Glycopeptide Synthesis

A General Strategy toward S-Linked Glycopeptides**

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Glycosylation, the most complex posttranslational modification of proteins, is involved in a number of biological processes. Such highly complex glycan structures have challenged present methods used in the functional study of glycoproteins.[1–4] Therefore, the development of new methods for the syntheses of natural and unnatural homogeneously glycosylated proteins[5–7] is needed for a systematic understanding of glycan function. In this context, the syntheses of natural and modified glycopeptides represent a means for additional glycoprotein studies and therapeutic developments.[8–11]

Naturally occurring glycopeptides most commonly incorporate an O- or N-glycosidic linkage between the saccharide moiety and the side chain of an appropriate amino acid residue. Replacement of the anomeric oxygen or nitrogen atom by sulfur results in the corresponding S-linked glycopeptide, which is known to be more stable chemically,[12] more resistant to the action of glycosidases, and tolerated by most biological systems. Furthermore, members of the closely related S-linked oligosaccharides have been used as enzyme inhibitors[13,14] and are suggested to be better immunogens than their natural O-linked analogues.[15–18] An alternate modification is the C-glycosyl linkage, for which several synthetic methods exist.[19]

The common synthetic approach for S-linked glycosyl amino acids uses an anomeric thiolate nucleophile in reaction with an alanine derivative equipped with a leaving group (Figure 1). The major side reaction is β-elimination, and the subsequent Michael addition results in a diastereomeric mixture at the α-carbon of the amino acid product. Several procedures have since emerged to overcome this problem.[20–24] The most advanced among these is a method reported by Schmidt and co-workers,[24–27] in which the reaction of the thiosugar **1** with β-bromoalanine was performed in an ethyl acetate/water two-phase system containing tetra-*n*-butylammonium hydrogen sulfate (TBAHS) and $NaHCO_3$ (Figure 2). We have used this protocol for the

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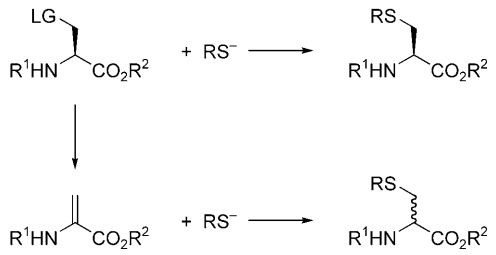


Figure 1. The common synthetic approach used for the synthesis of S-linked glycosyl amino acids.

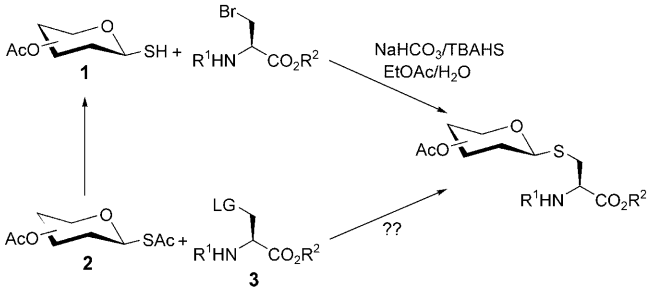


Figure 2. Reported procedure for the two-step synthesis of S-linked glycosyl amino acids and an alternative one-pot approach.

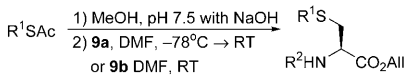
preparation of several different S-linked Boc- and Fmoc-modified glycosyl amino acids (Boc = *tert*-butoxycarbonyl, Fmoc = 9-fluorenylmethyloxycarbonyl). The Fmoc-protected compounds, however, can only be obtained indirectly from the S-linked Boc-modified glycosyl amino acids owing to the basicity of the reaction. The step for the generation of 1-thiosugars requires a special precaution, as it may give low yields due to the facile formation of disulfides during deacetylation of the sulfur atom and subsequent silica gel chromatography.

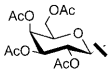
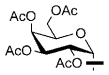
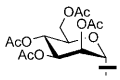
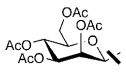
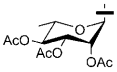
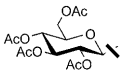
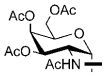
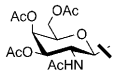
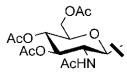
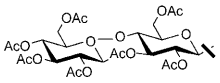
We therefore explored alternate reaction conditions in which thioacetate **2** and the alanine derivative **3** can be subjected to a one-pot reaction to generate the desired thio-linked glycosyl amino acid product (Figure 2). Such reaction conditions should meet several important criteria: first, deacetylation of the thioacetate group should be selective to generate the desired thiolate anion in situ, and to avoid β-elimination of the alanine derivative; second, dimethyl formamide (DMF) should be the solvent of choice, as solubility of large peptides in other solvents can be problematic;[24] furthermore, a set of optimal amino acid protecting groups should be employed to facilitate large-scale solid-phase peptide synthesis (SPPS).

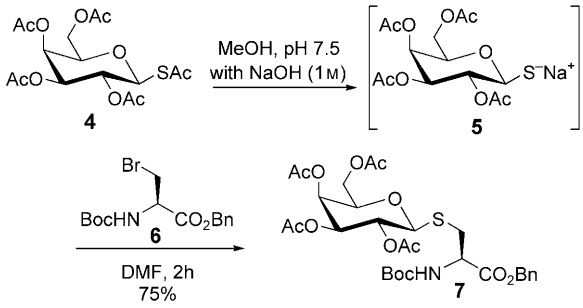
With these goals in mind, we first screened numerous one-pot reaction conditions (Table 1, Supporting Information). A simple "two-step one-pot" reaction emerged as the method of choice (Scheme 1). Therein, with 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-galactopyranose (**4**)[28] as a starting material, the anomeric thioacetate was selectively deprotected in situ with NaOH in anhydrous methanol (pH ≈7.5). After removing the methanol solvent, the resulting thiolate sodium salt **5** was subjected to nucleophilic substitution with β-bromoalanine **6** in dry DMF to give the desired S-linked galactosyl amino acid **7**. This reaction is simple yet very effective. It selectively deacetylates the sulfur atom, preserves the anomeric configuration,[29] and carries out the S_N2 displacement reaction effectively without disrupting the integrity of the α-carbon center. It is necessary to remove the methanol before the nucleophilic substitution reaction, as electrophile **6** would otherwise undergo β-elimination with sodium methoxide generated in situ.

As the Fmoc protecting group is labile in basic conditions, most of the procedures reported in the literature use Boc as

Table 1: General applicability of the synthesis of S-linked glycosyl amino acids.

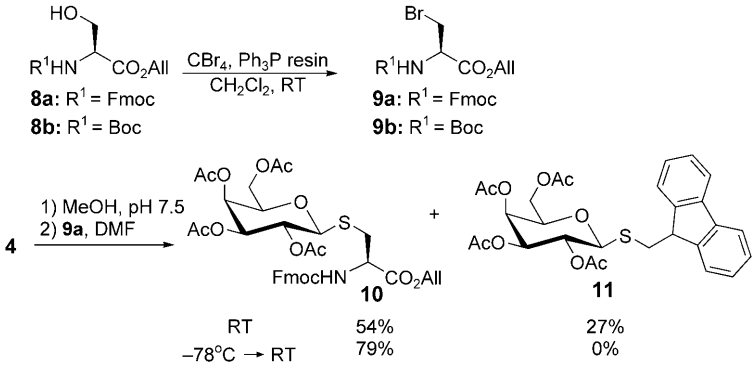


R^1SAc	R^1	R^2 = Fmoc (Yield [%])	R^2 = Boc (Yield [%])
4		**12** (79)	**19** (75)
20		**20a** (74)	
21		**21a** (77)	
22		**22a** (81)	
23		**23a** (75)	
24		**24a** (71)	
25		**25a** (66)	**25b** (75)
26		**26a** (68)	**26b** (70)
27		**27a** (71)	**27b** (78)
28		**28a** (78)	



Scheme 1. A two-step one-pot synthesis of a Boc-protected glycosyl amino acid (Bn = benzyl).

the temporary protecting group to sustain the alkaline conditions in the nucleophilic substitution reaction.[20,24] However, the Boc group must then be replaced with the Fmoc protecting group for the preferred Fmoc SPPS of glycopeptides.[30,31] After the reaction shown in Scheme 1 was carried out following this traditional method, we hypothesized that the basicity of intermediate **5** in DMF would be sufficiently mild to permit the S_N2 reaction with the Fmoc-protected β-bromoalanine species without affecting the Fmoc group itself. Thus, compound **8a** was prepared as reported in the literature (Scheme 2).[32] The allyl ester protecting group

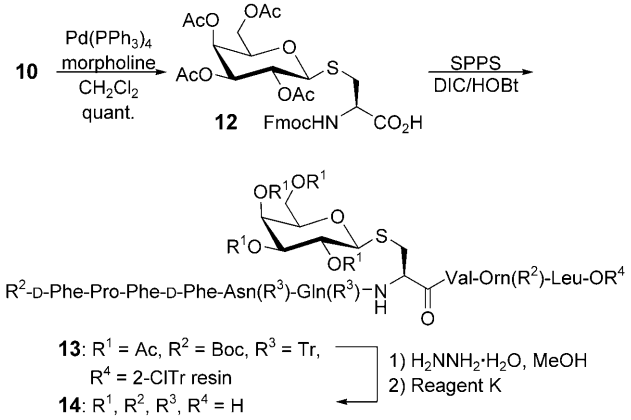


Scheme 2. A direct two-step one-pot synthesis of the S-linked Fmoc-modified glycosyl amino acid **10** with suppression of side product **11**.

was favored over the benzyl ester group, as it is easier to remove in high yield. Bromination of **8a** went smoothly with the solid-support Ph_3P resin and CBr_4 in dichloromethane to give **9a** (100%, TLC),[33] which was pure enough to carry out the next step without purification.[34] Upon performing the S_N2 reaction at ambient temperature, the desired amino acid **10** was obtained (54%), along with side-product **11** (27%). Thiolate **5** presumably attacks Fmoc-CH_2 to give **11**. To suppress the formation of this side-product, the reaction was performed at −78 °C to room temperature. We were gratified to observe that only compound **10** (79%) was formed without any trace of side product **11**.

S-linked glycosyl amino acid **10** was treated with tetrakis(triphenylphosphine)palladium(0) catalyst and morpholine in dichloromethane to give the Fmoc-modified building block **12** swiftly (100%, TLC), which was used without purification to afford the protected S-linked glycodecapeptide **13** (Scheme 3). Deacetylation of the sugar moiety was carried out on-resin by transesterification with hydrazine hydrate in methanol. Removal of the acid-labile amino acid protecting groups concomitant with cleavage from the 2-chlorotrityl resin by Reagent K afforded free S-linked glycopeptide **14** in pure form after HPLC. The whole process, starting from the readily available peracetylated 1-thio galactose to the final glycopeptide **14**, involved only two purifications (silica gel chromatography for **10** and HPLC for **14**). In fact, it is also possible to start with thiolate salt **5**, which can be stored under argon for up to a month without degradation.

Glycopeptide **14** is a linear analogue of tyrocidine A, which is a cyclic cationic decapeptide antibiotic produced in *Bacillus brevis* with an antiparallel amphipathic β-sheet

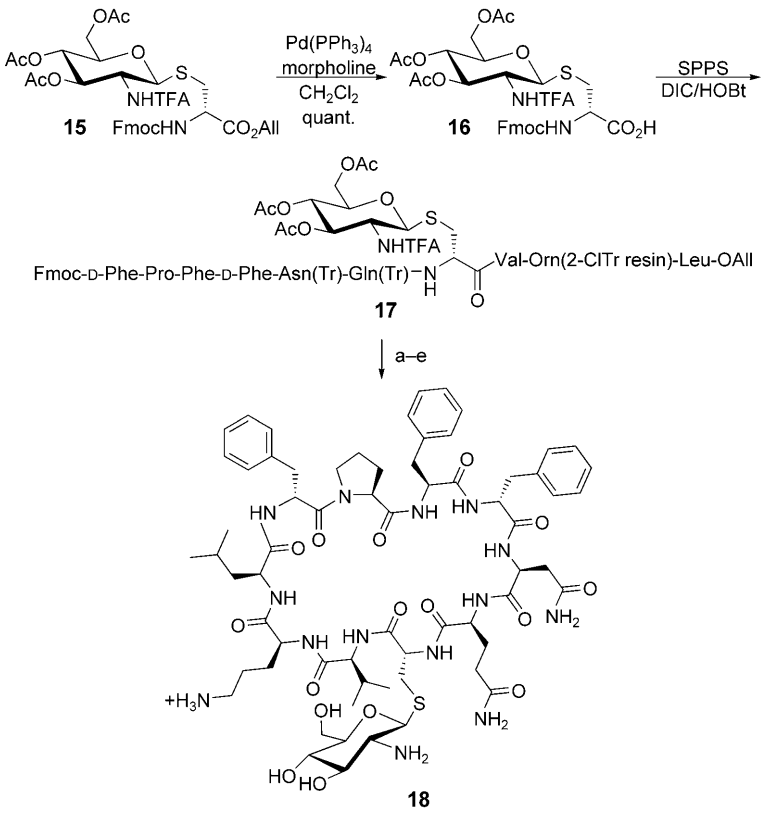


Scheme 3. Solid-phase peptide synthesis of glycopeptide **14** with Fmoc-modified glycosyl amino acid **12**. The yield from **12** to **14** is 60% (DIC = 1,3-diisopropylcarbodiimide, HOBt = 1-hydroxybenzotriazole, Tr = trityl).

conformation in solution.[35–38] A recent report has shown that several cyclic glycopeptide analogues of tyrocidine A retain biological activity as antibiotics.[39] To further test the method reported herein for the synthesis of S-linked glycopeptides, glycosyl amino acid **15** was synthesized and converted into **16**. After the removal of palladium by scavenger resin, the tyrocidine analogue precursor **17** was produced (Scheme 4). Conversion of **17** to cyclic glycopeptide **18** (an analogue of tyrocidine A in which the wild-type Tyr group at position 7 is replaced by a D-Ser(β-*S*-GlcNH_2) residue) was carried out in four steps on solid support followed by cleavage and amino acid side chain deprotection (29% overall yield from **16** to **18**). The biological activity of this glycosylated tyrocidine analogue was evaluated with the minimal inhibitory concentration (MIC) against *B. subtilis* as a measure of the antibiotic activity and the minimal hemolytic concentration (MHC) against human erythrocytes as an evaluation of eukaryotic membrane toxicity. The S-linked glycopeptide analogue **18** was shown to have a better antibiotic profile than tyrocidine A, with MIC values of 12.5 μM for both and MHC values of 100 and 50 μM, respectively.

Although Fmoc SPPS is preferred for glycopeptides, the synthetic approach reported herein should also be applicable for the synthesis of Boc-modified amino acid building blocks. Therefore, Boc-protected S-linked glycosyl amino acid building block **19** (Table 1) was synthesized from **4** and **9b** (Scheme 2) in a similar fashion.

To demonstrate that our approach is also applicable to other thiosugars, we synthesized several different peracetylated 1-thiosugars (Supporting Information). These represent all four possible classes of products: 1,2-*trans*-α (**21** and **23**[15]); 1,2-*trans*-β (**4**,[28] **24**,[40] **26**,[41] **27**,[42] and **28**[43]); 1,2-*cis*-α (**20** and **25**[23]); and 1,2-*cis*-β (**22**[29]). They are conveniently grouped based on the stereochemistry at C1 and its relationship to the functional group at C2 of the sugar ring. All compounds were subjected to the "two-step one-pot" conditions mentioned above to give the corresponding Fmoc- or Boc-protected S-linked glycosyl amino acid building blocks, in yields ranging from 66 to 81%.



Scheme 4. Solid-phase glycopeptide synthesis of the tyrocidine analogue **18**. Reagents and conditions: a) $[Pd(PPh_3)_4]$/4-methylmorpholine, $CHCl_3$; b) piperidine/DMF (20%); c) PyBOP/HOBt, DMF; d) $H_2NNH_2{\cdot}H_2O$, MeOH; e) TFA/CH_2Cl_2/triisopropylsilane (80:15:5); 29% overall yield from **16**. PyBOP = 1-benzotriazolyloxy-tris-(pyrollidino)phosphonium; TFA = trifluoroacetate.

In summary, we have developed a general strategy for S-linked glycopeptide synthesis. With a simple "two-step one-pot" reaction, peracetylated thiosugars are quickly converted into masked S-linked amino acid building blocks. Under such reaction conditions, deacetylation at the sulfur atom is selective, anomeric integrity is retained, and S_N2 substitution is stereoselective. Unmasking the building blocks by deallylation affords Fmoc- or Boc-protected amino acids, which are ready for SPPS. This strategy is applicable for large-scale S-linked glycopeptide synthesis and could also be used for other S-linked glycoconjugates.

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