Chemoenzymatic Synthesis of a PSGL-1 N-Terminal Glycopeptide Containing Tyrosine Sulfate and α-*O*-Linked Sialyl Lewis X

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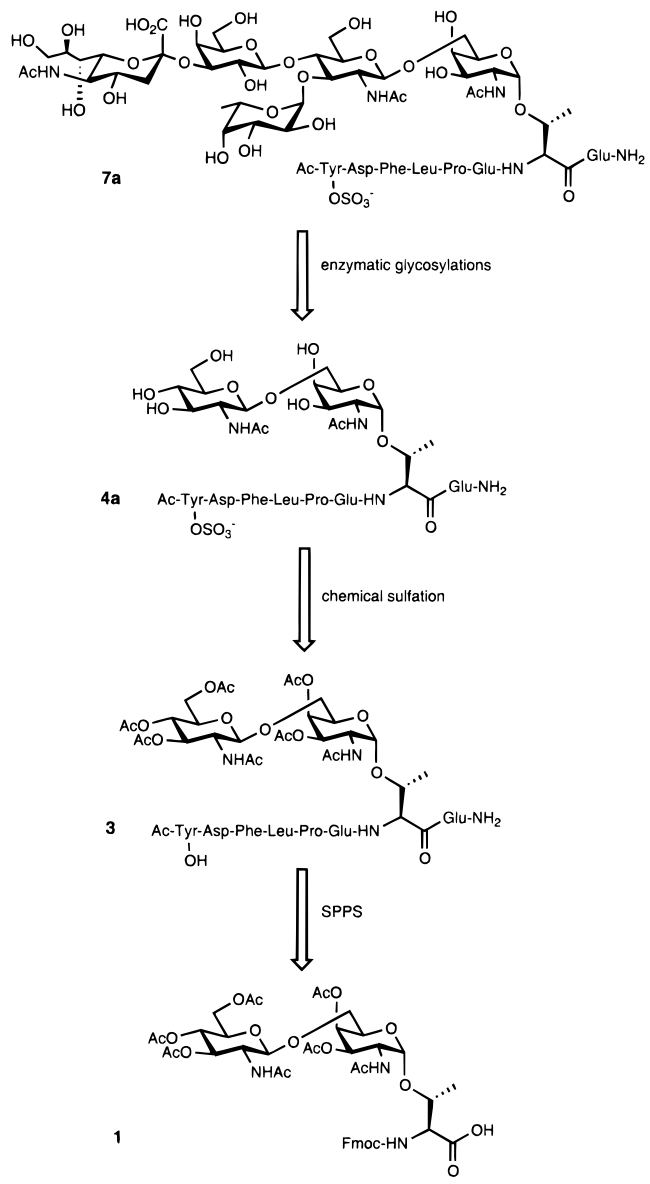
Tyrosine sulfation and *O*-linked glycosylation are post-translational modifications of P-selectin glycoprotein ligand-1 (PSGL-1) that are required for high-affinity binding interactions with P-selectin.[1] Interestingly, N-terminal fragments of PSGL-1 containing a core 2 *O*-linked glycan and tyrosine sulfate bind to P-selectin with affinity similar to that of the full-length homodimeric PSGL-1 protein.[2] This suggests that simple sulfated glycopeptide structures may be suitable for disruption of PSGL-1/P-selectin binding events during excessive leukocyte infiltration in the inflammatory response.[3] The PSGL-1 core 2 *O*-glycan attached to Thr16 displays sialyl Lewis X (sLe^x), the minimum selectin binding determinant, on the β-1,6-branch of an α-linked GalNAc.[4] Of greater interest is the role of the tyrosine sulfate. The isolated sLe^x tetrasaccharide binds to P-selectin only weakly ($K_d \approx 3-4$ mM), yet the N-terminal fragment of PSGL-1 containing sLe^x and tyrosine sulfate adheres with nanomolar affinity.[2]

The chemoenzymatic synthesis of sulfated glycopeptides has not been previously reported on a preparative scale. To develop methodology toward the synthesis of sulfated glycopeptides, as well as to investigate PSGL-1 biosynthesis and P-selectin interactions, a portion of the PSGL-1 N-terminus was selected as a synthetic target. Traditional chemical synthesis of sulfated glycopeptides has been prohibitive, due to lability of sulfate esters to the acid catalysts used in chemical glycosylation reactions. However, the utility of enzymes as catalysts provides another synthetic avenue. Enzymatic glycosylations with glycosyltransferases proceed at essentially neutral conditions and are therefore compatible with the presence of a sulfate ester. By combining chemical and enzymatic methodology, the synthesis of a PSGL-1 glycopeptide containing tyrosine sulfate and an α-linked sLe^x *O*-glycan was accomplished. The synthetic sequence outlined in Scheme 1 should be applicable to the chemoenzymatic synthesis of sulfated glycopeptides in general.

Initially, the chemical portion of the synthesis required a glycosylated threonine residue for incorporation into solid-phase peptide synthesis (SPPS). The disaccharide-linked threonine structure **1**[5] represents the (β-1,6)-branch of the core 2 *O*-glycan on which sLe^x is constructed. Utilizing glycoconjugate **1** in SPPS allowed the subsequent enzymatic synthesis to proceed with the readily available glycosyltransferases β-1,4-galactosyltransferase (β-1,4-GalT), α-2,3-sialyltransferase (α-2,3-SiaT), and α-1,3-fucosyltransferase-V (α-1,3-FucT V).

Scheme 1. Retro-synthetic Analysis of the PSGL-1 N-terminal Sulfated Glycopeptide



Glycosyl-threonine **1** was incorporated into an Fmoc-based SPPS strategy on Rink amide functionalized resin (Scheme 2). The iterative synthesis provided glycosylated octapeptide **2** attached to the solid support. Cleavage from the resin was then performed with TFA/H_2O, conditions that also resulted in deprotection of the *t*-Bu esters and ether, yielding **3**. With protected glycoconjugate **3** in hand, access to sulfated and unsulfated versions of the glycopeptide was possible. In the first case, tyrosine sulfation was achieved through reaction of **3** with SO_3−pyr complex. Subsequently, basic hydrolysis of the acetate protecting groups gave sulfated **4a**. Simple deprotection of **3** under identical conditions afforded unsulfated **4b**.

As previously reported, sulfation on tyrosine affected the ability of β-1,4-GalT to catalyze the addition of galactose to glycopeptide **4a**.[5] Under conditions successfully utilized for reaction with unsulfated **4b** (Scheme 3), reaction of β-1,4-GalT with sulfated **4a** proceeded slowly. As a further hindrance, proteolytic products

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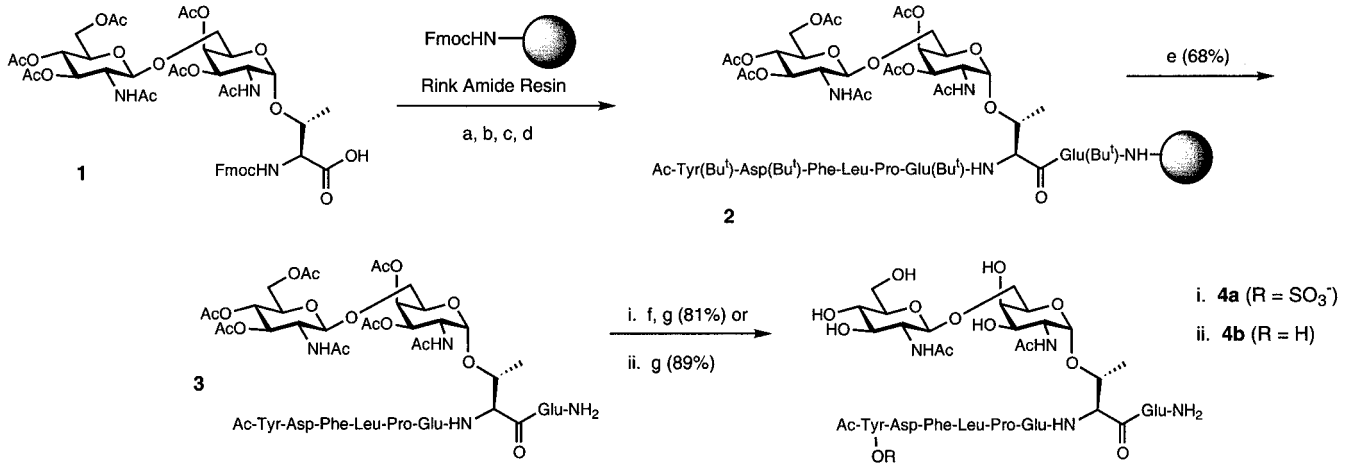
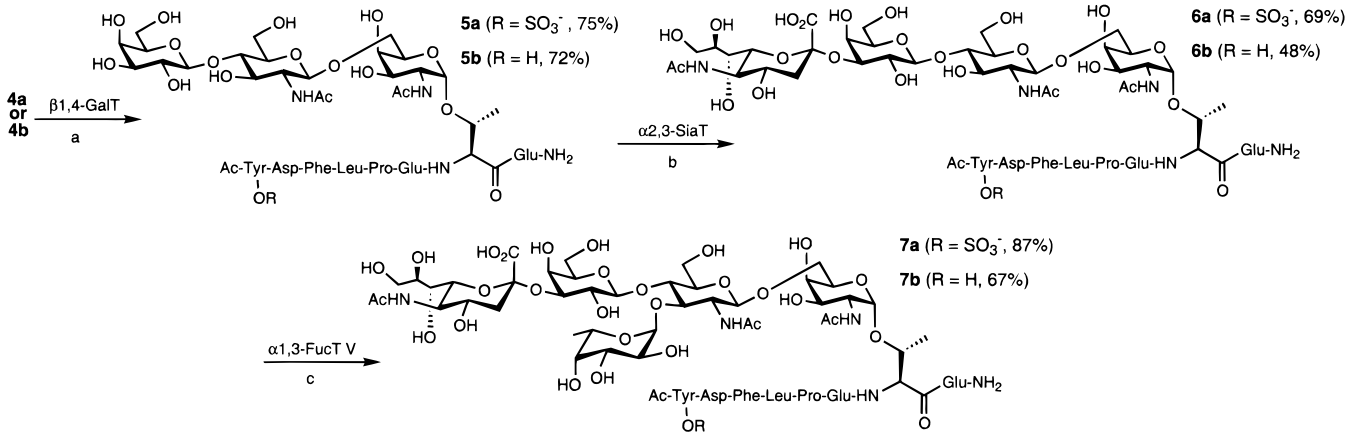
Scheme 2. Solid-phase Synthesis of Sulfated and Unsulfated Glycopeptides[a]



[a] (a) Fmoc-AA-OH, HBTU, HOBt, NMM, DMF; (b) Ac_2O, Pyr; (c) DMF/morpholine; (d)repeat a−c; (e) TFA/H_2O/EDT (68%, based on initial loading); (f) SO_3-pyr, pyr; (g) NaOH/MeOH (81%, 2 steps); or (g alone) NaOH/MeOH (89%, 1 step).

Scheme 3. Enzymatic Synthesis of Sulfated and Unsulfated Glycopeptides[a]



[a] (a) UDP-Gal, β-1,4-GalT (bovine), alkaline phosphatase, 130 mM HEPES, pH 7.4, 0.25% Triton X-100, $MnCl_2$, protease inhibitor cocktail ("PIC", Sigma) (**5a**: 75%, **5b**: 72%); (b) CMP-NeuAc, α-2,3-SiaT (**6a**: bacterial, **6b**: rat liver), alkaline phosphatase, 130 mM HEPES, pH 7.4, 0.25% Triton X-100, $MnCl_2$, (**6a**: +PIC, 69%, **6b**: −PIC, 48%); (c) GDP-Fuc, α-1,3-FucT V (human), alkaline phosphatase, 100 mM MES, pH 6.0, 0.25% Triton X-100, $MnCl_2$, protease inhibitor cocktail (Sigma) (**7a**: +PIC, 87%, **7b**: −PIC, 65%).

were observed to form at long reaction times. Fortunately, this problem was solved by the addition of a protease inhibitor cocktail into the reaction. The addition of this cocktail allowed the reaction to proceed for long duration without destruction of the reactant and product glycopeptides. Under the new conditions, it was possible to isolate and characterize sulfated glycopeptide **5a**. Moreover, addition of protease inhibitor cocktail into all glycosyltransferase reactions investigated was found to ease isolation and raise product yield. As such, the isolation of **5b** was also accomplished, a task which was not possible on prior occasions.

The commercially available rat liver α-2,3-SiaT was not reactive toward sulfated glycopeptide **5a** but did catalyze reaction with unsulfated **5b**, giving sialylated product **6b**.[5] The activity of rat liver derived α-2,3-SiaT appears to be dramatically affected by sulfation of its potential substrates, as it was also observed to be inactive toward certain sulfated chito-oligomers terminating in galactose.[6] Fortunately, examination of sialyltransferases from other sources proved beneficial. It was found that reaction of sulfated glycopeptide **5a** with CMP-NeuAc in the presence of α-2,3-SiaT from *Escherichia coli*[7] afforded product **6a**. Again, the addition of protease inhibitor cocktail was necessary to avoid the formation of cleavage products.

Finally, as described for the synthesis of unsulfated **7b**,[5] reaction of **6a** with GDP-Fuc in the presence of α-1,3-FucT V afforded **7a**, containing the complete sLe^x structure. As such, the synthesis of the PSGL-1 N-terminal sulfated glycopeptide containing both sLe^x and tyrosine sulfate was accomplished.

This is the first report of the chemoenzymatic synthesis of a sulfated glycopeptide on semipreparative scale and is the first in which the sulfate ester was introduced prior to the peripheral carbohydrates. This sulfated glycopeptide structure would be difficult to synthesize by chemical methods alone. The use of glycosyltransferases allowed the addition of peripheral sugars under neutral conditions, which did not result in destruction of the tyrosine sulfate ester. In the past, the combination of SPPS and enzymatic glycosylation has been a successful synthetic route to glycopeptides.[8] This methodology can now be extended to include sulfated glycopeptides as well.

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Supporting Information Available: Characterization data for **5a**, **5b**, **6a**, **7a**, as well as conditions for the cloning and overexpression of bacterial α2,3-SiaT (PDF). This information is available free of charge via the Internet at http://pubs.acs.org.

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(7) α2,3-SiaT from *Neisseria meningitidis* was cloned and overexpressed in *E. coli*. See the Supporting Information for details.

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