

# Synthesis of Oxime-Linked Mucin Mimics Containing the Tumor-Related T<sub>N</sub> and Sialyl T<sub>N</sub> Antigens

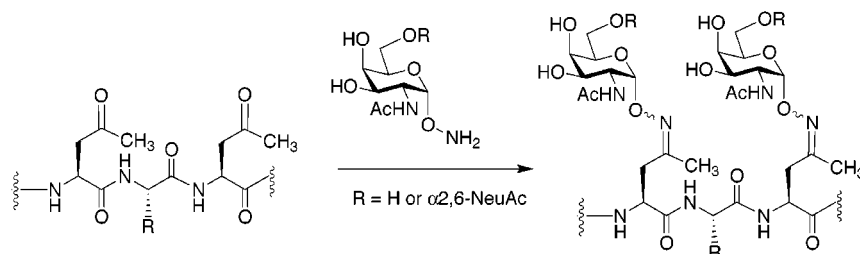
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## ABSTRACT



The synthesis of oxime-linked mucin mimics was accomplished via the incorporation of multiple ketone residues into a peptide followed by reaction with aminoxy sugars corresponding to the tumor-related T<sub>N</sub> and sialyl T<sub>N</sub> (ST<sub>N</sub>) antigens.

The site-specific attachment of oligosaccharides to proteins can be accomplished by reaction of nucleophilic sugar derivatives with aldehydes and ketones.<sup>1</sup> Previously, we reported on the use of ketone-amino acid **1** (Figure 1) for the synthesis of glycopeptide analogues containing unnatural sugar–peptide linkages.<sup>2</sup> Amino acid **1** can be prepared in one step via reductive ozonolysis of commercially available Fmoc-dehydroleucine (**2**)<sup>3</sup> and can be incorporated into peptides (**3**) by Fmoc-based solid-phase peptide synthesis (SPPS) without need for protection of the ketone group.<sup>4</sup> The ketone group is chemically orthogonal to all naturally

occurring amino acid side chain functional groups and thus can be selectively condensed with aminoxy sugars to give the corresponding oxime-linked products (**4**). Since glyco-

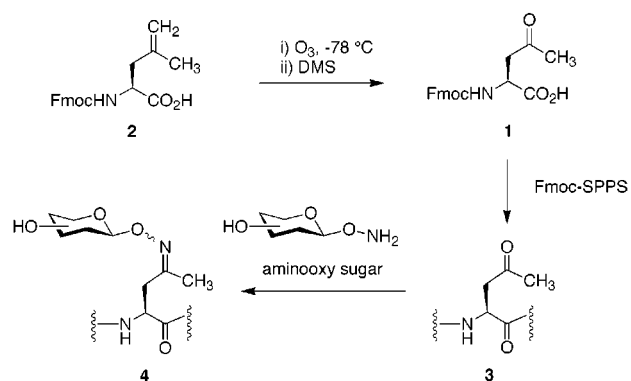


Figure 1. Synthesis of oxime-linked glycopeptides.

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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.

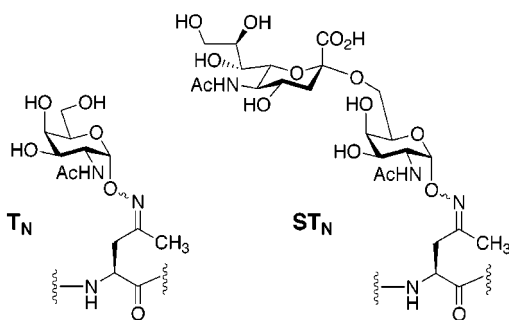
(2) (a) Marcaurelle, L. A.; Rodriguez, E. C.; Bertozzi, C. R. *Tetrahedron Lett.* **1998**, *39*, 8417. (b) Rodriguez, E. C.; Marcaurelle, L. A.; Bertozzi, C. R. *J. Org. Chem.* **1998**, *63*, 9614.

(3) Purchased from BACHEM.

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

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 epithelial cells specialized for mucus production.<sup>5</sup> They are rich in serine and threonine residues bearing  $\alpha$ -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.<sup>6</sup> Thus, mucin fragments and their cancer-associated oligosaccharides have attracted much attention as components of synthetic cancer vaccines.<sup>7</sup> In the present study we focused on the preparation of oxime-linked mucin mimics containing clusters of the T<sub>N</sub> and sialyl T<sub>N</sub> (ST<sub>N</sub>) antigens<sup>8</sup> (Figure 2), which are abundantly expressed in many types of



**Figure 2.** Oxime-linked analogues of the T<sub>N</sub> and ST<sub>N</sub> antigens.

cancer, including tumors of the breast, colon, liver, and pancreas.<sup>9</sup>

(5) Reviewed in: (a) Hanisch, F.-G. *Biol. Chem.* **2001**, 143. (b) Strous, G. J.; Dekker, J. *J. Crit. Rev. Biochem. Mol. Biol.* **1992**, 27, 57. (c) Carraway, K. L.; Hull, S. R. *Glycobiology* **1991**, 1, 131.

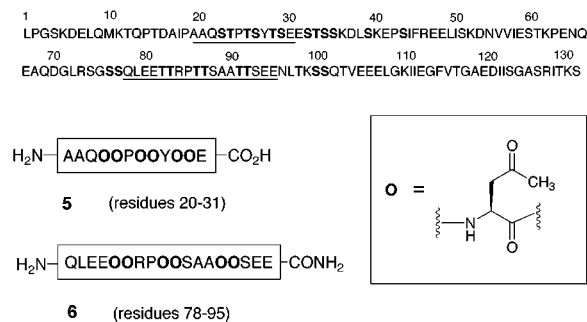
(6) Varki, A. Glycosylation "Changes in Cancer". In *Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Martin, J., Eds.; New York: Cold Spring Harbor Laboratory, 1999; pp 537–550.

(7) Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed.* **2000**, 39, 836.

(8) For the synthesis of glycopeptides containing the T<sub>N</sub> and ST<sub>N</sub> antigens, see: (a) Liebe, B.; Kunz, H. *Angew. Chem., Int. Ed.* **1997**, 36, 618. (b) Keil, S.; Claus, C.; Dippold, W.; Kunz, H. *Angew. Chem., Int. Ed.* **2001**, 40, 366. (c) Kuduk, S. D.; Schwarz, J. B.; Chen, X.-T.; Glunz, P. W.; Sames, D.; Ragupathi, G.; Livingston, P. O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, 120, 12474. (d) Schwarz, J. B.; Kuduk, S. D.; Chen, X.-T.; Sames, D.; Glunz, P. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, 121, 2662. (e) Elofsson, M.; Salvador, L. A.; Kihlberg, J. *Tetrahedron* **1997**, 53, 369. (f) George, S. K.; Holm, B.; Reis, C.; Schwientek, T.; Clausen, H.; Kihlberg, J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 880.

(9) (a) Brockhausen, I.; Yang, J. M.; Burchell, J.; Whitehouse, C.; Taylor-Papadimitriou, J. *Eur. J. Biochem.* **1995**, 233, 607. (b) Sasaki, M.; Yamato, T.; Nakanuma, Y. *Pathol. Int.* **1999**, 49, 325. (c) Itzkowitz, S. H.; Yaun, M.; Montgomery, C. K.; Kjeldsen, T.; Takahashi, H. K.; Bigbee, W. L.; Kim, Y. S. *Cancer Res.* **1989**, 49, 197. (d) Itzkowitz, S.; Kjeldsen, T.; Frieria, A.; Hakomori, S.-N.; Yang, U.-S.; Kim, Y. S. *Gastroenterology* **1991**, 100, 1691.

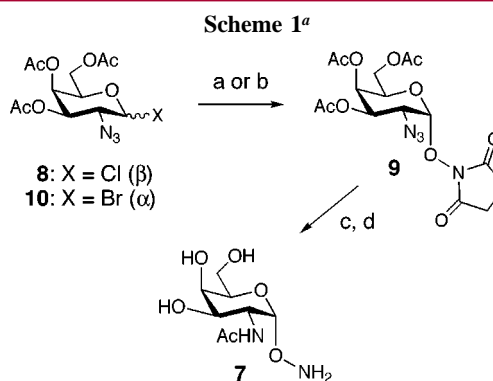
We chose fragments of the endothelial mucin GlyCAM-1 (**5** and **6**, Figure 3) as peptide scaffolds.<sup>10</sup> Peptides **5** and **6**



**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<sub>2</sub>O. Analysis of the crude peptides by ESI-MS showed the desired ketone-containing derivatives as the major products.<sup>11</sup> Purification of the peptides by reversed-phase HPLC yielded targets **5** and **6** in 40–46% yield.

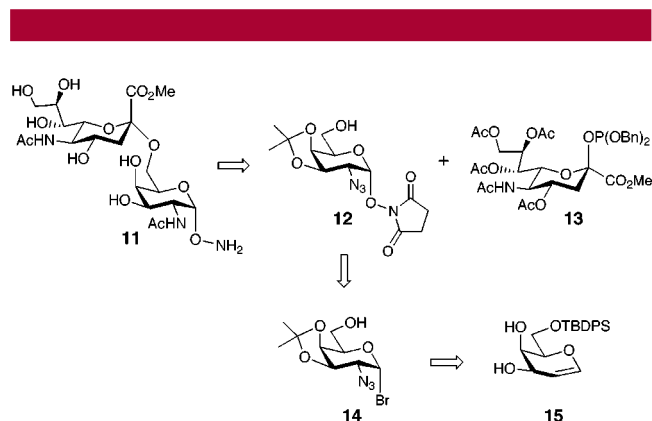
The synthesis of the aminoxy-T<sub>N</sub> antigen (**7**) was accomplished as previously described<sup>2</sup> using a phase transfer catalyzed (PTC)<sup>12</sup> reaction of *N*-hydroxysuccinimide (NHS) with glycosyl chloride **8**<sup>13</sup> (Scheme 1). Reductive acetylation



<sup>a</sup> Reagents: (a) NHS, (*n*Bu)<sub>4</sub>NHSO<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>, Na<sub>2</sub>CO<sub>3</sub>, 57% ( $\alpha$  only); (b) NHS, AgClO<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 4 Å MS, 67% (3:1  $\alpha/\beta$ ); (c) H<sub>2</sub>, Pd/C, Ac<sub>2</sub>O, 100%; (d) 10% aq N<sub>2</sub>H<sub>4</sub>, 71%.

of **9**, followed by mild hydrazinolysis, afforded the desired aminoxy sugar (**7**). We have recently found that the intermediate NHS glycoside (**9**) can also be accessed using glycosyl bromide **10**<sup>13</sup> 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  $\alpha$ -bromide is generally higher yielding than the  $\beta$ -chloride,<sup>13</sup> making this route an attractive alternative.

The synthesis of aminoxy-ST<sub>N</sub> **11** utilized the selectively protected glycosyl acceptor **12**, containing the preinstalled NHS glycoside, for reaction with known sialyl phosphite **13**<sup>14</sup> (Figure 4). For the installation of the NHS glycoside we



**Figure 4.** Retrosynthesis of aminoxy-ST<sub>N</sub> (**11**).

chose to use a Koenigs–Knorr glycosylation with glycosyl bromide **14**, which was obtained from 6-*O*-TBDPS-D-galactose (**15**).<sup>15</sup>

As depicted in Scheme 2, glycosyl bromide **14** was generated in three steps via isopropylidene formation, azidonitration<sup>12</sup> with CAN and NaN<sub>3</sub> and treatment with LiBr. Reaction of bromide **14** with NHS in the presence of AgClO<sub>4</sub> gave compound **16** in 65% yield as a mixture of anomers (3:1  $\alpha/\beta$ ). Isolation of the desired  $\alpha$ -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  $\alpha/\beta$ ). Subsequent deprotection and reductive acetylation of **17** over a series of steps afforded the target aminoxy-ST<sub>N</sub> **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,

(10) Imai, Y.; Singer, M. S.; Fennie, C.; Lasky, L. A.; Rosen, S. D. *J. Cell. Biol.* **1991**, *113*, 1213.

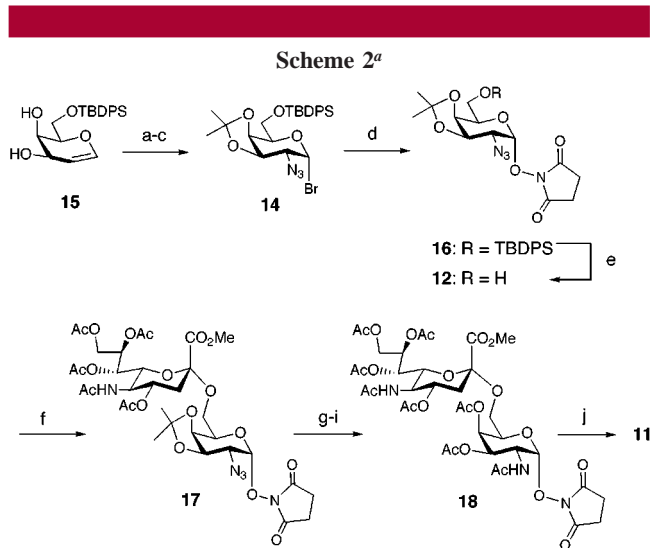
(11) ESI-MS (neg-ion mode): calcd for **5** 1356.1, found 1355.7; calcd for **6** 2021.8, found 2021.7.

(12) Cao, S.; Tropper, F. D.; Roy, R. *Tetrahedron* **1995**, *51*, 6679.

(13) Lemieux, R.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244.

(14) (a) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260. (b) Bhattacharya, S. K.; Danishefsky, S. J. *J. Org. Chem.* **2000**, *65*, 144.

(15) Gervay, J.; Peterson, J. M.; Oriyama, T.; Danishefsky, S. J. *J. Org. Chem.* **1993**, *58*, 5465.



<sup>a</sup> Reagents: (a) Me<sub>2</sub>C(OMe)<sub>2</sub>, PPTS, DMF, 50 °C, 1 h, 95%; (b) CAN, NaN<sub>3</sub>, CH<sub>3</sub>CN, -20 °C, 15 h, 71%; (c) LiBr, CH<sub>3</sub>CN, rt, 5 h, 96%; (d) NHS, AgClO<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 4 Å MS, rt, 2 d, 65% (3:1  $\alpha/\beta$ ); (e) TBAF, AcOH, THF, rt, 6 h, 45%; (f) **13**, TMSOTf, THF, 4 Å MS, -35 °C, 1 h, 44% (3:1  $\alpha/\beta$ ); (g) *p*-TsOH, MeOH, rt, overnight, 60%; (h) Ac<sub>2</sub>O, pyridine, DMAP, rt, overnight, 54%; (i) H<sub>2</sub>, Pd/C, Ac<sub>2</sub>O, rt, 2 h, 49% after HPLC; (j) (i) NaOMe, MeOH, rt, 24 h, (ii) LiOH, MeOH, H<sub>2</sub>O, 4 °C, overnight, (iii) 10% aq N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, 70% for 3 steps.

pH 5.5 (Scheme 3).<sup>16</sup> 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<sub>N</sub> and ST<sub>N</sub> antigens were purified by reversed-phase HPLC (60–70% yield), and their identity was confirmed by ESI-MS.<sup>17</sup>

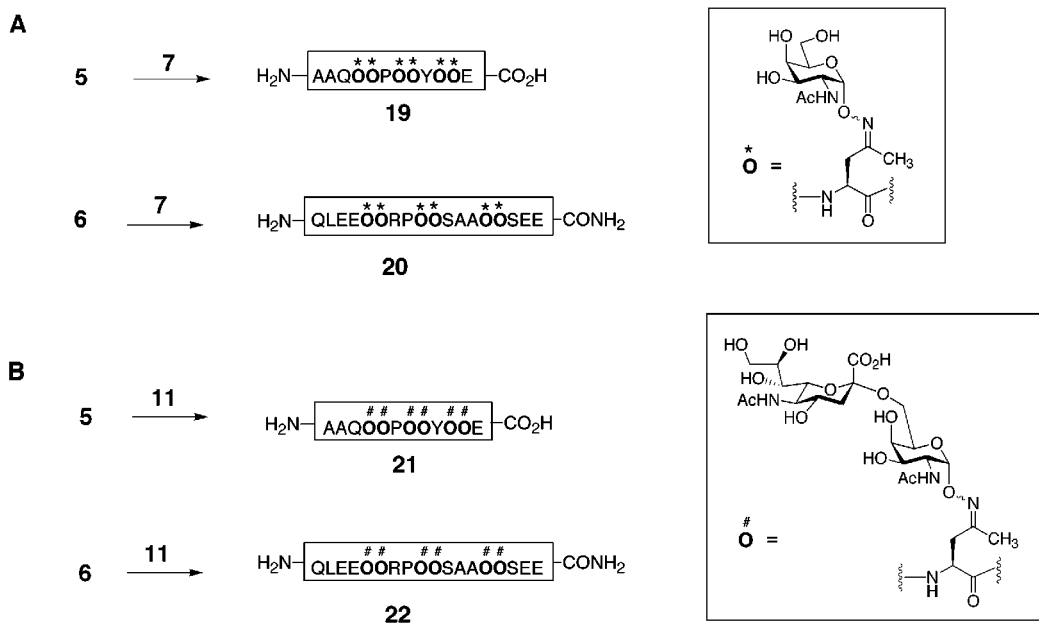
These syntheses illustrate that multiple clustered ketone residues can be incorporated into a peptide and reacted with aminoxy sugars. Such an approach circumvents the need to synthesize large quantities of complex glycosyl amino acids for use in peptide synthesis, a process that can be extremely labor intensive depending on the complexity of the pendant glycan. The oxime-based strategy benefits from convergent assembly of peptides and aminoxy 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,<sup>18</sup> should provide access to homogeneous mucin analogues for a variety of applications.

(16) **General Procedure for Oxime Formation.** To 200  $\mu$ L of peptide (50 mM with respect to ketone groups) was added 50  $\mu$ L of 1 M NaOAc buffer (pH 5.5) and 250  $\mu$ L of aminoxy 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<sub>3</sub>CN 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.

(18) (a) Macmillan, D.; Bertozzi, C. R. *Tetrahedron* **2000**, *56*, 9515. (b) Tilbert, T. J.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 5421. (c) Marcaurrelle, L. A.; Bertozzi, C. R. *Chem. Eur. J.* **2000**, *7*, 1129. (d) Dawson, P. E.; Kent, S. B. H. *Annu. Rev. Biochem.* **2000**, *69*, 923. (e) Muir, T. W.; Sondri, D.; Cole, P. A. *Proc. Natl. Acad. Sci.* **1998**, *95*, 6705.

**Scheme 3.** Synthesis of Mucin Mimics **19** and **20**, Containing the T<sub>N</sub> Antigen (A), and Mucin Mimics **21** and **22**, Containing the ST<sub>N</sub> Antigen (B)<sup>a</sup>



<sup>a</sup> All ligation reactions were performed by incubating the peptide with an excess of aminoxy sugar at 37 °C in NaOAc buffer, pH 5.5<sup>16</sup>.

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**Supporting Information Available:** Full experimental procedures and tabulated <sup>1</sup>H and <sup>13</sup>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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