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One-pot synthesis of triazole-linked glycoconjugates

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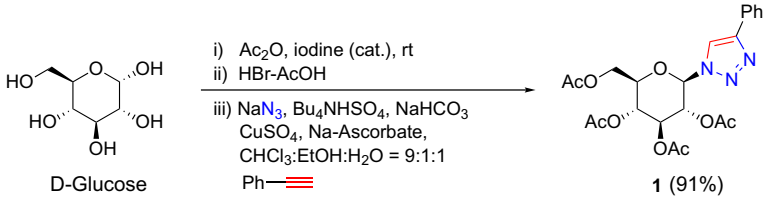
Abstract—Highly efficient one-pot synthesis of 1,2,3-triazole-linked glycoconjugates was presented involving a Cu(I) catalyzed 1,3-dipolar cycloaddition as the key step. It offers a convenient route to prepare neoglycoconjugates derived from unprotected saccharides or peracetylated saccharides.

Oligosaccharides and glycoconjugates exert important effects on many complex biological events,[1] including the cellular recognition in the processes of inflammation,[2] immune response,[3] tumor metastasis,[4] and bacterial and viral infections.[1a] In addition, glycosylation of proteins and lipids are key factors in modulating their structures and functions.[1] In order to study the functions of oligosaccharides in molecular detail, numerous glycosylation methods have been developed for the assembly of complex glycoconjugates.[5,6] However, the traditional protocol for regio- and stereo-control in glycoside bond-forming process often leads to laborious synthetic transformations and tremendous protecting group manipulations, which complicate the overall synthetic process and decrease the synthetic efficiency.[7] Hence, development of new strategies and tactics in glycosylation reactions are of growing importance.[8,9]

During our research in the synthesis of carbohydrate-based biosensors, we began exploring efficient and versatile methods that provide an easy access to carbohydrate conjugates from both chemical and biosynthetic approaches. Herein, we report a rapid one-pot synthesis of triazole-linked glycoconjugates from readily available unprotected saccharides or saccharide acetates on the basis of Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition reaction of azides and alkynes.[10]

As a prototype of 'click chemistry',[11] the recent advance of Cu(I) catalyzed condition affords superior regioselectivity, high tolerance of other functionalities, and almost quantitative transformation under mild conditions. The 1,3-dipolar cycloaddition reactions have been used in the synthesis of neoglycoconjugates[12] and the bioconjugation study of glycosides.[13] However, these compounds were designed by preparation of protected monosaccharides and their subsequent conversion into corresponding azides. Being inspired by the recent discovery of one-pot 1,3-dipolar cycloaddition with alkynes and in situ generated azides,[14] we designed a 'dovetailed' process to synthesize triazole-linked neoglycoconjugates.



Scheme 1.

Keywords: Click chemistry; 1,3-Dipolar cycloaddition; Glycosylation; Neoglycoconjugate; 1,2,3-Triazole; Carbohydrate; Bioconjugation.
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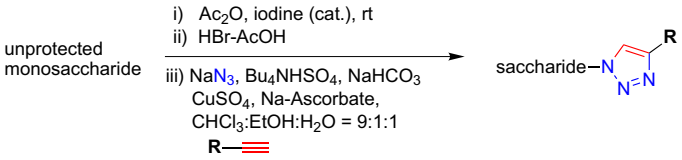
Table 1. One-pot synthesis of triazolylglycosides from unprotected monosaccharides



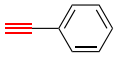
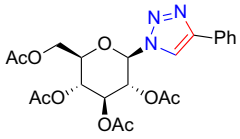
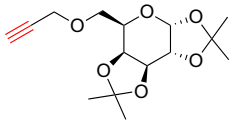
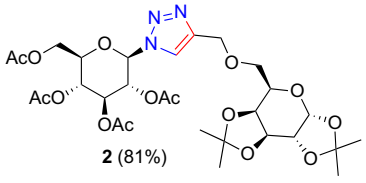
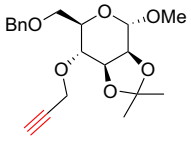
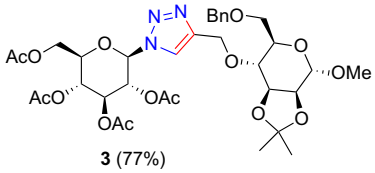
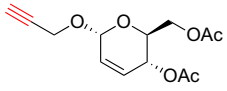
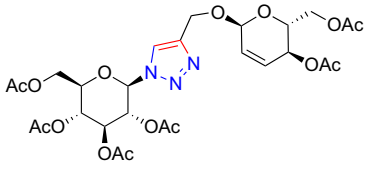
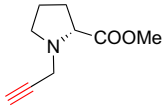
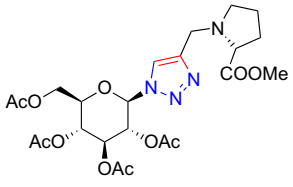
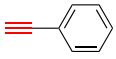
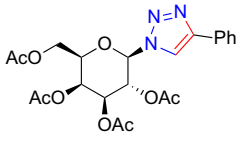
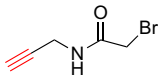
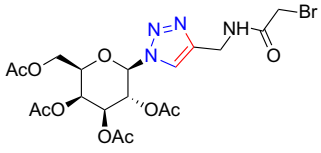
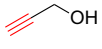
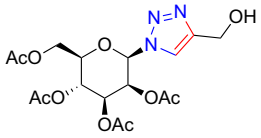
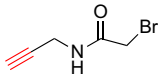
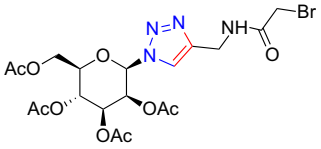
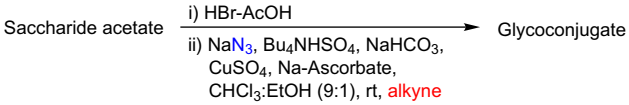
Entry	Saccharide	—R	Product[a,b]
1	D-Glucose		 **1** (95%)
2	D-Glucose		 **2** (81%)
3	D-Glucose		 **3** (77%)
4	D-Glucose		 **4** (71%)
5	D-Glucose		 **5** (72%)
6	D-Galactose		 **6** (91%)
7	D-Galactose		 **7** (71%)
8	D-Mannose		 **8** (71%) [c]

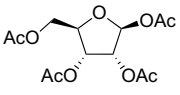
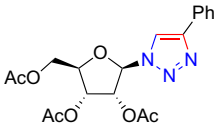
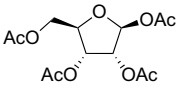
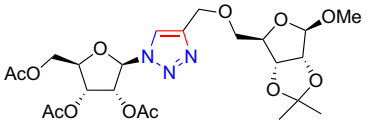
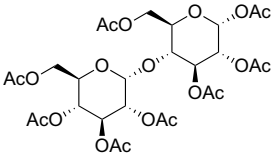
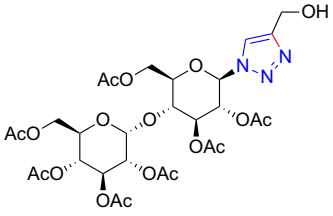
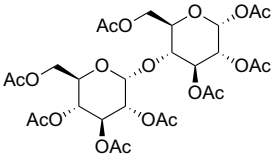
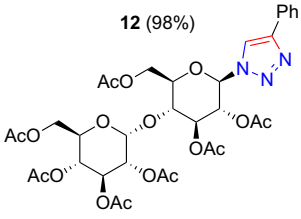
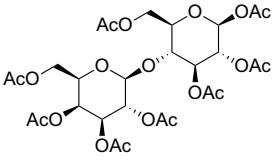
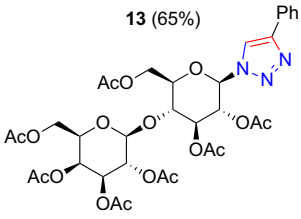
Table 1 (*continued*)

Entry	Saccharide	—R	Product[a,b]
9	D-Mannose		 **9** (98%) [c]

[a] Isolated yield. In most situations, the product will precipitate out. Due to the water solubility, some product was lost during filtration.
[b] Room temperature overnight reaction.
[c] Room temperature for 3 h, then incubation at 80 °C overnight.

Table 2. One-pot synthesis of triazolylglycosides from saccharide acetates



Entry	Saccharide acetate	Glycoconjugate[a,b]
1	 D-Ribose tetraacetate	 **10** (81%)
2	 D-Ribose tetraacetate	 **11** (74%)
3	 D-Cellobiose octaacetate	 **12** (98%)
4	 D-Cellobiose octaacetate	 **13** (65%)
5	 D-Lactose octaacetate	 **14** (76%)

[a] Isolated yield. Due to the water solubility, some product was lost during filtration.
[b] Room temperature overnight reaction.

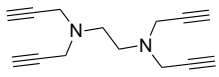
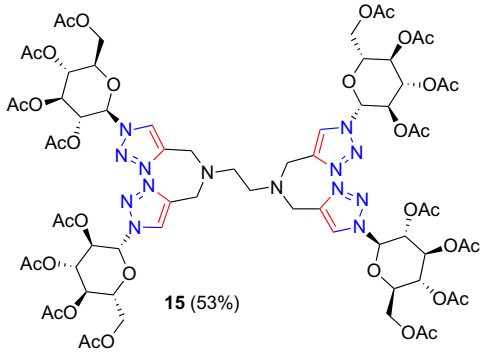
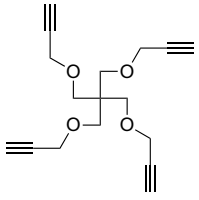
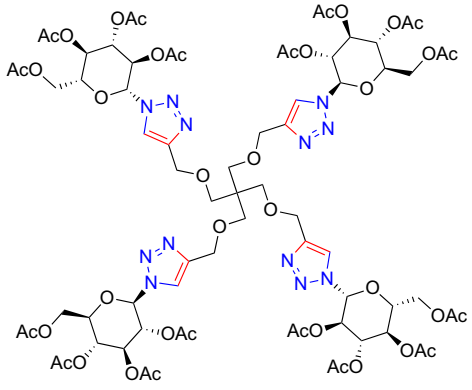
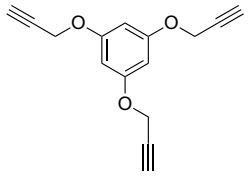
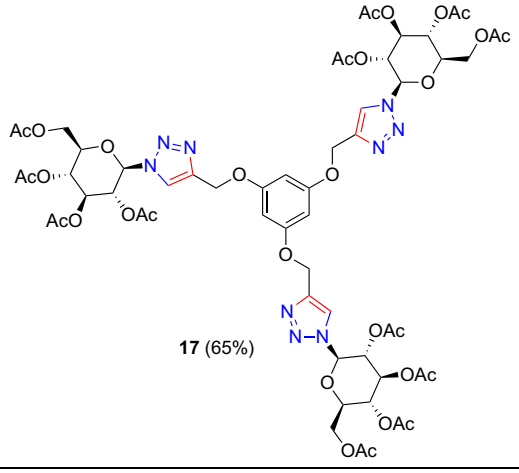
As shown in Scheme 1, unprotected D-glucose was acetylated with acetic anhydride catalyzed with trace amount of iodine, followed by brominolysis of the anomeric acetate. After removal of all volatiles, a subsequent azide conversion and in situ Cu(I) catalyzed 1,3-dipolar fusion reaction with phenyl acetylene afforded

the triazolylglycoside **1** in 91% yield. The product appeared as a white precipitate and a simple filtration afforded the product in high purity.

This reaction is remarkably efficient and can be used to link a variety of functionality to the anomeric position of monosaccharides (Table 1). With D-glucose and D-galactose as starting materials, the reaction was performed smoothly at room temperature (entries 1–7). D-Mannose was found to be less reactive than other carbohydrates due to the steric hindrance caused by the β-configuration of the 3-substituent. However, raising the temperature to 80 °C circumvented this problem and the product was isolated in reasonable yields (entries 8 and 9). Besides the simple alkynes, this reaction could also link carbohydrates with other carbohydrates (entries 2–4) or amino acid (entry 5). All reactions were highly regioselective and only 1,4-triazoles were produced. In most situations, quantitative transformations were observed and a simple filtration afforded the product in high purity. Due to the water solubility of some products, the isolated yields were from 71–98%.[15]

This reaction also enables the attachment of other linkers to carbohydrates, which may lead to further connection with biomacromolecules such as proteins via

Table 3. Synthesis of multivalent glycoconjugates

Entry	R–(≡)$_n$	Product[a,b]
1		 **15** (53%)
2		 **16** (61%)
3		 **17** (65%)

[a] Isolated yield.
[b] Overnight reaction at 40 °C.

traditional bioconjugation methods. For example, the conjugates of propargyl 2-bromoacetamide with D-galactose and D-mannose (entries 7 and 9 of Table 1) were synthesized with this one-pot protocol and consequent de-acetylation, which has been used to modify the cysteine residues of bionanoparticles for cell binding studies.[16]

Saccharide acetates can also be used as substrates for this reaction. As shown in Table 2, the acetates of D-ribose, D-cellobiose, and D-lactose have been employed as starting materials. With a similar brominolysis procedure followed by the in situ generation of glycosyl azide and 1,3-dipolar cycloaddition reaction, triazolyl glycoconjugates were synthesized in satisfactory yields.[17]

Polyvalent interactions between glycoconjugates and receptors play a very important role in biological systems.[18] To investigate the feasibility of our methods in the synthesis of polyvalent glycoconjugate clusters, a number of multivalent alkynes have been synthesized (Table 3). We were delighted to find that our one-pot process proceeded well with these substrates. A single product was detected in all the cases and purification by flash chromatography afforded reasonable yields. With this one-pot protocol, the synthesis of glycodendrimers is undergoing in our laboratory.

In conclusion, we have developed an efficient one-pot glycosylation method for the synthesis of 1,2,3-triazole bridged glycoconjugates. Both unprotected carbohydrates and carbohydrate acetates can be used as starting materials. By executing several reaction steps in a single pot and purifying only at the final stage, this procedure excludes the isolation of all the intermediates including labile glycosyl bromides, which significantly reduces the reaction time and improves the overall yield. Therefore, the reaction is very useful in the synthesis of oligosaccharides, glyco-peptides, glycosyl amino acids, and other glycoconjugates. Due to the efficient transformation of the reaction, it can also be used in the synthesis of polyvalent glycomolecules such as dendrimers.[19]

Furthermore, cell surface carbohydrates, as part of glycosylated proteins and lipids, are characteristic markers for different cell types. Therefore, for the emerging research in cell biology and carbohydrates-based vaccines development, it is important to develop efficient methods to conjugate carbohydrates with proteins or other biomolecules.[20] Our method affords a simple way to build a metabolically stable triazole linkage between carbohydrates and other functional groups, which can be used as a new strategy for the bioconjugation of carbohydrates.[21]

Acknowledgements

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Supplementary data

Spectral characterization of all products. Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.tetlet.2005.01.175.

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