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Synthesis of Oxime-Linked Mucin Mimics Containing the Tumor-Related T_N and Sialyl T_N Antigens

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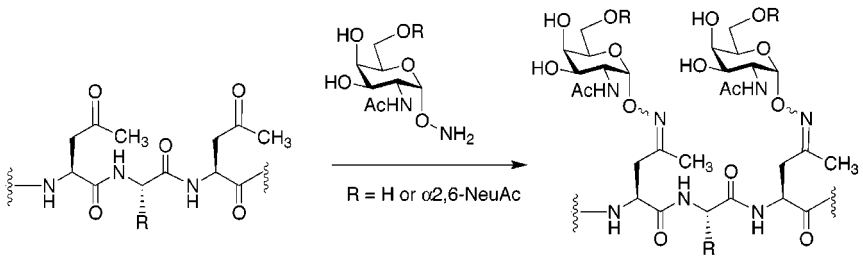
ABSTRACT



The synthesis of oxime-linked mucin mimics was accomplished via the incorporation of multiple ketone residues into a peptide followed by reaction with aminooxy sugars corresponding to the tumor-related T_N and sialyl T_N (ST_N) antigens.

The site-specific attachment of oligosaccharides to proteins can be accomplished by reaction of nucleophilic sugar derivatives with aldehydes and ketones.[1] Previously, we reported on the use of ketone-amino acid **1** (Figure 1) for the synthesis of glycopeptide analogues containing unnatural sugar−peptide linkages.[2] Amino acid **1** can be prepared in one step via reductive ozonolysis of commercially available Fmoc-dehydroleucine (**2**)[3] and can be incorporated into peptides (**3**) by Fmoc-based solid-phase peptide synthesis (SPPS) without need for protection of the ketone group.[4] The ketone group is chemically orthogonal to all naturally occurring amino acid side chain functional groups and thus can be selectively condensed with aminooxy sugars to give the corresponding oxime-linked products (**4**). Since glyco-

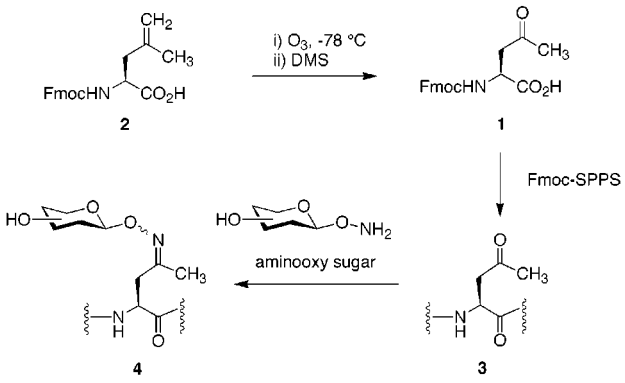


Figure 1. Synthesis of oxime-linked glycopeptides.

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(1) Reviewed in: (a) Marcaurelle, L. A.; Bertozzi, C. R. *Chem. Eur. J.* **1999**, *5*, 1384. (b) Hang, H.; Bertozzi, C. R. *Acc. Chem. Res.* **2001**, *34*, 727.

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(3) Purchased from BACHEM.

(4) Marcaurelle, L. A.; Bertozzi, C. R. *Tetrahedron Lett.* **1998**, *39*, 7279.

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proteins often contain more than one site of glycosylation, we were interested to see if this strategy could be applied to the synthesis of glycopeptides with clustered oxime-linked glycans.

Mucins are a class of heavily *O*-glycosylated proteins that are abundantly secreted by epthelial cells specialized for mucus production.[5] They are rich in serine and threonine residues bearing α-linked glycans initiated by *N*-acetylgalactosamine (GalNAc). A variety of carbohydrate ligands required for cell-surface interactions, including the Lewis and blood group antigens, can be presented on a mucin scaffold. In cancer cells, the glycosylation pattern of mucins is altered, leading to the expression of distinct tumor-related epitopes.[6] Thus, mucin fragments and their cancer-associated oligosaccharides have attracted much attention as components of synthetic cancer vaccines.[7] In the present study we focused on the preparation of oxime-linked mucin mimics containing clusters of the T_N and sialyl T_N (ST_N) antigens[8] (Figure 2), which are abundantly expressed in many types of

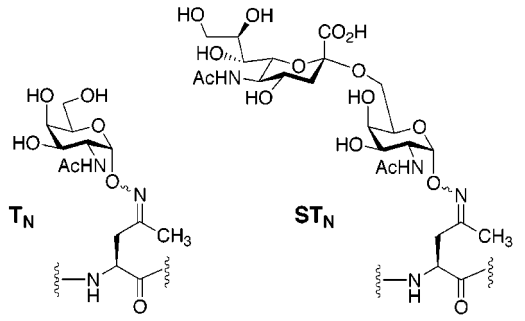


Figure 2. Oxime-linked analogues of the T_N and ST_N antigens.

cancer, including tumors of the breast, colon, liver, and pancreas.[9]

We chose fragments of the endothelial mucin GlyCAM-1 (**5** and **6**, Figure 3) as peptide scaffolds.[10] Peptides **5** and **6**

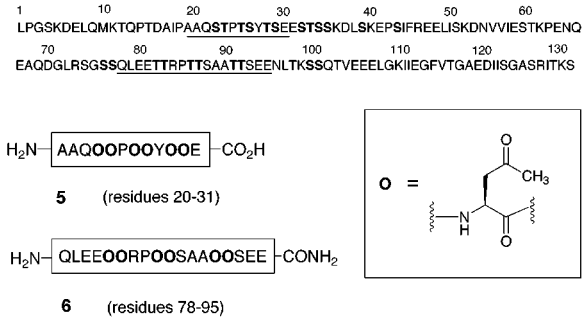
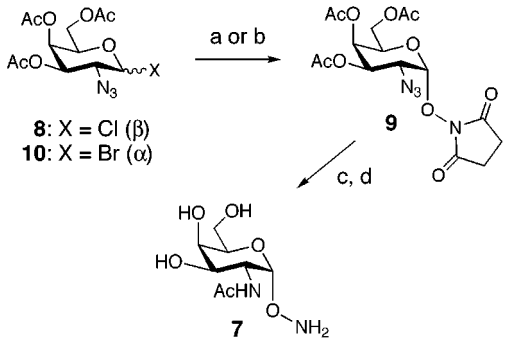


Figure 3. Amino acid sequence of GlyCAM-1 and corresponding peptide fragments **5** and **6** bearing ketone-amino acid **1** (single letter code "O") in place of Ser and Thr.

each replace six Ser or Thr residues within the 12- or 17-amino acid sequence with amino acid **1** (designated with the single letter code "O"). The syntheses of **5** and **6** were carried out on an automated peptide synthesizer using DCC/HOBt-mediated couplings, on Fmoc-Glu(*t*Bu)-Wang and MBHA resins, respectively. Amino acid **1** was used without protection of the ketone group. Following chain assembly the peptides were cleaved from the resin with 95% aqueous TFA for 5 h and precipitated from Et_2O. Analysis of the crude peptides by ESI-MS showed the desired ketone-containing derivatives as the major products.[11] Purification of the peptides by reversed-phase HPLC yielded targets **5** and **6** in 40−46% yield.

The synthesis of the aminooxy-T_N antigen (**7**) was accomplished as previously described[2] using a phase transfer catalyzed (PTC)[12] reaction of *N*-hydroxysuccinimide (NHS) with glycosyl chloride **8**[13] (Scheme 1). Reductive acetylation

Scheme 1[a]



[a] Reagents: (a) NHS, $(n\text{Bu})_4NHSO_4$, CH_2Cl_2, Na_2CO_3, 57% (α only); (b) NHS, $AgClO_4$, CH_2Cl_2, 4 Å MS, 67% (3:1 α/β); (c) H_2, Pd/C, Ac_2O, 100%; (d) 10% aq N_2H_4, 71%.

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of **9**, followed by mild hydrazinolysis, afforded the desired aminooxy sugar (**7**). We have recently found that the intermediate NHS glycoside (**9**) can also be accessed using glycosyl bromide **10**[13] as a donor in a Koenigs−Knorr glycosylation with NHS. While the stereoselectivity of this reaction is not as high as that achieved in the PTC reaction, the preparation of the α-bromide is generally higher yielding than the β-chloride,[13] making this route an attractive alternative.

The synthesis of aminooxy-ST_N **11** utilized the selectively protected glycosyl acceptor **12**, containing the preinstalled NHS glycoside, for reaction with known sialyl phosphite **13**[14] (Figure 4). For the installation of the NHS glycoside we

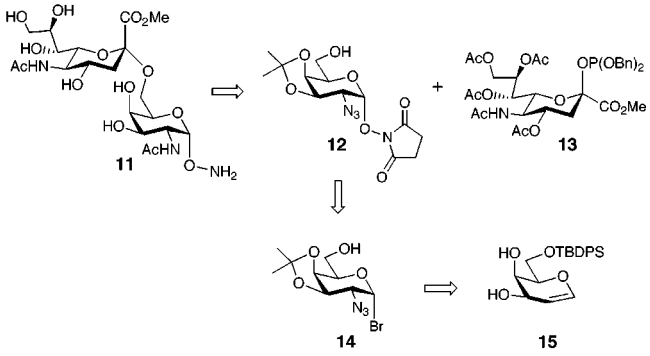


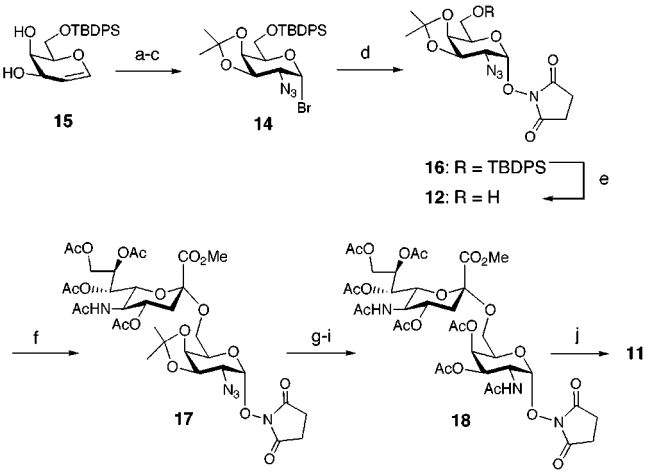
Figure 4. Retrosynthesis of aminooxy-ST_N (**11**).

chose to use a Koenigs−Knorr glycosylation with glycosyl bromide **14**, which was obtained from 6-*O*-TBDPS-D-galactal (**15**).[15]

As depicted in Scheme 2, glycosyl bromide **14** was generated in three steps via iosopropylidine formation, azidonitration[12] with CAN and NaN_3 and treatment with LiBr. Reaction of bromide **14** with NHS in the presence of $AgClO_4$ gave compound **16** in 65% yield as a mixture of anomers (3:1 α/β). Isolation of the desired α-glycoside (**12**) was achieved following removal of the TBDPS group with TBAF. Glycosylation of **12** with sialyl phosphite **13** using TMSOTf as the promoter gave disaccharide **17** in 44% yield as a mixture of anomers (3:1 α/β). Subsequent deprotection and reductive acetylation of **17** over a series of steps afforded the target aminooxy-ST_N **11**.

The ligation of **7** and **11** with peptides **5** and **6** was carried out at 37 °C with an excess of either sugar in NaOAc buffer,

Scheme 2[a]



[a] Reagents: (a) $Me_2C(OMe)_2$, PPTS, DMF, 50 °C, 1 h, 95%; (b) CAN, NaN_3, CH_3CN, −20 °C, 15 h, 71%, (c) LiBr, CH_3CN, rt, 5 h, 96%; (d) NHS, $AgClO_4$, CH_2Cl_2, 4 Å MS, rt, 2 d, 65% (3:1 α/β); (e) TBAF, AcOH, THF, rt, 6 h, 45%; (f) **13**, TMSOTf, THF, 4 Å MS, −35 °C, 1 h, 44% (3:1 α/β); (g) *p*-TsOH, MeOH, rt, overnight, 60%; (h) Ac_2O, pyridine, DMAP, rt, overnight, 54%; (i) H_2, Pd/C, Ac_2O, rt, 2 h, 49% after HPLC; (j) (i) NaOMe, MeOH, rt, 24 h, (ii) LiOH, MeOH, H_2O, 4 °C, overnight, (iii) 10% aq $N_2H_4 \cdot H_2O$, 70% for 3 steps.

pH 5.5 (Scheme 3).[16] The reactions were monitored by reversed-phase HPLC and judged to be complete after 24 h. The oxime-linked products (**19**−**22**) containing the T_N and ST_N antigens were purified by reversed-phase HPLC (60−70% yield), and their identity was confirmed by ESI-MS.[17]

These syntheses illustrate that multiple clustered ketone residues can be incorporated into a peptide and reacted with aminooxy sugars. Such an approach circumvents the need to synthesize large quantities of complex glycosyl amino acids for use in peptide synthesis, a process that can be extremely labor intensive depending on the complexity of the pendant glycan. The oxime-based strategy benefits from convergent assembly of peptides and aminooxy sugars, both of which are straightforward to prepare. The incorporation of these glycopeptides into larger, full-length proteins, by techniques such as native and expressed protein ligation,[18] should provide access to homogeneous mucin analogues for a variety of applications.

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(11) ESI-MS (neg-ion mode): calcd for **5** 1356.1, found 1355.7; calcd for **6** 2021.8, found 2021.7.
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(16) **General Procedure for Oxime Formation.** To 200 μL of peptide (50 mM with respect to ketone groups) was added 50 μL of 1 M NaOAc buffer (pH 5.5) and 250 μL of aminooxy sugar (100 mM). The reaction mixture was incubated at 37 °C for 24 h, and the oxime-linked product was isolated by reversed-phase HPLC using a gradient of 0−30% CH_3CN in water (0.1% TFA) over 20 min.
(17) ESI-MS (neg-ion mode): calcd for **19** 2665.3, found 2665.6; calcd for **20** 3331.3, found 3330.2; calcd for **21** 4412.9, found 4413.2; calcd for **22** 5078.9, found 5080.0.
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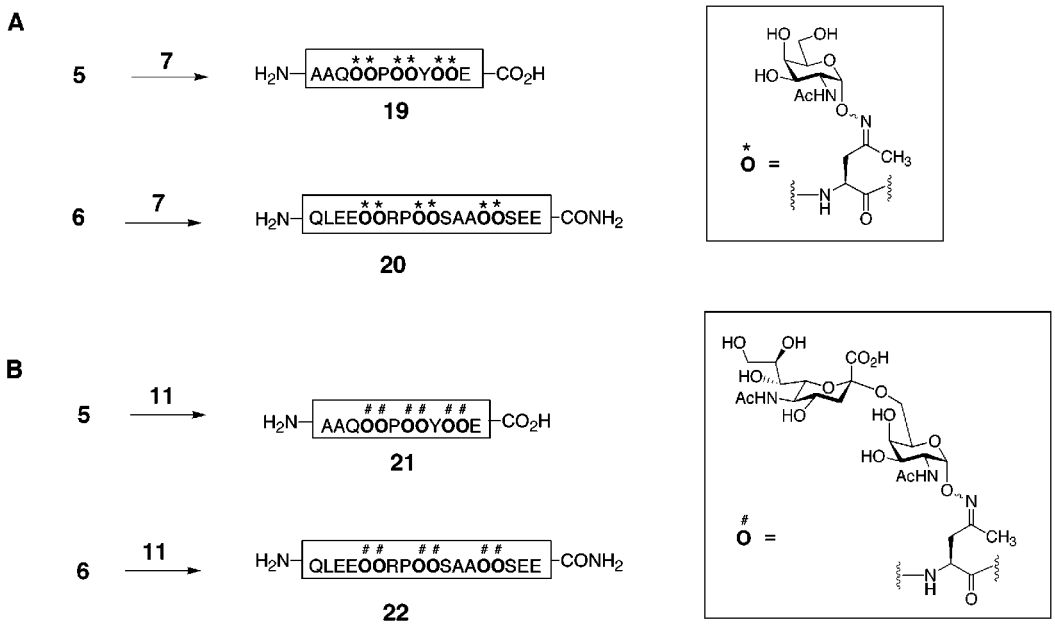
Scheme 3. Synthesis of Mucin Mimics **19** and **20**, Containing the T_N Antigen (A), and Mucin Mimics **21** and **22**, Containing the ST_N Antigen (B)[a]



[a] All ligation reactions were performed by incubating the peptide with an excess of aminooxy sugar at 37 °C in NaOAc buffer, pH 5.5[16].

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Supporting Information Available: Full experimental procedures and tabulated ^1H and ^{13}C NMR data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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