Synthesis of 2-Acetamido-2-deoxy-β-D-glucopyranose *O*-Glycopeptides from *N*-Dithiasuccinoyl-Protected Derivatives[1−3]

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Abstract: 2-Acetamido-2-deoxy-β-D-glucopyranose (β-D-Glc*p*NAc), in *O*-glycosidic linkage to the side chain hydroxyls of serine (Ser) and threonine (Thr) residues, is often found in nuclear and cytoplasmic proteins. The "active ester" approach for solid phase glycopeptide synthesis calls for the direct glycosylation of N^{α}-(9-fluorenylmethyloxycarbonyl)amino acid pentafluorophenyl esters (N^{α}-Fmoc-AA-OPfp's). The synthesis of the required Ser(β-D-Glc*p*NAc) and Thr(β-D-Glc*p*NAc) building blocks poses special problems arising from the 2-amino substituent in the corresponding glycosyl donors. Activation of donors with a 2-*N*-acyl group provides relatively unreactive oxazoline intermediates, whereas the otherwise promising phthaloyl (Phth) group requires prolonged base treatment at elevated temperatures for its removal, and incomplete deprotection is often encountered. The dithiasuccinoyl (Dts) group provides bivalent protection in the same way as the Phth group, but has the advantage of being removed rapidly under mild conditions by thiolysis or other reductive procedures. The novel donor 3,4,6-tri-*O*-acetyl-2-deoxy-2-(dithiasuccinoylamino)-D-glucopyranosyl bromide (**8**) was prepared from D-glucosamine in four steps and overall 58% yield; this compound served for the fast and efficient glycosylation of N^{α}-Fmoc-Ser-OPfp and N^{α}-Fmoc-Thr-OPfp. The resultant glycosylated building blocks N^{α}-Fmoc-Ser(Ac_3-β-D-Glc*p*NDts)-OPfp (**9**) and N^{α}-Fmoc-Thr(Ac_3-β-D-Glc*p*NDts)-OPfp (**10**) were applied in the solid phase synthesis of several glycopeptides. Following incorporation of the glycosylated residue, the Dts function was removed quantitatively by thiolysis, the resultant free 2-amino group was acetylated, and stepwise chain elongation by Fmoc chemistry continued. Alternatively, it was possible to achieve selective Dts removal from **9** and **10** (without affecting the Pfp ester) by reduction with zinc under acidic conditions; *in situ* acetylation of the exposed amine provided the alternative building blocks N^{α}-Fmoc-Ser(Ac_3-β-D-Glc*p*NAc)-OPfp (**11**) and N^{α}-Fmoc-Thr(Ac_3-β-D-Glc*p*NAc)-OPfp (**12**).

Introduction

Oligosaccharides linked covalently to proteins or lipids are found widely at the surfaces of cells, and are likely to play a key role in cell−cell recognition.[5,6] In many surface glycoproteins, a 2-acetamido-2-deoxy-α-D-galactopyranose (α-D-Gal*p*NAc) residue is attached as an *O*-glycoside to the side-chain hydroxyl of serine (Ser) or threonine (Thr), and additional sugar residues extend from the 3-position of α-D-Gal*p*NAc. It was reported recently that 2-acetamido-2-deoxy-β-D-glucopyranose [β-D-Glc*p*NAc, also referred to as *N*-acetylglucosamine; the β-anomer/C-4 epimer of D-Gal*p*NAc], attached to Ser and Thr, is found in proteins within the nuclear and cytoplasmic compartments of cells in eukaryotes from yeast to mammals;[7] this mode of glycosylation appears to be highly dynamic and responsive to extracellular stimuli in a fashion analogous to that of phosphorylation.[7e] In addition, many transcription factors for genes transcribed by RNA polymerase II are modified by glycosylation, probably by addition of *O*-D-GlcNAc moieties.[7d]

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(1) A preliminary account of portions of this work was presented at the 23rd European Peptide Symposium, Braga, Portugal, Sept 4−10, 1994: Hansen, P. R.; Jensen, K. J.; Barany, G. In *Peptides 1994: Proceedings of the Twenty-Third European Peptide Symposium;* Maia, H. L. S., Ed.; Escom Science Publishers: Leiden, The Netherlands, 1995; pp 729−730.

(2) (a) While this work was being prepared for publication, we became aware of similar studies focused on Dts-protected sugar derivatives; see: Meinjohanns, E.; Meldal, M.; Bock, K.; Paulsen, H. *XVIIth International Carbohydrate Symposium,* Ottawa, Canada, July 17−22, 1994; Abstract B 1.98. Meinjohanns, E.; Meldal, M.; Bock, K.; Paulsen, H. *J. Chem. Soc., Perkin Trans. 1* **1995**, 405−415. (b) A recent paper from the same research group, focused on GlcNAc glycopeptide synthesis, appeared subsequent to the submission of this paper; see: Meinjohanns, E.; Vargas-Berenguel, A.; Meldal, M.; Bock, K.; Paulsen, H. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2165−2175.

(3) Abbreviations used for amino acids and the designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* **1972**, *247*, 977−983. Abbreviations used for carbohydrates follow the Rules for Carbohydrate Nomenclature in *J. Org. Chem.* **1963**, *28*, 281−291 and *J. Chem. Soc.* **1962**, 5307−5312. The following additional abbreviations are used: Ac, acetyl; Al, allyl; Aloc, allyloxycarbonyl; BME, β-mercaptoethanol; Bn, benzyl; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMF, *N*,*N*-dimethylformamide; Dts, dithiasuccinoyl; HOBt, 1-hydroxybenzotriazole; Fmoc, 9-fluorenylmethyloxycarbonyl; MAc, *N*-methylmercaptoacetamide; PEG-PS, polyethylene glycol−polystyrene graft support; Pfp, pentafluorophenyl; SPPS, solid phase peptide synthesis; PAL, 5-(4-(Fmoc-aminomethyl)-3,5-dimethoxyphenoxy)valeric acid, tris(alkoxy)benzylamide handle suitable for synthesis of *C*-terminal peptide amides; Phth, phthaloyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran. Amino acid symbols denote the L-configuration. All solvent ratios are volume:volume unless stated otherwise.

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D-Glc*p*NAc glycosylation sites occur in protein regions that are rich in Ser and Thr, and often include a proline residue one to three positions from the attachment site.[7e]

Many naturally occurring glycoproteins show microheterogeneity in the glycan moiety, and isolation of pure glycopeptides is difficult. Reliable and convenient methods for the chemical synthesis of glycopeptides[8] are of interest since they will make available materials of well-defined structure in the amounts required for biological testing and spectroscopic studies of conformations. Synthetic glycopeptides may also have improved bioavailability characteristics and attenuated *in vivo* clearance, as well as increased stability toward proteases and reduced immunogenicity.[9] The synthesis of Ser and Thr glycosylated peptides is made challenging by their lability to strong acids, e.g., hydrogen fluoride,[10a] and their susceptibility to base-catalyzed β-elimination of the glycan.[10b] However, it has been demonstrated[11] that *O*-glycopeptides are stable to those bases, e.g., morpholine, piperidine, and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU), that are commonly used to remove the N^{α}-(9-fluorenylmethyloxycarbonyl) (Fmoc) protecting group for solid phase peptide synthesis (SPPS), and that acetyl (Ac) and benzoyl ester groups protecting the glycan hydroxyls can be cleaved from *O*-glycopeptides by mild base-catalyzed transesterification without significant β-elimination.[8] Furthermore, *O*-glycosidic linkages appear to be entirely stable to treatment with concentrated trifluoroacetic acid in the absence or presence of carbocation scavengers, particularly while the saccharide retains ester protecting groups.[12] Consequently, solid phase methods using N^{α}-Fmoc-protected glycosylated amino acid building blocks are emerging as potentially the most flexible and optimal way to prepare glycopeptides.[8]

Glycosylation of amino acids, with rare exceptions,[13] requires that both the N^{α}-amino and the C^{α}-carboxyl functions are blocked. Given N^{α}-amino protection with Fmoc, the C^{α}-carboxyl is generally protected during the glycosylation step with a group such as *tert*-butyl (*t*Bu), allyl (Al), or benzyl (Bn), orthogonal removal of which provides a building block suitable for activation and coupling in the context of Fmoc SPPS.[14,15] Alternatively, in the "active ester" approach, N^{α}-Fmoc-amino acid pentafluorophenyl esters (Fmoc-AA-OPfp's) can be glycosylated directly to provide building blocks for SPPS.[16] Pfp esters survive exposure to strong Lewis acids (equimolar) in organic solvents, are reasonably stable toward oxygen nucleophiles under weakly acidic and neutral conditions, and can be purified by silica gel chromatography with dry organic solvents[16a] or reversed-phase HPLC with water−acetonitrile mixtures;[16e] these properties make Pfp a suitable C^{α}-carboxyl protecting group for the glycosylation step.[17] Subsequently, Pfp esters are efficient acylating agents, especially in the presence of an auxiliary nucleophile such as 1-hydroxybenzotriazole (HOBt) or 3-hydroxy-2,3-dihydro-4-oxobenzotriazine. Thus, Pfp serves a dual role of protection and activation, and saves protecting group manipulation steps.

Stereocontrol in the syntheses of 1,2-*trans*-glycosides of carbohydrates by electrophilic activation of an anomeric (C-1) leaving group is usually accomplished through participation of the C-2 substituent.[18,19] For 2-(acylamino)-2-deoxyhexoses (e.g., **1**), such electrophilic activation forms a reactive oxocarbenium ion (**2**) intermediate, which often collapses rapidly to an oxazolinium ion (**3**) intermediate (Scheme 1, mechanism A). Intermediate **3** is expected to undergo nucleophilic attack at C-1 selectively from the β-face of the glycosyl donor to yield only 1,2-*trans*-glycosides [in those cases where an α/β mixture is observed, this is often attributed to reactions of **2**]. However, with the 2-amino group acylated, e.g., *N*-acetyl, the intermediate **3** can be stabilized further through abstraction of the amide proton to form a relatively stable oxazoline (**4**).[18c] Protected Ser derivatives (e.g., N^{α}-protection, benzyloxycarbonyl, Boc, Fmoc; C^{α}-protection, Bn, 4-nitrobenzyl, methyl) react with 2-methyl-(1,2-dideoxy-α-D-glycopyrano)[2.1-*d*]oxazoline (**4**, R = Me; prepared intentionally or assumed to be the true intermediate derived from **1** with L = Cl, R = Me) at elevated temperatures, typically ≥ 70 °C, to provide the corresponding 2-acetamido-2-deoxy-β-D-glucopyranosides of these Ser derivatives in marginal to adequate yields.[20] Alternatively, saccharides

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(14) For strategies that rely on N^{α}-Fmoc protection in combination with a temporary C^{α}-protecting group see: (a) Schultheiss-Reimann, P.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 62−63. (b) Paulsen, H.; Adermann, K.; Merz, G.; Schultz, M.; Weichert, U. *Starch* **1988**, *40*, 465−472. (b) Lüning, B.; Norberg, T.; Tejbrant, J. *J. Chem. Soc., Chem. Commun.* **1989**, 1267−1268. (c) Lüning, B.; Norberg, T.; Tejbrant, J. *Glycoconjugate J.* **1989**, 5−19. (d) de la Torre, B. G.; Torres, J. L.; Bardaji, E.; Clapes, P.; Xaus, N.; Jorba, X.; Calvet, S.; Albericio, F.; Valencia, G. *J. Chem. Soc., Chem. Commun.* **1990**, 965−967.

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(19) For the synthesis of 2-amino-2-deoxy-1,2-*cis*-hexosides, Lemieux and co-workers pioneered the use of an azido group at the 2-position; the azide is then reduced subsequent to glycosylation (reviewed in refs 8a and 18a). For recent examples see refs 11b, 14b, 14c, and 16d, as well as the following: (a) Andreotti, A. H.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 3352−3353. (b) Liang R. L.; Andreotti, A. H.; Kahne, D. *J. Am. Chem. Soc.* **1995**, *117*, 10395−10396.

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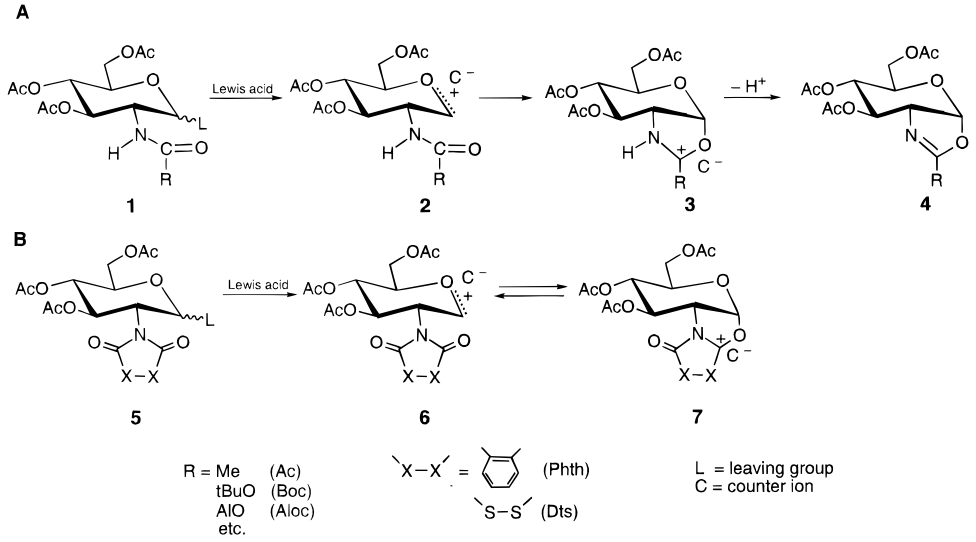
Scheme 1. Intermediates in Lewis Acid-Activated Glycosylation with D-Glucosamine Derivatives



with the 2-amino group blocked by chloroacetyl,[21] trichloroacetyl,[22] allyloxycarbonyl (Aloc),[23] and 2,2,2-trichloroethoxycarbonyl[24] have been explored for glycosylation reactions, but with each of these protected sugars, side reactions have been noted in their applications.

The formation of an oxazoline intermediate (**4**) upon electrophilic activation may be prevented when the abstractable amide proton is substituted by a protecting group that is not cleaved during the glycosylation step. This goal can be achieved by blocking the 2-amino group with two monovalent protecting groups,[25] or by application of the bivalent *N*-phthaloyl (Phth) group (Scheme 1, mechanism B).[26] Electrophilic activation of the bivalently protected glycosyl donor **5** yields an oxocarbenium ion (**6**) which can form an oxazolinium ion (**7**). The reactive intermediate **7** can only be attacked from the β-face by a nucleophile, and **7** cannot form a stable oxazoline. Glycosylation with Phth-protected glycosyl donors occurs under mild conditions at low temperatures and gives exclusively 1,2-*trans*-glycosides. However, removal of the Phth group requires relatively harsh conditions,[27] and incomplete deprotection is often encountered.[18c] Moreover, it seems likely that basic dephthaloylation conditions will be accompanied by β-elimination of the glycan from Ser and Thr.

With the goal to develop an efficient procedure for the synthesis of 1,2-*trans* glycosides of Ser and Thr, we sought an alternative imide-type protecting group for the saccharide 2-amino group.[1,2] We reasoned that the aforementioned limitations to Phth, which stem from problems with deprotection, would be circumvented by application of the *N*-dithiasuccinoyl (Dts) group, which can be removed readily and quantitatively under mild conditions by thiolysis or other reductive methods.[28] The present paper reports the preparation and use of the novel glycosyl donor 3,4,6-tri-*O*-acetyl-2-deoxy-2-(dithiasuccinoylamino)-D-glucopyranosyl bromide (**8**). Compound **8**, upon activation with silver salts, provided entry to the building blocks N^{α}-Fmoc-Ser(Ac_3-β-D-Glc*p*NDts)-OPfp (**9**) and N^{α}-Fmoc-Thr-(Ac_3-β-D-Glc*p*NDts)-OPfp (**10**), which were incorporated directly into peptides by Fmoc SPPS. Since Dts-protected amines react with secondary amines to form urea derivatives,[28a,d] it was next necessary to deblock selectively the 2-amino function by thiolysis. This was followed by acetylation of the released amine, resumption of chain elongation, and eventual release of the glycopeptide from the solid phase along with full deprotection of peptide side chains. For the present work, removal of the *O*-acetyl saccharide protecting groups was carried out as a final step on the solubilized peptide, although in principle this step could also be achieved earlier on the resin-bound peptide.[8c] Alternatively, it was possible to achieve selective Dts removal from **9** and **10** (without affecting the Pfp ester); *in situ* acetylation of the exposed amine provided the alternative building blocks N^{α}-Fmoc-Ser(Ac_3-β-D-Glc*p*NAc)-OPfp (**11**)[29] and N^{α}-Fmoc-Thr(Ac_3-β-D-Glc*p*NAc)-OPfp (**12**).[29]

Results and Discussion

Preparation of Glycosylated Building Blocks. The required glycosyl donor **8** was obtained in four facile steps and 58% overall isolated yield from D-glucosamine hydrochloride (**13**) (Scheme 2). The 2-amino group of the starting monosaccharide was converted to its ethoxythiocarbonyl (Etc) derivative by reaction with bis(ethoxythiocarbonyl) sulfide (**14**) in aqueous ethanol at pH 9−10,[30] and this step was followed by *in situ* acetylation with acetic anhydride in pyridine−CH_2Cl_2 to provide

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Scheme 2. Synthesis of Glycosyl Donor **8**

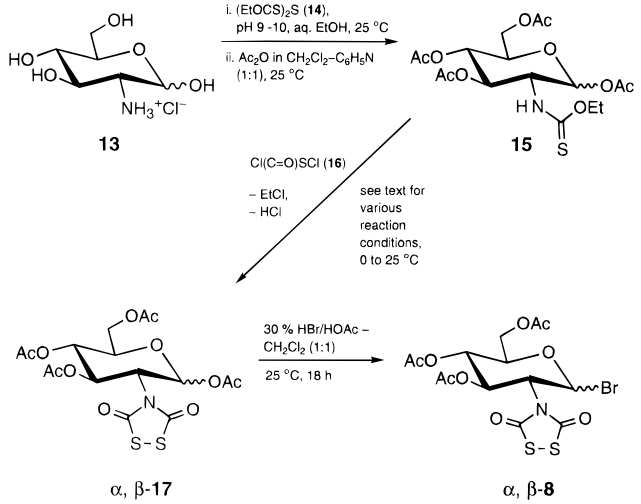


Table 1. ^{1}H NMR Data for the D-Glucosamine Moiety in Compounds α,*β*-**8** and α,*β*-**17**[a]

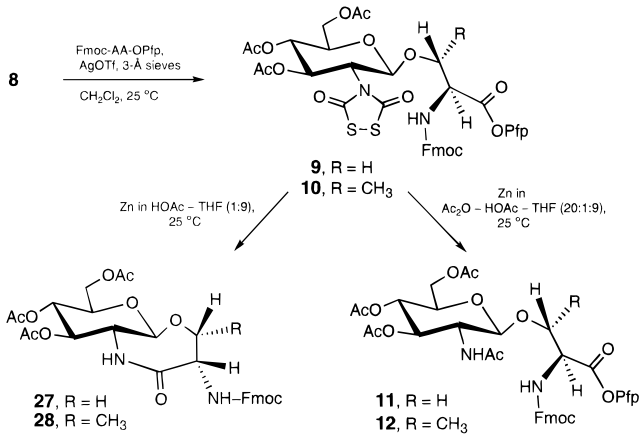
proton	α-**8**	*β*-**8**	α-**17**	*β*-**17**
H-1	6.46	6.33	6.23	6.45
($J_{1,2}$)	(3.4)	(9.5)	(3.1)	(8.6)
H-2	4.68	4.69	4.72	4.54
($J_{2,3}$)	(11.3)	(10.1)	(11.0)	(11.0)
H-3	6.54	5.71	6.48	5.84
($J_{3,4}$)	(9.2)	(9.5)	(9.2)	(8.6)
H-4	5.10	5.22	5.10	5.19
($J_{4,5}$)	(10.1)	(9.9)	(9.8)	(9.8)
H-5	4.35	3.87	4.25	3.93
($J_{5,6}$)	(na)	(4.6)	(4.9)	(4.9)
H-6	4.45	4.29	4.32	4.32
($J_{6,6'}$)	(na)	(12.5)	(12.2)	(12.2)
H-6′	na	4.15	4.09	4.12
($J_{5,6'}$)		(2.1)	(2.4)	(2.4)

[a] Chemical shifts (δ) are expressed in parts per million downfield from TMS. Coupling constants, in parentheses, are expressed in hertz. na = not assigned. *O*-Ac groups at ~2 ppm were sharp resolved singlets and are not reported.

the tetraacetylated Etc derivative **15** in a net 80% yield based on **13**. Next, the Dts heterocycle was established rapidly and cleanly (reaction ~2/3 complete within 5 min; >90% initial purity) by treatment of **15** with (chlorocarbonyl)sulfenyl chloride (**16**) in acetonitrile, providing the *N*-dithiasuccinoyl 1-*O*-acetate **17** as an anomeric mixture in an α:*β* ratio of 5.4 : 1 (Table 1). The purified yield of the anomer mix was in the 75−80% range, somewhat higher than a variation in which **15** was treated first with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) so that trimethylchlorosilane would replace hydrogen chloride as the coproduct of the reaction with **16**. Dts-forming reactions carried out in the presence of triethylamine as the HCl acceptor gave yet lower yields and poorer initial purities. Next, room temperature treatment of **17** with 30% HBr in HOAc−CH_2Cl_2 (1:1) provided[26] glucopyranosyl bromide **8** in essentially quantitative yield as an anomeric mixture in which the *β*-bromide predominated (α:*β* ratio 1 : 3.5; Table 1). The preferred formation of the *β*-bromide under these conditions indicates that the anomeric effect, which causes stabilization of the axial (α) anomeric substituent, is overcome substantially by a steric effect from the Dts moiety. As a precaution, conversion of glycosyl acetate **17** to glycosyl bromide **8** was carried out immediately prior to each glycosylation experiment; see the Experimental Section

(30) Barany, G.; Fulpius, B. W.; King, T. P. *J. Org. Chem.* **1978**, *43*, 2930−2932.

Scheme 3. Synthesis of Glycosylated Ser and Thr Derivatives **9**−**12**



for information about the stability of **8**. Independently of us, Meldal and co-workers[2] prepared **15**, **17**, and **8**, in ~48% overall yield; D-glucosamine (**13**) was converted to its Etc derivative with *S*-(carboxymethyl) *O*-ethyl dithiocarbonate;[30] and for **8** the minor α-anomer was not mentioned.

The key *β*-glycosidic building block N^{α}-Fmoc-Ser(Ac$_3$-*β*-D-Glc*p*NDts)-OPfp (**9**) was obtained in 86% purified yield by reaction of glycosyl bromide **8** (1.2 equiv) with N^{α}-Fmoc-Ser-OPfp (**18**)[16a,31] in the presence of silver triflate (1.4 equiv) and 3-Å molecular sieves at 25 °C (Scheme 3). No trace of the corresponding α-glycoside could be detected in either the isolated products or in any of the other chromatography fractions. In accounting for the overall yield, some of the starting amino acid derivative **18** was recovered, whereas excess starting **8** decomposed. However, when **18** was in slight excess over **8**, the overall yield was lowered. Further, conducting the reaction at −10 °C also resulted in a decreased yield (69%). In the absence of molecular sieves, the yield of **9** decreased to 30%, indicating the importance of dry conditions. The optimal conditions using donor **8** in excess (1.2 equiv) at 25 °C were extended to successfully glycosylate the *secondary* hydroxyl group of the Thr derivative **19**; the corresponding *β*-glycoside N^{α}-Fmoc-Thr(Ac$_3$-*β*-D-Glc*p*NDts)-OPfp (**10**) was obtained in a yield of 85%. The glycosides **9** and **10** both showed $^3J_{1,2}$ values of 8.5 Hz (at 5.40 and 5.52 ppm, respectively), which establishes firmly that the new glycosidic linkages have a *β*-configuration (Table 2).[26] The stereospecific formation of *β*-glycosides, i.e., 1,2-*trans*, is apparently due to the effectiveness of the Dts group in donor **8** in preventing nucleophilic attack from the α-face in the silver-activated intermediate [the same explanation has been invoked for stereospecificity in the phthaloyl precedent[26]]. Compounds **9** and **10** have been prepared also by Meldal and co-workers[2] in overall yields of 38% and 42%, respectively, based on **17**; two additional steps were used [bromide **8** was converted to the trichloroacetimidate, which then was used to glycosylate amino acid derivatives **18** and **19**].

Selective Reductive Deblocking of Dts-Protected Building Blocks. Selective removal of Dts from the complicated precursors **9** or **10** proved to be quite challenging (Scheme 3). Because the ultimate need was for Glc*p*NAc derivatives, the deprotection step was followed directly by *in situ* acylation of the freed C-2 amino group using acetic anhydride. Pilot reactions were modeled on literature precedents for Dts removal,[28] and were followed qualitatively by FABMS analysis of product mixtures. Although *β*-mercaptoethanol (BME) plus *N*,*N*-diisopropylethylamine (DIEA) in CH_2Cl_2, as well as

(31) (a) Kisfaludy, L.; Schön, I. *Synthesis* **1983**, 325−327. (b) Schön, I.; Kisfaludy, L. *Synthesis* **1986**, 303−305.

Table 2. ^{1}H NMR Data for the D-Glucosamine Moiety in Compounds **9**−**12**, **27**, and **28**[a]

proton	**9**	**10**	**11**	**12**	**27**	**28**
H-1	5.40	5.39	4.84	4.74	4.54	4.76
($J_{1,2}$)	(8.5)	(8.5)	(8.1)	(8.0)	(7.6)	(7.6)
H-2	4.38	4.38	3.74	3.79	3.70	3.70
($J_{2,3}$)	(11.0)	(11.0)	(10.1)	(10.5)	(9.8)	(9.9)
H-3	5.73	5.77	5.29	5.27	5.07	5.08
($J_{3,4}$)	(8.5)	(8.5)	(10.1)	(10.0)	(9.8)	(9.5)
H-4	5.15	5.15	5.07	5.07	5.15	5.16
($J_{4,5}$)	(10.0)	(9.8)	(9.5)	(10.0)	(9.9)	(9.8)
H-5	3.76	3.78	3.70	3.67	3.76	3.74
($J_{5,6}$)	(4.0)	(na)	(4.9)	(4.5)	(4.7)	(4.5)
H-6	4.23	4.23	4.22	4.20	4.27	4.27
($J_{6,6'}$)	(12.0)	(12.2)	(12.2)	(12.0)	(12.8)	(12.5)
H-6′	4.13	4.06	4.11	4.06	4.17	4.15
($J_{5,6'}$)	(2.0)	(2.4)	(1.8)	(2.5)	(2.1)	(2.1)
NH	N/A	N/A	5.59	5.58	6.69	6.60
($J_{NH,2}$)			(7.9)	(8.5)	(3.5)	(4.3)

[a] Chemical shifts (δ) are expressed in parts per million downfield from TMS. Coupling constants, in parentheses, are expressed in hertz. na = not assigned. N/A = not applicable. *O*-Ac and *N*-Ac groups at ~2 ppm were sharp resolved singlets and are not reported.

N-methylmercaptoacetamide (MAc) in DMF, were both effective in promoting rapid and quantitative Dts removal, the thiol nucleophiles used in these procedures also displaced the Pfp ester, resulting in significant levels of the corresponding thioesters.[32] Furthermore, the use of BME (but not MAc) gave rise to thiourethane products via thiolytic opening of the Dts ring carbonyl.[32] We reasoned that if reductive removal of Dts were carried out under acidic conditions, the freed amino group would be immediately protonated and hence less likely to react with the Pfp ester. In this regard, treatment of the *N*-Dts-protected derivatives **9** and **10** with zinc[33] *in the presence of acetic anhydride* gave N^{α}-Fmoc-Ser(Ac$_3$-β-D-Glc*p*NAc)-OPfp (**11**) and N^{α}-Fmoc-Thr(Ac$_3$-β-D-Glc*p*NAc)-OPfp (**12**) in 80% and 87% yields, respectively, after chromatography (Scheme 3). The $^3J_{1,2}$ values for **11** and **12** of 8.1 Hz (at 4.84 ppm) and 8.0 Hz (at 4.74 ppm), respectively, proved the β-configuration of the glycosidic linkages (Table 2).

Omission of Ac_2O from the zinc reductions, i.e., use of HOAc−THF (1:9) or TFA−THF (1:9) as solvent, gave the novel bicyclic lactams **27** and **28**, respectively, as the major products.[34] The seven-membered ring adjacent to the six-membered sugar forms by intramolecular displacement of Pfp by the freed C-2 amino group. This side reaction was also noted with other reductive methods; see the Experimental Section.

Peptide Synthesis. The glycosylated building blocks available through this work were tested on the synthesis of peptide fragments derived from serum response factor (SRF), a ubiquitous 508-residue transcription factor that binds the serum response element.[7d] Target sequences shown below were around the glycosylation site Ser^{316} [the analogue with Thr^{316} was also prepared]; the dodecapeptide is identical to a glycopeptide fragment isolated and characterized after treatment of SRF with cyanogen bromide followed by enzymatic digestion.[7d]

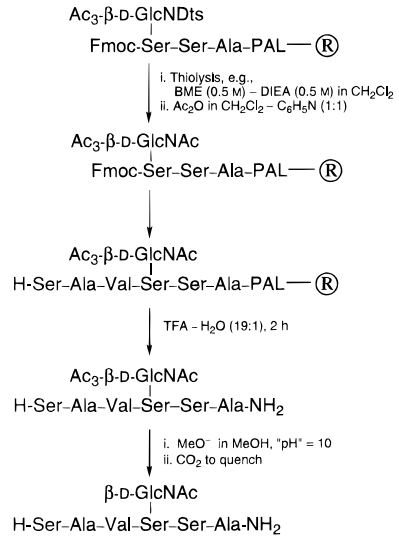
^{313}SAVS(GlcNAc)-SA^{318}

^{313}SAVT(GlcNAc)-SA^{318}

^{313}SAVS(GlcNAc)-SADGTVLK^{324}

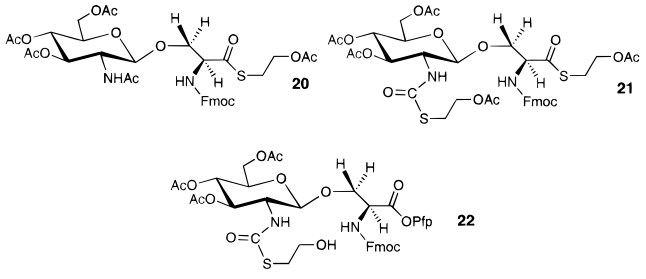
Glycopeptides were assembled smoothly by Fmoc solid phase synthesis[35] starting with acidolyzable tris(alkoxy)benzylamide

Scheme 4. Solid Phase Synthesis of H-Ser-Ala-Val-Ser(Ac$_3$-β-D-*O*-Glc*p*NAc)-Ser-Ala-NH$_2$ from Dts-Protected Glycosylated Ser and Thr Derivatives

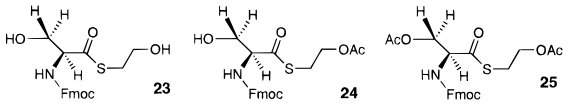


(PAL) anchoring[36] on a commercially available polyethylene glycol−polystyrene (PEG-PS) graft support[37] (e.g., Scheme 4). Couplings of all N^{α}-Fmoc-amino acid dervatives were mediated by *N*,*N*′-diisopropylcarbodiimide (DIPCDI)−HOBt in *N*,*N*-

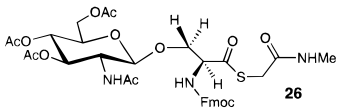
(32) Treatment of **9** with BME and DIEA in CH_2Cl_2, followed by acetylation, revealed the following major products (not quantitated) by FABMS:



As a control, treatment of N^{α}-Fmoc-Ser-OPfp (**18**) with BME gave **23** within 15 min; this was converted to **24** and **25** after acetylation



Finally, when MAc was used as the reducing agent, the thiourethane side reaction (analogous to compounds **21** and **22)** was not observed; however, displacement of the Pfp ester to form **26** was seen by FABMS.



(33) Such reactions are new with regard to applications for Dts removal. For reduction of aliphatic disulfides with Zn and hydrochloric acid, see: Zervas, L.; Photaki, I. *J. Am. Chem. Soc.* **1965**, *84*, 3887−3897.

(34) See ref 24 for a preparative experiment in which the lactam **27** was isolated by a different route.

(35) For reviews see: (a) Barany, G.; Kneib-Cordonier, N.; Mullen, D. G. *Int. J. Peptide Protein Res.* **1987**, *30*, 705-739. (b) Fields, G. B., Tian, Z., Barany, G. In *Synthetic Peptides, A User's Guide*; Grant, G. A., Ed.; W. H. Freeman: New York, 1991; pp 77−183.

(36) (a) Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730−3743. (b) Albericio, F.; Barany, G. *Int. J. Peptide Protein Res.* **1993**, *41*, 307−312 and references cited therein.

(37) (a) Zalipsky, S.; Albericio, F.; Barany, G. In *Proceedings of the Ninth American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; pp 257−260. (b) Zalipsky, S.; Chang, J. L.; Albericio, F.; Barany, G. *React. Polym.* **1994**, *22*, 243−258.

dimethylformamide (DMF), except for the Dts-protected Pfp ester building blocks **9** and **10** which were coupled in the sole presence of HOBt. Immediately following incorporation of **9** or **10**, and before continuing chain elongation, the Dts group was removed by brief treatment (3 × 2 min) with BME (0.5 M)−DIEA (0.5 M) in CH_2Cl_2. Next, the liberated 2-amino group was acetylated with acetic anhydride in CH_2Cl_2−pyridine (1:1). Acidolytic cleavage/deprotection of the completed peptide-resins with TFA−H_2O (19:1) at 25 °C for 2 h gave the desired *O*-acetylglycopeptide amides. These crude products were evaluated by analytical C-4 reversed-phase HPLC eluting with 0.1% aqueous TFA, and shown to be >80% pure [e.g., Figure 1; note that on C-18 HPLC, glycopeptides eluted with the solvent front]. Their identities were confirmed by FABMS. Without intermediate purification, a final solution deacetylation step was carried out by treatment with methanol in the presence of a catalytic amount of sodium methoxide (apparent pH of 10).[16c] The reaction mixture was neutralized after 15 min by addition of solid CO_2. The hydrophilic free glycopeptides eluted with the solvent front upon C-4 HPLC, making it difficult to estimate the purity. However, the proper structures were confirmed by FABMS, ESMS, and LC/MS,[38] and these techniques furthermore ruled out a variety of putative byproducts which might have been present due to incomplete deprotection, β-elimination, or side reactions at the Dts group.

Conclusions

Studies described herein demonstrate the use of Dts as a temporary amino protecting group in carbohydrate chemistry, and for the synthesis of *O*-β-D-Glc*p*NAc peptides. The Dts group is analogous to Phth, insofar as it covers two free valences of nitrogen at a point where this is critical for success. Both protecting groups convey stability to a range of acid and electrophilic conditions, but Dts has the further advantage that it can be removed under exceptionally mild and selective conditions. The novel glycosyl donor **8**, prepared in four convenient steps from D-glucosamine, served to efficiently glycosylate the primary and secondary hydroxyl groups, respectively, of appropriate serine and threonine derivatives. Resultant *N*-Dts derivatives **9** and **10** served directly as building blocks for peptide synthesis; they also provided convenient entries to *N*-acetylated derivatives **11** and **12** which could serve the same purpose. Generalizations of these concepts to other areas of sugar and glycopeptide chemistry are readily envisaged.

Experimental Section

General Procedures. Some of the materials and general synthetic and analytical procedures have been described in earlier publications from our laboratory.[36] Protected Fmoc-amino acid derivatives and resins for peptide synthesis were from the Biosearch Division of PerSeptive Biosystems (Framingham, MA), Bachem Bioscience (Philadelphia, PA), or Advanced Chemtech (Louisville, KY). Piperidine, TFA, DIEA, and HOBt were from Fisher (Pittsburgh, PA). DIPCDI, D-glucosamine hydrochloride, BSA, and BME were from Aldrich (Milwaukee, WI). MAc was obtained from Fluka (Ronkonkoma, NY). Bis(ethoxythiocarbonyl) sulfide (**14**) was prepared as described previously,[30] or the product as purchased from Fairfield Chemical (Blythewood, SC) was recrystallized from ethanol−water (3:1). (Chlorocarbonyl)-

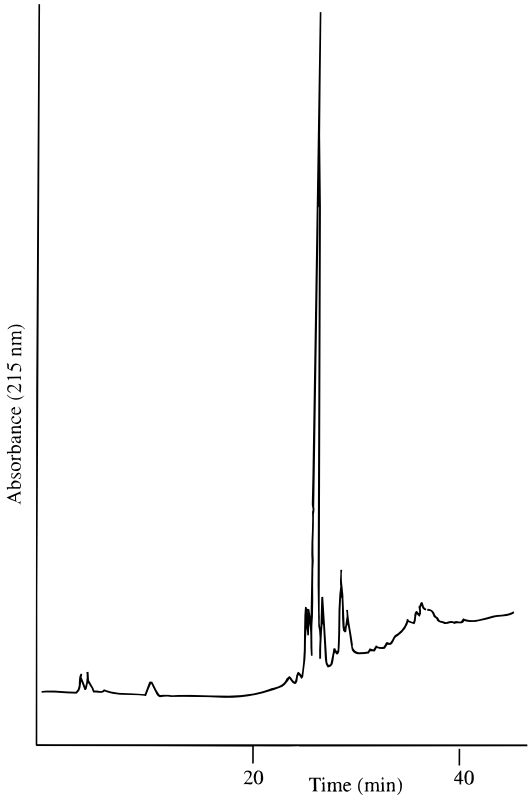


Figure 1. HPLC analysis of H-Ser-Ala-Val-Ser(Ac$_3$-β-D-*O*-Glc*p*NAc)-Ser-Ala-NH_2. HPLC conditions are given in the General Procedures.

sulfenyl chloride (**16**) was prepared as described previously.[39] All glycosylations were conducted under an argon atmosphere with the exclusion of moisture. Organic solvent extracts were dried over anhydrous $MgSO_4$ or Na_2SO_4, followed by solvent removal at reduced pressures and <40 °C. Silica gel chromatography was performed with Silica Gel 60 (230−400 mesh) from EM Science (Gibbstown, NJ); the gel used for purification of Pfp esters was dried for at least 24 h at 140 °C prior to use,[16a] and the ethyl acetate for this same purpose was dried over 3-Å molecular sieves for 24 h prior to use.[16a] Crystallizations specified in the text were carried out by dissolving in the minimum amount of ethyl acetate at 25 °C and adding hexane to incipient turbidity, and then chilling to 4 °C overnight. Elemental analyses were conducted by M-H-W Laboratories (Phoenix, AZ). Melting points were determined on a Fischer-Jones apparatus and are uncorrected.

Thin-layer chromatography was performed on either Polygram SIL G/UV$_{254}$ plates (250 μm, 40 × 80 mm) or Kieselgel 60 F_{254} (0.2 mm, 40 × 80 mm, Merck), developed by one of the following solvent systems: (A) EtOAc−hexane (1:2), (B) toluene−EtOAc (5:1), (C) toluene−EtOAc (3:2). Spots were visualized by UV and/or spraying with a dilute solution of H_2SO_4, followed by heating. Analytical HPLC was performed using either a Vydac analytical C-18 or C-4 reversed-phase column (0.46 × 25 cm and 0.46 × 15 cm, respectively) on a Beckman system configured with two 112 pumps and a 165 variable wavelength detector or a Nova Pak C-18 column (0.39 × 15 cm) on a Waters system configured with a 600E system controller, a 625 pump, a 700 satellite WISP autoinjector, and a 996 photodiode array detector. Samples were run at a flow rate of 1.2 mL/min starting with 0.1% aqueous TFA for 20 min, and increasing over 30 min to 0.1% aqueous TFA−0.1% TFA in CH_3CN (1:19), detection at 215 and 265 nm. Low-resolution fast atom bombardment mass spectroscopy (FABMS) was carried out in glycerol−H_2O or 3-nitrobenzyl alcohol (MNBA) matrices on a VG Analytical 707E-HF low-resolution double-focusing mass spectrometer equipped with a VG 11/250 data system, operated at a resolution of 2000. Liquid chromatography/mass spectrometry (LC/MS) was performed using a Beckman Ultrasphere analytical C-18 reversed-phase column (0.2 cm × 15 cm) on a Beckman system

(38) The mass spectra of the glycosylated peptides also show ions corresponding to the unglycosylated form. The question arises whether these latter ions (i) are diagnostic of the presence of glycosyl-free peptide or (ii) arise from mass spectrometric fragmentation. Since the peaks of mass corresponding to unglycosylated peptide were not observed by direct infusion ESMS of glycododecapeptide at lower orifice (cone) voltages (40, 50, and 60 V), we conclude that their presence can be explained by (ii). A similar phenomenon was noted recently: Peter-Katalanic, J.; Williger, K.; Egge, H.; Green, B.; Hanisch, F.-G.; Schindler, D. *J. Carbohydr. Res.* **1994**, *13*, 447−456.

(39) Barany, G.; Schroll, A. L.; Mott, A. W.; Halsrud, D. A. *J. Org. Chem.* **1983**, *48*, 4750−4761.

configured with a solvent 126 module and a 166 detector module controlled by Beckman System Gold Software and detection at 220 nm. This system was connected to a PE-Sciex API *III* triple quadrupole mass spectrometer equipped with an ionspray interface. Parameters were as follows: ionspray voltage 5000 V, interface temperature 55 °C, potential on first quadrupole 30 V, orifice voltage varied from 55 to 80 V. The curtain gas flow (N_2) and the nebulizer gas (ultrapure air) were set at 0.8−1.0 L/min. Molecular masses were calculated with the Sciex Macspec 3.22 program. ^{1}H NMR spectroscopy, at 21 °C, was performed on Varian VXR 300 and VXR 500 instruments operating at 300 and 500 MHz, respectively. Assignments of the spectra (Tables 1 and 2 in the main text, Table 3 in the supporting information) were based on 2D homonuclear chemical-shift correlation spectroscopy. ^{13}C NMR spectra were recorded on a Varian VXR 500 operating at 125.7 MHz and assignments (Tables 4 and 5 in the supporting information) were based on heteronuclear multiple quantum coherence (HMQC) spectroscopy. Optical rotations were measured on a JASCO DIP-370 digital polarimeter.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(dithiasuccinoylamino)-D-glucopyranosyl Bromide (8). A solution of glycosyl acetate **17** (mixture of both anomers; 2.0 g, 4.3 mmol) in CH_2Cl_2 (60 mL) was combined with 30% HBr−HOAc (60 mL), and stirred for 8 h at 25 °C while protected with a $CaCl_2$ drying tube. The homogeneous reaction mixture was then diluted with CH_2Cl_2 (150 mL), and washed with ice-cold water (3 × 50 mL), 10% aqueous $NaHCO_3$ (2 × 70 mL), and brine (70 mL). The clear light yellow solution was dried and concentrated to provide the title product as a light yellow solid (1.88 g, 90%). NMR: Tables 1 and 4. FABMS: *m/z* calcd for $C_{14}H_{16}NO_9S_2{}^{79}Br$ 485.0, found 485.9 and 487.9 [MH^+, corresponding to 1:1 Br isotopic distribution]. The title product was used for glycosylation within 24 h of being made, due to its limited stability. Ambient temperature storage for 2-week periods, either in a desiccator over KOH, or else under argon at −20 °C, revealed some degradation (∼10%), as judged by ^{1}H NMR.

***N*$^\alpha$-Fmoc-Ser(3,4,6-tri-*O*-acetyl-2-deoxy-2-(dithiasuccinoylamino)-*β*-D-glucopyranose)-OPfp (9).** A solution of *N*$^\alpha$-Fmoc-Ser-OPfp (**18**) (509 mg, 1.03 mmol) and the glycosyl bromide **8** (602 mg, 1.24 mmol) in dry CH_2Cl_2 (15 mL) was prepared in a flame-dried flask, and stirred under argon in the presence of a few pellets of 3-Å molecular sieves. After 15 min of stirring, freshly dried AgOTf (380 mg, 1.48 mmol) was added, and stirring continued for 3 h. The resultant dark brown mixture was neutralized with DIEA (0.19 mL, 1.2 equiv), diluted with CH_2Cl_2 (35 mL), filtered, and concentrated to provide a brown solid which was purified by silica gel chromatography developed with hexane−EtOAc (3:2). The title product [R_f(A) 0.15] was recrystallized from Et_2O−pentane (1:2) to give an amorphous white solid (801 mg, 86%). Mp: 85-88 °C; $[\alpha]^{25}_D$ −2.49° (*c* 0.80, DMF). NMR: Tables 2−5. FABMS: *m/z* calcd 898.1, found 899.4 [MH^+]. Anal. Calcd for $C_{38}H_{31}F_5N_2O_{14}S_2$: C, 50.78; H, 3.48; N, 3.12; S, 7.13. Found: C, 50.62; H, 3.53; N, 2.98; S, 6.98.

***N*$^\alpha$-Fmoc-Thr(3,4,6-tri-*O*-acetyl-2-deoxy-2-(dithiasuccinoylamino)-*β*-D-glucopyranose)-OPfp (10).** The title compound was prepared in the same manner already described for **9**, but starting with *N*$^\alpha$-Fmoc-Thr-OPfp (**19**) (436 mg, 0.86 mmol), **8** (500 mg, 1.02 mmol), and AgOTf (292 mg, 1.13 mmol). Chromatography [R_f (A) 0.14] and crystallization gave a pale white crystalline solid (666 mg, 85%). Mp: 91−92 °C. $[\alpha]^{25}_D$ −26.63° (*c* 0.86, DMF): NMR: Tables 2−5. FABMS: *m/z* calcd 912.1, found 913.2 [MH^+]. Anal. Calcd for $C_{39}H_{33}F_5N_2O_{14}S_2$: C, 51.32; H, 3.64; N, 3.07; S, 7.02. Found: C, 51.12; H, 3.80; N, 2.99; S, 7.27.

***N*$^\alpha$-Fmoc-Ser(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranose)-OPfp (11).** A solution of **9** (250 mg, 0.28 mmol) in HOAc−THF (1:9, 2 mL) was treated with Zn dust (72 mg, 1.1 mmol), followed 1 min later by Ac_2O (4 mL). After stirring for 17 h, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and filtered; the filtrate was washed with water (3 × 50 mL), 10% aqueous $NaHCO_3$ (4 × 50 mL), and brine (50 mL). This solution was next dried and concentrated to obtain a pale yellow solid which was purified by silica gel chromatography developed with hexane−EtOAc (1:1) [R_f(C) 0.12]. The title compound was obtained as a pale white solid (228 mg, 80%). Mp: 183-184 °C (lit.[23d] 207 °C). $[\alpha]^{25}_D$ −24.90° (*c* 0.39, DMF). NMR: Tables 2−5. FABMS: *m/z* calcd 822.2, found 823.1 [MH^+]. Anal. Calcd for $C_{38}H_{35}F_5N_2O_{13}$: C, 55.48; H, 4.41; N, 3.41. Found: C, 55.66; H, 4.58; N, 3.31.

***N*$^\alpha$-Fmoc-Thr(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-*β*-D-glucopyranose)-OPfp (12).** The title compound was prepared in the same manner already described for **11**, but starting with **10** (250 mg, 0.274 mmol). This provided a pale white solid (200 mg, 87%) [R_f(A) 0.24]. Mp: 179−180 °C (lit.[23d] 175 °C dec). $[\alpha]^{25}_D$ −28.49° (*c* 0.41, DMF). NMR: Tables 2−5. FABMS: *m/z* calcd 836.2, found 837.4 [MH^+]. Anal. Calcd for $C_{39}H_{37}F_5N_2O_{13}$: C, 55.92; H, 4.57; N, 3.34. Found: C, 55.99, H, 4.73; N, 3.50.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[(ethoxythiocarbonyl)amino]-D-glucopyranose (15). A suspension of D-glucosamine hydrochloride (**13**) (5.0 g, 23.2 mmol) and bis(ethoxythiocarbonyl) sulfide (**14**) (5.36 g, 25.5 mmol, 1.1 equiv) in EtOH−H_2O (1:1) (50 mL) was brought to a pH of 9−10 by addition of 4 N aqueous NaOH (7 mL). Additional 4 N aqueous NaOH (12 mL) was added dropwise over a period of 4 h in order to maintain the pH; at the end of this time, all of **14** had dissolved and the pH did not change more. The reaction mixture was stirred for a further 24 h, and then solvent was removed *in vacuo* followed by drying in a desiccator (1 mm vacuum) to provide a light yellow solid (8.15 g). This solid was suspended with vigorous stirring in CH_2Cl_2−C_5H_5N (1:1, total of 20 mL), and after 1 h, Ac_2O (21.9 mL, 232 mmol, 10 equiv) was added and stirring continued overnight. The reaction mixture was then diluted with CH_2Cl_2 (50 mL), washed with 3 N aqueous H_2SO_4 (3 × 50 mL), 5% aqueous $NaHCO_3$ (4 × 50 mL), and water (2 × 60 mL), and dried. Concentration provided a brown gum which was chromatographed on silica gel [eluent EtOAc−hexane (1:1), R_f(A) 0.28 (*β*) and 0.19 (α)] to provide a yellow foamy mass of the title compound, which on further drying *in vacuo* (1 mmHg) became a pale yellow crystalline solid (8.1 g, 80%). Mp: 72−75 °C. FABMS: *m/z* calcd 435.1, found 436.1 [MH^+]. ^{1}H NMR ($CDCl_3$) included characteristic resonances for the Et group at δ 4.45 and 1.30 (J = 7.2 Hz). Anal. Calcd for $C_{17}H_{25}NO_{10}S$: C, 46.89; H, 5.79; N, 3.22, S, 7.36. Found: C, 46.97, H, 5.66; N, 3.12; S,7.37.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(dithiasuccinoylamino)-D-glucopyranose (17). The procedure which follows is the most straightforward; numerous variations were tried as sketched in the text. A solution of thioamide **15** (3.0 g, 6.9 mmol) in dry CH_3CN (30 mL), placed in a flask protected by a $CaCl_2$ drying tube, was treated at 25 °C with (chlorocarbonyl)sulfenyl chloride (**16**) (0.69 mL, 8.25 mmol, 1.5 equiv, added via glass syringe). After 4 h of stirring, the reaction mixture was diluted with CH_2Cl_2 (60 mL) and washed with 0.6 N aqueous $NaHCO_3$ (3 × 60 mL), water (50 mL), and brine (50 mL). The solution was dried and concentrated to give a yellow gum, which was purified by silica gel chromatography [eluent toluene−EtOAc, 3:1]. The fractions containing product were combined, treated with activated charcoal (750 mg), filtered, and concentrated to provide the target compound as a light yellow solid (2.56 g, 80%). mp: 120-126 °C, a mixture of anomers (α:β = 5.4 : 1). NMR: Tables 1 and 4. Anal. Calcd for $C_{16}H_{19}NO_{11}S_2$: C, 41.13; H, 4.08; N, 3.01; S, 13.76. Found: C, 41.12; H, 4.15; N, 2.95; S, 13.66. In a separate experiment on a similar scale, chromatography with a gradient [eluent toluene−EtOAc, 5:1 to 3:1] provided the pure α-anomer (α-**17**) [34%, R_f(A) 0.19], whereas the *β*-anomer (*β*-**17**) [R_f(B) 0.23] could not be isolated completely pure. The α-anomer was recrystallized from ether−petroleum ether to yield a white solid. Mp: 147-150 °C. $[\alpha]^{25}_D$ +109.6° (*c* 0.92, DMF): FABMS: *m/z* calcd 465.0, found 466.1 [MH^+]. Anal. Calcd for $C_{16}H_{19}NO_{11}S_2$: C, 41.13; H, 4.08; N, 3.01; S, 13.76. Found: C, 41.24; H, 4.25 ; N, 2.86 ; S, 13.66.

Attempted Selective Deprotection and Acetylation of *N*$^\alpha$-Fmoc-Ser(3,4,6-tri-*O*-acetyl-2-deoxy-2-(dithiasuccinoylamino)-*β*-D-glucopyranose)-OPfp (9). Method A. A solution of **9** (20 mg, 22 μmol) in CH_2Cl_2 (1 mL) was treated with BME (5 μL, 3 equiv) and DIEA (3 μL, 3 equiv). Starting material [R_f(A) 0.15] disappeared within 5 min. Acetic anhydride (41 μL, 20 equiv) was added, and the reaction was monitored further for 18 h by TLC and HPLC (no starting material at t_R 48.0 min; three major peaks at t_R 37.0, 41.9, and 44.3 min). The complicated reaction mixture was evaluated by FABMS and shown to contain the following ions: *m/z* 759.5 due to **20**, 863.2 due to **21**, and 885.1 due to **22** (see ref 32 for structures).

Method B. A solution of **9** (5 mg, 5.5 μmol) was treated with MAc (3 μL, 5 equiv) in DMF. As in the previous experiment, starting

material disappeared within 5 min. Acetic anhydride (10 μL, 20 equiv) was added after 30 min, and the reaction was monitored further for 4 h by TLC and HPLC (new peaks at t_R 41.1 and 47.6 min). The reaction mixture was evaluated by FABMS and shown to be comprised of lactam **27** (structure in Scheme 3), *m/z* 597.1, and adduct **26** (structure in ref 32), *m/z* 744.0.

Bicyclic Lactam 27.[34] A solution of **9** (50 mg, 55 μmol) in HOAc−THF (1:9, 3 mL) was treated with Zn dust (14 mg, 0.22 mmol) for 1.5 h, at which point HPLC analysis showed a single major peak (92%), t_R 41.1 min [halftime for disappearance of **9** and appearance of **27**, ∼20 min]. The crude reaction mixture was filtered and diluted with CH_2Cl_2 (30 mL), washed with water (3 × 30 mL), dried, and evaporated to provide a light yellow gummy material. The title product precipitated as a white fluffy material (30 mg, 90%) from anhydrous ether−pentane at 4 °C overnight. Mp: 92−94 °C. NMR: Tables 2−5. FABMS: *m/z* calcd 596.5, found 597.3 [MH^+]. Anal. Calcd for $C_{30}H_{32}O_{11}N_2$: C, 60.40; H, 5.36; N, 4.69. Found: C, 58.56; H, 5.49; N, 4.59.

Bicyclic Lactam 28. The title compound was prepared in the same manner already described for **27**, but starting with **10** (50 mg, 54 μmol). HPLC analysis of the crude mixture showed a single major product (95%), t_R 41.8 min. The title product precipitated as a white fluffy material (31 mg, 93%). Mp: 113−115 °C. NMR: Tables 2−5. FABMS: *m/z* calcd 610.3, found 611.3 [MH^+]. Anal. Calcd for $C_{31}H_{34}O_{11}N_2$: C, 60.98; H, 5.57; N, 4.59. Found: C, 59.71; H, 5.15; N, 4.21.

H-Ser-Ala-Val-Ser(Ac$_3$-*β*-D-*O*-Glc*p*NAc)-Ser-Ala-NH$_2$. Manual chain assembly was carried out starting with PAL-Nle-PEG-PS resin (0.2 g, 0.26 mmol/g). Wash volumes were 2 mL. Side chain protection for the two nonglycosylated Ser residues was provided by *tert*-butyl ethers. Fmoc removal was accomplished with piperidine−DMF (1:4) (2 × 8 min), followed by washes with CH_2Cl_2−DMF (1:1, 4 × 2 min). Couplings of all N^{α}-Fmoc-amino acid derivatives (0.132 mmol, 3 equiv) were mediated by DIPCDI (3 equiv) and HOBt (1.5 equiv) in a minimal volume of DMF (∼ 1 mL) for 2 h, except for the Dts-protected glycosylated Pfp ester **9** (3 equiv) which was coupled for 2 h in the presence of only HOBt (1.5 equiv) in DMF. Following incorporation of Ser building block **9**, the Dts protecting group was cleaved with BME (0.5 M)−DIEA (0.5 M) in CH_2Cl_2 (3 × 2 min); immediately thereafter, the peptide-resin was treated with acetic anhydride (60 μL, 25 equiv) in CH_2Cl_2−C_5H_5N (1:1, 2 mL) for 2 h. Chain assembly continued in the usual way, and the completed peptide-resin was dried. For cleavage, a portion (50 mg) was treated first with piperidine−DMF (1:4) to remove Fmoc, followed by washings and then TFA−H_2O (19:1) for 2 h at 25 °C. The filtrate from the cleavage reaction was collected and combined with TFA washes (3 × 2 mL) of the cleaved peptide-resin, and the resultant solution was concentrated under a stream of N_2. The crude cleaved peptide was precipitated with cold diethyl ether (2 mL), collected by centrifugation, washed with more ether (3 × 2 mL), and dried to give material that showed a single major component by C-4 analytical HPLC (Figure 1, t_R 26.8 min, >80% purity). FABMS: *m/z* calcd for $C_{34}H_{56}N_8O_{17}$ 848.4, found 849.6 [MH^+].

H-Ser-Ala-Val-Ser(*β*-D-*O*-Glc*p*NAc)-Ser-Ala-NH$_2$. The dry acetylated intermediate (1.5 mg, 1.18 μmol) was dissolved in dry MeOH (1 mL), and 0.1 M NaOMe in MeOH was added dropwise (total 50 μL) until moist pH paper indicated a pH of 10. After 15 min, the reaction mixture was quenched by addition of solid CO_2. LC/MS: *m/z* calcd for $C_{28}H_{50}N_8O_{14}$ 722.4, found 723.0 [MH^+], 745.2 [MNa^+].

H-Ser-Ala-Val-Thr(*β*-D-*O*-Glc*p*NAc)-Ser-Ala-NH$_2$. Synthesis proceeded with Thr building block **10**, but otherwise was as just described for the corresponding Ser glycopeptide. FABMS of the intermediate after cleavage from the support: *m/z* calcd for $C_{35}H_{58}N_8O_{17}$ 862.4, found 863.4 [MH^+]. HPLC: t_R 26.4 min (>80% purity). LC/MS of the title glycopeptide after deacetylation: *m/z* calcd for $C_{29}H_{51}N_8O_{14}$ 736.4, found 737.1 [MH^+], 759.0 [MNa^+].

H-Ser-Ala-Val-Ser(*β*-D-*O*-Glc*p*NAc)-Ser-Ala-Asn-Gly-Thr-Val-Leu-Lys-NH$_2$. The methods followed those already described for the glycohexapeptides. Side chain protection was provided by *tert*-butyl-type derivatives (ethers, Boc-urethane); the Asn was incorporated as its N^{α}-Fmoc Pfp ester. FABMS of the intermediate after cleavage from the support: *m/z* calcd for $C_{61}H_{104}N_{16}O_{25}$ 1460.7, found: 1461.4 [MH^+]. C-4 analytical HPLC: one major peak, t_R 27.5 min. FABMS of the title glycopeptide after deacetylation: *m/z* calcd for $C_{55}H_{98}O_{22}N_{16}$ 1334.7, found: 1335.8 [MH^+], 1357.8 [MNa^+].

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Supporting Information Available: Tables 3−5 listing ^1H and ^{13}C NMR data (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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