

Glycosylation on the Merrifield Resin Using Anomeric Sulfoxides

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It is widely recognized that methods to synthesize oligosaccharides on the solid phase would revolutionize research in carbohydrate chemistry and biology. Spectacular advances in the use of enzymes to construct glycosidic linkages on the solid phase have recently been made by Paulson and Wong,¹ and enzymatic glycosylation methods hold great promise for the construction of certain classes of oligosaccharides.² Enzymes, however, have some inherent limitations because they are substrate specific. For many studies it may be desirable to have access to a broader range of oligosaccharide structures, including unnatural oligosaccharides. Chemical glycosylation methods to synthesize oligosaccharides on the solid phase would provide access to a potentially unlimited variety of carbohydrates, complementing the enzymatic methods nicely. Below we report the first chemical glycosylation method with the demonstrated ability to make not only (1-6)-linked oligosaccharides on the solid phase but also both α and β glycosidic linkages to biologically relevant secondary alcohols stereospecifically and in near quantitative yield.

Scattered reports have suggested that it should be possible to construct oligosaccharides on the solid phase using chemical methods.³ However, despite some initial successes by Gagnaire,⁴ van Boom,⁵ and Danishefsky,⁶ the efficient and stereospecific glycosylation of secondary alcohols on insoluble resins is still a problem. One reason for this is that glycosylation reactions go more slowly in the heterogeneous environment of a resin matrix than they do in solution. The slow reaction rates permit undesirable side reactions to predominate over glycosylation. Krepinsky has provided an elegant solution to the problem of slow reaction rates by using a soluble resin, which provides a more solution-like environment in which to carry out glycosylation reactions.^{7,8} However, insoluble resins have some strategic advantages over soluble resins, and interest in finding better ways to carry out glycosylation reactions on insoluble resins remains high.

In 1989, we reported a new method for glycosylation using anomeric sulfoxides.⁹ Upon activation under mild conditions,

anomeric sulfoxides generate extremely reactive glycosyl donors. We thought that the remarkable reactivity of these glycosyl donors augured well for the successful application of the sulfoxide glycosylation method to solid-phase oligosaccharide synthesis. Moreover, since the glycosyl sulfoxides and the activating agent (triflic anhydride) are relatively nonpolar, we anticipated favorable partitioning into the nonpolar matrix of an insoluble resin. We now report preliminary studies indicating that the sulfoxide glycosylation reaction has the potential to make the chemical synthesis of biologically important oligosaccharides on the solid phase a reality.

The construction of oligosaccharides on the solid phase involves a number of separate operations. Scheme 1, which outlines the synthesis of a β -(1-6)-linked trigalactose,¹⁰ illustrates our basic approach to solid-phase synthesis. We chose Merrifield's resin (1% cross-linked chloromethylated styrene/divinylbenzene copolymer, 200-400 mesh) as the solid support.¹¹ The first sugar was attached to the resin using the cesium salt of a *p*-hydroxythiophenyl glycoside.¹² The thiophenyl ether linkage is stable to the reaction conditions used during the synthesis but can be readily hydrolyzed at the end by treatment with mercuric trifluoroacetate. The attached sugar **2** was selectively deprotected to produce **3**.¹³ A solution of the protected galactosyl sulfoxide **4** (122.1 mg, 0.156 mmol, 4 equiv) and 2,6-di-*tert*-butyl-4-methylpyridine (98.2 mg, 0.478 mmol, 12 equiv) in anhydrous methylene chloride (7 mL) was added to the dried resin (95.4 mg, 0.405 mmol/g, 0.039 mmol, 1 equiv) in a reactor vessel. The suspension was cooled to -78 °C. Following a 10 min equilibration period, a solution of triflic anhydride (13 μ L, 0.078 mmol, 2 equiv) in methylene chloride (5 mL) was added to the suspension, which was agitated by a flow of argon through the reactor vessel. The mixture was warmed to -60 °C over 30 min, and the progress of the reaction was monitored by hydrolyzing small aliquots of resin with mercuric trifluoroacetate and analyzing the hydrolysis products by TLC. After 30 min, the resin was filtered and washed and glycosylation repeated. Subsequent deprotection of the resin-bound disaccharide followed by another coupling with **4** gave **7**, which was converted to **10** (28.5 mg, 52% overall yield).¹⁴ Independent experiments have shown that detachment of monosaccharides from the resin produces the corresponding lactols in 70-75% yield. If one therefore assumes a 70-75% yield for the detachment of the trisaccharide, then each of the other six steps must have proceeded to give better than a 94-95% yield on average. It should be emphasized that -60 °C is a remarkably low temperature for solid-phase synthesis of any kind, and highlights the reactivity of the donor species in the sulfoxide method.

Having established that we can make (1-6) linkages in close to quantitative yield on the solid phase using the sulfoxide glycosylation reaction, we turned our attention to some more challenging secondary alcohols. Le^x, one of the Lewis blood group antigens, contains a fuc- α -(1-3)-glcNac fragment. Le^s, another blood group antigen, contains a gal- β -(1-3)-glcNac fragment. Any glycosylation method with the potential to be used effectively

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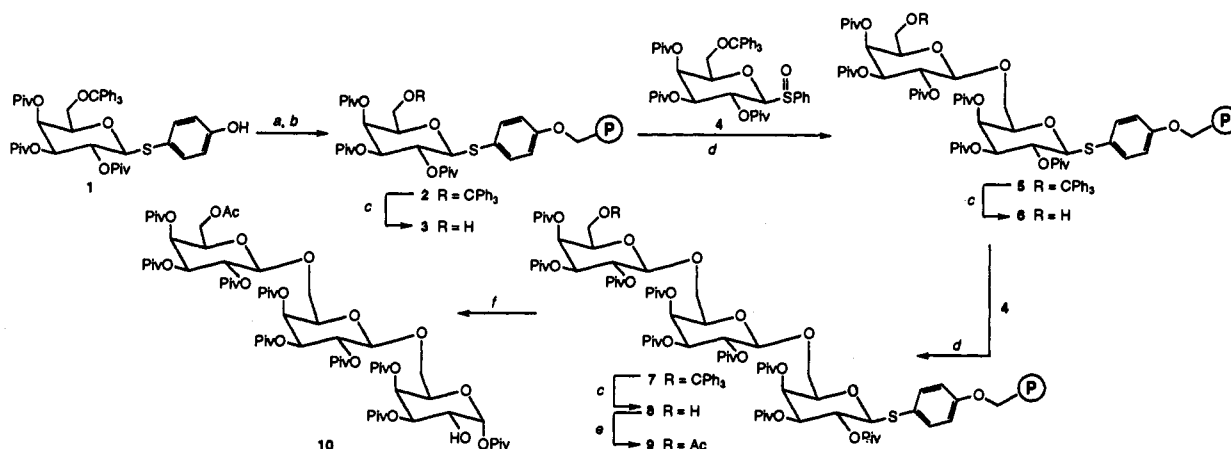
(12) Chiu and Anderson (ref 8 c) have used a somewhat different thioether linkage for attachment.

(13) The deprotection was followed by IR (KBr disc): 1734 [ν (C=O)] and 3430 [ν (OH)] cm^{-1} . For an excellent application of infrared spectroscopy to the monitoring of reactions on a resin: Crowley, J. I.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 3215.

(14) The yield is based on the amount of sugar estimated to be loaded onto the resin and the amount of chromatographically pure compound isolated after the cleavage from the resin. A crude estimate of the loading (mmol g⁻¹) of sugar on the resin can be calculated using the following formula:

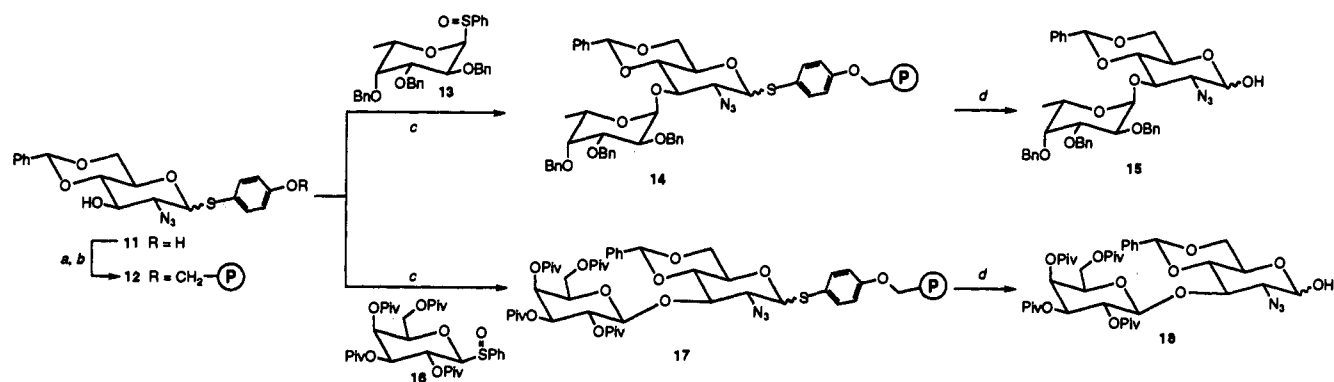
$$\frac{\Delta \text{mass}(\text{resin}) / [\text{mol wt sugar} - \text{mol wt Cl}]}{\text{mass}(\text{resin})}$$

Scheme 1



^a (a) Me_3SiCl , Et_3N , THF, 0 to 25 °C, 50 min; (b) CH_2Cl_2 , CsF , DMF, 60 °C, 24 h; (c) CF_3COOH , CH_2Cl_2 ; (d) Tf_2O , 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2 , -78 to -60 °C; (e) Ac_2O , pyridine; (f) $\text{Hg}(\text{OCOCF}_3)_2$, CH_2Cl_2 , H_2O , room temperature, 5 h.

Scheme 2



^a (a) Cs_2CO_3 , MeOH, room temperature, 3 h; (b) CH_2Cl_2 , *N*-methylpyrrolidinone, 55 °C, 24 h; (c) Tf_2O , 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2 , -60 to -30 °C; (d) $\text{Hg}(\text{OCOCF}_3)_2$, CH_2Cl_2 , H_2O , room temperature, 5 h.

on the solid phase must be able to make *both* α and β linkages stereospecifically to secondary alcohols of the type found in the Lewis blood group antigens.¹⁵ Accordingly, we focused on making disaccharides **15** and **18** on the solid phase (Scheme 2). The *p*-hydroxythiophenyl glycoside of 2-azido-2-deoxy-4,6-*O*-benzylidene-D-glucose was prepared and attached to the Merrifield resin.¹⁶

To make **15**, a solution of sulfoxide **13** (145.1 mg, 0.267 mmol, 4 equiv) and 2,6-di-*tert*-butyl-4-methylpyridine (164.7 mg, 0.802 mmol, 12 equiv) in methylene chloride (8 mL) was added to resin **12** (145.3 mg, 0.46 mmol/g, 0.067 mmol, 1 equiv), and the suspension was cooled to -60 °C. Triflic anhydride (23 μL , 0.134 mmol, 2 equiv) in methylene chloride (5 mL) was added dropwise. Twenty minutes after the addition was complete, the reaction mixture was warmed to -30 °C and kept at that temperature for 45 min. The glycosylation was repeated, and then the resin was treated with mercuric trifluoroacetate to give the desired α -linked disaccharide **15** as the only detectable sugar.¹⁷ The yield after purification was 32 mg. This represents an 89–96% yield for the glycosylation reaction if detachment gives a 70–75% yield (or 67% over the two steps). This yield is better than that which can

normally be obtained in solution for this type of glycosidic linkage. The high yield illustrates a key advantage of our strategy in which the glycosyl acceptor is attached to the resin and the glycosyl donor is in solution: one can do repetitive couplings to increase the yield of difficult glycosylations. Repetitive couplings are used routinely in peptide synthesis to obtain high yields.

To make **18**, resin **12** was treated with perpivaloylated galactose sulfoxide **16** in the same manner as described above. The desired β -linked disaccharide was isolated in 64% yield after cleavage from the resin, representing a glycosylation yield of 85–91%. The stereochemical outcome in this case was controlled by neighboring group participation of the pivaloyl group at C2.¹⁸

The work reported above establishes that the sulfoxide glycosylation reaction can be used to form *both* α and β glycosidic linkages stereospecifically and in high yield to secondary alcohols on an insoluble support. The yields we obtain are now close to those obtained in solid-phase peptide and nucleotide couplings. We believe that practical strategies for the chemical synthesis of biologically important oligosaccharides are now in sight.

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(16) A similar glucosamine derivative (with NHAc at C2) has been employed in solid-phase glycosylations; see ref 4b.

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