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Sugar-Assisted Glycopeptide Ligation

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The thiol capture strategy developed by Kemp and co-workers has inspired the development of several ligation-based methods for the preparation of large peptides.[1] Among them, native chemical ligation (NCL) represents one of the most useful tools for the chemical synthesis of proteins.[2] In this approach, an *N*-terminal cysteine residue is required for capturing the thioester peptide, followed by a spontaneous acyl transfer to form the amide bond at the ligation junction. To overcome the restriction imposed by the requirement for a cysteine residue at the ligation site, different strategies are adopted to extend the repertoire of peptide conjugation for non-cysteinyl ligation.[3] The use of removable thiol-based auxiliaries is an attractive approach to fulfill the same function as the side chain of the cysteine. Peptides with N^{α} -linked auxiliaries based on cysteine, such as 1-phenyl-2-mercaptoethyl and 2-mercaptobenzyl, have been investigated and successfully applied to the synthesis of large peptides.[4]

The synthesis of glycopeptides and glycoproteins from readily available materials represents an attractive route to homogeneous products for structural and functional studies.[5] Several approaches are currently being used for the synthesis; one of particular interest is the ligation method.[6] Our interest in obtaining homogeneous glycopeptides and glycoproteins via enzymatic and chemical methods[6d,7] prompted us to explore new ligation methods for cysteine-free glycopeptides. Here we report that the sugar moiety of a glycopeptide can mimic the cysteine function at the ligation site once it is modified with a thiol handle at the C-2 position (Figure 1). Moreover, the thiol handle can be reduced to the acetamide moiety, which is often found in this position, furnishing the unmodified glycopeptide (Figure 1).

Model studies were performed with small peptides to investigate the new approach. Initially, we tested glycopeptide **1**, in which the serine residue was modified with unprotected β-thioacetamido glucosamine (Figure 2). Reacting this peptide under NCL conditions (6 M guanidine·HCl, pH 8.0, 2% thiophenol, 2% benzyl mercaptan) with a model peptide thioester {AC-LYRAG-COS-$(CH_2)_2$-$CONH_2$} gave the ligated product after 6 h in ~15% conversion. A second model study was prepared in which glycopeptide **1** was extended with a glycine residue to give glycopeptide **2** (Figure 2). Using similar ligation conditions, we were pleased to see that the desired product was formed rapidly and appeared as the major product in the HPLC analysis (Figure 3). Preparative HPLC of the reaction mixture yielded the desired glycopeptide in 76% yield (Table 1, entry 1). Glycopeptide **3**, which lacks the thiol handle, was also prepared and tested under similar ligation conditions. However, only a trace of ligation product was observed, indicating the crucial role of the thiol handle in the reaction pathway and supporting the proposed mechanism shown in Figure 1.

To investigate the effect of the *C*-terminal amino acid of thioester peptide on the ligation rate, we performed initial studies by preparing four peptides thioester bearing glycine, histidine, alanine, and valine. The rate of the ligation reactions with glycopeptide **2**

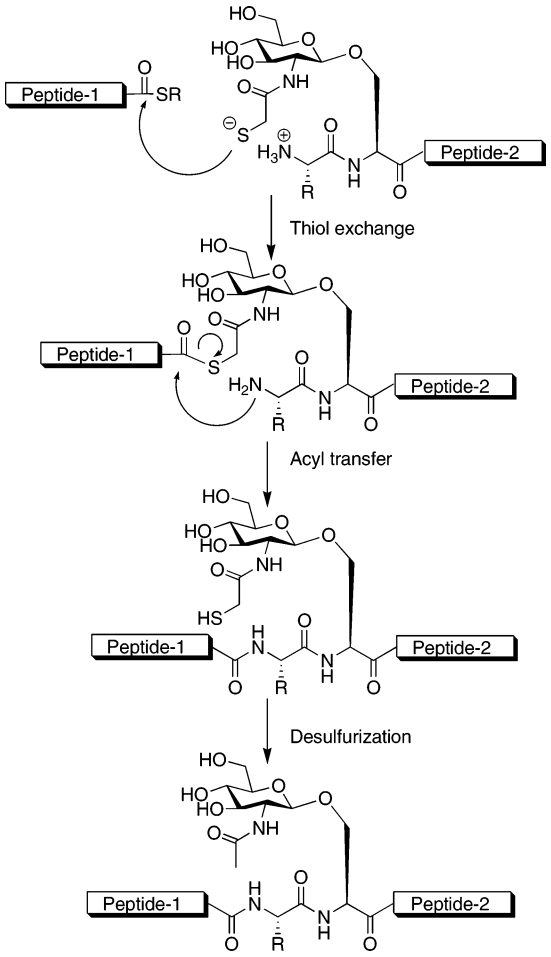

Figure 1. General strategy of sugar-assisted chemical synthesis of glycopeptide.

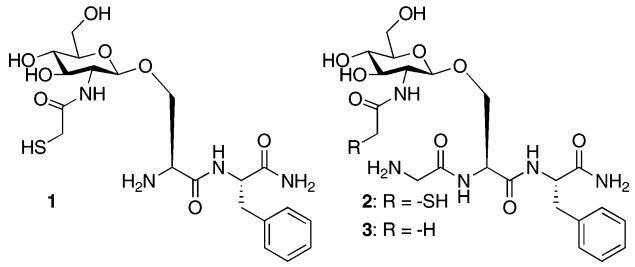

Figure 2. Glycopeptides that are used in the ligation reactions (for synthesis of **1**−**3**, see the Supporting Information).

is shown in Table 1.[8] In the case of valine thioester (entry 4), which reacted at a slower rate ($t_{1/2}$ > 48 h) compared to glycine, histidine, and alanine thioesters (entries 1−3), we were able to identify the proposed thioester intermediate by the mass spectrometry fragmentation pattern (see Supporting Information). Initial study on the

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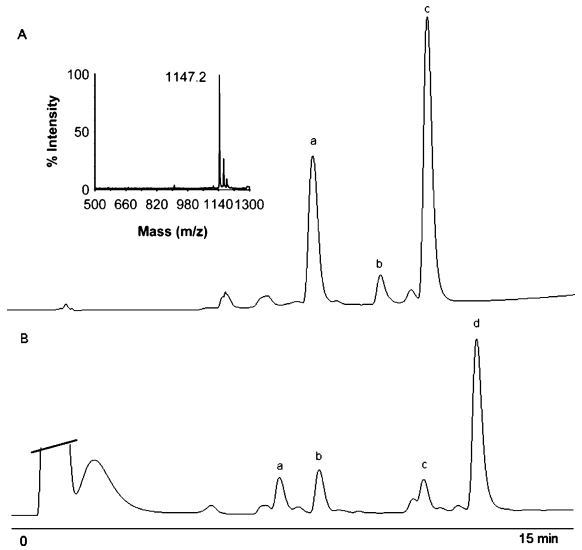
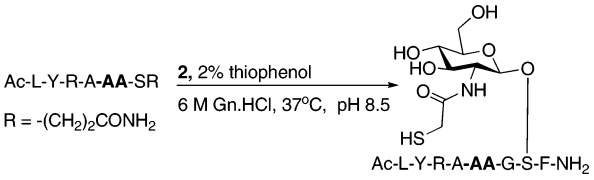


Figure 3. Analytical HPLC of the ligation reaction (Table 1, entry 1). (A) Reaction at 0 h: peak a, glycopeptide **2**; peak b, impurity from thioester peptide substrate; peak c, thioester peptide substrate. (B) Ligation reaction after 8 h: peak a, impurity from tris(2-carboxethyl)phosphine hydrochloride; peak b, unreacted glycopeptide **2**; peak c, thioester substrate hydrolysis product; peak d, the desired product with the expected mass of 1147.2 Da (see inset, MALDI-TOF/MS).

Table 1. Effect of the C-Terminal Amino Acid of Peptide Thioester on the Ligation Rate



entry	-AA-	ligation junction	$t_{1/2}$ (h)	mass obsd,[a] calcd (Da)
1	Gly	Gly-Gly	~5	1146.4 ± 0.2, 1146.52
2	His	His-Gly	~5	1126.5 ± 0.2, 1226.55
4	Ala	Ala-Gly	~10	1160.4 ± 0.2, 1160.53
4	Val	Val-Gly	>48	1188.5 ± 0.2, 1188.56

[a] Characterized by ESI-MS.

generality of our approach showed that the *N*-terminal amino acid is not limited only to a glycine residue. We have found that extending peptide **1** with alanine and serine gave results similar to those obtained in the glycine case.

By applying the desulfurization reaction developed by Dawson and co-workers,[3b] we reduced the acetamido thiol handle in the ligation product (Table 1, entry1) to the corresponding acetamide moiety. The reaction was completed within 1 h using Pd/Al_2O_3 under hydrogen to give the unmodified glycopeptide in 80% isolated yield. A mass decrease of 32 Da from the starting material was observed, as expected for the loss of one sulfur atom (see Supporting Information).

The results reported here are surprising, considering the size of the ring of the S−N acyl-transfer intermediate. It is plausible that the sugar moiety affects the proximity of the *N*-terminal amine to the thioester, which allows for the acyl transfer to occur despite the large ring size. Efforts to determine the effects of the configuration at the anomeric center and the *N*-linked sugar on the ligation rate are currently being pursued in our laboratory.

In summary, we have demonstrated that the sugar moiety of a glycopeptide modified with a thiol handle at the C-2 position can assist in the ligation of a cysteine-free glycopeptide with a thioester peptide. Upon completion, the thiol handle can be reduced to the acetamide moiety. Together, this sequence of reactions displays an attractive potential in glycopeptides and glycoproteins synthesis. The scope, mechanism, and applications of the sugar-assisted ligation in the synthesis of glycopeptides and glycoproteins are currently under investigation.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) We prepared the peptide α-phenyl thioester and used it directly in the ligation mixture, which was superior in terms of rate acceleration ($t_{1/2} \approx$ 2 h). However, we preferred preparing this peptide thioester in situ.

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