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SYNTHESIS AND INHIBITORY EFFECT OF A SIALYL LEWIS X-ACRYLAMIDE HOMOPOLYMER AT PREVENTING CELL ADHESION

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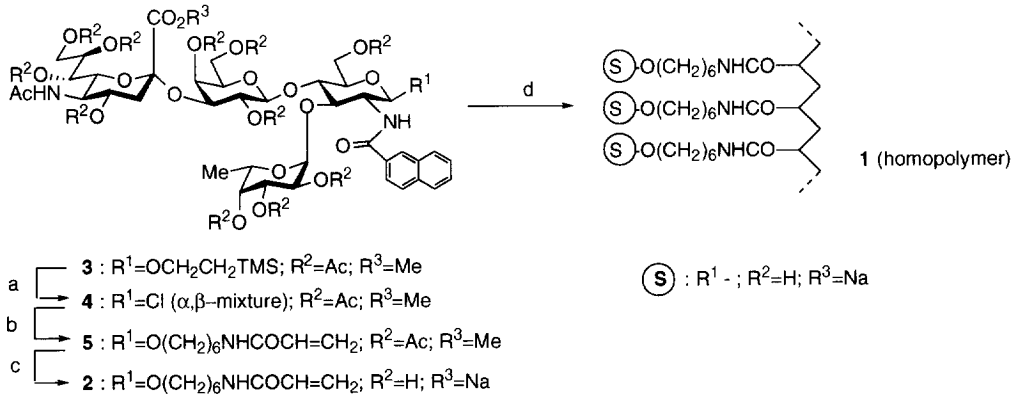
Abstract: A novel glycopolymer, a homopolymer of conjugated SLeX-acrylamide, was synthesized and evaluated for its inhibitory activity against E-selectin mediated cell adhesion both *in vitro* and *in vivo*. The homopolymer showed approximately 10-fold higher activity per SLeX unit than the corresponding SLeX-acrylamide monomer *in vitro* and was significantly more effective in an LTA-induced murine pleurisy model.

The tetrasaccharide sialyl Lewis X (SLeX) is known to be a ligand for E-selectin[1] and P-selectin.[2] SLeX exists at the nonreducing termini of several glycoproteins and glycolipids found on the surface of neutrophils and plays an important role in selectin-mediated cell adhesion during immune cell trafficking.[3] Many groups have investigated derivatizing the structure of SLeX including unnatural SLeX analogs with modified Gal, Fuc, NeuAc, and GlcN residues in an attempt to increase the potency of the native oligosaccharide.[4] The most potent result currently reported was obtained by *N*-naphthoylation of GlcN moiety.[4,5] The low affinity of carbohydrate-protein interactions has also been overcome by the use of multivalent glycoconjugates.[6] For example, mucins with multiple oligosaccharide moieties at very high surface densities have been identified as endogenous selectin ligands.[7] Moreover, multivalent forms of SLeX produced by conjugation to bovine serum albumin (BSA) have been reported to exhibit enhanced affinity for E-selectin relative to the affinity of the free monovalent SLeX.[8] An alternative approach to produce multivalent structures has been to prepare divalent,[9-11] trivalent,[11,12] tetravalent,[10] and polyvalent[13,14] forms of SLeX and/or LeX. Our interests have been directed to find new multivalent forms of SLeX that exhibit higher selectin-mediated cell adhesion inhibitory activity and that are well defined and easy to prepare in various shapes, orientations, and valencies. We describe herein the synthesis of a novel homopolymer of conjugated SLeX-acrylamide which contains a naphthamido group instead of a acetamido group on the GlcN moiety, and evaluation of the compound for its inhibitory activity in both a human E-selectin mediated cellular adhesion assay *in vitro*[9] and a lipoteichoic acid (LTA)-induced murine pleurisy model.[15]



Reagents and Conditions: (a)Cl_2CHOMe, $ZnCl_2$, CH_2Cl_2, r.t.; (b) $HO(CH_2)_6NHCOCH=CH_2$, $Sn(OTf)_2$, TMU, MS4Å, CH_2Cl_2, r.t., 80% yield from **3**; (c) NaOMe, MeOH, H_2O, r.t., 98% yield; (d) TEMED, APS, H_2O, r.t.;

Synthesis

In order to synthesize the naphthamido SLeX-acrylamide homopolymer (**1**) in a convenient manner, we selected the following synthetic route using the corresponding monomeric intermediate **2**.

Intermediate **2**, naphthamido SLeX-acrylamide monomer, was prepared by glycosylation of the oligosaccharide chloride **4**[4] with 6-(*N*-acryloylamino)-1-hexanol[16] which was followed by alkaline hydrolysis to deprotect the compound. Radical polymerization of monomer **2** was carried out in deionized water in the presence of *N,N,N',N'*-tetramethylethylenediamine (TEMED) and ammonium peroxodisulfate (APS).[17] The reaction proceeded efficiently at room temperature in the absence of acrylamide and afforded the desired homopolymer **1** in 40% yield after dialysis using a cellulose membrane of 2000 molecular weight cut-off (Spectra/Por®7, MWCO2000) followed by lyophilization. The molecular weight of **1** was determined to be > 300K by the GPC method.[14]

Biological activity

Homopolymer **1** and monomer **2** were evaluated for their ability to inhibit adhesion of HL-60 cells to recombinant human soluble E-selectin (rhsE) by a method reported previously.[9] The results are summarized in Table 1. Each data point is reported as the 50% inhibitory concentration (IC_{50}) using mg/ml instead of mM in order to more easily interpret the inhibitory effects of each SLeX. In the E-selectin mediated cell adhesion assay, the inhibitory activity per SLeX unit was improved approximately 10-fold by *N*-naphthoylation of GlcN moiety (**6** vs. **2**)[4,5] and a further 10-fold by homopolymerization (**2** vs. **1**).

The *in vivo* effects of the homopolymer **1** and monomer **2** were examined in an LTA-induced murine pleurisy model in which E-selectin has been demonstrated as playing a significant role.[15] Each compound was administered intravenously at a dose of 30 mg/kg. The effect was evaluated as an inhibition percent of the LTA-induced neutrophil accumulation into pleural cavity in mice. Monovalents **2** and **6** inhibited the neutrophil accumulation by 35 and 38%, respectively. Homopolymer **1** showed a much higher inhibitory effect (58%) at the same dose.

In conclusion, we synthesized a SLeX-acrylamide neoglycopolymer, homopolymer **1**, and have evaluated the structure for its inhibitory activity in both a human E-selectin mediated cellular adhesion assay *in vitro* and in an LTA-induced murine pleurisy model. The homopolymer **1** exhibited approximately 10-fold higher inhibitory activity per SLeX unit than the corresponding SLeX-acrylamide monomer **6** and showed higher inhibitory activity in the animal model. We are currently optimizing the molecular weight and the length of alkylene linker of SLeX-acrylamide homopolymers to improve the inhibitory effects in selectin mediated cell adhesion. An investigation on the corresponding SLeX-acrylamide copolymers is also underway.[18]

[Table 1] Inhibitory activity of naphthamido SLeX-acrylamide conjugates **1** and **2**

Compound	*in vitro**1 IC_{50} (mg/ml)	*in vivo**2 inhibition (%)
homopolymer **1**	0.010	58
monomer **2**	0.10	35
SLeX-OEt **6***3	1.5	38

*1; The IC_{50} value means the 50% inhibitory concentration for cell adhesion of HL-60 cells to recombinant human soluble E-selectin. For details, see reference 9.
*2; Each compound was administered intravenously at a dose of 30 mg/kg. Each data represents the mean of 7-8 determinations. For details, see reference 15.
*3; See reference 4.

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18) The corresponding copolymer could be obtained by a similar method described for the homopolymer **1**.[17] Namely, polymerization of monomer **2** with 4.0 equivalents of acrylamide yielded the corresponding copolymer in 33% yield after dialysis by the same conditions described above followed by lyophilization. The ratio of polymer composition (x:y) of the copolymer was determined to be 1:2 by a ^{1}H-NMR analysis. The molecular weight was determined to be 285K by the GPC method.[14]

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