ORGANIC LETTERS

2001
Vol. 3, No. 6
851−854

A Strategy for Probing the Autonomy of Cross-Domain Stereochemical Communication in Glycoconjugates

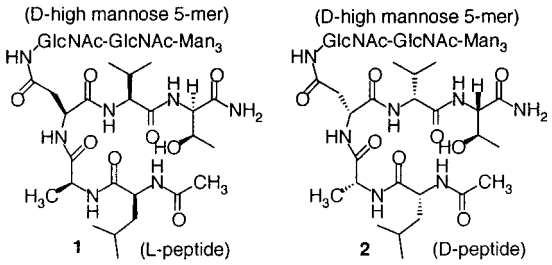
David H. Live,[†] Zhi-Guang Wang,[‡] Ulrich Iserloh,[‡] and Samuel J. Danishefsky*,[‡]

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, Minnesota 55455, and Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, New York, New York 10021

s-danishefsky@ski.mskcc.org

Received December 19, 2000

ABSTRACT



Glycoproteins contain carbohydrate and peptide sectors. As a model for studying whether there exists stereochemical "communication" between the two domains, we prepared two glycopeptides differing only in the absolute stereochemistry of the peptide domain (L-peptide vs D-peptide). High-field NMR spectroscopy revealed that there are distinct and measurable differences, indicating that the two domains are at some level interactive.

Glycopeptides are classified on the basis of the attachment mode of an oligosaccharide to the amino acid side chain of the peptide domain.[1,2] In *N*-linked glycopeptides, the asparagine γ-carboxamide is glycosylated with a conserved (high mannose) pentasaccharide core structure, whereas *O*-linked glycopeptides feature an α-*O*-GalNAc linkage to a serine (or threonine) side chain. Unfortunately, the exploration of structure and function of the *N*-linked constructs has been retarded by the scarcity of material from natural sources. A further impediment to detailed structural analysis of these glycoproteins arises from the microheterogeneity and increased disorder and flexibility of the sugar residues in the outer branches, which render X-ray crystallography rarely successful.[3] Much of the earlier NMR work has focused on interactions either within the carbohydrate or within the peptide domain, and relatively little is known about the cross-domain interactivity. Although it appears that glycosylation induces a turn structure, the precise origin of this phenomenon is difficult to ascertain because of the lack of characteristic NOE fingerprints.[4]

Given our continued interest in the synthesis of complex

[†] University of Minnesota.
[‡] Sloan-Kettering Institute for Cancer Research.

(1) (a) Montreuil, J.; Vliegenthart, J. F. G.; Schachter, H. In *Glycopeptides*; Neuberger, A., Van Deenen, L. L. M., Series Eds.; Elsevier: New York, 1995; Vol. 29a. (b) Rudd, P. M.; Wormald, M. R.; Stanfield, R. L.; Huang, M.; Mattsson, N.; Speir, J. A.; DiGennaro, J. A.; Fetrow, J. S.; Dwek, R. A.; Wilson, I. A. *J. Mol. Biol.* **1999**, *293*, 351−366. (c) Wormald, M. R.; Dwek, R. A. *Structure* **1999**, *7*, R155−R160.

(2) Effects of *O*-glycosylation on peptide backbone conformation: (a) Liang, R.; Hamilton Andreotti, A.; Kahne, D. *J. Am. Chem. Soc.* **1995**, *117*, 10395−10396. (b) Hamilton Andreotti, A.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 3352−3353.

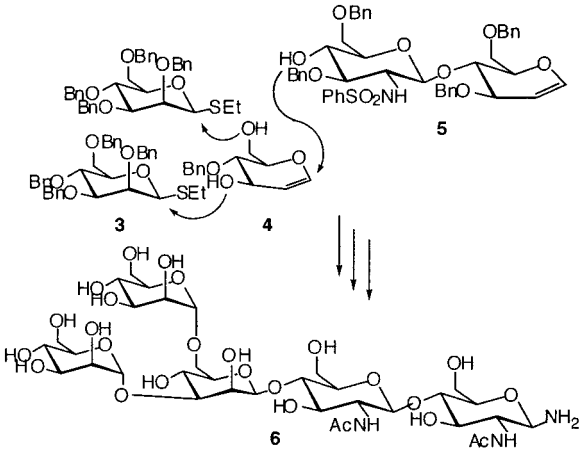
(3) X-ray structures for glycosylated proteins: (a) Ribonuclease B: Williams, R. L.; Greene, S. M.; McPherson, A. *J. Biol. Chem.* **1987**, *262*, 16020−16031. (b) extracellular domain of CD2: Bodian, D. L.; Jones, E. Y.; Harlos, K.; Stuart, D. I.; Davis, S. J. *Structure* **1994**, *2*, 755−766. Jones, E. Y.; Davis, S. J.; Williams, A. F.; Harlos, K.; Stuart, D. I. *Nature* **1992**, *360*, 232−239. (c) Human chorionic gonadotropin (partially deglycosylated): Lapthorn, A. J.; Harris, D. C.; Littlejohn, A.; Lustbader, J. W.; Canfield, R. E.; Machin, K. J.; Morgen, F. J.; Isaacs, N. W. *Nature* **1994**, *369*, 455−461. Wu, H.; Lustbader, J. W.; Liu, Y.; Canfield, R. E.; Hendrickson, W. A. *Structure* **1994**, *2*, 545−558. (d) IgG (F_c fragment): Deisenhofer, J. *Biochemistry* **1981**, *20*, 2361−2370.

10.1021/ol0070233 CCC: $20.00
Published on Web 02/23/2001

natural products and glycopeptides,[5] we wondered about the extent of the stereochemical communication between the peptide and carbohydrate domains. It seemed that an incisive way to probe whether the two domains are stereochemically autonomous would arise from the union of a fully synthetic, stereochemically defined carbohydrate domain with two "enantiomeric" peptide domains, composed of native L-amino acids (see matched glycopeptide **1**) and its enantiomeric D-amino acid ensemble (mismatched glycopeptide **2**). Herein, we report on the preparation of **1** and **2** and their high-field NMR characterization in aqueous solution. These measurements strongly point to the existence of stereochemical cross-talk between the carbohydrate and peptide domains.

Using the glycal assembly method established in our program to synthesize complex carbohydrates and their conjugates, pentasaccharide glycosylamine **6** was synthesized from components **3**−**5** (Scheme 1).[5]

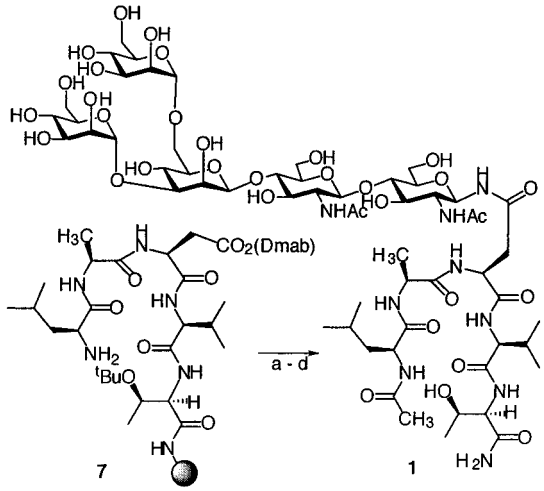
Scheme 1. Synthesis of Pentasaccharide via Glycal Assembly



Both L- and D-amino acid pentapeptides **7**/**8** were synthesized using standard Fmoc-based chemistry on a Rink amide MBHA resin (Scheme 2).[6]

The amino terminus of the pentapeptide was capped with acetic anhydride, followed by cleavage from the resin and removal of the threonine side chain protecting group. To reveal the aspartate carboxylate for the ensuing coupling with the glycosylamine, the Dmab protecting group was removed with 2% NH_2NH_2/MeOH,[7] followed by purification by reverse-phase HPLC. Subsequently, β-glycosylamine **6** was coupled to the aspartyl side chain using the standard Lansbury conditions.[8] After purification of the final glycopeptide constructs by reverse-phase HPLC, the structural assignment of **1**/**2** was confirmed both by ^{1}H NMR spectroscopy (800 MHz) and FAB mass spectrometry.

Scheme 2. Synthesis of the Two Analogues[a]



[a] (a) Ac_2O; (b) TFA; (c) NH_2NH_2, MeOH; (d) **6**, HOBt, HBTU, $^i Pr_2NEt$, DMSO, 25 °C, 48 h.

NMR studies of the glycopeptide **1** in H_2O revealed a complete set of sequential amide−amide NOEs along the peptide backbone. Further, the backbone proton couplings between α-CH and NH amide protons provide an indication of a preference for a type I β-turn (for coupling constants see Figure 1). The lower value for Ala followed by the higher one for Asn suggests such a turn preference, an arrangement that is analogous to what has been suggested by Imperiali for a different glycosylated peptide.[9] In addition to the peptide backbone amide couplings, values have been obtained for the NH bond in the glycosidic linkage and the two *N*-acetyl groups in the glycan.

For the γ-aspartamide NH-CH1 (GlcNAc1), the value is 9.2 Hz, while the two *N*-acetyl groups have NH-CH coupling constants of 9.8 and 9.1 Hz. Such high values indicate the absence of torsional averaging about the bonds in question. The glycosidic coupling constant itself does not allow one to distinguish between a *trans* or *cis* relationship of the protons, but NOE experiments clearly suggest a *trans* arrangement on the basis of the observation of a weak NOE between the NH and the anomeric proton and a strong NOE to H2 (Figure 2). Likewise, the NH proton of the *N*-acetyl group appears to be *trans* to H2 (GlcNAc1), based on a large coupling constant observed for NH (NAc)-H2 (GlcNAc1) and a strong NOE between NH (NAc)-H3 (GlcNAc1).[10] Furthermore, the pattern of NOEs between the proximal

(4) (a) Imperiali, B.; O'Connor, S. E. *Curr. Opin. Chem. Biol.* **1999**, *3*, 643−649. (b) Imperiali, B. *Acc. Chem. Res.* **1997**, *30*, 452−459. (c) Imperiali, B.; O'Connor, S. E. *Chem. Biol.* **1996**, *3*, 803−812.

(5) (a) Wang, Z.; Zhang, X.; Live, D.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 3652−3656. (b) Danishefsky, S. J.; Hu, S.; Cirillo, P. F.; Eckhardt, M.; Seeberger, P. H. *Chem. Eur. J.* **1997**, *3*, 1617−1628.

(6) Prepared by San San Yi, Microchemistry Laboratory, Sloan-Kettering Institute for Cancer Research.

(7) Dmab (4-[*N*-(1-(4,4-dimethyl-2,6-dioxacyclohexylidene)-3-methylbutyl)-amino]-benzyl ester): Chan, W. C.; Bycroft, B. W.; Evans, D. J.; White, P. D. *Peptides 1994, Proceedings of the 23rd European Peptide Symposium*, Braga, Portugal; ESCOM: Leiden, 1995; pp 153−154.

(8) (a) Cohen-Anisfeld, S. T.; Lansbury, P. T. *J. Am. Chem. Soc.* **1993**, *115*, 10531−10537. (b) Likhoshertstov, L. M.; Novikova, O. S.; Derkvitskaja, V. A.; Kochetkov, N. K. *Carbohydr. Res.* **1986**, *146*, C1−C5.

(9) O'Connor, S. E.; Imperiali, B. *J. Am. Chem. Soc.* **1997**, *119*, 2295−2296.

(10) This observation for the *N*-acetyl group is consistent with an earlier glycopeptide study: Davis, J. T.; Hirani, S.; Bartlett, C.; Reid, B. R. *J. Biol. Chem.* **1994**, *269*, 3331−3338. See also: O'Connor, S. E.; Imperiali, B. *Chem. Biol.* **1998**, *5*, 427−437.

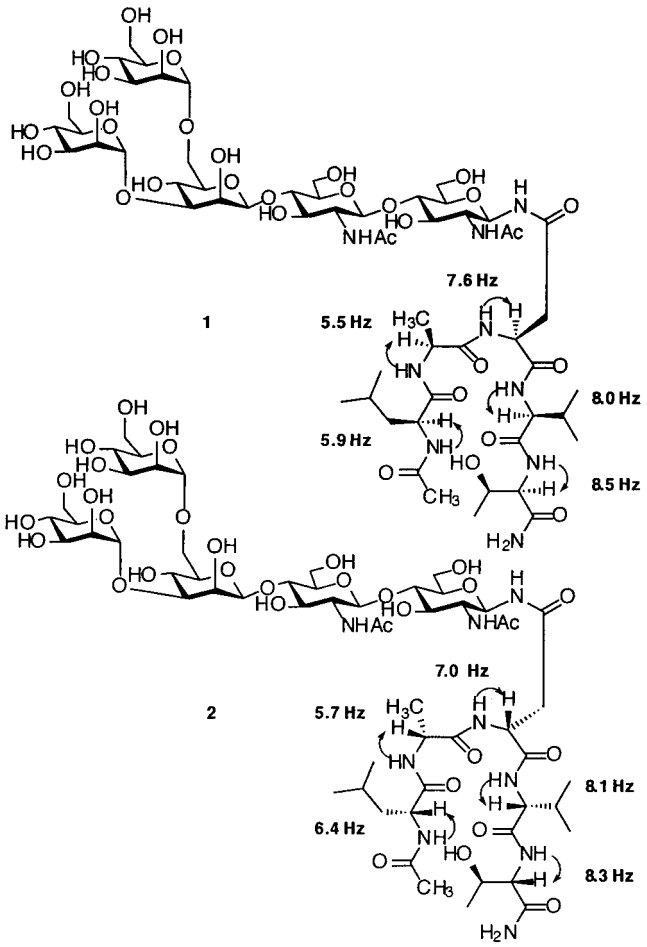


Figure 1. Coupling constants for glycopeptides **1** and **2** suggest a type I β-turn.

sugar and the asparagine residue is similar to that in glycosylated protein CD2, indicating the relevance of the glycopeptide model.[11]

Having prepared and characterized the matched glycopeptide **1** and its stereochemically "mismatched" analogue **2**, we compared their solution-phase conformations by high-field NMR spectroscopy. While the patterns in the NOESY spectra were very similar, distinct and measurable shift differences were observed for protons at the central amino acid residues (see Figure 3).

Significant shift differences occurred for the amide NH protons of asparagine, to which the carbohydrate is linked,

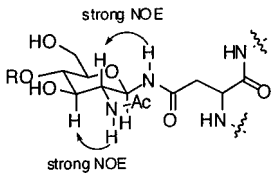


Figure 2. Observed NOEs for GlcNAc1-Asn (glycopeptide **1**).

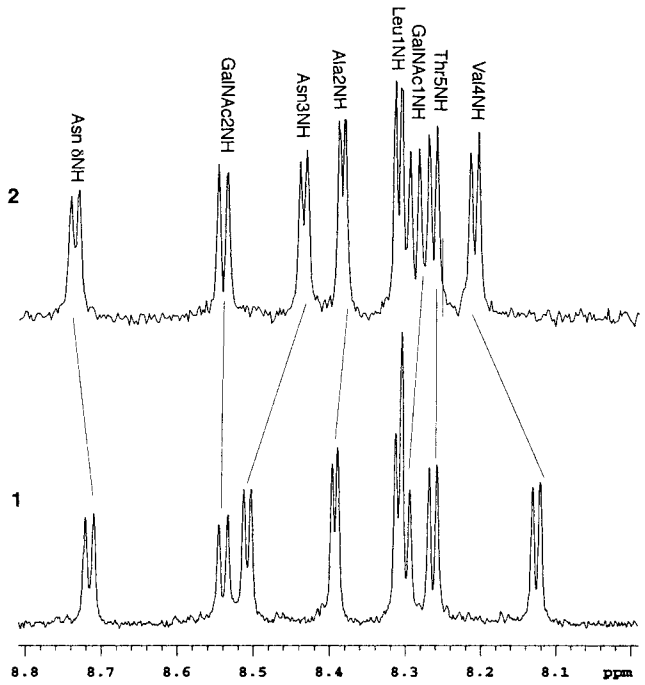


Figure 3. Amide proton spectra of **1** and **2** recorded at 800 MHz at 5 °C. Samples were dissolved in 90% H_2O/10% D_2O ([**1**] = 5 mM, [**2**] = 1 mM), and the pH was adjusted with a 10 mM phophate buffer (**1**, pH 3.5; **2**, pH 4.2). The H_2O signal was suppressed using the WATERGATE method.

and the neighboring valine (−0.1 ppm (Asn3NH) and +0.08 ppm (Val4NH), D → L respectively) and the α-CH proton of valine (+0.025 ppm, D → L), while the α- and β-CH protons of asparagine presented only a very minor change in shift or splitting pattern. The amide proton chemical shifts are sensitive indicators of peptide backbone interactions and conformations; thus, the comparison of high-field NMR data of **1** and **2** indicates that the global change in peptide stereochemistry has an impact on the peptide backbone conformation of the glycopeptide. While NOEs between the carbohydrate and the peptide have not been detected, there is nevertheless a distinct, stereochemistry-dependent communication between the two domains in this *N*-linked glycopeptide. In several examples of glycoproteins with *N*-linked carbohydrate attachments, specific evidence for interactions between the carbohydrate component and the polypeptide backbone have not been evident.[11−13] It has been shown for *N*-linked glycopeptides that changing the functional groups on the pendant carbohydrate does influence the arrangement of the peptide backbone.[4] Here we see that even a more subtle stereochemical change influences the

(11) (a) Wyss, D. F.; Choi, J. S.; Wagner, G. *Biochemistry* **1995**, *34*, 1622−1634. (b) Wyss, D. F.; Choi, J. S.; Li, J.; Knoppers, M. H.; Willis, K. J.; Arulanandam, A. R. N.; Smolyar, A.; Reinherz, E. L.; Wagner, G. *Science* **1995**, *269*, 1273−1278.

(12) Thijssen-van Zuylen, C. W. E. M.; de Beer, T., Leeflang, B. R.; Boelens, R.; Kaptein, R.; Kamerling, J. P.; Vliegenthart, F. G. *Biochemistry* **1998**, *37*, 1933−1940.

(13) Fletcher, C. M.; Harrison, R. A.; Lachman, P. J.; Neuhaus, D. *Structure* **1994**, *2*, 185−199.

peptide component. It is thus established that the communication between carbohydrate and the peptide domains can *not be attributed solely* to the bulk of the carbohydrate domain. These observations have prompted further ongoing studies. Already these results demonstrate the power of combined synthetic and structural approaches in delineating architecture in complex bi-domainal systems where direct contacts in the form of NOEs are not evident.

Acknowledgment. This work was supported by grants from the National Institutes of Health (Grants AI16943 and CA28824). We thank Dr. George Sukenick of the MSKCC NMR Core Facility for NMR and mass spectral analyses (NIH Grant CA08748). Z.-G. Wang gratefully acknowledges the U.S. Army for a Postdoctoral Fellowship (DAMD 17-97-1-7119).

OL0070233