Synthesis of Sulfated Neoglycopolymers: Selective P-Selectin Inhibitors

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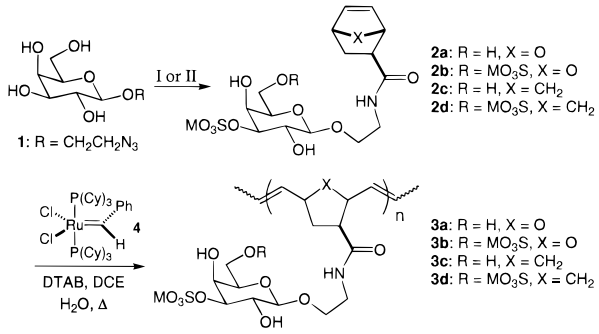
Sulfated saccharides mediate important physiological processes. For example, the proteins L- and P-selectin, which facilitate leukocyte trafficking to sites of inflammation, recognize sulfated carbohydrate residues.[1,2] Several densely *O*-glycosylated proteins, including PSGL-1, CD34, and GlyCAM-1, have been identified as high affinity, sulfate-containing selectin ligands.[3] Molecules that share the features of these naturally-occurring saccharide arrays could be used to modulate or promote physiological processes effected by the natural carbohydrate determinants. Generating structural analogs of these ligands is a significant challenge: not only are the saccharide epitopes replete with functional groups but they are exhibited in multivalent displays.[4] We report a strategy for the synthesis of ligands that present multiple copies of sulfated carbohydrate determinants and the application of this strategy to the creation of high affinity selective inhibitors of P-selectin.

Impetus to generate materials displaying multiple sulfated saccharide residues arose from our investigations of the role of carbohydrate sulfation in selectin recognition.[5,6] For monovalent saccharide derivatives, sulfation at a specific position can subtly alter the selectin-binding properties of a particular ligand. In studies with the lectin concanavalin A, small changes in the protein binding specificity of monovalent saccharide ligands were amplified when the interactions were multivalent.[7] If selectin−carbohydrate interactions benefit from multivalent binding, it was hypothesized that high-affinity, selective selectin ligands could be generated by displaying multiple copies of particular sulfated carbohydrate residues.[8] Multivalent saccharide derivatives have been synthesized by the ring-opening metathesis polymerization (ROMP), and potent, specific ligands for concanavalin A have been generated.[7] The application of this method to the synthesis of selectin ligands would provide the means to address the role of multivalent binding in selectin−carbohydrate interactions. The target selectin ligands, however, possess anionic sulfate substituents, and ROMP of monomers with sulfate groups is unknown. Therefore, we synthesized monomers bearing sulfated saccharide residues to develop an effective protocol for ROMP of these substrates.

Scheme 1[a]



[a] Method I (for **2a** and **2c**): (a) $PhB(OH)_2$, MeOH, PhH, Δ; (b) (i) Bu_2SnO, PhH, Δ, (ii) $SO_3{\cdot}NMe_3$, 1,3-dimethylimidazolidin-2-one; (c) H_2, $Pd(OH)_2$/C (77%); (d) (±)-7-oxabicyclo[2.2.1]hept-5-ene-*exo*-2-carboxylic acid pentafluorophenyl ester, *N*-methly morpholine, DMF (**2a**, 94%; **2c**, 98%). Method II: (for **2b** and **2d**) (a) (i) Bu_2SnO, MeOH, Δ, (ii) $SO_3{\cdot}$Pyr, pyridine; (b) H_2, $Pd(OH)_2$/C (71%); (c) (±)-bicyclo[2.2.1]hept-5-ene-*exo*-2-carboxylic acid pentafluorophenyl ester, *N*-methylmorpholine, DMF (**2b**, 92%; **2d**, 86%). DTAB = CH_3-$(CH_2)_{11}N(CH_3)_3Br$. Yields: **3a**, 74%; **3b**, 32%; **3c**, 70%; **3d**, 65%.

Our target monomers were based on two naturally-occurring sulfate-containing ligands that bind P- and L-selectin, glycolipid sulfatides,[9,10] and the glycoprotein GlyCAM-1.[11] Both sulfatides and GlyCAM-1 can function as multidentate ligands. For example, sulfatides can aggregate to form micelles, which are the likely selectin-binding species. GlyCAM-1 is a mucin that displays multiple copies of sulfated carbohydrate determinants, including 6′-sulfo sialyl Lewis x [6′-sulfo sLe^x: NeuNAcα2 → 3(6-*O*-SO_3)Galβ1 → 4(Fucα1 → 3)GlcNAc].[12] In designing selectin ligands, we sought to imitate the sulfatides by presenting multiple copies of 3-sulfated galactose, their saccharide substituent. To mimic the charge distribution of the determinant 6′-sulfo sLe^x, GlyCAM-1, our plan was to generate multivalent oligomers bearing 3,6-disulfo galactose residues. The sulfated saccharides were attached through an anomeric linker to either a norbornene or 7-oxanorbornene scaffold (Scheme 1). ROMP of such derivatives will afford linear polymers that display saccharide residues with a density of 1 epitope per repeat unit. Although the linear display of saccharides within the polymers mimics some features of sulfatide micelles, these substrates differ in that saccharide determinants of the neoglycopolymers are unable to reorganize dramatically. This mode of presentation and the high density of saccharide residues more closely parallel the features of mucins such as GlyCAM-1.

To synthesize the necessary saccharide monomers, a strategy that involved minimal protecting group manipulation was employed. Positional sulfate isomers were prepared by activation of (azidoethyl)glycoside **1** with dibutyltin oxide (Scheme 1). Stannylation of **1** followed by reaction with sulfur trioxide−pyridine complex afforded regioselective sulfation at O3 and O6. Initial attempts to generate the 3-monosulfate through the stannylene acetal failed to produce the desired sulfate in acceptable yield. This problem was solved by masking the 4- and 6-hydroxyl groups as a cyclic phenylboronate, which, when treated with Bu_2SnO and, $SO_3{\bullet}NMe_3$ afforded the 3-sulfo galactose derivative.[13] The resulting sulfated (azidoethyl)-glycosides were reduced to the corresponding amines. To

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synthesize the 7-oxanorbornene systems, the amino galactose derivatives were reacted with (±)-7-oxabicyclo[2.2.1]hept-5-ene-*exo*-2-carboxylic acid pentafluorophenyl ester to afford targets **2a** or **2b** as mixtures of diastereomers. Norbornene templates **2c** and **2d** were prepared similarly in high yields.

For the synthesis of the target neoglycopolymers by ROMP, we explored the ability of Grubbs' ruthenium alkylidene catalyst, $[(Cy)_3P]_2Cl_2Ru{=}CHPh$ **4**,[14] to polymerize the anionic monomers **2a**−**d**. In our previous syntheses of carbohydrate-substituted polymers, we employed from ruthenium trichloride.[15] Polymerizations that occur under these conditions are not living; consequently, many unique features of ROMP, such as the ability to vary the molecular mass of the polymers or synthesize block copolymers, cannot be exploited.[16] Given the properties of our anionic monomers, we chose catalyst **4** because of its high reactivity and its excellent functional group tolerance.[17] We anticipated, however, that the marked differences in solubility between the nonpolar catalyst and the anionic monomers might pose a serious problem. Initial polymerization studies were performed with triethylammonium salts of template **2a** in methanol/dichloromethane solutions. Although carbene **4** effected the transformation of **2a** into multivalent **3a**, the polymerization proceeded to low conversion. During the course of the reaction, the growing polymer chains became increasingly insoluble. Because these conditions did not produce satisfactory yields of polymer, emulsion polymerization conditions were explored.

Emulsion conditions have been used in ROMP of polar, neutral monomers.[17,18] For example, catalyst **4** promotes living polymerizations of these substrates in the presence of the detergent dodecyltrimethylammonium bromide (DTAB) in a 1,2-dichloroethane/water mixture.[17] This cationic surfactant might be expected to facilitate reaction of anionic monomers **2a**−**d**. Treatment of monomer **2a** with catalyst **4** under emulsion conditions (Scheme 1) followed by chain termination with ethyl vinyl ether[21] afforded product **3a**. This material was converted to its sodium salt and then purified by precipitation from absolute ethanol. Using this protocol, water-soluble polymers **3a**−**d** were obtained in isolated yields up to 74%.

To characterize the selectin-binding properties of the sulfated polymers, their abilities to inhibit binding of immobilized recombinant E-, L-, and P-selectin fusion proteins to a myeloid cell line were assayed.[20] Because the average molecular masses of the polymers are known, their activities are based on the concentration of saccharide residues needed to block 50% of HL60 cell binding to immobilized selectin rather than on molar concentrations. None of the monovalent ligands **2a**−**d** displayed activity against any selectin. Polymers **3a**−**d** exhibited little activity against E- or L-selectin in the HL60 cell assay; however, some inhibition of L-selectin was observed in an ELISA with immobilized heparin (data not shown).

The neoglycopolymers show unique properties in their interactions with P-selectin. For instance, neoglycopolymer **3a** was effective at blocking cell binding (Figure 1), a result consistent with our studies of monovalent saccharide recognition in which 3′-sulfo Lewis a displayed similar activity.[5] When a

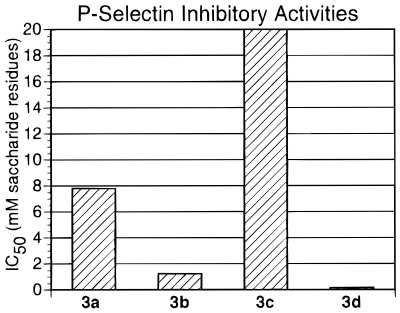

Figure 1. Sulfated polymers **3a**−**d** were tested for their ability to block binding of HL60 cells to immobilized selectin-Ig constructs. Reported IC_{50} values for the polymers represent the molar concentration of saccharide residues needed to inhibit cell binding by 50%.

sulfate group is added to the 6-position of the galactose residues, the resulting materials (compounds **3b** and **3d**) are potent and specific P-selectin inhibitors. Neoglycopolymer **3d** interferes with selectin-mediated cell adhesion at saccharide residue concentrations of 167 μM, corresponding to an IC_{50} of 7 μM based on the estimated molar concentration of polymer.[21] Thus, multidentate ligand **3d** is approximately 500-fold more effective than the monovalent inhibitor sLe^x (IC_{50} of 3.4 mM) at blocking P-selectin−cell interactions.

The high affinity and specificity for P-selectin manifested by polymer **3d** highlights the utility of ROMP for the synthesis of effective selectin ligands from readily accessible precursors. Our results indicate that multivalent arrays of galactose residues possessing anionic substituents at both the 3- and 6-positions exhibit dramatically different selectin-binding properties than those with a single charge at the 3-position. The efficacy of disulfated neoglycopolymer **3d** relative to its monosulfated counterpart is notable given the ability of GlyCAM-1 to bind P-selectin.[22] GlyCAM-1, which requires sulfation for high-affinity selectin interactions,[12] displays multiple copies of the determinant 6′-sulfo sLe^x. Compound **3d** may imitate P-selectin-binding features of GlyCAM-1, such as its distribution of anionic charges.

The unique features of ROMP can be used to further explore the molecular basis of the enhanced affinity and specificity exhibited by multivalent molecules. Mimetics of the GlyCAM-1 structure may be used to study not only the recognition properties of the selectins but also the physiological consequences of their aggregation at the cell surface. In addition, molecules that modulate selectin function have significant medical applications. In our pursuit of a new class of selectin ligands, we have demonstrated that ROMP provides access to new materials that can modulate physiologically important protein−carbohydrate interactions.

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Supporting Information Available: Experimental procedures and spectral data (32 pages). See any current masthead page for ordering and Internet access instructions.

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