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Synthesis of *S*-Linked Glycosyl Amino Acids in Aqueous Solution with Unprotected Carbohydrates

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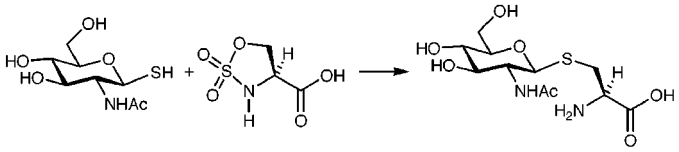
ABSTRACT

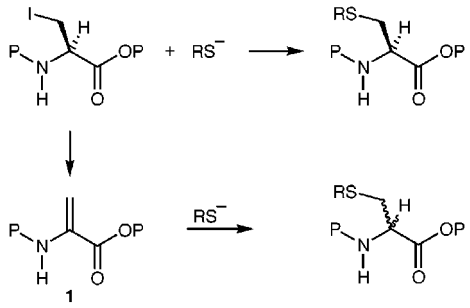


The cyclic sulfamidate 5 was synthesized in 60% overall yield from L-serine benzyl ester. Compound 5 reacted cleanly with the sodium thiolate salt of a variety of unprotected 1-thio sugars in aqueous buffer to afford the corresponding *S*-linked amino acid glycoconjugates in good yields after hydrolysis of the *N*-sulfates

O-Linked glycoproteins constitute a major class of glycoconjugates found in mammalian cells.[1] The corresponding *S*-linked glycoproteins are interesting synthesis targets as a result of their enhanced chemical stability and enzymatic resistance.[2] A major goal of our research is to develop a convergent approach for the synthesis of *S*-linked glycoproteins as stable analogues of *O*-linked wild types through the chemoselective ligation of unprotected carbohydrates with unprotected peptides in aqueous solution.[3] As a first step toward this goal, we chose to develop the principles for this concept within the framework of more simple glycosyl amino acids. This report describes the synthesis of a cyclic sulfamidate which was derived from serine and its reaction with a variety of unprotected 1-thio sugars.

Recent syntheses of *S*-linked amino acid glycosides utilized an anomeric thiolate nucleophile on a protected or support-bound sugar in a reaction with a protected β-iodoalanine (Scheme 1).[4,5] A potential limitation in the use of β-iodoalanyl derivatives as the electrophilic components is

Scheme 1



the propensity of these compounds to undergo elimination of HI, affording elimination product **1**. Subsequent Michael addition of the sulfur nucleophile to **1** results in a mixture of diastereomers that differ in configuration at the α-carbon

(1) Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317−11362.

(2) Horton, D.; Wander, J. D. *Carbohydrates: Chemistry and Biochemistry*, Vol. 4B; Pigman, W. W., Horton, D., Eds.; Academic Press: New York, 1990; pp 799−842.

(3) Chemoselective liagtions: (a) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1991**, *32*, 6739−6796. (b) Marcaurelle, L. A.; Rodriguez, E. C.; Bertozzi, C. R. *Tetrahedron Lett.* **1998**, *39*, 8417−8420. (c) Rodriguez, E. C.; Marcaurelle, L. A.; Bertozzi, C. R. *J. Org. Chem.* **1998**, *63*, 7134−7135. (d) Marcaurelle, L. A.; Bertozzi, C. R. *Chem. Eur. J.* **1999**, *5*, 1384−1390.

(4) *S*-Linked amino acid glycosides: (a) Jobron, L.; Hummel, G. *Org. Lett.* **2000**, *2*, 2265−2268. (b) Ohnishi, Y.; Ichikawa, M.; Ichikawa, Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1289−1291.

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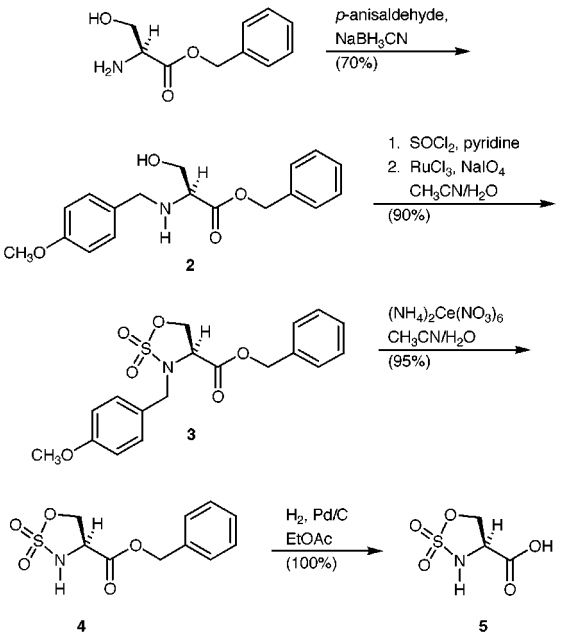
of the amino acid (Scheme 1). Under certain experimental conditions, such a pathway was in fact observed.[4b]

We hypothesized that constraining the β leaving group into a five-membered ring could reduce the rate of elimination due to poor orbital overlap between the developing enolate and the leaving oxygen atom (this transition state for elimination is the same as for a *5-endo-trig* cyclization).[6] Cyclic sulfamidate **5** (Scheme 2) was chosen as a suitable

Scheme 2

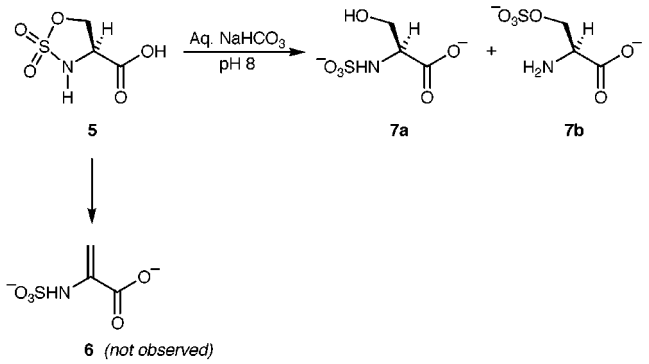


"β-alanyl" equivalent. Serine-derived cyclic sulfamidates were previously utilized in the synthesis of unnatural amino acids.[7] In a nonpeptidic system, cyclic sulfamidates were shown to react with a protected 1-thio sugar.[8]

Compound **5** was synthesized in five steps and 60% overall yield from L-serine benzyl ester (Scheme 2). First, the amine was protected by reductive amination with *p*-anisaldehyde. Cyclization of **2** with thionyl chloride cleanly afforded the corresponding cyclic sulfamidite, which was then oxidized to the protected sulfamidate **3** with catalytic Ru(III) and periodate.[9] Removal of the PMB protecting group was effected with ceric ammonium nitrate,[10] and subsequent hydrogenolysis of the benzyl ester of **4** to produce **5** was quantitative. Protection of the primary amine of serine benzyl ester, while retaining the sp^3 character of the nitrogen, was necessary to form the cyclic sulfamidate. Attempts to form a cyclic sulfamidate directly from serine benzyl ester or *N*-BOC-serine benzyl ester were unsuccessful.

Before the reactivity of **5** with sulfur nucleophiles was examined, a control experiment was performed to evaluate the stability of **5** toward hydrolysis in aqueous buffer and to determine the eagerness of **5** to provide the elimination product **6** (Scheme 3). Compound **5** was incubated in D_2O

Scheme 3



with sodium bicarbonate at pH 8 (23 °C), and its decomposition was followed by ^{1}H NMR. Loss of **5** proceeded slowly ($k = 0.034$ h^{-1}, $t_{1/2} = 20$ h) to form a mixture of the sulfamidate hydrolysis products **7a** and **7b** (Scheme 3). The elimination product **6** was not observed, suggesting that under these reaction conditions epimerization of the α-carbon did not occur.

A variety of 1-thio sugars were found to add rapidly to the β-carbon of **5**. Reaction of **5** with the sodium salt of commercially available 1-thio-β-D-glucose (**8**) in aqueous sodium bicarbonate proceeded with an initial half-life of less than 10 min to give the *N*-sulfatyl glycoconjugate intermediate in 95% yield (Scheme 4). The sulfamidate was hydrolyzed cleanly with aqueous HCl to provide **11** in 90% yield after purification by size exclusion chromatography (Biogel P-2, H_2O mobile phase). The same procedure using 1-thio-*N*-acetyl-β-D-glucosamine (**9**)[11] proceeded equally as well to afford **12**. Addition of 1-thio-α-D-glucose (**10**)[12] to **5** also afforded the corresponding *N*-sulfatyl adduct in 95%. After hydrolysis of the *N*-sulfate and size-exclusion chromatography to afford **13**, ^{1}H NMR indicated the presence of a minor component (ca. 3%), which was tentatively determined to be the product which was diastereomeric at the amino

(5) Thio-sugars: (a) Lu, P.; Hindsgaul, O.; Li, H.; Palcic, M. M. *Can. J. Chem.* **1997**, *75*, 790−800. (b) Witczk, Z. J.; Chhabra, R.; Chen, H.; Xie, X. *Carbohydr. Res.* **1997**, *301*, 167−175. (c) Eisele, T.; Windmuller, R.; Schmidt, R. R. *Carbohydr. Res.* **1998**, *306*, 81−91. (d) Fernandez, J. M. G.; Mellet, C. O.; Defaye, J. *J. Org. Chem.* **1998**, *63*, 3572−3580. (e) Auzanneau, F. I.; Bennis, K.; Fanton, E.; Prome, D.; Defaye, J.; Gelas, J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3629−3635. (f) Hummel, G.; Hindsgaul, O. *Angew. Chem., Int. Ed.* **1999**, *38*, 1782−1784. (g) Johnston, B. D.; Pinto, B. M. *J. Org. Chem.* **2000**, *65*, 4607−4617. (h) Xu, W.; Springfield, S. A.; Koh, J. T. *Carbohydr. Res.* **2000**, *325*, 169−176.

(6) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734−736.

(7) (a) Baldwin, J. E.; Spivey, A. C.; Schofield, C. J. *Tetrahedron: Asymmetry* **1990**, *1*, 881−884. (b) Boulton, L. T.; Stock, H. T.; Raphy, J.; Horwell, D. C. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1421−1429.

(8) Aguilera, B.; Fernandez-Mayoralas, A. *J. Org. Chem.* **1998**, *63*, 2719−2723.

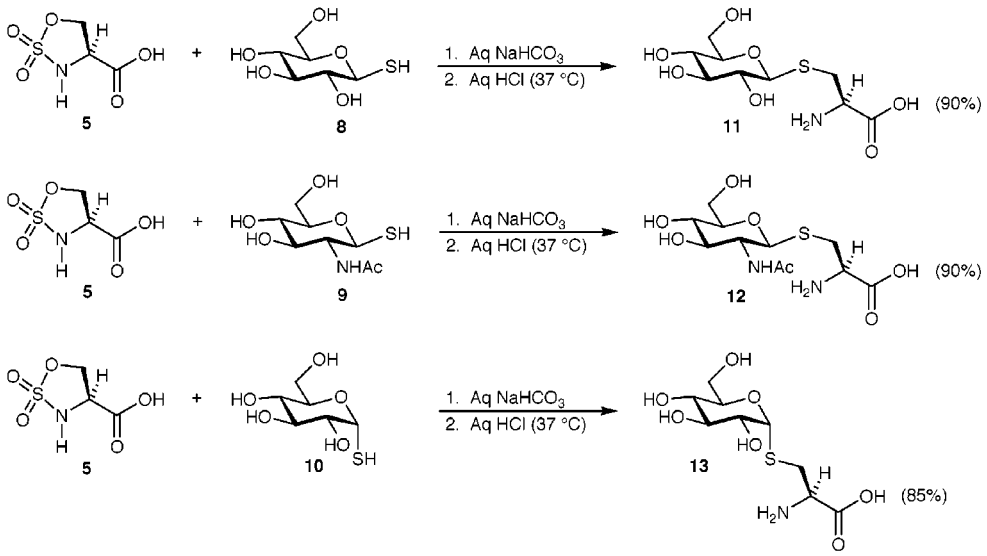
(9) Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538−7539.

(10) Yamaura, M.; Suzuki, T.; Hashimoto, H.; Yoshimura, J.; Okamoto, T.; Shin, C. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1413−1420.

(11) Obtained by deacylating the peracetylated derivative with sodium methoxide in methanol: Horton, D.; Wolfrom, M. L. *J. Org. Chem.* **1962**, *27*, 1794−1799.

(12) Obtained by deacylating the peracetylated derivative with sodium methoxide in methanol: Gadelle, A.; DeFaye, J.; Pedersen, C. *Carbohydr. Res.* **1990**, *200*, 497−498.

Scheme 4



acid α-carbon. We were unsuccessful in attempts to isolate this component in pure form for unambiguous confirmation of this assignment.

In summary, this report presents an efficient method for synthesizing *S*-linked glycosyl amino acids using unprotected carbohydrates in aqueous solution. An important feature of this method is the reduced risk of elimination and subsequent epimerization of the α-carbon by constraining the β leaving group within a ring as a cyclic sulfamidate. Current efforts in our laboratory focus on the incorporation of **5** into peptides to generate reactive peptide substrates for ligations with unprotected 1-thio sugars.

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Supporting Information Available: Full experimental procedures and tabulated ^{1}H and ^{13}C NMR and IR data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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