Solid-Phase Synthesis of O-Linked Glycopeptide Analogues of Enkephalin

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The synthesis of 18 N-α-FMOC-amino acid glycosides for solid-phase glycopeptide assembly is reported. The glycosides were synthesized either from the corresponding O'Donnell Schiff bases or from N-α-FMOC-amino protected serine or threonine and the appropriate glycosyl bromide using Hanessian's modification of the Koenigs–Knorr reaction. Reaction rates of D-glycosyl bromides (e.g., acetobromoglucose) with the L- and D-forms of serine and threonine are distinctly different and can be rationalized in terms of the steric interactions within the two types of diastereomeric transition states for the D/L and D/D reactant pairs. The N-α-FMOC-protected glycosides [monosaccharides Xyl, Glc, Gal, Man, GlcNAc, and GalNAc; disaccharides Gal-β(1–4)-Glc (lactose), Glc-β-(1–4)-Glc (cellobiose), and Gal-α(1–6)-Glc (melibiose)] were incorporated into 22 enkephalin glycopeptide analogues. These peptide opiates bearing the pharmacophore H-Tyr-c[DCys-Gly-Phe-DCys]- were designed to probe the significance of the glycoside moiety and the carbohydrate–peptide linkage region in blood–brain barrier (BBB) transport, opiate receptor binding, and analgesia.

Introduction

Advanced cellular processes such as cell–cell recognition and adhesion,[1] intercellular and intracellular transport of gene products,[2] secondary structure effects such as protein folding,[3] and control of membrane permeability and molecular recognition[4] are often directed by the extent and nature of carbohydrate moieties expressed on cell surfaces. Blood group determinants (ABH, etc.), tumor-associated antigens (Le$_x$, Le$_y$, etc.), and pathogen binding sites are some of the relevant glycoconjugates found on mammalian cells.[5] O-Linked glycosylation of proteins facilitates cellular communication by dictating cell-surface and cytosolic presentation of carbohydrate moieties and is essential for proper cellular homeostasis, hormonal balance, and immune response. Posttranslational modification of peptides and proteins is highly conserved, and microheterogeneity of proteo- and peptidoglycan may be involved in the regulation of many biological and physiological phenomena. Abnormalities in glycosylation are associated with disease states such as in the T- and T$_N$-epitopes related to cystic fibrosis and cancer[6] and in τ-protein in Alzheimer's disease.[7]

As predicted by Montreuil,[8] the field of glycobiology has grown explosively, and the interest in the development of methods for the synthesis of glycoconjugates is rapidly expanding as their biological roles come into focus. Synthetic glycopeptides (glycoprotein fragments) are required for the study of glycoprotein binding interactions, cell–cell adhesion, and receptor binding specificity. Recent progress in this area has been remarkable, and relatively complex carbohydrate moieties have been covalently attached to amino acids for incorporation into glycopeptide sequences, e.g., mucin fragments,[9] T- and T$_N$-antigens,[10] interleukin 8 receptor fragments,[11] helper T-cell haptens,[12] CD52 antigen fragments,[13] and oncofetal fibronectin sequences.[14] Recently, a complex glycopeptide of eel calcitonin was synthesized using an endo-β-N-acetylglucosaminidase to synthesize a 31-amino acid

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peptide with a disialyl biantennary complex-type saccharide moiety N-linked to asparagine.[15] Glycoconjugate vaccines against certain types of cancer have been synthesized.[16] Glycopeptides[17] and neoglycopeptides[18] can now be assembled once the required amino acid glycoside is in hand. Despite these advances, the pharmacology and biology of glycopeptides is still far less developed than peptides, largely because glycopeptides are considerably more difficult to synthesize.

Peptides have been discounted as drug candidates due to a perceived instability to hydrolytic enzymes and pH extremes and due to the blood-brain barrier (BBB), which blocks their entry into the central nervous system.[19] Glycosylation has been used as a method to penetrate the BBB, and enkephalin analogues glycosylated at or near the C-terminus were shown to elicit prolonged and profound analgesia in mice.[20] Similar results have been obtained with glycosylated vasopressin analogues[21] and with deltorphin and dermorphin glycopeptide analogues.[22]

Originally, it was envisaged that the glucose transporter GLUT-1 found at the BBB would transport the opiate message sequences to the targeted receptors within the brain. Subsequent work by Davis et al.[23] with ^{14}C-labeled glycopeptides showed that this mode of transport is unlikely and led to the conclusion that other mechanisms were responsible for the BBB permeability of these glycopeptides.[24] Paradoxically, the observed

transport rates, as shown by physicochemical data, do not correlate with lipophilicity,[25] which reinforces the premise that another transport mechanism is at work. This transport phenomenon may be related to the observation that glycosylation of peptides has been shown to improve absorption rates from the intestinal tract.[26]

Design Criteria. The "message sequence," or pharmacophore, used in these studies was borrowed from the work of Hruby et al.,[27] the δ-selective enkephalin parent sequence D-Pen^2,D-Pen^5-enkephalin (DPDPE).[28] The Tyr-DCys-Gly-Phe-DCys "message" was chosen as a relatively unselective ligand (μ vs δ) to maximize the possibility of interaction with a receptor within the brain, and to observe any influences of the glycoside moiety on receptor selectivity.[29] In addition, it was hoped that this would help establish a "pharmacological baseline" with which to compare the effectiveness of transport in vivo. Since D-amino acids have been used in several peptides to circumvent degradation by proteases and hydrolases,[30] D-serine and D-threonine were also used as glycoside-bearing amino acids. Surprisingly, these O-linked D-amino acid glycosides do not appear to have been examined previously in any context.

At this point, the precise role of the glycoside in peptide and protein transport processes remains enigmatic. Most O-linked glycoproteins found in nature have a core glycan structure initiated with a serine- or threonine-linked α-GalNAc, and it is believed that the glycan moiety plays a decisive role in intracellular transport (protein trafficking).[31] Thus, related glycopeptide structures may provide new insights on protein trafficking. Mannose-6-phosphate binding proteins are involved in the transport of glycoproteins to lysosomal compartments for enzymatic digestion.[32] Also, due to the importance of β-GlcNAc–Ser/Thr glycosides in both the β-amyloid precursor protein (APP)[33] and nuclear pore proteins,[34] β-GlcNAc was included as one of the glycosyl moieties. The disaccharides were incorporated to examine the effect of a larger glycan moiety on the pharmacology of these glycopeptides, and the xyloside (present in gel-forming mucins)[35] was included to see whether a pentose glycoside

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would display the same pharmacology as the hexose derivatives. Glucosides and galactosides commonly occur in mammalian glycosphingolipids and glycoproteins and were included on that basis.

The glycosidic linkage is unstable to strong acids such as HF, and thus BOC peptide strategy is not generally useful.[36] Although glycan susceptibility to β-elimination rules out the use of strong bases, the FMOC peptide strategy[37] can use 10% piperidine, 50% morpholine, or 2% DBU as a deprotection reagent without adverse affects on the stability of the sugar moiety.[38] Most *O*-glycosidic linkages have proven to be stable to acids such as TFA for brief periods, and since most FMOC-based resin linkers are cleaved with TFA, an FMOC protection scheme for amino acid glycosides has proven to be the most effective method for the preparation of glycopeptides on resin supports. Solution-phase methods are more flexible in terms of protection strategies, but are limited to relatively short glycopeptides.[39]

There have now been several reports of the synthesis of N-α-FMOC-amino acid glycosides using O'Donnell Schiff base esters,[40] N-α-FMOC-amino esters,[41,37c,37d,37e] N-α-FMOC pentafluorophenol esters,[42] and N-α-FMOC-amino acids,[43,37f] as aglycones. Many have displayed high α/β selectivity and yields in their respective glycosylations and have been incorporated into peptides using FMOC protection with various forms of activation. In this study, 18 glycosides were synthesized using Hanessian's modification[44] of the classical Koenigs–Knorr[45] reaction. Stereocontrol was imparted by the use of participating groups in the 2-position[46] to yield 1,2-trans glycosides, except for GalNAc in which a nonparticipating azido group was used in the 2-position to allow 1,2-cis glycoside formation in good yield. The glycosides were converted to their corresponding N-α-FMOC-amino acid glycosides and used for solid-phase glycopeptide synthesis (SPGPS) of several enkephalin analogues. Several resin linkers
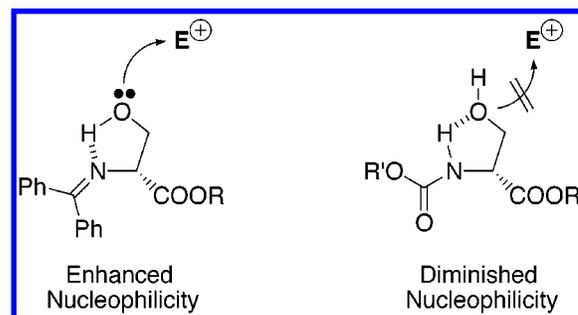

Figure 1. Hydrogen bonding patterns affect nucleophilicity of aglycones.

have been used in glycopeptide synthesis,[47] but for the synthesis of these glycopeptide amides, Rink resin[48] was employed. It is noteworthy that a choice of hydroxyl protection that is optimal for glycoside formation may become disastrous for peptide assembly and/or final deprotection of the glycopeptide (vide infra).

Aglycone Synthesis. The use of O'Donnell Schiff-bases has been shown to enhance the nucleophilicity of the aglycone in Koenigs–Knorr type glycosylations, and in Lemieux's in situ anomerization method.[40c,49] This is apparently due to a favorable hydrogen-bonding pattern versus an unfavorable pattern for *N*-acyl or *N*-carbamoyl protected aglycones (Figure 1). However, in concert with this enhancement, an equal if not greater effect on glycosylation yield and selectivity seems to be the carboxyl protection of the aglycone. It has been previously demonstrated that the use of a less sterically demanding ester protecting group (i.e., methyl) provides higher yields than the more crowded benzyl and benzhydryl esters.[40c] This effect may in part be due to improved chelation of silver ion with the aglycone prior to presentation to the glycosyl donor. In this study, glycosylations using both L- and D-forms of serine and threonine have been examined in order to provide additional information regarding the steric demands of the glycosylation reaction.

The FMOC-protected aglycone strategy was also examined. It was observed that N-α-FMOC-aglycones provide very reactive acceptors in the Koenigs–Knorr and related glycosylations in certain cases, while the Schiff base aglycones were more reactive in other cases. A hierarchy has been established to suggest the best conditions for each N-α-FMOC-amino acid glycoside synthesis. The aglycones **1a**–**8b** are displayed in Figure 2.

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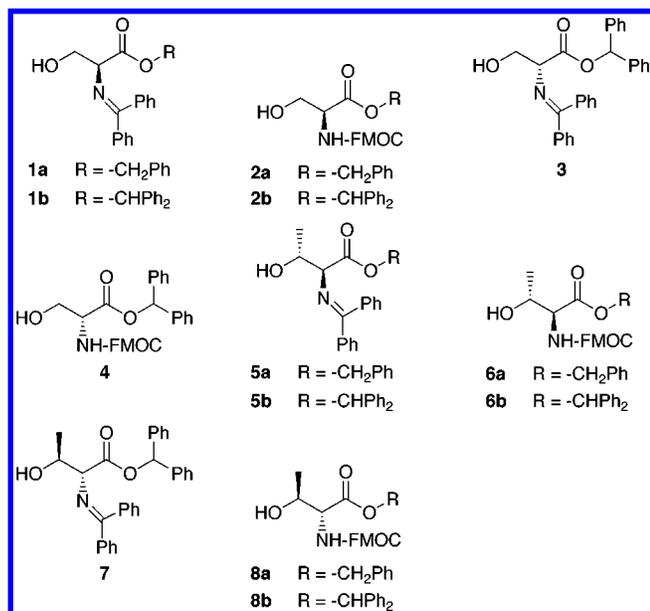(45) Koenigs, W.; Knorr, E. *Ber.* **1901**, *34*, 957.

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Figure 2. Aglycones used in this study.

Bromide Synthesis. Various donors have been used to produce oxonium ions. Schmidt's trichloroacetimidate method,[50] Fraser-Reid's pentenyl glycoside method,[51] and Kahn's sulfoxide activation[52] are all very effective strategies for block glycoside synthesis, but the classical Koenigs–Knorr method has also shown to be an effective and simple method for the synthesis of mono- and disaccharides. In this study, standardized Koenigs–Knorr reaction conditions were used in order to compare the glycosylation results. All peracetyl and perbenzoyl sugars were converted to their corresponding α-bromide using the established Fischer protocol[53] and were fully characterized prior to glycosylation. (Scheme 1)

Three bromides required several synthetic steps (Scheme 1). First was the synthesis of 2-deoxy-2-azido-1-bromo-D-Gal(OAc)₃ **19**. Readily available 3,4,6-triacetoxy-D-galactal was subjected to Lemieux's azidonitration conditions[54] to yield a mixture of azidonitrates in 60% yield. Direct treatment of the mixture with LiBr in CH₃CN produced the α-bromide **19** in 70% yield after removal of the talo-isomer by flash chromatography.[55] The "participating" 2-deoxy-2-trichloroethoxycarbonylamide-1-bromo-D-Glc(OAc)₃ **17** was synthesized from D-glucosamine using Schotten–Bauman conditions, followed by acylation and bromination. Similarly, the benzoyl-protected Troc-protected bromide **18** was produced by substituting benzoyl chloride for acetic anhydride. This synthetic route is considerably shorter than other established methods.[56] The glycosyl bromides **9**–**19** used in this study are shown in Figure 3.

(50) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.

(51) Udodong, U. E.; Rao, C. S.; Fraser-Reid, B. *Tetrahedron* **1992**, *48*, 4713–24.

(52) (a) Kahne, D.; Walker, S.; Cheng, Y.; van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881. (b) Kim, S. H.; Augeri, D.; Yang, D.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 1766–75.

(53) Fischer, E.; Zemplen, G. *Ber.* **1910**, *43*, 2534.

(54) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244–51.

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Scheme 1ᵃ
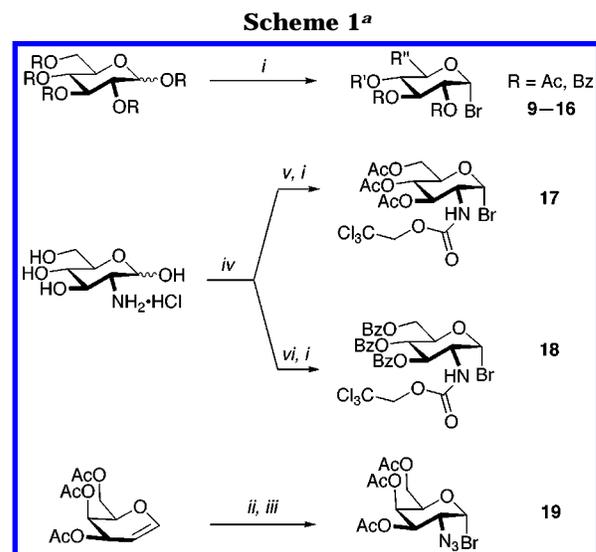
ᵃ Reagents: *i.* HBr/HOAc/CH₂Cl₂/0 °C → RT, *ii.* NaN₃/CAN/CH₃CN/−15 °C, *iii.* LiBr/CH₃CN, *iv.* Cl₃CCH₂OCOCl/NaHCO₃/H₂O, *v.* Ac₂O/pyridine, *vi.* PhCOCl/pyridine.

Glycosylation Results. Sizable quantities of N-α-FMOC-amino acid glycosides were required, and several problems emerged that prompted further investigation. Originally, due to high yields and selectivity in the glycosylation step, benzoate protection was used on the donors. However, peptide synthesis using the N-α-FMOC-amino acid glycosides **44**, **46**, **48**, **50**, and **58** (Figure 6) resulted in poor coupling yields and incomplete removal of the benzoates, both of which contributed to the heterogeneity of the resultant glycopeptides. Use of the corresponding acetates gave lower yields during glycosylation, but provided pure glycopeptides after assembly and cleavage.

Aglycone **1a** was glycosylated with bromide **11** and AgOTf promotion to provide the desired β-glycoside **21** in 92% yield (Scheme 2) and 5% of the α-product (18:1 β: α). However, when the same glycosylation was performed using bromide **10**, a 32% yield of **20b** was obtained with a 5:1 β: α ratio. The loss in yield was largely due to competing ortho ester formation, as evidenced by the recovery of the aglycone acetate (Scheme 3). Changes in concentration, molar excess of glycosyl donor, amount of molecular sieves, and temperature did not prevent this unwanted side-reaction. All the glycosylations using 2-acetoxy glycosyl bromides led to this degradation and occurred with both L- and D-forms of serine and threonine Schiff bases and with the N-α-FMOC-protected aglycones as well. However, this did not occur with the 2-benzoyloxy bromides, which displayed both high yields and excellent β-selectivity in the glycosylation reactions. (Table 1)

A comparison of L-/D-serine and L-/D-threonine as aglycones was accomplished using the Schiff-base benzhydryl esters **1b**, **3**, **5b**, **7** and the benzoyl glucosyl bromide **11** under identical glycosylation conditions. The same molarity, temperature, time, and molecular sieves were used in each case. The sterically accessible primary alcohol enantiomers L-serine **1b** and D-serine **3** showed little difference as aglycones; the yield of L-serine β-glycoside **22** was 61%, and the diastereomeric D-serine β-glycoside **24** was obtained in a comparable 71% yield. For the sterically hindered secondary threonine enantiomers, a more profound difference was observed, with
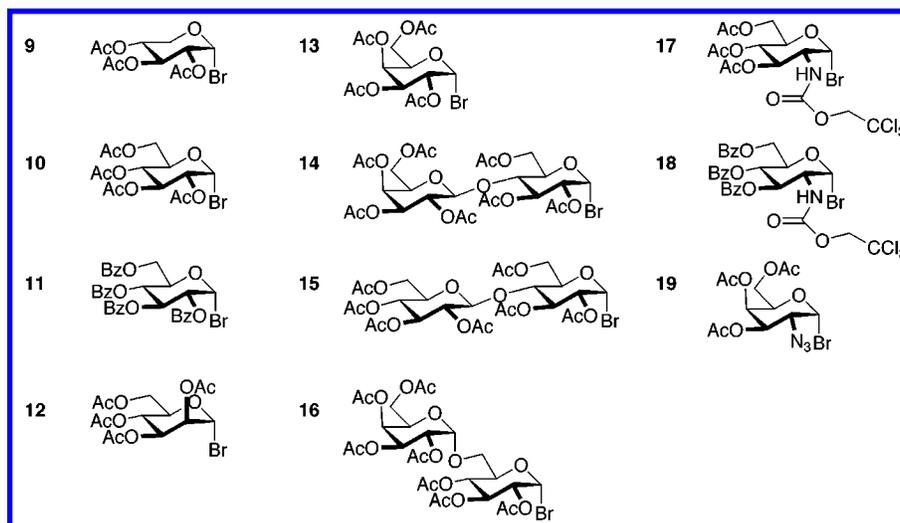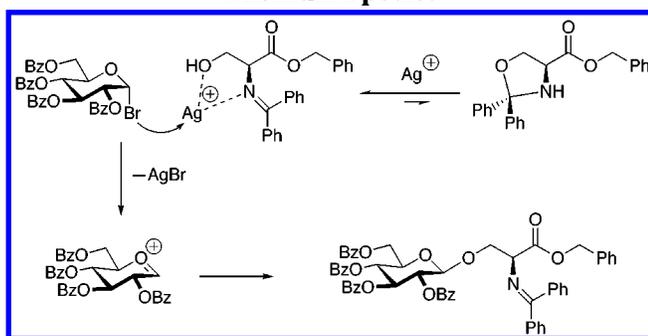
Figure 3. Glycosyl bromides used in this study.

Scheme 2. Ag-Directed Delivery of Aglycone to Oxonium Species

the L-threonine glycoside **26** obtained in only 20% yield and 1:8 α/β selectivity versus 50% yield and virtually complete β selectivity for the D-threonine glycoside **29**.

It is not surprising that the D- and L-serine aglycones **1b** and **3** provide very similar yields in the glycosylation reaction, since the electrophilic attack by the hydroxyl can occur from either face (Figure 4). On the other hand, the D- and L-threonine aglycones **5b** and **7** provide very

different steric environments for the glycosylation reaction with **11**, constituting a *matched* (D-amino acid−D-sugar) and a *mismatched* (L-amino acid−D-sugar) pair of reactants (Figure 5). While the D-threonine aglycone **7** has a relatively favorable anti-periplanar approach, the L-threonine enantiomer **5b** has no favorable approach due to interference of the methyl group. Delivery of the Schiff-base aglycones by silver ions to the oxonium species is intrinsically biased against the L-form.

To overcome the low yields of L-threonine glucosylation, the N-α-FMOC-amino protected L-threonine aglycone **6a** was used under the same glycosylation conditions using bromide **11**. This modification resulted in β-glycoside **27**, obtained in an 82% yield. While the benzyl ester **6a** reacted efficiently, the benzhydryl ester **6b** was glycosylated with **11** to provide a complex mixture. All serine and threonine glucosylation results are displayed in Tables 1 and 2. All yields were based on the purified glycosides after flash chromatography of the glycoside products.

When aglycone **1a** was glycosylated with acetobromo-mannose **12**, nearly quantitative yields of the α-glycoside **32** was obtained. When **1a** was reacted with acetobro-

Scheme 3. Acetate Protection Leads to Orthoester Formation and Transfer
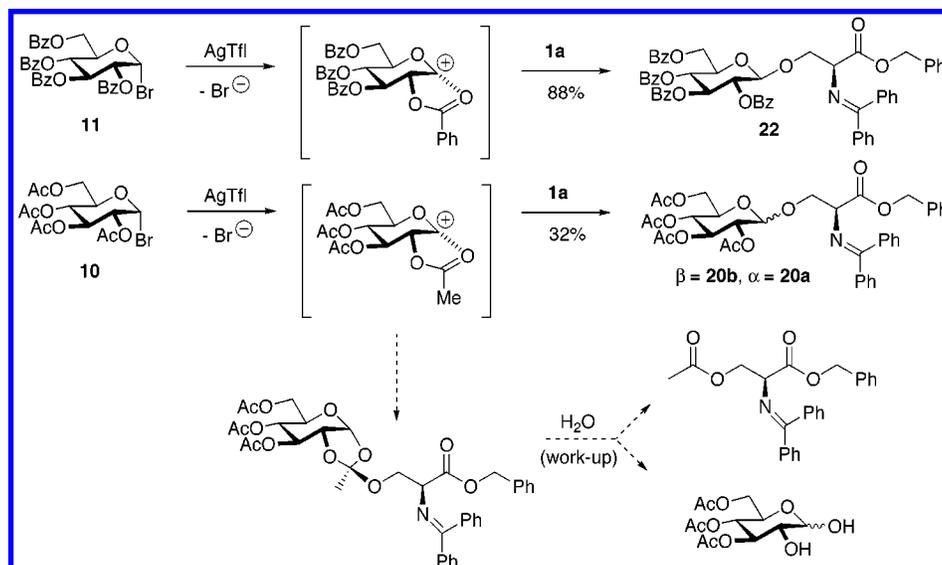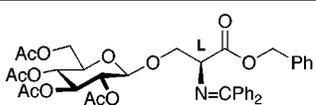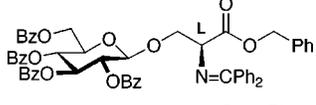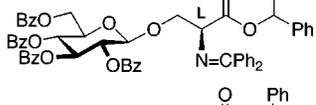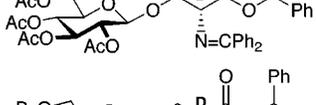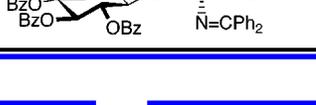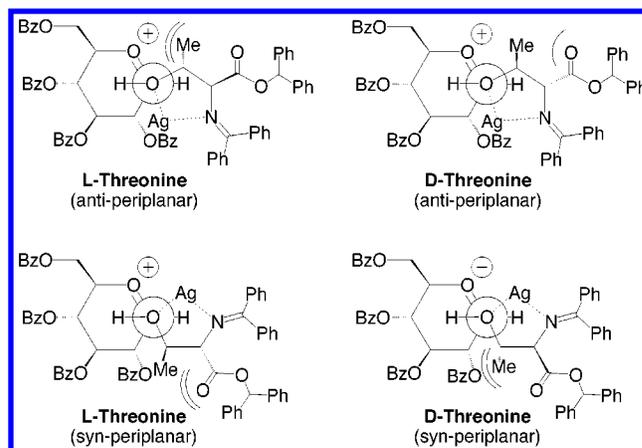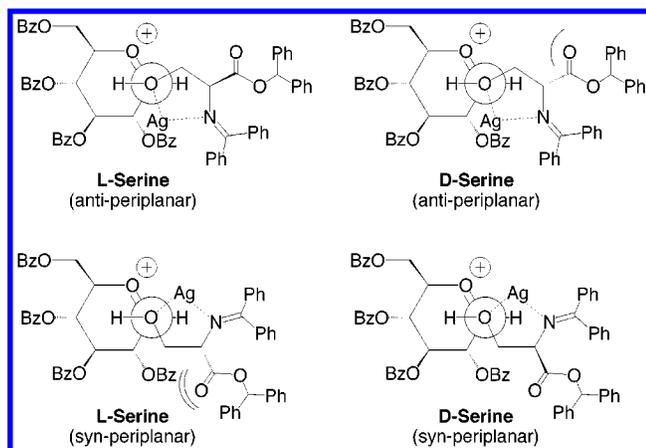
Table 1. All Glycosylations Were Performed Using 1.4−1.5 Equiv of AgOTFl in CH₂Cl₂ at 0 °C for 6−12 h. Ratios Were Established by NMR Analysis of Crude Material

Aglycone	Bromide	Yield	Glycoside		Selectivity (α:β)*
1a	10	32%		20b	1:5
1a	11	92%		21	1:18
1b	11	61%		22	1:10
3	10	26%		23	1:10
3	11	71%		24	<1:20

Figure 4. Serine can approach in either a syn- or antiperiplanar sense, thus avoiding steric strain. D-Serine and L-serine are roughly equivalent as aglycones.

Figure 5. L-Threonine encounters steric strain in both a syn- or antiperiplanar approach, while D-threonine can approach in an antiperiplanar sense to avoid strain. L-Threonine and D-threonine are quite different aglycones.

moxylose **9**, an 87% yield of β-glycoside **31** was obtained, and none of the corresponding α-product was observed. Thus, the Schiff-base methodology is ideal for the synthesis of serine α-mannosides and serine β-xylosides, displayed in Table 3. When acetobromoxylose and acetobromomannose were glycosylated with the N-α-FMOC-aglycone **2a**, drastically lower yields and selectivities resulted.

The glycosylation of lactose (β-D-Gal-(1→4)-D-Glc) and cellobiose (β-D-Glc-(1→4)-D-Glc) using bromides **14** and **15** and aglycone **1a** were effective, yielding glycosides **34** and **35** in 65% and 68%, respectively. The poorer yields were due to the formation of the respective aglycone acetates, which were isolated in both reactions. Only the β-form of each glycoside was observed after chromatography. For melibiose (α-D-Gal-(1→6)-D-Glc) only a 40% yield was obtained with a 15:1 β: α selectivity with **1a** and **16**. In this case β-attack can be sterically hindered due to the flexible Gal-α(1−6) linkage, as indicated by both the yield and selectivity.

Two methods were used to convert these glycosides to their N-α-FMOC-amino acid forms. In route 1 the Schiff-base glycosides were converted to their N-α-FMOC-ester forms via TFA hydrolysis in aqueous THF, followed by acylation with FMOC-Cl. Hydrogenation converted the esters to their final N-α-FMOC amino acid forms. This route required the removal of benzophenone by chromatography prior to acylation and may require an additional chromatography after hydrogenation. Although high yielding, this method was inefficient due to the number of purification steps. Route 2 subjected the Schiff-base glycosides to hydrogenation conditions to remove both amino and carboxyl protection groups in one step. Simple extraction followed by acylation yielded pure N-α-FMOC-amino acid glycosides after chromatography. Route 2 proved to be the most efficient conversion scheme for the gram-scale synthesis of these N-α-FMOC-amino acid glycosides. Both routes are displayed in Scheme 4 and all N-α-FMOC-amino acid glycosides are illustrated in Figure 6.

The syntheses of β-GlcNAc and α-GalNAc N-α-FMOC amino acids were optimized. A participating protecting group is required for nitrogen to obtain the β-GlcNAc

Table 2. All Glycosylations Were Performed Using 1.4−1.5 Equiv of AgOTFl in CH₂Cl₂ at 0 °C for 6−12 h. Ratios Were Established by NMR Analysis of Crude Material

Aglycone	Bromide	Yield	Glycoside		Selectivity (α:β)*
5b	11	20%		25	1:6
6a	10	40%		26	1:8
6a	11	82%		27	<1:20
7	10	40%		28	<1:20
7	11	50%		29	<1:20
8a	11	50%		30	<1:20

Table 3. All Glycosylations Were Performed Using 1.4−1.5 Equiv of AgOTFl in CH₂Cl₂ at 0 °C for 6−12 h. Ratios Were Established by NMR Analysis of Crude Material

Aglycone	Bromide	Yield	Glycoside		Selectivity (α:β)*
1a	9	87%		31	1:37
1a	12	99%		32	>20:1
1a	13	32%		33b	1:4
1a	14	65%		34	<1:20
1a	15	68%		35	<1:20
1a	16	40%		36	1:16

linkage. *N*-Acetyl itself cannot be used due to formation of the extremely stabile oxazoline upon activation. Several protecting groups have served this purpose. The 2,2,2-trichloroethyloxycarbonylamino (Troc) group was developed by Windholtz and Jonston[57] and has been used by many others.[58] The allyloxycarbonylamino (Aloc) group (Boullanger),[59] the phthalimido (Phth) group (Lemieux),[60] the tetrachlorophthalimido (TCP) group (Fraser-Reid),[61] the dithiasuccinylamino (DTS) group (Barany),[62] the *N,N*-diacetylamino group (Schmidt),[63] and most recently the

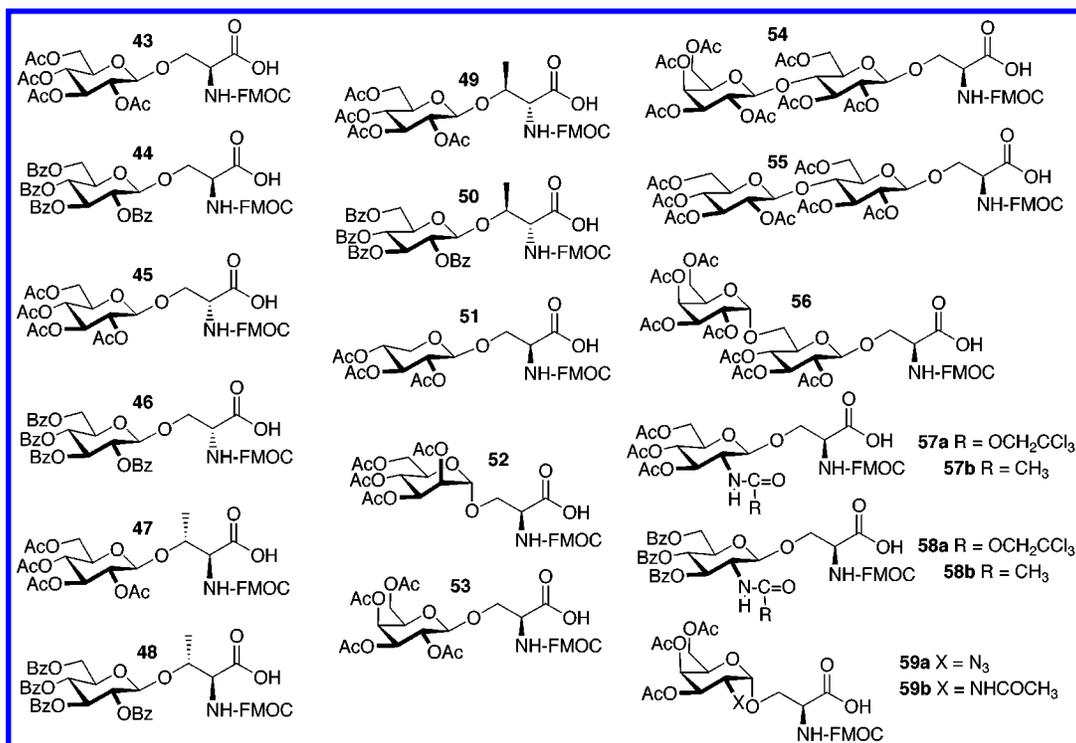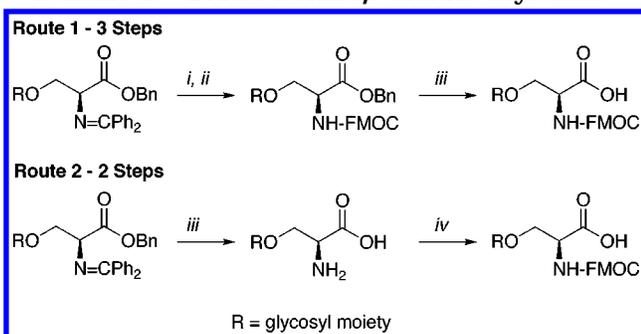(57) Windholz, T. M.; Jonston, B. R. *Tetrahedron Lett.* **1967**, 2555.

Figure 6. N-α-FMOC-amino acid glycosides.

Scheme 4. FMOC-Serine-β-GlcNAc Glycoside[a]

p-nitrobenzyloxycarbonyl (PNZ) group[64,59] have all been shown to favor β-glycosylation. The ease of removal and subsequent conversion to the acetamido motif in the presence of the completed peptide chain are important considerations that govern the choice of *N*-protection. Previous experience[65] indicated that Troc-amino protection provided excellent yields of β-GlcNAc N-α-FMOC amino acid glycosides. The α-GalNAc glycosides were synthesized via the nonparticipating 2-azido donor introduced by Lemieux (vide infra).

(58) (a) Kusumoto, S.; Yoshimura, H.; Imoto, M.; Shimamoto, T.; Shiba, T. *Tetrahedron Lett.* **1985**, *26*, 909. (b) Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135–47. (c) Saha, U.K.; Schmidt, R. R. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1855–60.

(59) Boullanger, P.; Banoub, J.; Descotes, G. *Can. J. Chem.* **1987**, *65*, 1343–48.

(60) Lemieux, R. U.; Takeda, T.; Chung, B. Y. *ACS Symp. Ser.* **1976**, *39*, 90–115.

(61) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302–3.

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(64) Qian, X.; Hindsgaul, O. *Chem. Commun.* **1998**, 1059–60.

(65) Szabó, L.; Ramza, J.; Langdon, C.; Polt, R. *Carbohydr. Res.* **1995**, *274*, 11–28.

Glycosylation of aglycone **1a** with either **17** or **18** with AgOTf promotion gave high yields of β-glycosides (Table 4). Conversion of the Schiff base glycoside to the N-α-FMOC-amino acid was initiated with catalytic hydrogenation to remove both the Schiff base and the benzyl ester in one step. Acylation with FMOC-Cl furnished the amino acid Troc-protected in an 80% yield. Treatment with zinc dust in acetic acid removed the Troc group to yield the free 2-amine. This conversion was moderate at best and required purification due to incomplete cleavage. Reaction with acetic anhydride and [i]Pr2NEt in CH2Cl2 afforded **57b** or **58b** in a 60% overall yield after chromatography. A faster and higher-yielding alternative was the procedure described by Dullenkopf,[66] where the N-α-FMOC ester Troc glycoside was treated with Zn dust and acetic anhydride to yield the acetamido form directly, accomplishing both cleavage of the Troc-group and acylation in one step in good yield. When this was attempted on **39**, the acetamido adduct was obtained after flash chromatography in 75% yield. Glycosylation using AgOTf and bromide **17** and **18** with the N-α-FMOC-protected aglycone **2a** furnished glycosides **39** and **40** in 66% and 82% yield, respectively. The yields were identical to those obtained using Schiff base-amino protection and thus proved to be the most efficient protocol. Thus, reaction of aglycone **2a** with bromide **17** to form glycoside **57b** required only three steps and resulted in a 49% overall yield, and reaction of aglycone **2a** with bromide **18** to furnish glycoside **58b** was accomplished in three steps with a 60% overall yield (Scheme 5).

The 2-amino 2-deoxy glycosyl bromides **17**–**19** are all highly active donors toward both Schiff-base and FMOC-based aglycones. However, for the bromides **9**–**16** the choice of the aglycone acceptor is critical for both glycosylation yield and α/β-selectivity. The protocol introduced

(66) Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135.

Table 4. All Glycosylations Were Performed Using 1.4–1.5 equiv AgOTFl in CH_2Cl_2 at 0 °C for 6–12 h, except in the GalNAc Glycosides with 2-Azido Donors Which Employed 1.3–1.5 Equiv of $AgClO_4$ at RT for 4–6 h. Ratios Were Established by ^1H NMR Analysis of Crude Material

Aglycone	Bromide	Yield	Glycoside		Selectivity (α:β)*
1a	17	72%		37	1:15
1a	18	72%		38	<1:20
2a	17	66%		39	1:12
2a	18	82%		40	<1:20
1a	19	73%		41	>20:1
2a	19	59%		42	>20:1

Scheme 5. FMOC-Serine-α-GalNAc Glycoside[a]
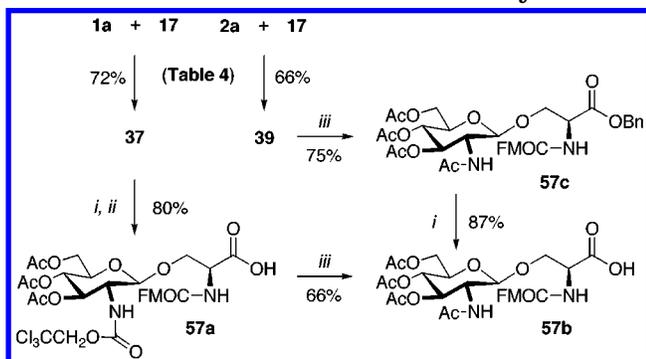
[a] Reagents: *i.* $CF_3COOH/H_2O/THF$, *ii.* FMOC-Cl/^{i}PrNEt$_2$/CH_2Cl_2, *iii.* H_2/Pd–C/EtOAc, *iv.* Ac_2O/pyridine/CH_2Cl_2.

Scheme 6[a]

[a] Reagents: *i.* 25% $C_5H_{10}NH/DMF$, *ii.* BOP/HOBt/EtNiPr$_2$, *iii.* $H_2NNH_2/H_2O/MeOH$, *iv.* 10% CF_3COOH/CH_2Cl_2, *v.* $K_3Fe(CN)_6$ or $O_2/NH_4^+ HCO_3^-$/pH 8.5.

by Lemieux and Ratcliffe[54] was used for the synthesis of glycosides **59a** and **59b**, utilizing azidonitration of D-galactal triacetate. Aglycone **1a** and bromide **19** with $AgClO_4$ promotion provided the α-glycoside **41** in a 73% yield after flash chromatography. This glycoside was then treated with TFA in THF:H_2O (9:1) to hydrolyze the Schiff base, and acylated with FMOC-Cl to yield glycoside **42** in a 71% overall yield. Hydrogenation of glycoside **42** simultaneously removed the benzyl ester and decomposed the azide to the free amine, which was treated with Ac_2O and ^{i}PrNEt$_2$ in CH_2Cl_2 to yield the glycoside **59b** in an overall yield of 84%. Glycosylation was also examined using the N-α-FMOC aglycone **2a** and bromide **19**. Glycoside **42** was obtained in a 59% yield after flash chromatography with the same glycosylation conditions used in the synthesis of **41**. Using this strategy, two steps were removed from the synthesis, and the overall yield was increased. Glycoside **42** was then converted to
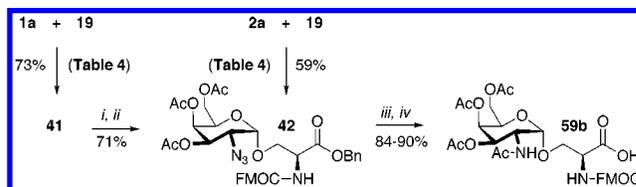
glycoside **59b** in a 90% yield over two steps (Scheme 6). Starting with the Schiff base aglycone, the final yield was 44% in five steps, which required four chromatographic purifications, while the N-α-FMOC aglycone provided the final product in 53% yield in only three steps and required only two chromatographies.

Peptide Assembly. The opioid glycopeptides were all C-terminal amides and were thus assembled on FMOC-amino protected Rink support. All peptide couplings were accomplished with equimolar amounts of Castro's BOP reagent,[67] 1-hydroxy-benzotriazole (HOBT), the desired amino acid, and 2 equiv of ^{i}PrNEt$_2$. Coupling times typically ranged from 20 min to 2 h for the more sterically encumbered sequences. For the glycoside-bearing residues, 1.25–1.50 equiv of the glycosylated amino acid were used due to their expense, and the coupling reactions were closely monitored with the Kaiser ninhydrin test.[68] For all other amino acids, 4.0 equiv were used. For tyrosine and D-cysteine, O-*tert*-butyl and S-trityl protect-

(67) Rivaille, P.; Gautron, J. P.; Castro, B.; Milhaud, G. *Tetrahedron* **1980**, *36*, 3413.

(68) Kaiser, E. T.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *84*, 595.
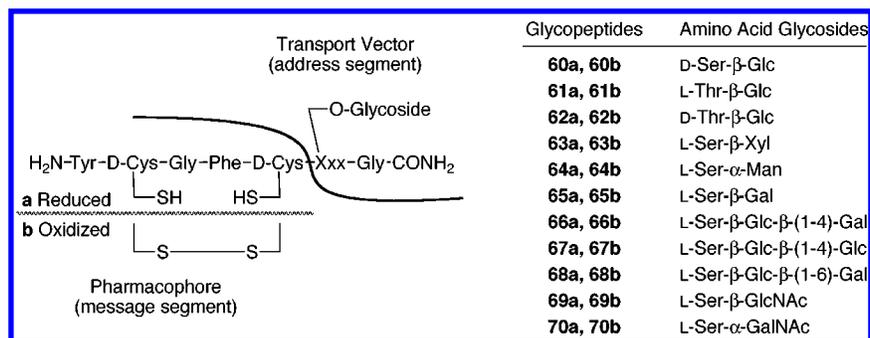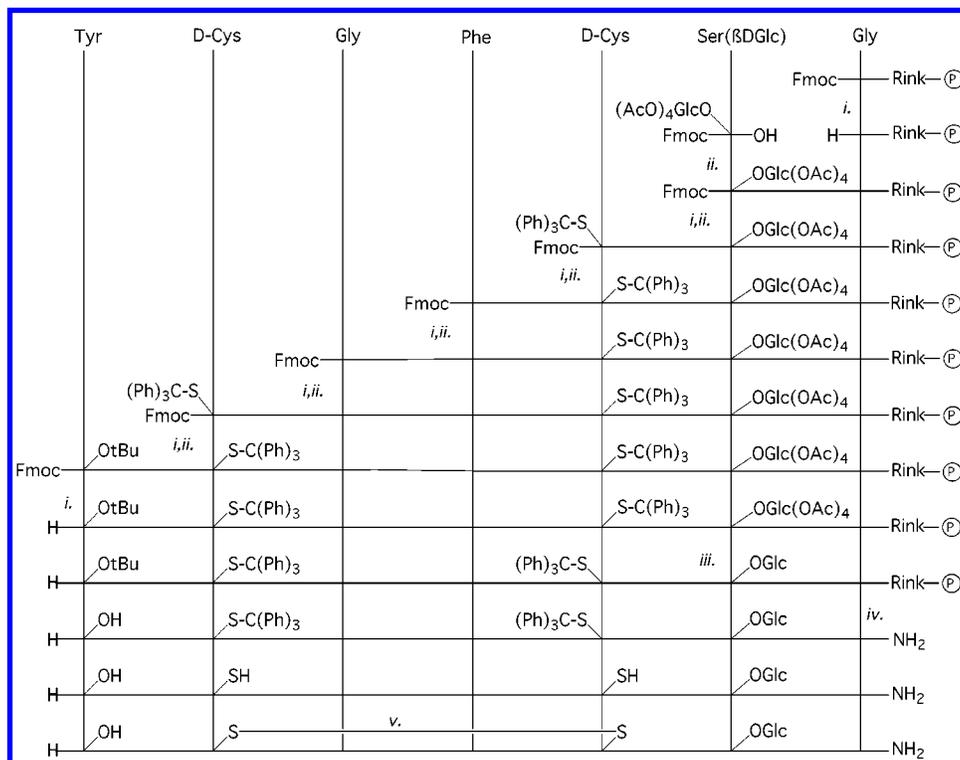
Glycopeptides	Amino Acid Glycosides
60a, 60b	D-Ser-β-Glc
61a, 61b	L-Thr-β-Glc
62a, 62b	D-Thr-β-Glc
63a, 63b	L-Ser-β-Xyl
64a, 64b	L-Ser-α-Man
65a, 65b	L-Ser-β-Gal
66a, 66b	L-Ser-β-Glc-β-(1-4)-Gal
67a, 67b	L-Ser-β-Glc-β-(1-4)-Glc
68a, 68b	L-Ser-β-Glc-β-(1-6)-Gal
69a, 69b	L-Ser-β-GlcNAc
70a, 70b	L-Ser-α-GalNAc

Figure 7. Glycopeptide opiates.

Scheme 7[a]

[a] Reagents: *i.* 25% $C_5H_{10}NH/DMF$, *ii.* BOP/HOBt/EtNiPr$_2$, *iii.* $H_2NNH_2/H_2O/MeOH$, *iv.* 10% CF_3COOH/CH_2Cl_2, *v.* $K_3Fe(CN)_6$ or $O_2/NH_4^+HCO_3^-$/pH 8.5.

ing groups were employed. Upon completion of the syntheses, the *N*-terminal FMOC-groups were removed, and the acetate or benzoate ester groups of the glycoside were removed with hydrazine hydrate in MeOH while on the solid phase.[69] After ester cleavage, the resin was washed several times to remove excess hydrazine and vacuum-dried to obtain an accurate mass determination.

The dried peptide resins were cleaved with a cocktail mixture (9.0 mL of TFA, 1.0 mL of CH_2Cl_2, 0.25 mL of Et$_3$SiH, 0.25 mL of H_2O, and 0.05 mL of anisole per 1.0 g of peptide resin) for 2 h at RT. After cleavage was complete, the solutions were filtered to remove the cleaved resin, and the resulting solution was concentrated to an oil in vacuo. After concentration, cold Et$_2$O was poured over the peptide solutions to precipitate the samples. The crude peptides were filtered, dried, and purified via preparative reverse-phase HPLC with a linear gradient of 10−60% CH_3CN:0.1% aqueous TFA to

yield the pure reduced compounds. These samples were cyclized with a solution of 3.0 equiv of $K_3Fe(CN)_6$ at pH 8.5 for roughly 8 h using a high-dilution, reverse-addition protocol. Once completed, these solutions were acidified to pH 4.0, anion-exchanged with Amberlite IRL-80 exchange resin, filtered, and lyophilized. The crude cyclic material was repurified with preparative HPLC to give the pure cyclic analogues, displayed in Figure 7. The overall synthetic approach is shown in Scheme 7.

Optimization of Glycopeptide Synthesis and Disulfide Bond Formation. In previous work, the synthesis of enkephalin glycopeptides was accomplished via the N-α-FMOC-amino acid glycoside **44**. Despite the higher yields during initial glycoside synthesis, removal of the benzoate esters was problematic after glycopeptide synthesis was completed. Detailed analysis of the post-cleavage crude reaction mixtures with FAB mass spectrometry and analytical HPLC showed undesired heterogeneity. This not only reduced the yield, but also made purification of the glycopeptides arduous. Benzoate cleav-

(69) Schultheiss-Reimann, P.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *1*, 62−63.
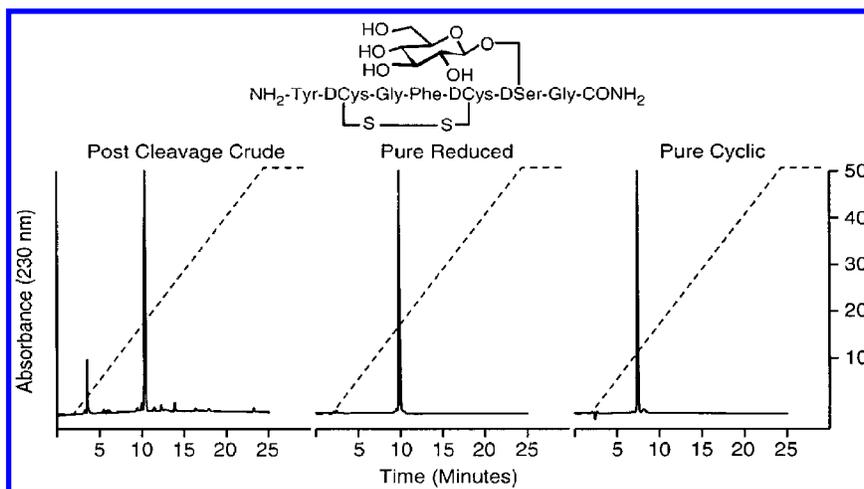
NH$_2$-Tyr-DCys-Gly-Phe-DCys-DSer-Gly-CONH$_2$

Figure 8. Analytical HPLC traces of glycopeptide **60b**.

age was slow on the solid-phase with hydrazine hydrate. Since we had no efficient method to monitor removal of the benzoates on the solid phase,[70] prolonged reaction times were required to ensure complete debenzoylation. Resin-bound glycopeptides, which contained either Ser-β-D-Glc(OBz)$_4$ or Ser-β-D-GlcNAc(OBz)$_3$, showed (MS, HPLC) significant amounts of mono-, di-, and tribenzoate protected glycopeptides after cleavage with 4:1 hydrazine hydrate:MeOH (4 × 30 min, 1 × 60 min). The amount of amino acid deletion was also high; the major impurity lacking D-Cys^5, which was adjacent to the bulky benzoyl glycoside.

However, when acetate protection was used, deprotection was quantitative and the post-cleavage crude material was very pure in all cases of manual glycopeptide synthesis. Mass spectra of all the crude peptides showed no remaining acetates and full incorporation of the bulky trityl-protected D-cysteine. With this enhancement, all of the glycopeptides were synthesized in fair to very good yields. Figure 8 displays HPLC traces of glycopeptide **60**.

Early in this work there was a question as to what form of D-cysteine would be optimal. In earlier work, acetamidomethyl (ACM) D-cysteine was employed.[40c] This was problematic for two reasons: (1) The ACM group was not removed with TFA during cleavage of the peptide and was removed afterward in a second step. (2) This conversion was modest at best and required the use of Hg(OAc)$_2$ and H$_2$S. Thus, trityl-protection for D-cysteine was examined. This group was easily cleaved with TFA, but it was essential to use an effective trityl cation scavenger. A cleavage cocktail of 9.0 mL of TFA, 1.0 mL of CH$_2$Cl$_2$, 0.25 mL of H$_2$O, and 0.25 mL of anisole was ineffective due to reversible retritylation. This was evident in the mass spectra, which showed up to 50% of mono- or ditritylation. However, when triethylsilane was used in the cleavage cocktail, quantitative cleavage of both trityl groups was achieved. Mass analysis verified the lack of any trityl-protected species. Thus, trityl was the protecting group of choice for the synthesis of cysteine-containing peptides using an N-α-FMOC-strategy.

Clearly, the most problematic step in each synthesis was the S–S cyclization. Several strategies were employed, all with moderate results. In previous work, a high-dilution syringe pump delivery method was used.

This involved dissolution of 100–200 mg of crude reduced peptide in distilled H$_2$O (10 mL) and dropwise addition over 12–20 h into 0.5–1.0 L solution of 3 equiv of K$_3$Fe-(CN)$_6$ at pH 8.8.[71] This was problematic for several reasons. After addition, there was a large volume of solvent after anion exchange, acidification, and filtration. This required removal of at least half of the solvent by rotary evaporation prior to lyophilization. When this was done, the integrity of the sample was altered, and much degradation was apparent by HPLC, apparently due to disulfide interchange. A method was needed whereby the total filtrate could be lyophilized directly without rotary evaporation. An alternate approach was attempted using a buffered ammonium bicarbonate solution at pH 8.5, purged with O$_2$ under medium dilution. Upon analysis of these samples, an undesired reaction occurred in which a more polar species (based on HPLC retention times) was observed. Mass spectral analysis confirmed that this product was the *mono*sulfide. These conditions apparently fostered a Ramberg–Backlund-like displacement of either a sulfoxide or sulfone intermediate[72] to furnish the sulfide (Scheme 8). Very little and sometimes none of the desired disulfide form was isolated. A method by Misicka and Hruby[73] was developed using a similar syringe pump addition, except a much lower volume of ferricyanide solution was used. The crude cleavage product (100 mg) was dissolved in water (10 mL), loaded into a syringe, and added over 8 h into 100 mL of pH 8.8 ferricyanide (3 equiv). After addition, the solution was acidified to pH 4.0, anion-exchanged, and filtered to a total volume of 200 mL, which was directly lyophilized. Both pre- and post-lyophilization HPLC traces were identical (Figure 8). This drastically increased both the yield and efficiency of the cyclization of these glycopeptides. Amino acid analysis and HPLC retention times are shown in Tables 5 and 6.

Conclusions

The efficient synthesis of 18 amino acid glycosides for solid-phase glycopeptide synthesis is reported. Use of D-amino acid aglycones uncovered a stereochemical bias

(70) Solid-phase FTIR is problematic due to the size of the overlapping peptide bond amide stretches.

(71) Rivier, J.; Kaiser, R.; Galyean, B. *Biopolymers* **1978**, *17*, 1927–38.

(72) Kimura, T.; Ishikawa, Y.; Ogawa, S.; Nishio, T.; Iida, I.; Furukawa, N. *Tetrahedron Lett.* **1992**, *33*, 6355–8.

(73) Misicka, A.; Hruby, V. *J. Pol. J. Chem.* **1994**, *68*, 1994.

Scheme 8. Disulfide Formation Using Various Conditions
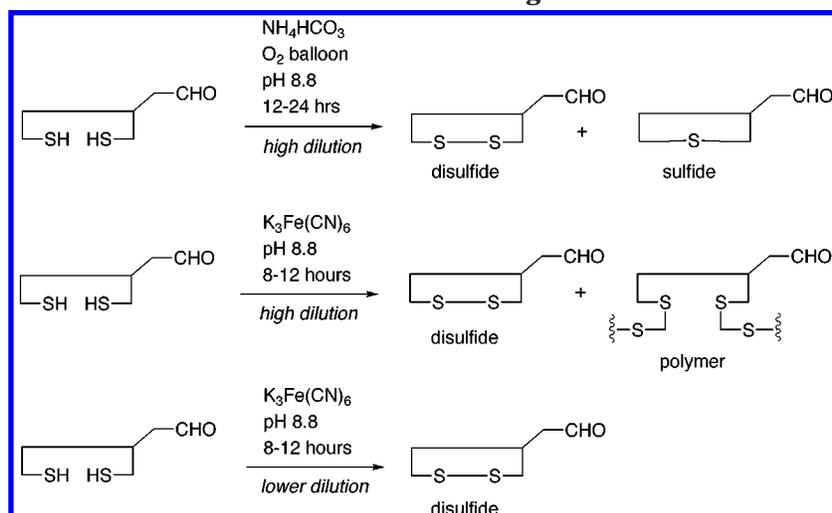
Table 5. Amino Acid Analysis, Normalized to Phenylalanine

glycopeptide	Tyr1[a] theor	found	D-Cys2,5 [b] theor	found	Gly3 theor	found	Phe4 theor	found	Ser/Thr6 glycoside theor	found
60a	1.00	0.466	2.00	0.599	2.00	1.792	1.00	1.00	1.00	0.874
61a	1.00	0.272	2.00	0.402	2.00	1.813	1.00	1.00	1.00	1.030
62a	1.00	0.412	2.00	0.438	2.00	1.807	1.00	1.00	1.00	0.989
63a	1.00	0.413	2.00	0.518	2.00	2.009	1.00	1.00	1.00	0.899
64a	1.00	0.305	2.00	0.439	2.00	1.960	1.00	1.00	1.00	0.919
65a	1.00	0.309	2.00	0.309	2.00	2.190	1.00	1.00	1.00	1.007
66a	1.00	0.279	2.00	0.273	2.00	1.979	1.00	1.00	1.00	0.910
67a	1.00	0.433	2.00	0.394	2.00	2.071	1.00	1.00	1.00	0.851
68a	1.00	0.408	2.00	0.408	2.00	2.406	1.00	1.00	1.00	1.014
69a	1.00	0.369	2.00	0.394	2.00	1.788	1.00	1.00	1.00	0.731
70a	1.00	0.397	2.00	0.483	2.00	2.067	1.00	1.00	1.00	0.910

[a] Tyr1 is terminal residue, adjacent to Cys2, and will provide low analyses. [b] D-Cys2,5 are degraded during hydrolysis and will provide very low analyses

Table 6. Glycopeptide Retention Times[a]

glycopeptide	retention time (min)	glycopeptide	retention time (min)
60a	9.69	**60b**	7.26
61a	10.05	**61b**	7.81
62a	10.12	**62b**	9.09
63a	10.53	**63b**	7.50
64a	10.36	**64b**	7.11
65a	10.29	**65b**	7.19
66a	9.43	**66b**	6.97
67a	10.10	**67b**	7.11
68s	10.01	**68b**	6.68
69a	9.81	**69b**	7.14
70a	10.46	**70b**	7.44

[a] Column: 4.6 mm × 250 mm C_{18} analytical column, 10−15 micron particle size, 300 Å pore size. Conditions used: 1.0 mL/min flow rate, 10−50% CH_3CN vs 0.1% CF_3COOH in H_2O over 20 min (2% per minute gradient), detected at 215, 254, and 278 nm wavelengths.

toward certain protecting group modalities in the cases of L- and D-threonine. The resultant yields from several glycosylations reveal a "match" vs "mismatch" aglycone/bromide pair that has to be taken into consideration when planning a threonine-based glycosylation. Both *N,N*-diphenylmethylene and *N*-FMOC temporary protecting groups provided adequate activation and direction in glycosylations of several glycan moieties. In certain cases, such as in GlcNAc and GalNAc glycosylations, either protecting group facilitated glycoside formation with high yield and selectivity. In addition, the xylose, mannose, lactose, and cellobiose glycosylation results reported here represent a highly efficient and scalable process for these types of glycosides.

The solid-phase glycopeptide synthesis of these en-kephalin-based glycopeptides has been highly optimized. The use of acetate protection on the glycan hydroxyls, the employment of trityl-D-cysteine, and utilization of an efficient high-dilution, low-volume cyclization protocol has afforded the synthesis of several glycopeptides in high overall yield and purity. These glycopeptide analogues have been subjected to further in vitro and in vivo pharmacological analysis.[23,25b]

Experimental Section

Representative examples are provided. Complete procedures are provided in the Supporting Information.

General Methods for Glycoside Synthesis. All air- and moisture-sensitive reactions were performed under an argon atmosphere in flame-dried reaction flasks. THF was dried and deoxygenated over $Ph_2C=O/Na°-K°$. CH_2Cl_2 and CH_3CN were dried over P_2O_5, and all solvents were freshly distilled under an argon atmosphere prior to use. All compounds described were >95% pure by ^1H and ^{13}C NMR, and purity was confirmed by high-resolution FAB mass spectrometry in most cases. The ^1H and ^{13}C NMR spectra were obtained at 250 or 500 MHz. ^{13}C spectra were recorded at 62.9 MHz in the form of APT's (attached proton test spectra). L-Serine benzyl ester HCl and L-threonine benzyl ester hemioxalate salt were purchased from commercial sources. All other aglycones were made via their TsOH amine salts. These salts were converted to their corresponding benzhydryl esters using Ph_2CN_2, which was freshly prepared from yellow HgO and $Ph_2C=N-NH_2$ in petroleum ether.[74] All peracetylated and perbezoylated sugars were made by reaction with either Ac_2O or $PhCOCl$ and pyridine. All ^1H and ^{13}C NMR data are provided in the Supporting Information.

Benzyl *N*-(Diphenylmethylene)-L-serinate (1a). The procedure used was that of O'Donnell.[40d] Benzyl L-serine HCl salt (15.00 g, 64.70 mmol, 1.0 equiv), $Ph_2C=NH$ (10.59 mL, 63.00 mmol, 0.975 equiv) and CH_2Cl_2 (100 mL) were stirred at RT for 24 h with the exclusion of moisture (drying tube). After TLC showed full conversion, the reaction mixture was diluted with 100 mL of CH_2Cl_2, and the product was washed $1 \times 1\%$ $NaHCO_3$, $1 \times$ concentrated $NaHCO_3$, $1 \times H_2O$, $1 \times$ brine, then dried over $MgSO_4$. The product was filtered and concentrated to a colorless oil. Recrystallization from cyclohexane yielded 12.71 g **1a** (84%), mp 78–80 °C; $[\alpha]_D = -151.4°$ ($c = 1.0$, $CHCl_3$); 20% EtOAc/hexanes (R_f 0.45); IR (NaCl plates) cm^{-1}: 3426, 1738, 1658, 1278, 699.

Benzyl *N*-[9-(Fluorenylmethoxycarbonyl)]-L-serinate (2a). Benzyl L-serine HCl salt (3.30 g, 14.24 mmol, 1.0 equiv) and $^iPrNEt_2$ (2.50 mL, 2.0 mmol) were dissolved in 100 mL of CH_2Cl_2 and cooled to 0 °C. Upon addition of base, the suspension became clear. FMOC-Cl (4.05 g, 15.67 mmol, 1.10 equiv) was dissolved in 25 mL of CH_2Cl_2 and added dropwise over 30 min. After 1 h, the reaction was complete by TLC. The solution was diluted with 100 mL of CH_2Cl_2 and washed $1 \times 1\%$ $NaHCO_3$, $1 \times$ concentrated $NaHCO_3$, $1 \times H_2O$, $1 \times$ brine, and dried over $MgSO_4$. The product was filtered and concentrated to a colorless oil. The product was recrystallized from EtOAc/hexanes to yield 5.65 g of **2a** (95%), mp 98–100 °C; $[\alpha]_D = +3.2°$ ($c = 0.9$, $CHCl_3$); 40% EtOAc/hexanes (R_f 0.43); IR (NaCl plates) cm^{-1}: 3423, 1720, 1519, 1338, 1203, 1080, 759, 738, 699.

Schiff Base Esters 1b, 3, 5a, 5b, and 7. See Supporting Information.

FMOC Amino Acid Esters 2b, 4, 6a, and 6b. See Supporting Information.

Benzyl *N*-[9-(Fluorenylmethoxycarbonyl)]-D-threoninate (8a). *N*-FMOC-*O*-^{t}butyl-D-threonine (1.0 g, 2.52 mmol, 1.0 equiv, Advanced ChemTech) was dissolved in 25 mL of CH_2Cl_2, and $^iPrNEt_2$ (1.05 equiv, 0.46 mL) was added and cooled to 0 °C under argon. DMAP (10 mol %, 31 mg) was added, and benzyl chloroformate (95%, 1.0 equiv, 0.38 mL) was subsequently added dropwise in 10 mL of CH_2Cl_2 over 20 min. After 35 min the reaction was complete, and the solution was concentrated and azeotroped 2×20 mL of toluene. Flash chromatography (20% EtOAc/hexanes, $R_f = 0.66$) yielded 1.07 g of a colorless syrup (87%). $[\alpha]_D = -3.8°$ ($c = 0.84$, $CHCl_3$). D-threonine-*O*-^{t}butyl-*N*-FMOC benzyl ester (0.92 g, 1.89 mmol) was dissolved in 15 mL of neat trifluoroacetic acid at RT and stirred under argon for 2 h. Upon completion of ether cleavage judged by TLC, the product was diluted with CH_2Cl_2 and concentrated. Excess TFA was azeotropically removed with 2×25 mL of toluene. The white solid was recrystallized from EtOAc/hexanes to give 0.75 g of a white solid (92%). mp 122–124 °C; $[\alpha]_D = +13.2°$ ($c = 1.2$, $CHCl_3$).

Benzhydryl *N*-[9-(Fluorenylmethoxycarbonyl)]-D-threoninate (8b).

2,3,4-Tri-*O*-acetyl-α-bromo-D-xylopyranose (9). 1,2,3,4-tetra-*O*-acetyl-D-xylopyranose (2.21 g, 6.94 mmol) was dissolved in 50 mL of CH_2Cl_2, and 5.0 mL of HBr (33% in AcOH) was added at 0 °C and was allowed to react for 1 h. The reaction was allowed to proceed for another hr at RT. After 2 h the reaction was complete by TLC and was worked up. The solution was diluted with 100 mL of CH_2Cl_2 and washed $1 \times$ ice water, $1 \times 1\%$ $NaHCO_3$, $1 \times$ concentrated $NaHCO_3$, $1 \times$ brine, and dried over $MgSO_4$. The product was filtered and concentrated to a colorless oil and then recrystallized from Et_2O/C_6H_{14} to yield 2.25 g of **9** (96%), mp 97–98 °C; $[\alpha]_D = +183.1°$ ($c = 2.5$, $CHCl_3$); 40% EtOAc/hexanes (R_f 0.45).

Bromosugars 10–16. See Supporting Information.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-[2,2,2-trichloroethoxycarbonyl(amino)]-α-bromo-D-glucopyranose (17). The procedure used was that of Imoto et al.[58] D-Glucosamine HCl salt (43 g, 0.20 mol, 1.0 equiv) and $NaHCO_3$ (28.65 g, 0.34 mol, 1.70 equiv) were dissolved in 450 mL of doubly distilled H_2O.

Cl_3CCH_2OCOCl (41 mL, 0.298 mol, 1.50 equiv) was added dropwise at 0 °C over 30 min and allowed to stir overnight at RT. The product precipitated and was filtered and washed with 2 L of doubly distilled H_2O until all the unreacted chloride was removed. The white solid product was recrystallized from EtOH to yield 23.88 g of waxy crystals (34%), mp 182–83 °C, $[\alpha]_D = +50.4$ ($c = 0.6$, MeOH). Troc-amino glucosamine (10 g, 28.20 mmol, 1.0 equiv) was dissolved in 50 mL of dry pyridine, Ac_2O (13.41 mL, 0.141 mol, 5 equiv) was added, and the mixture was stirred at RT for 12 h. The product was concentrated and azeotroped with toluene 3×50 mL. (Alternatively, the Troc-amino glucosamine was benzoylated to yield **18**, below.) The product was then washed 2×1 M HCl, $2 \times H_2O$, $1 \times$ brine, and dried over $MgSO_4$. Solvent removal and drying in vacuo provided a white foam 14.52 g (98%). Troc-amino peracetylglucosamine was brominated in the same manner as **9** to yield **17** as a white foam (96%), $[\alpha]_D = +64.5°$ ($c = 0.9$, $CHCl_3$); 40% EtOAc/hexanes (R_f 0.35).

3,4,6-Tri-*O*-benzoyl-2-deoxy-2-[(2,2,2-trichloroethyloxycarbonyl)amino]-α-bromo-D-glucopyranose (18). Troc-protected glucosamine, (10 g, 28.20 mmol, 1.0 equiv) was dissolved in 50 mL of pyridine, and benzoyl chloride (42.75 mL, 0.33 mol, 5 equiv) was added dropwise. The reaction was stirred at RT for 12 h. The product was concentrated and azeotroped with toluene 3×50 mL. The product was washed 2×1 M HCl, $2 \times H_2O$, $1 \times$ brine, and dried over $MgSO_4$. Solvent removal and drying in vacuo provided 38.72 g of white foam (76%), mp 172–75 °C. Troc-amino perbenzoylglucosamine was brominated in the same manner as **9** to yield **18** as a white foam (90%), $[\alpha]_D = +81.0°$ ($c = 0.5$, $CHCl_3$); 25% EtOAc/hexanes (R_f 0.65).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-azido-α-bromo-D-galactopyranose (19). The procedure is that of Lemieux and Radcliffe.[54] Bromide **13** (10 g, 24.33 mmol, 1.0 equiv) was dissolved in 160 mL of acetic acid. $CuSO_4$ (1.8 g dissolved in 20 mL of H_2O) and Zn dust (30 g) were added to the solution and allowed to react at 0 °C for 30 min and then stirred overnight at RT. The reaction was filtered through Celite and washed 3×100 mL of H_2O. The product was extracted out with 300 mL of $CHCl_3$, and the organic layer was washed $2 \times NaCO_3$, $1 \times H_2O$, $1 \times$ brine, and dried over $MgSO_4$. After concentration, the product was flash chromatographed with 40% EtOAc/hexanes to yield 4.64 g colorless oil (70%). Galactal(OAc)$_3$ (3.95 g, 14.50 mmol, 1.0 equiv), ceric ammonium nitrate (23.87 g, 43.54 mmol, 3 equiv), and NaN_3 (1.415 g, 21.77 mmol, 1.50 equiv) were dissolved in 100 mL of CH_3CN and allowed to react at −15 °C in the presence of 2.0 g of 4 Å molecular sieves for 3 h. After 3 h the glycal had been consumed (TLC), and the reaction mixture was diluted with 120 mL of cold Et_2O and 120 mL of cold H_2O. The product was filtered through Celite and washed 3×50 mL of Et_2O. The organic layer was separated and dried over Mg_2SO_4. Concentration yielded 3.26 g of yellowish oil (60%); 30% EtOAc/hexanes (R_f 0.67). The azidonitrate mixture (3.26 g) was dissolved in 20 mL of CH_3CN, and LiBr (2.257 g, 26 mmol, 3 equiv) was added and allowed to react at RT for 3 h. After TLC showed full conversion, the sample was diluted with 100 mL of CH_2Cl_2 and washed 2×100 mL of H_2O, $1 \times$ brine, and dried over $MgSO_4$. The product was filtered, concentrated, and flash chromatographed with 25% EtOAc/hexanes (R_f 0.52) to yield 1.93 g of a colorless oil (78%). $[\alpha]_D = +188.6°$ ($c = 2.0$, $CHCl_3$).

Benzyl *N*-Diphenylmethylene-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serinate (20b). Bromide **10** (11.44 g, 27.80 mmol, 1.25 equiv) and aglycone **1a** (8.00 g, 22.30 mmol, 1.0 equiv) were placed in a flame-dried flask in 100 mL of CH_2Cl_2 along with 3.0 g of powdered 4 Å molecular sieves under argon and cooled to 0 °C. AgOTf (8.00 g, 31.20 mmol, 1.40 equiv) was added in portions over 45 min. After 14 h the reaction was complete by TLC, and the reaction was quenched by the addition of $^iPr_2NEt$ (5.60 mL, 1.4 equiv) and stirred for 10 min. The solution was filtered through Celite, and the crude solution was washed $2 \times$ concentrated $Na_2S_2O_3$, $2 \times$ concentrated Na_2CO_3, $1 \times$ brine, and dried over $MgSO_4$. Chromatography with 30% EtOAc/hexanes ($R_f = 0.25$) provided 4.00 g of **20b**, and 0.95 g of **20a**, both as a white foams in (total yield

(74) Aboderin, A. A.; Delpierre, G. R.; Fruton, J. S. *J. Am. Chem. Soc.* **1965**, *87*, 5469–72.

32%). $[\alpha]_D = -56.3°$ ($c = 0.9$, CHCl$_3$). FAB HRMS (M + H) for C$_{37}$H$_{40}$O$_{12}$N, expected 690.2551, found 690.2558. IR (NaCl plates) cm^{-1}: 3444, 3061, 2969, 1755, 1626, 1367, 1223, 1040, 736, 700.

Benzyl *N*-Diphenylmethylene-*O*-(2,3,4,6-tetra-*O*-acetoxy-α-D-glucopyranosyl)-L-serinate (20a). Isolated as a minor isomer from the reaction for **20**, above. 30% EtOAc/hexanes ($R_f = 0.32$). $[\alpha]_D = +12.0°$ ($c = 0.7$, CHCl$_3$). FAB HRMS (M + H) for C$_{37}$H$_{40}$O$_{12}$N, expected 690.2551, found 690.2524. IR (NaCl plates) cm^{-1}: 3479, 3061, 2955, 1751, 1368, 1225, 1037, 737, 699.

Benzyl *N*-Diphenylmethylene-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-serinate (21).[75] Bromide **11** (7.15 g, 10.8 mmol, 1.3 equiv) and aglycone **1a** (3.00 g. 8.35 mmol, 1.0 equiv) were azeotroped with dry toluene. Powdered 4 Å molecular sieves and 110 mL of dry CH$_2$Cl$_2$ were added, and the reaction vessel was cooled to 0 °C under argon. AgOTf was added in small portions and the reaction stirred overnight at RT. The reaction was quenched by the addition of NEt$_3$, filtered, and washed 2 × concentrated Na$_2$S$_2$O$_3$, 2 × concentrated Na$_2$CO$_3$, 1 × brine, and dried over MgSO$_4$. The crude material was flash chromatographed with 25% EtOAc/hexanes to provide 7.20 g of the β-glycoside (92%), $R_f = 0.32$, and 0.40 g of the α-glycoside (5%), $R_f = 0.45$. This reaction was also performed at twice this scale with cooling to −15 °C during AgOTf addition to yield **21** in 75% yield and only traces of the α-product. ^{13}C δ: 172.22 (C=N), 169.28, 165.86, 165.61, 164.95, 164.83 (5 × O−C=O), 138.99, 135.55, 135.37, 133.23, 133.01, 132.88, 130.38 (7 × substituted aromatics), 130.00−127.67 (18 aromatics observed), 101.14 (C1), 72.88 (C3), 72.00 (C5), 71.70 (C2), 70.35 (Cβ), 69.48 (C4), 66.21 (CH$_2$Ph), 65.30 (Cα), 62.80 (C6); ^{1}H δ (selected): 5.87 (C3, t, 9.6 Hz), 5.65 (C4, t, 9.7 Hz), 5.50 (C2, dd, 7.9, 9.7 Hz), 5.03 (C1, d, 7.8 Hz), 4.89−4.78 (PhCH$_2$, AB system, 12.4 Hz).

Schiff Base Esters 22−25. See Supporting Information.

Benzyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-threoninate (26). Bromide **10** (3.60 g, 8.75 mmol, 1.7 equiv) and aglycone **6a** (2.90 g, 6.73 mmol, 1.0 equiv) were placed in a flame-dried flask in 100 mL of CH$_2$Cl$_2$ along with 3.0 g of powdered 4 Å molecular sieves under argon and cooled to 0 °C. AgOTf (2.42 g, 9.42 mmol, 1.40 equiv) was added in portions over 50 min. After 12 h the reaction was complete by TLC, and the reaction was quenched by the addition of ^{i}Pr$_2$NEt (1.76 mL, 1.5 equiv) and stirred for 10 min. The solution was filtered through Celite, and the crude solution was washed 2 × concentrated Na$_2$S$_2$O$_3$, 2 × concentrated Na$_2$CO$_3$, 1 × brine, and dried over MgSO$_4$. Chromatography with 40% EtOAc/hexanes ($R_f = 0.26$) provided 2.05 g pure **26** as a white foam (40%). $[\alpha]_D = -15.4°$ ($c = 0.75$, CHCl$_3$); FAB HRMS (M + H) for C$_{40}$H$_{44}$O$_{14}$N, expected 762.2762, found 762.2783. IR (NaCl plates) cm^{-1}: 3430, 3066, 1755, 1650, 1514, 1382, 1224, 1042, 737, 701.

Protected Amino Acid Glycosides 27−38. See Supporting Information.

Benzyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2-amino-3,4,6-tri-*O*-acetyl-2-deoxy-*N*-[(2,2,2-trichloroethyloxycarbonyl)amino]-β-D-glucopyranosyl)-L-serinate (39). From the Schiff base: Glycoside **37** (0.46 g, 0.56 mmol) was dissolved in 10 mL of THF, 0.50 mL of H$_2$O, and 1.0 mL of TFA and stirred at RT for 15 min. TLC showed completion, and the product was concentrated, azeotroped 2 × 25 mL of toluene, and then passed through a plug of silica gel first with CH$_2$Cl$_2$ to remove Ph$_2$C=O and then 20% MeOH/CH$_2$Cl$_2$ to elute the amine. The product was pooled, concentrated, and redissolved in 15 mL of CH$_2$Cl$_2$ with FMOC-Cl (0.16 g, 0.62 mmol, 1.10 equiv). ^{i}Pr$_2$NEt (0.30 mL, 1.68 mmol, 3.0 equiv) in 5 mL of CH$_2$Cl$_2$ was added dropwise to this mixture via addition funnel over 10 min at RT. After 30 min, the reaction was complete, and the product was concentrated. Flash chromatography with 40% EtOAc/hexanes ($R_f = 0.41$) yielded 0.44 g of **39** as a white foam in 89% over two steps. $[\alpha]_D = +5.5°$ ($c = 0.2$, CHCl$_3$); FAB HRMS (M + H) for C$_{40}$H$_{42}$O$_{14}$N$_2$-

Cl$_3$, expected 881.1683, found 881.1656. IR (NaCl plates) cm^{-1}: 3361, 3066, 2955, 2895, 1748, 1537, 1368, 1232, 1049, 738, 700.

By direct glycosylation: Bromide **17** (1.414 g, 2.60 mmol, 1.30 equiv), aglycone **2a** (0.835 g, 2.00 mmol, 1.0 equiv), 2.0 g of 4 Å powdered sieves, and 50 mL of CH$_2$Cl$_2$ were added to a flask under argon and cooled to 0 °C. AgOTf (0.72 g, 2.80 mmol, 1.40 equiv) was added in portions over 40 min. The reaction was allowed to proceed for 10 h. The reaction was then quenched with ^{i}Pr$_2$NEt (0.50 mL, 1.50 equiv), and the solution was filtered through Celite after 10 min. The crude solution was washed 2 × concentrated Na$_2$S$_2$O$_3$, 2 × concentrated Na$_2$-CO$_3$, 1 × brine, and dried over MgSO$_4$. Chromatography with 35% EtOAc/hexanes ($R_f = 0.35$) provided 1.168 g of **39** as an off-white foam (66%).

Benzyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2-amino-3,4,6-tri-*O*-benzoyl-2-deoxy-*N*-[(2,2,2-trichloroethyloxycarbonyl)amino]-β-D-glucopyranosyl)-L-serinate (40). Bromide **18** (0.63 g, 0.958 mmol, 1.30 equiv), aglycone **2a** (0.307 g, 0.737 mmol, 1.0 equiv), and 1.5 g 4 Å powdered sieves were added to a flask in 50 mL of CH$_2$Cl$_2$ under argon and cooled to 0 °C. AgOTf (0.265 g, 1.03 mmol, 1.40 equiv) was added in portions over 40 min. The reaction was allowed to proceed for 16 h. The reaction was quenched with ^{i}Pr$_2$NEt (0.20 mL, 1.50 equiv), and the solution was filtered through Celite after 10 min. The crude solution was washed 2 × concentrated Na$_2$S$_2$O$_3$, 2 × concentrated Na$_2$CO$_3$, 1 × brine, and dried over MgSO$_4$. Chromatography with 30% EtOAc/hexanes ($R_f = 0.28$) provided 0.60 g of **40** as a white foam (82%). $[\alpha]_D = -5.7°$ ($c = 0.35$, CHCl$_3$); FAB HRMS (M + H) for C$_{55}$H$_{48}$O$_{14}$N$_2$Cl$_3$, expected 1067.2159, found 1067.2180. IR (NaCl plates) cm^{-1}: 3412, 3074, 2956, 2895, 1728, 1536, 1271, 1107, 738, 711.

Benzyl *N*-Diphenylmethylene-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α-D-galactopyranosyl)-L-serinate (41). Bromide **19** (1.50 g, 3.81 mmol, 1.40 equiv), aglycone **1a** (1.00 g, 2.72 mmol, 1.0 equiv), and 2,4,6-collidine (0.61 mL, 4.62 mmol, 1,70 equiv) were dissolved in 10 mL of dry CH$_2$Cl$_2$. In a flask, AgClO$_4$ (0.959 g, 4.62 mmol, 1.70 equiv) and 1.0 g of 4 Å powdered sieves were dissolved in 10 mL of dry CH$_2$Cl$_2$ under argon. The reactant cocktail was cannulated to a dry addition funnel, and the solution was added dropwise at RT over 20 min. After 2 h the reaction was quenched with 0.25 mL of ^{i}-Pr$_2$NEt, and the solution was filtered through Celite after 10 min. The crude solution was washed 2 × concentrated Na$_2$S$_2$O$_3$, 2 × concentrated Na$_2$CO$_3$, 1 × brine, and dried over MgSO$_4$. Chromatography with 25% EtOAc/hexanes ($R_f = 0.30$) provided 1.310 g of **41** as a yellowish oil (73%). $[\alpha]_D = +6.1°$ ($c = 1.2$, CHCl$_3$); FAB HRMS (M + H) for C$_{35}$H$_{36}$O$_{10}$N$_4$, expected 673.2510, found 673.2527. IR (NaCl plates) cm^{-1}: 3426, 3068, 3037, 2950, 2111, 1749, 1228, 1047, 755, 698.

Benzyl *N*-(9-Fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α-D-galactopyranosyl)-L-serinate (42). Bromide **19** (0.40 g, 1.02 mmol, 1.40 equiv), aglycone **2a** (0.303 g, 0.725 mmol, 1.0 equiv), and 2,4,6-collidine (0.163 mL, 1.23 mmol, 1,70 equiv) were dissolved in 10 mL of dry CH$_2$Cl$_2$. AgClO$_4$ (0.255 g, 1.23 mmol, 1.70 equiv) and 0.5 g of 4 Å powdered sieves were dissolved in 10 mL of CH$_2$Cl$_2$ under argon. The reactant cocktail was cannulated to a dry addition funnel, and the solution was added dropwise at RT over 20 min. After 2 h the reaction was quenched with 0.25 mL of ^{i}-Pr$_2$NEt, and the solution was filtered through Celite after 10 min. The crude solution was washed 2 × concentrated Na$_2$S$_2$O$_3$, 2 × concentrated Na$_2$CO$_3$, 1 × brine, and dried over MgSO$_4$. Chromatography with 30% EtOAc/hexanes ($R_f = 0.35$) provided 0.310 g of **42** as a white foam (59%). $[\alpha]_D = +88.5°$ ($c = 0.13$, CHCl$_3$); FAB HRMS (M + H) for C$_{37}$H$_{39}$O$_{12}$N$_4$, expected 731.2564, found 731.2567. IR (NaCl plates) cm^{-1}: 3347, 3061, 2888, 2115, 1754, 1536, 1365, 1223, 1076, 1045, 737, 699.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-L-serine (43a).** Glycoside **20a** (0.72 g, 1.044 mmol) was dissolved in 100 mL of MeOH, and Pd−carbon (10%, 0.40 g) was added, and the flask was fitted with a H$_2$ balloon and hydrogenated for 3 h. TLC showed completion and the reaction was quenched with 100 mL of CH$_2$-Cl$_2$. After 10 min the solution was filtered through Celite and

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(75) Szabò, L. Z.; Polt, R. Unpublished results.

concentrated. The product was redissolved in 10% NaHCO$_3$ and washed 3 × 100 mL of Et$_2$O to remove Ph$_2$CH$_2$. The aqueous layer was adjusted to pH 8.5 and diluted with dioxane to the cloud point. FMOC-Cl (0.30 g, 1.15 mmol, 1.1 equiv) was dissolved in 25 mL of dioxane and added dropwise to the glycoside over 20 min. After 1 h the reaction was complete by TLC. The solution was diluted with 50 mL of concentrated NaHCO$_3$, and the solution was washed 3 × 100 mL of Et$_2$O. The mixture was then acidified to pH 2.0 with 1 M HCl, and the product was extracted with 3 × 100 mL of EtOAc. The sample was concentrated to yield 0.53 g of **43a** as a light yellow foam for 77% yield over two steps. TLC 10% MeOH/CH$_2$Cl$_2$ 0.1% AcOH (R_f = 0.86). [α]$_D$ = +97.3° (c = 0.24, CHCl$_3$); FAB HRMS (M + H) for C$_{32}$H$_{36}$O$_{14}$N, expected 658.2136, found 658.2119. IR (NaCl plates) cm^{-1}: 3420, 3055, 1751, 1265, 1228, 1040, 738, 705.

N-(9-Fluorenylmethoxycarbonyl)-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-serine (43b). Glycoside **20b** (3.42 g, 4.96 mmol) was dissolved in 150 mL of MeOH, Pd–carbon (10%, 0.50 g) was added, and the flask was fitted with a H$_2$ balloon and hydrogenated for 2 h. TLC showed completion, and the reaction was quenched with 100 mL of CH$_2$Cl$_2$. After 10 min the solution was filtered through Celite and concentrated. The product was redissolved in 10% NaHCO$_3$ and washed 3 × 100 mL of Et$_2$O to remove Ph$_2$CH$_3$. The aqueous layer was then adjusted to pH 8.5 and diluted with dioxane to the cloud point. FMOC-Cl (1.42 g, 5.45 mmol, 1.1 equiv) was dissolved in 25 mL of dioxane and added dropwise to the glycoside over 20 min. After 1 h the reaction was complete by TLC. The solution was diluted with 50 mL of concentrated NaHCO$_3$, and the solution was washed 3 × 100 mL of Et$_2$O. The product was then acidified to pH 2.0 with 1 M HCl, and the product was extracted with 3 × 100 mL of EtOAc. The product was concentrated to yield 3.23 g of **43b** as a white foam for 99% yield over two steps. TLC 5% MeOH/CH$_2$Cl$_2$ 0.1% AcOH (R_f = 0.54). [α]$_D$ = +13.7° (c = 0.32, CHCl$_3$); FAB HRMS (M + H) for C$_{32}$H$_{36}$O$_{14}$N, expected 658.2136, found 658.2119. IR (NaCl plates) cm^{-1}: 3435, 2963, 2932, 1754, 1383, 1225, 1042, 737.

Protected Amino Acid Glycosides 44–56. See Supporting Information.

N-(9-Fluorenylmethoxycarbonyl)-O-(2-amino-3,4,6-tri-O-acetyl-2-deoxy-N-[(2,2,2-trichloroethyloxycarbonyl)-amino]-β-D-glucopyranosyl)-L-serine (57a). Glycoside **37** (6.00 g, 7.30 mmol) was dissolved in 300 mL of MeOH, Pd–carbon (10%, 0.50 g) was added, and the flask was fitted with a H$_2$ balloon and hydrogenated for 2 h. TLC showed completion and the reaction was quenched with 100 mL of CH$_2$Cl$_2$. After 10 min the solution was filtered through Celite and concentrated. The product was redissolved in 10% NaHCO$_3$ and washed 3 × 100 mL of Et$_2$O to remove Ph$_2$CH$_2$. The aqueous layer was then adjusted to pH 8.5 and diluted with dioxane to the cloud point. FMOC-Cl (1.98 g, 7.66 mmol, 1.05 equiv) was dissolved in 25 mL of dioxane and added dropwise to the glycoside over 20 min. After 1 h the reaction was complete by TLC. The solution was diluted with 50 mL of concentrated NaHCO$_3$ and washed 3 × 100 mL of Et$_2$O. The product was then acidified to pH 2.0 with 1 M HCl and extracted with 3 × 100 mL of EtOAc. The product was concentrated to yield 4.72 g of **57a** as an off-white foam for 82% yield over two steps. TLC 5% MeOH/CH$_2$Cl$_2$ 0.1% AcOH (R_f = 0.43). [α]$_D$ = +13.7° (c = 0.47, CHCl$_3$); FAB HRMS (M + H) for C$_{33}$H$_{36}$O$_{14}$N$_2$Cl$_3$, expected 791.1211, found 791.1202. IR (NaCl plates) cm^{-1}: 3341, 3080, 2963, 1748, 1537, 1383, 1234, 1168, 1047, 739, 703.

N-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetoxy-2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-serine (57b). Glycoside **39** (0.418 g, 0.475 mmol) was dissolved in 5.0 mL of Ac$_2$O, 0.50 g zinc dust was added, and the reaction was stirred for 14 h. TLC showed completion, and the reaction was filtered through Celite, concentrated, and azeotroped 2 × 25 mL of toluene. The crude material was flash chromatographed with 80% EtOAc/hexanes (R_f = 0.41) to yield 0.265 g of **57c** as a white foam (75% yield). [α]$_D$ = −3.7° (c = 0.43, CHCl$_3$); FAB HRMS (M + H) for C$_{39}$H$_{43}$O$_{13}$N$_2$, expected 747.2765, found

747.2773. IR (NaCl plates) cm^{-1}: 3439, 3068, 2956, 1747, 1659, 1548, 1233, 1047, 760, 699.

The *N*-acetyl derivative (**57c**, 0.24 g, 0.324 mmol) was dissolved in 100 mL of MeOH, Pd–carbon (10%, 0.25 g) was added, and the flask was fitted with a H$_2$ balloon and hydrogenated for 30 min. When TLC showed completion, 50 mL of CH$_2$Cl$_2$ was added, and the product was filtered through Celite and concentrated. Chromatography with 10% MeOH/CH$_2$Cl$_2$ 0.1% AcOH (R_f = 0.58) yielded 0.185 g of **57b** as an off-white foam (87%). [α]$_D$ = −14.7° (c = 0.36, CHCl$_3$); FAB HRMS (M + H) for C$_{32}$H$_{37}$O$_{13}$N$_2$, expected 657.2296, found 657.2286. IR (NaCl plates) cm^{-1}: 3402, 2956, 2924, 2852, 1736, 1383, 1241, 1152, 1045, 739.

N-(9-Fluorenylmethoxycarbonyl)-O-(2-amino-3,4,6-tri-O-benzoyl-2-deoxy-N-[(2,2,2-trichloroethyloxycarbonyl)-amino]-β-D-glucopyranosyl)-L-serine (58a). Glycoside **40** (0.202 g, 0.19 mmol) was dissolved in 100 mL of MeOH, Pd–carbon (10%, 0.50 g) was added, and the flask was fitted with a H$_2$ balloon and hydrogenated for 30 min. TLC showed completion, and the reaction was quenched with 50 mL of CH$_2$Cl$_2$. After 10 min the solution was filtered through Celite and concentrated to yield 0.184 g of **58a** as an off-white foam (97%). TLC 5% MeOH/CH$_2$Cl$_2$ 0.1% AcOH (R_f = 0.40). [α]$_D$ = +4.4° (c = 0.64, CHCl$_3$); FAB HRMS (M + H) for C$_{48}$H$_{41}$O$_{14}$N$_2$Cl$_3$, expected 977.1687, found 977.1677. IR (NaCl plates) cm^{-1}: 3428, 3068, 2963, 1731, 1272, 1107, 739, 710.

N-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-benzoyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-serine (58b). Glycoside **40** (0.33 g, 0.31 mmol) was dissolved in 5.0 mL of Ac$_2$O, 0.50 g zinc dust was added, and the reaction was stirred for 14 h. TLC showed completion, and the reaction was filtered through Celite, concentrated, and azeotroped with 2 × 25 mL of toluene. The crude material was flash chromatographed with 80% EtOAc/hexanes (R_f = 0.41) to yield 0.20 g of a white foam (70%). [α]$_D$ = −17.8° (c = 0.3, CHCl$_3$); FAB HRMS (M + H) for C$_{54}$H$_{49}$O$_{13}$N$_2$, expected 933.3235, found 933.3232. IR (NaCl plates) cm^{-1}: 3064, 2956, 1727, 1681, 1451, 1270.

The *N*-acetyl derivative (0.20 g, 0.214 mmol) was dissolved in 100 mL of MeOH, Pd–carbon (10%, 0.25 g) was added, and the flask was fitted with a H$_2$ balloon and hydrogenated for 30 min. When TLC showed completion, 50 mL of CH$_2$Cl$_2$ was added, the reaction was filtered through Celite and concentrated. Chromatography with 10% MeOH/CH$_2$Cl$_2$ 0.1% AcOH (R_f = 0.58) yielded 0.160 g of **58b** as an off-white foam (89%). [α]$_D$ = −10.3° (c = 3.0, CHCl$_3$); FAB HRMS (M + H) for C$_{32}$H$_{37}$O$_{13}$N$_2$, expected 657.2296, found 657.2286. IR (NaCl plates) cm^{-1}: 3434, 2969, 2957, 1727, 1384, 1270, 1108, 1069, 1027, 710.

Benzyl N-(9-Fluorenylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-β-D-glucopyrano-syl)-L-serinate (58c). Glycoside **40** (0.3 3 g, 0.31 mmol) was dissolved in 5.0 mL of Ac$_2$O, 0.50 g of zinc dust was added, and the reaction was stirred for 14 h. TLC showed completion, and the reaction was filtered through Celite, concentrated, and azeotroped with 2 × 25 mL of toluene. The crude material was flash chromatographed with 80% EtOAc/hexanes (R_f = 0.41) to yield 0.20 g of **56** as a white foam (70%). [α]$_D$ = −17.8° (c = 0.3, CHCl$_3$); FAB HRMS (M + H) for C$_{54}$H$_{49}$O$_{13}$N$_2$, expected 933.3235, found 933.3232. IR (NaCl plates) cm^{-1}: 3064, 2956, 1727, 1681, 1451, 1270.

N-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (59b). From the Schiff base glycoside:[65] Glycoside **41** (2.50 g, 3.72 mmol) was dissolved in 20 mL of THF, 2.0 mL of CF$_3$COOH, and 0.50 mL of H$_2$O and allowed to react at RT for 15 min. After TLC showed complete hydrolysis, the solution was concentrated and azeotroped with toluene 3 × 50 mL. The product was passed through a plug of silica gel, first with CH$_2$Cl$_2$ to remove Ph$_2$C=O, followed by 20% acetone/CH$_2$Cl$_2$ to elute the product, which was immediately acylated with FMOC-Cl (0.64 g, 2.48 mmol, 1.05 equiv) in 20 mL of CH$_2$Cl$_2$ and ^{i}Pr$_2$NEt (0.85 mL, 4.95 mmol, 2 equiv). After 30 min, the reaction was complete, and the product was concentrated and flash chromatographed with 35% EtOAc/hexanes (R_f 0.49) to yield 1.22 g of the FMOC benzyl glycoside **42** as a white foam
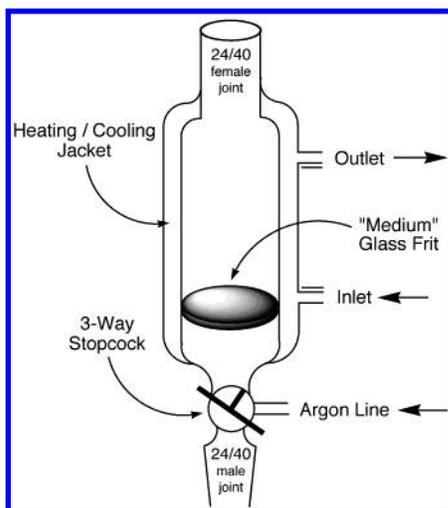
Figure 9. Apparatus used for peptide assembly.

in 71% over two steps. Glycoside **42** (1.17 g, 1.60 mmol) was hydrogenated and acetylated as above to yield 0.885 g of pure **59b** in 84% over two steps.

From the FMOC glycoside: Glycoside **42** (0.31 g, 0.42 mmol) was dissolved in 100 mL of MeOH, Pd–carbon (10%, 250 mg) was added under a balloon of H$_2$, andthe reaction was continued until completion. After 30 min the reaction was complete by TLC, 50 mL of CH$_2$Cl$_2$ was added, and the reaction was filtered through Celite, concentrated, and washed with toluene. The product was then dissolved in 10 mL of CH$_2$Cl$_2$, Ac$_2$O (6 μL, 0.64 mmol, 1.50 equiv), and ^{i}Pr$_2$NEt (0.20 mL, 1.27 mmol, 3.0 equiv) and stirred for 60 min. TLC showed completion, and the product was concentrated and flash chromatographed using 10% MeOH/CH$_2$Cl$_2$ with 0.1% AcOH (R_f = 0.20) to yield 0.250 g of **59b** as an off-white foam in 90% yield over two steps. [α]$_D$ = +81.5° (c = 1.6, CHCl$_3$); FAB HRMS (M + H) for C$_{32}$H$_{37}$O$_{13}$N$_2$, expected 657.2296; found 657.2286. IR (NaCl plates) cm^{-1}: 3360, 2957, 2926, 1747, 1537, 1373, 1235, 1050, 738.

General Methods for Glycopeptide Synthesis. All glycopeptides were assembled manually using a fritted glass reaction vessel with argon purging for effective mixing (Figure 9). Rink peptide resin was employed for each compound with a loading of 0.55–0.80 mmol/g active sites. Tyrosine was protected as the *tert*-butyl ether, D-Cys was protected as a triphenylmethyl (trityl) ether, and all glycosides were protected as acetate esters. Deprotection of the N-α-FMOC group was carried out using 25% piperidine/DMF (1 × 5 min + 1 × 20 min). The resin was then washed 2 × DMF followed by 4 × CH$_2$Cl$_2$. Deprotection and couplings were monitored using the Kaiser ninhydrin test. Normal coupling was performed in a 1:1 DMF/NMP mixture with 2.0 equiv of FMOC amino acid, BOP (Castro's reagent[67]), HOBt (1-hydroxy-benzotriazole), and 4.0 equiv ^{i}Pr$_2$NEt. For FMOC amino acid glycosides, 1.25 equiv of each reagent was employed. Coupling times were variable, ranging from 30 min for Gly, Phe, and Tyr, to 1 h for trityl-D-Cys, and 1.5–4 h for glycoside coupling. Similar results were obtained with the Protein Technologies Sonata equipment using the same reagents and solvent protocols. After completion of the synthesis, the acetates of the glycoside residue were removed while on the solid support using 80% H$_2$NNH$_2$ in CH$_3$-OH 2 × 30 min, and 1 × 1 h, followed by two washes with 1:1 MeOH/CH$_2$Cl$_2$ and six washes with CH$_2$Cl$_2$ and then drying in vacuo. The dried peptide resins were cleaved using a cocktail of 9.0 mL of CF$_3$COOH, 1.0 mL of CH$_2$Cl$_2$, 0.25 mL of Et$_3$SiH, 0.25 mL of H$_2$O, and 0.05 mL of anisole for 2 h at RT with agitation. The solution was then filtered to remove the cleaved resin, and the resultant solution was concentrated to a yellowish oil. The crude glycopeptide amides were precipitated with cold Et$_2$O, filtered, washed with more cold Et$_2$O, and dried in vacuo. These crude reduced samples were then purified on a Perkin-Elmer LC 250 system with a diode array detector

using a Vydak C$_{18}$ preparative reversed-phase column using a gradient of 10–50% CH$_3$CN vs 1% CF$_3$COOH in H$_2$O to give the reduced glycopeptides in pure form, assessed for purity by analytical HPLC on a Hewlett-Packard Series II 1090 LC system employing the same gradient.

Cyclization was performed immediately on the cleavage product. The crude reduced glycopeptide (∼100 mg) was dissolved in 10 mL of doubly distilled H$_2$O and placed into a clean syringe. In a 100 mL beaker, 2.5 equiv of K$_3$Fe(CN)$_6$ was dissolved in 50 mL of doubly distilled H$_2$O, and the pH was adjusted to 8.5 using 1M NH$_4$OH. The glycopeptide solution was then syringe-pumped into the K$_3$Fe(CN)$_6$ over 8 h. After the addition was complete, the pH was acidified with 1 M AcOH to pH 4. Amberlite IRL-60 anion-exchange resin was then added to sequester any remaining ferricyanide, and the solution was filtered, washed with 1 M AcOH, and lyophilized. The crude cyclic samples were immediately purified by HPLC using the conditions described above to yield the pure cyclic glycopeptides.

Glycopeptides 60a and 60b. FMOC-Rink resin (1.504 g, 0.60 mmol/g) was swollen in DMF for 30 min. FMOC deprotection and washes were followed by coupling of Gly[7] with FMOC-glycine (0.60 g, 2.0 mmol), BOP (0.89 g, 2.0 mmol), HOBt (0.31 g, 2.0 mmol), and ^{i}Pr$_2$NEt (0.70 mL, 4.0 mmol) in 25 mL of 1:1 DMF/NMP for 30 min. FMOC deprotection and washes were followed by coupling of Ser[6] with FMOC-D-Ser-[O-β-D-Glc(OAc)$_4$] **45** (0.76 g, 1.25 mmol), BOP (0.51 g, 1.25 mmol), HOBt (0.18 g, 1.25 mmol), and ^{i}Pr$_2$NEt (0.45 mL, 2.50 mmol) in 25 mL of 1:1 DMF/NMP for 3 h. FMOC deprotection and washes were followed by coupling of D-Cys[5] with FMOC-D-cysteine (trt) (1.17 g, 2.0 mmol), BOP (0.89 g, 2.0 mmol), HOBt (0.31 g, 2.0 mmol), and ^{i}Pr$_2$NEt (0.70 mL, 4.0 mmol) in 25 mL of 1:1 DMF/NMP for 3 h. FMOC deprotection and washes were followed by coupling of Phe[4] with FMOC-phenylalanine (0.77 g, 2.0 mmol), BOP (0.89 g, 2.0 mmol), HOBt (0.31 g, 2.0 mmol), and ^{i}Pr$_2$NEt (0.70 mL, 4.0 mmol) in 25 mL of 1:1 DMF/NMP for 60 min. FMOC deprotection and washes were followed by coupling of Gly[3] with FMOC-glycine (0.59 g, 2.0 mmol), BOP (0.89 g, 2.0 mmol), HOBt (0.31 g, 2.0 mmol), and ^{i}Pr$_2$NEt (0.70 mL, 4.0 mmol) in 25 mL of 1:1 DMF/NMP for 60 min. FMOC deprotection and washes were followed by coupling of D-Cys[2] with FMOC-S-trityl-D-cysteine (1.17 g, 2.0 mmol), BOP (0.89 g, 2.0 mmol), HOBt (0.31 g, 2.0 mmol), and ^{i}Pr$_2$NEt (0.70 mL, 4.0 mmol) in 25 mL of 1:1 DMF/NMP for 2.5 h. FMOC deprotection and washes were followed by coupling of Tyr[1] with FMOC-O^{t}Bu-L-tyrosine (0.92 g, 2.0 mmol), BOP (0.89 g, 2.0 mmol), HOBt (0.31 g, 2.0 mmol), and ^{i}Pr$_2$NEt (0.70 mL, 4.0 mmol) in 25 mL of 1:1 DMF/NMP for 2 h. N$^\alpha$-FMOC deprotection was followed by hydrazine treatment to remove sugar acetates, washing, and vacuum-drying to give 1.9625 g (36%) of protected peptide resin. Standard cleavage gave crude reduced **60a**, which yielded pure reduced **60a** after chromatography. [α]$_D$ = +38.3° (c = 0.08, H$_2$O); FAB HRMS (M + H) for C$_{37}$H$_{53}$O$_{14}$N$_8$S$_2$, expected 897.3123; found 897.3149. Cyclization was accomplished using the cleaved crude material under optimized conditions using K$_3$Fe(CN)$_6$ and yielded pure cyclic **60b** after chromatography. FAB HRMS (M + H) for C$_{37}$H$_{51}$O$_{14}$N$_8$S$_2$, expected 895.2966; found 895.2956.

Glycopeptides 61a–70a and 61b–70b. See Supporting Information.

Supporting Information Available: Complete experimental details are available. This material is available free of charge at http://pubs.acs.org.

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