Carbohydrates

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Protecting-Group-Free One-Pot Synthesis of Glycoconjugates Directly from Reducing Sugars**

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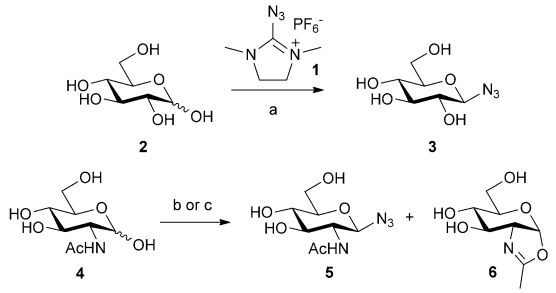
Abstract: *The conversion of sugars into glycomimetics typically involves multiple protecting-group manipulations. The development of methodology allowing the direct aqueous conversion of free sugars into glycosides, and mimics of oligosaccharides and glycoconjugates in a high-yielding and stereoselective process is highly desirable. The combined use of 2-azido-1,3-dimethylimidazolinium hexafluorophosphate and the Cu-catalyzed Huisgen cycloaddition allowed the synthesis of a range of glycoconjugates in a one-step reaction directly from reducing sugars under aqueous conditions. The reaction, which is completely stereoselective, may be applied to the convergent synthesis of triazole-linked glycosides, oligosaccharides, and glycopeptides. The procedure provides a method for the one-pot aqueous ligation of oligosaccharides and peptides bearing alkyne side chains.*



Scheme 1. Direct synthesis of glycosyl azides using ADMP. Reaction conditions: a) **1**, Et_3N, D_2O/MeCN (4:1), 90%; b) **1**, Et_3N, D_2O/MeCN (4:1), 50% of **5**, 50% of **6**; b) **1**, Et_3N, D_2O/MeCN (4:1), then HCl to pH 2, 86% of **5**.

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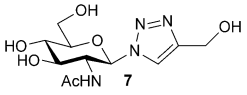
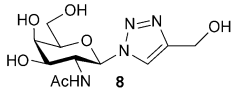
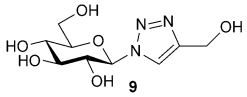
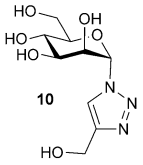
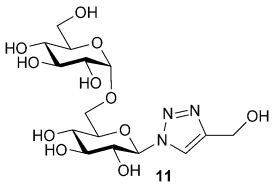
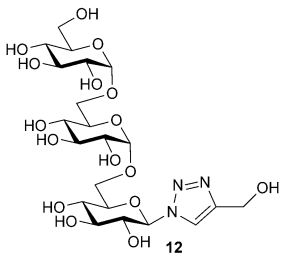
The number of chemical processes by which completely deprotected carbohydrates may be selectively converted into other materials is reasonably limited,[1] as differentiation between multiple hydroxy groups is difficult without the aid of either protecting groups or enzymatic catalysis. However exploitable differences do exist, and under certain circumstances, selective reactions of free sugars are feasible,[2] though very rarely in water.

In 2009, Shoda et al. reported the use of the reagent 2-chloro-1,3-dimethylimidazolinium chloride (DMC) for the

Table 1: One-pot synthesis of glycosyl triazoles directly from the corresponding reducing sugars in water.

Entry	Sugar	Product	Yield[a]	α/β
1	GlcNAc	 **7**	86%	β only
2	GalNAc	 **8**	75%	β only
3	glucose	 **9**	73%	β only
4	mannose	 **10**	73%	α only
5	isomaltose	 **11**	86%	β only
6	isomaltotriose	 **12**	88%	β only

[a] Sugar, Et_3N (5 equiv), ADMP (3 equiv) in D_2O/MeCN (4:1, 270 mM) at 0°C. After 3 h, alkyne (2 equiv), $CuSO_4 \cdot 5H_2O$ (1.5 mol%), and L-ascorbic acid (0.2 equiv) were added and the mixture heated to 50°C for 14 h.

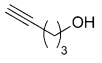
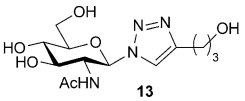
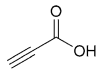
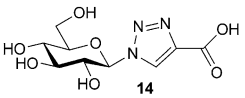
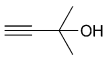
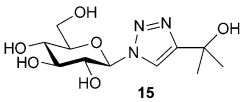
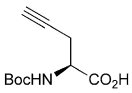
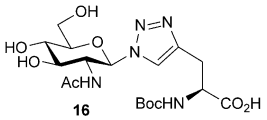
direct synthesis of glycosyl oxazolines from reducing sugars in water.[3] Subsequently, the use of DMC and derivatives was applied to the synthesis of glycosyl azides,[4] pyridyl thioglycosides,[5] peptides linked to carbohydrates through a sulphur atom,[6] and sugar nucleoside diphosphates.[7]

One of the most powerful and widely applied reactions of recent times is the modified Huisgen cycloaddition.[8] This high-yielding transformation may be performed in water, leading to numerous applications, as reaction conditions are compatible with biological systems.

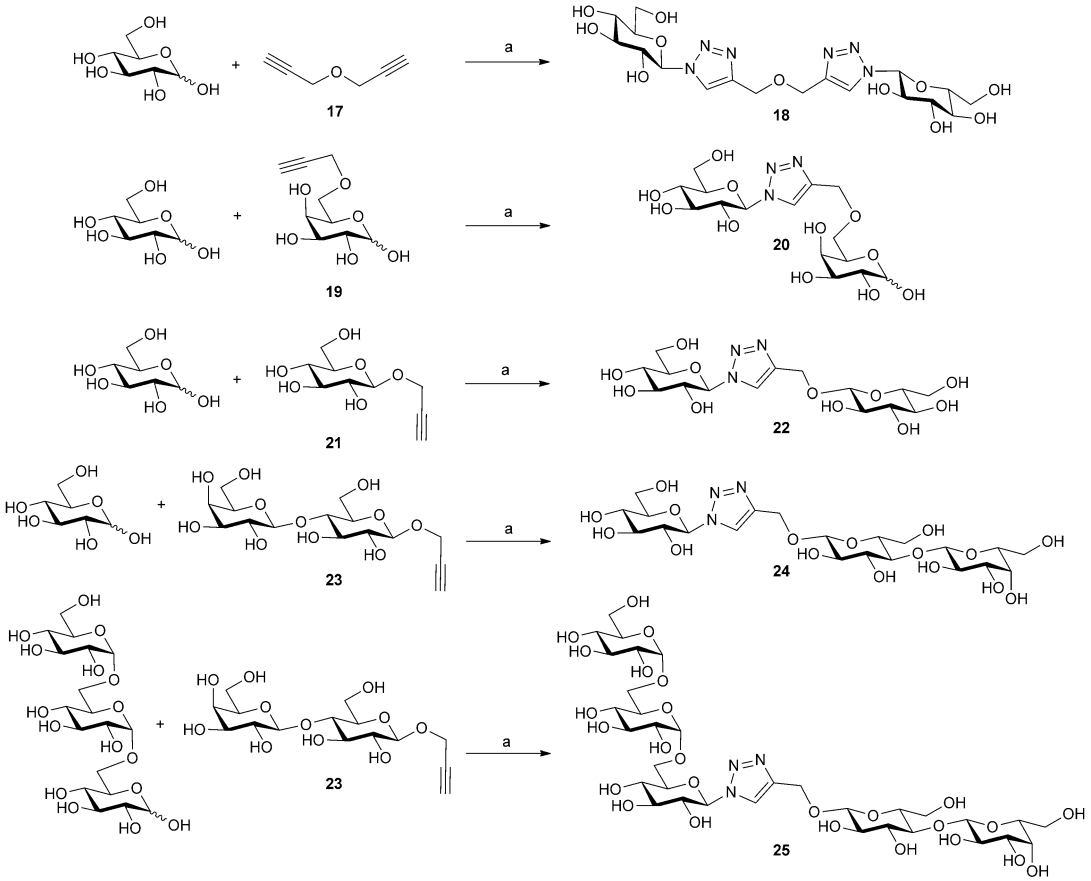
A logical development is the combination of these two processes, that is, selective reaction of the anomeric center and elaboration by "click" chemistry.[9] A single-step process would be particularly attractive, as it would allow the direct conjugation of free sugars with any material containing alkyne functionality, without the need for protecting groups, and using water as the reaction solvent.

The reported route to glycosyl azides using DMC involves the addition of ten or more equivalents of sodium azide, and the use of D_2O as the solvent.[10] Seeking a route that did not require a large excess of azide, we reasoned that 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (**1**, ADMP),[11,12] could be used as both activating agent for the anomeric hydroxy group and as the source of azide. ADMP was prepared by conversion of the hexafluorophosphate salt of DMC to ADMP by reaction with sodium azide, as reported by Kitamura et al.[13] The reaction of ADMP with glucose **2** in a mixture of D_2O and MeCN[14] (4:1), with an excess of Et_3N, gave the desired β-azide **3** in 93% yield (Scheme 1). However, when the reaction was applied to GlcNAc (**4**) a mixture of the β-azide **5** and oxazoline **6** was formed. Oxazoline **6** was not converted into azide **5** under the reaction conditions, indicat-

Table 2: Scope of alkyne substrate.

Entry	Sugar	Alkyne	Product[a]	Yield
1	GlcNAc		 **13**	95%
2	glucose		 **14**	97%
3	glucose		 **15**	81%
4	GlcNAc		 **16**	78%

[a] In all cases, only pure β products were formed.



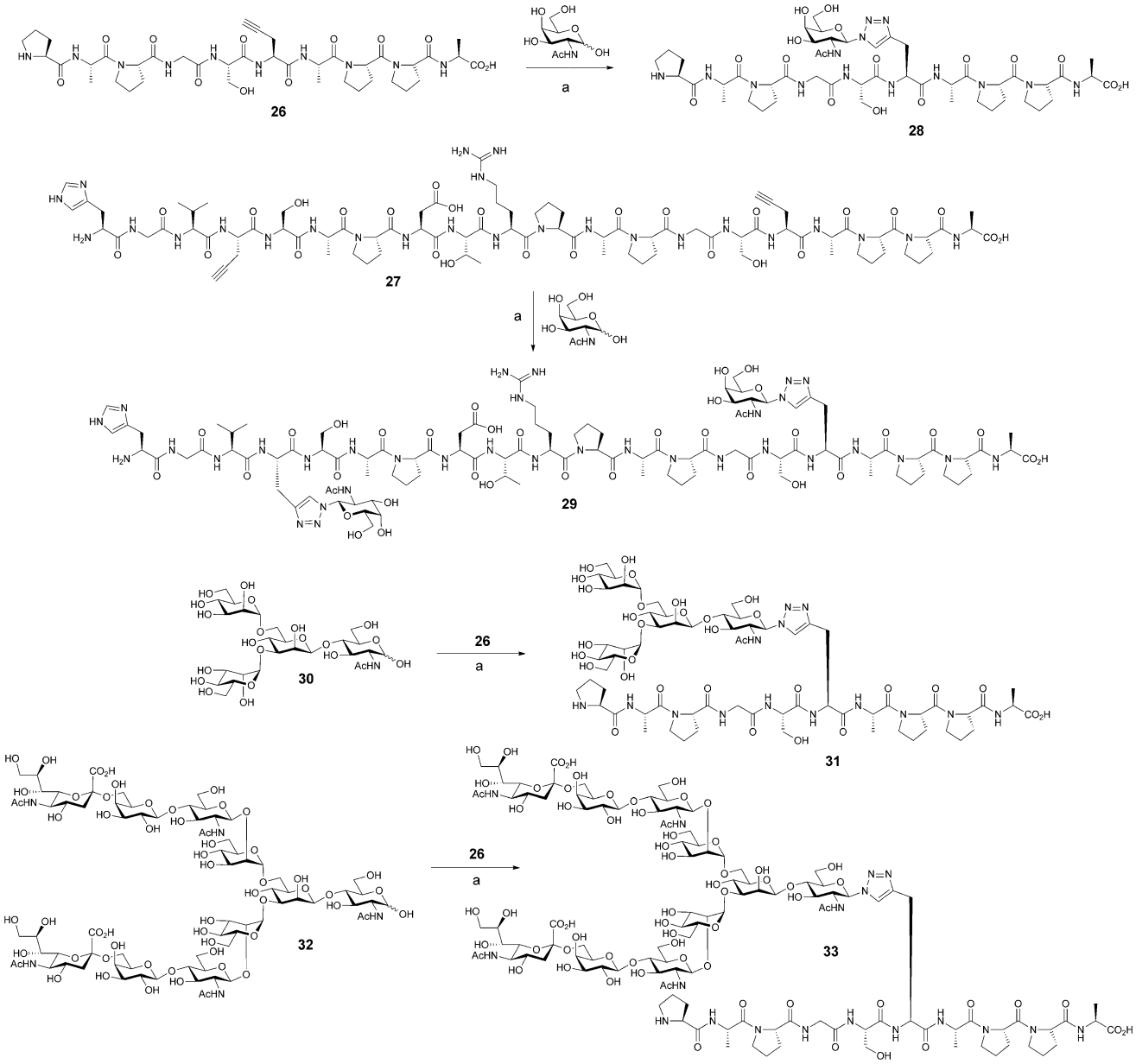
Scheme 2. Direct linking of deprotected carbohydrates to access mimics of oligosaccharides. Reaction conditions: a) sugar, ADMP, Et_3N, D_2O, MeCN, 0°C, 3 h, then add alkyne, $CuSO_4$, ascorbic acid, and heat to 50°C for 14 h.

ing that this material is not an intermediate on the pathway from reducing sugar to glycosyl azide in the Shoda procedure. Given the preponderance for competitive oxazoline formation, the requirement for a large excess of azide (10 equivalents or more) becomes evident, as in the Shoda process intermolecular nucleophilic attack by azide must outcompete oxazoline formation. As a large excess of azide may complicate the click reaction, conditions were sought under which the glycosyl azide **5** was formed in high yield using only three equivalents of **1**. A simple solution involved changing the pH value of the reaction mixture,[15] which effected the complete conversion of **4** into **5** as the sole product. Using this procedure, GlcNAc was converted into azide **5** in 86% yield.

We next turned our attention to the development of a one-pot click reaction. After the formation of the glycosyl azide was complete, the addition of propargyl alcohol, copper sulphate, and sodium ascorbate, and heating the mixture to 50°C led to the formation of the glycosyl triazole in excellent yield with complete stereoselectivity (Table 1). This simple procedure was applied to a number of mono-, di-, and trisaccharides (Table 1). The stereoselectivity of reactions was complete; all sugars except mannose gave the β product exclusively, while mannose gave only the α-glycosyl triazole.

Other alkynes were investigated to assay the generality of the process. Simple alkyl-substituted alkynes reacted in high yield. The efficacy of the reaction was not reduced by the use of carboxylic acid or tertiary alcohol substitution, and the process was equally applicable to more complex and potentially biologically interesting substrates (Table 2).

Triazole-linked oligosaccharides have been demonstrated to act as glycomimetics,[16] though published routes involve multiple-step syntheses and other difficulties, for example, the



Scheme 3. Synthesis of MUC1 glycopeptides in water through direct reaction with reducing sugars. Reaction conditions: a) sugar, ADMP, Et_3N, D_2O, MeCN, RT, 6 h, then add peptide, $CuSO_4$, ascorbic acid, and heat to 50°C for 14 h.

problematic removal of benzyl protecting groups.[17] The direct linking of carbohydrates through click reactions would allow rapid and efficient access to a wide range of such materials without the need for any protecting-group manipulations. The applicability of the approach was therefore assessed for the assembly of oligosaccharide structures. The conjugation of free reducing sugars to a variety of other carbohydrate materials containing an alkyne functionality gave rise to a variety of di-, tri-, and pentasaccharide mimics in a single operation (Scheme 2). In all cases, the reactions were high yielding and completely stereoselective. From these preliminary studies it would appear that there is little limitation as to the oligosaccharides structures that can be linked by this process.

Click chemistry has been applied to access glycopeptides[18,19] and glycoproteins,[20] though all of the reported procedures require multiple-step manipulations of the carbohydrate component. As further exemplification, this approach was applied to the direct synthesis of glycopeptides. Propargyl glycine (Pra) is readily incorporated into synthetic peptides using solid-phase peptide synthesis (SPSS). Glycosylated versions of the tandem repeat domain of the cancer-associated mucin MUC1[21] have recently shown significant potential as components of synthetic anticancer vaccines.[22] Two synthetic MUC1 peptides incorporating Pra were selected as alkyne-bearing peptides for glycoconjugation. Fragment **26**, corresponding to residues 11–20 in which T16 was replaced by Pra, and the full-length tandem repeat domain **27**, in which both T4 and T16 were replaced by Pra, were assembled as previously described.[23]

The reaction of **26** with GalNAc gave glycopeptide **28** in 47% yield (isolated product; Scheme 3). The reaction of GalNAc with peptide **27**, which contains two Pra residues, was equally efficient and gave diglycosylated peptide **29**. While these two processes are comparable in yield to those using a presynthesized glycosyl azide,[23] the current approach, in which the peptide undergoes direct reaction with a reducing sugar, is considerably more efficient.

The process could be used to attach *N*-glycan oligosaccharides to peptides, demonstrated by the use of the core *N*-glycan tetrasaccharide **30**.[24] The reaction of **26** with **30** gave glycopeptide **31** in 30% yield (isolated product). A significant attraction of the present approach is that naturally derived or commercially available oligosaccharides may be used without the need for any protection/deprotection sequences. Complex biantennary *N*-glycan **32** decasaccharide, readily accessed in significant quantities from egg yolks,[25] reacted smoothly with peptide **26** to give glycopeptide **33**, which bears a complex biantennary *N*-glycan, in 42% yield (isolated product).

The success of these reactions demonstrates the utility of the approach for the direct conjugation of reducing oligosaccharides to synthetic peptides under aqueous conditions. As proteins containing alkyne side chains, such as homopropargyl glycine,[26] may be expressed, a clear avenue for further application is the direct conjugation of oligosaccharides to proteins that contain alkyne tags, in order to produce homogenous neoglycoproteins bearing glycans of defined structure.

In conclusion, methodology has been developed that allows the direct conjugation of reducing sugars to alkynes under aqueous conditions in a single reaction vessel. The completely stereoselective procedure appears to have wide applicability, both in terms of carbohydrate and alkyne coupling partner. Reducing sugars may be linked directly to peptides and other carbohydrates, giving facile access to mimics of glycopeptides and oligosaccharides, respectively.

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