lower yield of **13** (20%) and was plagued by decomposition of **12**. Hydrogenolysis of **13** led to **14** which after purification by silica-gel column chromatography (AcOEt–MeOH, 1 : 1) and treatment with NaOMe afforded the targeted **4** which was then lyophilised to obtain an amorphous white powder. Hydrolysis of the methyl ester probably occurred during the work-up after methanolysis. The structure of **4** was fully characterized by NMR using a HMQC (Heteronuclear Multiple Quantum Correlation) experiment.

Trisaccharide **5** was easily obtained from the known intermediate **15**, which is prepared in four steps from methyl β-lactoside using highly selective reactions.[14] Sulfation of **15** gave **16** which after silica gel column chromatographic purification (AcOEt–hexane, 1 : 2) was hydrogenolysed and the resulting **5** was purified by silica-gel column chromatography (AcOEt–MeOH, 1 : 1) and then lyophilised to obtain an amorphous white solid.

In conclusion, the tri- and tetra-saccharides **5** and **4**, potential ligands of E-selectin, are efficiently prepared from methyl β-lactoside in six and seven steps respectively, the synthetic sequence constituting an effective route for the large-scale preparation of these compounds.

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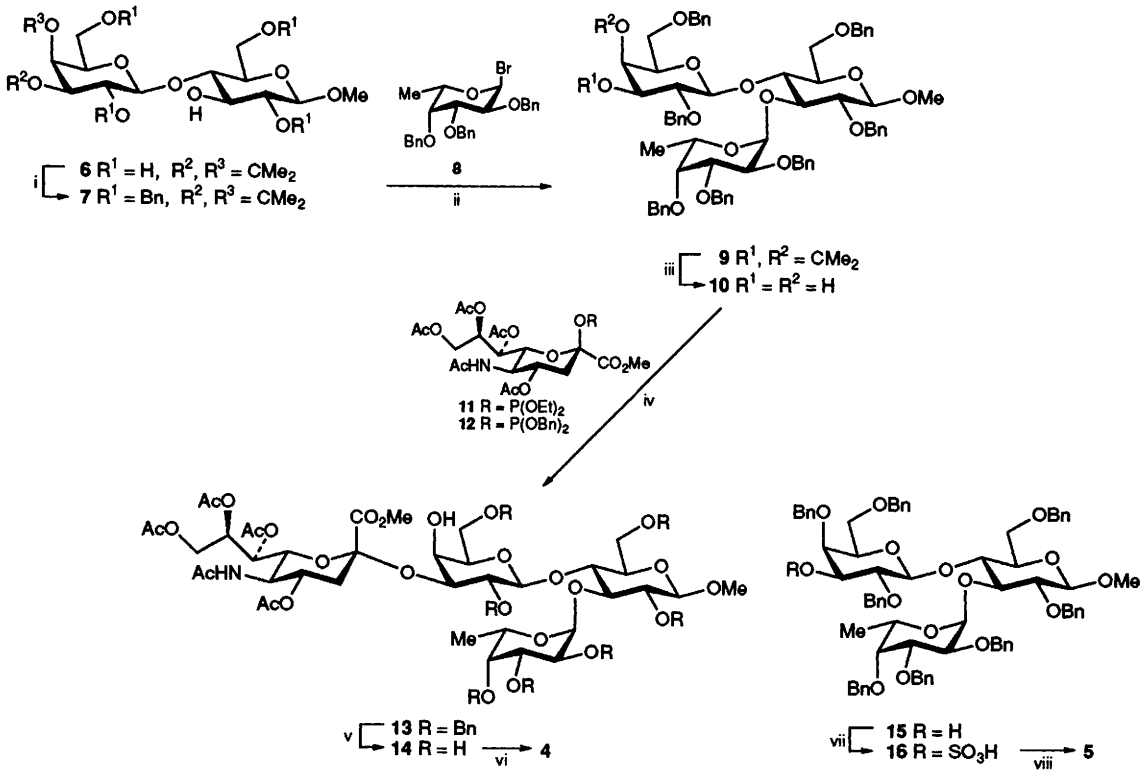
Footnote

† All new compounds gave satisfactory elemental analyses and depicted expected ^{13}C NMR data. *Physical and selected ^{1}H NMR data*: (*J*/Hz) **7**: $[\alpha]_D$ + 15 (*c* 0.9, $CHCl_3$). **9**: $[\alpha]_D$ −26 (*c* 1, $CHCl_3$). **10**: $[\alpha]_D$ −20.5 (*c* 1, $CHCl_3$), **13**: mp 85 °C, $[\alpha]_D$ −27.9 (*c* 0.55, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (d, 3H, *J* 7.0, CH_3, Fuc), 1.88 (s, 3H, NHAc, Neu), 1.90 (s, 3H, OAc), 1.96 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.45 (dd, 1H, $J_{3e,4}$ 4.7, $J_{3e,3a}$ 8.0, H-3e, Neu), 2.57 (br, 1H, D_2O exchangeable, OH), 3.46 (s, 3H, OCH_3), 3.79 (s, 3H, CO_2CH_3), 4.23 (d, 1H, *J* 8.0, H-1, Glc), 4.52 (d, 1H, *J* 8.0, H-1, Gal), 3.3–4.95 (m, 38 H), 5.1 (d, 1H, NH, N*H*Ac), 5.2 (d, 1H, *J* 4.0, H-1, Fuc), 7.0–7.4 (m, 35 H, ArH). **14**: mp 179 °C, $[\alpha]_D$ −28.5 (*c* 0.4, MeOH). **4**: mp 230 °C, $[\alpha]_D$ −31.4 (*c* 0.5, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 1.19 (d, 3H, *J* 7.0, CH_3, Fuc), 1.80 (t, 1H, *J* 12.3, H-3a, Neu), 2.04 (s, 3H, NHAc, Neu), 2.78 (dd, 1H, $J_{3e,4}$ 4.7 $J_{3e,3a}$ 12.3 H-3e, Neu), 3.58 (s, 3H, OCH_3), 3.94 (br d, $J_{3,4}$ 3.0, $J_{4,5}$ <1, H-4, Gal), 4.15 (dd, 1H, $J_{3,4}$ 3.0, $J_{2,3}$ 10.0, H-3, Gal), 4.40 (d, 1H, *J* 8.0, H-1, Glu), 4.50 (d, 1H, *J* 8.0, H-1, Gal), 4.79 (q, 1H, *J* 6.5, H-5, Fuc), 5.43 (d, 1H, *J* 4.0, H-1, Fuc). **16**: $[\alpha]_D$ −31 (*c* 0.5, MeOH), ^{1}H NMR (200 MHz, CD_3OD): δ 1.13 (d, 3H, *J* 7.0, CH_3, Fuc), 3.30 (s,



Scheme 1 *Reagents and conditions*: i, excess BnBr(Bn=CH_2Ph), benzene–NaOH (aq.) (20%), NBu^n_4Br, 25 °C, 24 h, 30%; ii, 3.3 equiv. of **8**, 0.6 equiv. of $HgBr_2$, 4 Å molecular sieves, 3.8 equiv. of 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2, 25 ° C, 31 h, 82%; iii, AcOH–H_2O (4 : 1), 25 °C overnight, 76%; iv, 0.66 equiv. of **11**, $Me_3SiOSO_2CF_3$ (catalyst), MeCN, −40 °C, 1 h, 36% (80% based upon consumed **10**); v, H_2, 10% Pd–C, EtOH–AcOEt, 3 d, 92%; vi, NaOMe (1 mol dm^{-3}), MeOH, 25 °C, 1 h, 78%; vii, 20 equiv. of SO_3·pyridine complex, pyridine, 25 °C, 15 h, 82%; viii, H_2, 10% Pd–C, EtOH, 25 °C, 92%

3H, OCH_3), 5.5 (d, *J* 4.0, H-1, Fuc), 7.0–7.48 (m, 40 H, ArH). **5**: mp 240 °C (decomp.), $[\alpha]_D$ −29 (*c* 0.4, H_2O); ^1H NMR (500 MHz, D_2O) δ 1.19 (d, 3H, *J* 7.0, CH_3, Fuc), 3.57 (s, 3H, OCH_3), 4.28 (br, 1H, $J_{3,4}$ 2.5, $J_{4,5}$ <1, H-4, Gal), 4.32 (dd, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 2.5, H-3, Gal), 4.40 (d, 1H, *J* 8.0, H-1, Glu), 4.54 (d, 1H, *J* 8.0, H-1, Gal), 4.78 (q, 1H, *J* 6.5, H-5, Fuc), 5.45 (d, 1H, *J* 4.0, H-1, Fuc).

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