

COMBINED GLYCOMIMETIC AND MULTIVALENT STRATEGIES FOR THE DESIGN OF POTENT SELECTIN ANTAGONISTS

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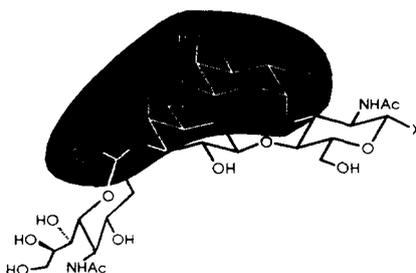
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Abstract: Stepwise large scale synthesis of 3'-sulfo-Lewis^X-Glc mimetic of the lead anti-inflammatory agent sialyl Lewis^X in a form suitable for copolymerization with acrylamide has been achieved. The resulting water-soluble copolyacrylamide showed inhibition of binding of both L- and E-selectins in the μ Molar range.
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The cascade of events leading to acute and chronic inflammation has its origin in the over recruitment of neutrophils at the sites of tissue injuries or infections.¹ The mechanism by which these events are initiated depends on cell adhesion molecules intrinsically present on leukocytes (L-selectins) or expressed on vascular endothelium (E-, P-selectins) and platelets (P-selectins) following the action of inflammatory factors.² Although there is some debate on the exact structures of the natural carbohydrate ligands responsible for these adhesions,³ sialyl Lewis^X (sLe^X), which is naturally present on cell surface glycoproteins and glycolipids of neutrophils, has been clearly established as a valid lead compound used by all the pharmaceutical industry. It is therefore expected that by blocking neutrophils adhesion by sLe^X or analogs, it would be possible to stop the adverse effects of inflammation.

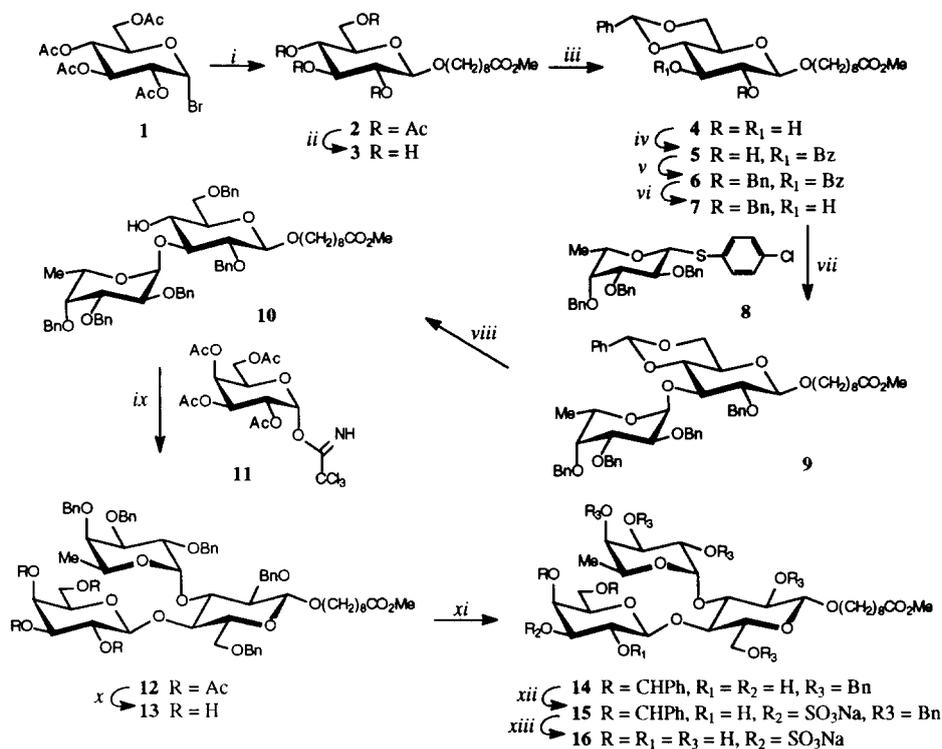
The numerous drawbacks of sLe^X as an anti-inflammatory agent reside in that it is an inhibitor of low affinity (IC₅₀ >1mM) and large scale synthesis is not viable for commercialization. To circumvent these drawbacks, a large number of simpler glycomimetics have been synthesized.⁴ One such promising candidate is an analog in which the sialic acid and the N-acetylglucosamine residues have been replaced by sulfate and glucose, respectively (3'-sulfo-Lewis^X-Glc).⁵



Structure of the natural selectin ligand Sialyl Lewis^X. The shaded area illustrates the E-selectin binding domain as determined by structure activity relationships.

The resulting analog is still however of low affinity ($IC_{50} < 1\text{mM}$). An alternative strategy which seems promising has been the design of small clusters of sLe^X .⁶ We describe herein a combined glycomimetic and multivalent strategy by synthesizing polymeric forms of the analog 3'-sulfo-Lewis^X-Glc.

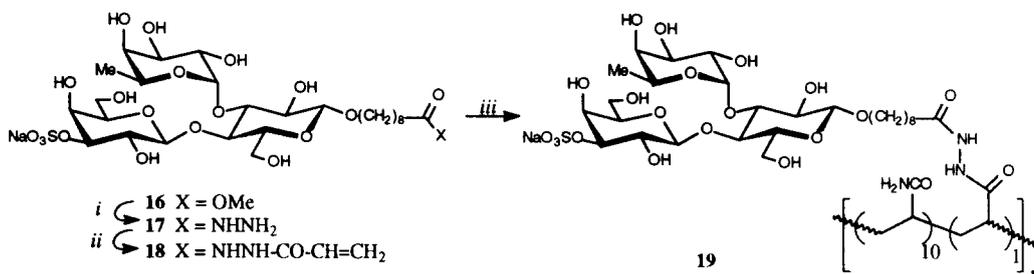
Acetobromoglucose (1) was glycosidated with 8-methoxycarboonyloctanol using silver oxide and silver trifluoromethanesulfonate (CH_2Cl_2) to provide β -glycoside 2 in 80% yield. Deacetylation of 2 under Zemplén conditions (NaOMe, MeOH) afforded tetraol 3 which was directly transformed into benzylidene acetal 4 in 85% yield ($PhCH(OMe)_2$, *p*-TsOH, CH_3CN). Regioselective benzylation of 4 (BzCl, 1.5 equiv, CH_2Cl_2 , pyridine, 3 h, $-50^\circ C$) provided 3-O-benzoate 5 (70%) which was benzylated at the remaining OH-2 using benzyl bromide and silver oxide as catalyst (CH_2Cl_2 , 72%). The resulting compound 6 (72%) was then deprotected at O-3 under Zemplén conditions to give 7 quantitatively (Scheme 1). Glycosylation of 7 with *p*-chlorophenyl 2,3,4-tri-O-benzyl-1-thio- β -L-fucopyranoside (8)⁷ using $CuBr_2$ -DMF complex and TEABr as promoters provided disaccharide 9 in 82% yield. Regioselective reductive ring opening of the benzylidene acetal of 9 ($NaBH_3CN$, THF, $HCl-Et_2O$) afforded 10 in 70% yield.⁸



Scheme 1. (i) Ag_2O , $AgOTf$, CH_2Cl_2 , 80%; (ii) NaOMe, MeOH; (iii) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN , 85%; (iv) BzCl, CH_2Cl_2 , C_5H_5N , $-50^\circ C$, Drierite, 70%; (v) BnBr, Ag_2O , CH_2Cl_2 , 72%; (vi) NaOMe, MeOH, quant.; (vii) 8, $CuBr_2$, TEABr, CH_2Cl_2 , DMF, Mol. Sieve, 82%; (viii) $NaBH_3CN$, THF, $HCl-Et_2O$, 70%; (ix) 11, $BF_3 \cdot Et_2O$, $Et_2O-CH_2Cl_2$, 1:1 (v/v), $-10^\circ C$, 67%; (x) NaOMe, MeOH, quant.; (xi) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN , 1 h, r.t., 83%; (xii) $SO_3 \cdot C_5H_5N$, 2 equiv, C_5H_5N , 5.5 h, $0-25^\circ C$ 80%; (xiii) H_2 , 20% $Pd(OH)_2$, MeOH, 86%.

The synthesis of the blocked trisaccharide **12** was then accomplished by dropwise addition of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2.5 eq.) to a stirred solution of disaccharide acceptor **10** and peracetylated galactosyl trichloroacetimidate **11**⁹ as glycosyl donor in a mixture of $\text{CH}_2\text{Cl}_2:\text{Et}_2\text{O}$ (1:1, v/v) at 0 °C (67% yield). The resulting trisaccharide **12** was transesterified under Zemplén conditions to give tetraol **13** which was directly converted into its 4,6-O-benzylidene derivative **14** ($\text{PhCH}(\text{OMe})_2$, p-TsOH, CH_3CN , 1 h, rt, 83%). Diol **14** was then selectively sulfated at the O-3' with SO_3 -pyridine complex (2.0 equiv) in pyridine (5.5 h, 0-25 °C, 80% yield). Only small amounts (7%) of 2',3'-O-bis-sulfate was obtained as side product. Complete removal of the remaining protecting groups, *i.e.* benzylidene acetal and benzyl ethers was effected in a single step using hydrogenolysis in the presence of 20% $\text{Pd}(\text{OH})_2$ to provide key precursor **16** in 86% yield.

Compound **16** was then transformed into N-acryloylated monomer **18** by hydrazinolysis of its methyl ester (5 equiv H_2NNH_2 , EtOH, reflux, 16 h) which provided intermediate hydrazide **17** quantitatively. Selective N-acryloylation of **17** ($\text{CH}_2=\text{CH-COCl}$, Na_2CO_3 , pH 8.5, $\text{MeOH}:\text{H}_2\text{O}$, 1:1 v/v, 0 °C, 16 h) afforded monomer **18** in 90% yield. Copolymerization of **18** with acrylamide (5 equiv) according to previously published procedure (ammonium persulfate cat., 95 °C, 15 min.)¹⁰ afforded water-soluble poly(acrylamide-co-3'-sulfo-Lewis^X-Glc) **19** in 56% yield after exhaustive dialysis against distilled water (MW cutoff 2 kDa) (Scheme 2). The copolymer was shown to have a molar ratio of acrylamide to 3'-sulfo-Lewis^X-Glc of 5.4:1 based on the integration of the ¹H-NMR (500 MHz, D_2O) signals attributed to the polymer backbone methine and methylene protons (δ 2.41 and 2.34, respectively) relative to that of the anomeric signal of the galactose residue at δ 4.54 ppm. Based on analogous glycopolymers and polyacrylamide standards (HPLC),¹⁰ copolymer **19** was shown to have an approximate molecular weight of ~150 kDa.



Scheme 2. (i) H_2NNH_2 5 equiv, EtOH, reflux, 16 h, quant.; (ii) $\text{CH}_2=\text{CH-COCl}$, Na_2CO_3 , pH 8.5, $\text{MeOH}:\text{H}_2\text{O}$, 1:1 v/v, 0 °C, 16 h, 90%; (iii) **18**, $\text{CH}_2=\text{CH-CONH}_2$, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, 95 °C, 15 min., then dialysis, 56%.

Preliminary inhibition of binding experiments of L- and E-selectin IgG chimera to 3'-sulfo-Lewis^X ceramide used as coating antigen¹¹ with **19** showed that copolyacrylamide **19** was very promising as inhibitor of both selectins with IC_{50} 's in the μMolar range. These results are similar to other inhibition results using glycopolymers containing solely sialic acid residues.^{4,12, 13} It is therefore possible to combine glycomimetic and multivalent strategies to design potent antagonists of cell adhesion molecules. Work is now in progress to synthesize even simpler multivalent sLe^X compounds. Although polymers such as **19** are unlikely to be of therapeutic value, it is obviously possible to use the above approach for targeting strategy. Parallel experiments with dendritic structures of 3'-sulfo-Lewis^X are being pursued.

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8. All new compounds exhibited consistent spectral ¹H- and ¹³C-NMR and MS data. Selected data: **16**: ¹H-NMR (500 MHz, D₂O), δ (ppm): 5.50 (d, 1H, J_{1,2}: 4.0 Hz, H-1'), 4.84 (q, 1H, J_{5,6}: 7.0 Hz, H-5), 4.59 (d, 1H, J_{1,2}: 8.0 Hz, H-1"), 4.52 (d, 1H, J_{1,2}: 8.5 Hz, H-1), 4.37 (dd, 1H, J_{3,4}: 3.5 Hz, J_{2,3}: 10.0 Hz, H-3"), 4.32 (bd, 1H, H-4"), 3.75 (s, 3H, OMe), 2.44 (t, 2H, J 7.5 Hz, CH₂CO₂), 1.23 (d, 3H, J_{5,6}: 7.0 Hz, H-6'); ¹³C-NMR: δ 101.6 (C-1), 100.9 (C-1"), 97.9 (C-1"); Ion Spray MS (neg.) calcd. for C₂₈H₄₉O₂₀S 737.3, found 737 (100%); **17**: 5.53 (d, 1H, J_{1,2}: 4.0 Hz, H-1', Fuc), 4.62 (d, 1H, J_{1,2}: 7.8 Hz, H-1", Gal), 4.54 (d, 1H, J_{1,2}: 8.1 Hz, H-1, Glc); ¹³C-NMR: δ 101.6 (C-1), 100.9 (C-1"), 97.9 (C-1"); Ion Spray MS (neg.) calcd. for C₂₇H₄₉N₂O₁₉S 737.3, found 737 (44.6%); **18**: 5.53 (d, 1H, J_{1,2}: 4.0 Hz, H-1', Fuc), 4.62 (d, 1H, J_{1,2}: 7.8 Hz, H-1", Gal), 4.54 (d, 1H, J_{1,2}: 8.1 Hz, H-1, Glc); ¹³C-NMR: δ 101.6 (C-1), 100.9 (C-1"), 97.9 (C-1"), 129.0, 126.7 (CH=CH₂); Ion Spray MS (neg.) calcd. for C₃₀H₅₁N₂O₂₀S 791.3, found 791 (100%); **19**: 5.53 (d, 1H, H-1'), 4.62 (d, 1H, H-1"), 4.54 (d, 1H, H-1), 2.20-2.45 (m, 9H, polymer backbone CH and H-α of spacer), 1.43-1.96 (m, 18H, polymer backbone CH₂ and spacer Hs).
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