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# Synthesis of Novel *S*-Neoglycopeptides from Glycosylthiomethyl Derivatives

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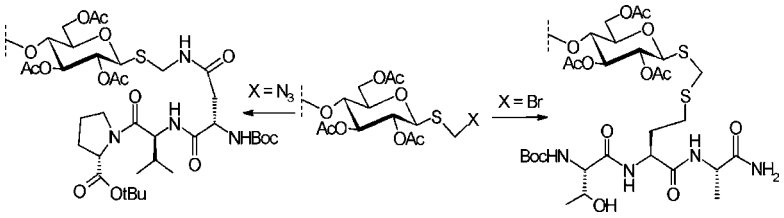
## ABSTRACT




**Reaction of glycosylthiomethyl azides with amino acid and peptide derivatives containing aspartate and glutamate thio acids gave the corresponding glycosylthiomethyl amides in excellent yields. Another type of neoglycopeptides was obtained via reaction of glycosylthiomethyl bromide with cysteine and homocysteine containing peptide derivatives, thus affording the corresponding *S*-(glycosylthiomethyl) peptides.**

Glycoproteins are widely distributed in nature and play a variety of biological roles.[1] Their biosynthesis is based on post-translational site-selective glycosylation of the peptide backbone. Glycopeptides,[2] which retain the carbohydrate−peptide linkage region of a glycoprotein but lack its size and complexity, are much more amenable to study and are important models for glycoproteins. As a consequence, significant attention has been paid to synthesize glycopeptides during the past two decades.[3] However, site-selective attachment of glycosyl residues to serine and threonine or asparagine residues in order to obtain natural *O*- and *N*-glycopeptides is generally not possible. This has led to considerable efforts toward the synthesis of modified glycopeptides, often termed neoglycopeptides or glycopeptide mimetics.[4] They possess non-native sugar−peptide linkages,[5] and importantly, the resulting neoglycopeptides have found use in various biological studies and demonstrated bioactivities comparable to the native glycopeptides.[5a,6,7]

For the desirable site-selective attachment of glycosyl residues to prepared peptides chemoselective reactions of sugars with hydroxylamine and hydrazine derivatives have been successfully employed (**I**−**III** in Figure 1). Additionally, several groups have exploited the unique reactivity of cysteine (Cys) or homocysteine (Hcy) residues. For example, *N*-glycosyliodoacetamide has been designed[7] and coupled

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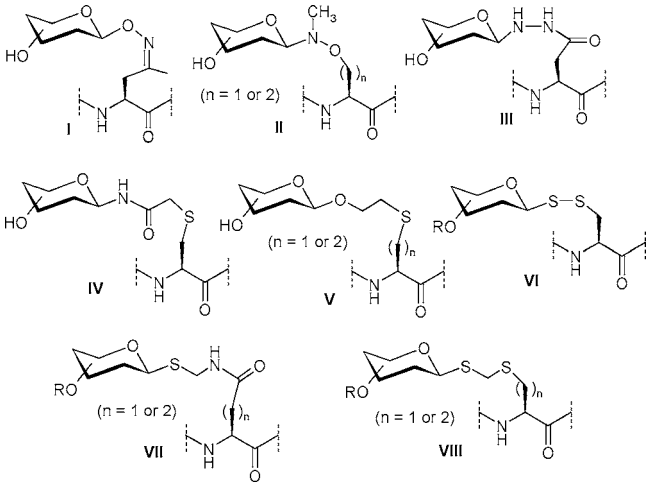


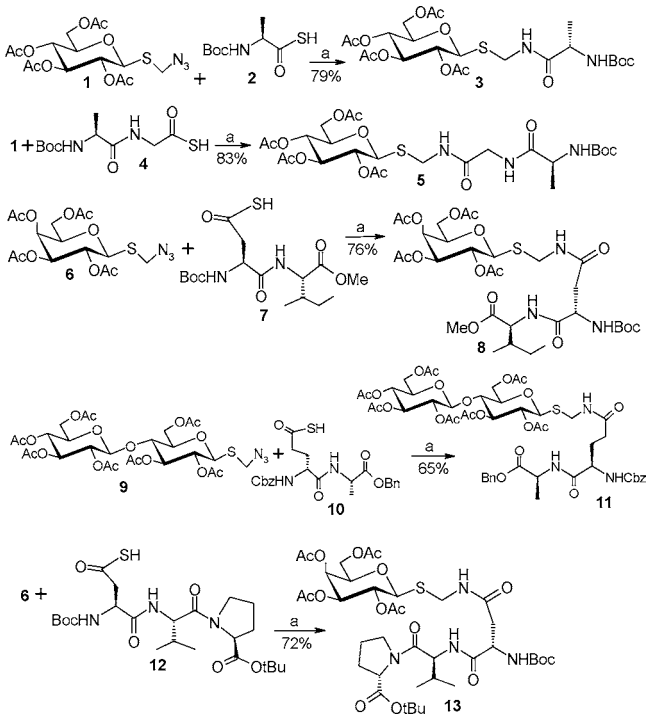
**Figure 1.** Some examples of non-native sugar−peptide linkages.

with cysteine sulfhydryl groups (**IV** in Figure 1) to synthesize, for instance, erythropoietin (EPO)-derived neoglycopeptides.[5h] Bromoethyl glycosides have been utilized to ligate with cysteine-containing peptides to build up neoglycopeptides derived from mucins and T-cell stimulating glycopeptides **V**.[5e] Only very few papers have been directed at the synthesis of *S*-linked neoglycopeptides containing sulfur atoms in their glycosidic linkage. Such molecules are not readily degraded by glycosidases, and they are also more stable under a number of chemical conditions. Recently, a new approach to the preparation of disulfide-linked neoglycopeptides **VI** was described wherein a 5-nitropyridine-2-sulfenyl-activated thioglycoside was conjugated with a preassembled peptide or protein sequence.[5b,8] As part of our ongoing interest in *S*-glycopeptides,[9] we report here the synthesis of two new types of neoglycopeptides, structures **VII** and **VIII**.

The azido group has gained significant attention recently due to its exceptional stability toward many synthetic conditions and its ready transformation into various nitrogen-containing functionalities.[10−12] Hence, the most convenient route to target structure **VII** would be through the conversion of the glycosylthiomethyl azide[12] (GTM-$N_3$) into the corresponding free amine followed by amide formation with Asp- or Glu-containing peptides in their side chains. Unfortunately, the reduction of GTM-$N_3$ failed under various conditions, giving rise to a mixture of byproducts after acetylation, thus indicating decomposition of the resultant glycosylthiomethylamines during the reduction. Another strategy based on direct conjugation of the azides with thio acids[11a] was therefore investigated.

To test the feasibility of this strategy, we reacted GTM-$N_3$ **1**[12] and Boc-Ala-SH **2**[13] in the presence of 2,6-lutidine to give amide **3** as shown in Scheme 1.[14] The yields are good

**Scheme 1.** Synthesis of *S*-Glycosides **3** and **5** and *S*-Neoglycopeptides **8**, **11**, and **13**[a]



[a] Reagents and conditions: (a) 2,6-lutidine, $CHCl_3$, reflux, 65−70 °C, 10−12 h.

for all five examples, which include three different GTM-$N_3$ partners and five different thio acids which were prepared in two steps from the corresponding acid according to known procedures.[15]

Recently, we reported a new protocol for the synthesis of *S*-glycopeptides in aqueous solution by direct *S*-glycosylation of Cys or Hcy containing peptides with glycosyl bromides, wherein chemical ligation techniques were utilized to prepare the required peptides.[9a] Based on this work, it occurred to us that ligation of GTM-Cl[12] with peptides containing Cys or Hcy residue would lead to the desired structure **VIII**. Hence, as a test, peracetyl glucosylthiomethyl chloride[12] was treated with Boc-Cys-OBn[16] under the same PTC conditions as used before,[9a] i.e., $Na_2CO_3$, TBAHS, and EtOAc−$H_2O$.

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(14) The yields in Scheme 1 were based on recovered starting materials; for details, see the Experimental Section (Supporting Information).

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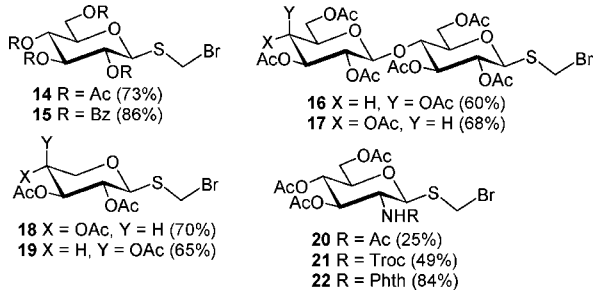
However, unexpectedly, no reaction took place even after extended reaction times.

To synthesize the desired *S*-neoglycopeptide **VIII** under these aqueous conditions, the reactivity of the sugar moiety had to be enhanced. For this purpose, the more reactive glycosylthiomethyl bromides (GTM-Br) were prepared from the corresponding glycosylthiols[17] by treatment with dibromomethane in the presence of $K_2CO_3$, as shown in Scheme 2. Glycosylthiomethyl bromides **14**−**19** were obtained in good to excellent yields, although under the same conditions, GlcNAc-derived bromide **20** was produced from the corresponding thiol only in 25% yield due to 2-acetamido group induced side reactions.[18] Compared to **20**, bromides **21** and **22** were obtained in much better yields, 49% and 84%, respectively.

**Scheme 2.** Synthesis of Glycosylthiomethyl Bromides (GTM-Br) from the Corresponding Glycosylthiol (Yield, %)

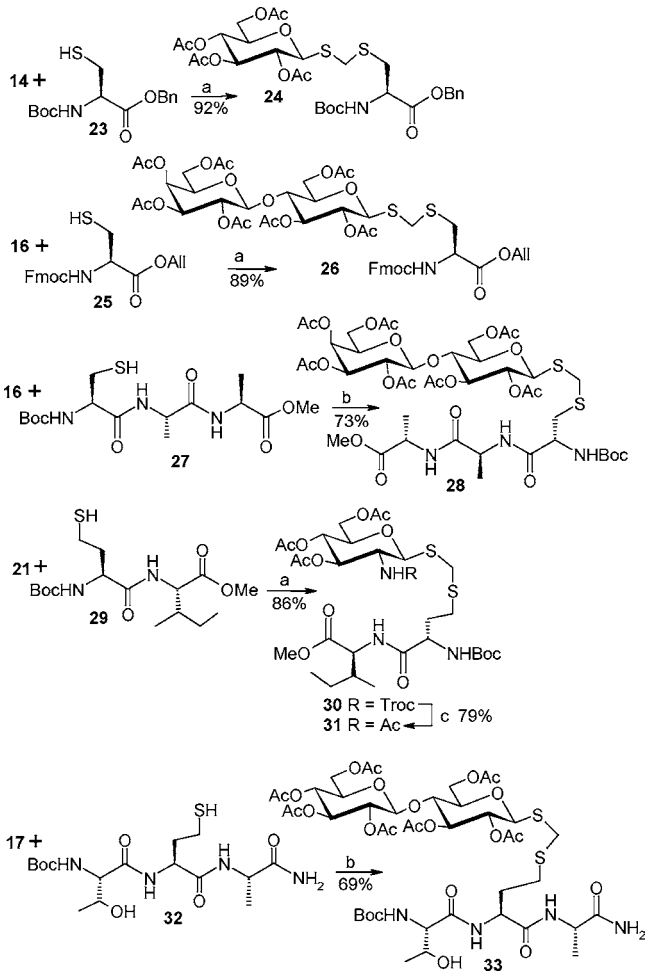



With these more reactive GTM-Br species in hand, reaction with amino acid and peptide derivatives containing Cys and Hcy to give neoglycopeptides of general structure type **VIII** was investigated. Bromide **14** was treated with Boc-Cys-OBn **23**[16] under the above-mentioned PTC conditions to give the desired *S*-glycoside **24** in very high yield (Scheme 3).

The yields with other investigated thiol-containing starting materials (**25**,[19] **27**,[9a] **29**,[9a] and **32**[20]) were invariably good, and the simplicity of the reaction conditions makes this an attractive way of preparing neoglycopeptides. For larger peptides (e.g., **27** and **32**) that are not soluble in ethyl acetate, a mixture of water and DMF proved to be a more effective solvent. The *N*-Troc group could be converted to the acetamide[21] to produce the GlcNAc glycoside **31** as shown. The formation of these tripeptide derivatives signals the potential utility of this method for the formation of more complex neoglycopeptides.[22]

In summary, we have presented efficient syntheses of two types of novel *S*-neoglycopeptides based on the new sugar species GTM-$N_3$ and GTM-Br that are useful synthons for anchoring sugars onto the side chain of Asp or Glu and Cys or Hcy, respectively. The reaction of GTM-$N_3$ with peptide-derived thiol acids gave rise to one type of *S*-neoglycopeptide **VII**. The ligation of GTM-Br with Cys- or Hcy-containing peptides led to another type of *S*-neoglycopeptide **VIII**. Both **VII** and **VIII** could be interesting mimetics of native *N*-glycopeptides with enhanced enzymatic resistance and chemical stability, thus providing good opportunities to probe biological properties of glycopeptides at the molecular level.

**Scheme 3.** Synthesis of *S*-Glycosides **24** and **26** and *S*-Neoglycopeptides **28**, **31**, and **33**[a]




[a] Reagents and conditions: (a) $Na_2CO_3$, EtOAc−$H_2O$, TBAHS, rt, 8 h; (b) $NaHCO_3$, DMF−$H_2O$, rt, 7 h; (c) Zn, $Ac_2O$, $Et_3N$, ultrasonic bath, rt, 3 h.

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**Supporting Information Available:** Experimental procedures and $^1H$ and $^{13}C$ data for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Presumably, polymerization took place in this reaction, giving rise to several byproducts as indicated by TLC.

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(22) (a) In view of the distance between the sugar and the peptide backbone, compounds **8** and **28** could be good mimetics of native *N*-glycopeptides.