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Stereoselective N-Glycosylation by Staudinger Ligation

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ABSTRACT

Stereoselective methods for the chemical synthesis of *β*-*N*-glycosyl amides are needed to generate glycopeptides and glycoproteins. Here, we report that the Staudinger ligation can be used to form glycosylated asparagine derivatives. The reaction proceeds with high stereoselectivity, and a variety of glycosyl azides can function as substrates. Our results provide precedence for the use of this powerful amide-bond-forming reaction for *N*-glycopeptide synthesis.

N-Glycosylation is an important posttranslational modification of proteins.[1] Most *N*-glycoproteins share a common core structure in which an oligosaccharide is attached via a *β*-glycosyl amide bond to an Asn residue located within the consensus sequence Asn-Xxx-Thr/Ser (Xxx is any amino acid except Pro).[2] *N*-Glycoproteins and glycopeptides obtained from physiological sources are typically heterogeneous, thereby complicating an analysis of their biological roles.[3] Chemical synthesis can provide access to homogeneous samples of these products.[4,5]

One challenge in the chemical synthesis of *N*-glycopeptides is the stereoselective formation of the glycosyl amide bond linking the glycan and the protein. The most convergent approach is to append the carbohydrate(s) of interest to a synthetic peptide. This approach is advantageous because it avoids carrying a protected glycosylated peptide sequence through multiple subsequent synthetic steps. The glycosyl amide is commonly synthesized by condensation of a glycosylamine and an activated carboxylic acid derivative.[6] Though valuable, this approach often results in the production of anomeric mixtures due to the propensity of the glycosylamine to isomerize. Other sugar-derived precursors have been explored as alternatives to glycosylamines, including glycosyl isothiocyanates,[7] pentenyl glycosides,[8] and glycosyl sulfoxides.[9] Each of these has some utility, yet milder methods for the stereoselective formation of glycosyl amides are still needed.

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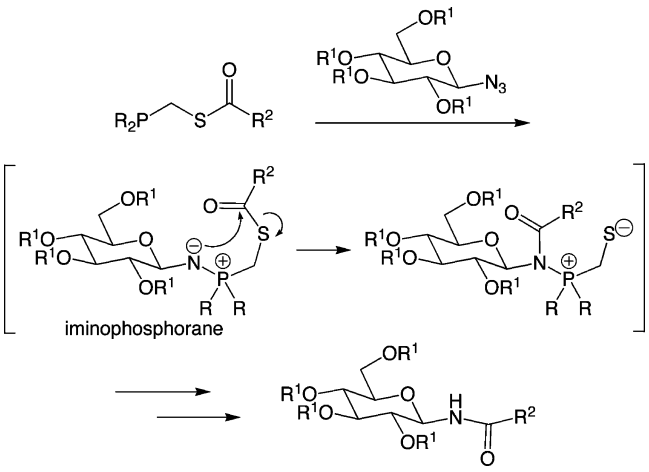
Two- and three-component Staudinger ligation reactions of different types have been used to modify carbohydrates. For example, three-component Staudinger ligation reactions that employ an azide, a carboxylic acid (or derivative), and a phosphine have been used to generate glycosyl amides,[10−13] but anomerization is often problematic.[14] Two-component Staudinger ligation reactions have been developed in which carbohydrate azide derivatives selectively engage in amide bond formation in complex environments.[15] Specifically, cells treated with a carbohydrate-containing azide can undergo reaction with a phosphinoester, thereby modifying the cell surface.[15d] In this version of the Staudinger ligation, an activated ester and phosphine reside in the same molecule. Simple glycosyl amide bonds have been formed using substrates of this type;[16] however, the products were obtained as isomeric mixtures. The Staudinger ligation had not been used to generate more complex *N*-glycopeptide precursors.

A version of the two-component Staudinger ligation reaction in which a phosphinothioester reacts with an azide-containing amino acid has been shown to afford peptide bonds.[17] Indeed, reactions of this type occur under mild conditions. Novel proteins of interest and small molecules have been immobilized on surfaces.[17e,18] We sought to examine the scope of the Staudinger ligation by evaluating the coupling of glycosyl azides and asparagine-derived phosphinothioesters.

Our initial goal was to generate an appropriate phosphinothioester that would serve as a precursor to glycosylated asparagine derivatives. We anticipated that the putative iminophosphorane intermediate (Scheme 1), which must undergo intramolecular transacylation to form the glycosyl amide, would be less nucleophilic than those generated in the peptide coupling reactions explored previously. The phosphine substituents will influence the steric and electronic properties of the resulting iminophosphorane intermediate and therefore the reaction outcome. Thus, we needed a convenient synthetic route to phosphinothioesters with alkyl as well as aryl phosphine substituents.

Scheme 1. Proposed Mechanism for the Formation of the Glycosyl Amide Bond via Staudinger Ligation



Diphenylphosphinothioesters were used in previous studies. We investigated whether the most effective route to diphenylphosphinothioesters[17c] could afford the more nucleophilic dialkylphosphine derivatives. Although the dialkyl species could be generated, the phosphinothiol precursors were very unstable, presumably because the dialkyl derivatives are more prone to oxidation. Thus, we developed a general route to phosphinothioesters.

Phosphine−boranes[19] and trialkylphosphonium salts[20] can protect oxygen-sensitive phosphine compounds. Air-stable diphenylphosphine−borane has been used to efficiently generate diphenylphosphinothioesters.[17c] We reasoned that this protecting group might prove to be valuable in the synthesis of a wide range of phosphinothioesters. To this end, we generated phosphine−borane complexes and added them to formaldehyde to yield alcohols **2a**−**c** (Scheme 2). These alcohols were converted to thioacetates **3a**−**c** in two steps. The acetyl groups were removed, and the resulting thiols were coupled to Boc-Asp-OBz to afford the borane-protected phosphinothioesters **4a**−**c** in six steps. The borane group was efficiently removed by heating **4a**−**c** with 1,4-diazabicyclo[2.2.2]octane (DABCO) in toluene under argon. The direct addition of the substituted phosphine−borane to formaldehyde, which occurs under mild conditions, is a key step in our scheme. We anticipate that this reaction will be useful for the synthesis of a wide range of functionalized phosphines. Overall, the revised synthetic route affords the dialkyl phosphinothioesters in higher yields than previous schemes. With phosphinothioesters **5a**−**c** in hand, we tested their reactivity in the coupling reaction.

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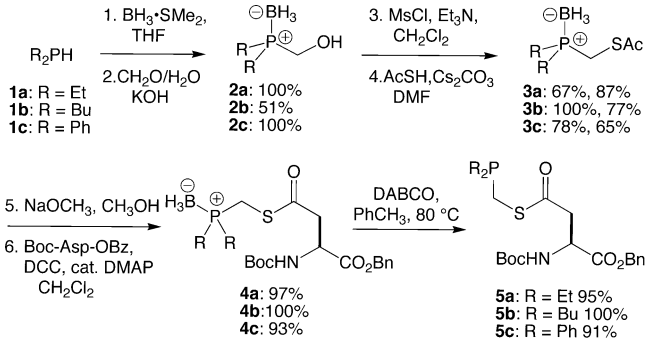
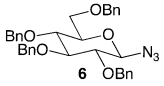
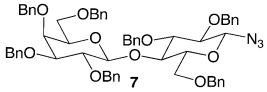
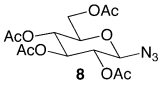
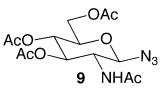
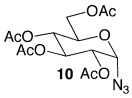
Scheme 2. Synthetic Route to Phosphinothioesters **5a**−**c**[a]



[a] Yields of each step are presented for each compound.

We initially explored the reaction of glycosyl azides **6**−**9** using phosphinothioesters **5a**−**c** (see: Scheme 1 and Table 1, entries 1−4). The coupling proceeded smoothly to afford the desired glycosylated amino acid in moderate yield. Solvent strongly influences the reaction yields. Transformations carried out in dimethylformamide (DMF) typically afforded the products in higher yields than did those conducted in tetrahydrofuran (THF). This solvent effect is pronounced for reactions of the electron-deficient glycosyl azides **8**−**10**; no product was observed when reactions were carried out in THF. Overall, the more nucleophilic phosphinothioesters **5a** and **5b** afforded the glycosylated asparagine derivatives in yields similar to those obtained with **5c**. Still, subtle differences in phosphinothioester reactivity are manifested in the outcome of the coupling reaction (Table 1).

Table 1. Staudinger Ligation of Glycosyl Azides **6**−**10** and Phosphinothioesters **5a**−**c**[a]

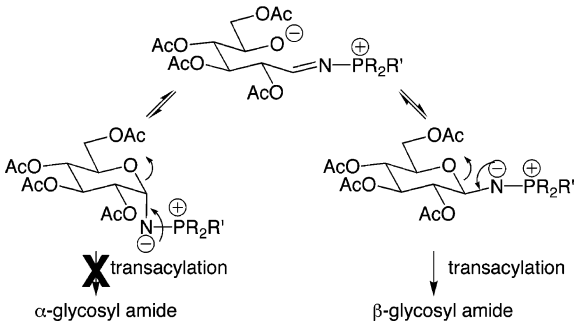
entry	azide	5 a	5 b	5 c
1	 **6**	54	54[b]	55
2	 **7**	51	30	40
3	 **8**	43	50	40
4	 **9**	32	>20	45
5	 **10**	42	0	0

[a] Yields of the β-glycosyl asparagine derivatives are for the isolated product. The α-isomer could not be detected by ^{1}H NMR spectroscopy. Conditions: DMF, rt. [b] THF, rt.

Strikingly, the β-glycosyl amide was the only product observed when β-glycosyl azides **6**−**9** were employed. This finding is advantageous, as this stereochemistry is that of natural *N*-glycoprotein linkages. Our results are consistent with those from related reactions in which conditions for reductive acylation that largely preserve glycosyl azide stereochemistry have been identified.[16] Still, the anomerization of glycosyl iminophosphoranes is known, and this anomerization can give rise to isomeric products.[21] Thus, we sought to explore further the basis for the observed stereochemical outcome.

There are two explanations for the selective production of β-glycosyl amide from compounds **6**−**9**: (1) anomerization of the β-iminophosphorane intermediate does not compete with transacylation or (2) anomerization occurs but transacylation of the β-iminophosphorane is favored (Scheme 3). To distinguish between these, we generated α-glycosyl

Scheme 3. Glycosyl Iminophosphorane Anomerization



azide **10** and exposed it to each of the phosphinothioesters **5a**−**c** (entry 5, Table 1). If **10** affords the α-glycosyl amide, we would conclude that transacylation occurs more rapidly than glycosyl iminophosphorane anomerization. Alternatively, if both isomeric glycosyl amides are generated, we would conclude that isomerization of the intermediate α-iminophosphorane occurs and that the isomeric intermediates both undergo transacylation. Finally, if the β-glycosyl amide is the sole product, from **10**, we would conclude that the anomerization of the α-iminophosphorane occurs and that only the β-iminophosphorane undergoes transacylation. We were surprised to find that the glycosyl amide was generated only when phosphinothioester **5a** was employed. Compound **10** reacted with **5a** to afford the β-glycosyl amide exclusively (Table 1). Thus, regardless of glycosyl azide configuration, all of the phosphinothioester coupling reactions examined proceed stereoselectively to deliver the desired β-glycosyl amide products.

The coupling of azide **10** provides insight into the ligation reaction. The process exhibits a marked dependence on phosphinothioester: only the diethyl derivative **5a** reacts. This phosphine is more nucleophilic than its diphenyl counterpart **5c**. The high electron density of the glycosyl iminophosphorane generated from **5a** may facilitate its

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anomerization (Scheme 3). The difference in reactivity between **5a** and **5b** may be due to the steric hindrance of the latter. Thus, the steric and electronic properties of the phosphinothioester can dramatically influence the reaction outcome.

Our findings extend the utility of the Staudinger ligation to generate a new class of amide bonds. The reaction of phosphinothioesters with glycosyl azides provides a general method for the stereoselective formation of β-glycosyl amides. In the course of these studies, we also developed a new route for the synthesis of functionalized phosphines. The stereoselectivity of the reaction provides insight into the requirements for ligation. Further mechanistic studies and their application to *N*-glycopeptide synthesis are underway.

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Supporting Information Available: Complete experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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