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Application of Serine- and Threonine-Derived Cyclic Sulfamidates for the Preparation of *S*-Linked Glycosyl Amino Acids in Solution- and Solid-Phase Peptide Synthesis

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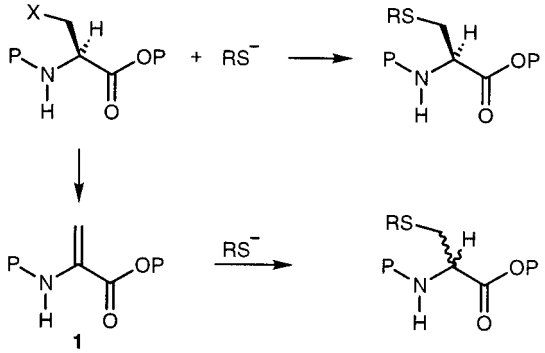
Abstract: Cyclic sulfamidates were synthesized in 60% yield from L-serine and *allo*-L-threonine, respectively. These sulfamidates reacted with a variety of unprotected 1-thio sugars in aqueous bicarbonate buffer (pH 8) to afford the corresponding *S*-linked serine- and threonine-glycosyl amino acids with good diastereoselectivity (≥97%) after hydrolysis of the *N*-sulfates. The serine-derived sulfamidate was incorporated into a simple dipeptide to generate a reactive dipeptide substrate that underwent chemoselective ligation with a 1-thio sugar to afford an *S*-linked glycopeptide. This sulfamidate was also incorporated into a peptide on a solid support in conjunction with solid-phase peptide synthesis. Chemoselective ligation of a 1-thio sugar with the cyclic sulfamidate was achieved on the solid support, followed by removal of the *N*-sulfate. Finally, the peptide chain of the resulting support-bound *S*-linked glycopeptide was extended using standard peptide synthesis procedures.

O-Linked glycoproteins constitute a major class of glycoconjugates found in mammalian cells.[1] The corresponding *S*-linked glycoproteins are desired synthesis targets as a result of their greater chemical stability and enzymatic resistance.[2] A major goal of our research is to develop a convergent approach for the synthesis of *S*-linked glycopeptides through chemoselective ligations of unprotected carbohydrates with unprotected peptides in aqueous solution or on a solid support.[3]

An intuitive method for preparing *S*-linked glycosyl amino acids is through a reaction of an anomeric thiolate with an alanine derivative containing a leaving group on the β-carbon (Scheme 1). Advantages of this method include avoiding the need for Lewis-acid mediated glycosylation protocols, and the problems inherent in such methods. A potential limitation of this route is the propensity of these alanine derivatives to eliminate HX, affording compound **1**. Subsequent Michael addition of the sulfur nucleophile to **1** results in a mixture of diastereomers that differ in configuration at the α-carbon of the amino acid.[3h] These competing pathways were in fact observed under certain experimental conditions using β-iodoalanine derivatives.[4b]

Scheme 1

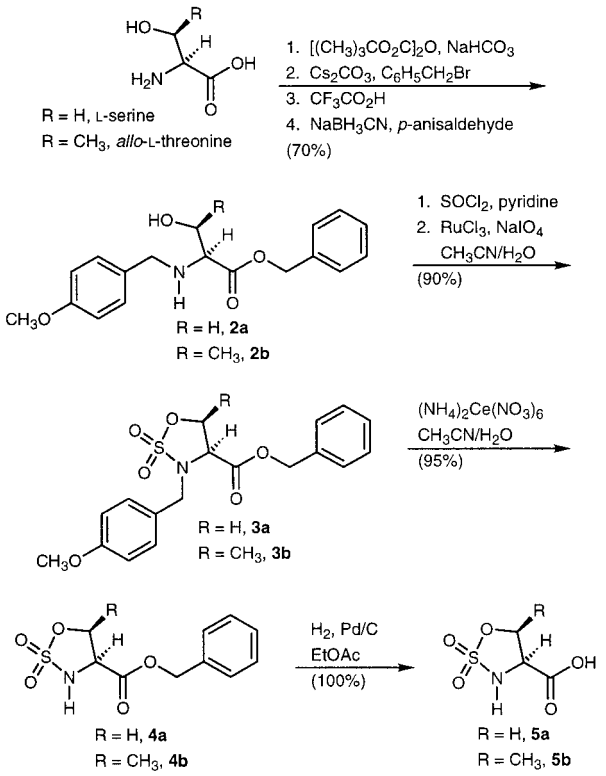


We reported the efficient preparation of *S*-linked glycosyl serine conjugates, employing the cyclic sulfamidate **5a** (Scheme 2) as the electrophilic component in reactions with a variety of unprotected 1-thio sugars in aqueous reaction solvents.[6] A cyclic sulfamidate was chosen as a useful "β-alanyl" equivalent with the hypothesis that constraining the leaving group in a five-membered ring would reduce the rate of elimination due to poor overlap between the developing enolate and the leaving oxygen

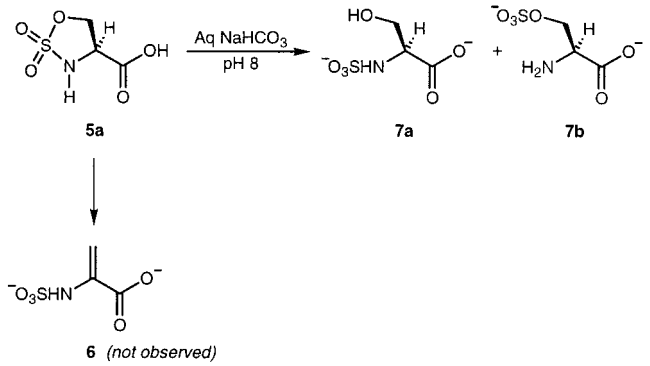
(1) Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317−11362.
(2) Horton, D.; Wander, J. D. *Carbohydrates: Chemistry and Biochemistry*; Pigman, W. W., Horton, D., Eds.; Academic Press: New York, 1990; Vol. 4B, pp 799−842.
(3) Chemoselective ligations: (a) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **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. (e) Macmillan: D.; Bill, R. M.; Sage, K. A.; Fern, D.; Flitsch, S. L. *Chem. Biol.* **2001**, *8*, 133−145. (f) Marcaurelle, L. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2001**, *123*, 1587−1595. (g) Shin, I.; Jung, H.; Lee, M. *Tetrahedron Lett.* **2001**, *42*, 1325−1328. (h) Zhu, Y.; Van der Donk, W. A. *Org. Lettt.* **2001**, *3*, 1189−1192.
(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. (c) Knapp, S.; Myers, D. S. *J. Org. Chem.* **2001**, *66*, 3636−3638.
(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) Cohen, S. B.; Halcomb, R. L. *Org. Lett.* **2001**, *3*, 405−407.

10.1021/ja011932l CCC: $22.00

Scheme 2



Scheme 3



atom (this transition state for elimination is the same as for a *5-endo-trig* cyclization).[7] Additionally, the use of aqueous reaction solvents avoids the need for complex protecting groups, particularly on the carbohydrate component. Cyclic sulfamidates had previously been utilized in the synthesis of unnatural amino acids[8] and, in a nonpeptidic system, were shown to react with a protected 1-thio sugar.[9]

In this report we provide a full account of our earlier results and report application of this method to the more difficult case of *S*-linked glycosyl threonine conjugates. Compound **5a** was also incorporated into a simple peptide to generate an electrophilic peptide substrate for chemoselective ligation with an unprotected 1-thio sugar. Finally, this chemistry was performed on a solid support in conjunction with solid-phase peptide synthesis to prepare *S*-linked glycopeptides.

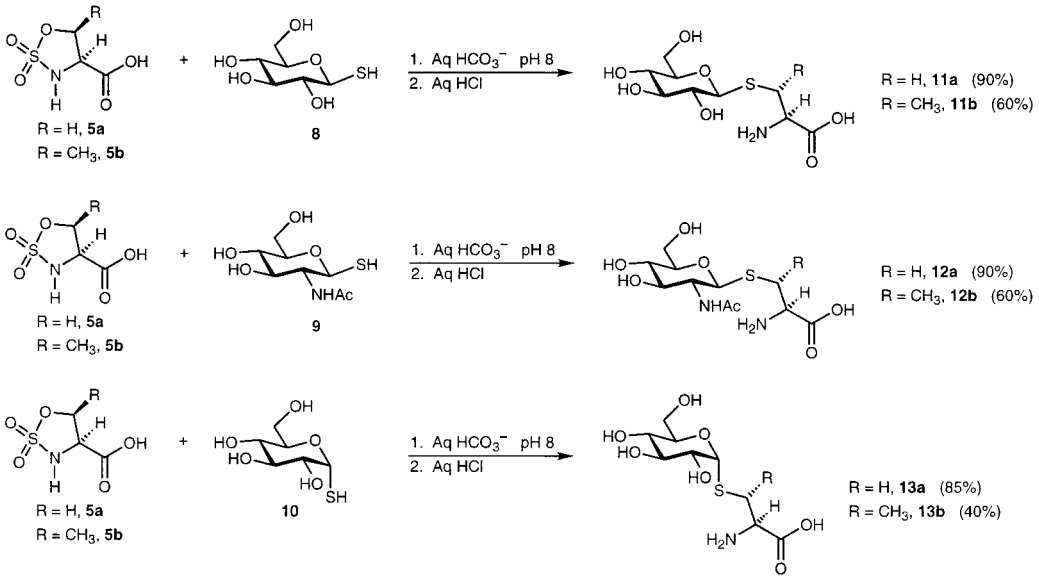
Synthesis and Characterization of Serine- and Threonine-Derived Cyclic Sulfamidates. Cyclic sulfamidates **5a** and **5b** were synthesized in 60% overall yield starting from L-serine and *allo*-L-threonine, respectively (Scheme 2). The reactivity of the cyclic sulfamidate required protection of the amino acid as a benzyl ester such that the carboxylate could be liberated under neutral conditions. The amino acid benzyl esters were protected as the *p*-methoxybenzylamines by reductive amination with *p*-anisaldehyde to provide **2a**/**2b**. Protected amino acids **2a**/**2b** reacted cleanly with thionyl chloride at −78 °C in the presence of excess pyridine to afford the corresponding cyclic sulfamidites, which were then oxidized to the sulfamidates **3a**/**3b** with catalytic Ru(III) and periodate.[10] Oxidative removal of the PMB-protecting group was effected with ceric ammonium nitrate,[11] and hydrogenolysis of the benzyl ester proceeded quantitatively to provide the desired substrates **5a** and **5b** (Scheme 2). The cheap availability of serine allowed preparation of products **3a**−**5a** on a multigram scale. Formation of the cyclic sulfamidate required protection of the primary amine while still retaining the sp^3 character of the nitrogen. Attempts to form a cyclic sulfamidate directly from serine benzyl ester or *N*-BOC-serine benzyl ester were unsuccessful.

Before the reactivity of **5a** and **5b** with sulfur nucleophiles was examined, control experiments were performed to evaluate the stability of the cyclic sulfamidates toward hydrolysis in aqueous buffer and to determine the eagerness of **5a**/**5b** to provide the undesired elimination product **6** (Scheme 3, illustrated for **5a**). Substrate **5a** (0.2 M) was incubated in D_2O with sodium bicarbonate (0.5 M) at pH 8 (23 °C), and its decomposition was followed by ^{1}H NMR. Loss of **5a** proceeded slowly ($k = 0.034$ h^{-1}, $t_{1/2} = 20$ h) to form a mixture of the hydrolysis products **7a** and **7b** (Scheme 3). Elimination product **6** was not observed, suggesting that under these conditions (pH 8), epimerization of the α-carbon does not occur. The same experiment with **5b** afforded an identical rate of hydrolysis ($k = 0.034$ h^{-1}), consistent with hydrolysis occurring at the sulfur atom rather than at the sulfamidate C−O bond. As with **5a**, the elimination product derived from **5b** was not observed.

Synthesis of *S*-Linked Glycosyl Amino Acids. After the stability of cyclic sulfamidates **5a**/**5b** in aqueous buffer was established, their reactivity with unprotected 1-thio sugars to generate *S*-linked glycosyl serine and threonine conjugates was explored (Scheme 4). A solution of substrate **5a** (0.2 M) in D_2O with sodium bicarbonate (0.5 M, pH 8, 23 °C) was treated with the sodium salt of 1-thio-β-D-glucose (**8**, 0.2 M), and the reaction was monitored by ^{1}H NMR. Thiolate addition to the β-carbon occurred with an initial half-life of ≤10 min, approximately 2 orders of magnitude faster than sulfamidate hydrolysis. The reaction was complete in 2−3 h, affording the *N*-sulfatyl glycoconjugate in ≥95% yield. The *N*-sulfate was hydrolyzed by incubation of the crude mixture in aqueous HCl (5 M) at 37 °C to provide glycoconjugate **11a** in 90% isolated yield after purification by size-exclusion chromatography (Scheme 4). Treatment of **5a** with 1-thio-*N*-acetyl-β-D-glucosamine (**9**)[12] proceeded equally as well, affording **12a** also in 90% yield. Reaction of **5a** with 1-thio-α-D-glucose (**10**)[13] proceeded 2−3-

(7) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734−736.
(8) (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.
(9) Aguilera, B.; Fernandez-Mayoralas, A. *J. Org. Chem.* **1998**, *63*, 2719−2723.
(10) Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538−7539.
(11) Yamaura, M.; Suzuki, T.; Hashimoto, H.; Yoshimura, J.; Okamoto, T.; Shin, C. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1413−1420.
(12) Obtained by deacylating the peracetylated derivative with sodium methoxide in methanol: Horton, D.; Wolfrom, M. L. *J. Org. Chem.* **1962**, *27*, 1794−1799.

Scheme 4

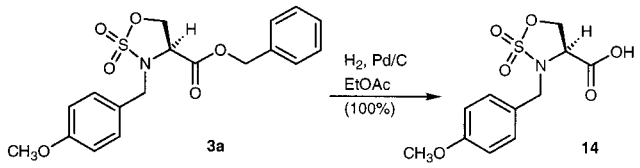


fold slower due to the lower reactivity of the more hindered axial nucleophile. However, glyconjugate **13a** was still obtained in good yield (85%, Scheme 4).

Changing the β-carbon from primary (serine) to secondary (threonine) slowed the rate of thiolate addition by 1000-fold. Treatment of **5b** (0.2 M) with **8** (0.2 M) at 23 °C (pH 8) did not afford any detectable addition product after 1 h. Increasing the temperature to 37 °C for 20 h provided a low yield (ca. 10%) of product **11b** after removal of the *N*-sulfate. The major product of the reaction sequence was *allo*-threonine, indicating that much of **5b** was lost to hydrolysis. Two changes were necessary for the thiolate addition (second-order) to better compete against hydrolysis (first-order). First, an excess (2 equiv) of 1-thio sugar was used. Second, the concentration of reactants was increased. By changing the monovalent counterion from sodium to cesium, concentrations up to 1 M could be attained. Treatment of **5b** (0.5 M) with the cesium thiolate salt of **8** (1 M) in aqueous cesium bicarbonate (1.5 M, pH 8) at 37 °C for 20 h afforded the desired conjugate as the major product. Conjugate **11b** was obtained in 60% isolated yield after removal of the *N*-sulfate (aq HCl, 37 °C) and purification by size-exclusion chromatography (Scheme 4). The *N*-acetylglucosamine conjugate **12b** was also obtained in 60% yield. The α-thio conjugate **13b** could not be obtained in higher than 40% yield.[14] Apparently the steric interactions between the β-carbon and the axial thiolate were too much for thiolate addition to effectively compete against hydrolysis. All six conjugates were obtained as a single diastereomer at the α-carbon as determined by ^{1}H NMR (≥97%).

Incorporation of the Cyclic Sulfamidate into Peptides. After a variety of *S*-linked glycosyl amino acids were successfully prepared, the cyclic sulfamidate was next incorporated into a simple peptide to generate an electrophilic peptide substrate. Carboxylic acid **14** was prepared quantitatively by hydrogenolysis of **3a** (Scheme 5). Activation of **14** (0.5 M) with PyBOP (0.5 M) in the presence of leucine benzyl ester (0.45 M), using *N*-methylmorpholine as a base, led to rapid (5 min) and complete consumption of the leucine (Scheme 6). Dipeptide **15** was obtained in 90% yield and was stable under the reaction conditions ($t_{1/2}$ > 4 h). The PMB-protecting group was cleanly removed with ceric ammonium nitrate, and hydrogenolysis of the benzyl ester was quantitative to provide the water-soluble dipeptide substrate **17**. Compound **16** was prepared directly from PyBOP-activation of **5a** in the presence of leucine benzyl ester. However, this reaction proceeded in poor and inconsistent yield (20−50%). Peptide **17** (0.1 M) underwent chemoselective ligation with **8** (0.11 M) in aqueous sodium bicarbonate (0.25 M, pH 8, 23 °C), affording the *S*-linked glycopeptide **18** in 80% isolated yield after removal of the *N*-sulfate and purification by size-exclusion chromatography (Scheme 6).

Scheme 5

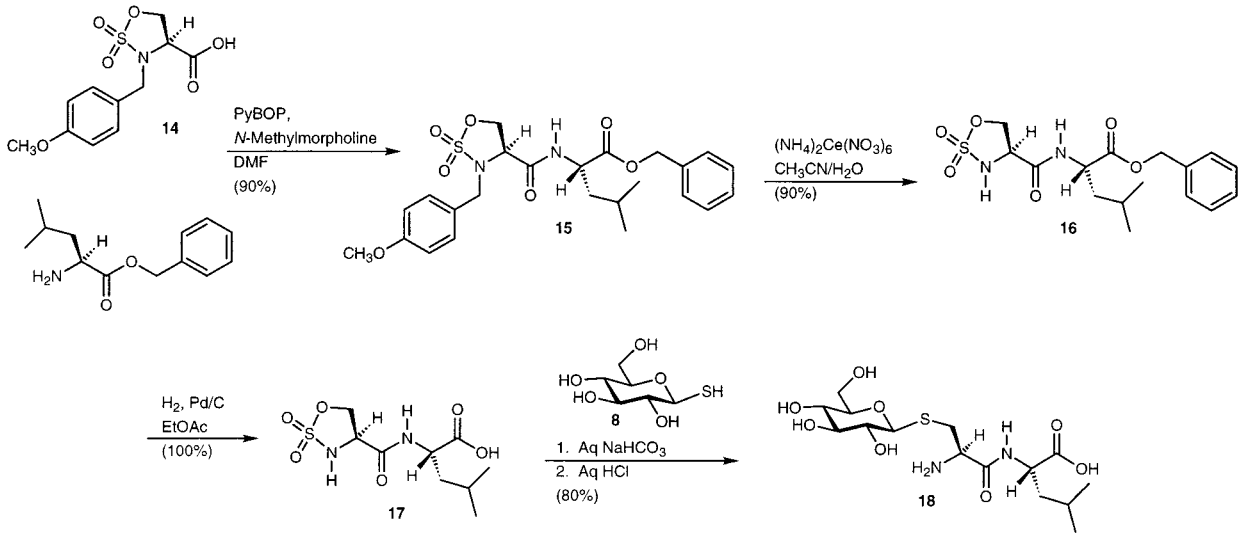


Extension of this chemistry to the synthesis of a peptide containing the cyclic sulfamidate within the interior rather than at the *N* terminus was investigated. Attempts to acylate the ring nitrogen of the sulfamidate were unsuccessful, probably because of the poor nucleophilicity of the nitrogen. In a model reaction, activation of *N*-BOC-glycine (1.5 equiv) with PyBOP (1.5 equiv) in the presence of **4a** (1 equiv) did not afford the desired *N*-acylated product after 4 h, during which time decomposition of **4a** became significant. The cyclic sulfamidate was also observed to be unstable under the basic conditions required for Fmoc removal in peptide synthesis (20% piperidine in DMF, 20 min per cycle). Compounds **3a** and **4a** had half-lives of ≤5 min in piperidine (20% in DMF) or DBU (10% in DMF). These results necessitate addition of the 1-thio sugar to the cyclic sulfamidate immediately after incorporation into the peptide

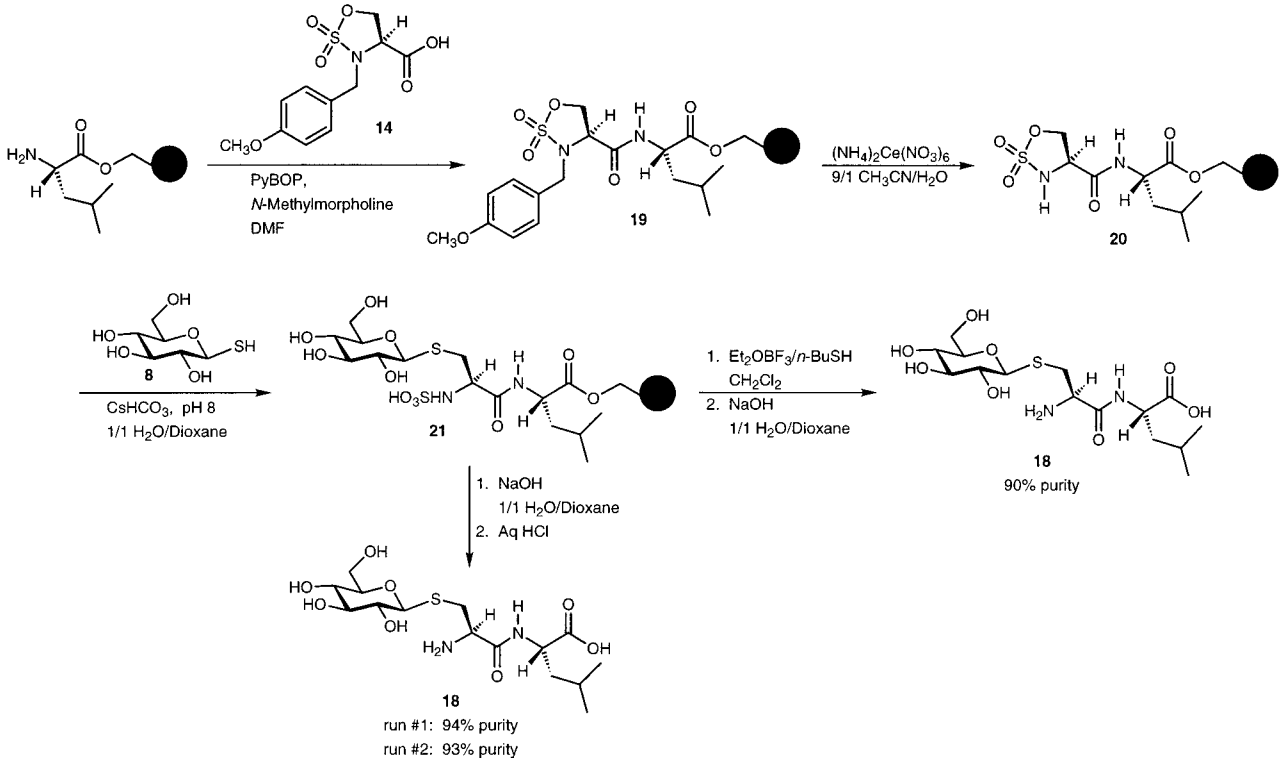
(13) Obtained by deacylating the peracetylated derivative with sodium methoxide in methanol: Gadelle, A.; DeFaye, J.; Pedersen, C. *Carbohydr. Res.* **1990**, *200*, 497−498.

(14) The preparation of **10** provided material that typically contained 1−2% of **8**. As a result of the greater reactivity of **8** relative to **10**, and the use of excess **10**, product **13b** was isolated with 4−5% **11b**, which was not separable by size-exclusion chromatography.

Scheme 6



Scheme 7



chain, followed by removal of the *N*-sulfate, to continue the peptide synthesis. This sequence was achieved on a solid support.

Synthesis of *S*-Linked Glycopeptides with Solid-Phase Peptide Synthesis. The efficiency of the cyclic sulfamidate chemistry described above prompted an investigation into employing a solid support in conjunction with Fmoc-based solid-phase peptide synthesis (SPPS). For this work, a polystyrene resin modified with poly(ethylene glycol) (3000−4000 MW PEG) was chosen for its ability to swell in both organic and aqueous solvents. The resin employed was NovaSyn TG-hydroxy resin, purchased from NovaBiochem. The success of the reactions on solid phase was evaluated in terms of the crude purity of the product rather than absolute yield, due to variations in resin loading. The initial target was compound **18**, whose solution-phase synthesis served as a standard.

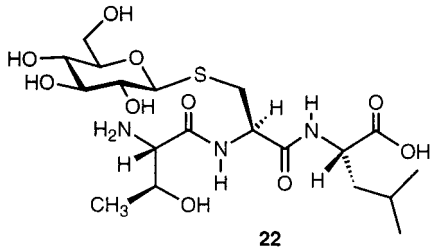
The terminal hydroxyl of the PEG was esterified with Fmoc−leucine using standard procedures.[15] Following Fmoc removal with piperidine, the resulting resin was treated with amino acid **14** (5 equiv, 0.3 M) and PyBOP (5 equiv, 0.3 M) in DMF with *N*-methylmorpholine for 30 min to provide **19** (Scheme 7). The PMB-protecting group was then removed with ceric ammonium nitrate (0.5 M) in 9/1 acetonitrile/water for 1 h to afford **20**. The addition of the cesium thiolate salt of **8** (0.5 M) was performed in 1/1 dioxane/water for 18 h. Initial runs evaluated these three transformations on solid support; the *N*-sulfate was

(15) Blankemeyer, B.; Nimtz, M.; Frank, R. *Tetrahedron Lett.* **1990**, *31*, 1701−1704.

hydrolyzed in solution. Thus, following opening of the cyclic sulfamidate with **8** to give **21**, the product was removed from the support with sodium hydroxide (0.2 M in 1/1 dioxane/water, 30 min) and the *N*-sulfate was then hydrolyzed in aqueous HCl. The reaction mixture was concentrated and analyzed directly by ^{1}H NMR, without purification (Scheme 7). Product **18** was obtained in 93% and 94% purity in two separate runs. This represents an average yield of ≥99% for each of the six transformations on the support.

The final issue that was addressed was removal of the *N*-sulfate on the solid support. Because of the slow rate of acid-catalyzed hydrolysis ($t_{1/2}$ ca. 4−6 h, 37 °C), this method was not an attractive one for solid phase, as the reaction times would likely have had to be extended severalfold. The limited solubility of the *N*-sulfatyl glycoconjugates in organic solvents necessitated use of aqueous conditions for removal of the *N*-sulfate. However, the use of a solid support allows for organic solvents as well. As an alternative to aqueous HCl, a Lewis acid-catalyzed removal of the *N*-sulfate with boron trifluoride and a thiol nucleophile was employed.[16] Support-bound intermediate **21** was treated with boron trifluoride etherate (1 M) and *n*-butanethiol (1 M) in dichloromethane for 20 h (Scheme 7). The product was then removed from the support with NaOH. Analysis by ^{1}H NMR revealed the crude purity of **18** at 90%.

The ability to remove the *N*-sulfate on the solid support enhanced the utility of the chemistry, as the peptide chain could now be extended past the site of glycosylation while still on the support. To demonstrate this point, support-bound **18** was treated with Fmoc-threonine and PyBOP. Subsequent Fmoc removal with piperidine, and cleavage from the support with NaOH, provided *S*-linked glycopeptide **22** in ca. 85% crude purity (nine steps total).



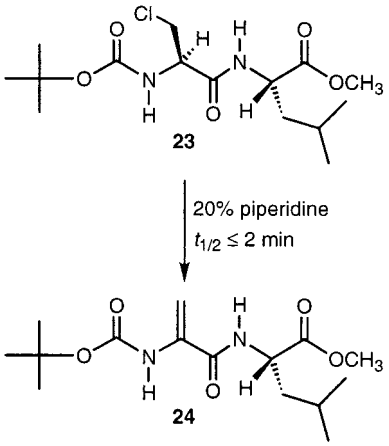
22

Scope and Future Directions

In this work exploring the chemistry of serine- and threonine-derived cyclic sulfamidates, we demonstrated their use for efficient and stereochemically controlled preparation of a variety of *S*-linked glycosyl amino acids using unprotected 1-thio sugars. The method was also extended to the construction of simple *S*-linked glycopeptides on a solid support in conjunction with SPPS. The use of the cyclic sulfamidate avoids potential drawbacks of previous methods, such as epimerization of the α-carbon; however, challenges still remain.

The primary limitation is that the cyclic sulfamidate cannot withstand the basic conditions of Fmoc removal used in peptide synthesis. Therefore, this electrophilic amino acid cannot be incorporated within the interior of a longer peptide and is currently limited to the *N* terminus. The 1-thio sugar must be incorporated immediately after the cyclic sulfamidate is installed. This places limitations on the overall convergence that can be attained in a synthesis. β-Haloalanine derivatives are not a viable solution to this problem. In early studies, we found that the β-chloroalanine dipeptide **23** was transformed to the elimination product **24** with a half-life of ca. 2 min, even faster than elimination of **3a** (Scheme 8). Derivatives with better leaving groups are anticipated to be even less stable under such conditions. Finding an electrophilic modification of an amino acid that will react with an anomeric thiolate nucleophile and yet withstand conditions of Fmoc removal and peptide synthesis has indeed been the biggest challenge in this area. The only example of this is the use of a dehydroalanine amino acid (e.g., **1**). However, the Michael addition of the 1-thio sugar was reported to afford a 1/1 mixture of diastereomers at the α-carbon.[3h] A powerful class of related electrophilic amino acids that was briefly investigated in this study consists of serine- and threonine-derived β-lactones.[17] These react readily with thiol nucleophiles, but we found cyclic sulfamidates to be more compatible with peptide synthesis under the conditions that were employed.

Scheme 8



23 **24**

Another limitation, and the subject of our current research, is that the methods described herein are compatible with monosaccharides and not di- or polysaccharides. The incompatibility arises from the conditions required to remove the *N*-sulfate. Protic and Lewis acidic reagents were observed to cleave the glycosidic linkage between saccharide units. Current work is directed at finding a milder method for removing the *N*-sulfate, perhaps by using a more specific Lewis acid. Finally, preparation of other *S*-linked glycopeptides with SPPS and a wider array of amino acids is in progress, and optimization with respect to protecting groups and deprotection schemes may be necessary. Resolving these issues should allow application of this chemistry to the synthesis of larger, more complex *S*-linked glycopeptides.

Experimental Section

General Procedures. Proton NMR (^{1}H NMR) spectra were recorded at 500 MHz. Chemical shifts are expressed in parts per million (δ) and are referenced to residual protium in the NMR solvent: $CD_3S(O)CD_2H$, δ 2.49; DOH, δ 4.80; C_6D_5H, δ 7.16; $CHCl_3$, δ 7.27. Carbon NMR (^{13}C NMR) spectra were recorded at 125 MHz. Chemical shifts (δ ppm) are referenced to the carbon signal for the solvent: DMSO-

(16) Kim, B. M.; So, S. M. *Tetrahedron Lett.* **1998**, *39*, 5381−5384.

(17) (a) Pansare, S. V.; Vederas, J. C. *J. Org Chem.* **1989**, *54*, 2311−2316. (b) Arnold, L. D.; Kalantar, T. H.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 7105−7109.

d_6, δ 39.5; $CDCl_3$, δ 77.3; C_6D_6, δ 128.4; carbon spectra recorded in D_2O are referenced to an external standard of DMSO-d_6.

***N*-(*p*-Methoxybenzyl)-L-serine Benzyl Ester (2a).** L-Serine (5.0 g, 48 mmol, 1.0 equiv) and $NaHCO_3$ (6.0 g, 72 mmol, 1.5 equiv) were dissolved in H_2O (96 mL). CH_3OH (96 mL) was added, followed by $[(CH_3)_3CO_2C]_2O$ (15.7 g, 72 mmol, 1.5 equiv). The solution was stirred at room temperature for ca. 8 h, after which TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O) showed complete conversion of serine ($R_f = 0.20$) to a less polar product ($R_f = 0.85$). The solvents were removed by rotary evaporation, and the product mixture was partitioned between HCl (1%) and EtOAc. The aqueous layer was extracted with EtOAc, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was dissolved in CH_3OH (200 mL), and Cs_2CO_3 (8.6 g, 26 mmol, 0.55 equiv) was added. Upon dissolution, the solution was concentrated. The product was dissolved in DMF (100 mL) and benzyl bromide (6.3 mL, 53 mmol, 1.1 equiv) was added. The solution was stirred at room temperature for 18 h, and the solvent was removed under reduced pressure at 40 °C. The crude mixture was partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was dissolved in CH_2Cl_2 (200 mL), and CF_3CO_2H (37 mL, 480 mmol, 10 equiv) was added. The reaction was stirred at room temperature for ca. 2 h, after which TLC (SiO_2, 1/1 hexane/EtOAc) showed complete conversion to a polar product ($R_f = 0.10$). The solution was concentrated, and the product was dissolved in CH_3OH (200 mL). CH_3CO_2H (5.7 mL, 96 mmol, 2 equiv) was added, and the reaction flask was placed in a water bath. $NaBH_3CN$ (4.5 g, 72 mmol, 1.5 equiv) was added, followed by *p*-anisaldehyde (6.3 mL, 52 mmol, 1.1 equiv). The reaction was stirred at room temperature for 12 h and was then quenched by the addition of $NaHCO_3$ (12 g, 144 mmol, 3 equiv). The suspension was concentrated, and the crude mixture was partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was purified by chromatography (silical gel, 250 mL), eluting with 1/1 hexane/EtOAc. The protected serine derivative **2a** was obtained as a clear, colorless oil (10.6 g, 70%). TLC (SiO_2, 100% EtOAc): $R_f = 0.45$. ^{1}H NMR (DMSO-d_6): δ 7.38−7.31 (m, 5 H), 7.19 (d, $J = 8.6$ Hz, 2 H), 6.85 (d, $J = 8.6$ Hz, 2 H), 5.15 (s, 2 H), 4.86 (t, $J = 5.8$ Hz, 1 H), 3.71 (s, 3 H), 3.66 (d, $J = 13.0$ Hz, 1 H), 3.62 (t, $J = 5.8$ Hz, 2 H), 3.55 (d, $J = 13.0$ Hz, 1 H), 3.31 (br t, $J = 5.0$ Hz, 1 H), 2.35 (br s, 1 H). ^{13}C NMR (DMSO-d_6): δ 173.1, 158.2, 136.3, 132.1, 129.2, 128.4, 127.9, 127.8, 113.5, 65.4, 62.6, 62.2, 55.0, 50.3. IR (cm^{-1}): 3200, 3042, 2954, 1733. HRFABMS: Calcd for $(M + H)^+$, 316.1549; found, 316.1536. Anal. calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.32; H, 6.43; N, 4.62.

(4*S*)-*N*-(*p*-Methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidinone-4-carboxylic Acid Benzyl Ester (3a). The protected serine derivative **2a** (7.7 g, 24.4 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (240 mL). Pyridine (9.8 mL, 122 mmol, 5 equiv) was added, and the solution was cooled to −78 °C. $SOCl_2$ (2.1 mL, 29.3 mmol, 1.2 equiv) was added over a period of 5 min, and the solution was stirred at −78 °C for 5 min. The dry-ice bath was removed and the reaction allowed to warm to room temperature. The reaction was quenched by the addition of HCl (1%). The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were washed with $NaHCO_3$, dried (Na_2SO_4), filtered, and concentrated. The sulfamidite was dissolved in CH_3CN (60 mL), and the solution was cooled to 0 °C. $NaIO_4$ (5.75 g, 26.8 mmol, 1.1 equiv) was added followed by $RuCl_3 \cdot xH_2O$ (50 mg, 0.24 mmol, 0.01 equiv). The reaction was initiated by addition of H_2O (60 mL), and the reaction was stirred at 0 °C for 5 min. The ice bath was removed, and the reaction was stirred for an additional 10 min. The reaction solution was partitioned between CH_2Cl_2 and $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was purified by chromatography (silica gel, 200 mL), eluting with 8/2 hexane/EtOAc to 6/4. The sulfamidate **3a** was obtained as a white solid (8.3 g, 90%). TLC (SiO_2, 1/1 hexane/EtOAc): $R_f = 0.55$. ^{1}H NMR (C_6D_6): δ 7.18−7.06 (m, 7 H), 6.69 (d, $J = 8.7$ Hz, 2 H), 4.85 (d, $J = 12.8$ Hz, 1 H), 4.81 (d, $J = 12.8$ H, 1 H), 4.27 (d, $J = 14.1$ Hz, 1 H), 4.20 (d, $J = 14.1$ Hz, 1 H), 4.15 (dd, $J = 4.4$, 9.0 Hz, 1 H), 3.74 (dd, $J = 7.5$, 9.0 Hz, 1 H), 3.46 (dd, $J = 4.4$, 7.5 Hz, 1 H), 3.30 (s, 3 H). ^{13}C NMR (C_6D_6): δ 168.1, 160.6, 135.6, 131.4, 129.2, 129.0, 126.5, 114.7, 68.1, 67.3, 58.8, 55.2, 50.7. IR (cm^{-1}): 2959, 1748, 1613, 1514. HREIMS: calcd for $(M)^+$, 377.0933; found, 377.0943. Anal. calcd for $C_{18}H_{19}NO_6S$: C, 57.28; H, 5.07; N, 3.71; S, 8.50. Found: C, 56.90; H, 4.77; N, 3.76; S, 8.82.

(4*S*)-2,2-Dioxo-1,2,3-oxathiazolidinone-4-carboxylic Acid Benzyl Ester (4a). The protected sulfamidate **3a** (8.1 g, 21.5 mmol, 1.0 equiv) was dissolved in CH_3CN (150 mL). H_2O (50 mL) was added with stirring, followed by $(NH_4)_2Ce(NO_3)_6$ (36 g, 65 mmol, 3 equiv). The reaction was stirred at room temperature for ca. 20 min, after which TLC (SiO_2, 1/1 hexane/EtOAc) showed complete conversion to a more polar product ($R_f = 0.45$). The reaction solution was partitioned between $NaHCO_3$ and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was purified by chromatography (silica gel, 200 mL), eluting with 9/1 hexane/EtOAc to 7/3. Sulfamidate **4a** was obtained as a clear, colorless oil (5.2 g, 95%). ^{1}H NMR (DMSO-d_6): δ 8.59 (d, $J = 6.0$ Hz, 1 H), 7.42−7.32 (m, 5 H), 5.22 (d, $J = 12.5$ Hz, 1 H), 5.19 (d, $J = 12.5$ Hz, 1 H), 4.81−4.70 (m, 3 H). ^{13}C NMR (DMSO-d_6): δ 168.4, 135.4, 128.5, 128.3, 128.0, 70.2, 67.0, 55.6. IR (cm^{-1}): 3269, 2958, 1746, 1188. HREIMS: calcd for $(M)^+$, 257.0358; found, 257.0346. Anal. calcd for $C_{10}H_{11}NO_5S$: C, 46.69; H, 4.31; N, 5.44; S, 12.46. Found: C, 46.74; H, 4.53; N, 5.38; S, 12.78.

(4*S*)-2,2-Dioxo-1,2,3-oxathiazolidinone Carboxylic Acid (5a). The protected sulfamidate **4a** (1.25 g, 4.86 mmol, 1.0 equiv) was dissolved in EtOAc (50 mL). Palladium-on-carbon (10 wt %, 260 mg, 0.24 mmol, 0.05 equiv) was added, and the suspension was stirred under 1 atm of hydrogen for ca. 30 min, after which TLC (SiO_2, 1/1 hexane/EtOAc) showed complete conversion to a product that did not migrate by TLC. The suspension was filtered through Celite and concentrated. The sulfamidate **5a** was used without further purification (810 mg, 100%). ^{1}H NMR (DMSO-d_6): δ 8.40 (br s, 1 H), 4.72 (dd, $J = 7.8$, 8.7 Hz, 1 H), 4.65 (dd, $J = 4.8$, 8.6 Hz, 1 H), 4.60 (dd, $J = 4.9$, 7.8 Hz, 1 H). ^{13}C NMR (DMSO-d_6): δ 170.0, 70.6, 55.7. HREIMS: calcd for $(M + H)^+$, 167.9967; found, 167.9959. Anal. calcd for $C_3H_5NO_5S$: C, 21.56; H, 3.02; N, 8.38; S, 19.18. Found: C, 21.52; H, 2.88; N, 8.33; S, 18.89.

***N*-(*p*-Methoxybenzyl)-L-*allo*-threonine Benzyl Ester (2b).** *allo*-L-Threonine (1.0 g, 8.4 mmol, 1.0 equiv) and $NaHCO_3$ (1.1 g, 12.6 mmol, 1.5 equiv) were dissolved in H_2O (17 mL). CH_3OH (17 mL) was added, followed by $[(CH_3)_3CO_2C]_2O$ (2.7 g, 12.6 mmol, 1.5 equiv). The solution was stirred at room temperature for ca. 8 h, after which TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O) showed complete conversion of *allo*-L-threonine ($R_f = 0.20$) to a less polar product ($R_f = 0.85$). The solvents were removed by rotary evaporation, and the product mixture was partitioned between HCl (1%) and EtOAc. The aqueous layer was extracted with EtOAc, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was dissolved in CH_3OH (50 mL), and Cs_2CO_3 (1.5 g, 4.6 mmol, 0.55 equiv) was added. Upon dissolution, the solution was concentrated. The product was dissolved in DMF (20 mL), and benzyl bromide (1.1 mL, 9.2 mmol, 1.1 equiv) was added. The solution was stirred at room temperature for 18 h, and the solvent was removed under reduced pressure at 40 °C. The crude mixture was partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was dissolved in CH_2Cl_2 (50 mL), and CF_3CO_2H (6.5 mL, 84 mmol, 10 equiv) was added. The reaction was stirred at room temperature for ca. 2 h, after which TLC (SiO_2, 1/1 hexane/EtOAc) showed complete conversion to a polar product ($R_f = 0.10$). The solution was concentrated, and the product was dissolved in CH_3OH (34 mL). CH_3CO_2H

(1.0 mL, 17 mmol, 2 equiv) was added, and the reaction flask was placed in a water bath. $NaBH_3CN$ (795 mg, 12.6 mmol, 1.5 equiv) was added, followed by *p*-anisaldehyde (1.1 mL, 9.2 mmol, 1.1 equiv). The reaction was stirred at room temperature for 12 h and was then quenched by the addition of $NaHCO_3$ (2.1 g, 25 mmol, 3 equiv). The suspension was concentrated, and the crude mixture was partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was purified by chromatography (silical gel, 100 mL), eluting with 8/2 hexane/EtOAc to 1/1. The protected *allo*-threonine derivative **2b** was obtained as a clear, colorless oil (2.0 g, 72%). TLC (SiO_2, 1/1 EtOAc/hexane): $R_f = 0.25$. ^1H NMR (C_6D_6): δ 7.20−7.02 (m, 7 H), 6.76 (d, $J = 8.7$ Hz, 2 H), 4.98 (d, $J = 12.3$ Hz, 1 H), 4.95 (d, $J = 12.3$ Hz, 1 H), 4.00 (m, 1 H), 3.66 (d, $J = 12.6$ Hz, 1 H), 3.45 (d, $J = 12.6$ Hz, 1 H), 3.35 (d, $J = 5.1$ Hz, 1 H), 3.30 (s, 3 H), 0.98 (d, $J = 6.4$ Hz, 3 H). ^{13}C NMR (C_6D_6): δ 173.1, 159.4, 136.2, 132.0, 129.8, 128.6, 128.4, 114.1, 67.6, 66.4, 65.8, 54.7, 52.2, 19.1. IR (cm^{-1}): 3446, 2935, 1732, 1513. HREIMS: calcd for $(M + H)^+$, 330.1705; found, 330.1698. Anal. calcd for $C_{19}H_{23}NO_4$: C, 69.28; H, 7.04; N, 4.25. Found: C, 69.58; H, 7.38; N, 4.44.

(4*S*,5*S*)-*N*-(*p*-Methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidinone-5-methyl-4-carboxylic Acid Benzyl Ester (3b). The protected *allo*-threonine derivative **2b** (2.0 g, 6.1 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (60 mL). Pyridine (2.4 mL, 31 mmol, 5 equiv) was added, and the solution was cooled to −78 °C. $SOCl_2$ (0.53 mL, 7.3 mmol, 1.2 equiv) was added over a period of 5 min, and the solution was stirred at −78 °C for 5 min. The dry ice bath was removed and the reaction allowed to warm to room temperature. The reaction was quenched by the addition of HCl (1%). The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were washed with $NaHCO_3$, dried (Na_2SO_4), filtered, and concentrated. The sulfamidite was dissolved in CH_3CN (30 mL), and the solution was cooled to 0 °C. $NaIO_4$ (1.44 g, 6.7 mmol, 1.1 equiv) was added followed by $RuCl_3 \cdot xH_2O$ (65 mg, 0.31 mmol, 0.05 equiv). The reaction was initiated by addition of H_2O (30 mL), and the reaction was stirred at 0 °C for 5 min. The ice bath was removed, and the reaction was stirred for an additional 10 min. The reaction solution was partitioned between CH_2Cl_2 and $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was purified by chromatography (silica gel, 50 mL), eluting with 8/2 hexane/EtOAc to 6/4. The sulfamidate **3a** was obtained as a clear, colorless oil (2.15 g, 90%). TLC (SiO_2, 8/2 hexane/EtOAc): $R_f = 0.20$. ^1H NMR (C_6D_6): δ 7.18−7.00 (m, 7 H), 6.65 (d, $J = 8.6$ Hz, 2 H), 4.81 (d, $J = 12.1$ Hz, 1 H), 4.77 (d, $J = 12.1$ Hz, 1 H), 4.27 (m, 1 H), 4.22 (d, $J = 14.3$ Hz, 1 H), 4.10 (d, $J = 14.3$ Hz, 1 H), 3.55 (d, $J = 6.7$ Hz, 1 H), 3.25 (s, 3 H), 0.84 (d, $J = 6.5$ Hz, 3 H). ^{13}C NMR (C_6D_6): δ 167.1, 160.6, 135.7, 131.3, 129.3, 129.2, 126.6, 114.7, 76.6, 67.6, 64.0, 55.1, 49.4, 15.8. IR (cm^{-1}): 2938, 1751, 1179. HREIMS: calcd for $(M)^+$, 391.1090; found, 391.1101. Anal. calcd for $C_{19}H_{21}NO_6S$: C, 58.30; H, 5.41; N, 3.58; S, 8.19. Found: C, 58.07; H, 5.55; N, 3.64; S, 8.12.

(4*S*,5*S*)-2,2-Dioxo-1,2,3-oxathiazolidinone-5-methyl-4-carboxylic Acid Benzyl Ester (4b). The protected sulfamidate **3b** (2.15 g, 5.5 mmol, 1.0 equiv) was dissolved in CH_3CN (40 mL). H_2O (15 mL) was added with stirring, followed by $(NH_4)_2Ce(NO_3)_6$ (9.0 g, 16.5 mmol, 3 equiv), thus affording the following concentrations of reactants at the onset of the reaction: **3b**, 0.1 M; $(NH_4)_2Ce(NO_3)_6$, 0.3 M. The reaction was stirred at room temperature for 30 min, and the solution was partitioned between $NaHCO_3$ and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was purified by chromatography (silica gel, 100 mL), eluting with 9/1 hexane/EtOAc to 7/3. Sulfamidate **4b** was obtained as a white solid (1.4, 95%). TLC (SiO_2, 8/2 hexane/EtOAc): $R_f = 0.20$. ^1H NMR ($CDCl_3$): δ 7.40 (m, 5 H), 5.33 (d, $J = 11.7$ Hz, 1 H), 5.29 (br d, $J = 7.2$ Hz, 1 H), 5.25 (d, $J = 11.7$ Hz, 1 H), 5.08 (m, 1 H), 4.58 (t, $J = 7.3$ Hz, 1 H), 1.31 (d, $J = 7.6$ Hz, 3 H). ^{13}C NMR ($CDCl_3$): δ 166.9, 134.0, 129.5, 129.3, 129.1, 79.7, 69.1, 60.3, 15.8. IR (cm^{-1}): 3243, 2989, 1727. HREIMS: calcd for $(M)^+$, 271.0514; found, 271.0505. Anal. calcd for $C_{11}H_{13}NO_5S$: C, 48.70; H, 4.83; N, 5.16; S, 11.82. Found: C, 48.84; H, 4.91; N, 5.28; S, 12.00.

(4*S*,5*S*)-2,2-Dioxo-1,2,3-oxathiazolidinone-5-methyl-4-carboxylic Acid (5b). The protected sulfamidate **4b** (590 mg, 2.18 mmol, 1.0 equiv) was dissolved in EtOAc (30 mL). Palladium-on-carbon (10 wt %, 230 mg, 0.22 mmol, 0.1 equiv) was added, and the suspension was stirred under 1 atm of hydrogen for ca. 30 min, after which TLC (SiO_2, 1/1 hexane/EtOAc) showed complete conversion to a product that did not migrate by TLC. The suspension was filtered through Celite and concentrated. The sulfamidate **5b** was used without further purification (390 mg, 100%). ^1H NMR (DMSO-d_6): δ 8.25 (br s, 1 H), 5.14 (m, 1 H), 4.53 (d, $J = 6.6$ Hz, 1 H), 1.34 (d, $J = 6.5$ Hz, 3 H). ^{13}C NMR (DMSO-d_6): δ 168.4, 80.1, 60.3, 15.5. HREIMS: calcd for $(M + H)^+$, 182.0123; found, 182.0111. Anal. calcd for $C_4H_7NO_5S$: C, 26.52; H, 3.89; N, 7.73 S, 17.70. Found: C, 26.72; H, 3.61; N, 7.57; S, 17.91.

1-Deoxy-1-thio-β-D-glucose, Sodium Thiolate Salt (8). 2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose (670 mg, 1.65 mmol, 1.0 equiv) was dissolved in CH_3OH (20 mL). Sodium methoxide (6.6 mL, 500 mM in CH_3OH, 3.3 mmol, 2.0 equiv) was added, and the reaction was stirred at room temperature for 2 h. The reaction was quenched by the addition of $NaHCO_3$ (280 mg, 3.3 mmol, 2.0 equiv). The solvent was removed by rotary evaporation, and the product was used without purification. (360 mg, 100%).

1-Deoxy-1-thio-β-D-glucose, Cesium Thiolate Salt (8). 2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose (940 mg, 2.32 mmol, 1.0 equiv) was dissolved in CH_3OH (25 mL). Cs_2CO_3 (1.5 g, 4.6 mmol, 2.0 equiv) was added, and the solution was stirred at room temperature for 18 h. The solvent was removed by rotary evaporation, and the product was used without purification. (760 mg, 100%).

2-Acetamido-1,2-dideoxy-1-thio-β-D-glucose, Sodium Thiolate Salt (9). 2-Acetamido-3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-β-D-glucopyranose[12] (655 mg, 1.62 mmol, 1.0 equiv) was dissolved in CH_3OH (25 mL). Sodium methoxide (6.5 mL, 500 mM in CH_3OH, 3.25 mmol, 2.0 equiv) was added, and the reaction was stirred at room temperature for 2 h. The reaction was quenched by the addition of $NaHCO_3$ (270 mg, 3.25 mmol, 2.0 equiv). The solvent was removed by rotary evaporation, and the product was used without purification. (420 mg, 100%). ^1H NMR (D_2O): δ 4.62 (d, $J = 9.6$ Hz, 1 H), 3.80 (dd, $J = 2.1$, 12.3 Hz, 1 H), 3.62 (dd, $J = 5.9$, 12.3 Hz, 1 H), 3.48 (m, 1 H), 3.40−3.30 (m, 3 H), 1.97 (s, 3 H). ^{13}C NMR (D_2O): δ 174.2, 82.3, 80.0, 76.6, 70.8, 62.0, 61.6, 22.9. Mass spectrometry (EI, ESI, FAB) afforded signals corresponding to the symmetric disulfide.

2-Acetamido-1,2-dideoxy-1-thio-β-D-glucose, Cesium Thiolate Salt (9). 2-Acetamido-3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-β-D-glucopyranose[12] (810 mg, 2.0 mmol, 1.0 equiv) was dissolved in CH_3OH (25 mL). Cs_2CO_3 (1.3 g, 4.0 mmol, 2.0 equiv) was added, and the solution was stirred at room temperature for 18 h. The solvent was removed by rotary evaporation, and the product was used without purification. (740 mg, 100%).

1-Deoxy-1-thio-α-D-glucose, Sodium Thiolate Salt (10). 2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose[13] (380 mg, 0.94 mmol, 1.0 equiv) was dissolved in CH_3OH (20 mL). Sodium methoxide (3.8 mL, 500 mM in CH_3OH, 1.9 mmol, 2.0 equiv) was added, and the reaction was stirred at room temperature for 2 h. The reaction was quenched by the addition of $NaHCO_3$ (160 mg, 1.9 mmol, 2.0 equiv). The solvent was removed by rotary evaporation, and the product was used without purification. (205 mg, 100%). ^1H NMR (D_2O): δ 5.56 (d, $J = 5.3$ Hz, 1 H), 4.09 (dt, $J = 3.6$, 9.9 Hz, 1 H), 3.75 (m, 3 H), 3.48 (dd, $J = 5.4$, 9.2 Hz, 1 H), 3.32 (t, $J = 9.6$ Hz, 1 H). ^{13}C NMR (D_2O): δ 83.7, 74.1, 72.2, 70.3, 70.1, 61.0. Mass spectrometry (EI, ESI, FAB) afforded signals corresponding to the symmetric disulfide.

1-Deoxy-1-thio-α-D-glucose, Cesium Thiolate Salt (10). 2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose[13] (810 mg, 2.0

mmol, 1.0 equiv) was dissolved in CH_3OH (30 mL). Cs_2CO_3 (1.3 g, 4.0 mmol, 2.0 equiv) was added, and the solution was stirred at room temperature for 18 h. The solvent was removed by rotary evaporation, and the product was used without purification. (655 mg, 100%).

***S*-β-D-Glucopyranosyl-L-cysteine (11a).** The sodium salt of 1-thio-β-D-glucose (**8**) (360 mg, 1.65 mmol, 1.0 equiv) was dissolved in H_2O (8 mL). In a second flask, $NaHCO_3$ (345 mg, 4.1 mmol, 2.5 equiv) was added to **5a** (275 mg, 1.65 mmol, 1.0 equiv). The reaction was initiated by addition of the sodium thiolate solution to the flask containing **5a**, thus affording the following concentrations of reactants at the onset of the reaction: **5a**, 0.2 M; **8**, 0.2 M; $NaHCO_3$, 0.5 M. The reaction was stirred at room temperature for 4 h and then concentrated by rotary evaporation under reduced pressure. To hydrolyze the sulfamidate, the crude material was dissolved in HCl (5 M, 20 mL). The hydrolysis reaction was incubated at 37 °C for 24 h and then concentrated by rotary evaporation under reduced pressure. The product was dissolved in H_2O (10 mL), and the pH was brought to neutral by the addition of $NaHCO_3$ (ca. 600 mg). The solution was passed through a nitrocellulose filter (0.45 μm). Product **11a** was purified over a column of Biogel P-2 (fine, column dimensions 2.5 cm i.d. × 70 cm L) eluting with H_2O (20 mL/h). Fractions containing the desired product were identified by TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O, $R_f = 0.25$) with ninhydrin staining. Product **11a** was obtained as a white solid (430 mg, 90%). ^{1}H NMR (D_2O, HCl salt): δ 4.40 (d, $J = 9.9$ Hz, 1 H), 4.23 (dd, $J = 4.2$, 7.6 Hz, 1 H), 3.70 (dd, $J = 2.3$, 12.5 Hz, 1 H), 3.52 (dd, $J = 5.6$, 12.7 Hz, 1 H), 3.36−3.30 (m, 3 H), 3.28 (dd, $J = 4.2$, 15.6 Hz, 1 H), 3.21 (dd, $J = 9.2$, 9.7 Hz, 1 H), 3.07 (dd, $J = 7.6$, 15.7 Hz, 1 H). ^{13}C NMR (D_2O, HCl salt): δ 170.2, 85.3, 80.3, 77.4, 72.2, 68.9, 61.4, 53.6, 30.2. HRFABMS: calcd for $(M + H)^+$, 284.0804; found, 284.0805.

***S*-2-Acetamido-2-deoxy-β-D-glucopyranosyl-L-cysteine (12a).** The sodium salt of 1-thio-*N*-acetyl-β-D-glucosamine (**9**) (420 mg, 1.62 mmol, 1.0 equiv) was dissolved in H_2O (8 mL). In a second flask, $NaHCO_3$ (340 mg, 4.1 mmol, 2.5 equiv) was added to **5a** (270 mg, 1.62 mmol, 1.0 equiv). The reaction was initiated by addition of the sodium thiolate solution to the flask containing **5a**, thus affording the following concentrations of reactants at the onset of the reaction: **5a**, 0.2 M; **9**, 0.2 M; $NaHCO_3$, 0.5 M. The reaction was stirred at room temperature for 4 h and then concentrated by rotary evaporation under reduced pressure. To hydrolyze the sulfamidate, the crude material was dissolved in HCl (5 M, 20 mL). The hydrolysis reaction was incubated at 37 °C for 24 h and then concentrated by rotary evaporation under reduced pressure. The product was dissolved in H_2O (10 mL), and the pH was brought to neutral by the addition of $NaHCO_3$ (ca. 600 mg). The solution was passed through a nitrocellulose filter (0.45 μm). Product **12a** was purified over a column of Biogel P-2 (fine, column dimensions 2.5 cm i.d. × 70 cm L) eluting with H_2O (20 mL/h). Fractions containing the desired product were identified by TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O, $R_f = 0.25$) with ninhydrin staining. Product **12a** was obtained as a white solid (470 mg, 90%). ^{1}H NMR (D_2O, HCl salt): δ 4.42 (d, $J = 10.4$ Hz, 1 H), 4.14 (dd, $J = 4.3$, 7.4 Hz, 1 H), 3.67 (dd, $J = 2.2$, 12.4 Hz, 1 H), 3.58 (t, $J = 10.2$ Hz, 1 H), 3.49 (dd, $J = 5.2$, 12.4 Hz, 1 H), 3.35 (t, $J = 9.2$ Hz, 1 H), 3.30−3.24 (m, 2 H), 3.21 (dd, $J = 4.3$, 15.6 Hz, 1 H), 2.95 (dd, $J = 7.4$, 15.6 Hz, 1 H), 1.79 (s, 3 H). ^{13}C NMR (D_2O, HCl salt): δ 174.8, 169.9, 83.9, 80.0, 74.8, 69.6, 60.8, 54.2, 53.1, 30.0, 22.3. HRFABMS: calcd for $(M + H)^+$, 325.1069; found, 325.1057.

***S*-α-D-Glucopyranosyl-L-cysteine (13a).** The sodium salt of 1-thio-α-D-glucose (**10**) (205 mg, 0.94 mmol, 1.0 equiv) was dissolved in H_2O (4.7 mL). In a second flask, $NaHCO_3$ (200 mg, 2.4 mmol, 2.5 equiv) was added to **5a** (157 mg, 0.94 mmol, 1.0 equiv). The reaction was initiated by addition of the sodium thiolate solution to the flask containing **5a**, thus affording the following concentrations of reactants at the onset of the reaction: **5a**, 0.2 M; **10**, 0.2 M; $NaHCO_3$, 0.5 M. The reaction was stirred at room temperature for 4 h and then concentrated by rotary evaporation under reduced pressure. To hydrolyze the sulfamidate, the crude material was dissolved in HCl (5 M, 15 mL). The hydrolysis reaction was incubated at 37 °C for 12 h, then concentrated by rotary evaporation under reduced pressure. The product was dissolved in H_2O (10 mL), and the pH was brought to neutral by the addition of $NaHCO_3$ (ca. 600 mg). The solution was passed through a nitrocellulose filter (0.45 μm). Product **13a** was purified over a column of Biogel P-2 (fine, column dimensions 2.5 cm i.d. × 70 cm L) eluting with H_2O (20 mL/h). Fractions containing the desired product were identified by TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O, $R_f = 0.25$) with ninhydrin staining. Product **13a** was obtained as a white solid (225 mg, 85%). ^{1}H NMR (D_2O, HCl salt): δ 5.38 (d, $J = 5.6$ Hz, 1 H), 4.37 (dd, $J = 4.0$, 6.2 Hz, 1 H), 3.89 (m, 1 H), 3.80 (dd, $J = 2.2$, 12.3 Hz, 1 H), 3.76 (dd, $J = 6.2$, 12.4 Hz, 1 H), 3.66 (dd, $J = 6.1$, 12.6 Hz, 1 H), 3.44 (t, $J = 9.6$ Hz, 1 H), 3.30 (t, $J = 9.6$ Hz, 1 H), 3.28 (dd, $J = 6.4$, 15.3 Hz, 1 H), 3.17 (dd, $J = 4.1$, 15.3 Hz, 1 H). ^{13}C NMR (D_2O, HCl salt): δ 170.1, 87.1, 73.5, 73.1, 70.9, 69.7, 60.7, 53.3, 31.0. HRFABMS: calcd for $(M + Na)^+$, 306.0623; found, 306.0631.

***S*-β-D-Glucopyranosyl-β-deoxy-β-thio-L-threonine (11b).** The cesium salt of 1-thio-β-D-glucose (**8**) (760 mg, 2.3 mmol, 2.0 equiv) was dissolved in H_2O (2 mL). In a second flask, $CsHCO_3$ (670 mg, 3.5 mmol, 3.0 equiv) was added to **5b** (210 mg, 1.16 mmol, 1.0 equiv). The reaction was initiated by addition of the cesium thiolate solution to the flask containing **5b**, thus affording the following concentrations of reactants at the onset of the reaction: **5b**, 0.5 M; **8**, 1.0 M; $CsHCO_3$, 1.5 M. The reaction was incubated at 37 °C for 20 h, then concentrated by rotary evaporation under reduced pressure. To hydrolyze the sulfamidate, the crude material was dissolved in HCl (5 M, 10 mL). The hydrolysis reaction was incubated at 37 °C for 24 h and then concentrated by rotary evaporation under reduced pressure. The product was dissolved in H_2O (10 mL), and the pH was brought to neutral by the addition of $NaHCO_3$ (ca. 600 mg). The solution was passed through a nitrocellulose filter (0.45 μm). Product **11b** was purified over a column of Biogel P-2 (fine, column dimensions 2.5 cm i.d. × 70 cm L) eluting with H_2O (20 mL/h). Fractions containing the desired product were identified by TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O, $R_f = 0.25$) with ninhydrin staining and were followed by fractions containing *allo*-threonine ($R_f = 0.30$). Fractions containing **11b** were pooled and concentrated. The product (ca. 90% purity) was dissolved in H_2O (4 mL) and eluted a second time over the Biogel column, affording a pure product. Product **11a** was obtained as a white solid (205 mg, 60%). ^{1}H NMR (D_2O, HCl salt): δ 4.49 (d, $J = 10.0$ Hz, 1 H), 4.13 (d, $J = 4.3$ Hz, 1 H), 3.72 (dd, $J = 1.2$, 12.3 Hz, 1 H), 3.65 (m, 1 H), 3.53 (m, 1 H), 3.32−3.24 (m, 3 H), 3.10 (dd, $J = 8.7$, 10.0 Hz, 1 H), 1.32 (d, $J = 7.4$ Hz, 3 H). ^{13}C NMR (D_2O, HCl salt): δ 169.8, 85.4, 79,6, 77.1, 72.5, 69.2, 60.7, 58.0, 41.0, 18.9. HRFABMS: calcd for $(M + H)^+$, 298.0960; found, 298.0962.

***S*-2-Acetamido-2-deoxy-β-D-glucopyranosyl-β-deoxy-β-thio-L-threonine (12b).** The cesium salt of 1-thio-*N*-acetyl-β-D-glucosamine (**9**) (740 mg, 2.0 mmol, 2.0 equiv) was dissolved in H_2O (2 mL). In a second flask, $CsHCO_3$ (580 mg, 3.0 mmol, 3.0 equiv) was added to **5b** (180 mg, 1.0 mmol, 1.0 equiv). The reaction was initiated by addition of the cesium thiolate solution to the flask containing **5b**, thus affording the following concentrations of reactants at the onset of the reaction: **5b**, 0.5 M; **9**, 1.0 M; $CsHCO_3$, 1.5 M. The reaction was incubated at 37 °C for 20 h, then concentrated by rotary evaporation under reduced pressure. To hydrolyze the sulfamidate, the crude material was dissolved in HCl (5 M, 10 mL). The hydrolysis reaction was incubated at 37 °C for 24 h, then concentrated by rotary evaporation under reduced pressure. The product was dissolved in H_2O (10 mL), and the pH was brought to neutral by the addition of $NaHCO_3$ (ca. 600 mg). The solution was passed through a nitrocellulose filter (0.45 μm). Product **12b** was purified over a column of Biogel P-2 (fine, column dimensions 2.5 cm i.d. × 70 cm L) eluting with H_2O (20 mL/h). Fractions containing the desired product were identified by TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O, $R_f = 0.25$) with ninhydrin staining and were followed by fractions containing *allo*-threonine ($R_f = 0.30$). Fractions

containing **12b** were pooled and concentrated. The product (ca. 90% purity) was dissolved in H_2O (4 mL) and eluted a second time over the Biogel column, affording a pure product. Product **12b** was obtained as a white solid (200 mg, 60%). ^{1}H NMR (D_2O, HCl salt): δ 4.41 (d, J = 10.5 Hz, 1 H), 3.92 (d, J = 4.1 Hz, 1 H), 3.53 (dd, J = 2.0, 12.6 Hz, 1 H), 3.41 (m, 1 H), 3.35 (dd, J = 4.7, 12.4 Hz, 1 H), 3.33 (t, J = 10.1 Hz, 1 H), 3.20 (t, J = 9.2 Hz, 1 H), 3.10 (m, 2 H), 1.66 (s, 3 H), 1.07 (d, J = 7.4 Hz, 3 H). ^{13}C NMR (D_2O, HCl salt): δ 174.7, 169.6, 85.0, 79.5, 74.8, 69.5, 60.6, 57.8, 54.8, 41.0, 22.3, 18.9. HRFABMS: calcd for $(M + H)^+$, 339.1226; found, 339.1220.

***S*-α-D-Glucopyranosyl-*β*-deoxy-*β*-thio-L-threonine (13b).** The cesium salt of 1-thio-α-D-glucose (**10**) (655 mg, 2.0 mmol, 2.0 equiv) was dissolved in H_2O (2 mL). In a second flask, $CsHCO_3$ (580 mg, 3.0 mmol, 3.0 equiv) was added to **5b** (180 mg, 1.0 mmol, 1.0 equiv). The reaction was initiated by addition of the cesium thiolate solution to the flask containing **5b**, thus affording the following concentrations of reactants at the onset of the reaction: **5b**, 0.5 M; **10**, 1 M; $CsHCO_3$, 1.5 M. The reaction was incubated at 37 °C for 20 h and then concentrated by rotary evaporation under reduced pressure. To hydrolyze the sulfamidate, the crude material was dissolved in HCl (5 M, 10 mL). The hydrolysis reaction was incubated at 37 °C for 12 h and then concentrated by rotary evaporation under reduced pressure. The product was dissolved in H_2O (10 mL), and the pH was brought to neutral by the addition of $NaHCO_3$ (ca. 600 mg). The solution was passed through a nitrocellulose filter (0.45 μm). Product **13b** was purified over a column of Biogel P-2 (fine, column dimensions 2.5 cm i.d. × 70 cm L) eluting with H_2O (20 mL/h). Fractions containing the desired product were identified by TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O, R_f = 0.25) with ninhydrin staining and were followed by fractions containing *allo*-threonine (R_f = 0.30). Fractions containing **13b** were pooled and concentrated. The product (ca. 90% purity) was dissolved in H_2O (4 mL) and eluted a second time over the Biogel column, affording a pure product. Product **13b** was obtained as a white solid (120 mg, 40%). ^{1}H NMR (D_2O, HCl salt): δ 5.40 (d, J = 5.5 Hz, 1 H), 4.25 (d, J = 4.0 Hz, 1 H), 3.91 (m, 1 H), 3.78 (dd, J = 2.2, 12.5 Hz, 1 H), 3.73 (dd, J = 5.7, 10.0 Hz, 1 H), 3.66 (m, 2 H), 3.44 (t, J = 9.6 Hz, 1 H), 3.30 (t, J = 9.5 Hz, 1 H), 1.40 (d, J = 7.3 Hz, 3 H). ^{13}C NMR (D_2O, HCl salt): δ 170.1, 85.1, 73.5, 73.0, 71.0, 69.7, 60.6, 58.1, 39.1, 19.2. HRFABMS: calcd for $(M + H)^+$, 298.0960; found, 298.0969.

(4*S*)-*N*-(*p*-Methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidinone-4-carboxylic Acid (14). The protected sulfamidate **3a** (3.0 g, 8.0 mmol, 1.0 equiv) was dissolved in EtOAc (100 mL). Palladium-on-carbon (10 wt %, 840 mg, 0.8 mmol, 0.1 equiv) was added, and the suspension was stirred under 1 atm of hydrogen for ca. 3 h, after which TLC (SiO_2, 1/1 hexane/EtOAc) showed complete conversion to a product that did not migrate by TLC. The suspension was filtered through Celite and concentrated. The sulfamidate **14** was used without further purification (2.3 g, 100%). ^{1}H NMR (DMSO-d_6): δ 7.31 (d, J = 8.7 Hz, 2 H), 6.92 (d, J = 8.7 Hz, 2 H), 4.75 (dd, J = 7.7, 9.1 Hz, 1 H), 4.67 (dd, J = 4.5, 9.1 Hz, 1 H), 4.39 (d, J = 14.3 Hz, 1 H), 4.35 (dd, J = 4.4, 7.7 Hz, 1 H), 4.30 (d, J = 14.3 Hz, 1 H), 3.74 (s, 3 H). ^{13}C NMR (DMSO-d_6): δ 169.7, 159.2, 130.4, 126.6, 113.9, 68.3, 59.2, 55.1, 49.9. HRFABMS: calcd for $(M)^+$, 287.0464; found, 287.0463. Anal. calcd for $C_{11}H_{13}NO_6S$: C, 45.99; H, 4.56; N, 4.88; S, 11.16. Found: C, 45.88; H, 4.83; N, 5.05; S, 11.38.

(4*S*)-*N*-(*p*-Methoxybenzyl)-2,2-Dioxo-1,2,3-oxathiazolidinone-4-carboxyl-leucine benzyl ester (15). Sulfamidate **14** (1.02 g, 3.55 mmol, 1.0 equiv) and PyBOP (1.85 g, 3.55 mmol, 1.0 equiv) were dissolved in DMF (7 mL). Leucine benzyl ester (700 mg, 3.20 mmol, 0.9 equiv) was added, and the reaction was initiated by the addition of *N*-methylmorpholine (0.97 mL, 8.9 mmol, 2.5 equiv). The reaction was stirred at room temperature for 5 min, and the DMF was then removed by rotary evaporation under reduced pressure at 30 °C. The product was immediately purified by chromatography (silica gel, 200 mL), eluting with 8/2 hexane/EtOAc to 6/4. Dipeptide **15** was obtained as a clear, colorless oil (1.4 g, 90%). TLC (SiO_2, 1/1 EtOAc/hexane): R_f = 0.45. ^{1}H NMR (C_6D_6): δ 7.25−7.00 (m, 7 H), 6.76 (d, J = 8.6 Hz, 2 H), 5.02 (d, J = 12.3 Hz, 1 H), 4.94 (d, J = 12.3 Hz, 1 H), 4.74 (m, 1 H), 4.30 (dd, J = 3.8, 9.0 Hz, 1 H), 4.28 (d, J = 13.9 Hz, 1 H), 4.00 (t, J = 8.6 Hz, 1 H), 3.88 (d, J = 13.9 Hz, 1 H), 3.75 (dd, J = 3.7, 8.1 Hz, 1 H), 3.32 (s, 3 H), 1.70−1.58 (m, 2 H), 1.48 (m, 1 H), 0.81 (d, J = 6.3 Hz, 3 H), 0.78 (d, J = 6.3 Hz, 3 H). ^{13}C NMR (C_6D_6): δ 172.5, 168.3, 160.8, 136.3, 131.7, 129.2, 128.9, 115.1, 69.2, 67.5, 62.1, 55.2, 53.8, 51.7, 41.6, 25.2, 23.2, 22.0. IR (cm^{-1}): 3562, 2958, 1747, 1514. HRFABMS: calcd for $(M + H)^+$, 491.1852; found, 491.1854. Anal. calcd for $C_{24}H_{30}N_2O_7S$: C, 58.76; H, 6.16; N, 5.71; S, 6.54. Found: C, 58.92; H, 6.36; N, 5.64; S, 6.86.

(4*S*)-2,2-Dioxo-1,2,3-oxathiazolidinone-4-carboxyl-leucine Benzyl Ester (16). Dipeptide **15** (1.10 g, 2.24 mmol, 1.0 equiv) was dissolved in CH_3CN (20 mL). H_2O (2 mL) was added with stirring, followed by $(NH_4)_2Ce(NO_3)_6$ (3.7 g, 6.7 mmol, 3.0 equiv). The reaction was stirred at room temperature for ca. 30 min, after which TLC (SiO_2, 1/1 hexane/EtOAc) showed complete conversion to a more polar product (R_f = 0.25). The reaction solution was partitioned between $NaHCO_3$ and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The product was purified by chromatography (silica gel, 50 mL), eluting with 8/2 hexane/EtOAc to 1/1. Dipeptide **16** was obtained as a clear, colorless oil (750 mg, 90%). ^{1}H NMR (C_6D_6): δ 7.19−7.03 (m, 6 H), 5.05 (br d, J = 7.9 Hz, 1 H), 5.03 (d, J = 12.2 Hz, 1 H), 4.96 (d, J = 12.2 Hz, 1 H), 4.83 (m, 1 H), 4.42 (dd, J = 4.0, 8.9 Hz, 1 H), 4.06 (t, J = 8.5 Hz, 1 H), 3.76 (m, 1 H), 1.73 (m, 1 H), 1.61 (m, 1 H), 1.50 (m, 1 H), 0.81 (d, J = 6.4 Hz, 3 H), 0.78 (d, J = 6.4 Hz, 3 H). ^{13}C NMR (C_6D_6): δ 173.0, 168.6, 136.2, 129.2, 129.0, 128.9, 71.6, 67.8, 57.5, 51.8, 41.3, 25.2, 23.2, 21.8. IR (cm^{-1}): 3273, 2960, 1738, 1667. HRFABMS: calcd for $(M + H)^+$, 371.1277; found, 371.1278. Anal. calcd for $C_{16}H_{22}N_2O_6S$: C, 51.88; H, 5.99; N, 7.56; S, 8.66. Found: C, 51.58; H, 6.13; N, 7.85; S, 9.05.

(4*S*)-2,2-Dioxo-1,2,3-oxathiazolidinone-4-carboxyl-leucine (17). The protected dipeptide **16** (270 mg, 0.73 mmol, 1.0 equiv) was dissolved in EtOAc (30 mL). Palladium-on-carbon (10 wt %, 75 mg, 0.07 mmol, 0.1 equiv) was added, and the suspension was stirred under 1 atm of hydrogen for ca. 30 min, after which TLC (SiO_2, 1/1 hexane/EtOAc) showed complete conversion to a product that did not migrate by TLC. The suspension was filtered through Celite and concentrated. The dipeptide **17** was used without further purification (205 mg, 100%). ^{1}H NMR (DMSO-d_6): δ 8.31 (d, J = 6.9 Hz, 1 H), 8.24 (d, J = 8.0 Hz, 1 H), 4.72 (t, J = 7.5 Hz, 1 H), 4.55−4.48 (m, 2 H), 4.25 (m, 1 H), 1.68−1.58 (m, 2 H), 1.51 (m, 1 H), 0.87 (d, J = 6.4 Hz, 3 H), 0.81 (d, J = 6.4 Hz, 3 H). ^{13}C NMR (DMSO-d_6): δ 173.4, 167.7, 71.0, 56.0, 50.5, 39.6, 24.1, 22.9, 21.2. HRFABMS: calcd for $(M + H)^+$, 281.0807; found, 281.0803.

***S*-*β*-D-Glucopyranosyl-L-cysteinyl-leucine (18).** The sodium salt of 1-thio-*β*-D-glucose (**8**) (205 mg, 0.95 mmol, 1.1 equiv) was dissolved in H_2O (4.8 mL). In a second flask, $NaHCO_3$ (180 mg, 2.15 mmol, 2.5 equiv) was added to **17** (240 mg, 0.86 mmol, 1.0 equiv). The reaction was initiated by addition of the sodium thiolate solution to the flask containing **17**, thus affording the following concentrations of reactants at the onset of the reaction: **17**, 0.18 M; **8**, 0.20 M; $NaHCO_3$, 0.45 M. The reaction was stirred at room temperature for 6 h and then concentrated by rotary evaporation under reduced pressure. To hydrolyze the sulfamidate, the crude material was dissolved in HCl (5 M, 15 mL). The hydrolysis reaction was incubated at 37 °C for 40 h and then concentrated by rotary evaporation under reduced pressure. The product was dissolved in H_2O (10 mL), and the pH was brought to neutral by the addition of $NaHCO_3$ (ca. 400 mg). The solution was passed through a nitrocellulose filter (0.45 μm). Product **18** was purified first over a column of Biogel P-2 (fine, column dimensions 2.5 cm i.d. × 70 cm L) eluting with H_2O (20 mL/h). Fractions containing the desired product were identified by TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O, R_f = 0.45) with ninhydrin staining. The fractions were

pooled, concentrated, and the product (ca. 95% purity) was dissolved in aqueous NH_4HCO_3 (50 mM, 5 mL). Final purification was achieved with reverse-phase HPLC: aliquots (0.5 mL) of the product solution were eluted through a Beckman Ultrasphere reverse-phase column (10 mm i.d. × 250 mm L, particle size 5 μm), eluting at 2.0 mL/min with 92/8 aqueous NH_4HCO_3 (50 mM)/CH_3OH. Peaks were detected by UV absorbance at 230 nm. Product **18** eluted with a retention time of approximately 15 min. Product **18** was obtained as a white solid (270 mg, 80%). ^{1}H NMR (D_2O, HCl salt): δ 4.76 (d, J = 9.8 Hz, 1 H), 4.49 (m, 1 H), 4.43 (dd, J = 4.9, 8.3 Hz, 1 H), 4.01 (dd, J = 2.3, 12.4 Hz, 1 H), 3.80 (dd, J = 6.0, 12.4 Hz, 1 H), 3.66 (m, 1 H), 3.62 (t, J = 9.0 Hz, 1 H), 3.50−3.56 (m, 3 H), 3.24 (dd, J = 8.3, 15.3 Hz, 1 H), 1.84−1.72 (m, 3 H), 1.01 (d, J = 6.0 Hz, 3 H), 0.97 (d, J = 6.0 Hz, 3 H). ^{13}C NMR (D_2O, HCl salt): δ 175.9, 168.5, 85.1, 80.2, 77.3, 72.2, 69.9, 61.3, 53.5, 52.1, 39.4, 31.3, 24.8, 22.7, 21.3. HRFABMS: calcd for $(M + H)^+$, 397.1645; found, 397.1640.

Solid-Phase Synthesis of Threoninyl-*S*-*β*-D-glucopyranosyl-L-cysteinyl-leucine (22). The synthesis was performed in a 25-mL manual peptide synthesis vessel. NovaSyn TG-hydroxy resin (1.0 g, 0.3 mmol/g, purchased from NovaBiochem) was swelled in CH_2Cl_2 (20 mL) overnight. (1) Esterification of the terminal hydroxyl: Fmoc-leucine (530 mg, 1.5 mmol, 5 equiv) was suspended in CH_2Cl_2 (5 mL). *N*-Methylimidazole (90 μL, 1.13 mmol, 3.75 equiv) was added to afford a clear solution upon stirring. 2,4,6-mesitylene-sulfonyl-3-nitro-1,2,4-triazolide (MSNT)[15] (445 mg, 1.5 mmol, 5 equiv) was then added, affording the following concentrations: Fmoc-leucine, 0.3 M; MSNT, 0.3 M. The solution was cannulated to the resin. The suspension was shaken at room temperature for 2 h, and then the resin was washed with CH_2Cl_2 followed by DMF. (2) Fmoc removal: The resin was shaken in piperidine/DMF (1/4 v/v, 15 mL) at room temperature for 20 min and was washed with DMF. (3) Peptide coupling with **14**: Amino acid **14** (430 mg, 1.5 mmol, 5 equiv) and PyBOP (730 mg, 1.5 mmol, 5 equiv) were dissolved in DMF (5 mL),, affording the following concentrations: **14**, 0.3 M; PyBOP, 0.3 M. The solution was cannulated to the resin. The reaction was initiated by addition of *N*-methylmorpholine (0.42 mL, 3.8 mmol, 12.5 equiv). The suspension was shaken at room temperature for 30 min, and then the resin was washed with DMF. (4) PMB-removal: The resin was swelled for 10 min in CH_3CN/H_2O (20 mL, 9/1 v/v). The resin was shaken in a solution of $(NH_4)_2Ce(NO_3)_6$ [0.5 M in 9/1 CH_3CN/H_2O (10 mL, 5 mmol, 16 equiv)] at room temperature for 1 h. The resin was washed thoroughly with 9/1 CH_3CN/H_2O follwed by washing with 1/1 dioxane/H_2O. (5) Addition of **8**: 2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-*β*-D-glucopyranose (1015 mg, 2.5 mmol, 8 equiv) was dissolved in CH_3OH (30 mL). Cs_2CO_3 (1.6 g, 5 mmol, 16 equiv) was added, and the solution was stirred at room temperature for 18 h. The solvent was removed by rotary evaporation, and the product was dissolved in H_2O (2.5 mL). Solid CO_2 was added with stirring until the pH of the solution had lowered to pH 8. Dioxane (2.5 mL) was added, affording [**8**] at 0.5 M. The solution was transferred to the resin, and the suspension was shaken at room temperature for 18 h. The resin was washed first with H_2O, followed by 1/1 dioxane/H_2O, and finally dioxane. (6) Removal of *N*-sulfate: The resin was washed with dry CH_2Cl_2. A solution (10 mL) of Et_2OBF_3 (1 M, 10 mmol) and *n*-butane thiol (1 M, 10 mmol) in CH_2Cl_2 was prepared and cannulated to the resin. The large excess of Lewis acid was necessary due to the Lewis basicity of the PEG. The suspension was shaken at room temperature for 20 h. The resin was washed with CH_2Cl_2 followed by 1/1 dioxane/H_2O, and finally dioxane. (7) Peptide coupling with Fmoc-threonine: The resin was swelled in DMF. Fmoc-threonine (510 mg, 1.5 mmol, 5 equiv) and PyBOP (730 mg, 1.5 mmol, 5 equiv) were added, followed by DMF (5 mL). The suspension was shaken briefly to dissolve the reagents. The reaction was initiated by addition of *N*-methylmorpholine (0.50 mL, 4.5 mmol, 15 equiv). The suspension was shaken at room temperature for 30 min, and then the resin was washed with DMF. (8) Fmoc removal: The resin was shaken in piperidine/DMF (1/4 v/v, 15 mL) at room temperature for 20 min, and the resin was washed with DMF. (9) Removal from support: The resin was washed with 1/1 dioxane/H_2O. The resin was shaken in a solution of NaOH [0.2 M in 1/1 dioxane/H_2O (7.5 mL, 1.5 mmol, 5 equiv)] at room temperature for 30 min. The filtrate was collected in a flask containing HCl (5 M, 3 mL). The solution was concentrated by rotary evaporation under reduced pressure. The product was dissolved in H_2O (7 mL), and the pH was brought to neutral by the addition of $NaHCO_3$ (ca. 300 mg). The solution was passed through a nitrocellulose filter (0.45 μm). Product **22** was purified over a column of Biogel P-2 (fine, column dimensions 2.5 cm i.d. × 70 cm L) eluting with H_2O (20 mL/h). Fractions containing the desired product were identified by TLC (SiO_2, 50/25/25 *n*-BuOH/HOAc/H_2O, R_f = 0.35) with ninhydrin staining. Product **22** was obtained as a white solid (70 mg). ^{1}H NMR (D_2O): δ 4.61 (dd, J = 5.8, 8.6 Hz, 1 H), 4.53 (d, J = 9.9 Hz, 1 H), 4.17 (m, 1 H), 3.92 (t, J = 6.0 Hz, 1 H), 3.88 (dd, J = 2.1, 10.4 Hz, 1 H), 3.68 (dd, J = 6.0, 12.5 Hz, 1 H), 3.48−3.25 (m, 6 H), 2.94 (dd, J = 8.6, 14.2 Hz, 1 H), 1.55 (m, 3 H), 1.15 (d, J = 6.5 Hz, 3 H), 0.87 (d, J = 6.2 Hz, 3 H), 0.82 (d, J = 6.2 Hz, 3 H). HRFABMS: calcd for $(M + H)^+$, 498.2121; found, 498.2137.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra for all products (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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