Efficient Synthesis of S-Linked Glycopeptides in Aqueous Solution by a Convergent Strategy

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Abstract: In naturally occurring glycopeptides and glycoproteins the glycan residues generally possess N- and O-linkages to the peptide backbone. Here we report the synthesis of the corresponding S-linked glycopeptides by a convergent strategy to provide compounds which should be quite stable to glycosidases. To this end, peptides that contain β-bromoalanine and γ-bromohomoalanine were generated either directly by bromination of serine and homoserine residues, respectively, or by standard ligation of the corresponding amino acids. 1-Thiosugars of O-acetyl protected GalNAc, GlcNAc, and lactose were prepared by known procedures. Reaction of the thiosugars with these peptides in an ethyl acetate/water two-phase system, which contained TBAHS and $NaHCO_3$, or in a one-phase system that consists of DMF/water and which contains $NaHCO_3$, led to the desired S-linked glycopeptides cleanly and in almost quantitative yield. This reaction also worked well for O-unprotected 1-thiosugars.

Keywords: carbohydrates · glycopeptides · substitution · thioglycosides

Introduction

Protein glycosylation is a ubiquitous posttranslational modification that is involved in a number of processes, both within cells and at cell surfaces. These include cell adhesion, cell differentiation, signal transduction, host–pathogen interactions, and immune response.[1] Aberrant glycosylation of proteins has often been correlated with specific disease states. However, the mechanism by which carbohydrates as protein constituents exert their function is poorly understood at the molecular level. This mainly stems from the microheterogeneity at the carbohydrate portions,[2] which yields an ensemble of glycoproteins that differ only in their glycan structure. For instance, erythropoietin (EPO), a clinically useful red-blood cell stimulant for anemia, is glycosylated by more than thirteen types of oligosaccharide chains when expressed in Chinese hamster ovary (CHO) cells.[3] This highly complex structure of glycoproteins has challenged existing analytical techniques with respect to separation and purification, and has restricted access to sufficient quantities of homogenous material for structural and functional analysis.

Therefore, chemical syntheses of glycoproteins[4] with well-defined structures are needed to gain an understanding of glycan function, as well as for the development of improved glycoprotein therapeutics. Moreover, since the most comprehensive studies of specific glycoprotein functions to date have been derived through the characterization of glycopeptide activities, chemists have also begun to explore various approaches to the construction of glycopeptides[5] and glycopeptide mimetics[6] that have superior properties for therapeutic application, or that permit a more facile synthesis.

Naturally occurring glycopeptides most commonly incorporate an O-glycosidic or an N-glycosidic linkage between the carbohydrate moiety and the side chain of an appropriate amino acid residue.[5a] Replacement of the anomeric oxygen of O-glycopeptides by sulfur, particularly those linked to serine or threonine, would give the corresponding S-linked glycopeptides; this is a modification tolerated by most biological systems. In addition, the stability of the peptide–sugar linkage against chemical degradation, as well as against enzymatic cleavage would be increased.[7] Therefore, investigations toward the synthesis of S-linked glycopeptides that may prove useful in biological studies and as potential therapeutic agents were initiated.[8,9]

In general, there are two distinct synthetic approaches for glycopeptide assembly. As depicted in Figure 1, one is a co-translational strategy wherein a glycosylated amino acid is incorporated into the desired peptide during solid-phase peptide synthesis (SPPS). The other is a posttranslational strategy, generally termed the "convergent strategy", in

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which the sugar is directly attached to the pre-established peptide structure. Most examples of glycopeptide synthesis described to date have employed the cotranslational strategy because it is normally compatible with the well-established SPPS.

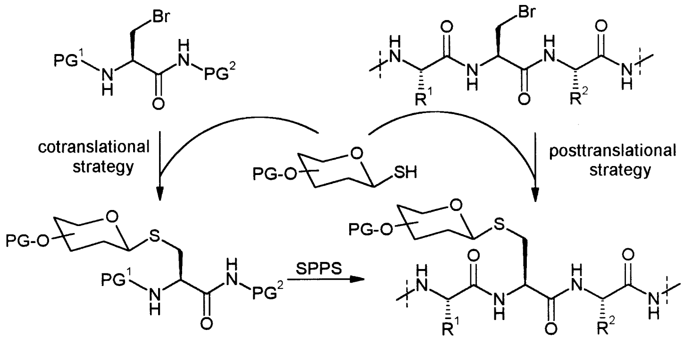


Figure 1. Two strategies for glycopeptide assembly.

In contrast, comparatively little work has been reported on posttranslational glycopeptide assembly even though this strategy presents a conceptually attractive alternative which could eventually allow a rapid and versatile means of forming glycopeptides libraries through the simple attachment of different carbohydrate structures. Moreover, the posttranslational strategy would circumvent any potential interference by the conjugate of interest that exists with SPPS.

Herein, we present an efficient method for the posttranslational assembly of S-linked glycopeptides using nucleophilic 1-thio sugars and electrophilic peptides that contain bromine.

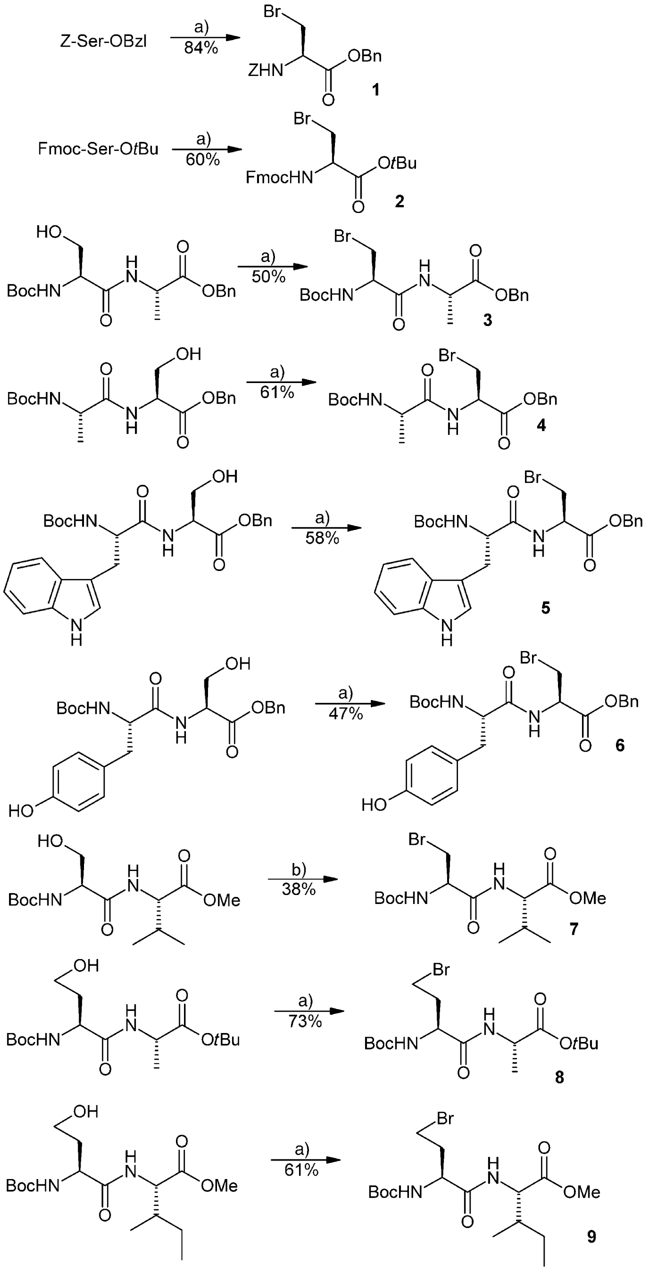
Prior to our work, several groups utilized acids that contain iodine to prepare S-linked glycosyl amino acids. Knapp et al. described the synthesis of Boc-Cys(α-GlcNAc)-OMe[10] and Boc-Cys(α-GalNAc)-OMe[11] in which β-iodoalanine derivatives were coupled with the corresponding α-GlcNAc or α-GalNAc thiols in the presence of a strong base in dry DMF. Milder conditions have also been employed in the preparation of Boc-Cys(β-GlcNAc)-OBn by a similar coupling reaction.[12] Preparation of S-glycosyl amino acids using solid-support sugar thiols and amino acids that contain iodine has also been reported.[13] However, these procedures have not all been extended to the convergent synthesis of S-linked glycopeptides presumably because of the unavailability of peptides that contain iodine. Indeed, there have only been two reports describing the convergent synthesis of S-linked glycopeptides. In the first report, 1-thio sugars were attached by Michael addition to peptides that contain dehydroalanine.[8b] Unfortunately, this addition was not diastereoselective. In the second report, serine- and threonine-derived cyclic sulfamidates were employed for the preparation of small S-linked glycopeptides.[8h] However, application of this method was limited because apart from other drawbacks, the reaction also resulted in removal of the N-sulfate group.

Results and Discussion

Most S-linked glycopeptides synthesized to date have capitalized on the inherent reactivity of the sulfhydryl group of cysteine or homocysteine.[8,9] However, the chemoselective linkage of an electrophilic sugar with a peptide sulfhydryl group may become problematic in the presence of multiple nucleophilic amino acid side chains, and could lead to the desired S-linked glycopeptides in unsatisfactory yields.[9] A more fashionable method to generate glycopeptide mimetics or neoglycopeptides proceeds by incorporation of a nonproteinogenic functional group into the peptide scaffold, that is, one that is absent from the side chains of natural amino acids but which reacts readily and specifically with special sugar derivatives.[14,8b] This technique, which involves the coupling of two mutually and uniquely reactive functional groups in an aqueous environment, actually has its origins in protein chemistry. Even in the presence of a multitude of potentially reactive functionalities, two chemoselective ligation partners will react only with each other. In this way, homogeneous glycoprotein mimetics have very recently been produced, for instance, by ligation of amino-oxy sugars with keto-containing proteins.[14e] Here we describe an alternative ligation based on the coupling of nucleophilic 1-thiol sugars with peptides that contain β-bromoalanine or γ-bromohomoalanine.[15] The S-linked glycopeptides produced in this fashion have structural motifs that are also shared by native O- or N-linked glycopeptides.

Synthesis of bromopeptides: Peptides that contain bromine have rarely been used in solution, although bromoamino acid derivatives have been used for quite some time.[16] Two approaches to the synthesis of bromopeptides exist: 1) a pre-prepared bromoamino acid is incorporated during the course of the peptide synthesis; and 2) a bromo group is directly introduced into the peptide. To determine the approach best adapted to the synthesis of any desired bromopeptides, both of these routes were investigated. Conceivably, the former approach exhibited the greater potential, and indeed, the latter approach was not successful in all cases.

Commercially available Z-Ser-OBzl and Fmoc-Ser-O*t*Bu were brominated under standard conditions[17] (CBr_4/PPh_3) to afford the corresponding bromides **1**[18] and **2** in 84 and 60% yields, respectively (Scheme 1). Although TLC indicated complete consumption of starting material, bromide **3** was only isolated in 50% yield under the same conditions. Similarly, treatment of dipeptides Boc-Ala-Ser-OBzl, Boc-Trp-Ser-Obzl, and Boc-Tyr-Ser-OBzl with CBr_4/PPh_3 in CH_2Cl_2 provided the corresponding bromodipeptides **4**, **5**, and **6** in 61, 58, and 47% yields, respectively, after flash chromatography. A two-step procedure[14f] was examined for the bromination of dipeptide Boc-Ser-Val-OMe (Scheme 1). This compound was firstly converted to its tosylate then treated with NaBr. Unfortunately, bromide **7** was obtained in only 38% yield. As we had feared,[8h] because α,β-unsaturated amides are so readily formed, the main side reaction involved the elimination of HBr to give dehydroalanine derivatives. Since the two-step procedure does not provide any advantage, we used the CBr_4/PPh_3 conditions for all the remaining bromination reactions. As shown in Scheme 1 bromides **8** and **9** were produced in good yields. This is due to the greatly reduced acidity of the carbonyl β protons (relative to the α protons), which means that any tendency towards β-elimination is limited.



Scheme 1. Preparation of bromoamino acids **1** and **2**, and bromodipeptides **3**–**9**. a) CBr_4, PPh_3; b) TsCl, Py, CH_2Cl_2, then NaBr, acetone.

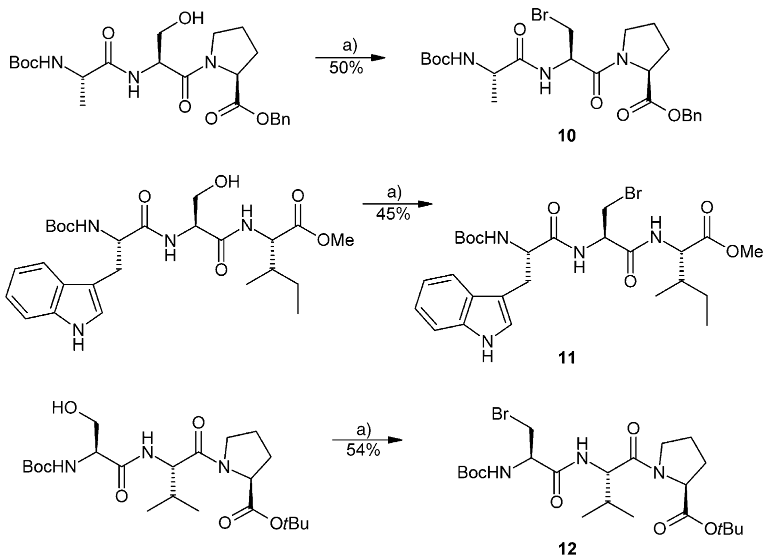
Bromotripeptides **10** and **11** were also readily produced in 50 and 45% yields, respectively, by direct bromination of Boc-Ala-Ser-Pro-OBzl and Boc-Trp-Ser-Ile-OMe with CBr_4/PPh_3 (Scheme 2). Tripeptide Boc-Ser-Val-Pro-O*t*Bu was also transformed to the corresponding bromide **12** in 54% yield by treatment with CBr_4/PPh_3. However, attempts to convert tripeptide Boc-Ala-Ser-Tyr-NH_2 to the corresponding bromide failed because of the poor solubility of this compound in suitable solvents.

Therefore, we turned our attention to an alternative approach by which bromopeptides can be prepared. This involves the elongation of β-bromoalanine or γ-bromohomoalanine derivatives at either the C- or N-termini to give more complex bromopeptides. To test the feasibility of elongation at the C-terminus of bromoamino acids we treated bromide **2** with TFA to give the free acid **13**, and then coupled this with H-Ala-OBzl·HCl in the presence of PyBOP[19] and diisopropylethylamine (DIPEA). The desired dipeptide **14** was obtained in 58% overall yield (Scheme 3) but a small amount of eliminated by-product **15** was also formed during the coupling reaction. Encouraged by this result, tripeptide **17** was prepared in good yield in two steps from dipeptide **4**. Dipeptide **4** was firstly debenzylated by catalytic hydrogenation to give acid **16**, and this was then coupled to H-Ser-OBzl to give **17**.

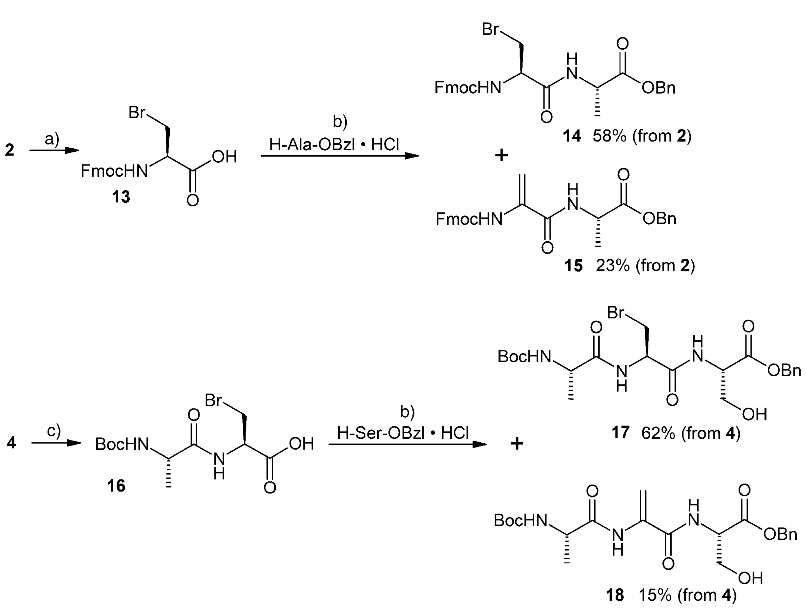
Not unexpectedly, by-product **18** was also isolated from the reaction mixture in 15% yield. It should be noted that by-products from an S_N2 reaction of **13** or **16** with amino or hydroxy compounds was not observed. Despite the formation of some elimination products, a noteworthy advantage of this route is that it allows the construction of peptides that contain both bromoamino acids and natural amino acids such as **17** that contain hydroxy groups. Such peptides are conceivably difficult to prepare by direct bromination of the corresponding tripeptide. Furthermore, the promising results obtained suggest that this approach could be applied to form more complex bromopeptides by the established SPPS method.

To obtain more information on the preparation of bromopeptides, we then turned to elongating peptide chains at the N-terminus. The results are summarized in Scheme 4. Exposure of dipeptide **7** to 25% TFA in CH_2Cl_2 led smoothly to the TFA salt of amine **19**, which was used without further purification in the following two coupling reactions. The use of a Boc protecting group is essential because the acidic conditions required to subsequently cleave it can be tolerated by the β-bromo structure motif.

Coupling of **19** with Boc-Ala-OH in the presence of PyBOP and DIPEA afforded the desired bromide **20** in 88% overall yield. Similarly, coupling of **19** with Boc-Ala-Gly-OH under the same conditions produced tetrapeptide **21** also in high yield. In this way, dipeptide **3** was converted to pentapeptide **23** in 83% yield over two steps, while treatment of dipeptide **9** with 25% TFA in CH_2Cl_2 followed by coupling with Boc-Ala-Gly-Gly-Gly-OH produced the desired hexapeptide **25** in 70% yield. The yields obtained for N-terminus elongation were significantly better than those obtained for the C-terminus elongation reactions. Moreover, N-terminus elongation did not afford any elimination by-products (like **15** and **18**) in the coupling reactions. A further benefit is that all the bromides prepared above were stable to silica-gel chromatography and routine purifications could be performed. The precise mechanistic details underlying the different behaviour observed when bromopeptides were elongated at the different termini are not yet clear. It is noteworthy that by conducting elongation at both termini



Scheme 2. Preparation of bromopeptides **10**, **11**, and **12**. a) CBr_4, PPh_3.

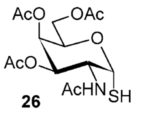


Scheme 3. Preparation of bromopeptides by elongation at the C-terminus. a) TFA, H_2O, CH_2Cl_2; b) PyBOP, DIPEA; c) H_2, Pd/C.

there seems to be, in principle, no limit to the structural complexity of the bromopeptides that can be formed.

Synthesis of 1-thio sugars: To accomplish the chemistry outlined in Figure 1 (posttranslational S-linked glycopeptide synthesis), suitably derivatized 1-thio sugars are also required. Moreover, in order to mimic the natural glycopeptides better the choice of glycosyl thiols, in particular the stereochemistry of the C–S bonds, is important. O-Linked glycoproteins found in nature often have a glycan core structure that is initiated with a serine or threonine-linked α-GalNAc.[20] Therefore, α-GalNAc thiol **26**[11] (see below) is essential to mimic the O-α-GalNAc glycopeptides. Known α-GlcNAc thiol **27**[10] was also prepared in order to construct S-α-GlcNAc glycopeptides; these mimic the corresponding O-α-GlcNAc-Ser linkage, which is a very common structural feature amongst O-linked glycoproteins.[21] Due to the importance of β-GlcNAc-Ser/Thr glycosides in both the β-amyloid precursor protein (APP)[22] and nuclear pore proteins,[23] β-GlcNAc thiol **28**[24] was included as one of the glycosyl moieties to be introduced into the glycopeptide. Peracetyl β-glucosyl thiol and β-lactosyl thiol **29**[25] were also investigated because β-glycosides commonly occur in natural glycoproteins.

Convergent assembly of S-linked glycopeptides: With the requisite building blocks in hand, the task that now confronted us was the development of appropriate conditions to achieve the desired post-translational assembly of the S-linked glycopeptides. Recently, we achieved the direct S-glycosylation of peptides that contain cysteine or homocysteine using glycosyl bromides in the presence of 10% Na_2CO_3.[9,26] On the basis of this work β-bromoalanine **1** was treated in ethyl acetate and an aqueous solution of $NaHCO_3$ at pH 8.5 with α-GlcNAc thiol **27** in the presence of tetra-*n*-butylammonium hydrogensulfate (TBAHS). Under these phase-transfer conditions (PTC), the desired α-thioglycoside **30** (Scheme 5) was smoothly obtained in 87% yield.[27] In this coupling, the thiolate anion is generated in situ by the action of $NaHCO_3$, and the α-thioglycoside **30** is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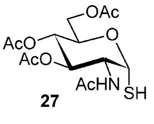
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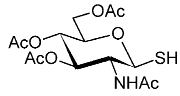
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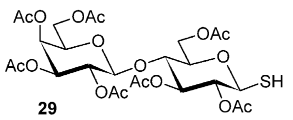
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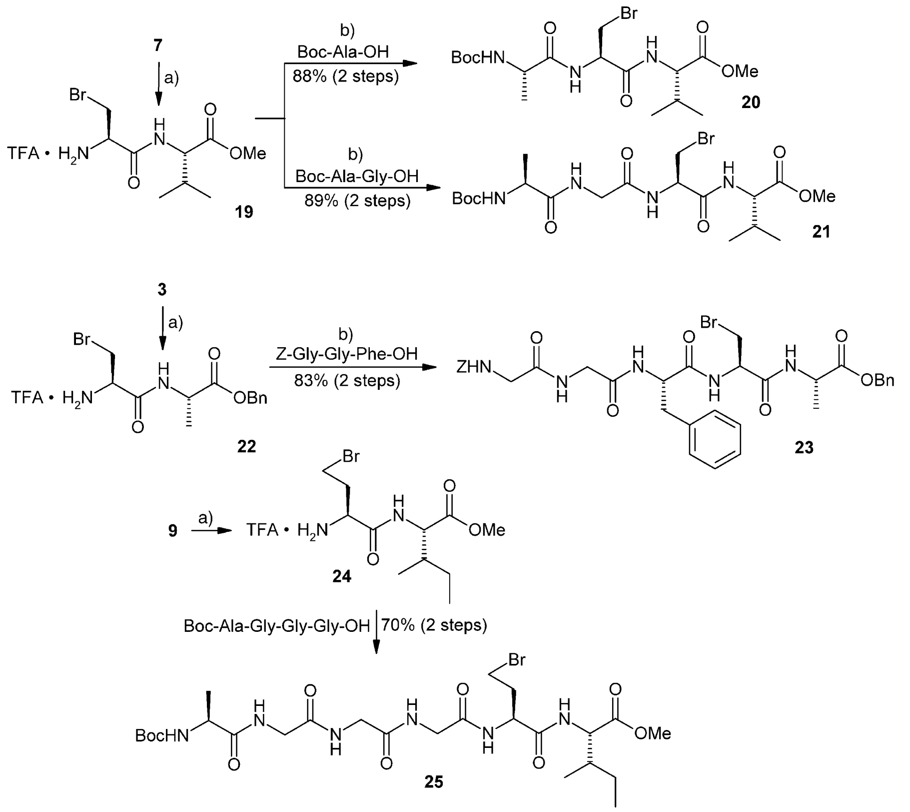


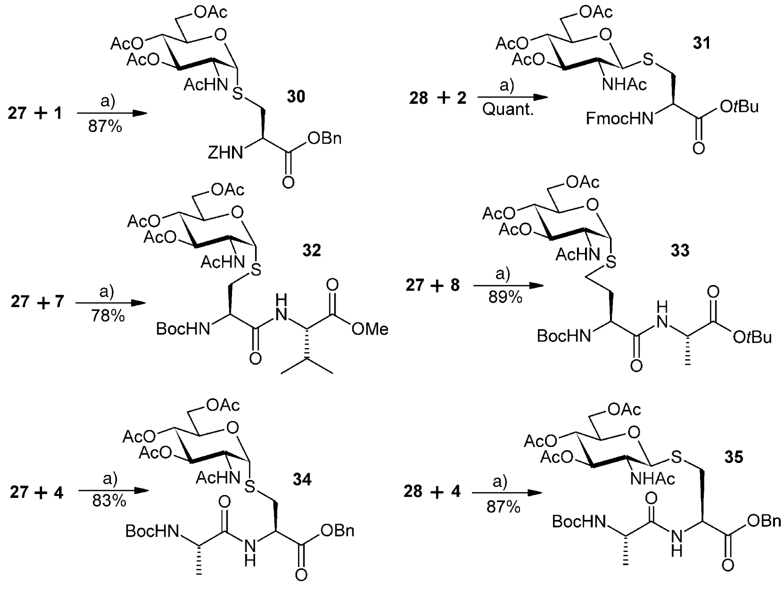
Scheme 4. Preparation of bromopeptides by elongation at the N-terminus. a) TFA, CH_2Cl_2; b) PyBOP, DIPEA.

then readily formed by nucleophilic displacement of the β-bromo atom in **1**. A solution of $NaHCO_3$ at pH 8.5 was used rather than 10% Na_2CO_3 in order to reduce the possibility of the bromoalanine derivatives undergoing β-elimination. Had this occurred, subsequent Michael addition would give rise to a mixture of diastereomers that only differ in configuration at the carbon α to cysteine. Fortunately, this did not take place in our system, although it has been observed under different experimental conditions.[8h,12] A further advantage of this methodology is that the stereochemistry at the important anomeric center is established at an early stage in the synthetic sequence. Even under more basic conditions, the stereochemical integrity at the anomeric center of the 1-thiosugars is maintained.[28]

Gratifyingly, when β-bromoalanine **2** was treated with thiol **28** according to the same procedure, β-GlcNAc glycoside **31** was isolated in quantitative yield (Scheme 5).[29] It should be noted that recently Ichikawa et al.[12] used the Mitsunobu reaction to prepare a similar compound in only modest yield (53%). The excellent yields obtained, as well as the extremely simple conditions required by our method make this an attractive alternative in the preparation of S-glycosylated cysteine derivatives such as **31**. Furthermore, these can subsequently be incorporated into glycopeptide syntheses (cotranslational strategy).

Encouraged by these results, our attention then turned toward applying this procedure to the convergent assembly of glycopeptide arrays. As expected, S-glycodipeptide **32** was produced smoothly in 78% yield by treatment of dipeptide **7** with α-GlcNAc thiol **27** under phase-transfer conditions. More importantly, careful inspection of the ^1H NMR spectra of **32** did not reveal any epimerized glycopeptide. We subsequently investigated the use of bromodipeptide **8** as an electrophile. When **8** was treated with thiol **27** under PTC, the desired S-glycodipeptide **33** was efficiently obtained in 89% yield as shown in Scheme 5. This was also a promising result as it demonstrated the feasibility of utilizing our procedure in the synthesis of N-linked glycopeptide mimetics.[30]

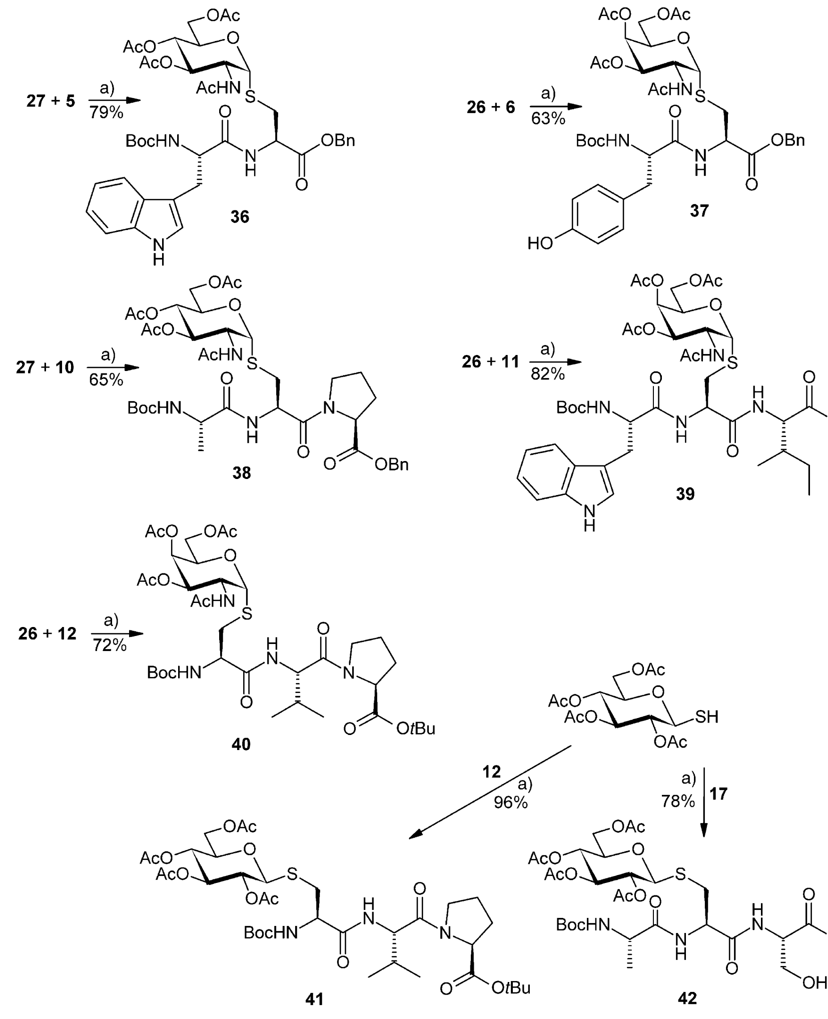
Since it has been demonstrated during the synthesis of O-glycopeptides that the hydroxyl group at the C-terminus has



Scheme 5. Synthesis of S-glycopeptides **30**–**35**. a) $NaHCO_3$, TBAHS, EtOAc/H_2O.

a much lower reactivity[31] than the hydroxyl group at the N-terminus, we thought it was important to examine the reactivity of a bromo group at the C-terminus. For this purpose, dipeptide **4** was treated with thiol **27** under the above described conditions, and interestingly, the α-thioglycoside **34** was produced in 83 % yield. The high reactivity of the bromine atom at the C-terminus was further confirmed when **4** was effectively coupled with β-GlcNAc thiol **28** to give the desired product **35** in an even higher yield (87 %). This demonstrates that the bromo group has a similar reactivity at either terminus and can be effectively attacked by sulfur nucleophiles.

The scope of this novel procedure in the synthesis of S-glycopeptides is further illustrated in Scheme 6. Treatment of tryptophan (Trp) dipeptide **5** with thiol **27** in the presence of aqueous $NaHCO_3$ (pH 8.5) and TBAHS afforded the desired α-thioglycoside **36** in 79 % yield. Again, under the same conditions, ligation between tyrosine (Tyr) dipeptide **6** and α-GalNAc thiol **26** occurred smoothly and led to the desired α-thioglycoside **37** in good yield (63 %).[32] It is important to note that although the indole ring of Trp and the hydroxyl group of Tyr are nucleophilic, they did not participate in the ligation because the highly nucleophilic thiolate group was present.



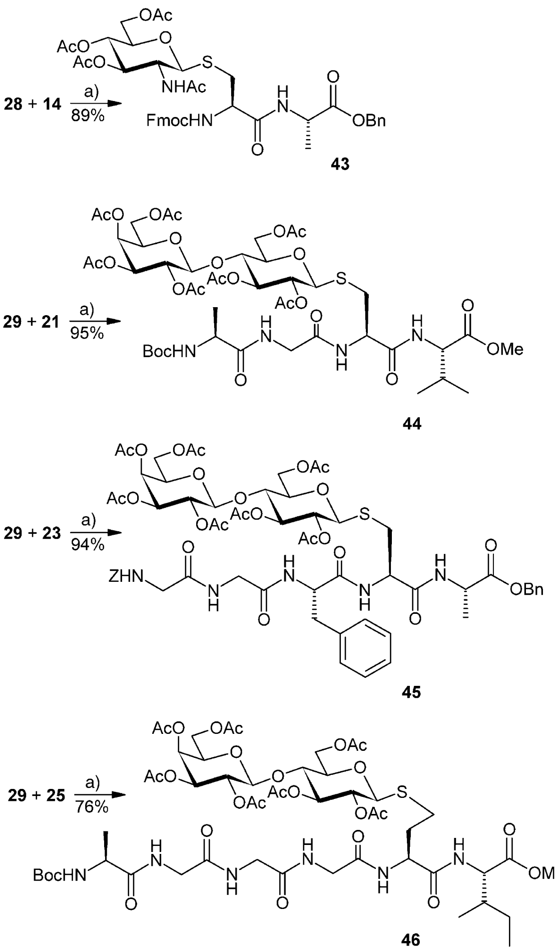
Scheme 6. Synthesis of S-glycopeptides **36**–**42**. a) $NaHCO_3$, TBAHS, EtOAc/H_2O.

To further explore the scope of this methodology, we proceeded to use bromotripeptides **10**, **11**, **12**, and **17** as electrophiles in the convergent synthesis of S-linked glycopeptides. Thus, reaction of **10** with thiol **27** under the above described conditions gave solely the desired glycotripeptide **38** in 65 % yield. As shown in Scheme 6, tripeptide **11** also underwent effective thioglycosylation with thiol **26** under PTC to give S-α-glycotripeptide **39** in 82 % yield. The above smooth formation of glycotripeptides **38** and **39** further demonstrates the effectiveness of this methodology and lends credence to the notion that more complex S-linked glycopeptides could be produced in this fashion.

Similarly, thioglycosylation of tripeptide **12** with α-GalNAc thiol **26** in a mixture of EtOAc/aqueous $NaHCO_3$ and in the presence of TBAHS (pH 8.5) also proceeded smoothly to give S-α-glycotripeptide **40** in good yield (72 %). As depicted in Scheme 6, exposure of **12** to peracetyl β-glucosyl thiol under the above conditions furnished the S-β-glycotripeptide **41**[9] in almost quantitative yield (96 %). This result is in sharp contrast to the modest yield obtained for the same compound by a previously reported procedure.[9] Ligation of tripeptide **17** and glucosyl thiol (Scheme 6) was also carried out under PTC and the desired S-glycopeptide **42** was isolated from the reaction mixture in 78 % yield.

Despite the above achievements there is an apparent limit to the present procedure. In particular, the procedure is dependent upon the bromopeptide being soluble in ethyl acetate. In view of the fact that protected or partially protected peptide chains frequently exhibit only limited solubility in organic solvents, it was essential to adjust the present conditions used if we were to attain our goal of synthesizing more complex S-linked glycopeptides.

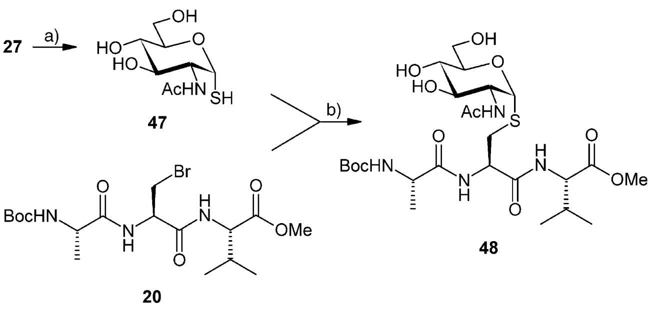
We decided to trial DMF as a solvent for this purpose. Hence, Fmoc-protected dipeptide **14**, which is only very slightly soluble in EtOAc, was treated in DMF and in the presence of aqueous $NaHCO_3$ (pH 8.5) with thiol **28** (Scheme 7). To our delight, the desired β-thioglycoside **43** was smoothly produced in this homogeneous solution in high yield (89 %) without observable epimerization of the cysteine α-carbon. This is an especially promising result as it forebodes the feasibility of performing this ligation on more complex peptides. Indeed, tetrapeptide **21** underwent a highly effective thioglycosylation with β-lactosyl thiol **29** in DMF/H_2O and in the presence of $NaHCO_3$ to give the desired S-linked glycotetrapeptide **44** in almost quantitative yield (95%) (Scheme 7). Moreover, under these optimized conditions pentapeptide **23** and thiol **29** underwent chemoselective ligation to afford **45** in excellent yield after purification.



Scheme 7. Synthesis of S-glycopeptides **43**–**46**. a) $NaHCO_3$, DMF/H_2O.

To demonstrate the power of this method in the synthesis of complex S-linked glycopeptides, hexapeptide **25** was subjected to thioglycosylation with sugar **29** under the above conditions ($NaHCO_3$, DMF/H_2O). Once again, the desired S-linked glycohexapeptide **46** was obtained in high yield (76%).

Finally, we reasoned that unprotected sugar thiols could also be used in this procedure, and indeed, as demonstrated by the synthesis of S-glycopeptide **48** (Scheme 8), this proved to be the case. Deacetylation of **27** afforded the unprotected sugar **47**, and this was then selectively ligated with bromotripeptide **20** under the same reaction conditions to give the desired glycotripeptide **48** in 70% yield.



Scheme 8. Synthesis of glycotripeptide **48**. a) NaOMe, MeOH; b) $NaHCO_3$, DMF/H_2O.

Conclusion

In summary, we have presented a highly efficient method for the synthesis of S-linked glycopeptides in aqueous solution by chemoselective ligation of bromopeptides with 1-thio sugars. All the S-linked glycopeptides were prepared by a convergent strategy. This is particularly notable because biological studies would benefit from the modular nature of this strategy. An advantage of this procedure is that due to the extremely mild reaction conditions employed for the ligation, epimerization at the α-carbon of the amino acid was not observed. Notably, both S-α- and S-β-glycopeptides[33] can be efficiently generated by this procedure from the corresponding α- or β-glycosyl thiols, respectively. A further advantage of this protocol is that unprotected 1-thio sugars can also be utilized to provide partially protected glycopeptides. The high yields attained under extremely mild reaction conditions means that the method described herein has considerable potential in the synthesis of large S-linked glycopeptides or even S-linked glycoproteins.

Experimental Section

General remarks: Unless otherwise stated, all moisture-sensitive reactions were performed in oven-dried glassware under a nitrogen atmosphere using dry solvents. Solvents were evaporated under reduced pressure at temperatures below 40°C. All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 F_{254}, and the compounds were visualized with UV light (254 nm), iodine, or by treatment with either 0.2% ninhydrin in ethanol or 10% H_2SO_4 in methanol followed by heating at 150°C. Flash chromatography was performed with the indicated solvent system using 30–60 µm silica gel at a pressure of 0.3–0.4 bar. Melting points were determined in an open capillary and are reported in degrees Celsius (uncorrected). Optical rotations were measured at 25°C with a Perkin–Elmer 241/MC polarimeter (1 dm^3 cell). ^1H NMR spectra were recorded with Bruker AC 250 (250 MHz) or Bruker DRX (600 MHz) instruments using tetramethylsilane as an internal standard, while ^{13}C NMR spectra were recorded on a Bruker AC 250 (72.9 MHz) spectrometer. MS spectra were recorded with a MALDI-kompakt (Kratos) instrument in the positive mode using 2,5-dihydroxybenzoic acid in dioxane as the matrix. Elemental analyses were performed in the microanalysis unit at Fachbereich Chemie, Universität Konstanz. Yields refer to chromatographically pure compounds and are calculated based on consumed reagents.

All commercially obtained reagents were used as received. Amino acid derivatives, Z-Gly-Gly-Phe-OH and Boc-Ala-Gly-Gly-Gly-OH were purchased from Novabiochem or Bachem. All other dipeptides and tripeptides used were prepared from commercially available materials by normal PyBOP-mediated peptide coupling.

Benzyl *N*-benzyloxycarbonyl-β-bromo-L-alaninate (1):[18] Ph_3P (1.16 g, 4.44 mmol) was added portionwise to a solution of Z-Ser-OBzl (730 mg, 2.22 mmol) and CBr_4 (1.25 g, 3.77 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C. The mixture was stirred at 0 °C for 20 min and was then concentrated under reduced pressure at room temperature. The residue was purified by flash-column chromatography (petroleum ether/EtOAc 12:1→3:1) to give **1** (730 mg, 84 %) as a white solid. $R_f = 0.28$ (petroleum ether/EtOAc 4:1); $[\alpha]_D = -19.0$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.35$ (m, 10H), 5.75 (d, $J = 7.7$ Hz, 1H), 5.21 (s, 2H), 5.12 (s, 2H), 4.84 (dt, $J = 7.9$, 3.3 Hz, 1H), 3.83 (dd, $J = 10.6$, 3.2 Hz, 1H), 3.72 ppm (dd, $J = 10.6$, 3.4 Hz, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 168.7$, 155.5, 135.9, 134.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 68.0, 67.2, 54.3, 33.6 ppm; MS (MALDI): *m*/*z*: 414, 416 $[M+Na]^+$, 430, 432 $[M+K]^+$; elemental analysis calcd (%) for $C_{18}H_{18}BrNO_4$ (392.2): C 55.12, H 4.63, N 3.57; found: C 55.21, H 4.50, N 3.58.

***tert*-Butyl *N*-(9-fluorenylmethoxycarbonyl)-β-bromo-L-alaninate (2)**: The reaction procedure was identical to that described for **1** except that Fmoc-Ser-O*t*Bu (218 mg, 0.57 mmol) was used instead of Z-Ser-OBzl. Compound **2** (152 mg, 60 %) was isolated as a colourless syrup. $R_f = 0.32$ (petroleum ether/EtOAc 2.5:1); $[\alpha]_D = +16.0$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.78$ (d, $J = 7.3$ Hz, 2H), 7.62 (d, $J = 7.3$ Hz, 2H), 7.36 (m, 4H), 5.75 (d, $J = 7.3$ Hz, 1H), 4.69 (dt, $J = 7.4$, 3.1 Hz, 1H), 4.38 (m, 2H), 4.25 (t, $J = 7.2$ Hz, 1H), 3.84 (dd, $J = 10.5$, 2.9 Hz, 1H), 3.77 (dd, $J = 10.5$, 3.3 Hz, 1H), 1.52 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 167.6$, 155.4, 143.6, 143.5, 141.1, 127.6, 127.0, 125.0, 119.9, 83.4, 67.2, 54.4, 46.9, 34.3, 27.8 ppm; MS (MALDI): *m*/*z*: 468, 470 $[M+Na]^+$; elemental analysis calcd (%) for $C_{22}H_{24}BrNO_4 \cdot H_2O$ (464.3): C 56.91, H 5.64, N 3.02; found: C 56.98, H 5.42, N 2.65.

***N*-*tert*-Butoxycarbonyl-β-bromo-L-alanyl-L-alanine benzyl ester (3)**: The reaction procedure was identical to that described for **1** except that Boc-Ser-Ala-OBzl (1.5 g, 4.1 mmol) was used instead of Z-Ser-OBzl. Compound **3** (880 mg, 50 %) was isolated as a white solid. $R_f = 0.29$ (petroleum ether/EtOAc 2:1); $[\alpha]_D = -9.6$ ($c = 0.7$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.36$ (m, 5H), 6.90 (d, $J = 6.9$ Hz, 1H), 5.27 (d-like, 1H), 5.19 (AB quartet, $J = 12.3$ Hz, 2H), 4.63 (dt, $J = 14.4$, 7.2 Hz, 1H), 4.52 (brs, 1H), 3.89 (dd, $J = 10.3$, 4.3 Hz, 1H), 3.56 (dd, $J = 10.4$, 4.9 Hz, 1H), 1.47 (s, 9H), 1.45 ppm (d, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 172.1$, 168.5, 155.0, 153.1, 128.4, 128.2, 127.9, 80.5, 67.0, 54.6, 48.2, 33.1, 28.1, 18.0 ppm; MS (MALDI): *m*/*z*: 451, 453 $[M+Na]^+$; elemental analysis calcd (%) for $C_{18}H_{25}BrN_2O_5$ (429.3): C 50.36, H 5.87, N 6.53; found: C 50.17, H 5.70, N 6.53.

***N*-*tert*-Butoxycarbonyl-L-alanyl-β-bromo-L-alanine benzyl ester (4)**: The reaction procedure was identical to that described for **1** except that Boc-Ala-Ser-OBzl (439 mg, 1.2 mmol) was used instead of Z-Ser-OBzl. Compound **4** (314 mg, 61 %) was isolated as a colourless oil. $R_f = 0.37$ (petroleum ether/EtOAc 1:1); $[\alpha]_D = -12.0$ ($c = 1.4$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.37$ (m, 5H), 7.14 (brs, 1H), 5.23 (m, 2H), 5.09 (d, $J = 6.8$ Hz, 1H), 5.02 (dt, $J = 7.3$, 3.4 Hz, 1H), 4.25 (m, 1H), 3.84 (dd, $J = 10.6$, 3.3 Hz, 1H), 3.76 (dd, $J = 10.6$, 3.5 Hz, 1H), 1.45 (s, 9H), 1.37 ppm (d, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 172.7$, 168.6, 155.4, 134.7, 128.6, 128.4, 80.2, 67.9, 52.7, 50.0, 33.0, 28.2, 18.0 ppm; MS (MALDI): *m*/*z*: 451, 453 $[M+Na]^+$, 467, 469 $[M+K]^+$; elemental analysis calcd (%) for $C_{18}H_{25}BrN_2O_5$ (429.3): C 50.36, H 5.87, N 6.53; found: C 50.31, H 6.46, N 6.50.

***N*-*tert*-Butoxycarbonyl-L-tryptophanyl-β-bromo-L-alanine benzyl ester (5)**: The reaction procedure was identical to that described for **1** except that Boc-Trp-Ser-OBzl (246 mg, 0.51 mmol) was used instead of Z-Ser-OBzl. Compound **5** (161 mg, 58 %) was isolated as a white solid. $R_f = 0.47$ (petroleum ether/EtOAc 1:1); $[\alpha]_D = -4.2$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 8.20$ (brs, 1H), 7.63 (d, $J = 7.7$ Hz, 1H), 7.35 (m, 5H), 7.21–7.04 (m, 4H), 6.77 (d, $J = 6.7$ Hz, 1H), 5.18 (m, 1H), 5.13 (s, 2H), 4.88 (m, 1H), 4.50 (m, 1H), 3.71 (dd, $J = 10.6$, 3.1 Hz, 1H), 3.62 (m, 1H), 3.34 (dd, $J = 14.5$, 4.6 Hz, 1H), 3.19 (dd, $J = 14.6$, 6.9 Hz, 1H), 1.42 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 171.8$, 168.3, 155.4, 136.2, 134.7, 128.6, 128.4, 127.4, 123.2, 122.2, 119.7, 118.7, 111.2, 110.2, 80.3, 67.9, 55.1, 52.8, 33.0, 28.2 ppm; MS (MALDI): *m*/*z*: 566, 568 $[M+Na]^+$, 582, 584 $[M+K]^+$; elemental analysis calcd (%) for $C_{26}H_{30}BrN_3O_5$ (544.4): C 57.36, H 5.55, N 7.72; found: C 57.35, H 5.78, N 8.05.

***N*-*tert*-Butoxycarbonyl-L-tyrosinyl-β-bromo-L-alanine benzyl ester (6)**: The reaction procedure was identical to that described for **1** except that Boc-Tyr-Ser-OBzl (174 mg, 0.38 mmol) was used instead of Z-Ser-OBzl. Compound **6** (93 mg, 47 %) was isolated as a white solid. $R_f = 0.50$ (petroleum ether/EtOAc 1:1.2); $[\alpha]_D = +0.5$ ($c = 0.5$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.35$ (m, 5H), 7.02 (d, $J = 8.3$ Hz, 2H), 6.92 (d, $J = 6.9$ Hz, 1H), 6.73 (d, $J = 8.3$ Hz, 2H), 5.20 (s, 2H), 5.04 (d-like, 1H), 4.95 (m, 1H), 4.36 (m, 1H), 3.79 (dd, $J = 10.8$, 3.1 Hz, 1H), 3.72 (dd, $J = 10.8$, 3.6 Hz, 1H), 3.00 (d, $J = 6.4$ Hz, 2H), 1.42 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 171.9$, 168.3, 155.5, 155.3, 134.6, 130.3, 128.6, 128.4, 127.3, 115.7, 80.6, 68.0, 55.7, 52.9, 37.2, 32.7, 28.2 ppm; elemental analysis calcd (%) for $C_{24}H_{29}BrN_2O_6$ (521.4): C 55.29, H 5.61, N 1.34; found: C 55.23, H 5.80, N 1.32.

***N*-*tert*-Butoxycarbonyl-β-bromo-L-alanyl-L-valine methyl ester (7)**: Pyridine (1.3 mL, 15.9 mmol) was added to a stirred solution of Boc-Ser-Val-OMe (1.07 g, 3.4 mmol) and toluene-*p*-sulfonyl chloride (1.3 g, 6.8 mmol) in dry CH_2Cl_2 (25 mL). The mixture was stirred at ambient temperature for 48 h. Removal of the volatiles in vacuo gave rise to a residue which was purified by flash-column chromatography (petroleum ether/EtOAc 3:1) to afford the tosylate (900 mg, 56 %) as a colorless oil. $R_f = 0.33$ (petroleum ether/EtOAc 1.5:1); $[\alpha]_D = +8.1$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.78$ (d, $J = 8.2$ Hz, 2H), 7.35 (d, $J = 8.3$ Hz, 2H), 6.95 (d, $J = 8.4$ Hz, 1H), 5.46 (d, $J = 6.9$ Hz, 1H), 4.48 (dd, $J = 8.8$, 5.0 Hz, 1H), 4.39 (m, 2H), 4.21 (m, 1H), 3.73 (s, 3H), 2.45 (s, 3H), 2.13 (m, 1H), 1.46 (s, 9H), 0.90 (d, $J = 6.8$ Hz, 3H), 0.88 ppm (d, $J = 6.8$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 171.6$, 168.1, 155.2, 145.2, 132.1, 129.9, 128.0, 80.9, 68.7, 57.3, 53.5, 52.1, 31.2, 28.1, 21.6, 18.8, 17.6 ppm; MS (MALDI): *m*/*z*: 495 $[M+Na]^+$, 511 $[M+K]^+$; elemental analysis calcd (%) for $C_{21}H_{32}N_2O_8S$ (472.6): C 53.38, H 6.83, N 5.93; found: C 53.48, H 6.72, N 5.62.

Bromination of the tosylate (640 mg, 1.35 mmol) was performed under nitrogen with NaBr (556 mg, 5.4 mmol) in dry acetone (20 mL). The suspension was heated under reflux at 60 °C for 20 h, the solvent was removed in vacuo, and the residue was diluted with 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 5:1→3:1) to give **7** (350 mg, 68 %) as a white solid after lyophilization with dioxane. $R_f = 0.36$ (petroleum ether/EtOAc 2:1); $[\alpha]_D = -3.1$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 6.86$ (d, $J = 8.3$ Hz, 1H), 5.33 (d, $J = 7.0$ Hz, 1H), 4.54 (m, 2H), 3.93 (dd, $J = 10.4$, 4,4 Hz, 1H), 3.75 (s, 3H), 3.60 (dd, $J = 10.4$, 4.8 Hz, 1H), 2.20 (m, 1H), 1.49 (s, 9H), 0.94 ppm (dd, $J = 8.6$, 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$): $\delta = 171.8$, 168.8, 155.1, 81.1, 57.3, 55.0, 52.2, 33.2, 31.4, 28.2, 18.9, 17.7 ppm; MS (MALDI): *m*/*z*: 403, 405 $[M+Na]^+$, 419, 420 $[M+K]^+$; elemental analysis calcd (%) for $C_{14}H_{25}BrN_2O_5$ (381.3): C 44.10, H 6.61, N 7.35; found: C 44.41, H 6.70, N 7.58.

***N*-*tert*-Butoxycarbonyl-γ-bromo-L-homoalanyl-L-alanine *tert*-butyl ester (8)**: The reaction procedure was identical to that described for **1** except that Boc-Hse-Ala-O*t*Bu (122 mg, 0.35 mmol) was used instead of Z-Ser-OBzl. Compound **8** (105 mg, 73 %) was isolated as a colourless syrup. $R_f = 0.53$ (petroleum ether/EtOAc 1.5:1); $[\alpha]_D = -8.9$ ($c = 0.6$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 6.72$ (d, $J = 6.2$ Hz, 1H), 5.33 (d, $J = 8.1$ Hz, 1H), 4.43 (m, 1H), 4.37 (m, 1H), 3.50 (t, $J = 6.8$ Hz, 2H), 2.30 (m, 2H), 1.47 (s, 9H), 1.45 (s, 9H), 1.38 ppm (d, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 171.5$, 170.7, 155.5, 81.8, 80.0, 53.0, 48.7, 35.8, 28.9, 28.2, 27.8, 17.9 ppm; MS (MALDI): *m*/*z*: 431, 433 $[M+Na]^+$, 447, 449 $[M+K]^+$; elemental analysis calcd (%) for $C_{16}H_{29}BrN_2O_5$ (409.3): C 46.95, H 7.14, N 6.84; found: C 47.07, H 7.07, N 6.54.

***N*-*tert*-Butoxycarbonyl-γ-bromo-L-homoalanyl-L-isoleucine methyl ester (9)**: The reaction procedure was identical to that described for **1** except that Boc-Hse-Ile-OMe (208 mg, 0.6 mmol) was used instead of Z-Ser-OBzl. Compound **9** (150 mg, 61 %) was isolated as a white solid. $R_f = 0.63$ (petroleum ether/EtOAc 1:1); $[\alpha]_D = -10.0$ ($c = 0.6$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 6.70$ (d, $J = 8.6$ Hz, 1H), 5.24 (d, $J = 8.4$ Hz, 1H), 4.56 (dd, $J = 8.5$, 4.7 Hz, 1H), 4.38 (q, $J = 7.8$ Hz, 1H), 3.75 (s, 3H), 3.51 (t, $J = 6.5$ Hz, 2H), 2.30 (m, 2H), 1.95 (m, 1H), 1.45 (s, 9H), 1.42 (m, 1H), 1.22 (m, 1H), 1.92 ppm (m, 6H); ^{13}C NMR ($CDCl_3$): $\delta = 171.9$, 170.9, 155.5, 80.3, 56.6, 53.0, 52.1, 37.5, 35.0, 29.3, 28.2, 24.9, 15.4, 11.5 ppm; MS (MALDI): *m*/*z*: 431, 433 $[M+Na]^+$, 447, 449 $[M+K]^+$; elemental analysis calcd (%) for $C_{16}H_{29}BrN_2O_5$ (409.3): C 46.95, H 7.14, N 6.84; found: C 46.81, H 7.22, N 6.67.

N-tert**-Butoxycarbonyl-L-alanyl-β-bromo-L-alanyl-L-proline benzyl ester (10)**: The reaction procedure was identical to that described for **1** except that Boc-Ala-Ser-Pro-OBzl (600 mg, 1.29 mmol) was used instead of Z-Ser-OBzl. Compound **10** (339 mg, 50 %) was isolated as a white solid. $R_f = 0.25$ (petroleum ether/EtOAc 1:2); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta =$ 7.36 (m, 5 H), 7.14 (d, $J = 8.1$ Hz, 1 H), 5.17 (AB quartet, $J = 12.3$ Hz, 2 H), 5.14 (m, 1 H), 5.05 (m, 2 H), 4.57 (dd, $J = 8.5$, 4.3 Hz, 1 H), 4.20 (m, 1 H), 3.75 (m, 2 H), 3.63 (dd, $J = 10.5$, 6.1 Hz, 1 H), 3.46 (dd, $J = 10.5$, 6.3 Hz, 1 H), 2.21 (m, 1 H), 2.04 (m, 3 H), 1.44 (s, 9 H), 1.37 ppm (d, $J = 7.1$ Hz, 3 H); ^{13}C NMR ($CDCl_3$): $\delta = 172.6$, 171.1, 167.6, 155.3, 135.5, 128.5, 128.3, 128.2, 80.2, 66.9, 59.2, 51.2, 50.2, 47.3, 30.7, 28.9, 28.2, 24.7, 18.2 ppm; MS (MALDI): m/z: 548, 550 $[M+Na]^+$, 564, 566 $[M+K]^+$. This compound was immediately used in the next step.

N-tert**-Butoxycarbonyl-L-tryptophanyl-β-bromo-L-alanyl-L-isoleucine methyl ester (11)**: The reaction procedure was identical to that described for **1** except that Boc-Trp-Ser-Ile-OMe (187 mg, 0.36 mmol) was used instead of Z-Ser-OBzl. Compound **11** (94 mg, 45 %) was isolated as a white solid. $R_f = 0.47$ (petroleum ether/EtOAc 1:2); $[\alpha]_D = -7.2$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 8.42$ (brs, 1 H), 7.64 (d, $J = 7.8$ Hz, 1 H), 7.38 (d, $J = 8.0$ Hz, 1 H), 7.24–7.10 (m, 3 H), 7.03 (d-like, $J = 8.2$ Hz, 1 H), 6.82 (d, $J = 7.9$ Hz, 1 H), 5.12 (d, $J = 6.0$ Hz, 1 H), 4.79 (dt, $J = 7.7$, 4.4 Hz, 1 H), 4.54 (dd, $J = 8.5$, 5.3 Hz, 1 H), 4.48 (q, $J = 6.1$ Hz, 1 H), 3.74 (overlapped, 1 H), 3.73 (s, 3 H), 3.32 (dd, $J = 11.9$, 6.1 Hz, 1 H), 3.24 (dd, $J = 11.4$, 6.1 Hz, 1 H), 3.23 (m, 1 H), 1.89 (m, 1 H), 1.41 (s, 9 H), 1.20 (m, 2 H), 0.90 ppm (m, 6 H); ^{13}C NMR ($CDCl_3$): $\delta = 172.0$, 171.6, 168.2, 155.7, 136.3, 127.2, 123.2, 122.4, 119.8, 118.7, 111.3, 110.0, 80.7, 57.0, 55.3, 53.4, 52.1, 37.5, 32.8, 28.2, 27.6, 25.0, 15.3, 11.4 ppm; MS (MALDI): m/z: 603, 605 $[M+Na]^+$, 619, 621 $[M+K]^+$; elemental analysis calcd (%) for $C_{26}H_{37}BrN_4O_6$ (581.5): C 53.70, H 6.41, N 9.63; found: C 53.98, H 6.64, N 9.29.

N-tert**-Butoxycarbonyl-β-bromo-L-alanyl-L-valinyl-L-proline *tert*-butyl ester (12)**: The reaction procedure was identical to that described for **1** except that Boc-Ser-Val-Pro-O*t*Bu (213 mg, 0.47 mmol) was used instead of Z-Ser-OBzl. Compound **12** (131 mg, 54 %) was isolated as a white solid. $R_f = 0.42$ (petroleum ether/EtOAc 1:1); $[\alpha]_D = -55.4$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.38$ (d, $J = 7.0$ Hz, 1 H), 5.49 (d, $J = 7.4$ Hz, 1 H), 4.65 (dd, $J = 8.7$, 6.9 Hz, 1 H), 4.58 (m, 1 H), 4.39 (dd, $J = 8.8$, 4.8 Hz, 1 H), 3.83 (m, 2 H), 3.69 (m, 1 H), 3.62 (dd, $J = 10.4$, 4.4 Hz, 1 H), 2.22–1.90 (m, 5 H), 1.45 (s, 18 H), 1.05 (d, $J = 6.7$ Hz, 3 H), 0.96 ppm (d, $J = 6.7$ Hz, 3 H); ^{13}C NMR ($CDCl_3$): $\delta = 170.9$, 170.0, 168.7, 154.9, 81.3, 80.6, 59.7, 55.7, 54.8, 47.3, 34.1, 31.3, 29.0, 28.1, 27.8, 24.7, 19.3, 17.7 ppm; MS (MALDI): m/z: 541, 543 $[M+Na]^+$, 557, 559 $[M+K]^+$; elemental analysis calcd (%) for $C_{22}H_{38}BrN_3O_6$ (520.5): C 50.77, H 7.36, N 8.07; found: C 50.80, H 7.62, N 7.80.

N**-(9-Fluorenylmethoxycarbonyl)-β-bromo-L-alanine (13)**: *tert*-Butyl ester **2** (140 mg, 0.31 mmol) was dissolved in a mixture of TFA (1.2 mL), CH_2Cl_2 (4 mL), and water (0.1 mL). The resultant mixture was stirred for 5 h at room temperature. Evaporation of the solvent afforded acid **13**, which was directly used in the next reaction without purification.

N**-(9-Fluorenylmethoxycarbonyl)-β-bromo-L-alanyl-L-alanine benzyl ester (14)**: PyBOP (162 mg, 0.31 mmol) and DIPEA (70 μL, 0.4 mmol) were added to a stirred solution of acid **13** and H-Ala-OBzl·HCl (70 mg, 0.32 mmol) in dry DMF (3.5 mL) at 0 °C. The reaction solution was removed from the cooling bath, stirred at room temperature for 1 h, diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash-column chromatography (petroleum ether/EtOAc 4:1→2:1) to give **14** (99 mg, 58 % over two steps). $R_f = 0.21$ (petroleum ether/EtOAc 3:1); $[\alpha]_D = -10.1$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.77$ (d, $J = 7.4$ Hz, 2 H), 7.59 (d, $J = 7.2$ Hz, 2 H), 7.37 (m, 9 H), 6.89 (d, $J = 6.9$ Hz, 1 H), 5.64 (d, $J = 8.0$ Hz, 1 H), 5.17 (m, 2 H), 4.62 (m, 2 H), 4.45 (m, 2 H), 4.27 (t, $J = 6.8$ Hz, 1 H), 3.80 (m, 1 H), 3.56 (m, 1 H), 1.43 ppm (d, $J = 7.2$ Hz, 3 H); ^{13}C NMR ($CDCl_3$): $\delta = 172.1$, 167.9, 155.7, 143.6, 143.5, 141.3, 135.1, 128.6, 128.5, 128.2, 127.8, 127.1, 125.0, 120.0, 67.8, 67.4, 55.2, 48.6, 47.1, 32.9, 18.3 ppm; MS (MALDI): m/z: 572, 574 $[M+Na]^+$, 588, 590 $[M+K]^+$.

N-tert**-Butoxycarbonyl-L-alanyl-β-bromo-L-alanine (16)**: Benzyl ester **4** (195 mg, 0.45 mmol) was hydrogenated for 4 h at room temperature with 10 % palladium on charcoal (96 mg) as catalyst in a mixture of EtOH (5 mL), EtOAc (4 mL), and HOAc (2 drops). The reaction mixture was filtered through Celite and concentrated in vacuo to afford the free acid **16** as a white foam. This was used without further purification in the next coupling reaction.

N-tert**-Butoxycarbonyl-L-alanyl-β-bromo-L-alanyl-L-serine benzyl ester (17)**: PyBOP (254 mg, 0.48 mmol) and DIPEA (95 μL, 0.54 mmol) were added to a stirred solution of acid **16** and H-Ser-OBzl·HCl (112 mg, 0.48 mmol) in dry DMF (5 mL) at 0 °C. The reaction solution was removed from the cooling bath, stirred at room temperature for 1 h, diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash-column chromatography (petroleum ether/EtOAc 2:1→1:1.5) to give **17** (145 mg, 62 % over two steps). $R_f = 0.13$ (petroleum ether/EtOAc 1:1.5); $[\alpha]_D = -26.2$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.35$ (m, 5 H), 5.20 (s, 2 H), 4.78 (m, 1 H), 4.65 (dt, $J = 7.9$, 3.3 Hz, 1 H), 4.12 (q, $J = 7.0$ Hz, 1 H), 4.01 (dd, $J = 11.8$, 3.9 Hz, 1 H), 3.89 (dd, $J = 11.8$, 3.2 Hz, 1 H), 3.80 (m, 1 H), 3.68 (dd, $J = 10.5$, 4.6 Hz, 1 H), 1.43 (s, 9 H), 1.39 ppm (d, $J = 7.2$ Hz, 3 H); MS (MALDI): m/z: 538, 540 $[M+Na]^+$; elemental analysis calcd (%) for $C_{21}H_{30}BrN_3O_7$ (516.4): C 48.85, H 5.86, N 8.14; found: C 48.62, H 5.59, N 8.32.

N-tert**-Butoxycarbonyl-L-alanyl-β-bromo-L-alanyl-L-valine methyl ester (20)**: TFA (1.2 mL) was added to a solution of Boc-protected dipeptide **7** (153 mg, 0.40 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C. The cooling bath was removed, the mixture was stirred at room temperature for 3 h, concentrated in vacuo, and azeotroped with toluene to remove excess TFA to give **19** as a white solid. The solid was dried and then added to a solution of Boc-Ala-OH (91 mg, 0.48 mmol) in DMF (4 mL). PyBOP (250 mg, 0.48 mmol) and DIPEA (84 μL, 0.48 mmol) were added to the mixture and the reaction was stirred at room temperature for 45 min, after which time the reaction was diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash-column chromatography (petroleum ether/EtOAc 3:1→1:1) to afford title compound **20** (159 mg, 88 % over two steps) as a white amorphous solid after lyophilization with dioxane. $R_f = 0.40$ (petroleum ether/EtOAc 1:1); $[\alpha]_D = -32.1$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.31$ (t-like, 2 H), 5.29 (d, $J = 5.3$ Hz, 1 H), 4.94 (m, 1 H), 4.54 (dd, $J = 8.7$, 5.3 Hz, 1 H), 4.26 (m, 1 H), 3.84 (dd, $J = 7.7$, 1.9 Hz, 1 H), 3.75 (s, 3 H), 3.63 (dd, $J = 10.4$, 5.1 Hz, 1 H), 2.20 (m, 1 H), 1.45 (s, 9 H), 1.42 (d, $J = 7.2$ Hz, 3 H), 0.95 (d, $J = 6.8$ Hz, 3 H), 0.93 ppm (d, $J = 6.8$ Hz, 3 H); ^{13}C NMR ($CDCl_3$): $\delta = 173.0$, 171.7, 168.5, 155.7, 80.5, 57.6, 53.5, 52.1, 50.4, 32.6, 31.1, 28.2, 18.9, 17.8 ppm; MS (MALDI): m/z: 473, 475 $[M+Na]^+$, 489, 491 $[M+K]^+$; elemental analysis calcd (%) for $C_{17}H_{30}BrN_3O_6\cdot0.5\,C_4H_8O_2$ (496.4): C 45.97, H 6.90, N 9.29; found: C 45.95, H 7.25, N 9.06.

N-tert**-Butoxycarbonyl-L-alanyl-L-glycyl-β-bromo-L-alanyl-L-valine methyl ester (21)**: Product **21** was prepared following the procedure described for the synthesis of **20**. Compound **21** was obtained, after purification by flash-column chromatography (petroleum ether/EtOAc 1:1→0:100) and lyophilization with dioxane, as a white amorphous solid (89 % over two steps). $R_f = 0.13$ (petroleum ether/EtOAc 1:2); $[\alpha]_D = -23.5$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.72$ (d, $J = 8.3$ Hz, 1 H), 7.61 (t, $J = 5.3$ Hz, 1 H), 7.50 (d, $J = 9.1$ Hz, 1 H), 5.68 (d, $J = 7.1$ Hz, 1 H), 4.99 (m, 1 H), 4.52 (dd, $J = 8.7$, 5.4 Hz, 1 H), 4.25 (m, 1 H), 4.04 (d, $J = 5.4$ Hz, 2 H), 3.75 (s, 3 H), 3.68 (d, $J = 5.6$ Hz, 2 H), 2.19 (m, 1 H), 1.44 (s, 9 H), 1.38 (d, $J = 7.1$ Hz, 3 H), 0.94 (d, $J = 6.8$ Hz, 3 H), 0.91 ppm (d, $J = 6.8$ Hz, 3 H); ^{13}C NMR ($CDCl_3$): $\delta = 174.1$, 172.1, 169.4, 168.6, 155.7, 80.1, 57.6, 53.8, 52.2, 50.2, 53.2, 32.3, 31.1, 28.3, 18.9, 18.5, 17.9 ppm; MS (MALDI): m/z: 530, 532 $[M+Na]^+$, 546, 548 $[M+K]^+$; elemental analysis calcd (%) for $C_{19}H_{33}BrN_4O_7\cdot0.5\,C_4H_8O_2$ (553.4): C 45.57, H 6.74, N 11.00; found: C 45.55, H 6.74, N 10.98.

N**-Benzyloxycarbonyl-L-glycyl-L-glycyl-L-phenylalanyl-β-bromo-L-alanyl-L-alanine benzyl ester (23)**: TFA (1.5 mL) was added to a solution of Boc-protected dipeptide **3** (210 mg, 0.49 mmol) in dry CH_2Cl_2 (6 mL) at 0 °C. The cooling bath was removed, the mixture was stirred at room temperature for 3 h, concentrated in vacuo, and azeotroped with toluene to remove excess TFA to give **22** as a white solid. PyBOP (285 mg, 0.55 mmol) and DIPEA (102 μL, 0.58 mmol) were added to a solution of **22** and Z-Gly-Gly-Phe-OH (207 mg, 0.50 mmol) in dry DMF (4 mL) at 0 °C. The mixture was stirred for 45 min at room temperature, then diluted with EtOAc, washed successively with 0.5 N HCl and brine, dried over $MgSO_4$, and concentrated. The residue was crystallized from EtOAc to give the title compound **23** (295 mg, 83 % over two steps) as a white solid. $R_f = 0.11$ (EtOAc); $[\alpha]_D = -23.4$ ($c = 0.6$ in $CHCl_3$/MeOH 1:1);

^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 8.58$ (d, $J = 6.9$ Hz, 1H), 8.46 (d, $J = 8.0$ Hz, 1H), 7.34–7.15 (m, 15H), 5.10 (s, 2H), 5.01 (s, 2H), 4.60 (m, 2H), 4.32 (m, 1H), 3.76–3.38 (m, 6H), 3.02 (dd, $J = 14.1$, 4.9 Hz, 1H), 2.74 (dd, $J = 13.8$, 9.5 Hz, 1H), 1.31 ppm (d, $J = 7.2$ Hz, 3H); MS (MALDI): m/z: 747, 749 $[M+Na]^+$; elemental analysis calcd (%) for $C_{34}H_{38}BrN_5O_8$ (724.6): C 56.36, H 5.29, N 9.67; found: C 56.57, H 5.78, N 9.76.

***N-tert*-Butoxycarbonyl-L-alanyl-L-glycyl-L-glycyl-L-glycyl-γ-bromo-L-homoalanyl-L-isoleucine methyl ester (25)**: TFA (1 mL) was added to a solution of Boc-protected dipeptide **9** (110 mg, 0.27 mmol) in dry CH_2Cl_2 (4 mL) at 0°C. The cooling bath was removed, the mixture was stirred at room temperature for 2 h, concentrated in vacuo, and azeotroped with toluene to remove excess TFA to give **24** as a colourless oil which was used directly in the next reaction. PyBOP (143 mg, 0.27 mmol) and DIPEA (70 µL, 0.40 mmol) were added to a solution of **24** and Boc-Ala-Gly-Gly-Gly-OH (96 mg, 0.27 mmol) in dry DMF (2.4 mL). The mixture was stirred for 3 h at room temperature, diluted with EtOAc, washed successively with 0.5 N HCl and brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash-column chromatography ($CHCl_3$/MeOH 30:1→10:1) to afford hexapeptide **25** (122 mg, 70%) as a white amorphous solid. $R_f = 0.49$ ($CHCl_3$/MeOH 10:1); $[\alpha]_D = -8.6$ ($c = 0.5$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 4.88$ (overlapped with water peak, 1H), 4.65 (m, 1H), 4.39 (m, 1H), 4.04 (m, 1H), 3.90 (m, 6H), 3.70 (m, 3H), 3.49 (m, 1H), 2.31 (m, 2H), 1.92 (m, 1H), 1.44 (s, 9H), 1.40 (m, 2H), 1.33 (d, $J = 7.1$ Hz, 3H), 0.91 ppm (m, 6H); MS (MALDI): m/z: 672, 674 $[M+Na]^+$; elemental analysis calcd (%) for $C_{25}H_{43}BrN_6O_9$ (651.5): C 46.09, H 6.65, N 12.90; found: C 46.48, H 6.88, N 12.97.

***N*-Benzyloxycarbonyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-cysteine benzyl ester (30)**: A pH 8.5 solution of $NaHCO_3$ (3 mL) followed by TBAHS (203 mg, 0.6 mmol) were added to a solution of bromide **1** (60 mg, 0.15 mmol) and α-GlcNAc thiol **27** (70 mg, 0.19 mmol) in EtOAc (3 mL). The mixture was vigorously stirred at room temperature for 5 h, and was 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 residue was purified by flash-column chromatography (CH_2Cl_2/MeOH 40:1→30:1) to afford the α-GlcNAc glycoside **30** (88 mg, 87%) as a white amorphous solid. $R_f = 0.063$ (petroleum ether/EtOAc 1:1); $[\alpha]_D = +66.6$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.35$ (m, 10H), 6.16 (d, $J = 8.6$ Hz, 1H), 5.69 (d, $J = 8.8$ Hz, 1H), 5.30 (d, $J = 5.4$ Hz, 1H), 5.19 (d-like, 2H), 5.11 (d-like, 2H), 5.06 (t, $J = 9.4$ Hz, 1H), 4.96 (dd, $J = 11.2$, 9.4 Hz, 1H), 4.78 (m, 1H), 4.49 (m, 1H), 4.25–4.06 (m, 3H), 3.32 (dd, $J = 14.6$, 4.8 Hz, 1H), 3.04 (dd, $J = 14.6$, 3.4 Hz, 1H), 2.05, 2.04, 2.01, 1.95 ppm (4s, 12H); ^{13}C NMR ($CDCl_3$): $\delta = 171.5$, 170.6, 169.8, 169.1, 155.8, 136.0, 134.8, 128.7, 128.6, 128.5, 128.2, 128.0, 86.5, 70.9, 69.0, 67.8, 67.7, 67.1, 61.8, 54.3, 52.4, 36.0, 23.2, 20.6, 20.5 ppm; MS (MALDI): m/z: 697 $[M+Na]^+$, 713 $[M+K]^+$; elemental analysis calcd (%) for $C_{32}H_{38}N_2O_{12}S$ (674.7): C 56.96, H 5.68, N 4.15; found: C 56.67, H 5.64, N 4.18.

***N*-(9-Fluorenylmethoxycarbonyl)-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-cysteine *tert*-butyl ester (31)**: The reaction procedure was identical to that described for **30** except that bromide **2** was used instead of **1** and β-GlcNAc thiol **28** was used instead of **27**. Column chromatography was performed with petroleum ether/EtOAc (1:1→1:2). The β-thioglycoside **31** was isolated as a white solid in quantitative yield. $R_f = 0.13$ (petroleum ether/EtOAc 1:2); $[\alpha]_D = -41.1$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.78$ (d, $J = 7.3$ Hz, 2H), 7.63 (d, $J = 6.7$ Hz, 2H), 7.41 (t, $J = 7.2$ Hz, 2H), 7.33 (t, $J = 7.1$ Hz, 2H), 5.95 (dd, $J = 13.7$, 7.8 Hz, 2H), 5.18 (t, $J = 9.7$ Hz, 1H), 5.08 (t, $J = 9.5$ Hz, 1H), 4.71 (d, $J = 10.4$ Hz, 1H), 4.45 (m, 2H), 4.36–4.03 (m, 5H), 3.68 (m, 1H), 3.32 (dd, $J = 14.2$, 3.3 Hz, 1H), 2.86 (dd, $J = 14.2$, 8.1 Hz, 1H), 2.04, 2.03, 2.02, 1.88 (4s, 12H), 1.48 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 170.7$, 170.5, 170.3, 169.3, 169.1, 156.0, 143.6, 143.5, 141.0, 127.6, 127.0, 125.0, 124.9, 119.8, 83.2, 82.6, 75.8, 73.2, 68.3, 66.9, 62.1, 53.8, 52.7, 46.9, 27.8, 22.9, 20.5, 20.4 ppm; MS (MALDI): m/z: 751 $[M+Na]^+$, 767 $[M+K]^+$; elemental analysis calcd (%) for $C_{36}H_{44}N_2O_{12}S$ (728.8): C 59.33, H 6.08, N 3.84; found: C 59.08, H 6.15, N 4.06.

***N-tert*-Butoxycarbonyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-cysteinyl-L-valine methyl ester (32)**: The reaction procedure was identical to that described for **30** except that bromide **7** was used instead of **1**. Column chromatography was performed with petroleum ether/EtOAc (1:1→1:2). The α-thioglycoside **32** was isolated as a white solid in 78% yield. $R_f = 0.13$ (petroleum ether/EtOAc 1:2); $[\alpha]_D = +71.8$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 6.88$ (d, $J = 7.7$ Hz, 1H), 5.88 (d, $J = 8.6$ Hz, 1H), 5.56 (d, $J = 7.3$ Hz, 1H), 5.42 (d, $J = 4.2$ Hz, 1H), 5.14 (t, $J = 9.5$ Hz, 1H), 5.02 (dd, $J = 10.8$, 9.7 Hz, 1H), 4.53 (m, 2H), 4.40 (brs, 1H), 4.33 (m, 2H), 4.13 (d, $J = 10.6$ Hz, 1H), 3.76 (s, 3H), 3.24 (dd, $J = 14.1$, 5.9 Hz, 1H), 2.94 (dd, $J = 14.2$, 5.3 Hz, 1H), 2.19 (m, 1H), 2.13, 2.04, 1.98 (3s, 12H), 1.46 (s, 9H), 0.95 (d, $J = 6.8$ Hz, 3H), 0.91 ppm (d, $J = 6.8$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 178.4$, 172.0, 171.4, 170.7, 170.1, 169.2, 155.4, 86.4, 80.8, 71.1, 68.9, 68.0, 62.0, 57.2, 54.3, 52.2, 34.8, 31.2, 28.2, 23.1, 20.7, 20.5, 18.9, 17.6 ppm; MS (MALDI): m/z: 686 $[M+Na]^+$, 702 $[M+K]^+$; elemental analysis calcd (%) for $C_{28}H_{45}N_3O_{13}S$ (663.7): C 50.67, H 6.83, N 6.33; found: C 50.86, H 6.91, N 6.42.

***N-tert*-Butoxycarbonyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-homocysteinyl-L-alanine *tert*-butyl ester (33)**: The reaction procedure was identical to that descirbed for **30** except that bromide **8** was used instead of **1**. Column chromatography was performed with petroleum ether/EtOAc (1:1→1:2). The α-thioglycoside **33** was isolated as a white solid in 89% yield. $R_f = 0.037$ (petroleum ether/EtOAc 1.5:1); $[\alpha]_D = +78.8$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 6.69$ (d, $J = 7.1$ Hz, 1H), 5.80 (d, $J = 8.9$ Hz, 1H), 5.45 (d, $J = 4.9$ Hz, 1H), 5.17 (d, $J = 8.1$ Hz, 1H), 5.13 (t, $J = 9.8$ Hz, 1H), 5.05 (t, $J = 9.4$ Hz, 1H), 4.54 (ddd, $J = 14.4$, 9.2, 5.4 Hz, 1H), 4.41 (t-like, 2H), 4.28 (dd-like, 2H), 4.13 (dd, $J = 12.5$, 2.1 Hz, 1H), 2.79 (dt, $J = 13.8$, 7.0 Hz, 1H), 2.73 (dt, $J = 13.8$, 7.1 Hz, 1H), 2.13 (m, 1H), 2.09, 2.05, 2.03, 1.98 (4s, 12H), 1.92 (m, 1H), 1.46 (s, 9H), 1.44 (s, 9H), 1.37 ppm (d, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 171.7$, 171.4, 170.63, 170.55, 169.6, 169.3, 155.4, 85.6, 82.1, 80.2, 71.3, 68.7, 68.1, 62.0, 53.0, 52.2, 48.7, 33.3, 28.5, 27.9, 23.2, 20.7, 20.6, 18.2 ppm; MS (MALDI): m/z: 715 $[M+Na]^+$, 731 $[M+K]^+$; elemental analysis calcd (%) for $C_{30}H_{49}N_3O_{13}S$ (691.8): C 52.09, H 7.14, N 6.07; found: C 52.05, H 7.36, N 5.98.

***N-tert*-Butoxycarbonyl-L-alanyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-cysteine benzyl ester (34)**: The reaction procedure was identical to that described for **30** except that bromide **4** was used instead of **1**. Column chromatography was performed with EtOAc/MeOH (60:1). The α-thioglycoside **34** was isolated as a white solid in 83% yield. $R_f = 0.59$ (EtOAc/MeOH 20:1); $[\alpha]_D = +65.4$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.36$ (m, 5H), 7.25 (d, $J = 8.2$ Hz, 1H), 5.73 (d, $J = 8.8$ Hz, 1H), 5.29 (d, $J = 5.2$ Hz, 1H), 5.18 (s, 2H), 5.09 (t, $J = 9.6$ Hz, 1H), 4.98 (m, 2H), 4.91 (dd, $J = 11.2$, 9.3 Hz, 1H), 4.49 (m, 1H), 4.30 (dd, $J = 12.1$, 1.6 Hz, 1H), 4.20 (m, 4H), 3.16 (m, 1H), 2.13, 2.07, 2.04, 1.96 (4s, 12H), 1.45 (s, 9H), 1.36 ppm (d, $J = 7.1$ Hz, 3H); MS (MALDI): m/z: 734 $[M+Na]^+$, 750 $[M+K]^+$; elemental analysis calcd (%) for $C_{32}H_{45}N_3O_{13}S$ (711.8): C 54.00, H 6.37, N 5.90; found: C 53.82, H 6.25, N 5.72.

***N-tert*-Butoxycarbonyl-L-alanyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-cysteine benzyl ester (35)**: The reaction procedure was identical to that described for **34** except that β-GlcNAc thiol **28** was used instead of α-GlcNAc thiol **27**. β-Thioglycoside **35** was isolated as a white solid in 87% yield. $R_f = 0.037$ (petroleum ether/EtOAc 1:2); $[\alpha]_D = -41.8$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.37$ (m, 5H), 7.04 (d, $J = 6.8$ Hz, 1H), 6.29 (d, $J = 8.2$ Hz, 1H), 5.47 (d, $J = 7.4$ Hz, 1H), 5.29 (t, $J = 9.7$ Hz, 1H), 5.18 (s, 2H), 5.05 (t, $J = 9.5$ Hz, 1H), 4.84 (d, $J = 10.4$ Hz, 1H), 4.82 (m, 1H), 4.24 (dd, $J = 12.6$, 5.0 Hz, 1H), 4.19 (t, $J = 7.3$ Hz, 1H), 4.09 (dd, $J = 12.6$, 1.7 Hz, 1H), 3.84 (q, $J = 10.2$ Hz, 1H), 3.68 (m, 1H), 3.32 (dd, $J = 14.5$, 4.1 Hz, 1H), 3.06 (dd, $J = 14.8$, 5.9 Hz, 1H), 2.05, 2.02, 2.01, 1.99 (4s, 12H), 1.46 (s, 9H), 1.41 ppm (d, $J = 7.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 173.0$, 170.8, 170.7, 170.5, 169.9, 169.3, 155.5, 134.8, 128.6, 128.3, 84.0, 80.1, 75.8, 73.5, 68.4, 67.7, 62.0, 53.9, 51.6, 50.4, 31.8, 28.3, 23.2, 20.7, 20.6, 18.3 ppm; MS (MALDI): m/z: 734 $[M+Na]^+$, 750 $[M+K]^+$; elemental analysis calcd (%) for $C_{32}H_{45}N_3O_{13}S$ (711.8): C 54.00, H 7.37, N 5.90; found: C 53.99, H 6.44, N 6.08.

***N-tert*-Butoxycarbonyl-L-tryptophanyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-cysteine benzyl ester (36)**: The reaction procedure was identical to that described for **30** except that bromide **5** was used instead of **1**. The crude product was purified by column chromatography using petroleum ether/EtOAc (1:1→1:1.2) as eluent to give α-thioglycoside **36** as a colorless syrup in 79% yield. $R_f = 0.54$ (EtOAc/MeOH 25:1); $[\alpha]_D = +58.2$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 9.11$ (brs, 1H), 7.65 (d, $J = 8.0$ Hz, 1H), 7.34 (m, 5H), 7.28 (m, 2H), 7.21 (d, $J = 7.1$ Hz, 1H), 7.16 (t, $J = 6.9$ Hz, 1H), 7.09 (t, $J =$

7.5 Hz, 1H), 7.02 (brs, 1H), 5.53 (d, J=9.4 Hz, 1H), 5.18 (m, 1H), 5.09 (AB quartet, J=12.0 Hz, 2H), 4.99 (t-like, J=3.6 Hz, 1H), 4.72 (brs, 1H), 4.66 (m, 2H), 4.24 (m, 2H), 3.86 (t-like, J=8.0 Hz, 1H), 3.73 (brt, 1H), 3.66 (dd, J=14.6, 3.6 Hz, 1H), 3.62 (brs, 1H), 3.01 (dd, J=14.7, 2.8 Hz, 2H), 2.07, 2.04, 2.033, 2.030 (4s, 12H), 1.49 ppm (s, 9H); ^{13}C NMR (CDCl$_3$): δ=171.2, 170.8, 170.4, 169.2, 168.9, 155.4, 136.1, 134.8, 128.7, 128.5, 127.7, 123.0, 122.1, 119.5, 111.2, 109.9, 87.0, 80.6, 70.8, 69.9, 67.9, 67.5, 67.0, 61.7, 55.3, 52.6, 52.0, 36.9, 28.2, 23.4, 20.8, 20.54, 20.50 ppm; MS (MALDI): m/z: 850 [M+Na]$^+$; elemental analysis calcd (%) for $C_{40}H_{50}N_4O_{13}S$ (826.9): C 58.04, H 6.09, N 6.78; found: C 57.64, H 6.06, N 6.43.

***N-tert*-Butoxycarbonyl-L-tyrosinyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-cysteine benzyl ester (37)**: The reaction procedure was identical to that described for **30** except that bromide **6** was used instead of **1** and α-GalNAc thiol **26** was used instead of **27**. The crude product was purified by column chromatography using petroleum ether/EtOAc (1:1→1:2) as eluent to give the title compound **37** as a white solid after lyophilization with dioxane in 63% yield. R_f=0.10 (petroleum ether/EtOAc 1:2); $[\alpha]_D$=+69.5 (c=1.0 in CHCl$_3$); ^{1}H NMR (250 MHz, CDCl$_3$): δ=7.68 (brs, 1H), 7.36 (m, 5H), 7.27 (d, J=7.1 Hz, 1H), 6.99 (d, J=8.4 Hz, 2H), 6.75 (d, J=8.4 Hz, 2H), 5.62 (d, J=9.9 Hz, 1H), 5.17 (AB quartet, J=11.9 Hz, 2H), 5.16 (d-like, 1H), 5.00 (m, 1H), 4.82 (d-like, J=10.2 Hz, 1H), 4.62 (m, 1H), 4.60 (dd, J=11.4, 2.9 Hz, 1H), 4.46 (dd, J=10.1, 5.0 Hz, 1H), 4.31 (m, 2H), 4.03 (m, 1H), 3.83 (dd, J=10.6, 7.9 Hz, 1H), 3.48 (dd, J=13.6, 2.4 Hz, 1H), 3.28 (dd, J=14.9, 4.2 Hz, 1H), 2.90 (dd, J=14.8, 1.6 Hz, 1H), 2.78 (dd, J=13.7, 5.9 Hz, 1H), 2.23, 2,11, 2.05, 1.99 (4s, 12H), 1.49 ppm (s, 9H); ^{13}C NMR (CDCl$_3$): δ=170.8, 170.7, 170.47, 170.43, 170.1, 168.9, 156.0, 155.4, 134.7, 131.0, 128.9, 128.8, 128.6, 127.3, 116.1, 88.5, 80.8, 68.9, 68.7, 67.8, 66.9, 61.6, 55.2, 53.1, 47.7, 36.7, 36.0, 28.2, 23.6, 20.7, 20.6, 20.5 ppm; MS (MALDI): m/z: 827 [M+Na]$^+$, 843 [M+K]$^+$; elemental analysis calcd (%) for $C_{38}H_{49}N_3O_{14}S$ (803.9): C 56.78, H 6.14, N 5.23; found: C 56.56, H 6.40, N 5.05.

***N-tert*-Butoxycarbonyl-L-alanyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-cysteinyl-L-proline benzyl ester (38)**: The reaction procedure was identical to that described for **30** except that bromide **10** was used instead of **1**. The crude product was purified by column chromatography using EtOAc/MeOH (100:1) as eluent to give the title compound **38** as a white solid after lyophilization with dioxane in 65% yield. R_f=0.46 (EtOAc/MeOH 20:1); $[\alpha]_D$=+35.3 (c=1.2 in CHCl$_3$); ^{1}H NMR (250 MHz, CDCl$_3$): δ=7.36 (m, 5H), 7.08 (d, J=8.2 Hz, 1H), 5.91 (d, J=8.8 Hz, 1H), 5.44 (t-like, J=5.4 Hz, 1H), 5.24–4.96 (m, 6H), 4.53 (m, 2H), 4.41–4.15 (m, 4H), 3.67 (m, 2H), 3.04 (dd, J=14.2, 3.8 Hz, 1H), 2.89 (dd, J=14.5, 7.1 Hz, 1H), 2.10 (m, 4H), 2.10, 2.04, 1.97 (3s, 12H), 1.46 (s, 9H), 1.36 ppm (d, J=7.1 Hz, 3H); ^{13}C NMR (CDCl$_3$): δ=172.5, 171.4, 171.3, 170.6, 170.1, 169.2, 168.3, 155.4, 135.5, 128.5, 128.3, 128.1, 86.5, 80.2, 71.4, 68.7, 67.9, 67.0, 61.6, 58.8, 52.2, 50.6, 50.2, 47.0, 34.5, 28.8, 28.2, 24.7, 23.1, 20.7, 20.64, 20.56, 17.9 ppm; MS (MALDI): m/z: 831 [M+Na]$^+$, 847 [M+K]$^+$; elemental analysis calcd (%) for $C_{37}H_{52}N_4O_{14}S$ (808.9): C 54.94, H 6.48, N 6.93; found: C 55.09, H 6.80, N 7.20.

***N-tert*-Butoxycarbonyl-L-tryptophanyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-cysteinyl-L-isoleucine methyl ester (39)**: The reaction procedure was identical to that described for **30** except that bromide **11** was used instead of **1** and α-GalNAc thiol **26** was used instead of **27**. The crude product was purified by column chromatography using petroleum ether/EtOAc (1:2) → EtOAc/MeOH (100:1) as eluent to give the α-glycotripeptide **39** as a white solid after lyophilization with dioxane in 82% yield. R_f=0.38 (EtOAc/MeOH 25:1); $[\alpha]_D$=+62.6 (c=1.0 in CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$): δ=9.03 (brs, 1H), 7.69 (d, J=7.6 Hz, 1H), 7.37 (d, J=7.8 Hz, 1H), 7.19 (t, J=7.4 Hz, 1H), 7.11 (dd, J=15.0, 7.5 Hz, 2H), 7.05 (d, J=6.5 Hz, 1H), 6.86 (d, J=6.7 Hz, 1H), 5.78 (brs, 1H), 5.21 (brs, 1H), 5.19 (d, J=8.2 Hz, 1H), 4.87 (dd, J=11.7, 2.9 Hz, 1H), 4.72 (brs, 1H), 4.65 (d-like, J=3.9 Hz, 3H), 4.51 (dd, J=8.5, 5.0 Hz, 1H), 4.09 (brs, 1H), 3.92 (m, 1H), 3.73 (s, 3H), 3.71 (d, J=4.3 Hz, 1H), 3.49 (dd, J=14.5, 3.6 Hz, 1H), 3.12 (dd, J=14.5, 6.6 Hz, 1H), 3.04 (brd-like, J=7.6 Hz, 1H), 2.48 (d, J=10.3 Hz, 1H), 2.12, 2.04, 2.03, 2.02 (4s, 12H), 1.88 (m, 1H), 1.46 (s, 9H), 1.18 (m, 2H), 0.90 (t, J=7.3 Hz, 3H), 0.88 ppm (d, J=6.8 Hz, 3H); ^{13}C NMR (CDCl$_3$): δ=172.1, 171.9, 171.1, 170.5, 170.3, 170.1, 168.8, 155.4, 136.2, 127.7, 123.1, 122.3, 119.7, 119.1, 111.4, 110.0, 86.4, 80.5, 68.3, 67.9, 67.0, 61.7, 56.6, 55.3, 53.3, 52.2, 48.1, 37.7, 34.0, 28.2, 25.1, 23.4, 20.7, 20.6, 15.4, 11.5 ppm; MS (MALDI): m/z: 886 [M+Na]$^+$, 902 [M+K]$^+$; elemental analysis calcd (%) for $C_{40}H_{57}N_5O_{14}S$ (864.0): C 55.61, H 6.65, N 8.11; found: C 55.45, H 6.82, N 7.83.

***N-tert*-Butoxycarbonyl-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-cysteinyl-L-valinyl-L-proline *tert*-butyl ester (40)**: The reaction procedure was identical to that described for **30** except that bromide **12** was used instead of **1** and α-GalNAc thiol **26** was used instead of **27**. The crude product was purified by column chromatography using petroleum ether/EtOAc (1:2) → EtOAc/MeOH (100:1) as eluent to give the *S*-glycotripeptide **40** as a white solid after lyophilization with dioxane in 72% yield. R_f=0.04 (petroleum ether/EtOAc 1:2.7); $[\alpha]_D$=+50.2 (c=0.5 in CHCl$_3$); ^{1}H NMR (250 MHz, CDCl$_3$): δ=6.98 (d, J=8.5 Hz, 1H), 6.09 (brs, 1H), 5.66 (d, J=8.4 Hz, 1H), 5.38 (m, 2H), 4.97 (dd, J=11.8, 2.9 Hz, 1H), 4.88 (dd, J=8.7, 4.9 Hz, 1H), 4.64 (dd, J=8.7, 5.6 Hz, 1H), 4.52 (t, J=6.8 Hz, 1H), 4.39 (m, 2H), 4.14 (m, 2H), 3.70 (m, 2H), 3.39 (dd, J=14.5, 4.4 Hz, 1H), 2.84 (dd, J=14.5, 5.9 Hz, 1H), 2.17, 2.11 (2s, 6H), 2.00 (s, 6H), 2.30–1.90 (m, 5H), 1.44 (s, 18H), 1.05 (d, J=6.8 Hz, 3H), 0.94 ppm (d, J=6.7 Hz, 3H); ^{13}C NMR (CDCl$_3$): δ=171.1, 170.7, 170.5, 170.4, 170.3, 169.8, 169.7, 155.3, 87.3, 81.3, 80.6, 68.4, 67.9, 67.3, 62.0, 59.8, 55.5, 54.0, 48.0, 47.3, 35.2, 31.4, 29.1, 28.2, 27.9, 24.9, 23.2, 20.7, 19.6, 17.3 ppm; MS (MALDI): m/z: 825 [M+Na]$^+$, 841 [M+K]$^+$; elemental analysis calcd (%) for $C_{36}H_{58}N_4O_{14}S$ (802.9): C 53.85, H 7.28, N 6.98; found: C 53.64, H 7.49, N 6.51.

***N-tert*-Butoxycarbonyl-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-cysteinyl-L-valinyl-L-proline *tert*-butyl ester (41)**: A pH 8.5 solution of NaHCO$_3$ (3 mL) followed by TBAHS (136 mg, 0.40 mmol) were added to a solution of bromotripeptide **12** (54 mg, 0.10 mmol) and peracetyl β-glucosyl thiol (80 mg, 0.20 mmol) in EtOAc (3 mL). The mixture was vigorously stirred at room temperature for 5 h, and was 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 which was purified by flash-column chromatography (petroleum ether/EtOAc 2:1→1:1) to afford the β-thioglycoside **41** (78 mg, 96%) as a white amorphous solid. R_f=0.13 (petroleum ether/EtOAc 1.3:1); $[\alpha]_D$=−50.3 (c=1.2 in CHCl$_3$); ^{1}H NMR (250 MHz, 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 ppm (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 ppm; MS (MALDI): m/z: 827 [M+Na]$^+$, 843 [M+K]$^+$; elemental analysis calcd (%) for $C_{36}H_{57}N_3O_{15}S$ (803.9): C 53.79, H 7.15, N 5.23; found: C 54.22, H 7.52, N 4.92.

***N-tert*-Butoxycarbonyl-L-alanyl-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-cysteinyl-L-serine benzyl ester (42)**: The reaction procedure was identical to that described for **41** except that bromide **17** was used instead of **12**. The crude product was purified by column chromatography using petroleum ether/EtOAc (1:1) and then EtOAc as eluent to give the *S*-glycotripeptide **42** as a white solid after lyophilization with dioxane in 78% yield. R_f=0.44 (EtOAc); $[\alpha]_D$=−10.8 (c=1.0 in CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$): δ=7.44 (d, J=6.9 Hz, 1H), 7.38 (m, 5H), 7.19 (d, J=7.0 Hz, 1H), 5.26 (t, J=9.3 Hz, 1H), 5.23 (q-like, 2H), 5.16 (d, J=6.0 Hz, 1H), 5.07 (t, J=9.7 Hz, 1H), 4.99 (t, J=9.6 Hz, 1H), 4.81 (q, J=4.9 Hz, 1H), 4.74 (d, J=10.1 Hz, 1H), 4.69 (t-like, J=3.7 Hz, 1H), 4.37 (d, J=12.1 Hz, 1H), 4.20 (dd, J=12.5, 5.1 Hz, 1H), 4.16 (t, J=6.2 Hz, 1H), 3.98 (dd, J=11.7, 3.6 Hz, 1H), 3.90 (m, 2H), 3.04 (dd, J=13.7, 3.6 Hz, 1H), 2.83 (dd, J=13.8, 8.7 Hz, 1H), 2.053, 2.048, 2.03, 2.02 (4s, 12H), 1.43 (s, 9H), 1.37 ppm (d, J=7.1 Hz, 3H); ^{13}C NMR (CDCl$_3$): δ=172.8, 171.4, 170.0, 169.6, 169.4, 155.3, 135.2, 128.6, 128.4, 128.3, 85.5, 80.4, 76.2, 73.7, 69.7, 68.1, 67.4, 62.8, 61.9, 55.3, 52.5, 50.5, 34.1, 28.2, 20.6, 20.5, 18.3 ppm; MS (MALDI): m/z: 822 [M+Na]$^+$, 838 [M+K]$^+$; elemental analysis calcd (%) for $C_{35}H_{49}N_3O_{16}S$ (799.8): C 52.56, H 6.17, N 5.25; found: C 52.48, H 6.31, N 5.50.

***N*-(9-Fluorenylmethoxycarbonyl)-*S*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-cysteinyl-L-alanine benzyl ester (43)**: A pH 8.5 solution of NaHCO$_3$ (0.8 mL) followed by H$_2$O (1.7 mL) were added to a solution of bromodipeptide **14** (82 mg, 0.15 mmol) and β-

GlcNAc thiol **28** (93 mg, 0.26 mmol) in DMF (4 mL). The resultant mixture was stirred at room temperature for 3 h, and was 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 which was purified by flash-column chromatography (petroleum ether/EtOAc 1:1→0:1) to afford the title compound **43** (110 mg, 89%) as a white amorphous solid. $R_f=0.44$ (CH_2Cl_2/MeOH 12:1); $[\alpha]_D=-30.2$ ($c=0.9$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta=$ 7.78 (d, $J=7.2$ Hz, 2H), 7.59 (d, $J=7.3$ Hz, 2H), 7.37 (m, 9H), 7.09 (d, $J=8.5$ Hz, 1H), 5.90 (t, $J=9.2$ Hz, 2H), 5.22 (m, 2H), 5.10 (m, 2H), 4.66–4.38 (m, 5H), 4.23–4.08 (m, 4H), 3.73 (brs, 1H), 2.96 (d, $J=6.8$ Hz, 2H), 2.04, 2.00, 1.92 (3s, 12H), 1.45 ppm (d, $J=6.9$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta=172.3$, 170.9, 170.6, 170.3, 169.9, 169.2, 155.8, 143.5, 141.2, 135.2, 128.5, 128.3, 128.1, 127.7, 127.0, 124.9, 119.9, 85.3, 75.8, 73.6, 68.3, 67.2, 67.0, 62.2, 54.4, 52.7, 48.5, 46.9, 32.8, 23.1, 20.57, 20.55, 20.51, 17.7 ppm; MS (MALDI): m/z: 857 $[M+Na]^+$, 873 $[M+K]^+$; elemental analysis calcd (%) for $C_{42}H_{47}N_3O_{13}S$ (833.9): C 60.49, H 5.68, N 5.04; found: C 60.13, H 6.07, N 4.85.

***N-tert*-Butoxycarbonyl-L-alanyl-L-glycyl-*S*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl]-L-cysteinyl-L-valine methyl ester (44)**: The reaction procedure was identical to that described for **43** except that bromide **21** was used instead of **14** and β-lactosyl thiol **29** was used instead of **28**. The crude product was purified by column chromatography using petroleum ether/EtOAc (1:1) and then EtOAc as eluent to give the β-thioglycoside **44** as a white solid in 95% yield. $R_f=0.40$ (EtOAc); $[\alpha]_D=-14.0$ ($c=0.5$ in $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta=7.12$ (d, $J=8.6$ Hz, 1H), 7.11 (d, $J=5.7$ Hz, 1H), 6.98 (brs, 1H), 5.36 (d, $J=3.1$ Hz, 1H), 5.23 (t, $J=9.1$ Hz, 1H), 5.17 (d, $J=6.2$ Hz, 1H), 5.12 (dd, $J=10.3$, 8.0 Hz, 1H), 4.98 (dd, $J=10.1$, 2.7 Hz, 1H), 4.93 (t, $J=9.6$ Hz, 1H), 4.78 (q, $J=6.3$ Hz, 1H), 4.72 (d, $J=11.4$ Hz, 1H), 4.65 (d, $J=10.2$ Hz, 1H), 4.53 (d, $J=7.9$ Hz, 1H), 4.45 (dd, $J=8.5$, 5.4 Hz, 1H), 4.20 (brt-like, 1H), 4.15 (dd, $J=11.1$, 6.3 Hz, 1H), 4.08 (m, 2H), 4.00 (dd, $J=16.9$, 5.2 Hz, 1H), 3.94 (dd, $J=16.9$, 5.3 Hz, 1H), 3.91 (brt-like, 1H), 3.80 (t, $J=9.1$ Hz, 1H), 3.77 (dd, $J=5.9$, 0.9 Hz, 1H), 3.74 (s, 3H), 2.99 (dd, $J=14.4$, 5.5 Hz, 1H), 2.93 (t-like, 1H), 2.16, 2.10, 2.08, 2.07, 2.05, 1.97 (6s, 21H), 1.94 (m, 1H), 1.45 (s, 9H), 1.39 (d, $J=7.1$ Hz, 3H), 0.94 ppm (d, $J=6.8$ Hz, 6H); ^{13}C NMR ($CDCl_3$): $\delta=173.2$, 171.7, 170.6, 170.4, 170.13, 170.05, 169.8, 169.7, 169.6, 169.0, 168.5, 155.6, 101.0, 84.7, 80.4, 76.0, 73.5, 71.0, 70.7, 70.2, 69.1, 66.6, 61.9, 60.8, 57.7, 53.0, 52.1, 50.3, 42.9, 33.4, 31.0, 28.3, 20.74, 20.69, 20.63, 20.5, 18.9, 18.2, 17.9 ppm; MS (MALDI): m/z: 1103 $[M+Na]^+$, 1119 $[M+K]^+$; elemental analysis calcd (%) for $C_{45}H_{68}N_4O_{24}S$ (1081.1): C 49.99, H 6.34, N 5.18; found: C 50.06, H 6.44, N 4.97.

***N*-Benzyloxycarbonyl-L-glycyl-L-glycyl-L-phenylalanyl-*S*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl]-L-cysteinyl-L-alanine benzyl ester (45)**: The reaction procedure was identical to that described for **43** except that bromide **23** was used instead of **14** and β-lactosyl thiol **29** was used instead of **28**. The crude product was purified by column chromatography using petroleum ether/EtOAc (1:1) → EtOAc/MeOH (20:1) as eluent to give the glycopentapeptide **45** as a white solid in 94% yield. $R_f=0.71$ (EtOAc/MeOH 10:1); $[\alpha]_D=-14.5$ ($c=1.0$ in $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta=7.90$ (brs, 1H), 7.63 (brs, 1H), 7.49 (brs, 1H), 7.32 (m, 13H), 7.18 (t, $J=7.3$ Hz, 1H), 7.14 (d, $J=7.1$ Hz, 1H), 7.07 (d, $J=7.3$ Hz, 1H), 6.32 (brs, 1H), 5.35 (d, $J=3.4$ Hz, 1H), 5.19 (t, $J=9.2$ Hz, 1H), 5.15 (AB quartet, $J=12.7$ Hz, 2H), 5.10 (m, 4H), 4.98 (dd, $J=10.3$, 3.2 Hz, 1H), 4.88 (t, $J=9.7$ Hz, 1H), 4.83 (d-like, $J=6.1$ Hz, 1H), 4.63 (d, $J=10.3$ Hz, 2H), 4.59 (t, $J=7.1$ Hz, 1H), 4.51 (d, $J=7.8$ Hz, 1H), 4.14 (dd, $J=11.1$, 6.1 Hz, 1H), 4.07 (m, 3H), 3.97 (m, 3H), 3.89 (t, $J=6.8$ Hz, 1H), 3.77 (t, $J=9.4$ Hz, 1H), 3.68 (m, 1H), 3.06 (dd, $J=12.9$, 5.2 Hz, 1H), 3.00 (dd, $J=12.9$, 5.5 Hz, 1H), 2.92 (dd, $J=12.9$, 5.7 Hz, 1H), 2.88 (dd, $J=13.4$, 5.4 Hz, 1H), 2.14, 2.052, 2.045, 2.03, 2.02, 1.97, 1.96 (7s, 21H), 1.38 ppm (d, $J=7.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta=172.0$, 170.6, 170.5, 170.4, 170.1, 170.0, 169.8, 169.6, 169.3, 169.1, 168.3, 156.8, 136.4, 136.1, 135.4, 129.4, 128.6, 128.5, 128.4, 128.2, 128.1, 127.9, 126.9, 100.9, 84.7, 76.0, 73.5, 71.0, 70.6, 70.4, 69.1, 67.0, 66.7, 61.8, 60.8, 54.1, 52.9, 48.4, 44.3, 43.2, 39.1, 34.3, 20.8, 20.6, 20.5, 17.9 ppm; MS (MALDI): m/z: 1318 $[M+Na]^+$, 1334 $[M+K]^+$; elemental analysis calcd (%) for $C_{60}H_{73}N_5O_{25}S$ (1296.3): C 55.59, H 5.68, N 5.40; found: C 55.16, H 5.79, N 5.37.

***N-tert*-Butoxycarbonyl-L-alanyl-L-glycyl-L-glycyl-L-glycyl-*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 (46)**: The reaction procedure was identical to that described for **43** except that bromide **25** was used instead of **14** and β-lactosyl thiol **29** was used instead of **28**. The crude product was purified by column chromatography using EtOAc/MeOH (20:1→5:1) as eluent to give the glycohexapeptide **46** as a white solid in 76% yield. $R_f=0.48$ (EtOAc/MeOH 5:1); $[\alpha]_D=-14.0$ ($c=0.1$ in $CHCl_3$); ^1H NMR (600 MHz, CD_3OD): $\delta=5.37$ (d, $J=3.5$ Hz, 1H), 5.24 (t, $J=9.2$ Hz, 1H), 5.13 (dd, $J=10.4$, 3.4 Hz, 1H), 5.02 (dd, $J=10.4$, 7.9 Hz, 1H), 4.87 (overlapped with water peak, 2H), 4.74 (dd, $J=10.1$, 3.3 Hz, 1H), 4.71 (t, $J=7.9$ Hz, 1H), 4.53 (m, 2H), 4.38 (dd, $J=10.9$, 6.3 Hz, 1H), 4.15 (m, 4H), 4.09 (q, $J=7.1$ Hz, 1H), 3.98 (dd, $J=16.9$, 8.8 Hz, 1H), 3.90 (m, 7H), 3.79 (m, 1H), 3.73 (m, 3H), 2.72 (m, 2H), 2.14, 2.13 (2s, 6H), 2.10 (overlapped m, 1H), 2.064, 2.056, 2.03, 1.93 (4s, 15H), 1.45 (s, 9H), 1.44 (overlapped, 1H), 1.35 (d, $J=7.2$ Hz, 3H), 1.32 (m, 1H), 0.94 (d, $J=7.0$ Hz, 3H), 0.93 ppm (t, $J=6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$): $\delta=173.6$, 173.5, 172.5, 172.1, 172.0, 171.9, 171.7, 171.6, 171.4, 171.3, 171.2, 102.1, 84.9, 80.9, 77.9, 77.2, 75.4, 72.5, 71.7, 70.7, 68.6, 63.7, 62.3, 58.3, 53.7, 52.6, 52.1, 43.9, 43.6, 38.3, 34.1, 28.8, 28.0, 26.3, 21.1, 20.9, 20.7, 20.6, 20.5, 17.9, 16.0, 11.8 ppm; MS (MALDI): m/z: 1245 $[M+Na]^+$; elemental analysis calcd (%) for $C_{51}H_{78}N_6O_{26}S$ (1223.3): C 50.08, H 6.43, N 6.87; found: C 50.01, H 6.60, N 6.81.

***N-tert*-Butoxycarbonyl-L-alanyl-*S*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-cysteinyl-L-valine methyl ester (48)**: α-GlcNAc thiol **27** (230 mg, 0.63 mmol) was dissolved in MeOH (12 mL). NaOMe (1.3 mL, 1.0 M in MeOH) was added and the reaction was stirred at room temperature for 2 h, after which time $NaHCO_3$ (114 mg) was added and the solvent was evaporated. The resultant solid was dried and used in the next reaction without purification. The sodium salt of **47** was dissolved in a pH 8.5 solution of $NaHCO_3$ (4 mL). To this solution was added a solution of bromotripeptide **20** (158 mg, 0.35 mmol) in DMF (4 mL), then a small amount of water was added until the mixture became clear. After stirring at room temperature for 3 h the mixture was neutralized with 0.5 N HCl and the solvent was evaporated under reduced pressure. The residue was purified by flash-column chromatography ($CHCl_3$/MeOH 6:1→4:1) to afford the title compound **48** (149 mg, 70%) as a white amorphous solid. $R_f=0.16$ ($CHCl_3$/MeOH 8:1); $[\alpha]_D=+58.7$ ($c=0.5$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta=7.85$ (m, 2H), 7.20 (d, $J=6.4$ Hz, 1H), 5.93 (d, $J=5.5$ Hz, 1H), 5.50 (d, $J=5.1$ Hz, 1H), 4.70 (brs, 1H), 4.42 (m, 1H), 4.19 (brs, 2H), 3.97 (m, 2H), 3.81–3.45 (m, 10H), 3.02 (m, 1H), 2.15 (m, 1H), 2.03 (s, 3H), 1.44 (s, 9H), 1.35 (d, $J=7.0$ Hz, 3H), 0.92 ppm (d, $J=6.8$ Hz, 6H); MS (MALDI): m/z: 632 $[M+Na]^+$, 648 $[M+K]^+$; elemental analysis calcd (%) for $C_{25}H_{44}N_4O_{11}S$ (608.7): C 49.33, H 7.29, N 9.20; found: C 49.29, H 7.42, N 8.98.

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[26] This procedure is a significant advance in the convergent assembly of S-linked glycopeptides since there have not been any reports on direct S-glycosylation prior to this work. An advantage of this procedure is the ready availability of peptides that contain cysteine or homocysteine. For instance, through native chemical ligation techniques, see: ref. [9].

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[30] Compound **33** and **46** are considered to mimic native N-linked glycopeptides based on the viewpoint that the distance between the sugar and the peptide backbone in **33** and **46** is similar to the N-glycan linkage in asparagines.

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[32] It is worth mentioning that α-thioglycosides **37**, **39**, and **40** mimic the important Tn antigen structure.

[33] β-S-Linked glycopeptides can also be prepared by our previous procedure, see ref. [9].

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