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Convergent Synthesis of Sulfated Bivalent Glycopeptides as Selectin Ligands

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Abstract: Non-natural bivalent N-linked glycopeptides, designed to probe selectin recognition, were synthesized by HBTU-promoted coupling of a sulfated lactoside derivative to aspartic acid residues.

Efforts to understand and modulate protein-carbohydrate interactions have been galvanized by the discovery of their involvement in the inflammatory response. In the early stages of inflammation, the proteins E-, P- and L-selectin faciliate leukocyte rolling along the vascular endothelium.[1] Each selectin contains a C-type lectin domain, a feature that suggests these proteins mediate leukoctye tethering through recognition of carbohydrate epitopes on apposing cell surfaces. Carbohydrate ligands for the selectins have been identified, and these include various sialylated and sulfated derivatives of Lewis a and Lewis x. Monovalent Le^a and Le^x saccharides, however, bind weakly ($K_a \approx 10^4\ M^{-1}$)[2] to all of the selectins. In contrast, glycoproteins have been isolated that bind tightly and specifically to individual selectins, such as the highly glycosylated mucins GlyCAM-1 and CD34 which bind tightly to L-selectin.[1] The molecular features responsible for these high affinity interactions are unknown.

Two characteristics of glycoprotein selectin ligands that may contribute to tight, specific binding are: first, such ligands can display multiple copies of individual saccharide recognition elements and second, their peptide backbones can also contribute direct contacts to the recognition event. In the case of multidentate binding, the functional affinity of a multivalent ligand will be influenced by several features. These include the distance and orientation between saccharide residues and the flexibility and structure of the template upon which the carbohydrate groups are displayed.[3] Several scaffolds for the display of divalent and tetravalent selectin ligand have been investigated.[4] These backbones, however, do not address the possibility that protein-protein and protein-carbohydrate interactions may be involved in selectin recognition. For example, both components are needed for the P-selectin binding to its physiological ligand, PSGL-1.[5] To elucidate the importance of multipoint binding and protein-protein interactions in selectin recognition, we set out to develop a flexible, systematic method to generate multivalent glycopeptide ligands.

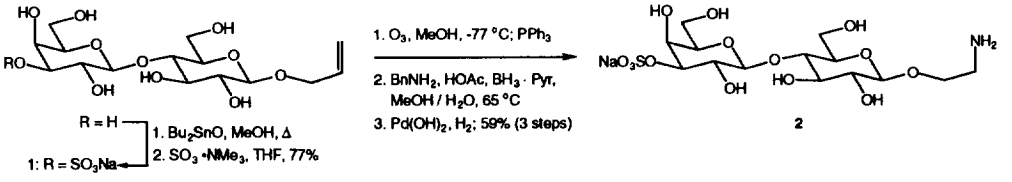
Our design for the synthesis of bivalent selectin ligands relies on simultaneous attachment of monovalent carbohydrate residues to terminal aspartic acid residues within a peptide. The saccharide precursors contain a short linker at the reducing end. The linker terminates in an amino group that serves as a point of attachment to activated aspartic acid residues within the peptides. In this convergent synthetic strategy, it was anticipated that such carbohydrate substrates would be more reactive than the

glycosylamines commonly used in glycopeptide synthesis. By avoiding the use of glycosylamines, peptide amidation side reactions can be circumvented. Similarly to natural selectin-binding glycoproteins, the carbohydrate epitopes are presented on a peptide backbone in our non-natural ligands, a feature that facilitates the exploration of different spatial relationships between carbohydrate residues. Moreover, additional selectin-peptide interactions may be engineered into these bivalent glycopeptides either through design or through selection.

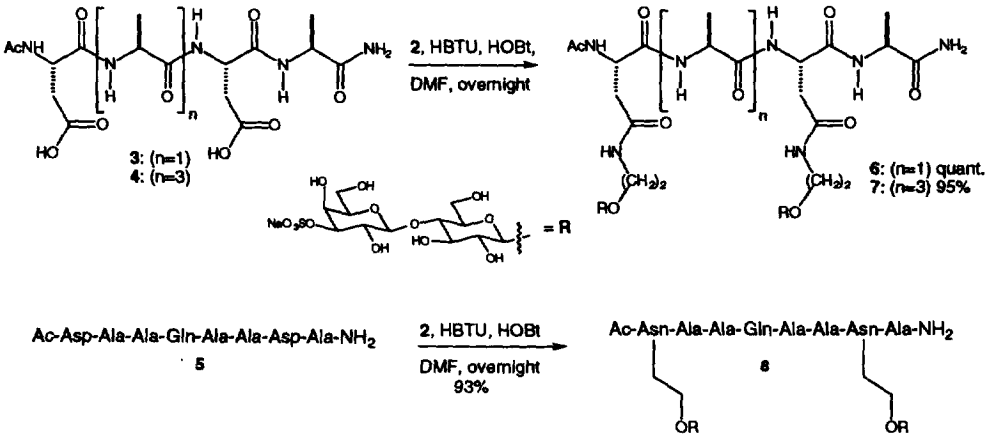
Asparagine-linked glycopeptides have been synthesized by amide bond formation between glycosylamines and peptides in solution[6] or on a solid support.[7] The preparation of defined glycopeptides bearing more than one saccharide determinant by this approach has not been described. In addition, no efficient protocols for the attachment of sulfated saccharides have been developed.

The saccharide component of the synthetic glycopeptides is based on 3'-sulfo allyllactoside **1**, a compound that interacts with L-selectin.[8] Additionally, this determinant serves as a readily accessible model compound for the more complex Lewis a and Lewis x derivatives prepared in our laboratory (Scheme 1).[9,10] Intermediate **1** was obtained by conversion of allyllactoside[11] into the dibutylstannylene acetal, which was regioselectively sulfated with $SO_3 \cdot N(CH_3)_3$.[12] The amino substituent was introduced by ozonolysis of the allyl group and reductive amination of the resulting aldehyde, which yielded the (2-aminoethyl)-3'-sulfo-lactoside **2**.[13] It should be noted that the sulfate functional group was stable to the ozonolysis and reductive amination procedures employed here.

Scheme 1



To investigate the role of multipoint binding in selectin recognition, the peptides **3-5** (Scheme 2) were designed to have little interaction with the selectins. The corresponding glycopeptides can therefore be used to elucidate the effects of different spatial relationships between the carbohydrate units on selectin binding. The peptide sequences, which differ in the number of amino acid residues between the terminal aspartic acids, were generated by standard solid phase procedures. The peptides were deprotected and purified before the the sulfated saccharide substituents were appended. The final step, coupling of the sulfolactoside **2** to the peptides **3-5**, proceeded in excellent yields in the presence of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt)[6] (Scheme 2). The structures of glycopeptides **6-8** were confirmed by ^{1}H-NMR and electrospray mass spectroscopy.[14] All substrates examined afforded high yields of the glycopeptide products, and no aspartimide formation was observed under these conditions.[6] The efficiency of this strategy for the synthesis of sulfated glycopeptides compares favorably with those previously reported.[7]



Scheme 2

In conclusion,we have synthesized selectin-targeted glycopeptides carrying multiple carbohydrate residues by coupling of sulfated lactoside amines to aspartic acid residues. This approach provides the means to generate new glycopeptides that can be used for the study of multipoint recognition in selectin-mediated cell adhesion. In addition, this strategy can be used to dissect contributions from the peptide backbone sequence and carbohydrate sequence to selectin recognition affinity and specificity.

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13. Preparation of (2-aminoethyl)-3'-sulfo-lactoside **2**: Ozone was bubbled for 3 min through a solution of allyl-3'-sulfolactoside **1** (48 mg, 99 μmol) in anhydrous MeOH (10 mL) at -77 °C. After purging with N_2 for 1 h, polymer supported PPh_3 (68 mg, 20 μmol) was added. The reaction mixture was allowed to reach r.t. and stirred for 16 h. Filtration and evaporation of the solvent yielded crude product (53 mg). To a solution of the crude product in methanol/water (1:1, 4 mL) benzylamine (54 μL, 4.9 μmol), glacial acetic acid (28 μL, 49 μmol) and Pyr•BH_3 (8 M, 65 μL, 52 μmol) were added. The mixture was heated to 65-70 °C for 19 h, then cooled to r.t. and extracted with chloroform (2 x 10 mL). The aqueous layer was concentrated and dried in vacuo. The material obtained from the reductive amination was dissolved in methanol/water (1:1, 4 mL) and hydrogenated in the presence of $Pd(OH)_2$ (72 mg) on carbon (20% Pd, Pearlmans catalyst) in a Parr apparatus (55 psi). After filtration through cellulose, the crude product was purified through anion exchange chromatography (Dowex® 1x2-400; pH 7.37, 0-0.5 M $Et_3NH^+HCO_3^-$), cation exchange chromatography (Sephadex®SP C-25) and flash chromatography (SiO_2, chloroform/methanol/water 5:5:1) to afford (2-aminoethyl)-3'-sulfolactoside **2** (29 mg, 5.9 μmol, 59%).
^{1}H-NMR (300 MHz, D_2O, external reference):δ 3.18 (t, J = 5.15 Hz, 2H), 3.37 (t, J = 8.5 Hz, 1H), 3.58-4.01 (m, 10H), 4.20 (dt, J = 5.15, 11.77 Hz, 1H), 4.28 - 4.35 (m, 2H), 4.53 (d, J = 7.35 Hz, 1H) and 4.61 (d, J = 7.36 Hz, 1H); ^{13}C-NMR (75 MHz, D_2O, TSP insert): δ 44.0 (CH_2), 64.5 (CH_2), 65.5 (CH_2), 71.0 (CH_2), 71.4, 73.6, 77.3, 78.8, 79.3, 79.5, 82.8, 84.5, 106.5 and 107.1; LRMS (LSIMS, 3-NBA, negative ion mode, *m/z*) for $C_{14}H_{26}NO_{14}S^-$, calcd 464.1, found 464.0.
14. Glycopeptide **6**: ^{1}H-NMR (300 MHz, D_2O, external reference): δ 1.15-1.25 (m, 6H), 1.82 (s, 3H), 2.45-2.75 (m, 4H), 3.10-3.70 (m, 26H), 3.75-3.85 (m, 4H), 4.05-4.20 (m, 6H), 4.32 (d, J = 8.09 Hz, 2H), 4.39 (d, J = 8.09 Hz, 2H), 4.45-4.55 (m). LRMS (electrospray, H_2O/CH_3OH, negative ion mode, *m/z*) for $C_{44}H_{73}N_7O_{35}S_2^{2-}$, calcd 661.6, found 661.5.
Glycopeptide **7**: ^{1}H-NMR (300 MHz, D_2O, TSP insert): δ 1.35-1.45 (m, 12H), 2.23 (s, 3H), 2.65-2.90 (m, 4H), 3.30-3.52 (m, 5H), 3.55-3.85 (m, 18H), 3.90-4.03 (m, 4H), 4.20-4.37 (m, 9H), 4.49 (d, J = 8.09 Hz, 2H), 4.56 (d, J = 8.1 Hz, 2H) and 4.62-4.72 (m, 2H). LRMS (electrospray, H_2O/CH_3OH, negative ion mode, *m/z*) for $C_{55}H_{83}N_9O_{37}S_2^{2-}$, calcd 732.7 found 732.6.
Glycopeptide **8**: ^{1}H-NMR (300 MHz, D_2O , TSP insert):δ 1.39-1.42 (m, 15H), 1.95-2.15 (m, 2H), 2.03 (s, 3H), 2.39 (m, 2H), 2.65-2.90 (m, 4H), 3.30-3.50 (m, 5H), 3.56-3.85 (m, 19H), 3.92-4.02 (m, 4H), 4.2-4.47 (m, 10 H), 4.50 (d, J = 7.7 Hz, 2H), 4.57 (d, J = 7.7 Hz, 2H) and 4.65-4.70 (m, 2H). LRMS (electrospray, H_2O/CH_3OH, negative ion mode, *m/z*) for $C_{58}H_{96}N_{12}O_{40}S_2^{2-}$, calcd 832.2, found 832.1.

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