

This article was downloaded by:

On: 23 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713617200>

The Peptide Can Influence Sugar Conformation in Small Asn Glycopeptides

Margaret L. Hindley^a; Kwun-Chi Lee^a; Jeffery T. Davis^a

^a Department of Chemistry and Biochemistry, University of Maryland, MD, USA

To cite this Article Hindley, Margaret L. , Lee, Kwun-Chi and Davis, Jeffery T.(2005) 'The Peptide Can Influence Sugar Conformation in Small Asn Glycopeptides', *Journal of Carbohydrate Chemistry*, 24: 3, 261 – 273

To link to this Article: DOI: 10.1081/CAR-200060393

URL: <http://dx.doi.org/10.1081/CAR-200060393>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

The Peptide Can Influence Sugar Conformation in Small Asn Glycopeptides

Margaret L. Hindley, Kwun-Chi Lee, and Jeffery T. Davis

Department of Chemistry and Biochemistry, University of Maryland, MD, USA

The Asn glycopeptide **1**, when compared to analogs Gln **2** and (D)Thr **3**, displays intramolecular sugar-peptide interactions in a DMSO- d_6 /CD₂Cl₂ organic solvent mixture. A comparative ¹H NMR study between Asn **1** and Gln **2** shows sequence-specific changes in the sugar's C5–C6 rotamer population. The diastereomeric glycopeptide **3** containing a (D)-Thr3 residue also shows some significantly different sugar ¹H and ¹³C NMR chemical shifts compared to the parent glycopeptide **1**. The GlcNAc sugar attached to the Asn1 sidechain is able to sense the stereochemistry at the remote Thr3 residue.

Keywords Glycopeptide, Sugar conformation, Peptide-carbohydrate interactions

In this paper we describe conformational studies of 3 N-linked glycopeptides that carry a GlcNAc monosaccharide on an Asn or Gln sidechain. The study's main objective is to determine whether intrinsic features of the Asn-Xaa-Ser/Thr N-glycosylation site might influence the attached sugar's conformation. Protein glycosylation can modulate structure and dynamics by altering the conformational space available to the connected peptide and glycan units, or by providing opportunities for direct peptide-sugar interactions. These conformational effects typically occur for the oligosaccharide's internal sugar units and for those amino acid residues closest to the glycosylation site.^[1–5] These local peptide-sugar interactions may well influence such crucial processes as protein folding and presentation of carbohydrate ligands to their receptors. Small glycopeptides are, therefore, useful models for investigating how

Received October 9, 2003; accepted March 17, 2004.
Address correspondence to Prof. Jeffery T. Davis, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA. Tel.: 301-405-1845; Fax: 301-314-9121; E-mail: jd140@umail.umd.edu

intramolecular sugar-peptide interactions influence glycoprotein structure and dynamics. While many studies have shown that glycosylation can affect peptide conformation,^[6–15] less attention has focused on how the peptide influences carbohydrate conformation.^[16–21] We present solution NMR evidence that sugar conformation can be modulated, in a sequence-selective manner, by the neighboring peptide. Specifically, the peptide's sequence and stereochemistry influence the sugar's C5–C6 rotamer population.

Asparagine glycosylation (N-glycosylation) of the Asn-Xaa-Ser/Thr sequence is a cotranslational process that can affect protein structure and function.^[1,2] We are interested in understanding how glycosylation of the Asn sidechain influences peptide and carbohydrate conformation.^[7,22] Our main goal in this study was to determine if there are intrinsic sugar-peptide interactions that occur within the context of the tripeptide N-glycosylation site.

We describe NMR studies on three related tripeptides: Ac-Asn(β 1-N-GlcNAc)LeuThr-NH₂ **1**, Ac-Gln(β 1-N-GlcNAc)LeuThr-NH₂ **2**, and Ac-Asn(β 1-N-GlcNAc)Leu(D)Thr-NH₂ **3** (Chart 1). Glycopeptides **2** and **3** differ from **1** due to changes within the N-glycosylation consensus sequence. Glycopeptide **2** has an Asn1 \rightarrow Gln1 mutation, while glycopeptide **3** contains the unnatural (D)-Thr at residue 3 rather than (L)-Thr3. We carried out these comparative NMR studies in d₆-DMSO/CD₂Cl₂ mixtures. NMR studies in organic solvents have merit. For example, intramolecular hydrogen bonds that are possible between the peptide and sugar domains, particularly for short and flexible glycopeptides like **1–3**, should be best manifested in a less competitive organic solvent. This allows one to gain insight into the fundamental interactions that are possible between the Asn-Xaa-Ser tripeptide and sugar domains. Furthermore, there is also a growing interest in how glycopeptides might interact with biomembranes, and organic solvents are potential models for better understanding how a membrane's hydrophobic environment might influence glycopeptide conformation.^[23–25]

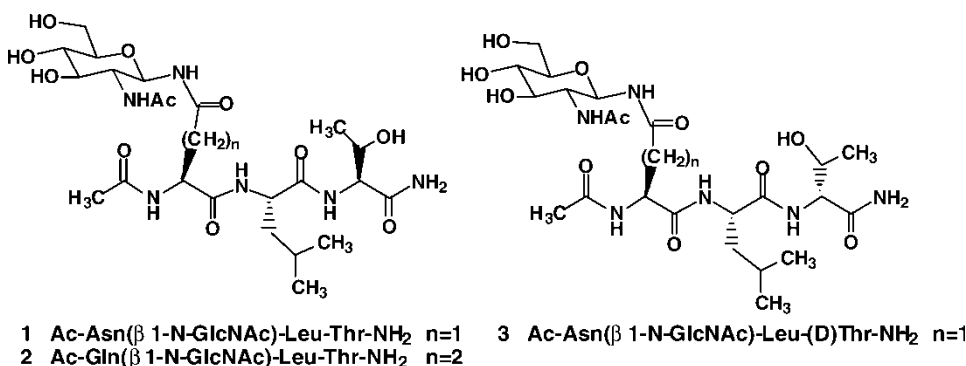


Chart 1: Structures of the N-linked glycopeptides **1–3** compared in this study.

We previously reported that Asn glycopeptide **1** is structured in DMSO- d_6 : CD_2Cl_2 .^[22] Our conclusion was based on 1H NMR data for glycopeptides Asn **1**, Gln **2**, and the corresponding aglycosyl peptides Ac-AsnLeuThr-NH₂ (**4**) and Ac-GlnLeuThr-NH₂ (**5**). Comparison of 1H NMR chemical shifts, NH temperature coefficients, Asn α,β 3J coupling constants and 1H - 1H NOEs indicated that glycopeptide **1** had sequence-specific secondary structure that the other compounds lacked. NOEs between the peptide's C-terminal NH protons and the GlcNAc H1, H5, and upfield-shifted H6 protons provided compelling evidence that the peptide chain folded back on the sugar unit. Energy-minimized structures showed that the Thr3 NH hydrogen bonds to the Asn1 $\gamma C=O$ to form an "Asx-turn."^[26,27] We also concluded that N-glycosylation stabilized this Asx-turn in glycopeptide **1**, relative to the aglycosyl peptide **4**. The schematic model in Figure 1 shows that the GlcNAc's O6 hydroxyl group is close to the peptide's C-terminus. The sugar's O6 hydroxyl, by functioning either as an H-bond donor or acceptor, could well stabilize the Asx-turn in glycopeptide **1** by providing intramolecular peptide-sugar interactions that would not be possible for the aglycosyl peptide. Thus, we became interested in detecting potential peptide-sugar interactions in Asn **1** by studying the GlcNAc C5-C6 hydroxymethylene conformation in the glycopeptides **1-3**. Our hypothesis was that modification of the peptide's amino acid sequence might show changes in the sugar sidechain conformation, especially if peptide-sugar interactions were important for stabilizing the solution structure in the parent glycopeptide Asn **1**.

Glycopeptide Synthesis

Glycopeptides **1-3** were prepared by coupling β 1-N-amino-GlcNAc with the Asp- or Glu-tripeptides.^[22,28] After HPLC purification, **1-3** were

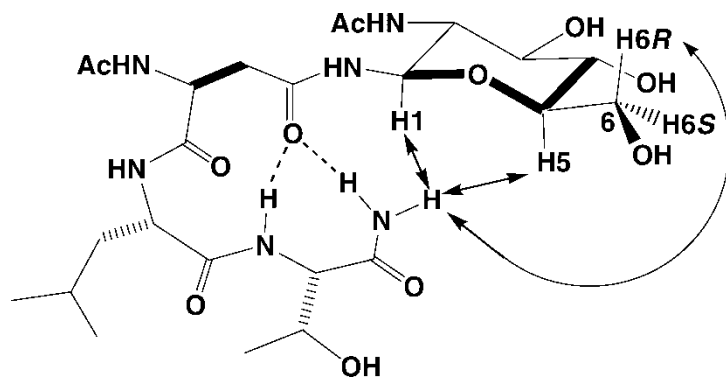


Figure 1: Schematic representation of secondary structure for Asn glycopeptide **1** (see ref. 22). This model shows an Asx-turn with hydrogen bond between the Asn1 sidechain's C=O and the Thr3 NH and a C-terminal amide NH. Peptide-sugar NOEs are indicated by the double-headed arrows. The GlcNAc C6-C5 sidechain is close in space to the peptide's C-terminus.

characterized by ^1H and ^{13}C NMR spectroscopy and FAB mass spectrometry. The ^1H and ^{13}C NMR resonance assignments for **1–3** in 30% DMSO- d_6 /70% CD_2Cl_2 were obtained from 2D ^1H - ^1H TOCSY and DQF-COSY and ^1H - ^{13}C HMQC and HMBC experiments, as previously described.^[22]

Comparison of ^1H NMR Data for Glycopeptides Asn **1** and Gln **2** Indicate that the GlcNAc C5–C6 Rotamer Population Depends on Peptide Sequence

Figure 2 shows Newman projections for the three major conformers about the GlcNAc C5–C6 bond. These rotamers are designated gt (gauche-trans), gg (gauche-gauche), and tg (trans-gauche), where the first letter indicates relative O6–O5 orientation and the second letter indicates the O6–C4 orientation. By measuring chemical shifts and $^3J_{5,6}$ coupling constants as a function of temperature and solvent we hoped to learn whether the peptide influences the equilibrium populations of these GlcNAc C5–C6 rotamers.^[29–33]

The ^1H NMR chemical shifts for the GlcNAc H6R and H6S protons in glycopeptides **1** and **2**, in a 30% DMSO- d_6 :70% CD_2Cl_2 solvent mixture, are presented in Table 1. We assigned the H6S resonance as the more downfield shifted of the two C6 diastereotopic signals. This assignment is strongly supported by the following information: (1) we previously used selective ^2H labeling at C6 to make stereospecific assignments for H6R and H6S in the parent GlcNAc sugar^[34] and (2) glucopyranose derivatives with unsubstituted O4 and O6 hydroxyl groups always show a H6S signal at a higher frequency than the H6R resonance, irrespective of solvent.^[35] Even if this glycopeptide system is the exception to this rule and the stereospecific assignments are reversed, the comparative 3J and chemical shift data below still show that the GlcNAc sugar on the native Asn glycopeptide **1** undergoes a temperature- and solvent-induced change in conformation that is not observed for the unnatural Gln glycopeptide **2**.

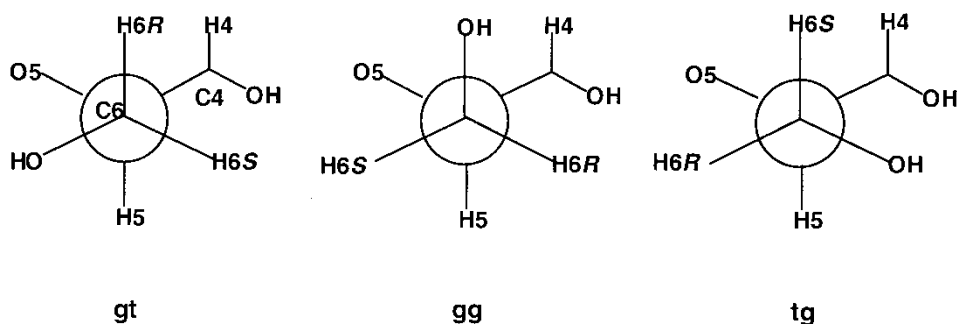


Figure 2: Newman projections for the three main rotamers about the GlcNAc C5–C6 bond.

Table 1: ^1H chemical shifts (ppm) for C6 protons in Asn **1** and Gln **2** in 30% DMSO- d_6 /70% CD_2Cl_2 .

Glycopeptide	Temp.	δ H6S	δ H6R	$\Delta\delta$ (ppm)
Asn 1	298 K	3.79	3.48	0.31 (0.15) ^a
	233 K	3.78	3.33	0.45 (0.15) ^a
Gln 2	298 K	3.66	3.48	0.14 (0.14)
	233 K	3.64	3.49	0.15 (0.15)

^a Values in parentheses determined in 100% DMSO- d_6 at both 298 and 233 K.

The important point from Table 1 is that the chemical shift difference ($\Delta\delta$) between the diastereotopic sugar protons H6R and H6S in glycopeptides **1** and **2** is significantly in the organic solvent mixture. This was the first indication that the peptide's amino acid sequence must influence the sugar's C5–C6 rotamer population. For Asn **1**, $\Delta\delta = 0.31$ ppm at 298 K, while Gln **2** has a value of $\Delta\delta = 0.14$ ppm under identical conditions. In addition, the $\Delta\delta_{\text{H6S,H6R}}$ value shows significant temperature dependence for the Asn glycopeptide **1**, increasing from $\Delta\delta = 0.31$ ppm at 298 K to $\Delta\delta = 0.45$ ppm at 233 K. In marked contrast, the H6S/H6R chemical shift separation is temperature independent for Gln **2**. In this mixed organic solvent the data imply that the GlcNAc sugar is able to interact with the Asn peptide, but not with the Gln peptide. Moreover, both Asn **1** and Gln **2** have identical $\Delta\delta$ values for GlcNAc's H6R, and H6S, at all temperatures, in 100% DMSO- d_6 , a solvent in which neither glycopeptide **1** or **2** is structured. In summary, the NMR data in Table 1 are consistent with the GlcNAc C5–C6 sidechain being involved in intramolecular interactions with the peptides that stabilize secondary structure in Asn **1**, but not in the homologous Gln glycopeptide **2**.

GlcNAc $^3\text{J}_{5,6}$ Values Indicate Peptide-Sugar Interactions in Asn **1**

More strong evidence for the GlcNAc C5–C6 sidechain's participation in structure in glycopeptide **1** was obtained from comparing $^3\text{J}_{5,6}$ coupling constants for Asn **1** and Gln **2**.^[29–33] Table 2 shows that the $^3\text{J}_{5,6}$ values, and therefore the GlcNAc C5–C6 rotamer population, in 30% DMSO- d_6 :70% CD_2Cl_2 depends on the amino acid sequence. These $^3\text{J}_{5,6}$ coupling constants for Asn glycopeptide **1** change significantly with temperature, indicating a shift in the equilibrium population of sidechain rotamers (Table 2). In contrast, the $^3\text{J}_{5,6\text{S}}$ coupling constants for Gln **2** are temperature invariant. We note that the corresponding $^3\text{J}_{5,6\text{R}}$ values for Gln **2** could not be determined because the H6S resonance could not be clearly resolved between 298 and 253 K. These comparative ^3J data indicate that sugar C5–C6 conformation in Asn **1** must be

Table 2: GlcNAc $^3J_{5,6}$ coupling constants for glycopeptides Asn **1** and Gln **2**.^a

Temp	Asn 1		Gln 2	
	$^3J_{5,6R}$	$^3J_{5,6S}$	$^3J_{5,6R}$	$^3J_{5,6S}$
298	6.5	2.5	— ^b	5.0
283	7.0	1.9	—	5.0
273	7.5	1.5	—	5.0
263	8.1	—	—	5.0
253	8.7	—	—	—

^a The uncertainty in 3J values, measured from 500 MHz spectra, is 0.3 Hz.

^b For Asn **1**, H_{6R} and H_{6S} were resolved from other signals at most temperatures.

For Gln glycopeptide **2** only H_{6S} was resolved in the 1D spectra.

modulated by the neighboring peptide, whereas the same sugar is not at all influenced by the peptide domain in the homologous Gln glycopeptide **2**.

As shown in Table 3, we calculated the GlcNAc C5–C6 rotamer populations for Asn **1** using the measured $^3J_{5,6R}$ and $^3J_{5,6S}$ coupling constants in Table 2 and equations (1)–(3) shown in footnote 36.^[36] The data indicate that more of the gt rotamer is favored at lower temperatures in this organic solvent mixture. Figures 1 and 2 show that the gt rotamer is that conformation that would point the O6 hydroxyl group back toward the attached peptide chain. As the temperature is lowered, a new rotamer population is established for Asn glycopeptide **1**, due to enhanced intramolecular peptide-sugar interactions. In marked contrast, the C5–C6 sidechain in Gln **2** is not involved in peptide-sugar interactions, as its $^3J_{5,6}$ coupling constants are temperature independent (Table 2).

Thr3 Stereochemistry Affects Sugar C5–C6 Conformation

To determine whether more subtle aspects of peptide primary structure might impact N-glycopeptide secondary structure, we compared ^1H and ^{13}C NMR data for Asn glycopeptide **1** with that for Ac-Asn(β 1-NGlcNAc)Leu(D)Thr-NH₂ **3**.

Table 3: Calculated GlcNAc C5–C6 rotamer populations in glycopeptide Asn **1**.^a

	298 K	273 K
X _{gt} (%) ^b	58	73
X _{gg} (%)	31	26
X _{tg} (%)	11	1

^a See refs. 33 and 36 for method used to determine rotamer percentages.

^b See Figure 2 for rotamer definitions.

Glycopeptides **1** and **3** have identical functional groups, but differ in their Thr3 configuration. Below, we show that the stereochemistry at this terminal peptide residue does indeed influence the conformation of the remote sugar C5–C6 sidechain in Asn glycopeptide **1**. Due to the solubility properties of **3**, comparative NMR experiments with diastereomeric glycopeptides **1** and **3** were done in 50% DMSO- d_6 /50% CD $_2$ Cl $_2$. In this more competitive solvent, containing higher percentages of DMSO- d_6 , glycopeptide **1** still showed peptide-sugar NOEs that were diagnostic of folded structure. As in our previous study,^[22] ROESY experiments at 273 K showed strong NOEs between the C-terminal trans NH and GlcNAc H1, as well as between the C-terminal cis NH and GlcNAc H5. In contrast to the intramolecular dipolar interactions for **1**, no peptide-sugar NOEs were observed for the analogous (D)-Thr3 glycopeptide **3** in this 50% DMSO- d_6 /50% CD $_2$ Cl $_2$ solvent, indicating that the (D)-Thr3 is likely to be unstructured under these conditions. We note that increasing the DMSO- d_6 concentration to approximately 70% leads to the disappearance of NOES in glycopeptide **1**.

Thr3 Stereochemistry Influences ^1H Chemical Shifts for GlcNAc H6R, H6S

The ^1H NMR chemical shifts for the H6R/H6S protons of glycopeptides **1** and **3** in 50% DMSO- d_6 /50% CD $_2$ Cl $_2$ at 298 K are presented in Table 4. The $\bullet\delta$ value for the H6R/H6S pair in glycopeptide **1** is $\bullet\delta = 0.39$ ppm. In contrast, $\bullet\delta$ is much smaller ($\bullet\delta = 0.11$ ppm) for the H6R/H6S protons in (D)-Thr glycopeptide **3**. In this solvent, the GlcNAc sugar's H6R/H6S chemical shifts clearly depend on Thr3 stereochemistry. Such a long-range effect, in terms of primary sequence, suggests that the Thr3 absolute configuration has an important influence on the preferred GlcNAc C5–C6 rotamer. This would occur only for folded conformations of Asn glycopeptide **1** where the sugar attached to Asn1 could interact with the Thr3 residue. Again, the solvent was crucial for chemical shift dispersion. The $\bullet\delta$ values ($\bullet\delta = 0.15$ ppm) are identical for glycopeptides **1** and **3** in 90% H $_2$ O–10% D $_2$ O, a solvent that does not stabilize structures in such small glycopeptides. The sensitivity of the GlcNAc H6R, H6S chemical

Table 4: ^1H NMR chemical shifts (ppm) for C6 protons in glycopeptides **1** and **3**.^a

Glycopeptide	δ H6S	δ H6R	$\Delta\delta$ (ppm)
1	3.54	3.15	0.39 (0.15) ^b
3	3.46	3.35	0.11 (0.15)

^aIn 50% DMSO- d_6 /50% CD $_2$ Cl $_2$ at 298 K.

^bValues in parentheses determined in 90% H $_2$ O/10% D $_2$ O.

shifts to temperature and solvent polarity is entirely consistent with the GlcNAc C5–C6 sidechain stabilizing the Asx-turn in glycopeptide **1**, but not in the diastereomeric glycopeptide **3**. Live et al. recently reported another example of an N-glycopeptide that shows stereochemical communication between its carbohydrate and peptide domains.^[37]

GlcNAc ¹³C Resonances Differ in Glycopeptides **1** and **3**

Isotropic ¹³C NMR chemical shifts in solution, particularly for carbohydrates, can be quite sensitive to conformation.^[38–42] We assigned ¹³C resonances for glycopeptides **1** and **3** through the use of 2D homonuclear (TOCSY and DQF-COSY) and heteronuclear (¹H-¹³C HMQC and ¹H-¹³C HMBC) NMR experiments as previously described.^[22] Figure 3 shows a region of the ¹³C NMR spectra for glycopeptides **1** and **3** at 298 K in 50% DMSO-d₆/50% CD₂Cl₂. This region (δ 60–85) shows resonances for the GlcNAc sugar carbons (C1, C3–C6) and for the Thr3 β -carbon. Under identical conditions of concentration, solvent, and temperature, these diastereomeric glycopeptides have much different chemical shifts for GlcNAc C6 ($\Delta\delta$ 0.97 ppm) and C4 ($\Delta\delta$ 0.85 ppm). In contrast, the GlcNAc C1, C2, C3, and C5 chemical shifts are essentially the same for the two compounds. These different ¹³C chemical shifts for GlcNAc C4 and C6 in glycopeptides **1** and **3** clearly indicate that the sugar

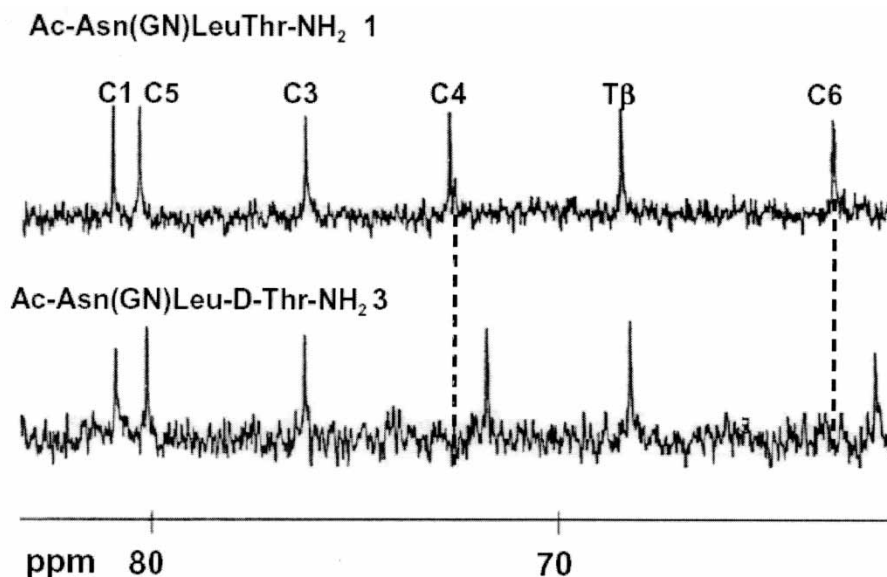


Figure 3: A region of ¹³C NMR spectra comparing glycopeptides **1** and **3** at 298 K in 50% DMSO-d₆/50% CD₂Cl₂. The sugar's carbon resonances are labeled. The chemical shifts for the GlcNAc C4 and C6 resonances differ significantly for the two diastereomeric peptides, as portrayed by the dashed lines.

attached to Asn1 can sense the Thr3 stereochemistry, once again consistent with a much different GlcNAc C5–C6 rotamer population for the two diastereomeric glycopeptides **1** and **3**.

The Newman projections in Figure 2 show that the ^{13}C chemical shift for GlcNAc C4 should be influenced by the sugar's C5–C6 rotamer population. The C6 resonance is also sensitive to sidechain rotamer populations, most likely due to involvement of the 6-OH hydroxyl group in an intramolecular sugar-peptide hydrogen bond.

Summary

As summarized in Figure 4, comparison of ^1H and ^{13}C NMR data for glycopeptides **1–3** confirm that only the structured Asn glycopeptide **1** has peptide-sugar interactions in a mixed DMSO- CD_2Cl_2 solution. In particular, the GlcNAc hydroxymethylene conformation is sensitive to the peptide's amino acid sequence, indicating that this sugar C5–C6 sidechain is involved in intramolecular interactions with the peptide domain. Modifications to the Asn1 and Thr3 residues in glycopeptide **1** result in significant changes in the equilibrium populations of GlcNAc C5–C6 rotamers. These local peptide-sugar interactions seem to be an intrinsic property of the Asn-Xaa-Thr/Ser glycosylation site, suggesting that they may also be important in stabilizing local structure in N-linked glycoproteins as well.

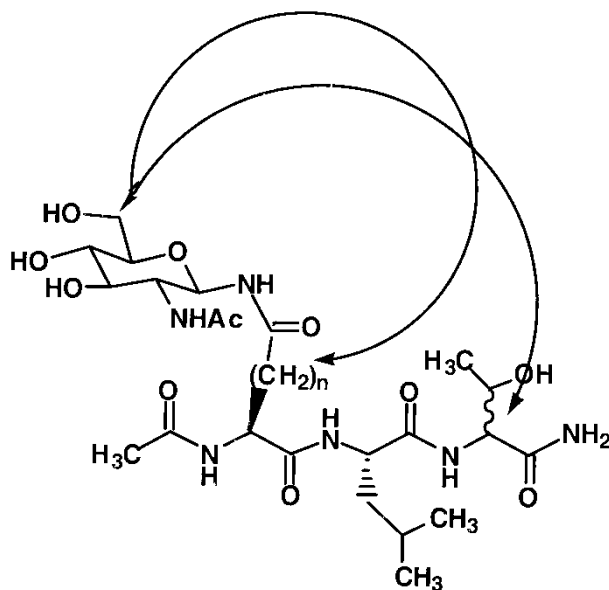


Figure 4: Schematic showing that changes to the amino sequence within the N-glycosylation site influences the GlcNAc C5–C6 sidechain conformation of glycopeptides **1–3** in the mixed organic solvent system.

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra were recorded on a 500 MHz spectrometer. The 2D-homonuclear (TOCSY and DQF-COSY) and heteronuclear NMR (^{13}C - ^1H HMBC and ^{13}C - ^1H HMQC) experiments used to assign ^1H and ^{13}C NMR chemical shifts were done as described in the supporting information to reference 22. The 1D spectra for glycopeptide **3** are included as supporting information to this paper. The 2D NMR spectra for glycopeptides **1–3** can be obtained by contacting the corresponding author.

Peptide Synthesis

Peptides were synthesized by solid-phase synthesis using t-Boc protocols. Peptides were cleaved from the resin with HF, using anisole (10:1) as a scavenger. Peptides and glycopeptides were purified by HPLC, detected at 214 nm with a UV/VIS detector, and separated on a C-18 reverse phase column (2.2 × 25 cm, 300 Å) using a 5 to 35% gradient of 80% acetonitrile in 0.1% TFA against 0.1% TFA in water.

Glycopeptide Synthesis

Compounds **1** and **2** were prepared as described in reference 22. **Ac-Asn (β 1-N GlcNAc)-Leu-(D)Thr-NH₂ (**3**)**. To a solution of Ac-Asp-Leu-(D)Thr-NH₂ (20 mg, 0.052 mmol) and BOP (98 mg, 0.156 mmol) in 1.5 mL of DMSO-*d*₆ was added 1-amino-2-acetamido- β -D-glucose (34 mg, 0.156 mmol, Sigma) in 1.5 mL of DMSO-*d*₆. The reaction was stirred at rt for 18 hr and monitored by ^1H NMR until the glycopeptide's anomeric H1 signal (δ 4.75) had reached a constant intensity. Glycopeptide **3** was purified by HPLC to yield 12.5 mg (41%) of a white solid after lyophilization. ^1H NMR (500 MHz, DMSO-*d*₆): δ 8.24 (d, 1H, J = 8.7 Hz, GlcNAc NH1), 8.14 (d, 1 H, J = 8.9, Asn NH), 8.05 (d, 1 H, J = 7.9 Hz, Leu NH), 7.78 (d, 1 H, J = 9.0, GlcNAc NHAc), 7.62 (d, 1H, J = 8.7, Thr NH), 7.14 (s, 1 H, term NH_E), 7.08 (s, 1H, term NH_Z), 4.98 (m, 2 H, C3 OH & C4 OH), 4.75 (t, 1H, J = 8.7, H1), 4.74 (m, 1 H, Thr3 OH), 4.58 (m, 1 H, C6 OH) 4.51 (dd, 1 H, J = 7.2, 8.9 Asn α), 4.27 (m, 1 H, Leu α), 4.02 (m, 2H, Thr α,β), 3.63 (m, 1H, H6S), 3.51 (dd, 1H, J = 9.0, 10.9, H2), 3.48 (m, 1H, H6R) 3.43 (d, 1H, J = 10.9, H3), 3.08 (d, 1H, J = 5.9, H5), 2.64 (dd, 1 H, J = 6.4, 15.6, Asn β), 2.32 (dd, 1 H, J = 8.9, 15.6, Asn β), 1.81 (s, 3H, NHAc), 1.79 (s, 3H, NHAc), 0.98 (d, 3H, J = 6.4, Thr Me), 1.60–1.40 (m, 3H, Leu α,β) 0.87 (d, 3 H, J = 5.8, Leu Me), 0.81 (d, 3 H, J = 5.8, Leu Me); ^{13}C NMR (125 MHz, DMSO-*d*₆): δ 172.3, 172.0, 171.1, 170.0, 169.5, 88.5, 78.8, 78.6, 74.5, 70.3, 66.2, 60.7, 58.2, 54.5, 51.3, 49.4, 36.9, 24.1, 23.0, 22.8, 22.4, 21.5, 20.0; FAB MS m/z (%) (M^+) 590.9 (24.1), 371.2 (17.8), 225.1 (38.9), 204.1 (77.2); HRMS m/z ($M^{+1} + 1$) calcd. for C₂₄H₄₃N₆O₁₁ 591.2990, found 591.2972.

SUPPORTING INFORMATION AVAILABLE:

Copies of ^1H and ^{13}C NMR spectra for glycopeptide **3**.

ACKNOWLEDGMENT

We thank the NIH (R29 CA16644) for funding this work. JD also thanks the Dreyfus Foundation for a Teacher-Scholar Award.

REFERENCES

- [1] Imperiali, B.; O'Connor, S.E. Effect of N-linked glycosylation on glycopeptide and glycoprotein structure. *Curr. Opin. Chem. Biol.* **1999**, *3*, 643–649.
- [2] Wormald, M.R.; Petrescu, A.J.; Pao, Y.L.; Glithero, A.; Elliott, T.; Dwek, R.A. Conformational studies of oligosaccharides and glycopeptides: complementarity of NMR, X-ray crystallography, and molecular modelling. *Chem. Rev.* **2002**, *102*, 371–386.
- [3] 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. Conformation and Function of the N-linked glycan in the adhesion domain of human CD2. *Science* **1995**, *269*, 1273–1278.
- [4] O'Connor, S.E.; Pohlmann, J.; Imperiali, B.; Saskiawan, I.; Yamamoto, K. Probing the effect of the outer saccharide residues of N-linked glycans on peptide conformation. *J. Am. Chem. Soc.* **2001**, *123*, 6187–6188.
- [5] Coltart, D.M.; Royyuru, A.K.; Williams, L.J.; Glunz, P.W.; Sames, D.; Kuduk, S.D.; Schwarz, J.B.; Chen, X.T.; Danishefsky, S.J.; Live, D.H. Principles of mucin architecture: structural studies on synthetic glycopeptides bearing clustered mono-, di-, tri- and hexasaccharide glycodomains. *J. Am. Chem. Soc.* **2002**, *124*, 9833–9844.
- [6] Wormald, M.E.; Wooten, E.W.; Bazzo, R.; Edge, C.J.; Feinstein, A.; Rademacher, T.W.; Dwek, R.A. The conformational effects of N-glycosylation on the tailpiece from serum IgM. *Eur. J. Biochem.* **1991**, *198*, 131–139.
- [7] Davis, J.T.; Hirani, S.; Bartlett, C.; Reid, B.R. H-1 NMR studies on an Asn-linked glycopeptide—GlcNAc-1 C2-N2 bond is rigid in H_2O . *J. Biol. Chem.* **1994**, *269*, 3331–3338.
- [8] Liang, R.; Andreotti, A.H.; Kahne, D. Sensitivity of glycopeptide conformation to carbohydrate chain-length. *J. Am. Chem. Soc.* **1995**, *117*, 10395–10396.
- [9] Live, D.H.; Kumar, R.A.; Beebe, X.; Danishefsky, S.J. Conformational influences of glycosylation of a peptide: a possible model for the effect of glycosylation on the rate of protein folding. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12759–12761.
- [10] O'Connor, S.E.; Imperiali, B. Conformational switching by asparagine-linked glycosylation. *J. Am. Chem. Soc.* **1997**, *119*, 2295–2296.
- [11] Imperiali, B.; Rickert, K.W. Conformational implications of asparagine-linked glycosylation. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 97–101.
- [12] Imperiali, B.; O'Connor, S.E. A molecular basis for glycosylation-induced conformational switching. *Chem. Biol.* **1996**, *3*, 803–812.
- [13] Huang, X.L.; Barchi, J.J.; Lung, E.D.T.; Roller, P.P.; Nara, P.L.; Muschik, J.; Garrity, R.R. Glycosylation affects both the three-dimensional structure and

- antibody binding properties of the HIV-1(IIIB) gp120 peptide RP135. *Biochemistry* **1997**, *36*, 10846–10856.
- [14] McManus, A.M.; Otvos, L.; Hoffman, R.; Craik, D.J. Conformational studies by NMR of the antimicrobial peptide, drosocin, and its non-glycosylated derivative: effects of glycosylation on solution conformation. *Biochemistry* **1999**, *38*, 704–714.
- [15] Huang, X.L.; Smith, M.C.; Berzofsky, J.A.; Barchi, J.J. Structural comparison of a 15 residue peptide from the V3 loop of HIV-1(IIIb) and an O-glycosylated analogue. *FEBS Lett.* **1996**, *393*, 280–286.
- [16] Brockbank, R.L.; Vogel, H.J. Structure of the oligosaccharide of hen phosphatase as determined by 2-dimensional H-1 NMR of the intact glycoprotein. *Biochemistry* **1990**, *29*, 5574–5583.
- [17] Lommerse, J.P.M.; Kroon-Batenburg, L.M.J.; Kroon, J.; Kamerling, J.P.; Vliegthart, J.F.G. Conformations and internal mobility of a glycopeptide derived from bromelain using molecular dynamics simulations and NOESY analysis. *J. Biomol. NMR* **1995**, *6*, 79–94.
- [18] Lu, J.Y.; van Halbeek, H. Complete H-1 and C-13 resonance assignments of a 21-amino acid glycopeptide prepared from human serum transferrin. *Carbohydr. Res.* **1996**, *296*, 1–21.
- [19] Weller, C.T.; Lustbader, J.; Seshradi, K.; Brown, J.M.; Chadwick, C.A.; Kolthoff, C.E.; Ramnarain, S.; Pollack, S.; Canfield, R.; Homans, S.W. Structural and conformational analysis of glycan moieties in situ on isotopically C-13,N-15-enriched recombinant human chorionic gonadotropin. *Biochemistry* **1996**, *35*, 8815–8823.
- [20] Bailey, D.; Renouf, D.V.; Large, D.G.; Warren, C.D.; Hounsell, E.F. Conformational studies of the glycopeptide Ac-Tyr-[Man(5)GlcNAc-beta-(1 - → 4)GlcNAc-beta-(1 - → N-delta)]-Asn-Leu-Thr-Ser-OBz and the constituent peptide and oligosaccharide. *Carbohydrate Res.* **2000**, *324*, 242–254.
- [21] Kindahl, L.; Sandstrom, C.E.; Norberg, T.; Kenne, L. H-1 NMR studies of hydroxy protons of the V[beta-Gal(1 - → 3)-alpha-GalNAc(1 - → O)THPGY glycopeptide. *Carbohydrate Res.* **2001**, *336*, 319–323.
- [22] Lee, K.C.; Falcone, M.F.; Davis, J.T. Sequence-specific peptide-carbohydrate interactions in an asparagine-linked glycopeptide. *J. Org. Chem.* **1996**, *61*, 4198–4199.
- [23] Hashimoto, Y.; Toma, K.; Nishikido, J.; Yamamoto, K.; Haneda, K.; Inazu, T.; Valentine, K.G.; Opella, S.J. Effects of glycosylation on the structure and dynamics of eel calcitonin in micelles and lipid bilayers determined by nuclear magnetic resonance spectroscopy. *Biochemistry* **1999**, *38*, 8377–8384.
- [24] Stella, L.; Venanzi, M.; Carafa, M.; Maccaroni, E.; Straccamore, M.E.; Zanotti, G.; Palleschi, A.; Pispisa, B. Structural features of model glycopeptides in solution and in membrane phase: a spectroscopic and molecular mechanics investigation. *Biopolymers* **2002**, *64*, 44–56.
- [25] Palian, M.M.; Boguslavsky, V.I.; O'Brien, D.F.; Polt, R. Glycopeptide-membrane interactions: glycosyl enkephalin analogues adopt turn conformations by NMR and CD in amphipathic media. *J. Am. Chem. Soc.* **2003**, *125*, 5823–5831.
- [26] Baker, E.N.; Hubbard, R.E. Hydrogen-bonding in globular proteins. *Prog. Biophys. Mol. Biol.* **1984**, *44*, 97–179.
- [27] Abbadi, A.; McHarfi, M.; Aubry, A.; Premilat, S.; Boussard, G.; Marraud, M. Involvement of side functions in peptide structures—the Asx turn-occurrence and conformational aspects. *J. Am. Chem. Soc.* **1991**, *113*, 2729–2735.

- [28] Cohen-Anisfeld, S.T.; Lansbury, P.T. A practical, convergent method for glycopeptide synthesis. *J. Am. Chem. Soc.* **1993**, *115*, 10531–10537.
- [29] Nishida, Y.; Ohruji, H.; Meguro, H. H-1-NMR studies of (6R)-deuterated and (6S)-deuterated D-hexoses—assignment of the preferred rotamers about C5-C6 bond of D-glucose and D-galactose derivatives in solution. *Tetrahedron Lett.* **1984**, *25*, 1575–1578.
- [30] Nishida, Y.; Hori, H.; Ohruji, H.; Meguro, H. H-1 NMR analyses of rotameric distribution of C5-C6 bonds of D-glucopyranoses in solution. *J. Carbohydr. Chem.* **1988**, *7*, 239–250.
- [31] Bock, K.; Duus, J.Ø. A conformational study of hydroxymethyl groups in carbohydrates investigated by H-1 NMR spectroscopy. *J. Carbohydr. Chem.* **1994**, *13*, 513–543.
- [32] Tvaroska, I.; Taravel, F.R.; Utille, J.P.; Carver, J.P. Quantum mechanical and NMR spectroscopy studies on the conformations of the hydroxymethyl and methoxymethyl groups in aldohexosides. *Carbohydrate. Res.* **2002**, *337*, 353–367.
- [33] Stenutz, R.; Carmichael, I.; Widmalm, G.; Serianni, A.S. Hydroxymethyl group conformation in saccharides: structural dependencies of (2)J(HH), (3)J(HH), and (1)J(CH) spin-spin coupling constants. *J. Org. Chem.* **2002**, *67*, 949–958.
- [34] Falcone-Hindley, M.L.; Davis, J.T. Stereoselective preparation of deuterium-labeled sugars: (6R)-(6-H-2(1))-N-acetylglucosamine derivatives. *J. Org. Chem.* **1998**, *63*, 5555–5561.
- [35] Rockwell, G.D.; Grindley, T.B. Effect of solvation on the rotation of hydroxymethyl groups in carbohydrates. *J. Am. Chem. Soc.* **1998**, *120*, 10953–10963.
- [36] Percentages of gt, gg and tg rotamers were calculated by solving the following three equations simultaneously: ${}^3J_{H5,H6R} = 9.9 \text{ pgt} + 0.8 \text{ pgg} + 4.5 \text{ ptg}$, ${}^3J_{H5,H6S} = 1.5 \text{ pgt} + 1.3 \text{ pgg} + 10.8 \text{ ptg}$, and $\text{pgt} + \text{pgg} + \text{ptg} = 1$, where p is the percentage of a particular rotamer. The coefficients used in the equations, standard coupling constant values, were obtained from Table 5 in Serianni's recent study of glucosyl hydroxymethylene conformation; see reference 33.
- [37] Live, D.H.; Wang, Z.G.; Iserloh, U.; Danishefsky, S.J. A strategy for probing the autonomy of cross-domain stereochemical communication in glycoconjugates. *Org. Lett.* **2001**, *3*, 851–854.
- [38] Barfield, M. Ab-Initio IGLO studies of the conformational and substituent dependencies of alpha-effects, beta-effects, gamma-effects and delta-effects in the C-13 NMR spectra of 1-substituted butanes. *J. Am. Chem. Soc.* **1995**, *117*, 2862–2876.
- [39] deDios, A.C.; Oldfield, E. Chemical shifts of carbonyl carbons in peptides and proteins. *J. Am. Chem. Soc.* **1994**, *116*, 5307–5314.
- [40] Mazeau, K.; Taravel, F.R.; Tvaroska, I. Angular dependence of the C-6 chemical shift and the conformation of the hydroxymethyl group in carbohydrates. *Chem. Papers* **1996**, *50*, 77–83.
- [41] Yates, E.A.; Santini, F.; De Cristofano, B.; Payre, N.; Cosentino, C.; Guerrini, M.; Naggi, A.; Torri, G.; Hricovini, M. Effect of substitution pattern on H-1, C-13 NMR chemical shifts and (1)J(CH) coupling constants in heparin derivatives. *Carbohydr. Res.* **2000**, *329*, 239–247.
- [42] Batta, G.; Gervay, J. Solution-phase C-13 and H-1 chemical-shift anisotropy of sialic acid and its homopolymer (colominic acid) from cross-correlated NMR. *J. Am. Chem. Soc.* **1995**, *117*, 368–374.