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Synthesis of α-*S*-linked glycopeptides in water containing solution

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Abstract—α-Thiols of *N*-acetylglucosamine and of *N*-acetylgalactosamine react with β-bromoalanine containing peptides at pH 8.5 under phase transfer conditions or, alternatively, in aqueous DMF, cleanly to α-*S*-linked glycopeptides. Thus, mimetics of important *O*-glycopeptides can be readily prepared.

It has been well established that glycopeptides and glycoproteins play a central role in various biological functions.[1] However, glycopeptides and particularly glycoproteins are often difficult to obtain in homogeneous form from biological sources because they appear as a population of different glycoforms. The synthesis of pure native glycopeptides and finally glycoproteins is therefore necessary in order to further explore the function of these compounds in biological systems.[2] On the other hand, the field of glycopeptide mimetics[3] is driven by the urgency of certain biological problems, which overrides the preference for a native structure. For example, a native *O*-linked glycopeptide may lack the stability or bioavailability required for a specific therapeutic application or a basic scientific investigation. As a result, more and more glycopeptide mimetics have been designed and synthesized as potential therapeutic agents, including *S*-linked glycopeptides.[4,5] Replacement of the anomeric oxygen of *O*-glycopeptides by sulfur (Fig. 1), thus leading to *S*-linked glycopeptides is a modification tolerated by most biological systems which, in addition, increases the stability of the peptide-sugar linkage against chemical degradation as well as against enzymatic cleavage.[6] Thus, particularly valuable compounds for biological studies are provided.

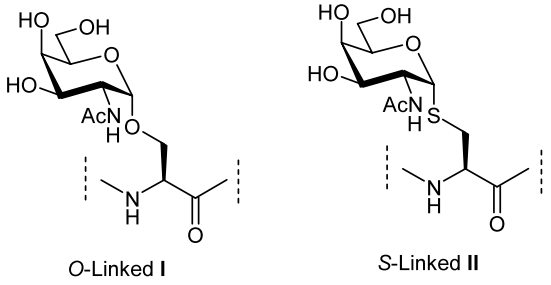

Figure 1. Comparison of a native *O*-linked glycopeptide (**I**) and *S*-linked glycopeptide (**II**).

The synthesis of several *S*-linked glycopeptides has been recently reviewed.[2a] Most of these compounds were obtained by the *co-translational approach*, wherein the *S*-linked glycosyl amino acid was pre-prepared and then incorporated during the course of peptide assembly. Only one paper was devoted to the synthesis of *S*-linked glycopeptides in a *post-translational strategy*: 1-Thio sugars were attached to dehydroalanine containing peptides by Michael addition; however, no diastereoselectivity was observed in this addition.[4b] In view of the advantage of the post-translational strategy[7] in synthesizing peptide conjugates, we described very recently a convenient synthesis of *S*-linked glycopeptides from glycosyl bromides and cysteine or homocysteine containing peptides in aqueous solution.[5] This procedure is a significant advance in the convergent assembly of *S*-linked glycopeptides because it demonstrates that under mild basic, aqueous conditions peptides can be *S*-glycosylated effectively. By this procedure, as mimetics of native β-*O*- and β-*N*-linked glycopeptides, β-*S*-linked glycopeptides were obtained efficiently, as shown in reaction 1 in Figure 2.

Amongst *O*-linked glycoproteins α-linkage between a GlcNAc and a Ser or Thr residue, respectively, is very common.[8] In addition, the Tn antigen,[9] featuring GalNAc α-*O*-linkage to a Ser or a Thr residue, is a

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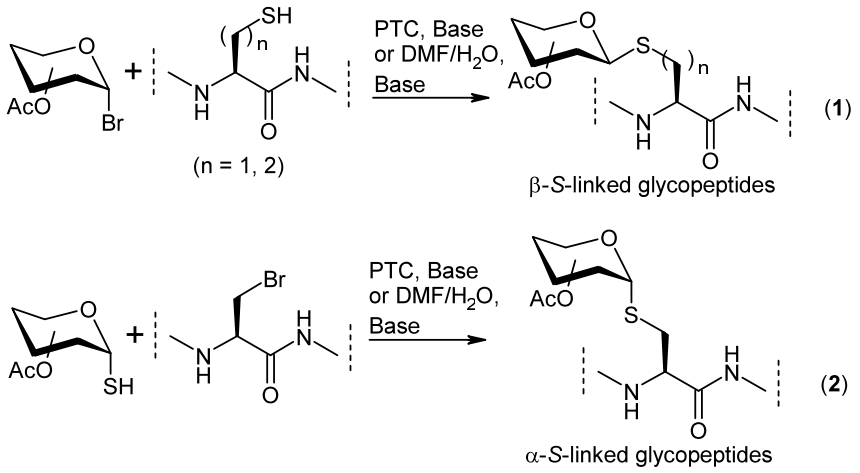


Figure 2. Strategies for synthesizing -*S*-linked glycopeptides and α-*S*-linked glycopeptides.

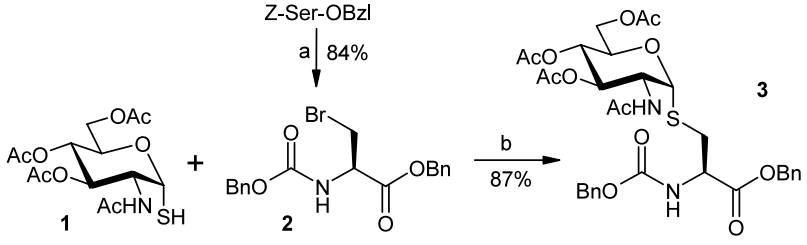
common human tumor cell surface motif, which is typical for all *O*-linkages in mucins. Therefore, the corresponding α-*S*-linked glycopeptides are important targets for biological studies also because of the expected enhanced chemical stability and enzymatic resistance. However, attempts to use our earlier procedure for the synthesis of α-*S*-linked glycopeptides were met by difficulties in the preparation of the required β-glycosyl bromides.

In order to overcome this problem, we planned to exchange the electrophile and nucleophile, i.e. electrophilic bromide containing peptides and nucleophilic 1-thio sugars were devised to construct the desired α-linkage to sulfur (reaction 2 in Fig. 2). We present here this new and effective strategy for the synthesis of α-*S*-linked glycopeptides in aqueous solution.

To the best of our knowledge, the synthesis of *S*-linked glycopeptides with exclusively α-glycosidic linkage particularly under aqueous conditions has not been reported, though α-*S*-glycosyl cysteines have previously been prepared in different laboratories.[10] Among them, Knapp and his co-workers firstly used α-GlcNAc[10c] and α-GalNAc[10d] thiols to prepare the corresponding Boc-Cys(α-GlcNAc)-OMe and Boc-Cys(α-GalNAc)-OMe by coupling with β-iodoalanine with a strong base in dry DMF.

Our initial efforts to test the feasibility of the new route focused at first on amino acid β-bromoalanine **2**[11] which was simply prepared by bromination of commercially available Z-Ser-OBzl with CBr_4/Ph_3P[12] in 84% yield, as shown in Scheme 1. α-GlcNAc thiol **1** was prepared as a white foam from glucosamine according to Knapp's procedure.[10c] Based on our earlier work,[5] the subsequent coupling between **1** and **2** was performed in the presence of tetra-*n*-butylammonium hydrogen sulfate (TBAHS) in ethyl acetate and an aqueous solution of $NaHCO_3$ at pH 8.5, i.e. phase transfer conditions (PTC); expectedly, the Z-Cys(α-GlcNAc)-OBzl **3** was smoothly produced in 87% yield without observable epimerization of the α-carbon of cysteine, which has been detected under different experimental conditions.[13] In this coupling, the thiolate anion was generated in situ by the action of $NaHCO_3$, and the α-thioglycoside **3** was then readily formed via nucleophilic displacement of the β-bromo atom of **2** in one pot. The corresponding iodoalanine[10c] turned out to be much less stable than **2** and exhibited some tendency to elimination;[14,15] however, generation of bromoalanine **2** or of peptide containing **2** led to stable and pure compounds which underwent clean reactions with glycosyl thiols. The same reaction could also be performed with **1** and **2** in a mixture of DMF/H_2O in the presence of $NaHCO_3$ at pH 8.5; also de-*O*-acetylated **1** reacted under these conditions.[15]

Encouraged by this result, the generality of the present procedure for the synthesis of α-*S*-linked glycopeptides was examined. The required β-bromoalanine containing peptides were prepared from the corresponding serine

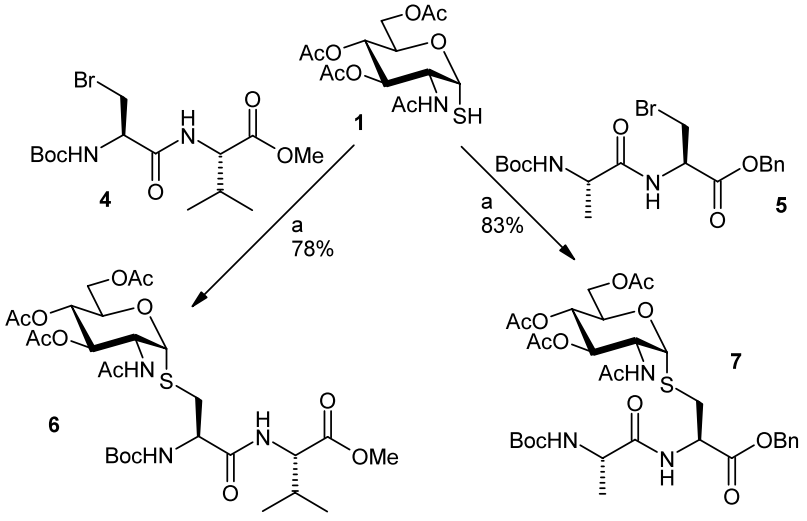


Scheme 1. Synthesis of *N*,*O*-protected (L)-bromoalanine and its reaction with glycosylthiol **1**. *Reagents and conditions*: (a) CBr_4, PPh_3; (b) $NaHCO_3$, TBAHS, $EtOAc/H_2O$.

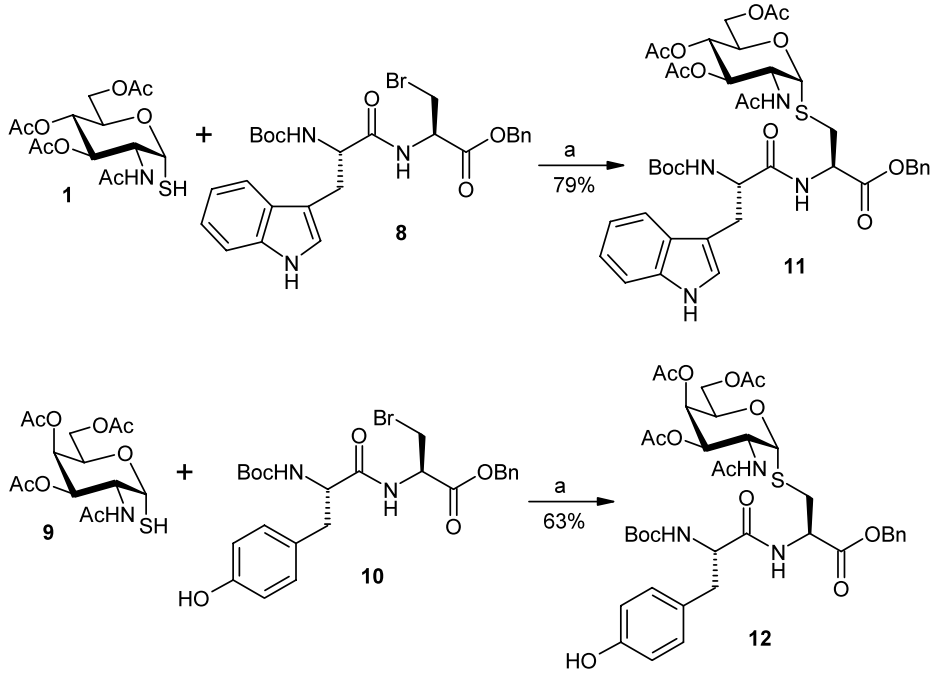
containing peptides using the same condition as for **2** or by ligating the peptides by using *N*-Boc-protected (L)-bromoalanine. Dipeptide bromide **4** was also prepared in a two-step procedure,[16] i.e. tosylation and then bromination, but the yield was not better compared with the other procedures. As expected, reaction of thiol **1** with dipeptide bromides **4** and **5** under the above PTC conditions provided the corresponding α-*S*-linked glycodipeptides **6** and **7** in 78 and 83% yields, respectively (Scheme 2).

Tryptophan (Trp) containing dipeptide **8** was also thioglycosylated effectively with **1** under the same reaction conditions to give the desired α-thioglycoside **11** in 79% yield, as shown in Scheme 3. Similarly, glycodipeptide **12** was produced from the corresponding α-GalNAc thiol **9**, which was prepared following literature procedure,[10d] and tyrosine (Tyr) containing dipeptide **10** in 63% yield. It is noteworthy that the indole ring of Trp and the hydroxyl group of Tyr did not compete as nucleophiles because of the presence of the highly nucleophilic thiol group.

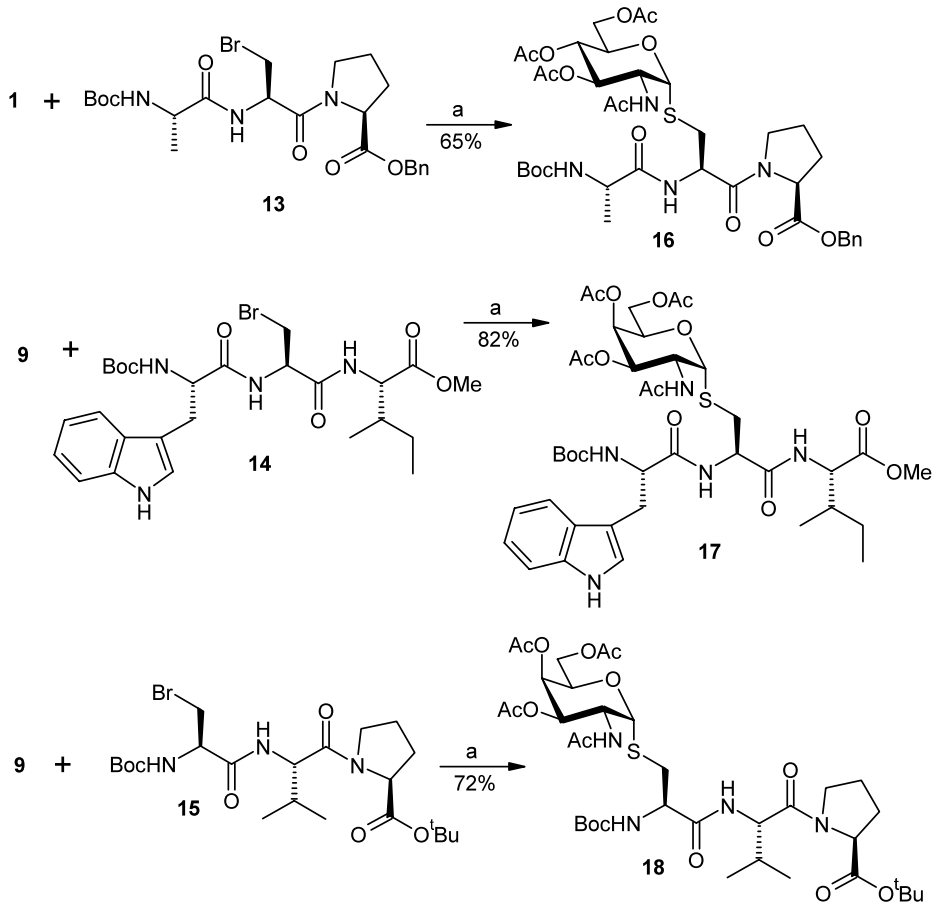
In order to further exploit the above procedure, tripeptide bromides **13**, **14** and **15** were prepared and subjected to the thioglycosylation with **1** and **9**, as shown



Scheme 2. Reaction of bromoalanine containing dipeptides with glycosyl thiol **1**. *Reagents and conditions*: (a) solution of $NaHCO_3$ (pH 8.5), TBAHS, EtOAc.



Scheme 3. Reaction of glycosyl thiols **1** and **9** with dipeptides **8** and **10**. *Reagents and conditions*: (a) solution of $NaHCO_3$ (pH 8.5), TBAHS, EtOAc.



Scheme 4. Reaction of glycosyl thiols **1** and **9** with tripeptides **13**–**15**. *Reagents and conditions*: (a) solution of $NaHCO_3$ (pH 8.5), TBAHS, EtOAc.

in Scheme 4. Interestingly, all the couplings worked well and afforded the corresponding α-*S*-linked glycotripeptides **16**, **17** and **18** in good yields (Scheme 4).[17,18] **18** was also fully deprotected.[19]

In summary, we have developed a convenient and efficient synthesis of α-*S*-linked glycopeptides using α-glycosyl thiols and peptides containing (L)-β-bromoalanine as building blocks. An advantage of this procedure is that peptide derivatives were not exposed to strong basic conditions because of the use of a two-phase system; the risk of elimination and subsequent epimerization of the amino acid α-carbon were therefore greatly reduced. In addition, it is noteworthy that the α-linked coupling products with GalNAc, such as **12**, **17** and **18** mimic the important Tn antigen structure. We believe that the current study, together with our earlier report,[5] is of considerable potential for the synthesis of *S*-linked glycopeptides. Studies on the application of these procedures to specific, biorelevant glycopeptide targets are in progress.

Acknowledgements

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17. General procedure for the synthesis of α-*S*-linked glycopeptides **3**, **6**, **7**, **11**, **12**, **16**, **17** and **18**. To a solution of appropriate bromide (0.2 mmol) and the corresponding sugar thiol (0.34 mmol) in EtOAc (3 mL) was added pH 8.5 solution of $NaHCO_3$ (3 mL) followed by addition of TBAHS (272 mg, 0.8 mmol). The mixture was vigorously 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 which was purified by flash column chromatography to afford the corresponding thioglycoside.
18. All synthesized compounds gave satisfactory elemental analyses and were identified by optical rotations, ^1H, ^{13}C NMR, MALDI-MS spectroscopy. Selected data compound **17**: $[\alpha]_D = +63$ (*c* 1.0 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.01 (br s, 1H), 7.70 (d, $J = 7.6$ Hz, 1H), 7.37 (d, $J = 7.9$ Hz, 1H), 7.16 (m, 3H), 7.04 (d, $J = 6.5$ Hz, 1H), 6.78 (d, $J = 8.0$ Hz, 1H), 5.68 (d, $J = 6.6$ Hz, 1H), 5.19 (m, 2H), 4.86 (dd, $J = 11.3$, 2.9 Hz, 1H), 4.65 (m, 4H), 4.52 (dd, $J = 8.5$, 4.9 Hz, 1H), 4.05 (m, 1H), 3.90 (m, 1H), 3.73 (s, 3H), 3.71 (m, 1H), 3.50 (dd, $J = 14.5$, 3.6 Hz, 1H), 3.11 (dd, $J = 14.6$, 6.6 Hz, 1H), 3.04 (m, 1H), 2.45 (m, 1H), 2.12, 2.04, 2.03, 2.02 (4s, 12H), 1.88 (m, 1H), 1.47 (s, 9H), 1.30 (m, 2H), 0.90 (m, 6H); ^{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; MALDI-MS *m/z* 886.3 [M+Na^+], 902.2 [M+K^+]. Anal. 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.

Compound **18**: $[\alpha]_D = +50$ (*c* 0.5 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.98 (d, $J = 8.5$ Hz, 1H), 6.09 (br s, 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 (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; MALDI-MS *m/z* 822.9 [M+Na^+], 839.3 [M+K^+]. Anal. 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.
19. **18** was smoothly fully deprotected to give the corresponding glycotripeptide by treatment with 6 mM NaOMe/MeOH solution and 40% trifluoroacetic acid in CH_2Cl_2 successively: $[\alpha]_D = +93$ (*c* 1.0 MeOH); MALDI-MS *m/z* 521.4 (M+H^+), 543.7 (M+Na^+), 559.7 (M+K^+).