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3-Mercaptopropanol as a Traceless Linker for Chemical and Enzymatic Synthesis of Oligosaccharides

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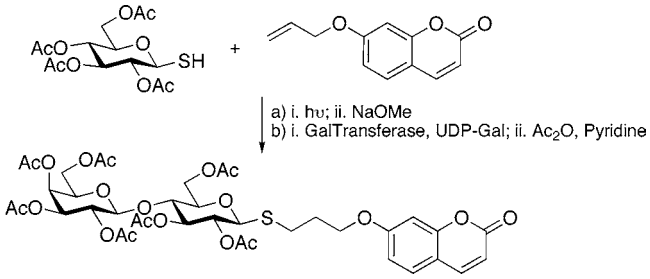
ABSTRACT



The reducing end of protected carbohydrates can be equipped with a series of aglycones via the photochemical installation of a 3-mercaptoethanol linker. This linker is stable during chemical and enzymatic glycosylation reactions but can be activated readily and efficiently to couple oligosaccharides with different nucleophiles. This approach provides straightforward access to a range of molecules that serve as probes for carbohydrate modifying enzymes.

The increasing interest in the biology of glycoconjugates has resulted in a rapidly rising demand for defined carbohydrates and their analogues to serve as tools in the study of biological processes.[1] Chemical carbohydrate syntheses in solution and on solid support have been greatly accelerated, and diverse target compounds can be prepared faster and more reliably than ever before.[2] Still, serious synthetic challenges have to be addressed and enzymatic methods constitute a viable alternative to chemical synthesis, particularly for production scale-up.[3] The combination of solution- or solid-phase chemical synthesis and enzymatic assembly holds significant opportunities for the synthesis of complex oligosaccharides.

The superb substrate- and stereospecificity of glycosyl transferases is of great benefit in the construction of glycosidic linkages but is limiting in the incorporation of different aglycones. Because enzymatic oligosaccharide syntheses typically are carried out in aqueous solutions, different aglycons have been used to mark the substrate to facilitate purification and separation. Useful aglycons include hydrophobic groups, chromophores, and fluorophores that remain in place throughout the synthesis and appear in the final product. Traceless replacement of the aglycons that are incorporated to facilitate purification, by any other group in the final product, would be desirable.[4]

Here, we describe a traceless 3-mercaptopropanol linker to connect different aglycons to the reducing end of a carbohydrate that serves as a substrate in enzymatic elonga-

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(1) (a) *Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H. H., Hart, G.W., Marth, J., Eds.; Cold Spring Laboratory Press: Plainview, New York, 1999. (b) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357. (c) Wong, C.-H. *J. Org. Chem.* **2005**, *70*, 4219.

(2) (a) *Carbohydrate-based Drug Discovery*; Wong, C.-H., Ed.; Wiley-VCH Verlag GmbH & Co. KgaA: Weinheim, 2003. (b) Routenberg, L. K.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 602.

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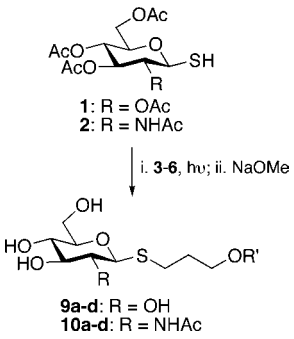
Scheme 1. Photochemical Installation of Allyl Ethers and Esters on Thio-glucose and Thio-glucosamine



tion steps. Following the synthesis of the oligosaccharide, the linker can be activated to introduce different aglycons without a trace of the original linker.

3-Mercaptopropanol has previously been used as a spacer for the construction of carbohydrate-containing dendrimers.[5] Installation of an anomeric 3-mercaptopropanol linker at the reducing end of an oligosaccharide can be readily achieved by the photochemical reaction of allyl ethers with carbohydrates bearing a C1 thiol group.[6] The ease of installation of the 3-mercaptopropanol attracted our attention to introduce different aglycons into oligosaccharide substrates for enzymatic elongation. The stable thioglycoside linkage provides a spacer to separate the often bulky aglycon from the sugar. This linker can be readily severed as the thioglycoside is prone to selective alkylation and thereby acts as a glycosylating agent. Compounds **1**[5] and **2**[7] were reacted with a series of allyl ethers (**3** and **5**−**7**) and allyl esters (**4** and **8**) (Scheme 1 and Table 1).

Table 1. Photochemical Installation of Allyl Ethers and Esters on 1-Thio-glucose and 1-Thio-glucosamine

carbohydrate	allyl derivative	product	yield[a]
1	**3**	**9a**	65%
1	**4**	**9b**	75%
1	**5**	**9c**	72%
1	**6**	**9d**	75%
1	**7**	**9e**	75%[b]
1	**8**	**9f**	55%[b]
2	**3**	**10a**	68%
2	**4**	**10b**	70%
2	**5**	**10c**	65%
2	**6**	**10d**	75%

[a] Yield of final, deacetylated compound. [b] Compounds isolated as acetates.

Allyl derivatives **3**−**7** are chromophores, and the biotin derivative **8** enables simple product isolation (Figure 1). Interestingly, stoichiometric amounts of **1** and **2** were sufficient for the reaction with coumarin (**7**) or fluorescein (**8**) derivatives, and several equivalents of the thio-carbohydrates were required for efficient reactions with naphthalene derivatives.

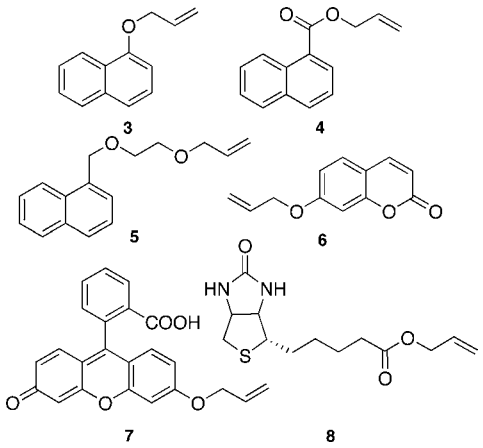
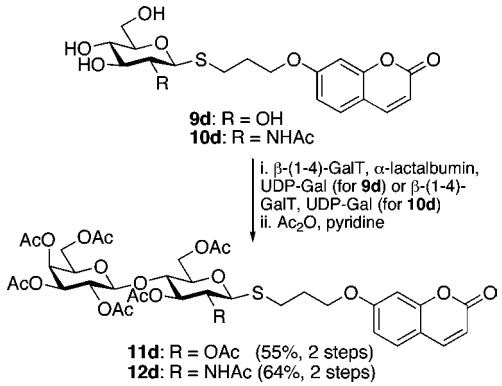


Figure 1. Allyl ethers and allyl esters that were photochemically coupled to 1-thio sugars.

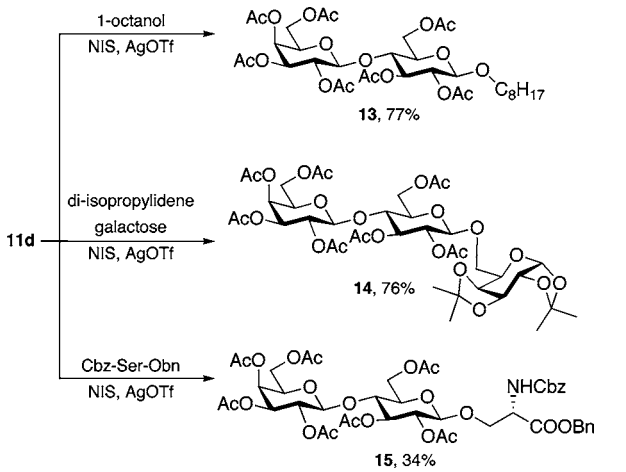
UV irradiation at 250 nm of a solution containing the per-*O*-acetylated 1-thio monosaccharides and the allyl ethers or esters in methanol yielded the desired thioglycosides in 4 h (Table 1). After complete conversion, sodium methoxide was added to cleave all *O*-acetates. Continuous extraction with methylene chloride afforded the desired compounds in good yield and purity. The biotinylated and fluorescein-containing compounds **9e** and **9f** were not deacetylated. The one-pot coupling/deprotection sequence described here overcomes separation problems caused by mixtures of α/β stereoisomers, commonly associated with the installation of other anomeric linkers.

To determine the compatibility of the mercaptopropanol linker with enzymatic glycosylations, glucose **9d** and glucosamine **10d** were enzymatically extended to form lactose (**11d**) and lactosamine (**12d**) derivatives. Treatment of the monosaccharides with β-galactosyltransferase and UDP-galactose readily afforded the desired disaccharides (Scheme 2). Solid-phase extraction using silica cartridges functionalized with C-18 alkane chains, followed by acetylation and flash chromatography, yielded pure **11d** and **12d**.

Scheme 2. Enzymatic Galactosylation of Glucosamine and Glucose Derivatives **9d** and **10d**



Scheme 3. Glycosylations with Chemoenzymatic Derived Lactose Derivative **11d**



After demonstrating that enzymatic glycosylations can be achieved on the mercaptoethanol linker system, we had to show that different aglycones can replace the linker. Activation of acetylated lactose thioglycoside **11d** by treatment with *N*-iodosuccinimide (NIS) and silver triflate (AgOTf) readily installed 1-octanol, diisopropylidene galactose, or protected serine (Scheme 3).[8] The low yield for the serine derivative was accompanied by a substantial amount of hydrolyzed glycosylating agent, presumably due to the poor nucleophilicity of the serine hydroxyl group.[9]

In conclusion, we have developed an efficient method to photochemically install a 3-mercaptoethanol linker at the reducing end of protected carbohydrates. Sugars containing this linker were enzymatically elongated before different nucleophiles replaced the linker by chemical glycosylation. This method is currently employed in the combined chemical and enzymatic construction of carbohydrate libraries containing different aglycones to be tested as enzyme inhibitors.

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

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(8) The use of benzyl protecting groups instead of acetates gave an α/β-mixture of 1:1. Lactosamine **12d** was not a good glycosyl donor except during hydrolysis.

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