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

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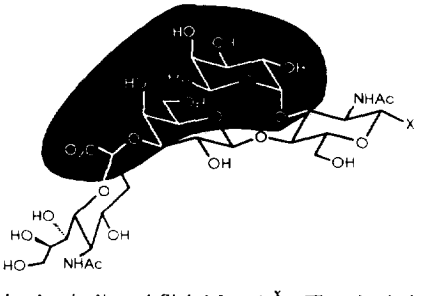
Abstract: Stepwise large scale synthesis of 3'-sulfo-Lewis^X-Glc mimetic of the lead anti-inflammatory agent sialyl Lewis^X in a form suitable for copolymerization with acrylamide has been achieved. The resulting water-soluble copolyacrylamide showed inhibition of binding of both L- and E-selectins in the μMolar range.

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.

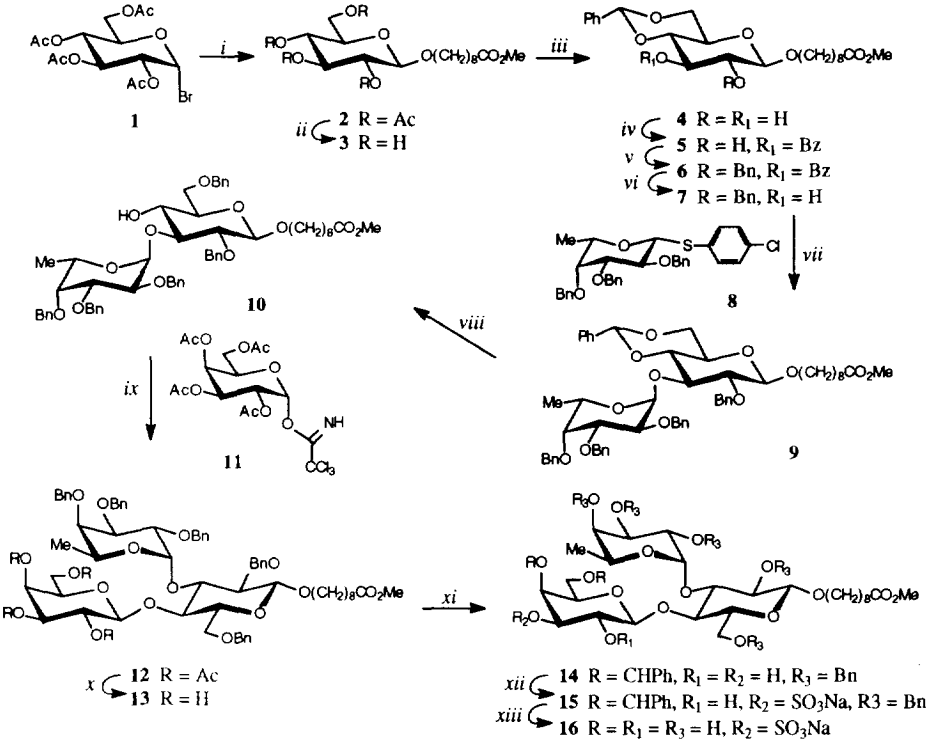
The numerous drawbacks of sLe^X as an anti-inflammatory agent reside in that it is an inhibitor of low affinity (IC_{50} >1mM) and large scale synthesis is not viable for commercialization. To circumvent these drawbacks, a large number of simpler glycomimetics have been synthesized.[4] One such promising candidate is an analog in which the sialic acid and the N-acetylglucosamine residues have been replaced by sulfate and glucose, respectively (3'-sulfo-Lewis^X-Glc).[5]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} < 1mM). 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.

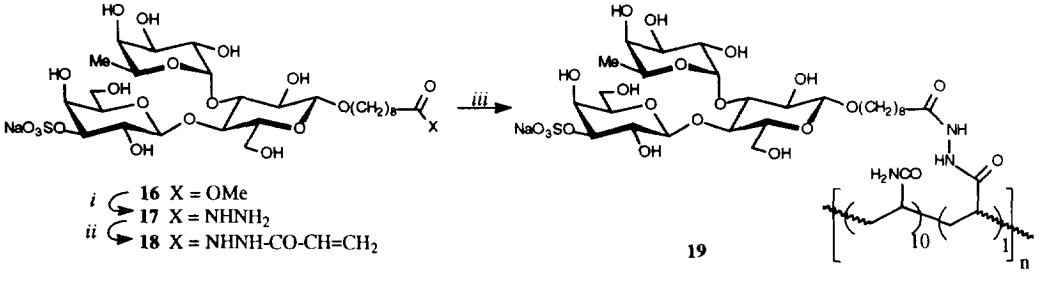
Acetobromoglucose (**1**) was glycosidated with 8-methoxycarbonyloctanol using silver oxide and silver trifluoromethanesulfonate (CH_2Cl_2) to provide β-glycoside **2** in 80% yield. Deacetylation of **2** under Zemplén conditions (NaOMe, MeOH) afforded tetraol **3** which was directly transformed into benzylidene acetal **4** in 85% yield ($PhCH(OMe)_2$, *p*-TsOH, CH_3CN). Regioselective benzoylation of **4** (BzCl, 1.5 equiv, CH_2Cl_2, pyridine, 3 h, -50 °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 promotors 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 °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•Et_2O, Et_2O-CH_2Cl_2, 1:1 (v/v), -10 °C, 67%; (*x*) NaOMe, MeOH, quant.; (*xi*) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN, 1 h, r.t., 83%; (*xii*) SO_3•C_5H_5N, 2 equiv, C_5H_5N, 5.5 h, 0-25 °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 BF_3•Et_2O (2.5 eq.) to a stirred solution of disaccharide acceptor **10** and peracetylated galactosyl trichloroacetimidate **11**[9] as glycosyl donor in a mixture of CH_2Cl_2:Et_2O (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** ($PhCH(OMe)_2$, 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% $Pd(OH)_2$ 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** (CH_2=CH-COCl, Na_2CO_3, pH 8.5, MeOH:H_2O, 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 ^{1}H-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*) CH_2=CH-COCl, Na_2CO_3, pH 8.5, MeOH:H_2O, 1:1 v/v, 0 °C, 16 h, 90%; (*iii*) 18, CH_2=CH-$CONH_2$, $(NH_4)_2S_2O_8$, 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 antigen[11] 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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References and Notes

1. (a) Springer, T. A; Larsky, L. A. *Nature*, **1991**, *349*, 196; (b) Paulson, J. C. In *Adhesion: Its Role in Inflammatory Diseases*; Harlan, J. M.; Liu, D. Y., Eds.; WH Freeman: New York, 1992, pp 19-42; (c) Larsky, L. A. *Ann. Rev. Biochem.* **1995**, *64*, 113; (d) Carlos, T. M.; Harlan, J. M. *Blood* **1994**, *84*, 2068.
2. Dasgupta, F.; Rao, B. N. N. *Exp. Opin. Invest. Drugs* **1994**, *3*, 709.
3. (a) Springer, T. A. *Cell* **1994**, *76*, 301; (b) Rosen, S. D.; Bertozzi, C. R. *Curr. Opin. Cell Biol.* **1994**, *6*, 663.
4. For a review on sialyl Lewis^x analogs and their biological activity see: Roy, R. In *Carbohydrates: Targets for Drug Design*; Witczak, Z. J., Ed.; Marcel Dekker, Inc.: New York, 1996, pp 82-134.
5. Brandley, B. K.; Kiso, M.; Abbas, S.; Nikrad, P.; Srivastava, O.; Foxall, C.; Oda, Y.; Hasegawa, A. *Glycobiology* **1993**, *3*, 633.
6. (a) DeFrees, S. A.; Kosch, W.; Way, W.; Paulson, J. C.; Sabesan, S.; Halcomb, R. L.; Huang, D.-H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 66; (b) Kretschmar, G.; Sprengard, U.; Kunz, H.; Bartnik, E.; Schmidt, W.; Toepfer, A.; Hörsch, B.; Krause, M.; Seiffge, D. *Tetrahedron* **1995**, *51*, 13015; (c) Zemlyanukhina, T. V.; Nifant'ev, N. E.; Shashkov, A. S.; Tsvetkov, Y. E.; Bovin, N. V. *Carbohydr. Lett.* **1995**, *1*, 277; (d) Spevak, W.; Foxall, C.; Charych, D. H.; Dasgupta, F.; Nagy, J. O. *J. Med. Chem.* **1996**, *39*, 1018.
7. Ippolito, R. M.; Haque, W.; Jiang, C.; Venot, A. P.; Nikrad, P. V.; Kashem, M. A.; Smith, R.; Srivastava, O. P. Patent, PTC/US, 1993/04909.
8. All new compounds exhibited consistent spectral ^{1}H- and ^{13}C-NMR and MS data. Selected data: **16**: ^{1}H-NMR (500 MHz, D_2O), 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, CH_2CO_2), 1.23 (d, 3H, $J_{5',6'}$ 7.0 Hz, H-6'); ^{13}C-NMR: δ 101.6 (C-1), 100.9 (C-1"), 97.9 (C-1"); Ion Spray MS (neg.) calcd. for $C_{28}H_{49}O_{20}S$ 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); ^{13}C-NMR: δ 101.6 (C-1), 100.9 (C-1"), 97.9 (C-1"); Ion Spray MS (neg) calcd. for $C_{27}H_{49}N_2O_{19}S$ 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); ^{13}C-NMR: δ 101.6 (C-1), 100.9 (C-1"), 97.9 (C-1"), 129.0, 126.7 (CH=CH_2); Ion Spray MS (neg.) calcd. for $C_{30}H_{51}N_2O_{20}S$ 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 CH_2 and spacer Hs).
9. Toepfer, A.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 5161.
10. Roy, R.; Tropper, F. D.; Romanowska, A. *Bioconjugate Chem.* **1992**, *3*, 256.
11. Foxall, C.; Watson, S. R.; Dowbenko, D.; Fennie, C.; Larsky, L.; Kiso, M.; Hasegawa, A.; Asa, D.; Brandley, B. K., *J. Cell Biol.* **1992**, *117*, 895.
12. (a) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1869; (b) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *ACS Symposium Series* **1994**, *560*, 104.
13. Roy, R. In *Modern Methods in Carbohydrate Synthesis*; Khan, S. H., O'Neil, R., Eds.; Harwood Academic: Amsterdam, 1996, pp 378-402.

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