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. 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. 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 glycosaccharides and to develop glycoproteins as potential pharmaceutical agents.

Recently, several approaches have been explored to introduce carbohydrate moieties into proteins or peptides. As the first step to develop new methodology to prepare nonnative via nonnative mutagenesis studies, it appears that Ser20 corresponds to address the biological functions of carbohydrate moieties. Based on NetOGlyc 2.0 prediction server data and preliminary mutagenesis studies, we were intrigued to prepare the glycosylated Fas and/or FasL model peptide and efficiently converted to the corresponding glycosylated products. The 1-maleimidosugars were conjugated to model peptides containing a single cysteine residue to produce the corresponding glycosylated products (Table 1).

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.

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. 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 ZnCl₂ in good yields (60-80%).

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. 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% CH₃CN-water. Subsequent to the removal of CH₃CN from the reaction mixture, the unreacted 4a and 4b were extracted with EtOAc. The glycosylated glutathiones 5a and 5b were characterized by FAB MS.

Next, a synthetic Fas peptide (Ac₁₅VARLSCKSVMQA₉-NH₂, 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. 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. 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.

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.
Acknowledgment. This work was supported by a grant from the Korea Research Foundation (1999-015-DP0218), Ministry of Education, Korea. Shin thanks the Korea Basic Science Institute for synthesizing Fas peptide and providing us with mass spectra of glycosylated peptides. Shin also thanks Dr. Srikanth Dakoji (University of California, San Francisco) for his critical reading of the manuscript.

References and Notes

11. Ellman, G. L. Arch. Biochem. Biophys. 1959, 82, 70. After different time intervals, an aliquot of the reaction mixture was removed and reacted with DTNB, and UV absorbance at 412 nm was recorded.
12. Selected data for 5a (LR FAB-MS): calcd for C_{28}H_{39}N_{4}O_{17}S [M+H]+ 735.2, found 735.2. 5b: calcd for C_{40}H_{55}N_{4}O_{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.
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_{48}H_{132}N_{10}O_{10}S [M]^{+} 2030.9, found 2031.6. 6c: calcd for C_{48}H_{132}N_{10}O_{10}S [M]^{+} 2030.9, found 2031.8. 6d: calcd for C_{48}H_{132}N_{10}O_{10}S [M]^{+} 2030.9, found 2031.1.

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

<table>
<thead>
<tr>
<th>Protein/Peptide</th>
<th>Maleimidosugar</th>
<th>Reaction time (min)</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. γ-Glu Cys Gly</td>
<td>4a 20</td>
<td>5a γ-Glu Cys Gly</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4b 20</td>
<td>5b</td>
<td></td>
</tr>
<tr>
<td>2. Ac-VARLSCKSVPNQ-NH₂</td>
<td>4a 20</td>
<td>6a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4b 20</td>
<td>6b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4c 20</td>
<td>6c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4d 20</td>
<td>6d</td>
<td></td>
</tr>
</tbody>
</table>

The Experimental details are described in the text.