Pergamon

Tetrahedron Letters, Vol. 38, No. 20, pp. 3487-3490, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00703-X

Oligomeric Glycopeptidomimetics Bearing the Cancer Related T_N-Antigen

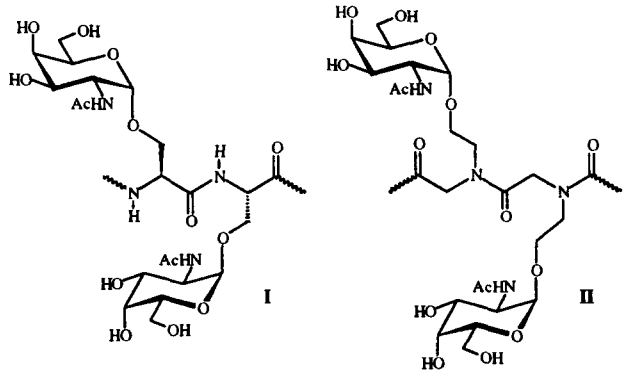
Jin Mi Kim and René Roy*

Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

Abstract: α-*O*-Linked N-acetylgalactosamine clusters with 2, 3, 4, 6, and 8 repeating units were synthesized using a reiterative blockwise approach and *N*-substituted oligoglycine peptoid backbones. Orthogonally protected glycopeptide **5** and **7** were used as key building blocks.
© 1997 Elsevier Science Ltd.

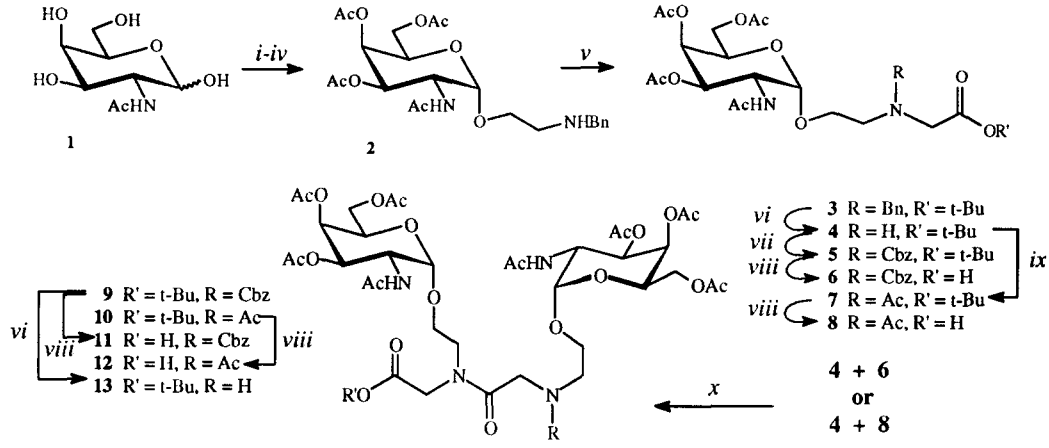
Malignant cells express abnormally substituted mucin glycoproteins as a result of incomplete or aberrant glycosylation. In adenocarcinomas, these mucins expose tumor-associated carbohydrate antigens that are cryptic in healthy tissues.[1] Of particular interest are the blood group antigens T_N (GalNAcα-*O*-Ser), T (Galβ-(1,3)-GalNAcα-*O*-Ser), and sialosyl-T_N (Neu5Ac-α(2,6)-GalNAcα-*O*-Ser) of epithelial cancers.[2] These antigens appear as clusters of glycopeptide repeating units and a synthetic vaccine (neoglycoprotein) is now in clinical trials.[3] Moreover, a dimeric T_N-antigen glycopeptidolipid has been shown to be highly immunogenic.[4] These vaccines were not derived from natural glycopeptide and since there is still no concluding evidence as to the antigenic participation of the peptide backbone, it became of interest to generate glycomimetics. To evaluate the role of multivalency in antigen presentation and to generate metabolically stable glycopeptide analogs, the synthesis of T_N-antigen glycopeptidomimetic clusters is presented herein.

As scaffolding peptide surrogates, *N*-substituted oligoglycines (peptoids) have been considered.[5] In previous work from this group, a number of "glycopeptoid" libraries representing both *N*-linked[6] and *O*-linked[7] glycans have been successfully generated. Scheme 1 below illustrates structural similarities between representative divalent analogs.



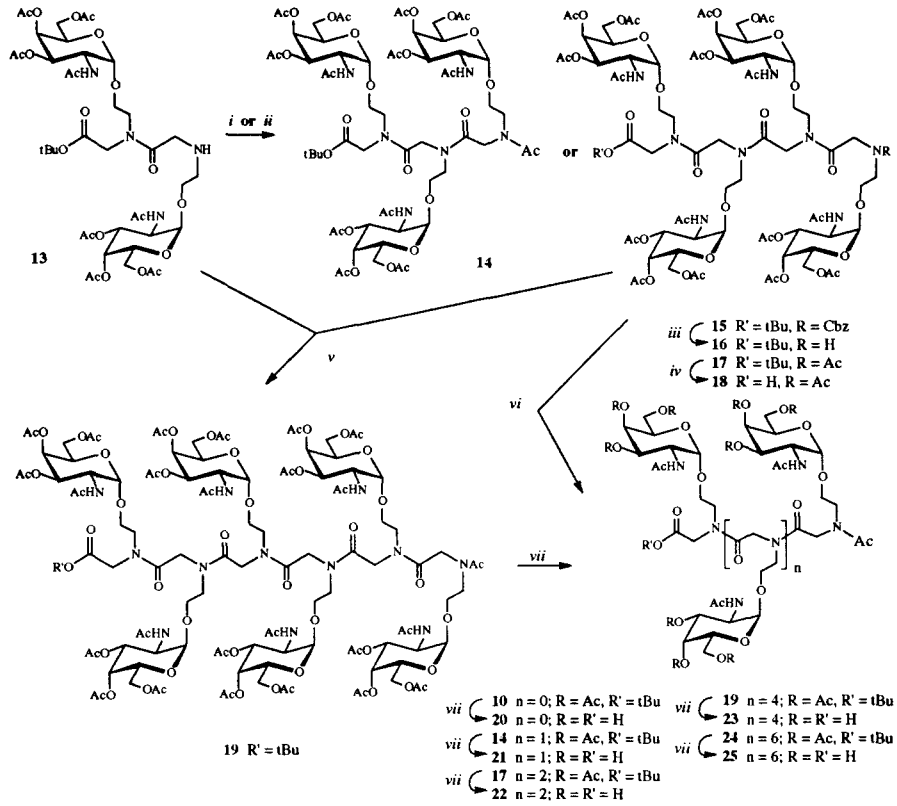
Scheme 1. Structural similarities between dimeric α-D-GalNAc-O-Ser dipeptide (**I**) and α-D-GalNAc-O-(homo)Ser dipeptoid mimetic (**II**).

The strategy described herein was based on the reiterative scaffolding of an orthogonally protected key building block **3** that was prepared from readily available[8] allyl α-*N*-acetylgalactopyranoside derived from commercial GalNAc (**1**) by treatment with allyl alcohol and BF_3Et_2O (67%) (Scheme 2). Ozonolysis and reductive amination of the resulting peracetylated aldehyde (Ac_2O, pyridine, 94%) with benzylamine afforded secondary amine **2** (74%). *N*-Alkylation of **2** with *tert*-butylbromoacetate provided **3** in 97% yield. After standard hydrogenolysis of the benzyl group (quant.), the resulting *C*-terminal amino ester unit **4** was transformed into either an internal *N*-Cbz-protected acid **6** (CbzCl, DIPEA, CH_2Cl_2, 77%; 20% TFA in CH_2Cl_2, quant.) or into an *N*-terminal *N*-Ac-protected acid **8** (AcCl, DIPEA, CH_2Cl_2, 98%, 20% TFA in CH_2Cl_2, quant.). Coupling of amine **4** with either acid **6** or **8** (TBTU coupling) afforded intermediate dimer **9** or **10**, respectively (81%). Divalent *N*-Cbz-protected ester **9** was further transformed into acid **11** or amine **13** following the procedure just described above, while dimeric t-butyl ester **10** was converted into acid **12** in similar yields (20% TFA).



Scheme 2. Reagents: *i)* CH_2=$CHCH_2OH$, BF_3Et_2O, 67%; *ii)* Ac_2O, C_5H_5N, 94%; *iii)* O_3, CH_2Cl_2, -76°C, 10 min, then Me_2S; *iv)* $BnNH_2$, $NaBH_3CN$, THF, r.t., o.n., 74%; *v)* $BrCH_2CO_2tBu$, CH_2Cl_2, 25°C, o.n., 97%; *vi)* H_2, 10% Pd-C, MeOH, HOAc, o.n., quant.; *vii)* CbzCl, DIPEA, CH_2Cl_2, 0°C, 1.5 h, 77%; *viii)* 20% TFA in CH_2Cl_2, r.t., 2 h, quant.; *ix)* CH_3COCl, DIPEA, CH_2Cl_2, 0°C, 30 min, 98%; *x)* TBTU, DIPEA, CH_2Cl_2, MeCN, 2 h., r.t., 81% **9** and **10**.

Then, dimeric amine **13** was coupled (BOP, *N*-methylmorpholine) to either monomeric **8** or dimeric acids **11** or **12** to provide protected trimer **14** and tetramers **15** or **17**, in 60, 68, and 69% yields, respectively (Scheme 3). By reiteration of the deprotection-coupling process, dimer **13** and tetramer **18** afforded hexamer **19** (62%). Alternatively, peptide coupling between tetrameric amine **16** with its analogous tetrameric acid **18** furnished octamer **24** in 59% yield. All of the above well-structured oligomers **20** (dimer), **21** (trimer), **22** (tetramer), **23** (hexamer), and **25** (octamer) were fully deprotected in essentially quantitative yields by treatment with a catalytic amount of NaOMe in MeOH (Zemplén conditions) followed by trifluoroacetolysis as above (20% TFA, CH_2Cl_2).



Scheme 3. Reagents. *i)* **8**, BOP, NMM, CH_2Cl_2, r.t., 2 h, 60%; *ii)* **11** or **12**, TBTU, DIPEA, CH_2Cl_2, MeCN, r.t., 2 h, 68% (**15**), 69% (**17**); *iii)* H_2, 10% Pd-C, MeOH, quant.; *iv)* 20% TFA in CH_2Cl_2, r.t., 2 h, quant.; *v)* **18**, TBTU, DIPEA, CH_2Cl_2, MeCN, r.t., 2 h, 62%; *vi)* **16** + **18**, TBTU, DIPEA, CH_2Cl_2, MeCN, r.t., o.n., 59%; *vii)* 20% TFA in CH_2Cl_2, then NaOMe, MeOH.

All compounds provided spectroscopic data in agreement with their structures.[9] Per-*O*-acetylated intermediates were readily purified by silica gel chromatography, while final deprotected oligopeptoids were purified my size exclusion chromatography (LH-20, H_2O) since they were derived from a blockwise approach, each fragment having twice the size of its predecessors. It is worth mentioning that by virtue of their secondary amide contents, these oligomers gave mixtures of rotameric isomers which could be detected on the NMR time scale. Integration of key signals, together with MS were used to confirm purity. These slowly interconverting stereoisomers are capable of induced fit, a properties which can be exploited to scan an ensemble of receptors. Moreover, these glycopeptidomimetics showed MS-fragmentation patterns similar to peptides, thus enabling easy structural determination. The strategy is potentially amenable to solid-phase synthesis and can thus be used to generate combinatorial libraries by changing the nature of the sugar, the interspacing distances between both the sugar residues and the sugar to backbone distances. Work is now in progress towards this goal. Given the importance of hepatic Gal/GalNAc receptors, these oligomers may find application in liver targeting. Preliminary binding studies with model plant lectins are encouraging and will be presented in due course.

Acknowledgments: We thank the Natural Science and Engineering Research Council of Canada (NSERC) for financial support.

References and Notes

1. a) Hakomori, S. *Adv. Cancer Res.* **1989**, *52*, 2214-2220; Hakomori, S. *Curr. Opin. Immunol.* **1991**, *3*, 646-653; c) Singhal, A.; Hakomori, S. *BioEssays* **1990**, *12*, 223-230.
2. Toyokuni, T.; Singhal, A.K. *Chem. Soc. Rev.* **1995**, 231-242.
3. Longenecker, B.M.; Reddish, M.; Miles, D.; MacLean, G.D. *Vaccine Res.* **1993**, *2*, 151-162.
4. Toyokuni, T.; Dean, B.; Cai, S.; Boivin, D.; Kakomori, S.; Singhal, A.K. *J. Am. Chem. Soc.* **1994**, *116*, 395-396.
5. a) Simon, R.J.; Kania, R.S.; Zuckermann, R.N.; Huebner, V.D.; Jewell, D.A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C.K.; Spellmeyer, D.C.; Tan, R.; Frankel, A.D.; Santi, D.V.; Cohen, F.E.; Bartlett, P.A. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9367-9371; b) Zuckermann, R.N.; Kerr, J.M.; Kent, S.B.H.; Moos, W.H. *J. Am. Chem. Soc.* **1992**, *114*, 10646-10647.
6. a) Saha, U.K.; Roy, R. *Tetrahedron Lett.* **1995**, *36*, 3635-3638; b) Saha, U.K.; Roy, R. *J. Chem. Soc., Chem. Commun.* **1995**, 2571-2573; c) Saha, U.K.; Roy, R. *Chem. Commun.* **1996**, 210-202.
7. a) Kim, J.M.; Roy, R. *Carbohydr. Lett.* **1996**, *1*, 465-468; b) Kim, J.M.; Roy, R. *Carbohydr. Res.* **1997**, in press.
8. Wong, T.C.; Townsend, R.R.; Lee, Y.C. *Carbohydr. Res.* **1987**, 170, 27-46.
9. Selected spectroscopic data: **4:** ^{1}H NMR ($CDCl_3$) δ ppm: 7.35 (2H, bs, NH_2), 6.98 (1H, d, J=9.9 Hz, NH), 5.35 (1H, dd, $J_{3,4}$=3.3 Hz, $J_{4,5}$=1.0 Hz, H-4), 5.17 (1H, dd, $J_{2,3}$=11.4 Hz, $J_{3,4}$=3.3 Hz, H-3), 4.86 (1H, d, $J_{1,2}$=3.5 Hz, H-1), 4.60 (1H, ddd, $J_{2,3}$=11.4 Hz, $J_{2,NH}$=9.9 Hz, $J_{1,2}$=3.5 Hz, H-2), 4.16 (1H, ddd, $J_{4,5}$=1.0 Hz, $J_{5,6}$=6.0 Hz, H-5), 4.06-4.03 (2H, m, H-6), 3.88 (1H, td, J=11.3 Hz, J=4.9 Hz, OCH), 3.58 (1H, td, J=11.3 Hz, J=4.9 Hz, OCH), 3.49 (2H, s, O_2CCH_2), 3.03 (2H, t, J=4.8 Hz, NCH_2), 2.11, 2.00, 1.97, 1.92 (4×3H, s, OAc), 1.43 (9H, s, CMe_3); ^{13}C NMR ($CDCl_3$) δ ppm: 170.9, 170.6, 170.4, 170.3, 168.8 (C=O), 98.1 (C-1), 82.9, 68.3, 67.3, 66.9, 64.9, 62.0, 49.1, 47.2, 28.0, 23.0, 20.7; MS (m/z) FAB+ 506.05 ($M+1^+$, 100%), 448.4 (26.7%), 330.1 (33.5%). **5:** ^{1}H NMR ($CDCl_3$) δ ppm: 7.35-7.27 (5H, m, Ph), 6.43, 6.15 (1H, 2d, J=9.7 Hz, NH), 5.34, 5.30 (1H, 2d, J=2.2 Hz, H-4), 5.16, 5.10 (2H, 2s, CH_2Ph), 5.06, 5.02 (1H, 2dd, $J_{2,3}$=11.2 Hz, $J_{3,4}$=3.2 Hz, H-3), 4.85, 4.79 (1H, 2s, $J_{1,2}$=3.5 Hz, H-1), 4.61-4.57 (1H, m, H-2), 4.16-4.01 (3H, m, H-5, H-6), 3.93, 3.87 (2H, 2s, O_2CCH_2N), 3.84-3.72, 3.65-3.54, 3.52-3.45, 3.37-3.32 (4H, m, OCH_2CH_2N), 2.13, 2.01, 2.00, 1.96, 1.95, 1.93 (12H, 5s, OAc), 1.44, 1.36 (9H, 2s, t-Bu); MS (m/z) FAB+ 639.3 ($M+1^+$, 12.7%), 583.2 (4.0%), 505.3 (3.6%); ratio of two rotamers = 2:1. **8:** ^{1}H NMR ($CDCl_3$) δ ppm: 6.92, 6.70 (1H, 2d, J=9.6 Hz, NH), 5.37, 5.34 (1H, 2d, J=2.2 Hz, H-4), 5.10, 5.09 (1H, 2dd, $J_{2,3}$=11.2 Hz, $H_{3,4}$=3.2 Hz, H-3), 4.93, 4.81 (1H, 2d, J1,2=3.5 Hz, H-1), 4.54-4.50 (1H, m, H-2), 4.26-4.00 (5H, m, O_2CCH_2N, H-5, H-6), 3.89-3.74, 3.69-3.54, 3.48-3.42 (4H, m, NCH_2CH_2O), 2.26, 2.18, 2.15, 2.14, 2.04, 1.99, 1.98, 1.97 (12H, 8s, OAc); MS (m/z) FAB+ 491.2 ($M+1^+$, 83.2%), 449.2 (27.8%); ratio of two rotamers = 1:1.3. **9:** ^{1}H NMR ($CDCl_3$) δ ppm: 7.55, 6.57 (2H, 2d, J=9.4 Hz, J=8.4 Hz, NH), 7.34-7.26 (5H, m, Ph), 5.39, 5.27 (2H, 2d, J=2.0 Hz, H-4), 5.19, 5.01 (2H, 2dd, $J_{2,3}$=11.9 Hz, $J_{3,4}$=3.1 Hz, H-3), 4.96, 4.83 (2H, 2d, $J_{1,2}$=3.5 Hz, H-1), 4.57-4.50 (2H, m, H-2), 5.11, 5.09, 4.98, 4.91, 4.88 (3H, 5s), 4.22-3.98, 3.93-3.68, 3.64-3.32 (17H, m), 2.11, 2.10, 2.01, 2.00, 1.94, 1.93, 1.91, 1.77 (24H, 8s, OAc), 1.43, 1.40 (9H, 2s, t-Bu); MS (m/z) FAB+ 1069.3 ($M+1^+$, 3.4%), 935.3 (1.5%), 565.2 (1.9%); $[\alpha]_D^{23}$ = +56.7 (c 1.0, $CHCl_3$); ratio of two rotamers = 1:1. **10:** $[\alpha]_D^{23}$ = +54.6 (c 0.88, $CHCl_3$); MS (m/z) FAB+ 977.35 ($M+1^+$, 2.9%), 935.3, 630.3, 574.2, 473.2. **11:** MS (m/z) FAB+ 1013.4 ($M+1^+$, 7.5%), 971.4 (2.0%), 879.4 (1.7%). **12:** MS (m/z) FAB+ 921.2 (6.1%), 574.5 (26.6%), 473.2 (19.3%). **14:** $[\alpha]_D^{23}$ = +126.5 (c 1.1, $CHCl_3$). MS (m/z) FAB+ 1407.9 (4.8%), 935.3, 473.2. **15:** $[\alpha]_D^{23}$ = +134.6 (c 1.1, $CHCl_3$); MS (m/z) FAB+ 1929.7 ($M+1^+$, 0.6%), 1795.7(0.2%), 1582.6 (1.6%), 1425.5 (0.5%), 995.3 (2.8%), 935.4 (3.0%). **17:** $[\alpha]_D^{23}$ = +79.0 (c 1.1, $CHCl_3$); MS (m/z) FAB+ 1838.8 (1.4%), 1366.2 (21.0%), 935.4 (2.4%), 903.4 (5.1%). **18**: MS (m/z) FAB+ 1782.2 ($M+1^+$, 0.7%), 1309.4 (0.4%). **19:** $[\alpha]_D^{23}$ = +58.9 (c 1.6, $CHCl_3$); MS (m/z) FAB+ 2698.7 ($M+1^+$, 0.1%), 2227.0 (1.2%), 1366.4 (31.3%).

(*Received in USA* 20 *February* 1997; *revised* 7 *April* 1997; *accepted* 8 *April* 1997)