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Chemoselective ligation of maleimidosugars to peptides/protein for the preparation of neoglycopeptides/neoglycoprotein

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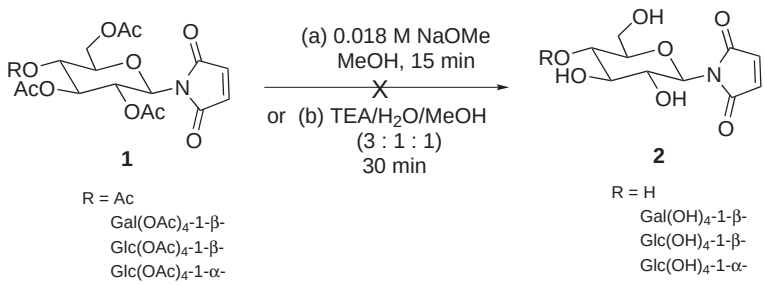
Abstract—Two types of maleimidosugars as thiol-selective carbohydrates, 1-maleimidosugars and acetyl-linked maleimidosugars, were efficiently synthesized. They were coupled to glutathione, Fas peptide and bovine serium albumin (BSA) to prepare the corresponding glycosylated peptides and a protein via stable thioether linkages in a chemoselective manner.

It is known that oligosaccharides in glycoconjugates such as glycoproteins and glycolipids play important roles in many biological processes and thus their biological functions have been extensively investigated by chemists, biochemists and biologists.[1] In general, glycoproteins in eukaryotic cells are expressed as heterogeneous mixtures of glycoforms, namely, proteins bearing heterogeneous carbohydrate moieties. As a consequence, their purification from natural sources is difficult and their biological roles 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.

Several approaches to introduce carbohydrate moieties into proteins or peptides at specific positions via nonnative glycosidic linkage have been reported.[2] For example, iodoacetamide-containing sugars were employed for site-specific glycosylation of a cysteine residue in proteins/peptides.[3] Carbohydrates bearing an asymmetric disulfide linkage were also used as thiol-reactive reagents.[4] *O*-Linked *N*-acetylgalactosaminyl peptides after treatment with galactose oxidase were coupled to aminooxy sugars to prepare *O*-linked glycopeptides.[5] In a different approach, a lysine residue was glycosylated using a designed helix–loop–helix motif.[6]

In an attempt to develop new methodology to prepare homogeneous glycoproteins, we have investigated the



Scheme 1.

Abbreviations: DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; ESI MS, electrospray ionization mass spectrometry; TBSOTf, *tert*-butyldimethylsilyl trifluoromethanesulfonate; TEA, triethylamine; TFA, trifluoroacetic acid; TMSOTf, trimethylsilyl trifluoromethanesulfonate.

Keywords: carbohydrates; thioethers; glycopeptides/glycoproteins; glycosylations.

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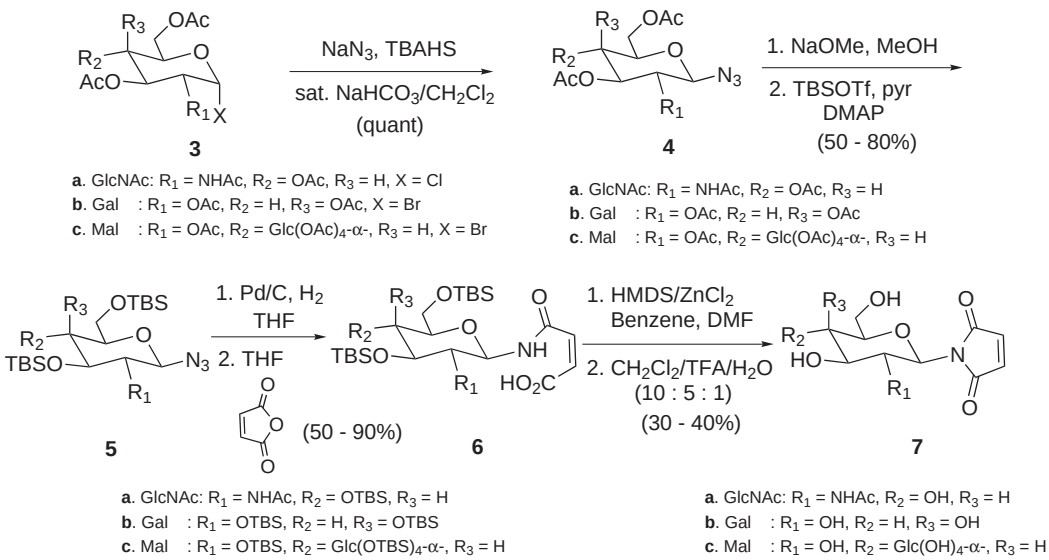
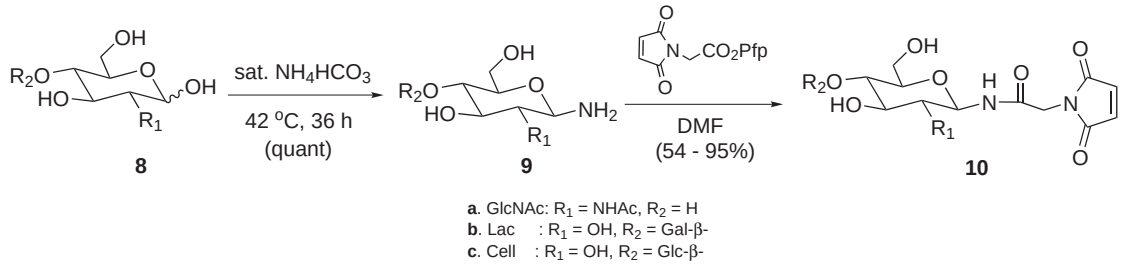
chemoselective ligation of carbohydrates containing a maleimide group to peptides or proteins. Maleimide functionality has been widely used for the selective modification of thiol in the presence of other nucleophiles such as amine, alcohol, carboxylate and guanidine.[7] Two types of thiol-reactive carbohydrates, 1-maleimidosugars (**7**) and acetyl-linked maleimidosugars (**10**), were synthesized to produce neoglycosylated peptides/proteins. For the preparation of anomeric maleimidosugars (**7**), we first made attempts to remove acetyl groups in peracetylated maleimidosugars **1**[8] under mild basic conditions such as 0.018 M NaOMe and TEA–H_2O–MeOH (3:1:1) (Scheme 1).[4a] However, it was found that a maleimide group was hydrolyzed with concomitant deacetylation under these conditions. Therefore, we protected hydroxyl groups by an acid-labile group such as TBS or TMS. Acetohalosugars **3** were converted to the corresponding acetylated glycosyl azides **4** by substitution of chloro (**3a**) and bromo (**3b**, **c**) groups with azide under biphasic conditions (Scheme 2). Removal of acetyl groups and reprotection of the exposed alcohols by TBS or TMS with TBSOTf or TMSOTf in the presence of DMAP gave TBS or TMS-protected sugars. However, it was discovered that a TMS protecting group was not suitable since it was partially cleaved during the next reduction of azide with Pd/C and H_2. Reduction of TBS-protected azide **5** and reaction of the resultant glycosylamines with maleic anhydride in THF afforded amic acids **6** in moderate to high yields. Cyclization of amic acids **6** with hexamethyldisilazane (HMDS) in the presence of $ZnCl_2$[9] and a subsequent deprotection of TBS by CH_2Cl_2–TFA–H_2O (10:5:1) furnished the desired products **7**. On the other hand, we also prepared acetyl-linked maleimidosugars (**10**) that could be synthesized by a simple reaction step. One-pot amination of carbohydrates[10] and a subsequent coupling with pentafluorophenyl *N*-maleoylacetate[11] in DMF produced the acetyl-linked maleimidosugars **10** in 54–95% yield (Scheme 3).

We then examined the potential of synthesized maleimidosugars as thiol-reactive oligosaccharides to generate glycosylated peptides/protein. First, glutathione (γ-GluCysGly) was efficiently glycosylated at a cysteine residue to yield the corresponding carbohydrate-adducts **7a$_1$**–**7c$_1$** and **10a$_1$**–**10c$_1$** by 1 molar equiv. of **7a**–**c** and **10a**–**c**, respectively, in H_2O (Table 1).[12,13] Next, a synthetic Fas peptide (Ac-VARLS**C**KSVNAQ-NH_2, Table 1) was also glycosylated according to the similar procedure. The interaction between glycoproteins Fas and FasL is known to be involved in apoptosis (programmed cell-death) and thus has been extensively studied.[14] However, the function and nature of oligosaccharide chains on Fas and FasL remain unclear. Based on preliminary mutagenesis studies, it appears that Ser[4] corresponds to an *O*-glycosylation site in the Fas protein.[15] We synthesized a Fas peptide using standard solid phase peptide synthesis and reacted with 1 molar equiv. of **7a**–**c** and **10a**–**c** in DMSO–H_2O to give the corresponding glycosylated products. Characterization of the Fas peptide by ESI MS following ligation revealed selective incorporation of the oligosaccharides into the Fas peptide.[16] The thiol-selective glycosylation was investigated using a Fas peptide whose thiol was blocked by 5-thio-2-nitrobenzoic acid (TNB). The TNB-protected peptide was not glycosylated, suggesting that maleimidosugars are thiol-specific.

Bovine serum albumin (BSA), possessing a single reduced cysteine at position 58, which provides a unique site for attachment of thiol-reactive maleimidosugars, was employed as a model protein. BSA was incubated with 40 molar equiv. of **7** and **10** in 50 mM sodium phosphate buffer (pH 7). Subsequently, the reaction mixture was passed through gel filtration column (PD-10, Amersham Pharmacia) to remove an excess of maleimidosugars. The resultant glycosylated products of BSA were characterized by ESI MS, which gave a



Scheme 2.



Scheme 3.

Table 1. Chemoselective ligation of maleimidosugars to peptides and a protein

Protein/Peptide	Maleimidosugar	Reaction time (min)	Product	
1. γ-Glu Cys Gly	**7a ~ 7c**	30 ~ 60	**$7a_1$~ $7c_1$**	γ-Glu Cys Gly
	10a ~ 10c	30	**$10a_1$~ $10c_1$**	
2. Ac-VARLS**C**KSVNAQ-NH_2	**7a ~ 7c**	30 ~ 60	**$7a_2$ ~ $7c_2$**	Ac-VARLS**C**KSVNAQ-NH_2
	10a ~ 10c	30	**$10a_2$ ~ $10c_2$**	
3. BSA - Cys^{58}	**7a ~ 7c**	30 ~ 60	**$7a_3$ ~ $7c_3$**	BSA - Cys^{58}
	10a ~ 10c	30	**$10a_3$ ~ $10c_3$**	

The experimental details are described in the text.

mass change of 322.3, 228.7 and 487.7 following reaction with **7a**–**c**, and 386.3, 477.1 and 536.1 with **10a**–**c**, respectively. Chemoselective ligation of maleimidosugars to a cysteine residue in BSA was also investigated using TNB-protected BSA. It was found that BSA blocked by TNB at a cysteine residue was not glycosylated. These results suggest that a single carbohydrate moiety was incorporated into a cysteine in BSA. We believe that this methodology may be useful in the synthesis of a variety of glycoconjugates.

Acknowledgements

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References

1. (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683; (b) Varki, A. *Glycobiology* **1993**, *3*, 97.
2. Reviews: (a) Stowell, C. P.; Lee, Y. C. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225; (b) Lemieux, G. A.; Bertozzi, C. R. *TIBTECH*. **1998**, *16*, 506.
3. (a) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett*. **1991**, *32*, 6793; (b) Wong, S. Y. C.; Guile, G. R.; Dwek, R. A.; Arsequell, G. *Biochem. J*. **1994**, *300*, 843.
4. (a) Macindoe, W. M.; van Oijen, A. H.; Boons, G.-J. *Chem. Commun*. **1998**, 847; (b) Davis, B. G.; Maughan, M. A. T.; Green, M. P.; Ullman, A.; Jones, J. B.2 *Tetrahedron: Asymmetry* **2000**, *11*, 245.
5. Rodriguez, E. C.; Winans, K. A.; King, D. S.; Bertozzi, C. R. *J. Am. Chem. Soc*. **1997**, *119*, 9905.
6. Andersson, L.; Stenhagen, G.; Baltzer, L. *J. Org. Chem*. **1998**, *63*, 1366.
7. Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: New York, 1996; p. 148.
8. Shin, I.; Jung, H.-j.; Cho, J. *Bull. Korean Chem. Soc*. **2000**, *21*, 845.
9. Reddy, P. Y.; Kondo, S.; Fujita, S.; Toru, T. *Synthesis* **1998**, 999.
10. Lubineau, A.; Augé, J.; Drouillat, B. *Carbohydr. Res*. **1995**, *266*, 211.
11. Oishi, T.; Kagawa, K.; Fujimoto, M. *Macromolecules* **1993**, *26*, 24.
12. The progress of ligation reactions was directly monitored by a decrease in absorbance at 220 nm or the unreacted

SH was determined by 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) using the Ellman method. After different time intervals, an aliquot of the reaction mixture was removed and reacted with DTNB, and UV absorbance at 412 nm was recorded. Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70.

13. Selected data for **7a$_1$** (ESI): calcd for $C_{22}H_{35}N_5O_{13}S$ $[M+H]^+$ 608.4, found 608.5. **7b$_1$**: calcd for $C_{20}H_{31}N_4O_{13}S$ $[M+H]^+$ 567.5, found 567.4. **7c$_1$**: calcd for $C_{26}H_{41}N_4O_{18}S$ $[M+H]^+$ 729.6, found 729.5. **10a$_1$** (ESI): calcd for $C_{24}H_{38}N_6O_{14}S$ $[M+H]^+$ 665.4, found 665.4. **10b$_1$**: calcd for $C_{28}H_{45}N_5O_{19}S$ $[M+H]^+$ 786.4, found 786.4. **10c$_1$**: calcd for $C_{28}H_{45}N_5O_{19}S$ $[M+H]^+$ 786.4, found 786.4.
14. Nagata, S.; Golstein, P. *Science* **1995**, *267*, 1449.
15. The N-terminal dipeptide, Val-Ala, is included in the C-terminus of the lead sequence of the Fas protein.
16. Selected data for **7a$_2$** (ESI): calcd for $C_{66}H_{115}N_{21}O_{24}S$ $[M+H]^+$ 1616.8, found 1616.5. **7b$_2$**: calcd for $C_{64}H_{110}N_{20}O_{24}S$ $[M]^+$ 1575.9, found 1575.5. **7c$_2$**: calcd for $C_{70}H_{120}N_{20}O_{29}S$ $[M]^+$ 1737.1, found 1737.4. **10a$_2$** (ESI): calcd for $C_{68}H_{118}N_{22}O_{25}S$ $[M+H]^+$ 1673.8, found 1673.7. **10b$_2$**: calcd for $C_{72}H_{125}N_{21}O_{30}S$ $[M+H]^+$ 1794.8, found 1794.5. **10c$_2$**: calcd for $C_{72}H_{125}N_{21}O_{30}S$ $[M+H]^+$ 1794.8, found 1794.9.