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Tin–Mediated Phosphorylation: Synthesis and Selectin Binding of a Phospho Lewis a Analog

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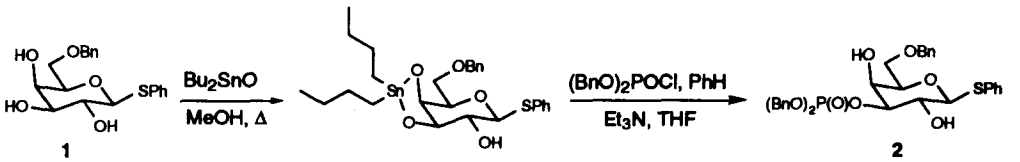
Abstract: Tin–mediated phosphorylation of a galactose residue regioselectively generates the 3'-*O*-phosphotriester. The application of this strategy to a Lewis a analog affords an effective selectin ligand.

The discovery that selectin–saccharide interactions are critical components of the inflammatory response has launched investigations into the determinants of carbohydrate recognition by these proteins.[1,2] It is well known that oligosaccharides that possess sialylated and/or sulfated Lewis a (Le^a:Galβ1→3(Fucα1→4)GlcNAc) or Lewis x (Le^x:Galβ1→4(Fucα1→3)GlcNAc) trisaccharide core structures are ligands of E-, P-, and L-selectin. Moreover, several simple phosphorylated molecules are surprisingly effective selectin inhibitors. For example, mannose-6-phosphate binds to L–selectin with affinity comparable to that of sialyl Le^x, but it does not interact with E– or P–selectin.[3,4] Additionally, phosphorylated inositols are potent inhibitors of L– and P–selectin but fail to inhibit E–selectin.[5] These results indicate that the placement of phosphate groups on a carbohydrate template can confer selectivity in selectin binding. The differential binding of these phosphorylated derivatives may be due to unique features of the selectin binding sites; however, with no available structure of a selectin–carbohydrate complex, the origins of these differences remain obscure. We envisioned that the substitution of a phosphate for a sulfate or carboxylate on the Le^a core structure would afford a ligand with potential to reveal detailed structural information about the selectin binding sites. With its additional NMR active nucleus, a phosphorylated derivative could act as a probe to identify key residues involved in carbohydrate binding. Because the protonation state of phosphomonoesters is sensitive to pH changes in the physiological range, a phosphorylated analog could be used to examine the relation of charge density to affinity and specificity in carbohydrate–protein interactions. We report herein the synthesis and selectin binding properties of the 3'-*O*-phospho Le^a analog **8** (Scheme 3).[6]

Our efforts to probe the importance of sites of sulfation on the galactose ring of Le^a–type saccharides led us to tetraol **7b**,[7] a synthetic intermediate with potential for elaboration into phosphorylated analogs. Phosphorylation reactions of unprotected polyhydroxylated systems, however, are inherently problematic. The regioselectivity of such reactions is generally poor and migration of the phosphate group often occurs in the case of *cis*–diols. Elaborate protecting group schemes are commonly employed to combat these difficulties, rendering the syntheses of phosphorylated adducts complex and laborious. For these reasons, methods to selectively phosphorylate specific hydroxyl groups in an unprotected polyhydroxylated system would be beneficial. Reagents such as

bis(tributyltin) oxide and dibutyltin oxide have been successfully used in carbohydrate chemistry to effect highly regioselective substitution reactions of polyhydroxylated systems.[7-9] To extend this methodology to phosphorylation of carbohydrates,[10, 11] reactions of thiogalactoside triol derivative **1** were explored (Scheme 1). Treatment of **1** with dibutyltin oxide in refluxing methanol afforded the 3,4-*O*-dibutylstannylene acetal. Evaporation of the solvent and subsequent reaction with dibenzyl phosphorochloridate[12] afforded the 3'-*O*-phosphotriester **2** (51%) as the sole product. Phosphorylation occurred at the three position of the thiogalactoside as judged by a ^{31}P decoupled ^{1}H NMR experiment.

Scheme 1

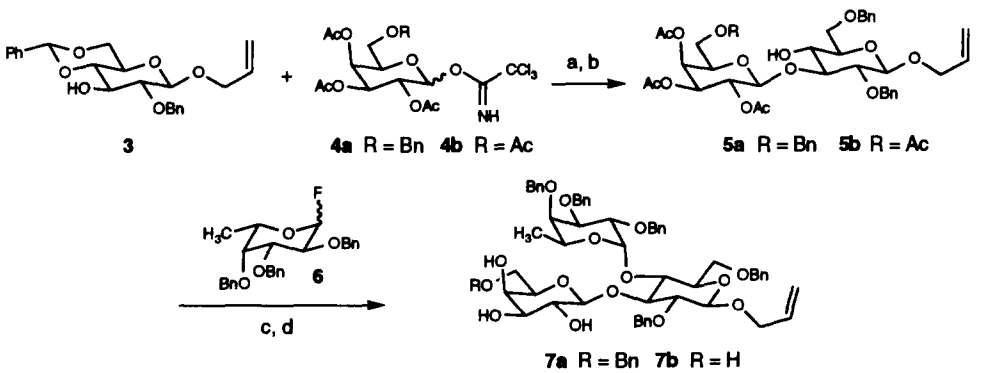


1 **2**

Furthermore, the ^{31}P NMR spectrum exhibited a single resonance at -3.1 ppm, a signal typical for phosphotriesters. These results demonstrate high regioselectivity among three secondary hydroxyls and confirm that the phosphorylated adduct does not undergo phosphate migration in the *cis*–diol system.[10]

The success of the model reaction prompted us to investigate the phosphorylation of the more complex Le^a template **7**. An appropriately functionalized Le^a scaffold was constructed from the corresponding carbohydrate residues **3**, **4**, and **6** (Scheme 2)[7]. Stannylation of triol **7a** with Bu_2SnO in

Scheme 2:



3 **4a** R = Bn **4b** R = Ac **5a** R = Bn **5b** R = Ac

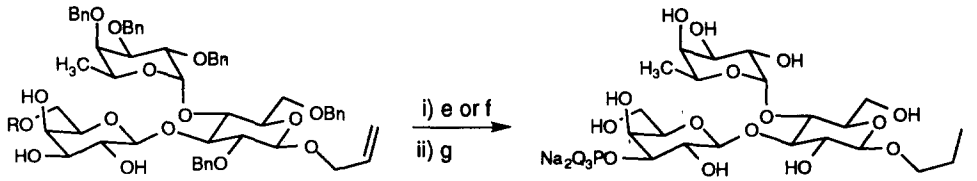
7a R = Bn **7b** R = H

Conditions: (a) 1 eq. **3**, 1.1 eq. **4a** or **4b**, 0.1 M TMSOTf, CH_2Cl_2 (87%); (b) **4a**: $NaCNBH_3$, Et_2O•HCl, (72%); **4b**: 5 eq Et_3SiH, 5 eq TFA, 0 °C (77%); (c) 1 eq **5a** or **5b**, 2.8 eq of **6**, 1.6 eq Bu_2SnCl_2, 3.1 eq AgOTf, 1.5 eq 2,6-di-*tert*-butyl-4-methylpyridine, 4 Å ms, toluene, 0 °C (**5a**, **5b** 90%); (d) K_2CO_3, 1:1 MeOH : THF (**7a**: 86% **7b** 91%)

benzene followed by phosphorylation with dibenzyl phosphorochloridate afforded 3'-phospho Le^a **8** after hydrogenolysis of the benzyl protecting groups.[13] A useful extension of this methodology would be to mediate phosphorylations at other hydroxyls of the galactose residue. Selective activation of a primary hydroxyl may be accomplished using bis(tributyltin) oxide, but attempts to phosphorylate the

primary hydroxyl of **7b** with dibenzyl phosphorochloridate following activation with bis(tributyltin) oxide led to several products. Unexpectedly, reaction of the tributylstannyl ether of **7b** with dibenzyl

Scheme 3:

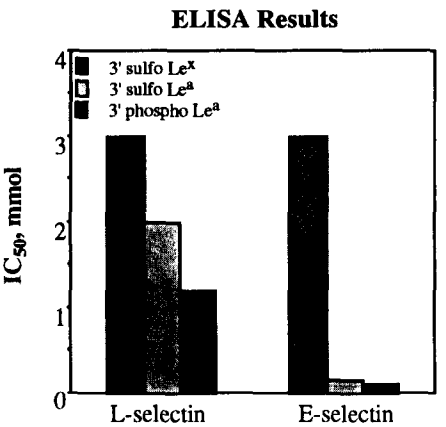


7a R =Bn **7b** R = H **8**

Conditions: i) **7a**: (e) Bu_2SnO, PhH, Δ; $(BnO)_2POCl$, PhH, Et_3N or **7b**: (f) $(Bu_3Sn)_2O$, PhH, Δ; $(BnO)_2POI$, CH_2Cl_2, pyridine; ii) H_2, $Pd(OH)_2/C$ (from **7a**: 52%, **7b**: 57%)

phosphoroiodate[14] cleanly afforded the 3'-*O*-phosphotriester instead of the 6'-*O*-phosphotriester. Subsequent hydrogenolysis of protecting groups provided **8** as a single regioisomer from tetraol **7b**.[15] Thus, trisaccharides **7a** or **7b** may be activated by dibutyltin oxide or bis(tributyltin) oxide respectively, for selective phosphorylation at the 3'-position of the galactose residue.

Phosphate **8** was tested in an ELISA for its ability to block binding of an IgG–selectin chimera to immobilized GlyCAM–1,[16] a physiological L–selectin ligand with affinity for all three selectins.[7] To evaluate the relative ability of the phosphorylated Le^a template to block selectin binding, we compared the affinities of phosphate derivative **8** with 3'-*O*-sulfo Le^x and a structurally similar 3'-*O*-sulfo Le^a analog.[7] We previously found that the sulfated derivatives of Le^x and Le^a were comparably effective at blocking L-selectin binding to GlyCAM–1 (3.0 and 2.0 mmol, respectively). Replacement of the sulfate functionality with a phosphate resulted in enhanced affinity for L-selectin. E–selectin displays a significant preference between the 3'-*O*-sulfo Le^x (3 mmol) and the 3'-*O*-sulfo Le^a (140 μmol) controls, a twentyfold enhancement.[7, 17] Likewise , the phosphorylated Le^a adduct exibits an analogous effect with an IC_{50} of 100 μmol. The similarity in IC_{50} values among the sulfated and phosphorylated Le^a derivatives suggests a common mode of binding. Indeed, our findings indicate that 3' phospho Le^a derivative **8**, which is a more effective inhibitor of E- and L-selectin than the corresponding sulfated or sialylated derivatives, can serve as a useful NMR probe of the carbohydrate binding site.



Acknowledgments

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15. Phosphorylation procedure: In a Dean-Stark apparatus, bis(tributyltin) oxide (11 μL, 0.021 mmol) and tetraol **7b** (33 mg, 0.033 mmol)) were refluxed in benzene (2.5 mL) for one hour then concentrated to afford an oil. The oil was dissolved in CH_2Cl_2 (100 μL) and pyridine (11 μL). A solution of $(BnO)_2POI$ (0.039 mmol) in CH_2Cl_2 (200 μL) was freshly prepared[14] then cannulated into the solution of stannylated **7b** at -40 °C. After 15 minutes, the reaction was quenched with 1 mL saturated aqueous $NaHCO_3$ then extracted with ethyl acetate (2 x 2 mL). The extract was dried (Na_2SO_4), filtered through Celite and concentrated. Chromatography (silica, 30 → 40% ethyl acetate:hexane) afforded the phosphotriester (21 mg, 0.024 mmol) in 73% yield. The phosphotriester was dissolved in methanol (1 mL) and 1 M NaOH(aq) (50 μL). Pearlman's catalyst was added (20 mg, 20% $Pd(OH)_2$/C), and the reaction was treated with H_2 at 1000 psi for two hours. The reaction was filtered through Cellulose eluting with methanol, concentrated then subjected to ion exchange chromatography (Dowex 1X2-400 anion exchange resin, linear gradient 0 → 0.5 M $Et_3NH_2CO_3$(aq); Sephadex SP C-25 400 mesh cation exchange resin, H_2O). Concentration afforded **8** (12 mg,0.019 mmol) in 57% over two steps.
16. Serial dilutions of the test inhibitors in PBS buffer were incubated with a selectin–IgG/antibody/streptavidin–alkaline phosphatase complex for 30 minutes at 4 °C then transferred to wells containing immobilized GlyCAM-1. After 30 minutes at room temperature, the wells were washed three times with PBS/0.1% TWEEN. Bound selectin complex was monitored at 405 nm after addition of *p*-nitrophenyl phosphate in ethanolamine/$MgCl_2$ buffer. For further details see: Bertozzi, C. R.; Fukuda, S.; Rosen, S. D. *Biochemistry* **1995**, *34*, 14271
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