Chemoselective Ligation of Acetylated 1-Maleimidosugars to Peptides for the Preparation of Neoglycopeptides

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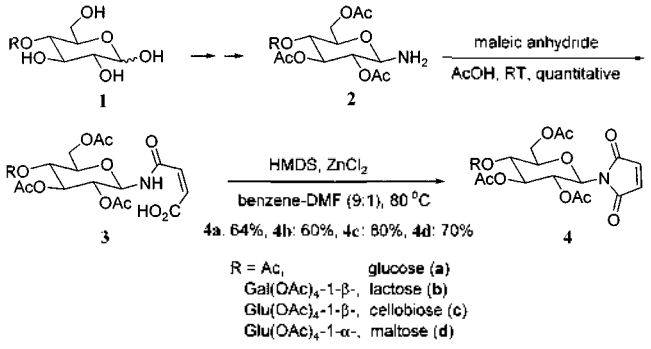
The biological functions of oligosaccharides in glycoproteins and glycolipids have been extensively investigated by chemists, biochemists and biologists. The cell-surface oligosaccharides are known to contribute to many important biological roles including cell-cell communication, cell adhesion, cell growth, bacterial and viral infection.[1] Carbohydrates of glycoproteins also influence the intrinsic properties of proteins and thus result in the proper folding of proteins, increased thermal stability and resistance to proteases.[1] In general, glycoproteins contain microheterogeneous carbohydrate moieties, hence rendering their purification from natural sources difficult and time-consuming. As a consequence, the biological roles of carbohydrates in glycoproteins remain elusive. To better understand the molecular basis of oligosaccharides and to develop glycoproteins as potential pharmaceutical agents, it is imperative to readily access glycoproteins with well-defined oligosaccharide chains. Recently, several approaches have been explored to introduce carbohydrates moieties into proteins or peptides *via* nonnative glycosidic linkage.[2-7]

As the first step to develop new methodology to prepare homogeneous glycoproteins, we have investigated the chemoselective ligation of peracetylated 1-maleimidosugars to peptides. Maleimido functionality has been widely used for the selective modification of cysteine residues of proteins in the presence of other amino acids with nucleophilic side chains such as Lys, Arg, His, Glu, Asp, Ser, Thr and Tyr.[8]

Synthesis of peracetylated 1-maleimidosugars **4** was effectively achieved in two steps from acetylated glycosylamines **2** obtained from corresponding mono- and disaccharides **1** as shown in Scheme 1.[2,9] The protected glucosylamine (**2a**), lactosylamine (**2b**), cellobiosylamine (**2c**) and maltosylamine (**2d**) were reacted quantitatively with maleic anhydride in AcOH. The resultant amic acids **3** were then converted to the desired acetylated 1-maleimidoglucose (**4a**), 1-maleimidolactose (**4b**), 1-maleimidocellobiose (**4c**) and 1-maleimidomaltose (**4d**) by hexamethyldisilazane (HMDS) and $ZnCl_2$ in good yields (60-80%).[10]



Scheme 1

We then tested the potential of peracetylated 1-maleimidosugars as thiol-selective oligosaccharides to generate glycosylated peptides. The 1-maleimidosugars were conjugated to model peptides containing a single cysteine residue to produce the corresponding glycosylated products (Table 1). The progress of ligation reactions was directly monitored by decrease in absorbance at 270 nm characteristic of a maleimido group or the unreacted SH was determined by 5,5`-dithiobis(2-nitrobenzoic acid) (DTNB) using Ellman method.[11] First, glutathione (γ-GluCysGly) was employed as a simple model peptide and efficiently converted to the corresponding carbohydrate-adducts **5a** and **5b** by 2 molar equivalents of **4a** and **4b**, respectively, in 20% CH_3CN-water. Subsequent to the removal of CH_3CN from the reaction mixture, the unreacted **4a** and **4b** were extracted with EtOAc. The glycosylated glutathiones **5a** and **5b** were characterized by FAB MS.[12]

Next, a synthetic Fas peptide (Ac-^{15}VARLSCKSVNAQ^{26}-NH_2, Table 1) was glycosylated according to a similar procedure. Although it is well documented that the interaction between glycoproteins Fas and FasL induces apoptosis (programmed cell-death), the function and nature of oligosaccharide chains on Fas and FasL remain elusive.[13] Thus, we were intrigued to prepare the glycosylated Fas and/or FasL to address the biological functions of carbohydrate moieties. Based on NetOGlyc 2.0 prediction server data and preliminary mutagenesis studies, it appears that Ser20 corresponds to *O*-glycosylation site in Fas protein.[14] We synthesized Fas peptide (15-26, Ser20Cys) using standard solid phase peptide synthesis and reacted with 1 molar equivalent of **4a-d** in DMSO to give the corresponding glycosylated products **6a-d**. Characterization of Fas peptide by ESI MS following ligation revealed selective incorporation of the oligosaccharides into the Fas peptide.[15]

In conclusion, we described the facile synthesis of peracetylated 1-maleimidosugars as new thiol-selective oligosaccharides and their efficient chemoselective ligation to glutathione and Fas peptide. We believe that this methodology may be useful in the synthesis of a variety of glycoconjugates.

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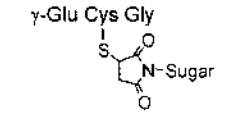
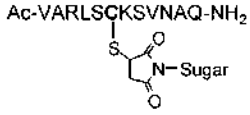
Table 1. Chemoselective ligation of peracetylated 1-maleimidosugars to glutathione and Fas peptide

Protein/Peptide	Maleimidosugar	Reaction time (min)		Product
1. γ-Glu Cys Gly	**4a**	20	**5a**	
	4b	20	**5b**	
2. Ac-VARLSCKSVNAQ-NH_2	**4a**	20	**6a**	
	4b	20	**6b**	
	4c	20	**6c**	
	4d	20	**6d**	

The Experimental details are described in the text

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12. *Selected data* for **5a** (LR FAB-MS): calcd for $C_{28}H_{39}N_4O_{17}S$ [M+H] 735.2, found 735.2. **5b**: calcd for $C_{34}H_{55}N_4O_{25}S$ [M+H] 1023.3, found 1023.4. The structure of two products was identified by ^1H NMR. Although ^1H NMR spectra of products were complicated due to the formation of two diastereomers by coupling of a maleimido group to SH functionality, it was shown that the clean ligation reaction occurred.
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15. *Selected data* for **6a**: (ESI MS): calcd for $C_{72}H_{118}N_{20}O_{28}S$ $[M]^+$ 1742.8, found 1742.9. **6b**: calcd for $C_{84}H_{134}N_{20}O_{36}S$ $[M]^+$ 2030.9, found 2031.6. **6c**: calcd for $C_{84}H_{134}N_{20}O_{36}S$ $[M]^+$ 2030.9, found 2031.8. **6d**: calcd for $C_{84}H_{134}N_{20}O_{36}S$ $[M]^+$ 2030.9, found 2031.1.