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Synthesis of *S*-Linked Glycopeptides in Aqueous Solution

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Investigation of direct *S*-glycosylation of homocysteine and cysteine containing peptides with *O*-acetyl protected glycosyl halides led under two-phase conditions in the presence of sodium carbonate as base to excellent results. Thus, from glucosyl bromide, galactosyl bromide, lactosyl bromide, sialyl chloride, and *N*-Troc protected 2-amino-2-deoxyglucosyl bromide *S*-glycosylated dipeptides **15**, **18**−**21**, **23**, **24**, and **26**−**29**, respectively, were obtained in excellent yields. Alternatively, depending on the solubility of the peptide moiety, mixtures of DMF and water could be employed for successfully carrying out this reaction. Thus, *S*-glycosylated tripeptides **42**−**45** could be obtained. Combination of this method with chemical ligation was also successfully carried out.

Introduction

Glycoproteins[1] are ubiquitous in all forms of life from Archaebacteria to humans and play a variety of biological roles. The glycosylation of proteins directs protein folding, endows conformational stability, grants resistance to proteolytic degradation, regulates the protein's serum half-life, and provides unique epitopes for molecular recognition.[2] Altered protein glycosylation patterns often modify intercellular recognition processes through modulation of protein−protein or protein−carbohydrate interaction. The most comprehensive studies of specific glycoprotein function to date have been derived through the characterization of glycopeptide activity.

However, understanding how and why these highly specific interactions come about has always been hindered by the difficulties associated with the "microheterogeneity" of glycopeptides.[3] Therefore, glycopeptides are relatively difficult to obtain from biological sources in homogeneous form, rendering the synthesis of isomerically pure glycopeptides[4,1] and neoglycopeptides[5] a necessity to further explore the function of these compounds in biological systems.

Although the most relevant glycosyl−peptide linkages occurring in glycoproteins are the *N*- and *O*-linked structures,[1a] the corresponding *S*-linked glycoproteins have been characterized as well[6] and they have been the subject of significant interest as a result of their enhanced chemical stability and enzymatic resistance.[7] This has stimulated the development of simpler structural analogues (glycopeptide mimetics) as fundamental tools for biological research and as potential agents for therapeutic intervention.[8]

Usually, direct glycosylation of peptides has been shown to be unsatisfactory due to the presence of competing functional groups. *S*-Linked glycopeptides have been obtained by direct coupling between sugar and peptide only in a few cases. In the procedure reported by van der Donk,[9] the stereochemistry of products has not been controlled. In a few examples,[10−12] most notably by Kessler and co-workers,[10,11] *S*-glycopeptides were synthesized by peptide synthesis with use of *S*-linked glycosyl amino acids as building blocks. Difficulties with the glycosylation of cysteine containing di- and tripeptides have been described by Kessler et al.[10] They attributed this problem to steric hindrance by the *tert*-butyl group and to the decreased reactivity of the thiol function of tripeptides due to unfavorable hydrogen bondings, respectively. In contrast, *S*-linked glycosyl amino acids,[13] which contain sulfur in the glycosidic linkage, have previously been prepared by some methods

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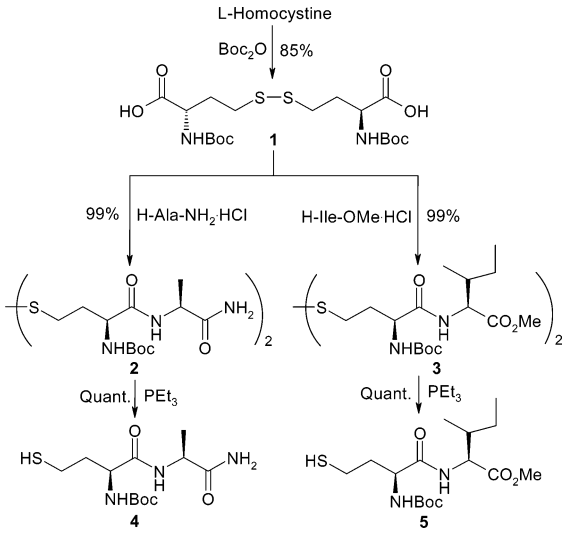
including Koenigs−Knorr conditions,[13b] S_N2 displacement of an iodide[13k] or a cyclic sulfamidate[13j] by 1-thioglycopyranose derivatives, or Lewis acid-catalyzed glycosylation with glycosyl fluorides,[13d] trichloroacetimidates,[13g] or peracetylated sugars[13e] as donors. However, most of these methods have suffered from two problems that limit their further applicability: usually dry conditions were required (when sugar was used as electrophile) or indirect building blocks had to be synthesized first (when peptide was used as electrophile). In addition, most solvents used in the above procedures were only organic solvents, but protected or partially protected peptide chains frequently exhibit only limited solubility in the organic phase, which made these procedures impractical for the synthesis of glycopeptides. Therefore, a method allowing the direct glycosylation of cysteinyl or homocysteinyl peptides to produce the corresponding product would be welcome in the context of the synthesis of *S*-linked glycopeptides. This is of particular interest because in terms of distance between the oligosaccharide and the peptide backbone ligation of sugar residues to cysteine mimics *O*-glycan linkage to serine and threonine and linkage to homocysteine (Hcy) mimics *N*-glycan linkage to aspargine, respectively.

The chemical ligation method,[14] which was developed in the early 1990s, has proven to be highly effective and generally applicable to the synthesis of a range of protein targets. In particular, the technique of "native chemical ligation" developed by the laboratories of Kent and Tam[15] permits the convergent coupling of peptide fragments to generate polypeptides of over 100 residues in length. In this method, one peptide fragment possesses a *C*-terminal thioester, and the other fragment bears an *N*-terminal cysteine residue; their selective and reversible transthioesterification is followed by an irreversible rearrangement that forms an amide bond at the site of ligation. The spontaneous *S*- to *N*-acyl migration occurs to form a peptide bond through a five-membered-ring intermediate. Chemical ligation of a thioester at a peptide *C*-terminus with a homocysteine residue at the *N*-terminus of a second peptide was also reported.[16,17] The convergent character, the simplicity of a one-pot procedure, the tolerance to most free functional groups, as well as the access to large polypeptides are among the described advantages of native chemical ligation.

SCHEME 1



As part of our ongoing project on the synthesis of *O*- and *N*-linked glycopeptides containing methionine,[17] we decided to pursue approaches to *S*-linked glycopeptides because of their aforementioned significance. We wish to report here that cysteinyl and homocysteinyl peptides, which also could be prepared via chemical ligation, can be glycosylated effectively by glycosyl halides in the presence of Na_2CO_3 in aqueous solution. This procedure provides *S*-linked glycopeptides chemoselectively and in excellent to reasonable chemical yield independent of the *N*-protection and also with tripeptides.

Results and Discussion

Recently, we developed a procedure for the synthesis of methionine-containing peptides related to native chemical ligation where the free sulfhydryl group was produced after the ligation step.[17] Upon chemoselective methylation of the sulfhydryl group in the presence of methyl iodide in NH_3/MeOH the methionine-containing peptides were produced. This prompted us to explore the reactivity of the sulfhydryl group with sugar halides to produce glycopeptides through S_N2 displacement. For this purpose, Boc-Hcy-Ala-NH_2 (**4**) was envisaged as a model compound, as shown in Scheme 1. It was prepared by coupling of *N*-Boc protected homocystine[18] with H-Ala-NH_2 in the presence of PyBOP[19] and DIPEA in DMF (→ **2**, 99% yield) followed by reduction of its disulfide bond with PEt_3[20] in MeOH−THF−H_2O (quant.). Similarly, from **1** dipeptide disulfide **3** was prepared, which was transformed into Hcy containing dipeptide **5**.

Before reacting the dipeptide thiol **4** with sugar halides, we performed some preliminary work on the *S*-alkylation of **4** to find appropriate conditions for its glycosylation (Scheme 2). Reaction of **4** with iodoacetamide[21] in the presence of Et_3N afforded the desired

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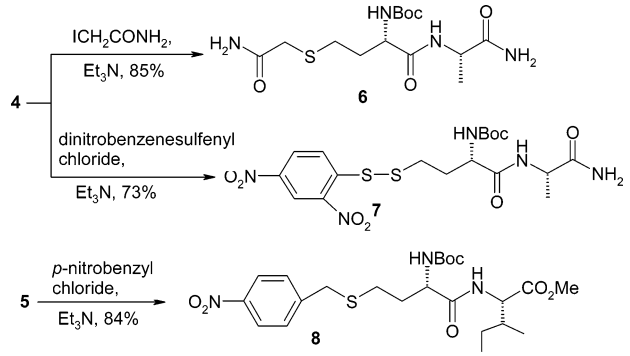
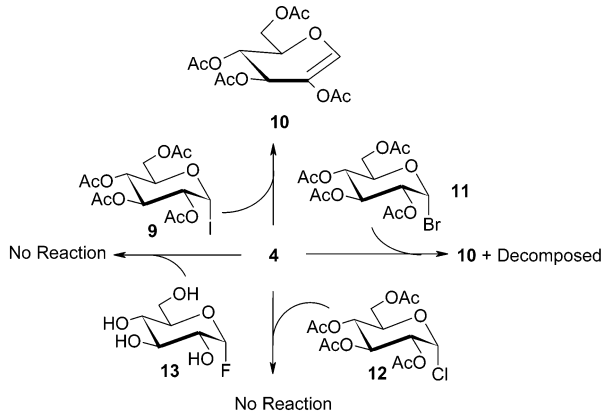
SCHEME 2



SCHEME 3[a]

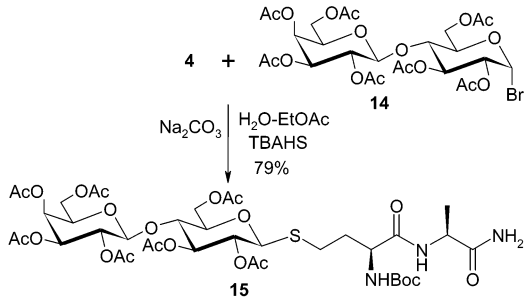


[a] Note: For **9**, **11**, and **12**, Et_3N was used as base; for **13**, Et_3N, or NH_3 was used as base.

S-carbamoylmethyl derivative **6** in 85% yield. Next we employed dinitrobenzenesulfenyl chloride as the electrophile under the same conditions; expectedly, disulfide product **7** was produced in 73% yield. Also the *p*-nitrobenzyl group was introduced smoothly to dipeptide thiol **5** by using Et_3N as base, thus giving sulfide **8** in 84% yield.

We then extended these reaction conditions to investigate the base-promoted coupling of dipeptide thiol **4** with sugar halides. Unfortunately, all the coupling reactions failed; formation of undesirable side products was observed. First, glycosylation of thiol **4** was performed with glucosyl iodide **9**[22] in the presence of Et_3N, but only elimination of HI took place to yield the glucal **10**;[23] no desired product was formed. The same problem existed by replacement of Et_3N by a weaker base like imidazole. To avoid this elimination reaction, it was necessary to use less reactive sugar halides as donors (e.g. fluoride, chloride, or bromide). However, use of glucosyl bromide **11**[24] as the donor with Et_3N as base produced, as shown in Scheme 3, no desired product and only elimination and decomposition of the starting material took place. We also investigated glucosyl chloride **12**[25] and fluoride **13**[26] to react with thiol **4** under basic conditions, but no product

SCHEME 4



was formed and donors were not affected even after prolonged reaction time.

Roy and co-workers demonstrated the stereoselective synthesis of various glycosyl derivatives including *S*-aryl glycosides from glycosyl halides under phase-transfer catalysis (PTC) conditions with complete anomeric inversion.[27]

Therefore, we applied these PTC conditions to synthesize *S*-linked glycopeptides, and indeed it worked very well with dipeptides. Treatment of thiol **4** with lactosyl bromide **14** at room temperature in the presence of tetra-*n*-butylammonium hydrogen sulfate (TBAHS) in ethyl acetate and a 10% solution of Na_2CO_3 afforded the corresponding β-thioglycoside **15** in 79% yield (Scheme 4). Encouraged by this result, the generality of the present procedure for the glycosylation of other peptides was examined. Indeed, reaction of **5** with glucosyl bromide **11**, galactosyl bromide **16**,[28] lactosyl bromide **14**,[28] and neuraminic acid chloride **17**[29] under PTC conditions provided the corresponding β-thioglycosides **18**−**21** in yields of 86, 87, 83, and 76%, respectively.

To further exploit the above PTC conditions, cysteinyl thiol **22** was also glycosylated with glycosyl bromides **11** and **16** under the same reaction conditions to give the corresponding *S*-linked glycodipeptides **23** and **24** in 94% and 92% yield, respectively (Scheme 6). It is noteworthy that the negative effect of the *N*-Boc protecting group on the nucleophilicity of the β-SH group, as described before,[10,30] was not observed in our cases. Dipeptide thiol **22** was prepared by coupling of *N*-Boc protected cystine with H-Ile-OMe followed by reductive cleavage of the disulfide bond with Et_3P.

In view of the importance of β-linked GlcNAc residues in intracellular glycoproteins,[31] we decided to use 2-azi-

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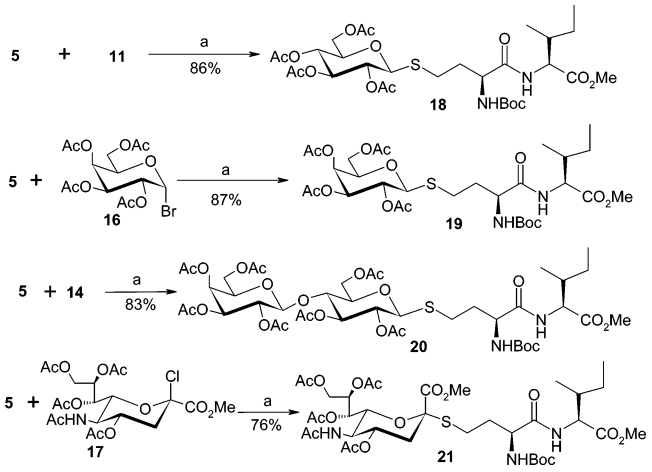
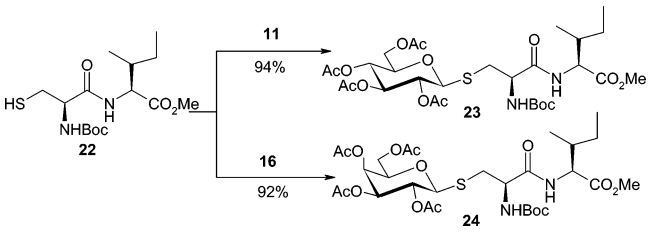
(30) An unfavorable hydrogen bond pattern between the acyl-protected (cf. Boc, Z, etc.) NH and the β-OH (or in our case the β-SH) was claimed to be the reason for the reduced reactivity of acyl-protected glycosyl acceptors. See: (a) Szabo, L.; Ramza, J.; Langdon, C.; Polt, R. *Carbohydr. Res.* **1995**, *274*, 11. (b) Polt, R.; Szabo, L.; Treiberg, J.; Li, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **1992**, *114*, 10249. (c) Peterson, M. A.; Polt, R. *J. Org. Chem.* **1993**, *58*, 4309. (d) Yamada, K.; Matsumoto, S.; Nishimura, S. I. *J. Chem. Soc., Chem. Commun.* **1999**, 507. See also refs 13g and 28.

SCHEME 5[a]



[a] Reagents and conditions: (a) Na_2CO_3, $H_2O-EtOAc$, TBAHS.

SCHEME 6[a]

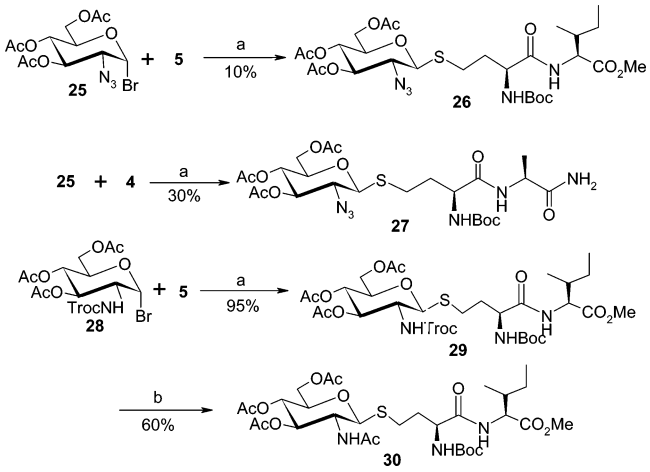


[a] Reagents and conditions: (a) Na_2CO_3, $H_2O-EtOAc$, TBAHS.

doglucosyl bromide **25** as a glycosyl donor to prepare some *S*-linked *β*-GlcNAc glycosides. The preparation of donor **25** was carried out by established procedures,[32] starting from commercially available glucosamine. However, subsequent treatment of **25** with the dipeptide **4** and **15** under the PTC conditions afforded the desired *β*-thioglycosides **26** and **27** presumably due to reduction[33] of the azido group by the presence of free sulfhydryl groups in quite low yields. Changes in concentration, molar excess of glycosyl donor, and amount of Na_2CO_3 did not prevent this unwanted side reaction. Therefore, the azido group had to be changed to another amino group protection. In view of its wide application in amino sugars, *N*-Troc protected 2-amino glucosyl bromide **28**[28] was prepared to couple with dipeptide **5** under the same glycosylation conditions, and happily, the desired *β*-thioglycoside **29** was produced in 95% yield. Then **29** was treated with Zn dust in the presence of Ac_2O,[34] accomplishing both cleavage of the Troc group and acetylation in one pot to yield the *β*-GlcNAc glycoside **30** in good yield (60%), as shown in Scheme 7.

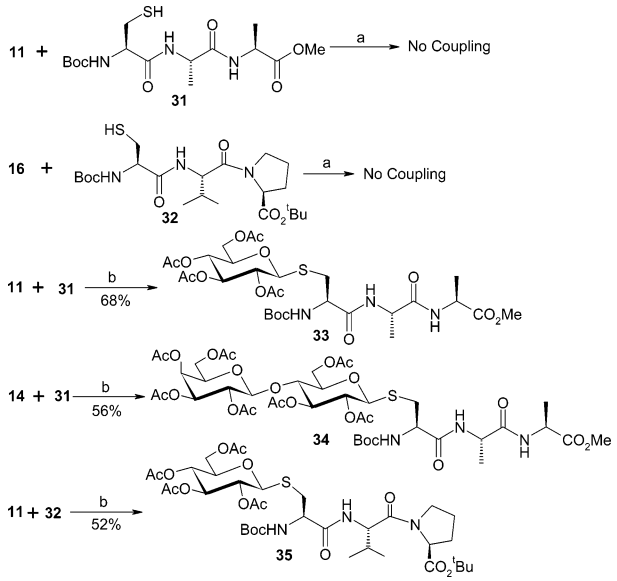
Then the two-phase glycosylation of the sulfhydryl group was applied to tripetides **31** and **32** (Scheme 8), which were prepared by coupling of *N*-Boc protected cystine with H-Ala-Ala-OMe and H-Val-Pro-O^{t}Bu, re-

SCHEME 7[a]



[a] Reagents and conditions: (a) Na_2CO_3, $EtOAc-H_2O$, TBAHS; (b) Zn dust, Ac_2O.

SCHEME 8[a]



[a] Reagents and conditions: (a) Na_2CO_3, $EtOAc-H_2O$, TBAHS; (b) Na_2CO_3, DMF/H_2O.

spectively, followed by reduction of the disulfide bond with Et_3P.[27] However, the coupling of tripeptide thiols **31** and **32** with glucosyl bromide **11** and galactosyl bromide **16** failed to provide the desired glycosylated glycotripeptides even after prolonged reaction times under PTC conditions. A variety of attempts to bring about the glycosylation reaction led to destruction of donors and recovery of acceptors. This result was in sharp contrast with the smooth glycosylation of related dipeptides **4** or **5**. We surmised that the different behavior of tripeptides and dipeptides might be caused by their different solubility and not, as suggested, by hydrogen bonding effects.[10] We then investigated whether another system would succeed where the PTC conditions had apparently failed. Therefore, the procedure was modified by changing the solvent EtOAc to DMF/H_2O, as shown in Scheme 8. Gratifyingly, tripeptide **31** reacted with glucosyl bromide **11** and lactosyl bromide **14** in the presence of Na_2CO_3 in DMF/H_2O to give the desired

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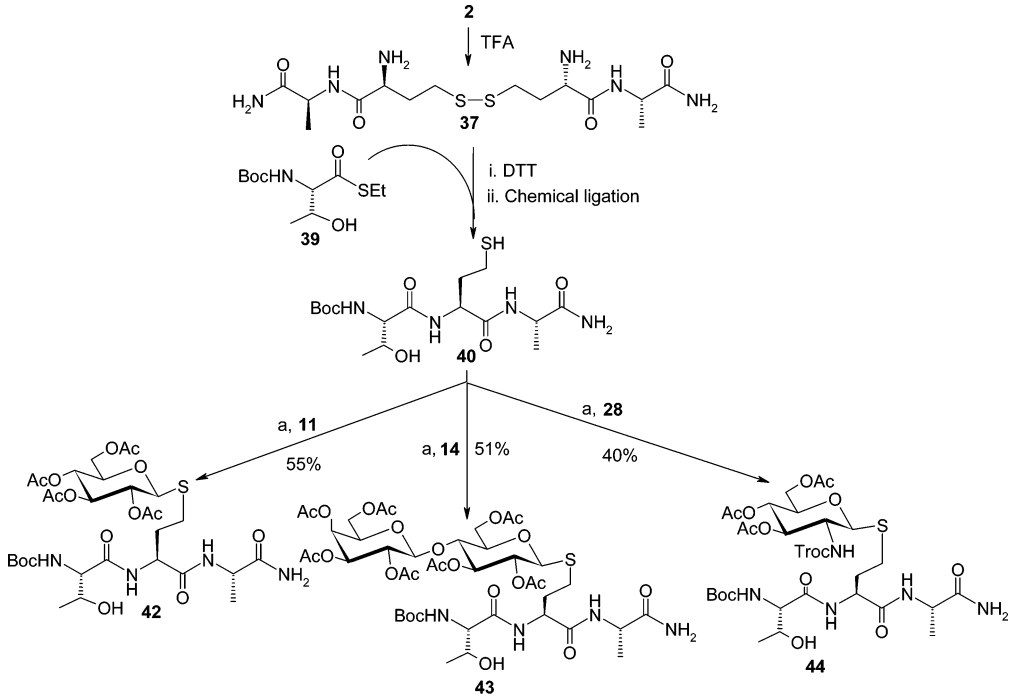
SCHEME 9[a]



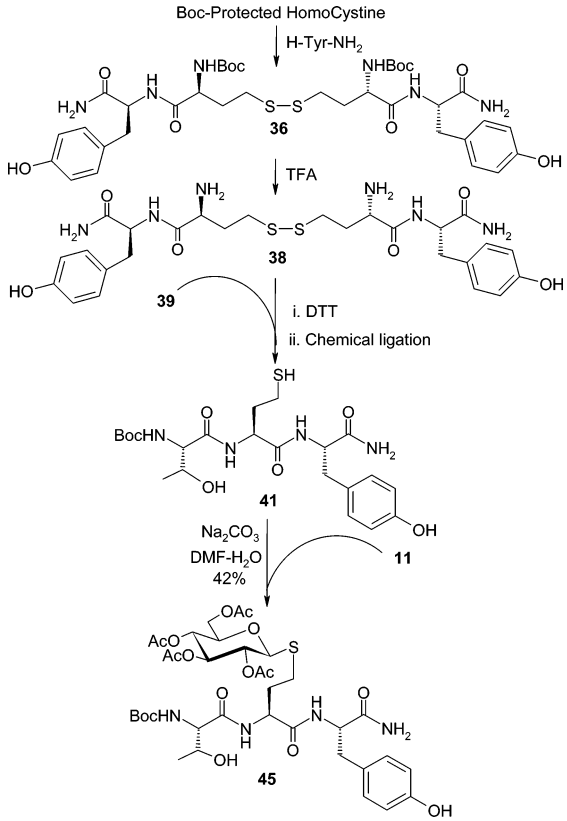
[a] Reagents and conditions: (a) Na_2CO_3, DMF−H_2O.

S-glycosylated tripeptides **33** and **34** in 68% and 56% yield, respectively. Tripeptide **32** was also *S*-glycosylated effectively under the same conditions to furnish the *β*-thioglycoside **35** in 52% yield.

In all couplings in Scheme 8, tripeptides possessing the sulfhydryl group in the terminal position were employed; it was essential to investigate also the reactivity of sulfhydryl groups at other positions. For this purpose, tripeptides **40** and **41** were synthesized by using the chemical ligation strategy[15,17] (Schemes 9 and 10) to further test the feasibility of the convergent synthesis of *S*-linked glycopeptides under the present aqueous conditions. In practice, peptide **36** was prepared in the same manner as **2** from Boc-protected homocystine by amidation with H-Tyr-NH_2 in the presence of PyBOP and DIPEA in DMF. Removal of the Boc protecting group from the corresponding homocystine residue in **2** and **36** was accomplished by the action of 20% TFA in dry CH_2Cl_2 to give the peptides **37** and **38**, respectively. The disulfide bonds of these two unprotected peptides were cleaved by DTT[35] in Tris buffer (pH 8) at room temperature, and ensuing addition of the threonine thioester **39** to the reaction mixture produced the desired tripeptides **40** and **41** in a one-pot procedure in 70% and 72% yield, respectively.

Then, tripeptide **40** was treated with glucosyl bromide **11** in the presence of Na_2CO_3 in DMF/H_2O; thus, the desired coupling product **42** was obtained in 55% yield. This reaction was a significant advance for the convergent synthesis of *S*-linked glycopeptides in that it demonstrated that under suitable conditions peptides could be *S*-glycosylated effectively. Similarly, treatment of **40**

SCHEME 10



with glycosyl bromides **14** and **28** afforded the desired *S*-glycosylated products **43** and **44** in 51% and 40% yield, respectively. Tripeptide **41** containing a tyrosine residue was also *S*-glycosylated with glucosyl bromide **11** under

(35) Zahler, W. L.; Cleland, W. W. *J. Biol. Chem.* **1968**, *243*, 716.

the same conditions in reasonable yield (42%). No tyrosine *O*-glycosylation product of **41** was observed. However, because of sluggish reactions of **40** and **41**, some glycosyl donor underwent hydrolysis before reaction; also adsorption to silica gel led to less product during purification, therefore lower yields were obtained for **42**−**45**. Yet, it should be noted that exploiting the reactivity differences between the different functional groups in **41** provided access to the desired product **45**.

Conclusions

In this report, a new protocol for the preparation of *S*-linked glycopeptides in aqueous solution has been developed, and to the best of our knowledge, this is the first report on direct glycosylation of sulfhydryl groups on peptides. Eight *S*-linked glycodipeptides and seven *S*-linked glycotripeptides were efficiently synthesized from the corresponding glycosyl halides and peptides. An additional advantage of this strategy is the ready availability of cysteine- or homocysteine-containing peptides, for instance via native chemical ligation techniques. This methodology is of considerable potential for the synthesis of *S*-linked glycopeptides, since the glycosylation reaction is highly chemoselective and extremely simple in execution and in workup, and the products are mimics of *O*- and *N*-glycans.

Experimental Section

General Methods and Materials The solvents were purified according to the standard procedures. All reactions were performed under argon unless otherwise stated. Melting points were reported in degrees Celsius (uncorrected). TLC was performed on plastic plates, silica gel 60 F_{254}. Detection was achieved by treatment with a solution of 20 g of ammonium molybdate and 0.4 g of cerium(IV) sulfate in 400 mL of 10% H_2SO_4 or with 15% H_2SO_4, and heating at 150 °C. Flash chromatography was carried out on silica gel (30−60 mm) at a pressure of 0.3−0.4 bar. Optical rotations were determined at 21 °C (1-dm cell). NMR spectra were recorded at 250 MHz by using tetramethylsilane as internal standard. MS spectra were recorded in the positive mode by using 2,5-dihydroxybenzoic acid in dioxane as matrix.

Chemicals used were reagent grade, used as supplied except where noted. L-Homocystine and *N*,*N*′-di-*tert*-butoxycarboxyl-L-cystine were purchased. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl iodide **9** was prepared from 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose as described previously.[22] All the sugar bromides except bromide **25** were prepared from the corresponding peracetylated sugars by treatment with HBr in HOAc at 0 °C, and **25** was prepared from commercially available D-glucosamine·HCl according to the procedures described by Wong[32a] and Paulsen.[32b] 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl chloride **12** was prepared according to Montero's procedure.[25] α-D-Glucopyranosyl fluoride **13** was synthesized from peracetylated glucose in two steps as described by Noyori[26a] and Albert.[26b] *N*-Acetyl-4,7,8,9-tetra-*O*-acetyl-2-chloro-2-deoxy neuraminic acid methyl ester **17** was prepared from Neu5Ac according to the procedure described by Wong.[29] All the thiols prepared were used immediately after flash column chromatography.

***N*,*N*′-Di-*tert*-butoxycarbonyl-L-homocystine (1).** To a stirred solution of L-homocystine (5.0 g, 18.6 mmol) in aqueous 10% Na_2CO_3 (70 mL) and THF (60 mL) was added di-*tert*-butyl pyrocarbonate (10.2 g, 46.7 mmol) in one portion. The mixture was refluxed at 80 °C for 3.5 h, then diluted with H_2O (120 mL). The pH of the mixture was adjusted to 6 with 1 N HCl, the aqueous solution was extracted with $CHCl_3$ (3 × 100 mL), and the combined organic layers were washed with brine and water, dried over $MgSO_4$, and evaporated to give the product **1** (7.4 g) in 85% yield. An analytical sample was obtained by crystallization from CH_2Cl_2−petroleum ether: TLC R_f 0.20 (CH_2Cl_2/MeOH, 4:1); $[\alpha]_D$ −28 (*c* 1.05 MeOH); mp 152−154 °C; ^{1}H NMR ($CDCl_3$) δ 5.28 (br s, 1H), 4.43 (br s, 1H), 4.28 (br s, 1H), 2.76 (br s, 4H), 2.20 (m, 4H), 1.44 (s, 18H); ^{13}C NMR ($CDCl_3$/CD_3OD) δ 179.0, 174.0, 155.7, 52.4, 34.5, 32.3, 28.2; MALDI-MS *m*/*z* 491.1 [M + Na^+], 507.1 [M + K^+].

(Boc-Hcy-Ala-NH_2)$_2$ (2). To a stirred solution of Boc-protected homocystine **1** (434 mg, 0.93 mmol) and H-Ala-NH_2·HCl (248 mg, 2.0 mmol) in dry DMF (6 mL) were added PyBOP (1.1 g, 2.1 mmol) and DIPEA (0.6 mL, 3.4 mmol). The mixture was stirred at room temperature for 5 h, diluted with EtOAc, washed with brine, dried with $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (EtOAc/MeOH, 15:1) to afford **2** (560 mg) as a white solid in 99% yield: TLC R_f 0.35 (EtOAc/MeOH, 12:1); $[\alpha]_D$ −3.8 (*c* 0.48 MeOH); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 7.83 (d, J = 7.0 Hz, 2H), 4.27 (m, 2H), 4.06 (t, J = 6.1 Hz, 2H), 3.89 (s, 4H), 2.56 (t, J = 7.8 Hz, 4H), 1.92 (m, 4H), 1.28 (s, 18H), 1.23 (d, J = 7.1 Hz, 6H); MALDI-MS *m*/*z* 630.9 [M + Na^+]. Anal. Calcd for $C_{24}H_{44}N_6O_8S_2$ (608.77): C, 47.35; H, 7.28; N, 13.80. Found: C, 47.28; H, 7.41; N, 13.47.

(Boc-Hcy-Ile-OMe)$_2$ (3). To a stirred solution of Boc-protected homocystine **1** (249 mg, 0.53 mmol) and H-Ala-OMe·HCl (224 mg, 1.2 mmol) in dry DMF (3 mL) were added PyBOP (0.67 g, 1.2 mmol) and DIPEA (0.5 mL, 2.9 mmol). The mixture was stirred at room temperature for 5 h, diluted with EtOAc, washed with brine, dried with $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (petroleum ether/EtOAc, 1:1) to afford **3** (380 mg) as a white solid in 99% yield: TLC R_f 0.67 (petroleum ether/EtOAc, 1:1); $[\alpha]_D$ +12.0 (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.93 (br s, 2H), 5.28 (d-like, 2H), 4.60 (dd, J = 8.7, 5.0 Hz, 2H), 4.32 (m, 2H), 3.76 (s, 6H), 2.77 (t, J = 7.4, 4H), 2.21 (m, 2H), 2.00 (m, 4H), 1.44 (s, 18H), 1.43 (m, 2H), 1.19 (m, 2H), 0.91 (m, 12H); MALDI-MS *m*/*z* 744.8 [M + Na^+], 760.9 [M + K^+]. Anal. Calcd for $C_{32}H_{58}N_4O_{10}S_2$ (722.95): C, 53.16; H, 8.09; N, 7.75. Found: C, 52.59; H, 7.96; N, 7.63.

Boc-Hcy-Ala-NH_2 (4). Reduction of **2** (320 mg, 0.53 mmol) was performed under argon with a 1.0 M solution of Et_3P in THF (2.5 mL, 2.5 mmol) in MeOH/THF/H_2O (9:9:1, 15 mL). After 3 h, the solvents were evaporated, and the residue was dissolved in EtOAc, washed with water, and dried with $MgSO_4$. Evaporation of the solvent yielded the crude product, which was purified by flash column chromatography (CH_2Cl_2/MeOH, 15:1 → 8:1) to give **4** (323 mg, Quant.) as a colorless viscous oil, which was used immediately for the next reaction: TLC R_f 0.55 (CH_2Cl_2/MeOH, 5:1).

Boc-Hcy-Ile-OMe (5). Disulfide **3** (380 mg, 0.52 mmol) was treated under argon with a 1.0 M solution of Et_3P in THF (2.4 mL, 2.4 mmol) in MeOH/THF/H_2O (9:9:1, 15 mL). After 3 h, the solvents were evaporated, and the residue was dissolved in EtOAc, washed with water, and dried with $MgSO_4$. Evaporation of the solvent yielded the crude product, which was purified by flash column chromatography (petroleum ether/EtOAc, 3:2) to give **5** (376 mg, Quant.) as a white solid, which was used immediately for the next reaction: TLC R_f 0.56 (petroleum ether/EtOAc, 1.3:1).

***N*-*tert*-Butoxycarbonyl-*S*-carbamoylmethyl-L-homocysteinyl-L-alanine Amide (6).** To a stirred solution of Boc-Hcy-Ala-NH_2 **4** (62 mg, 0.20 mmol) in MeOH (3 mL) were added Et_3N (0.2 mL) and iodacetamide (74 mg, 0.4 mmol) with stirring at room temperature for 12 h, then the solvent was evaporated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 3:1) to give **6** (62 mg, 85%): TLC R_f 0.10 (CH_2Cl_2/MeOH, 3:1); $[\alpha]_D$ −27.3 (*c* 0.9 MeOH); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 7.80 (d-like, 1H), 4.40 (m, 1H), 4.28 (s, 4H), 4.18 (dd, J = 7.8, 5.2 Hz, 1H), 3.36 (m, 1H), 3.20 (m, 2H), 2.65 (t, J = 7.3 Hz, 2H), 2.00 (m, 2H), 1.45 (s, 9H), 1.39 (d, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$/CD_3OD) δ 175.4, 173.2, 172.0, 156.2,

80.1, 53.4, 34.9, 31.7, 28.6, 27.9, 17.3; MALDI-MS *m/z* 384.9 [M + Na^+], 401.0 [M + K^+]. Anal. Calcd for $C_{14}H_{26}N_4O_5S$ (362.45): C, 46.39; H, 7.23. Found: C, 46.09; H, 7.30.

***N-tert*-Butoxycarbonyl-*S*-(2,4-di-nitro-benzenesulfenyl)-L-homocysteinyl-L-alanine Amide (7).** To a solution of Boc-Hcy-Ala-NH_2 **4** (80 mg, 0.26 mmol) in $CHCl_3$ (5 mL) containing 2,4-di-nitro-benzenesulfenyl chloride (123 mg, 0.52 mmol) was added Et_3N (0.15 mL) at 0 °C. The reaction solution was removed from the cooling bath after 1 h and the reaction was monitored by TLC. After another 4 h, the solution was concentrated under reduced pressure, diluted with EtOAc, washed with brine, and dried with $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 15:1) to afford **7** (96 mg, 73%): TLC R_f 0.48 (CH_2Cl_2/MeOH, 10:1); $[\alpha]_D$ −21.6 (*c* 1.1 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.07 (s, 1H), 8.48 (s, 2H), 6.96 (d, J = 6.5 Hz, 1H), 6.16 (br s, 1H), 5.68 (br s, 1H), 5.21 (br s, 1H), 4.43 (m, 1H), 4.24 (m, 1H), 2.84 (t, J = 7.4 Hz, 2H), 2.20 (m, 1H), 1.99 (m, 1H), 1.40 (s, 9H), 1.36 (d, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 174.0, 170.8, 155.6, 145.9, 145.7, 145.2, 128.8, 127.5, 121.6, 80.9, 53.4, 48.6, 34.7, 32.2, 28.3, 18.2; MALDI-MS *m/z* 526.3 [M + Na^+], 542.3 [M + K^+]. Anal. Calcd for $C_{18}H_{25}N_5O_8S_2 \cdot 3H_2O$ (557.59): C, 38.77; H, 5.60; N, 12.55. Found: C, 39.37; H, 5.09; N, 12.11.

***N-tert*-Butoxycarbonyl-*S*-(*p*-nitro-benzyl)-L-homocysteinyl-L-isoleucine Methyl Ester (8).** Et_3N (0.3 mL) was added to a stirred solution of Boc-Hcy-Ile-OMe **5** (32 mg, 0.088 mmol) in CH_2Cl_2 (3 mL), containing *p*-nitro-benzyl chloride (45 mg, 0.26 mmol) at room temperature. The reaction was stirred overnignt. The solvent was removed and the crude material was purified by flash column chromatography (petroleum ether/EtOAc, 2:1) to give **8** (36 mg, 84%): TLC R_f 0.44 (petroleum ether/EtOAc, 1.3:1); $[\alpha]_D$ +13.3 (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.18 and 7.50 (m, 4H), 6.62 (d, J = 8.5 Hz, 1H), 5.14 (d, J = 8.2 Hz, 1H), 4.55 (dd, J = 8.7, 4.8 Hz, 1H), 4.29 (m, 1H), 3.82 (s, 2H), 3.73 (s, 3H), 2.51 (t, J = 7.5 Hz, 2H), 2.00 (m, 3H), 1.44 (s, 9H), 1.21 (m, 2H), 0.92 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.0, 171.0, 155.4, 147.0, 146.0, 129.7, 123.8, 80.3, 77.2, 56.5, 53.1, 52.1, 37.7, 35.3, 31.5, 28.2, 27.5, 25.0, 15.5, 11.5; MALDI-MS *m/z* 519.6 [M + Na^+]. Anal. Calcd for $C_{23}H_{35}N_3O_7S$ (497.60): C, 55.52; H, 7.09; N, 8.44. Found: C, 55.43; H, 7.11; N, 8.39.

***N-tert*-Butoxycarbonyl-*S*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl]-L-homocysteinyl-L-alanine amide (15).** To a solution of dipeptide **4** (91 mg, 0.30 mmol) in 4 mL of 10% Na_2CO_3 was added a solution of bromide **14** (384 mg, 0.55 mmol) in EtOAc (4 mL). To this mixture was added tetrabutylammonium hydrogen sulfate TBAHS (403 mg, 1.2 mmol). The two-phase reaction mixture was vigorously stirred at room temperature and the progress of the reaction was monitored by TLC. After 8 h, EtOAc was added and the organic phase was washed sequentially with saturated aqueous $NaHCO_3$ and brine. The organic extracts were then dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product, which was purified by flash column chromatography (CH_2Cl_2/MeOH, 15:1 → 10:1) to afford **15** (216 mg, 79%) as a white amorphous solid: TLC R_f 0.28 (petroleum ether/EtOAc, 1:5); $[\alpha]_D$ −23.9 (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.02 (d, J = 7.6 Hz, 1H), 6.50 (br s, 1H), 5.60 (br s, 1H), 5.41 (d, J = 6.9 Hz, 1H), 5.32 (dd, J = 3.3, 0.8 Hz, 1H), 5.13 (t, J = 9.3 Hz, 1H), 5.08 (dd, J = 10.4, 7.8 Hz, 1H), 4.95 (dd, J = 10.4, 3.4 Hz, 1H), 4.91 (t, J = 9.6 Hz, 1H), 4.49 (m, 4H), 4.21 (m, 2H), 4.10 (m, 2H), 3.90 (t, J = 6.8 Hz, 1H), 3.83 (t, J = 9.4 Hz, 1H), 3.61 (m, 1H), 2.64 (m, 2H), 2.12 (s, 3H), 2.11 (s, 3H), 2.03 (s, 3H), 2.02 (s, 6H), 2.01 (s, 3H), 1.94 (s, 3H), 1.41 (s, 9H), 1.38 (d, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 174.2, 171.5, 170.8, 170.4, 170.1, 170.09, 170.02, 169.7, 169.6, 169.0, 155.8, 100.9, 83.5, 80.4, 77.2, 75.9, 73.8, 71.0, 70.7, 69.9, 69.2, 66.7, 61.8, 60.9, 53.9, 48.5, 33.0, 28.3, 26.2, 21.0, 20.8, 20.7, 20.6, 20.5, 17.2; MALDI-MS *m/z* 946.2 [M + Na^+], 962.5 [M + K^+]. Anal. Calcd for $C_{38}H_{57}N_3O_{21}S \cdot 2H_2O$ (959.96): C, 47.55; H, 6.19; N, 4.38. Found: C, 47.90; H, 6.49; N, 4.15.

***N-tert*-Butoxycarbonyl-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-homocysteinyl-L-isoleucine Methyl Ester (18).** To a solution of dipeptide **5** (82 mg, 0.23 mmol) in 3.2 mL of 10% Na_2CO_3 was added a solution of bromide **11** (176 mg, 0.43 mmol) in EtOAc (3.2 mL). TBAHS (311 mg, 0.92 mmol) was then added to the above mixture. The reaction mixture was vigorously stirred at room temperature for 8 h, after which time EtOAc (30 mL) was added. The mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ and concentrated. Flash column chromatography (petroleum ether/EtOAc, 1:1) of the residue gave **18** (134 mg, 86%) as a white amorphous solid: TLC R_f 0.11 (petroleum ether/EtOAc, 1.5:1); $[\alpha]_D$ −11.9 (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.68 (d, J = 8.4 Hz, 1H), 5.20 (t, J = 9.2 Hz, 1H), 5.17 (d, J = 7.8 Hz, 1H), 5.07 (t, J = 9.8 Hz, 1H), 5.00 (t, J = 9.3 Hz, 1H), 4.53 (d, J = 9.9 Hz, 1H), 4.50 (d, J = 8.6 Hz, 1H), 4.25 (m, 2H), 4.11 (dd, J = 12.4, 2.2 Hz, 1H), 3.73 (m, 1H), 3.71 (s, 3H), 2.73 (m, 2H), 2.06, 2.03, 1.99, 1.97 (4s, 12H), 2.00 (m, 3H), 1.41 (s, 9H), 1.40 (m, 1H), 1.19 (m, 1H), 0.90 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.1, 171.2, 170.7, 170.1, 169.40, 169.36, 155.6, 83.8, 80.3, 77.2, 76.0, 73.9, 69.9, 68.3, 62.0, 56.5, 53.3, 52.1, 37.6, 32.6, 28.3, 26.6, 25.0, 20.7, 20.5, 15.5, 11.5; MALDI-MS *m/z* 715.4 [M + Na^+], 731.6 [M + K^+]. Anal. Calcd for $C_{30}H_{48}N_2O_{14}S$ (692.77): C, 52.01; H, 6.98; N, 4.04. Found: C, 52.46; H, 7.11; N, 3.71.

***N-tert*-Butoxycarbonyl-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-homocysteinyl-L-isoleucine Methyl Ester (19).** To a solution of dipeptide **5** (86 mg, 0.24 mmol) in 3.3 mL of 10% Na_2CO_3 was added a solution of bromide **16** (167 mg, 0.41 mmol) in EtOAc (3.3 mL). To this mixture was added TBAHS (319 mg, 0.94 mmol). After being stirred vigorously for 8 h at room temperature, the mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc, 1:1) to give **19** (143 mg, 87%) as a white amorphous solid: TLC R_f 0.14 (petroleum ether/EtOAc, 1.5:1); $[\alpha]_D$ −4.0 (*c* 1.1 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.64 (d, J = 8.5 Hz, 1H), 5.40 (dd, J = 3.3, 0.8 Hz, 1H), 5.20 (t, J = 9.9 Hz, 1H), 5.12 (d, J = 8.2 Hz, 1H), 5.03 (dd, J = 10.0, 3.3 Hz, 1H), 4.53 (d, J = 9.0 Hz, 1H), 4.51 (d, J = 8.6 Hz, 1H), 4.24 (m, 1H), 4.12 (m, 2H), 3.93 (t, J = 6.6 Hz, 1H), 3.69 (s, 3H), 2.76 (m, 2H), 2.13, 2.04, 2.01, 1.95 (4s, 12H), 2.00 (m, 3H), 1.41 (s, 9H), 1.38 (m, 1H), 1.18 (m, 1H), 0.89 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.0, 171.2, 170.3, 170.2, 170.0, 169.6, 155.6, 84.3, 80.3, 77.2, 74.5, 71.8, 67.3, 67.2, 61.5, 56.5, 53.2, 52.1, 37.7, 32.3, 28.2, 26.9, 25.0, 20.8, 20.6, 15.5, 11.5; MALDI-MS *m/z* 715.6 [M + Na^+]. Anal. Calcd for $C_{30}H_{48}N_2O_{14}S$ (692.77): C, 52.01; H, 6.98; N, 4.04. Found: C, 52.53; H, 7.00; N, 3.91.

***N-tert*-Butoxycarbonyl-*S*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl]-L-homocysteinyl-L-isoleucine Methyl Ester (20).** To a solution of dipeptide **5** (80 mg, 0.22 mmol) in 3.2 mL of 10% Na_2CO_3 was added a solution of bromide **14** (320 mg, 0.46 mmol) in EtOAc (3.2 mL). To this mixture was added TBAHS (310 mg, 0.91 mmol) with vigorous stirring at room temperature until TLC indicated completion of the reaction (8 h), then the solution was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (toluene/acetone, 2:1 → 1:1) to give **20** (179 mg, 83%) as a white amorphous solid: TLC R_f 0.21 (petroleum ether/EtOAc, 1:1); $[\alpha]_D$ −10.3 (*c* 1.3 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.72 (d, J = 8.5 Hz, 1H), 5.32 (dd, J = 3.3, 0.9 Hz, 1H), 5.19 (t, J = 9.1 Hz, 1H), 5.12 (t, J = 7.8 Hz, 1H), 5.07 (d, J = 7.8 Hz, 1H), 4.93 (d, J = 10.4 Hz, 1H), 4.90 (t, J = 9.7 Hz, 1H), 4.50 (m, 4H), 4.25 (m, 1H), 4.09 (m, 3H), 3.84 (t, J = 6.8 Hz, 1H), 3.75 (d, J = 9.2 Hz, 1H), 3.71 (s, 3H), 3.62 (m, 1H), 2.70 (m, 2H), 2.13, 2.10, 2.04, 2.03, 2.02, 2.01, 1.94 (7s, 21H), 2.00

(m, 3H), 1.41 (s, 9H), 1.20 (m, 2H), 0.90 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.1, 171.2, 170.4, 170.3, 170.06, 169.97, 169.64, 169.58, 169.0, 155.6, 101.1, 83.7, 80.2, 77.2, 76.8, 76.1, 73.7, 71.0, 70.7, 70.4, 69.1, 66.6, 62.0, 60.8, 56.5, 53.2, 52.0, 37.6, 32.6, 29.6, 28.2, 25.0, 20.8, 20.72, 20.67, 20.55, 20.4, 15.4, 11.5; MALDI-MS *m/z* 1004.5 [M + Na^+]. Anal. Calcd for $C_{42}H_{64}N_2O_{22}S\cdot0.5C_7H_8$ (1027.09): C, 53.21; H, 6.67; N, 2.73. Found: C, 53.43; H, 6.90; N, 2.67.

***N-tert*-Butoxycarbonyl-*S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-L-homocysteinyl-L-isoleucine Methyl Ester (21).** To a solution of dipeptide **5** (86 mg, 0.24 mmol) in 3.3 mL of 10% Na_2CO_3 was added a solution of freshly prepared *β*-acetochloroneuraminic acid **17** (160 mg, 0.31 mmol) in EtOAc (3.3 mL). To this mixture was added TBAHS (338 mg, 0.99 mmol). The mixture was stirred vigorously at room temperature for 8 h and next diluted with EtOAc, washed successively with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and evaporated to a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 1:2 → 1:4) to afford **21** (150 mg, 76%) as a white amorphous solid: TLC R_f 0.10 (petroleum ether/EtOAc, 1:1.5); $[\alpha]_D$ +11.8 (c 1.3 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.03 (d, J = 8.4 Hz, 1H), 5.84 (d, J = 8.1 Hz, 1H), 5.40 (m, 1H), 5.31 (dd, J = 9.6, 1.4 Hz, 1H), 5.18 (d, J = 9.9 Hz, 1H), 4.85 (m, 1H), 4.53 (dd, J = 8.6, 5.3 Hz, 1H), 4.24 (dd, J = 12.2, 1.6 Hz, 1H), 4.02 (m, 2H), 3.80 (m, 1H), 3.77 (s, 3H), 3.68 (s, 3H), 2.66 (m, 3H), 2.20, 2.11, 2.03, 2.00, 1.85 (each s, each 3H), 1.42 (s, 9H), 0.87 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.0, 171.3, 171.0, 170.9, 170.2, 170.1, 168.7, 155.7, 83.2, 79.8, 77.2, 73.8, 69.5, 68.1, 66.8, 62.1, 56.5, 54.3, 53.0, 51.9, 49.5, 38.0, 37.7, 33.4, 29.7, 28.3, 25.4, 25.1, 23.2, 21.3, 20.8, 20.7, 15.4, 11.5; MALDI-MS *m/z* 859.1 [M + Na^+], 875.4 [M + K^+]. Anal. Calcd for $C_{36}H_{57}N_3O_{17}S$ (835.91): C, 51.73; H, 6.87; N, 5.03. Found: C, 51.32; H, 6.90; N, 4.83.

Boc-Cys-Ile-OMe (22). 22 was prepared from commercially available *N*-Boc protected cystine in two steps as described for Boc-Hcy-Ile-OMe **5**, and it was used immediately after purification.

***N-tert*-Butoxycarbonyl-*S*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-cysteinyl-L-isoleucine Methyl Ester (23).** To a solution of dipeptide **22** (51 mg, 0.15 mmol) in 2 mL of 10% Na_2CO_3 was added a solution of bromide **11** (112 mg, 0.27 mmol) in EtOAc (2 mL). TBAHS (190 mg, 0.56 mmol) was then added to the above mixture. The reaction mixture was vigorously stirred at room temperature until TLC indicated complete consumption of the thiol, then diluted with EtOAc. The mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ and concentrated. Flash column chromatography (petroleum ether/EtOAc, 1:1) of the residue gave **23** (93 mg, 94%) as a white amorphous solid: TLC R_f 0.20 (petroleum ether/EtOAc, 1.5:1); $[\alpha]_D$ −13.5 (c 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.10 (d, J = 7.9 Hz, 1H), 5.65 (d, J = 7.1 Hz, 1H), 5.23 (t, J = 9.3 Hz, 1H), 5.05 (t, J = 10.0 Hz, 1H), 5.01 (t, J = 9.3 Hz, 1H), 4.63 (d, J = 10.0 Hz, 1H), 4.50 (dd, J = 8.4, 5.1 Hz, 1H), 4.43 (m, 1H), 4.26 (dd, J = 12.3, 2.3 Hz, 1H), 4.13 (dd, J = 12.3, 6.2 Hz, 1H), 3.86 (m, 1H), 3.72 (s, 3H), 3.06 (dd, J = 14.3, 5.4 Hz, 1H), 2.85 (dd, J = 14.3, 7.9 Hz, 1H), 2.04, 2.03, 2.02, 1.99 (each s, each 3H), 1.90 (m, 1H), 1.43 (s, 9H), 1.20 (m, 2H), 0.91 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 171.6, 170.5, 170.1, 170.0, 169.5, 155.3, 84.7, 80.3, 77.2, 76.2, 73.6, 69.6, 68.4, 62.3, 56.8, 53.9, 52.0, 37.8, 33.5, 28.3, 25.1, 20.6, 20.5, 15.4, 11.5; MALDI-MS *m/z* 701.6 [M + Na^+]. Anal. Calcd for $C_{29}H_{46}N_2O_{14}S$ (678.75): C, 51.32; H, 6.83; N, 4.12. Found: C, 51.11; H, 6.87; N, 3.85.

***N-tert*-Butoxycarbonyl-*S*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-L-cysteinyl-L-isoleucine Methyl Ester (24).** To a solution of dipeptide **22** (70 mg, 0.20 mmol) in 3 mL of 10% Na_2CO_3 was added a solution of bromide **16** (153 mg, 0.37 mmol) in EtOAc (3 mL) followed by addition of TBAHS (260 mg, 0.77 mmol). The resulting mixture was vigorously stirred at room temperature until TLC indicated complete consumption of the thiol, then diluted with EtOAc. The organic phases were successively washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$, then concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc, 1:1) to give **24** (125 mg, 92%) as a white amorphous solid: TLC R_f 0.21 (petroleum ether/EtOAc, 1.5:1); $[\alpha]_D$ −12.5 (c 1.7 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.12 (d, J = 7.9 Hz, 1H), 5.72 (d, J = 7.2 Hz, 1H), 5.43 (d, J = 3.0 Hz, 1H), 5.22 (t, J = 10.0 Hz, 1H), 5.03 (dd, J = 10.0, 3.3 Hz, 1H), 4.56 (d, J = 9.9 Hz, 1H), 4.50 (dd, J = 8.4, 5.0 Hz, 1H), 4.39 (m, 1H), 4.27−4.00 (m, 3H), 3.71 (s, 3H), 3.09 (dd, J = 14.4, 5.6 Hz, 1H), 2.88 (dd, J = 14.4, 8.0 Hz, 1H), 2.15, 2.04, 2.01, 1.96 (each s, each 3H), 1.89 (m, 1H), 1.43 (s, 9H), 1.20 (m, 2H), 0.90 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 171.6, 170.3, 170.2, 170.1, 169.9, 169.7, 155.6, 84.6, 80.3, 77.2, 75.1, 71.7, 67.3, 66.8, 61.7, 56.7, 53.8, 52.0, 37.8, 32.8, 29.7, 28.3, 25.1, 20.7, 20.5, 15.4, 11.5; MALDI-MS *m/z* 701.5 [M + Na^+], 717.9 [M + K^+]. Anal. Calcd for $C_{29}H_{46}N_2O_{14}S$ (678.75): C, 51.32; H, 6.83; N, 4.13. Found: C, 51.36; H, 7.08; N, 4.05.

***N-tert*-Butoxycarbonyl-*S*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-*β*-D-glucopyranosyl)-L-homocysteinyl-L-isoleucine Methyl Ester (26).** To a solution of dipeptide **5** (84 mg, 0.23 mmol) in 3.2 mL of 10% Na_2CO_3 was added a solution of bromide **25** (168 mg, 0.43 mmol) in EtOAc (3.2 mL) followed by addition of TBAHS (316 mg, 0.93 mmol). The mixture was vigorously stirred for 8 h at room temperature, then diluted with EtOAc, washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (petroleum ether/EtOAc, 1:1) to afford **26** (15 mg, 10%), which was redissolved in a small amount of dioxane and lyophilized to give **26** as a white solid: TLC R_f 0.30 (petroleum ether/EtOAc, 1:1); $[\alpha]_D$ +39.9 (c 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.85 (d, J = 7.9 Hz, 1H), 5.58 (d, J = 8.2 Hz, 1H), 5.15 (d, J = 10.6 Hz, 1H), 5.09 (dd, J = 10.2, 8.3 Hz, 1H), 4.63 (d, J = 6.9 Hz, 1H), 4.51 (dd, J = 8.5, 5.0 Hz, 1H), 4.26 (t, J = 7.8 Hz, 1H), 4.18 (dd, J = 12.2, 4.4 Hz, 1H), 4.03 (dd, J = 12.2, 2.2 Hz, 1H), 3.96 (m, 1H), 3.71 (s, 4H), 2.83 (m, 2H), 2.11, 2.03, 1.94 (4s, 12H), 1.90 (m, 3H), 1.40 (s, 9H), 1.20 (m, 2H), 0.88 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.3, 171.3, 170.9, 170.6, 169.3, 155.4, 80.2, 77.2, 74.8, 74.7, 71.2, 69.1, 62.0, 56.7, 52.7, 52.1, 45.0, 37.4, 32.3, 28.3, 27.7, 25.1, 20.9, 20.7, 20.5, 15.6, 11.5. Anal. Calcd for $C_{28}H_{45}N_5O_{12}S$ (675.75): C, 49.77; H, 6.71; N, 10.36. Found: C, 49.28; H, 7.03; N, 10.05.

***N-tert*-Butoxycarbonyl-*S*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-*β*-D-glucopyranosyl)-L-homocysteinyl-L-alanine Amide (27).** To a solution of dipeptide **4** (70 mg, 0.23 mmol) in 3.2 mL of 10% Na_2CO_3 was added a solution of bromide **25** (193 mg, 0.49 mmol) in EtOAc (3.2 mL) followed by addition of TBAHS (316 mg, 0.93 mmol). The mixture was vigorously stirred for 8 h at room temperature, then diluted with EtOAc, washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 40:1 → 25:1) to afford **27** (43 mg, 30%), which was lyophilized with dioxane to yield **27** as a white solid: TLC R_f 0.21 (EtOAc); $[\alpha]_D$ −24.0 (c 1.1 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.84 (d, J = 7.5 Hz, 1H), 6.25 (br s, 1H), 5.47 (br s, 1H), 5.31 (d, J = 5.9 Hz, 1H), 5.08 (t, J = 9.4 Hz, 1H), 5.01 (t, J = 9.4 Hz, 1H), 4.45 (m, 2H), 4.26 (m, 1H), 4.20 (d, J = 3.4 Hz, 2H), 3.71 (m, 1H), 3.51 (t, J = 9.6 Hz, 1H), 2.81 (t, J = 7.3 Hz, 2H), 2.10 (m, 2H), 2.08, 2.07, 2.01 (3s, 9H), 1.43 (s, 9H), 1.39 (d, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 174.0, 171.4, 170.8, 169.9, 169.6, 156.4, 84.8, 80.7, 75.9, 74.5, 68.2, 63.6, 61.9, 53.8, 48.6, 32.9, 28.3, 27.6, 20.8, 20.64, 20.56, 17.6; MALDI-MS *m/z* 641.5 [M + Na^+], 657.6 [M + K^+]. Anal. Calcd for $C_{24}H_{38}N_6O_{11}S\cdot C_4H_8O_2$ (706.77): C, 47.58; H, 6.56; N, 11.89. Found: C, 47.66; H, 6.77; N, 11.92.

***N-tert*-Butoxycarbonyl-*S*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethyloxycarbonylamino)-*β*-D-glucopyranosyl]-L-cysteinyl-L-isoleucine Methyl Ester (29).** To a

solution of dipeptide **5** (83 mg, 0.23 mmol) in 3.2 mL of 10% Na_2CO_3 was added a solution of bromide **28** (168 mg, 0.43 mmol) in EtOAc (3.2 mL). After TBAHS (314 mg, 0.92 mmol) was added, the mixture was vigorously stirred at room temperature for 8 h, at which time TLC indicated no trace of **5**. The mixture was diluted with EtOAc, washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to give a crude product that was purified by flash column chromatography (petroleum ether/EtOAc, 2:1 → 1:1). Compound **29** was obtained as a white solid (179 mg, 95%): TLC R_f 0.17 (petroleum ether/EtOAc, 1.3:1); $[\alpha]_D$ −18.2 (1.1 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.77 (d, J = 8.3 Hz, 1H), 5.91 (d, J = 8.5 Hz, 1H), 5.26 (t, J = 10.1 Hz, 1H), 5.07 (m, 2H), 4.90 (d, J = 12.1 Hz, 1H), 4.66 (d, J = 12.1 Hz, 2H), 4.54 (m, 1H), 4.37 (m, 1H), 4.23 (dd, J = 12.6, 5.0 Hz, 1H), 4.10 (d, J = 12.6 Hz, 1H), 3.76 (s, 3H), 3.72 (m, 2H), 2.80 (m, 2H), 2.06, 2.00, 1.99 (3s, 9H), 2.00 (m, 3H), 1.41 (s, 9H), 1.30 (m. 2H), 0.90 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.8, 171.4, 170.7, 170.4, 169.4, 155.5, 154.3, 95.5, 85.4, 80.3, 77.2, 76.0, 74.5, 73.3, 68.6, 62.2, 56.7, 55.3, 52.7, 52.4, 37.5, 31.9, 28.3, 27.6, 24.9, 20.7, 20.6, 15.6, 11.5; MALDI-MS m/z 846.5 [M + Na^+], 862.5 [M + K^+]. Anal. Calcd for $C_{31}H_{48}Cl_3N_3O_{14}S$ (825.14): C, 45.12; H, 5.86; N, 5.09. Found: C, 45.24; H, 6.70; N, 5.12.

***N-tert*-Butoxycarbonyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-L-homocysteinyl-L-isoleucine Methyl Ester (30).** Glycoside **29** (40 mg, 0.048 mmol) was dissolved in Ac_2O (1.4 mL), freshly activated Zinc dust (31 mg) was added, and the suspension was stirred for 5 h. Then the mixture was filtered through Celite and concentrated under vacuo to give a crude product, which was purified by flash column chromatography (CH_2Cl_2/MeOH, 30:1) to afford **30** (20 mg, 60%) as a white amorphous solid: TLC R_f 0.15 (petroleum ether/EtOAc, 1:1); $[\alpha]_D$ −23.5 (c 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.97 (d, J = 8.0 Hz, 1H), 6.16 (d, J = 8.8 Hz, 1H), 5.15 (m, 4H), 4.70 (d, J = 10.4 Hz, 1H), 4.52 (dd, J = 8.4, 4.9 Hz, 1H), 4.22 (dd, J = 12.4, 4.8 Hz, 1H), 4.10 (dd, J = 12.4, 2.3 Hz, 1H), 3.74 (s, 3H), 3.70 (m, 2H), 2.77 (t, J = 6.6 Hz, 2H), 2.06, 2.00, 1.99, 1.96 (4s, 12H), 2.00 (m, 3H), 1.41 (s, 9H), 1.40 (m, 1H), 1.20 (m, 1H), 0.90 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.6, 171.6, 170.8, 170.7, 170.4, 169.3, 155.8, 84.5, 76.0, 73.8, 68.6, 62.2, 56.7, 53.6, 52.2, 37.6, 31.8, 28.3, 26.9, 25.0, 23.2, 20.7, 20.64, 20.58, 15.6, 11.5; MALDI-MS m/z 714.5 [M + Na^+], 730.6 [M + K^+]. Anal. Calcd for $C_{30}H_{49}N_3O_{13}S$ (691.79): C, 52.09; H, 7.14; N, 6.07. Found: C, 51.73; H, 7.60; N, 5.63.

Boc-Cys-Ala-Ala-OMe (31). To a stirred solution of (Boc-Cys-OH)$_2$ (323 mg, 0.73 mmol) and H-Ala-Ala-OMe·HCl (358 mg, 1.7 mmol) in dry DMF (5 mL) were added PyBOP (890 mg, 1.7 mmol) and DIPEA (0.63 mL, 3.6 mmol). The mixture was stirred at room temperature for 5 h, then diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 10:1) to give a white solid, the disulfide of **31** (527 mg, 96%): TLC R_f 0.25 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D$ +26.3 (c 1.0 MeOH); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 7.78 (d, J = 7.4 Hz, 2H), 6.86 (d, J = 7.8 Hz, 2H), 5.50 (d, J = 8.9 Hz, 2H), 4.70 (m, 2H), 4.52 (q-like, J = 7.1 Hz, 4H), 3.71 (s, 6H), 1.79 (m, 4H), 1.43 (s, 18 H), 1.38 (m, 12H); MALDI-MS m/z 776.3 [M + Na^+].

The disulfide of **31** was reduced by Et_3P as described for **2** to give the title compound **31**, which was immediately used in the next step.

Boc-Cys-Val-Pro-O^{t}Bu (32). To a stirred solution of (Boc-Cys-OH)$_2$ (211 mg, 0.48 mmol) and H-Val-Pro-O^{t}Bu·HCl (299 mg, 0.97 mmol) in dry DMF (3 mL) were added PyBOP (525 mg, 1.0 mmol) and DIPEA (0.41 mL, 2.3 mmol). The mixture was stirred at room temperature for 5 h, then diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (petroleum ether/EtOAc, 1:1 → 1:1.5) to give the disulfide of **32** (426 mg, 94%): TLC R_f 0.23 (petroleum ether/EtOAc, 1:1); $[\alpha]_D$ −90.1 (c 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.10 (d, J = 8.2 Hz, 2H), 5.53 (d, J = 7.5 Hz, 2H), 4.55 (t, J = 8.0 Hz, 2H), 4.42 (m, 4H), 3.89 (m, 2H), 3.63 (m, 2H), 3.22 (dd, J = 13.1, 4.8 Hz, 2H), 2.85 (dd, J = 13.4, 4.1 Hz, 2H), 2.42−1.82 (m, 10H), 1.39 (s, 36H), 1.00 (d, J = 6.7 Hz, 6H), 0.93 (d, J = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.6, 170.0, 154.9, 81.2, 79.8, 59.8, 56.3, 53.5, 47.5, 43.5, 31.1, 29.2, 28.3, 27.9, 24.9, 19.2, 18.2; MALDI-MS m/z 967.9 [M + Na^+], 984.0 [M + K^+]. Anal. Calcd for $C_{44}H_{76}N_6O_{12}S_2$·H_2O (963.25): C, 54.86; H, 7.95; N, 8.72. Found: C, 54.64; H, 7.81; N, 8.52.

The disulfide of **32** was reduced by Et_3P as described for **2** to give the title compound **32**, which was immediately used in the next step.

***N-tert*-Butoxycarbonyl-*S*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-cysteinyl-L-alanyl-L-alanine Methyl Ester (33).** To a solution of tripeptide **31** (22 mg, 0.058 mmol) and bromide **11** (41 mg, 0.10 mmol) in 2 mL of DMF was added 1.8 mL of 5% Na_2CO_3. The mixture was stirred at room temperature for 5 h, then diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, concentrated in vacuo to give a residue that was purified by flash column chromatography (toluene/acetone, 2:1) to afford the title compound **33** (28 mg, 68%) as a white amorphous solid: TLC R_f 0.31 (toluene/acetone, 1:1); $[\alpha]_D$ −15.6 (c 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.05 (d, J = 7.7 Hz, 1H), 6.92 (d, J = 7.0 Hz, 1H), 5.60 (d, J = 7.5 Hz, 1H), 5.23 (t, J = 9.3 Hz, 1H), 5.01 (m, 2H), 4.67 (d, J = 10.1 Hz, 1H), 4.51 (m, 3H), 4.35 (dd, J = 12.3, 1.8 Hz, 1H), 4.12 (dd, J = 12.3, 6.4 Hz, 1H), 3.88 (m, 1H), 3.71 (s, 3H), 3.05 (dd, J = 14.3, 5.0 Hz, 1H), 2.88 (dd, J = 14.3, 9.1 Hz, 1H), 2.04, 2.03, 2.02, 1.98 (each s, each 3H), 1.41 (s, 9H), 1.37 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 173.1, 171.1, 170.5, 169.9, 169.8, 169.4, 169.3, 155.1, 85.4, 80.4, 77.2, 73.5, 69.6, 68.3, 62.2, 54.2, 52.4, 49.3, 48.1, 34.6, 28.3, 20.6, 20.5, 18.3, 17.7; MALDI-MS m/z 731.0 [M + Na^+]. Anal. Calcd for $C_{29}H_{45}N_3O_{15}S$ (707.75): C, 49.21; H, 6.41; N, 5.94. Found: C, 49.54; H, 6.79; N, 5.41.

***N-tert*-Butoxycarbonyl-*S*-[2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl]-L-homocysteinyl-L-alanyl-L-alanine Methyl Ester (34).** To a solution of tripeptide **31** (62 mg, 0.16 mmol) and bromide **14** (195 mg, 0.28 mmol) in 5 mL of DMF was added 4.5 mL of 5% Na_2CO_3. The resulting suspension was stirred at room temperature for 5 h, then diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude compound was purified by flash column chromatography (petroleum ether/EtOAc, 1:1 → 1:3) to give the title compound **34** (91 mg, 56%) as a white amorphous solid: TLC R_f 0.15 (petroleum ether/EtOAc, 1:2); $[\alpha]_D$ −9.7 (c 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.09 (d, J = 7.8 Hz, 1H), 6.87 (d, J = 7.0 Hz, 1H), 5.50 (d, J = 8.0 Hz, 1H), 5.33 (d, J = 2.7 Hz, 1H), 5.19 (t, J = 9.0 Hz, 1H), 5.10 (dd, J = 10.4, 7.8 Hz, 1H), 4.95 (dd, J = 10.4, 3.4 Hz, 1H), 4.87 (t, J = 9.7 Hz, 1H), 4.69 (AB peak, J = 11.5, 10.2 Hz, 2H), 4.54−4.38 (m, 4H), 4.08 (m, 3H), 3.86 (t, J = 6.9 Hz, 1H), 3.76 (d, J = 5.5 Hz, 2H), 3.71 (s, 3H), 3.00 (dd, J = 14.2, 5.0 Hz, 1H), 2.85 (dd, J = 14.2, 9.0 Hz, 1H), 2.13 (s, 3H), 2.07 (s, 3H), 2.04 (s, 6H), 2.01 (s, 6H), 1.94 (s, 3H), 1.41 (s, 9H), 1.38 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 173.0, 171.1, 170.5, 170.3, 170.1, 170.0, 169.9, 169.6, 169.5, 169.1, 154.9, 101.1, 85.7, 80.3, 77.3, 77.2, 76.0, 73.5, 71.0, 70.8, 70.0, 69.1, 66.6, 62.0, 60.8, 54.4, 52.4, 49.1, 48.2, 35.1, 28.3, 20.7, 20.6, 20.5, 18.3, 17.5; MALDI-MS m/z 1019.3 [M + Na^+]. Anal. Calcd for $C_{41}H_{61}N_3O_{23}S$ (996.00): C, 49.44; H, 6.17; N, 4.22. Found: C, 49.39; H, 6.37; N, 3.95.

***N-tert*-Butoxycarbonyl-*S*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-cysteinyl-L-valinyl-L-proline *tert*-Butyl Ester (35).** To a solution of tripeptide **32** (62 mg, 0.13 mmol) and bromide **11** (91 mg, 0.22 mmol) in 4 mL of DMF was added 3.6 mL of 5% Na_2CO_3. The resulting suspension was stirred at room temperature for 5 h, then diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give a residue that was purified by flash

column chromatography (petroleum ether/EtOAc, 1:1) to give the title compound **35** (54 mg, 52%) as a white amorphous solid: TLC R_f 0.13 (petroleum ether/EtOAc, 1.3:1); $[\alpha]_D$ −50.3 (*c* 1.2 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.10 (d, J = 8.6 Hz, 1H), 5.66 (d, J = 7.0 Hz, 1H), 5.21 (t, J = 9.2 Hz, 1H), 5.05 (t, J = 9.8 Hz, 1H), 4.99 (t, J = 9.3 Hz, 1H), 4.54 (m, 2H), 4.38 (m, 2H), 4.21 (m, 2H), 3.72 (m, 3H), 3.04 (dd, J = 14.3, 5.9 Hz, 1H), 2.92 (dd, J = 14.3, 6.5 Hz, 1H), 2.07, 2.03, 2.01, 1.98 (4s, 12H), 2.00 (m, 5H), 1.43 (s, 18H), 1.00 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 171.2, 170.6, 170.1, 170.0, 169.5, 169.4, 169.3, 155.3, 83.8, 81.2, 80.4, 77.2, 76.2, 73.7, 69.4, 68.3, 62.1, 59.6, 55.7, 54.2, 47.2, 32.8, 31.4, 29.6, 29.1, 28.2, 27.9, 24.8, 20.6, 20.5, 19.4, 17.6; MALDI-MS *m*/*z* 827.1 [M + Na^+], 843.1 [M + K^+]. Anal. Calcd for $C_{36}H_{57}N_3O_{15}S$ (803.92): C, 53.79; H, 7.15; N, 5.23. Found: C, 54.22; H, 7.52; N, 4.92.

(Boc-Hcy-Tyr-NH$_2$)$_2$ (36). To a stirred solution of (Boc-Hcy-OH)$_2$ **1** (106 mg, 0.23 mmol) and H-Tyr-NH_2·HCl (108 mg, 0.5 mmol) in dry DMF (2 mL) were added PyBOP (259 mg, 0.5 mmol) and DIPEA (0.25 mL, 1.43 mmol). The mixture was stirred at room temperature for 5 h, then diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (EtOAc/MeOH, 15:1) to give the title compound **36** (176 mg, 98%) as an off-white solid: TLC R_f 0.55 (EtOAc/MeOH, 10:1); $[\alpha]_D$ −11.3 (*c* 1.0 MeOH); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 6.94 (d, J = 8.4 Hz, 4H), 6.65 (d, J = 8.4 Hz, 4H), 4.48 (t, J = 6.4 Hz, 2H), 4.04 (t-like, 2H), 2.90 (m, 4H), 2.44 (t, J = 7.3 Hz, 4H), 1.88 (m, 4H), 1.33 (s, 18H); ^{13}C NMR (CD_3OD) δ 176.0, 174.3, 158.0, 157.4, 131.4, 128.8, 116.4, 81.2, 55.6, 37.8, 35.5, 32.7, 28.8; MALDI-MS *m*/*z* 815.4 [M + Na^+]. Anal. Calcd for $C_{36}H_{52}N_6O_{10}S_2$ (792.97): C, 54.53; H, 6.61; N, 10.60. Found: C, 54.03; H, 7.02; N, 10.32.

Ethylthio *N*-*tert*-Butoxycarbonyl-L-threoninate (39). To a stirred solution of Boc-Thr-OH (219 mg, 1.0 mmol) and EtSH (0.22 mL, 3.0 mmol) in dry DMF (5 mL) were added DCC (206 mg, 1.0 mmol) and HOBt (135 mg, 1.0 mmol). The mixture was stirred at room temperature for 8 h, filtered, and concentrated under reduced pressure to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 4:1) to afford the title compound **39** (231 mg, 88%): TLC R_f 0.45 (petroleum ether/EtOAc, 2:1); $[\alpha]_D$ −70.5 (*c* 1.0 MeOH); ^{1}H NMR ($CDCl_3$) δ 5.40 (d, J = 9.4 Hz, 1H), 4.50−4.40 (m, 1H), 4.25 (d, J = 9.3 Hz, 1H), 2.90 (q, J = 7.3 Hz, 2H), 1.86 (d, J = 4.1 Hz, 1H), 1.48 (s, 9H), 1.26 (t, J = 7.5 Hz, 3H), 1.25 (d, J = 7.0 Hz, 3H); ^{13}C NMR (CD_3OD) δ 201.2, 155.8, 79.7, 66.8, 65.2, 27.9, 22.8, 19.5, 13.9. Anal. Calcd for $C_{11}H_{21}NO_4S$ (263.35): C, 50.17; H, 8.04; N, 5.32. Found: C, 50.03; H, 8.16; N, 5.17.

Boc-Thr-Hcy-Ala-NH$_2$ (40). (Boc-Hcy-Ala-NH_2)$_2$ **2** (90 mg, 0.15 mmol) was dissolved in dry CH_2Cl_2 (3 mL), to which TFA (0.6 mL) was added. The mixture was stirred at room temperature for 3 h, concentrated in vacuo, and azeotroped with toluene to remove excess TFA. The product was dried and dissolved in 0.1 M Tris buffer (2 mL, pH 8.0) containing 6 M guanidinium hydrochloride. DTT (46 mg, 0.3 mmol) was added to the mixture and the reaction was stirred at room temperature for 2 h, after which time a solution of thioester **39** (79 mg, 0.3 mmol) in THF (1 mL) was added to the reaction mixture. When the ligation was deemed complete (TLC monitoring), the reaction was diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude product, which was purified by flash column chromatography to afford title compound **40** (85 mg, 70%), which was immediately used in the next step.

Boc-Thr-Hcy-Tyr-NH$_2$ (41). 41 was prepared from disulfide **36** (118 mg, 0.15 mmol) in three steps following the procedures described for the synthesis of **40** and the product was used immediately in the next reaction after purification.

***N*-*tert*-Butoxycarbonyl-L-threonyl-*S*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-homocysteinyl-L-alanine Amide (42).** Tripeptide **40** (38 mg, 0.093 mmol) and bromide **11** (66 mg, 0.16 mmol) were dissolved in DMF (4 mL). To this solution was added 3.6 mL of 5% Na_2CO_3 and the mixture was stirred at room temperature for 5 h, after which time the mixture was diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give a residue that was purified by flash column chromatography (EtOAc/MeOH, 30:1 → 15:1) to afford **42** (37 mg, 55%) as a white amorphous solid: TLC R_f 0.45 (EtOAc/MeOH, 10:1); $[\alpha]_D$ −31.1 (*c* 1.1 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.37 (d, J = 7.6 Hz, 1H), 7.23 (d, J = 6.9 Hz, 1H), 6.56 (br s, 1H), 5.64 (m, 2H), 5.21 (t, J = 9.3 Hz, 1H), 5.08 (t, J = 9.7 Hz, 1H), 5.02 (t, J = 9.4 Hz, 1H), 4.46 (m, 3H), 4.32 (m, 1H), 4.24 (d, J = 5.0 Hz, 1H), 4.16 (m, 1H), 4.05 (dd, J = 6.6, 2.8 Hz, 1H), 3.75 (m, 1H), 3.62 (d, J = 3.8 Hz, 1H), 2.82 (m, 1H), 2.65 (m, 1H), 2.10 (m, 2H), 2.07, 2.04, 2.02, 1.98 (4s, 12H), 1.43 (s, 9H), 1.39 (d, J = 7.2 Hz, 3H), 1.23 (d, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 175.4, 172.1, 171.1, 171.0, 170.1, 169.8, 169.5, 156.5, 83.3, 80.6, 77.2, 76.0, 73.7, 69.6, 68.4, 67.2, 62.0, 59.8, 53.3, 49.1, 31.8, 28.3, 26.2, 20.8, 20.7, 20.6, 19.2, 17.5; MALDI-MS *m*/*z* 759.8 [M + Na^+], 776.1 [M + K^+]. Anal. Calcd for $C_{30}H_{48}N_4O_{15}S$ (736.79): C, 48.91; H, 6.57; N, 7.60. Found: C, 48.99; H, 7.01; N, 6.89.

***N*-*tert*-Butoxycarbonyl-L-threonyl-*S*-[2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl]-L-homocysteinyl-L-alanine Amide (43).** Product **43** was prepared following the procedure described for the synthesis of **42**. **43** was obtained, after purification by flash column chromatography (EtOAc/MeOH, 20:1 → 12:1) as a white amorphous solid in 51% yield: TLC R_f 0.40 (EtOAc/MeOH, 10:1); $[\alpha]_D$ −26.4 (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.33 (d, J = 7.4 Hz, 1H), 7.24 (d, J = 6.5 Hz, 1H), 6.53 (br s, 1H), 5.63 (br s, 1H), 5.55 (d, J = 7.1 Hz, 1H), 5.33 (d, J = 2.7 Hz, 1H), 5.15 (t, J = 9.2 Hz, 1H), 5.09 (dd, J = 10.4, 7.7 Hz, 1H), 4.95 (dd, J = 10.4, 3.4 Hz, 1H), 4.91 (t, J = 9.6 Hz, 1H), 4.50 (m, 4H), 4.30 (m, 1H), 4.22−4.01 (m, 4H), 3.89 (t, J = 6.7 Hz, 1H), 3.83 (t, J = 9.4 Hz, 1H), 3.60 (m, 2H), 2.72 (m, 1H), 2.58 (m, 1H), 2.10 (m, 2H), 2.13, 2.11, 2.04 (3s, 9H), 2.03 (s, 6H), 2.02 (s, 3H), 1.94 (s, 3H), 1.43 (s, 9H), 1.39 (d, J = 7.2 Hz, 3H), 1.20 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 174.5, 171.9, 171.0, 170.8, 170.4, 170.1, 170.0, 169.9, 169.7, 169.1, 156.4, 101.0, 83.1, 80.7, 77.2, 75.9, 73.8, 71.0, 70.7, 69.8, 69.2, 67.1, 66.7, 61.8, 60.8, 59.5, 53.0, 48.9, 32.2, 28.3, 25.9, 21.0, 20.8, 20.7, 20.6, 20.5, 19.1, 17.3; MALDI-MS *m*/*z* 1049.1 [M + Na^+]. Anal. Calcd for $C_{42}H_{64}N_4O_{23}S$ (1025.04): C, 49.21; H, 6.29; N, 5.47. Found: C, 49.34; H, 6.75; N, 5.24.

***N*-*tert*-Butoxycarbonyl-L-threonyl-*S*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethyloxycarbonylamino)-*β*-D-glucopyranosyl]-L-homocysteinyl-L-alanine Amide (44).** Product **44** was prepared following the procedure described for the synthesis of **42**. **44** was obtained, after purification by flash column chromatography (EtOAc/MeOH, 30:1 → 15:1) and lyophilization with dioxane, as a white amorphous solid in 40% yield: TLC R_f 0.48 (EtOAc/MeOH, 8:1); $[\alpha]_D$ −1.3 (*c* 1.1 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.36 (d, J = 8.8 Hz, 1H), 7.17 (d, J = 6.8 Hz, 1H), 6.60 (br s, 1H), 5.98 (d, J = 8.9 Hz, 1H), 5.79 (br s, 1H), 5.65 (d, J = 7.3 Hz, 1H), 5.31 (br s, 1H), 4.90 (t, J = 11.8 Hz, 1H), 4.75 (AB peak, J = 12.0 Hz, 2H), 4.40 (m, 4H), 4.15 (m, 5H), 2.96 (t, J = 11.3 Hz, 2H), 2.70 (m, 1H), 2.56 (m, 1H), 2.10 (s, 3H), 2.07 (s, 6H), 1.44 (s, 9H), 1.40 (d, J = 7.0 Hz, 3H), 1.23 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 175.4, 172.2, 171.2, 171.0, 170.3, 162.6, 156.5, 154.9, 95.5, 91.3, 80.7, 77.2, 74.7, 68.5, 67.7, 67.3, 62.9, 59.9, 53.0, 52.5, 49.2, 47.6, 31.5, 29.7, 28.3, 20.8, 19.3, 19.0, 17.5; MALDI-MS *m*/*z* 867.8 [M^+]. Anal. Calcd for $C_{31}H_{48}Cl_3N_5O_{15}S$·$C_4H_8O_2$ (957.27): C, 43.92; H, 5.90; N, 7.32. Found: C, 44.48; H, 6.26; N, 7.33.

***N*-*tert*-Butoxycarbonyl-L-threonyl-*S*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-homocysteinyl-L-tyrosine Amide (45).** Tripeptide **41** (27 mg, 0.054 mmol) and bromide **11** (42 mg, 0.10 mmol) were dissolved in DMF (2.5 mL). To this solution was added 2 mL of 5% Na_2CO_3 and the mixture was stirred at room temperature for 5 h, after which time the mixture was diluted with EtOAc and washed successively with

saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give a residue that was purified by flash column chromatography (EtOAc/MeOH, 40:1 → 20:1) to afford **45** (18 mg, 42%) as a white amorphous solid: TLC R_f 0.50 (EtOAc/MeOH, 10:1); $[\alpha]_D$ −45.2 (*c* 0.5 $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.29 (d, J = 7.2 Hz, 1H), 7.01 (d, J = 8.2 Hz, 1H), 6.94 (d, J = 12.5 Hz, 1H), 6.73 (d, J = 8.2 Hz, 2H), 6.51 (br s, 1H), 5.85 (br s, 1H), 5.61 (d, J = 7.2 Hz, 1H), 5.21 (t, J = 9.3 Hz, 1H), 5.07 (t, J = 9.5 Hz, 1H), 4.98 (t, J = 9.5 Hz, 1H), 4.62 (m, 1H), 4.46 (d, J = 10.2 Hz, 1H), 4.41 (m, 1H), 4.20 (m, 5H), 3.69 (m, 2H), 3.10 (m, 1H), 2.91 (m, 1H), 2.69 (m, 1H), 2.50 (m, 1H), 2.05, 2.04, 2.02, 1.99 (each s, each 3H), 1.43 (s, 9H), 1.17 (d, J = 6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.8, 171.7, 171.1, 171.0, 170.3, 169.9, 169.6, 156.4, 155.5, 130.2, 127.7, 115.5, 83.3, 80.5, 75.8, 73.8, 69.8, 68.3, 67.4, 61.9, 58.7, 54.1, 52.9, 36.6, 31.6, 28.2, 26.0, 20.62, 20.56, 20.5, 18.5; MALDI-MS *m/z* 852.2 [M + Na^+], 868.3 [M + K^+]. Anal. Calcd for $C_{36}H_{52}N_4O_{16}S$ (828.88): C, 52.17; H, 6.32; N, 6.76. Found: C, 52.41; H, 6.71; N, 6.73.

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Supporting Information Available: ^{1}H NMR spectra of all new compounds as well as ^{13}C NMR sprectra of selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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