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An *N*-acetylglucosamine containing glycopeptide — synthesis and structure assignment

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Abstract

A nonapeptide sequence alignment known to be O-glycosylated at serine and/or threonine residues by *N*-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) was chemically synthesized with and without *O*-GlcNAc residues. A detailed conformational analysis was performed which displays the preferred conformation of the peptide backbone and the influence of the sugar residues. © 1997 Elsevier Science Ltd.

Keywords: O-Glycopeptides; O-Linked *N*-acetylglucosamine; Synthesis; Conformational analysis

1. Introduction

The novel intracellular carbohydrate modification by O-glycosidically linked *N*-acetylglucosamine (GlcNAc) has been shown to be ubiquitous among eucaryotes [1], on cytoplasmic [2], nuclear membrane [3], and plasma membrane associated [4] proteins. This modification occurs on serine (Ser) or threonine (Thr) residues in phosphoproteins and occurs frequently at consensus sites for proline (Pro) directed kinases [5]. The specific function of *O*-GlcNAc attachment to proteins has not yet been fully elucidated; indirect evidence led to the hypothesis that *O*-GlcNAc linkages may often have a reciprocal relationship to the regulatory effect of protein phosphorylation [3,6]. The *O*-GlcNAc expression is also significantly upregulated in Alzheimer brains over that of age-matched control brains [7]. Therefore, access to glycopeptides carrying β-linked *O*-GlcNAc at Ser and/or Thr residues became of great interest. Based on an efficient approach to *O*-GlcNAc-Ser and -Thr building blocks **1** and **2** [8] (Scheme 1) for glycopeptide synthesis, potential substrates for *O*-GlcNAc-transferases and -hydrolysases for in vitro and cellular investigations (transport into APP_{695} producing CHO cell [1]) were prepared and their conformations assigned. The selection of the peptide sequence

Ala-Val-Ser-Thr-Glu-Pro-Phe-Gly-Arg-NH_2 (**3**)

is based on sequence alignments of peptide sequences known to be O-glycosylated by GlcNAc [5,9]. Attachment of GlcNAc to Ser and/or Thr residues of **3**

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[1] B. Schmitz, University of Bonn, private communication.

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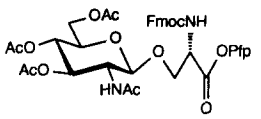

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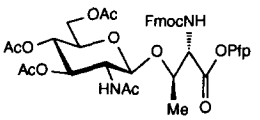

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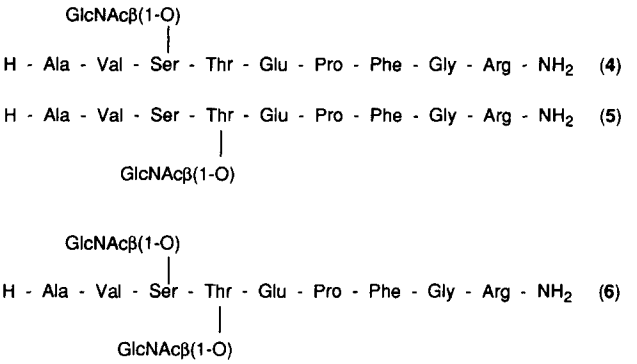
Scheme 1.

led to glycopeptides **4–6** as promising target molecules for biological studies (Scheme 2).

2. Results and discussion

Solid-phase peptide synthesis was carried out using a poly(ethyleneglycol)-poly(styrene) resin [10]



Scheme 2.

Table 1
^{1}H chemical shifts (δ, ppm) and backbone coupling constants (J, Hz) of peptides **3–6** (600 MHz, 300 K, 9:1 H_2O–D_2O)

		3	**4**	**5**	**6**
Ala	H^{α}	4.21 [a]	4.04	4.05	4.04
	H^{β}	1.25	1.41	1.43	1.41
Val	NH	8.07	8.37	8.33	8.32
	$J_{NH\text{-}H\alpha}$	7.7	6.6	(br) [b]	7.8
	H^{α}	4.07	4.08	4.12	4.10
	H^{β}	1.96	1.97	2.00	1.97
	H^{γ}	0.84	0.85, 0.86	0.85, 0.86	0.83, 0.84
Ser	NH	8.34	8.38	8.30	8.33
	$J_{NH\text{-}H\alpha}$	6.5	6.8	6.3	6.6
	H^{α}	4.43	4.45	4.52	4.51
	H^{β}	3.72, 3.97	3.71, 3.80	3.89, 3.94	3.89
Thr	NH	8.09	8.11	7.83	8.03
	$J_{NH\text{-}H\alpha}$	8.1	8.3	7.9	7.8
	H^{α}	4.26	4.35	4.22	4.33
	H^{β}	4.15	4.25	4.10	4.10
	H^{γ}	1.09	1.05	1.09	1.02
Glu	NH	8.15	8.07	8.09	8.10
	$J_{NH\text{-}H\alpha}$	7.1	7.1	6.6	7.1
	H^{α}	4.54	4.54	4.50	4.54
	H^{β}	1.77, 1.92	1.74, 1.92	1.77, 1.91	1.79, 1.90
	H^{γ}	2.28	2.32	2.20	2.22
Pro	H^{α}	4.26	4.26	4.26	4.26
	H^{β}	1.64, 2.08	1.65, 3.09	1.64, 2.08	1.62, 2.07
	H^{γ}	1.82	1.84	1.84	1.83
	H^{δ}	3.6, 3.52	3.65, 3.5	3.67, 3.5	3.63, 3.5
Phe	NH	8.15	8.15	8.09	8.15
	$J_{NH\text{-}H\alpha}$	n.d. [b]	n.d.	6.6	6.3
	H^{α}	4.45	4.46	4.46	4.44
	H^{β}	3.00–3.05	3.02	3.02	2.99–3.01
Gly	NH	8.18	8.16	8.13	8.17
	$J_{NH\text{-}H\alpha}$	n.d.	n.d.	n.d.	[c]
	H^{α}	3.70, 3.80	3.72, 3.79	3.72, 3.78	3.70, 3.81
Arg	NH	7.96	7.97	7.94	7.99
	$J_{NH\text{-}H\alpha}$	7.4	7.5	7.4	7.3
	H^{α}	4.21	4.20	4.20	4.18
	H^{β}	1.60, 1.77	1.64, 1.80	1.64, 1.80	1.60, 1.78
	H^{γ}	1.53	1.53	1.53	1.52
	H^{δ}	3.09	3.09	3.09	3.07

[a] $Ac(CH_3)$: 1.90 ppm.
[b] br = broad; n.d. = not determined.
[c] Gly: $J_{NH\text{-}H\alpha proS}$ 2.0 Hz; $J_{NH\text{-}H\alpha proR}$ 5.4 Hz.

Table 2
^{13}C chemical shifts (δ, ppm) (150 MHz, 300 K, 9:1 H_2O–D_2O)

		3	4	5	6
Ala	C^{α}	49.57	48.77	48.78	48.81
	C^{β}	16.38	16.58	16.61	16.65
	C′	173.86 [a]	171.14	171.00	170.52
Val	C^{α}	59.22	59.47	59.36	59.23
	C^{β}	30.19	30.04	30.32	30.40
	C^{γ}	17.50, 18.35	17.70, 18.30	17.46, 18.36	17.53, 18.43
	C′	173.51	173.06	173.29	172.98
Ser	C^{α}	55.30	55.1	53.79	53.63
	C^{β}	60.94	61.09	67.75	67.72
	C′	171.55	171.20	171.22	170.68
Thr	C^{α}	58.82	57.74	58.81	57.50
	C^{β}	66.75	74.17	66.99	75.03
	C^{γ}	18.80	16.04	18.85	16.08
	C′	171.20	170.82	170.83	170.33
Glu	C^{α}	51.12	51.20	51.50	51.27
	C^{β}	26.03	25.99	26.75	26.63
	C^{γ}	30.78	30.52	32.54	31.57
	C^{δ}	171.97	178.02	n.d.	179.11
	C′	178.39	172.10	171.85	171.40
Pro	C^{α}	60.56	60.52	60.56	60.49
	C^{β}	29.08	29.12	29.10	29.16
	C^{γ}	24.52	24.52	24.51	24.50
	C^{δ}	47.85	47.83	47.87	47.86
	C′	173.98	173.81	173.90	173.78
Phe	C^{α}	55.15	55.10	55.16	55.18
	C^{β}	36.38	36.41	36.41	36.50
	C′	173.68	173.68	173.71	173.65
Gly	C^{α}	42.32	42.51	42.52	42.52
	C′	171.67	171.44	171.27	171.16
Arg	C^{α}	52.88	52.90	52.94	52.95
	C^{β}	28.00	28.01	27.97	28.02
	C^{γ}	24.32	24.32	24.32	24.35
	C^{δ}	40.41	40.41	40.41	40.42
	C^{ζ}	156.64	156.67	156.66	156.64
	C′	176.09	176.11	176.15	176.11

[a] Ac(Me): 21.44 ppm.

functionalized with the acid labile 4-[4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid [11]. Excess of all activated amino acids was employed; in the case of pentafluorophenyl esters, two equivalents of 1-hydroxybenzotriazole were added to accelerate the coupling reaction [12]. The completion of all coupling reactions was monitored by a UV/VIS-spectrophotometer and a flow cell. In the case of the Pfp esters, Acid Violet 17 was added as counterion. For the cleavage of the Fmoc groups, 50% morpholine in *N*,*N*-dimethylformamide was used [13]. Cleavage from the resin was achieved with aq 95% trifluoroacetic acid. The resulting glycopeptides were then fully deprotected with a catalytic amount of sodium methoxide in methanol (pH 8.5) to yield compounds **4–6**.

NMR and conformational analysis.—^{1}H and ^{13}C chemical shifts for the parent peptide **3** and for the glycopeptides **4–6** are listed in Tables 1–3, ^{1}H NMR expansions are shown in Fig. 1. Chemical shift variations are restricted to the glycosylated amino acids, indicating that changes in peptide structure and dynamics are also limited to the O-glycosylation sites [2]. This is further proven by a nearly identical NOE pattern for **3**, **4**, **5**, and **6**. Cross-peak volumes in a ROESY [14] spectrum with a mixing time of 200 ms

[2] Compounds **3**, **4**, **5**, and **6** exhibit a second conformer (5%) which is probably caused by *cis*/*trans* isomerism about the Glu–Pro bond. No exchange peaks in the ROESY spectra could be detected.

Table 3
^{1}H and ^{13}C chemical shifts (δ, ppm) and coupling constants (J, Hz) of the monosaccharide moieties (300 K, 9:1 H_2O–D_2O)

	4 (Thr-*O*-GlcNAc)	**5** (Ser-*O*-GlcNAc)	**6** (Ser-*O*-GlcNAc)	**6** (Thr-*O*-GlcNAc)
J_{1H-2H}	8.4	8.4	8.5	8.4
1-H	4.40	4.43	4.46	4.42
2-H	3.55	3.59	3.59	3.56
NH	8.16	8.09	8.05	8.13
J_{NH-2H}	n.d.	n.d.	9.4	9.6
Ac(Me)	1.93	1.89	1.90	1.93
3-H	3.42	3.44	3.43	3.42
4-H	3.28	3.33	3.33	3.30
5-H	3.30	3.36	4.34	4.30
6-H	3.59, 3.71	3.63, 3.82	3.63, 3.77	3.62, 3.75
1-C	99.38	100.68	100.59	99.84
2-C	55.48	55.29	55.29	55.53
Ac (Me, CO)	22.17, 174.79	22.20, 174.45	22.21, 174.42	22.26, 174.63
3-C	73.64	73.80	73.81	73.69
4-C	70.02	69.80	69.84	69.92
5-C	75.73	75.95	75.94	75.82
6-C	61.09	60.69	60.72	61.01

were integrated, offset corrected, and proton–proton distances were determined from the r^{-6} dependence of cross relaxation rates [15] on the assumption of an isotropic tumbling molecule and by using the isolated two-spin approximation [16]. Experimental proton–proton distances of the peptide backbone of **6** are listed in Table 4.

A linear nonapeptide is not expected to exhibit a rigid secondary structure, but the ROEs are in accordance with an extended backbone conformation. NH $\rightarrow H^{\alpha}$ ROEs within each amino acid are weak and correlate with average distances of about 300 pm; $H^{\alpha} \rightarrow$ NH ROEs between adjacent amino acids are very intense and correlate with ca. 220 pm. NH $\rightarrow$ NH ROEs, detected for helical conformations, are absent. The cross relaxation rates may be averaged by peptide conformers interconverting rapidly. Therefore, we investigated the structural homogeneity with a molecular dynamics simulation of glycopeptide **6** with the proton–proton distances being included as

Table 4
Experimentally derived distance restraints (column ROE) and proton–proton distances from an energy minimized molecular dynamics simulation of **6** (column MD [3]). The average deviation is about 10% and well within the range of experimental error. The mean backbone torsions and the standard deviations (in brackets) were determined. Val: $\phi = -99.5°$ (15.4°), $\psi = 172.1°$ (9.9°);Ser: $\phi = -137.8°$ (24.4°); $\psi = 151.7°$ (8.3°);Thr: $\phi = -138.1°$ (23.7°); $\psi = 172.6°$ (11.7°);Glu: $\phi = -44.3°$ (35.5°); $\psi = 147.7°$ (21.5°);Pro: $\phi = -74.9°$ (6.6°); $\psi = -166.2°$ (44.7°);Phe: $\phi = -53.6°$ (78.2°); $\psi = 116.0°$ (45.9°);Gly: $\phi = -75.7°$ (8.9°); $\psi = 60.6°$ (13.1°);Arg: $\phi = -69.5°$ (12.8°); $\psi = 23.7°$ (64.8°)

		ROE [pm]	MD [pm]			ROE [pm]	MD [pm]
Ala-H^{α}	Val-NH	240	238	Thr-H^{β}	Glu-$H^{\beta proS}$	340	367
Val-NH	Val-H^{α}	330	302	Glu-NH	Glu-H^{α}	280	296
Val-H^{α}	Ser-NH	220	229	Glu-H^{α}	Pro-$H^{\delta proR}$	220	259
Val-H^{β}	Ser-NH	250	255	Glu-H^{α}	Pro-$H^{\delta proS}$	220	255
Ser-NH	Ser-H^{α}	280	304	Pro-$H^{\beta proS}$	Phe-NH	320	344
Ser-H^{α}	Thr-NH	220	246	Phe-H^{α}	Gly-NH	220	210
Ser-NH	Thr-NH	400	452	Gly-NH	Gly-$H^{\alpha proR}$	250	295
Thr-NH	Thr-H^{α}	280	303	Gly-NH	Gly-$H^{\alpha proS}$	250	231
Thr-H^{α}	Thr-H^{β}	230	256	Gly-NH	Arg-NH	350	382
Thr-NH	GN-2H	340	402	Gly-$H^{\alpha proR}$	Arg-NH	270	255
Thr-NH	Glu-NH	400	438	Gly-$H^{\alpha proS}$	Arg-NH	270	365
Thr-H^{α}	Glu-NH	230	266	Arg-NH	Arg-H^{α}	280	298

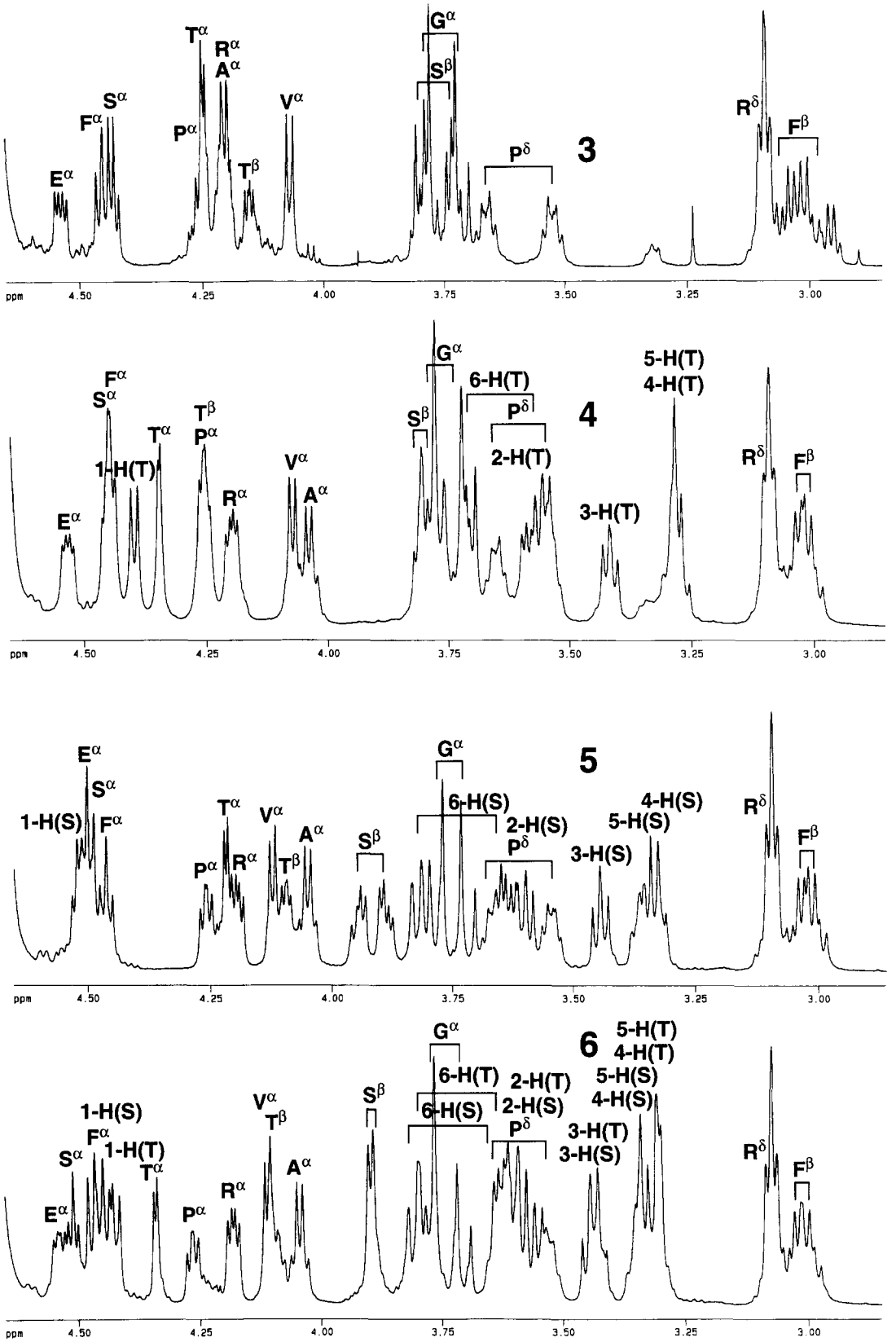


Fig. 1. Expansions from the ^{1}H NMR spectra (300 K, 600 MHz, D_2O) of the acetylated parent peptide **3** and the glycopeptides **4**–**6**. Upon glycosylation, the α-protons of Ser and Thr exhibit a minor low field shift of 0.05 to 0.1 ppm, no coherent variations in chemical shifts are observable for Ser H_2^{β} and Thr H^{β}, and the Thr γ-protons resonate at 0.05 ppm higher field. Larger variations are found for the ^{13}C resonances. α-Carbons are shifted to higher field by 1 to 1.5 ppm in the glycosylated amino acids, β-carbons show, as expected, the largest low field shifts of up to 7 ppm, and the Thr methyl group is high field shifted by 2.8 ppm.

experimental restraints [3]. The backbone proves to be well defined at the glycosylation sites but a considerable amount of flexibility is found towards the carboxy terminus. Table 4 contains the proton–proton distances of an energy minimized average structure.

Since the peptide backbone conformation is not altered upon glycosylation, we examined the rotamer distribution of the χ_1 side-chain torsions (χ_1 = N–C$^{\alpha}$–C$^{\beta}$–O) of the O-glycosylated amino acids. The percentage of each staggered rotamer about χ_1 was estimated from the degree of averaging of the experimental $^3J_{\alpha\text{-}\beta}$ coupling constants [4]. A stereospecific assignment of Ser-H$^{\beta}_2$ cannot be performed when the chemical shift variations are too small or when cross relaxation rates and coupling constants are severely averaged by rotation about χ_1. Therefore, even detailed NMR investigations of O-glycopeptides lack the diastereotopic assignments of Ser-H$^{\beta}_2$ and the rotamer distribution about χ_1 is not determined [18]. Fast averaging between two or more rotamers is also found for the Ser β-protons in **3**, **4**, and **5** (**6** Ser-H$^{\beta}_2$ are not resolved); experimental $^3J_{\alpha\text{-}\beta}$ coupling constants (Table 5) and ROEs [5] are obviously time averaged values. The HMBC spectra show correlations between Ser-CO and both Ser β-protons, indicating the averaging of $^3J_{\text{CO-H}\beta}$ couplings. Without clear differences in between the ^3J couplings and in between the ROEs, a diastereotopic assignment of the Ser-H$^{\beta}_2$ methylene group is not possible. Still we can estimate the rotamer distribution about χ_1, since only a close to equal population of the three staggered rotamers is in harmony with the abovementioned experimental data. The Pachler equations [17] yield rotamer populations of ca. 30% about χ_1, no matter which of the Ser β-protons is assigned H$^{\text{proR}}$ or H$^{\text{proS}}$ (Fig. 2).

Table 5
Ser and Thr side-chain coupling constants (J, Hz). A maximal difference of 1 Hz in between the $^3J_{\alpha\text{-}\beta}$ couplings of the four peptides correlates to ca. 10% difference in rotamer population about χ_1

	3	**4**	**5**	**6**
Ser $J_{\alpha\text{-}\beta\,\text{low field}}$	5.7	5.9	6.6	$\Delta\delta < J$
Ser $J_{\alpha\text{-}\beta\,\text{high field}}$	6.4	5.4	5.4	$\Delta\delta < J$
Thr $J_{\alpha\text{-}\beta}$	4.0	3.2	4.3	4.3

Experimental data are easier to interpret for Thr where a clear preference for one of the three rotamers was detected in all peptides **3**–**6**. A small $^3J_{\alpha\text{-}\beta}$ coupling (Table 5), an intense ROE Thr-H$^{\alpha}$ → Thr-H$^{\gamma}_3$, and a ROE Glu-NH → Thr-H$^{\beta}$ of about 270 pm clearly define the $\chi_1 = 60°$ rotamer as the dominantly populated one (> 70%).

In conclusion, O-glycosylation of **3** is accompanied only by minor variations of the rotamer distribution about the Ser χ_1 and Thr χ_1 torsions (Table 5), and the carbohydrate moieties in **4**, **5**, and **6** do not interfere with the peptide backbone (absence of ROEs between GlcNAc and amino acids other than Ser or Thr). No general rules apply for the conformational influences of O-glycosylation on peptide structure. Since strong influences of a single monosaccharide moiety are the exception [19], there are probably additional aspects of glycosylation like the shielding of the peptide backbone from undesired binding interactions [20]. This is effectively performed by a polar and therefore highly solvated Ser(-OGlcNAc) modification which also occupies several rotamers and opposes aggregation for entropic reasons.

3. Experimental

General methods.—All N^{α}-(9-fluorenylmethoxycarbonyl)amino acid pentafluorophenyl esters (Fmoc-AA-Ppf's) and the N$^{\alpha}$-(9-fluorenylmethoxycarbonyl) amino acid 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazines (Fmoc-AA-ODhbt's) were purchased from

[3] The molecular dynamics simulation was performed with the MM+ force field in the *HyperChem* program package (Hypercube, Ontario, Canada, 1994). ROE distances were incorporated with force constants of 7 kcal/(mol Å^2). After 100 ps of equilibration, a second 100-ps molecular dynamics run was taken for analysis. Ten structure snapshots were stored in intervals of 10 ps; the average structure was energy minimized without experimental restraints.

[4] The rotamer distribution was determined on the assumption that only the three staggered conformers are populated ($\chi_1 = -60°$, 60°, and 180°) and that the averaging proceeds between the *gauche* $^3J_{\text{H-H}}$ coupling of 2.6 Hz and the *trans* $^3J_{\text{H-H}}$ coupling of 13.6 Hz as described in ref. [17]. The side-chains of Glu and Arg in **6** mainly (ca. 60%) populate the $\chi_1 = -60°$ conformer; they are shown with their dominating rotamers in Fig. 2. Glu: $^3J_{\alpha\text{-}\beta}$(proS) 4.9 Hz, $^3J_{\alpha\text{-}\beta(\text{proR})}$ 9.2 Hz; Arg: $^3J_{\alpha\text{-}\beta(\text{proS})}$ 5.1 Hz, $^3J_{\alpha\text{-}\beta(\text{proR})}$ 9.2 Hz. No diastereospecific assignment was possible for Phe: $^3J_{\alpha\text{-}\beta(\text{low field})}$ 7.3 Hz, $^3J_{\alpha\text{-}\beta(\text{high field})}$ 7.9 Hz.

[5] The ROEs Ser-H$^{\alpha}$ → Ser-H$^{\beta(\text{low field})}$ and Ser-H$^{\alpha}$ → Ser-H$^{\beta(\text{high field})}$ are both of similar averaged intensity and correspond to 220–230 pm. The ROEs Ser-NH → Ser-H$^{\beta(\text{low field})}$ and Ser-NH → Ser-H$^{\beta(\text{high field})}$ correspond to 270–280 pm, and the ROEs Thr-NH → Ser-H$^{\beta(\text{low field})}$ and Thr-NH → Ser-H$^{\beta(\text{high field})}$ correspond to 330–350 pm.

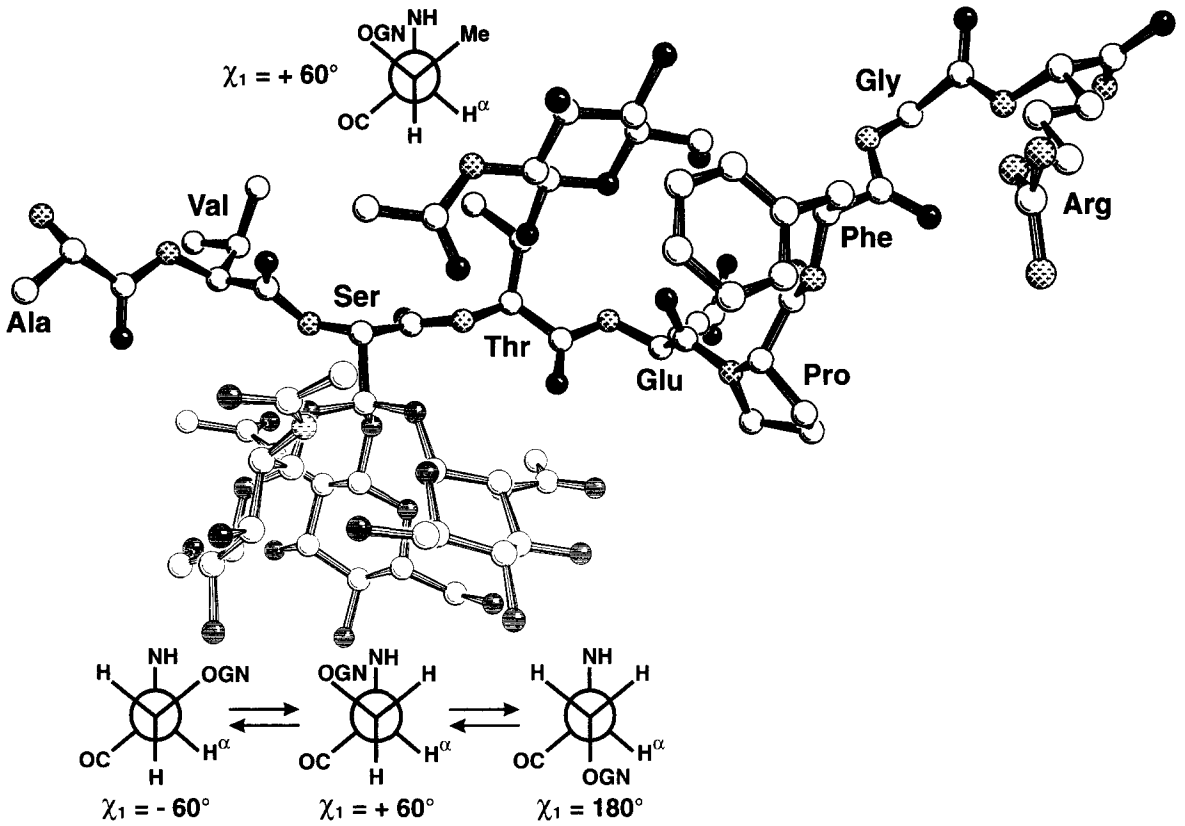


Fig. 2. Averaged and energy minimized conformation of glycopeptide **6**. The preferred rotamers about Ser χ_1 are shown as Newman projections and indicate the range of flexibility of the carbohydrate moiety (GN = *N*-acetylglucosamine). Thr χ_1 shows a clear preference of the +60° rotamer. Intense transglycosidic NOEs (GlcNAc-1H → Ser-H_2^β, GlcNAc-1H → Thr-H^β) define the *syn*-orientation of the glycosidic linkage which is preferred for steric and electronic reasons.

Novabiochem. Compounds **1** and **2** were prepared as previously reported [8]. Poly(ethylene glycol)-poly(styrene) resin, which was functionalized with acid labile Fmoc-protected peptide amide linker (Fmoc-PAL-PEG-PS, 0.23 mmol/g) was obtained from Rapp Polymere. Dry DMF (< 0.01% H_2O, purity > 99.5%) was purchased from Fluka. Preparative HPLC was performed on Lichrosorb RP-18 (Fluka). ^1H and ^{13}C NMR spectra were obtained with a Bruker DRX-600 spectrometer at a proton resonance frequency of 600.13 MHz. Spectra were recorded at 300 K in 9:1 H_2O–D_2O and in pure D_2O; chemical shifts are calibrated to internal HDO (δ 4.67). ^1H spin systems were identified from TOCSY, DQF-COSY, and E.COSY spectra; ^{13}C resonances were assigned from inverse ^{13}C–^1H shift correlations (HMQC); HMBC spectra allowed the assignment of the carbonyl resonances [6].

[6] General NMR procedures for biopolymer characterization are described in the chapters *Peptides and polypeptides* (H. Kessler and W. Schmitt) and *Carbohydrates and glycoconjugates* (H. van Halbeek) in the *Encyclopedia of Nuclear Magnetic Resonance* [21].

Peptide and glycopeptide synthesis.—For each glycopeptide sequence, poly(styrene)-poly(ethylene glycol) resin (0.5 g), functionalized with primary amine (0.23 mmol/g) containing acid labile linker (PAL), was transferred into the continuous flow reactor. In all acylation reactions DMF was used as a solvent. All amino acids were coupled as their *N*-Fmoc *O*-Pfp esters (3 eq), except Ser and Thr which were used as *N*-Fmoc *O*-Dhbt esters. Of the *N*-Fmoc *O*-Pfp glycosylated amino acids, two equivalents were used. Fmoc group cleavage was effected by 1:1 morpholine–DMF. Completion of the acylation reaction was monitored by using dye Acid Violet 17 at 600 nm in the case of Pfp esters and at 360 nm for the Dhbt esters. All normal peptide bond formations were completed within 2–3 h. Attachment of glycosylated active esters required 24 h. Reaction time for the amino acids which are next to the glycosylated amino acids required 6–8 h. After attachment of the last amino acid, the resin was thoroughly washed with CH_2Cl_2 and dried under reduced pressure. It was then treated with 95:5 CF_3CO_2H–H_2O in the case of compound **3** and 95:5 CF_3CO_2H–thioanisol in the case of **4**, **5**, and **6** for 3 h at room temperature.

The mixtures were filtered and washed with CF_3CO_2H. The filtrates were concentrated under reduced pressure and the residues were washed with dry Et_2O 3–4 times in order to get white solids. The solid compounds were dissolved in dry MeOH (5 mL), and NaOMe (1 M in MeOH) was added dropwise till the pH reached 8.5. The solutions were allowed to stir for 1 h; then they were deionized with IR-120 (H^+) resin. Filtration and concentration gave crude products, which were purified by preparative RP-HPLC (9:1 water–acetonitrile containing 0.1% CF_3CO_2H) to afford the fully deprotected peptide and glycopeptides: **3** (74 mg, 78%), **4** (87 mg, 65%), **5** (91 mg, 68%), and **6** (90 mg, 57%). The structures of these compounds were characterized by ^1H NMR, ^{13}C NMR and FABMS: **4** and **5**: Anal. Calcd for $C_{50}H_{80}N_{14}O_{18}$: 1166. Found: *m/z* 1166 (M^+). **6**: Anal. Calcd for $C_{58}H_{92}N_{15}O_{23}$: 1370. Found: *m/z* 1370 (M^+). ^1H and ^{13}C data are presented in Tables 1–5.

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