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^{1}H and ^{13}C Nuclear Magnetic Resonance Studies of Leucine-Enkephalin Glucoconjugates

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Synthetic leucine-enkephalin glucoconjugates (**1–3**) were studied by several one– and two–dimensional ^{1}H and ^{13}C nuclear magnetic resonance (NMR) methods. Although all the values of the temperature coefficients of amide protons were larger than expected for protons involved in hydrogen bonds, using ROESY spectra, it was possible to detect several inter- and intra-residual nuclear Overhauser effects. The results do not support the existence of a single folded conformation, indicating that the structures of glucoconjugates **1–3** in DMSO–d_6 solution are not completely random. The inter-residual ROESY cross-peaks **1** point to the existence of at least one conformer with two turns in **1**, involving the Tyr^1 and Gly^2, and the Leu^5 and glucose residues, while, in the glucoconjugates **2** and **3**, inter-residual ROESY cross-peaks indicate the presence of a conformer with a different compact structure.

INTRODUCTION

Enkephalins (Tyr^1–Gly^2–Gly^3–Phe^4–Leu^5/Met^5) are endogenous opioid peptides found to mediate a remarkable number of physiological functions.[1] Many experimental and theoretical analyses have shown that enkephalin molecules are flexible and capable of adopting various folded and extended conformations.[2] It is, therefore, not surprising that these pentapeptides are recognized by both δ– and μ–opiate receptor sites. However, an insight into the receptor-bound conformation of a flexible peptide like enkephalin can be gained by studying the properties of analogues obtained through incorporation of conformational restrictions.

Evidence that glycosylation affects the structure and conformational motions of small linear peptides in solution[3–6] prompted us to prepare the following series of [Leu^5]-enkephalin related glycoconjugates: 1–*O*–([Leu^5]enkephalyl)–β–D–glucopyranose (**1**) 6–*O*–([Leu^5]enkephalyl)–D–glucopyranose (**2**) and 1,2,3,4–tetra–*O*–acetyl–6–*O*–([Leu^5]enkephalyl)–β–D–glucopyranose (**3**), in which D–glucose has been linked to the opioid pentapeptide

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through an ester bond involving the carboxyl function of the C-terminal leucine residue and the hydroxyl function at C–1 or C–6 of the D–glucopyranose moiety.[7,8] The enkephalin derivatives **1–3** were assayed for opioid activity[7,8] and found to be δ–receptor selective; in particular, the acetylated analogue **3** was three times more δ–receptor selective than [Leu^5]enkephalin.

Most NMR studies on enkephalin derivatives have been performed in very polar media[9–21] and there is no general agreement on the conformations adopted in these media. For example, in older literature, there are many claims[11,12,16] in favor of the presence of folded structures in solutions of enkephalins, while Higashijima *et al.*[15] and Dhingra and Saran[18] demonstrated that Met^5– and Leu^5–enkephalin amides in DMSO–d_6 solutions prefer an extended–backbone conformation. Motta *et al.*[20] found that the structure of [Leu^5]enkephalin is not completely random, but the presented NMR data did not support the existence of a single folded conformation. Vesterman *et al.*[22] claimed that the [Leu^5]enkephalin molecule in DMSO–d_6 solution is represented by a mixture of at least two peptide-backbone conformers.

In order to find out whether the carbohydrate moiety in glucopeptides **1–3** exerts some influence on the flexibility of the parent peptide chain, we report in this paper the results of NMR analysis in DMSO–d_6 and D_2O solutions. Corresponding data for the related peptides, [Leu^5]enkephalin methyl ester (**4**) and [Leu^5]enkephalin (**5**), are provided for comparison.

MATERIALS AND METHODS

Preparation of [Leu^5]enkephalin Glucoconjugates

The glycosylated [Leu^5]enkephalin derivatives **1–3** were synthesized and purified as previously reported.[7,8] After lyophilization, derivative **1** contained 5.99% of water (2.5 molecules of H_2O per one molecule of glucoconjugate), derivative **2** 13.58% (6.3 molecules) and **3** 9.56% (5.2 molecules), respectively.

[Leu^5]enkephalin methyl ester (**4**) was prepared as described previously[23] while [Leu^5]enkephalin (**5**) was purchased from Sigma (St. Louis, MO, U.S.A.).

NMR Techniques

^{13}C–NMR spectra were recorded at room temperature, in 99.75% D_2O using a Varian Gemini 300 spectrometer operating at 75.0 MHz. The sweep width used was 18000 Hz, the pulse width was 4.5 μs (30°), the acquisition time was 1.25 s. Chemical shifts were measured relative to internal 1,4–dioxane, set at 66.6 p.p.m. downfield of Me_4Si.

^{1}H–NMR spectra were obtained at temperatures ranging from 25 to 55 °C in DMSO–d_6 on a Bruker AM-400 spectrometer operating at 400.1 MHz (^{1}H), equipped with an Aspect 3000 computer. The spectra were referenced to residual DMSO (2.49 p.p.m.). Typically, each one-dimensional spectrum was collected using a single 90° pulse, 2 s relaxation delay time, and a total sweep width of 4000 Hz sampled with 16384 points (16 k). Data processing employed a Lorentz line-broadening factor of 0.6 to 1.0 Hz.

Two-dimensional experiments: COSY,[24] relayed COSY,[25] ROESY,[26,27] and double quantum two-dimensional experiments for protons[28] were all performed at 25 ± 1 °C. The COSY and relayed COSY spectra were obtained in the magnitude mode, while ROESY spectra were obtained in the phase-sensitive mode.[29] The COSY, relayed COSY, and ROESY experiments had 1024 points in F2 dimension and 256 slices in F1, which were

TABLE I

Assignment of signals in ^{13}C—NMR spectra of compounds **1–5**[a]

Residue	Carbon(s)	Compound				
		1	**2**[b]	**3**	**4**	**5**
pH of solution		3.85	5.13	5.25	6.10	4.27
Tyr-1	α	54.6	54.7	54.4	54.8	54.8
	β	35.95	36.0	36.1	36.6	36.0
	1	125.45	125.45	125.45	126.0	125.4
	2,6	130.9	130.9	130.8	130.9	130.9
	3,5	115.9	115.9	116.0	115.8	116.0
	4	155.25	152.25	155.5	155.1	155.2
	CO	169.9	169.9	171.45	170.6	170.0
Gly-2	α^1	42.2	42.2	42.3	42.2	42.1
	CO^2	171.2	170.05	171.9	171.2	171.1
Gly-3	α^1	42.4	42.4	42.5	42.4	42.5
	CO^2	170.7	170.7	171.1	170.1	170.7
Phe-4	α	54.6	54.7	54.4	54.8	54.6
	β	37.0	37.2	37.4	37.1	37.0
	1	136.2	136.2	136.4	136.1	136.5
	2,6	128.8	128.7	128.6	128.7	128.7
	3,5	129.2	129.2	129.3	129.3	129.3
	4	127.2	127.2	127.0	127.2	127.1
	CO	172.4	172.8	172.2	172.9	172.2
Leu-5	α	51.3	51.4	51.3	51.4	53.8
	β	39.05	39.2	39.8	39.4	40.7
	γ	24.3	24.3	24.4	24.2	24.5
	δ	20.5	20.8	21.1	20.7	20.9
	δ'	22.2	22.0	21.95	22.1	22.4
	CO	173.0	173.5	173.6	174.4	179.0
D-Glc	C–1	94.8	96.05.	91.7		
	C–2	71.45	71.5	70.5		
	C–3	75.4	72.7	72.1		
	C–4	69.1	69.7	68.0		
	C–5	76.9	73.4	72.9		
	C–6	60.4	64.1	62.25		
OMe					52.9	
O-acetyl	CH_3			20.0		
	CO			171.85[3](2x)		
				172.2[3]		
				172.5[3]		

[a] In D_2O at 25 °C. Values are chemical shifts relative to 1,4–dioxane (66.6 p.p.m.)
[b] Chemical shifts given for the α–anomer of **2**.
[1,2,3] Assignation of signals can be interchangeable.

zero-filled to 1024 points. Each slice was obtained using 16 averages, a relaxation delay of 2 s, and a spectral width of 4000 Hz. The resolution in the F2 domain was 4.0 Hz/point and in F1 8.0 Hz/point. The ROESY experiment was conducted as a continuous spin lock pulse with the lowest useful power, and employing a mixing time of 300 ms. In the relayed COSY experiment, the delay for relay transfer of magnetization was adjusted to 30 ms to optimize the sensitivity for the coupling constants of interest (*ca.* 8 Hz).

The double quantum two-dimensional experiment for protons was obtained with a sweep width of 4000 Hz in F2 and 8000 Hz in F1 dimension. The double quantum coherence pulse was set to 135°. The number of points in both dimensions, the zero-filling, the pulse recovery time, and the number of averages per slice were the same as in the COSY experiment.

RESULTS AND DISCUSSION

^{13}C-NMR Assignments in Aqueous Medium

The ^{13}C-NMR data for **1–5** in D_2O are given in Table I. Most assignments of the ^{13}C chemical shifts to specific carbons of **1–4** were made by comparison with ^{13}C spectra of [Leu^5]enkephalin (**5**) and on the basis of literature data for enkephalins[8,12,16,17] and free or selectively O-acetylated carbohydrates.[30–32]

As seen from Table I, the ^{13}C resonances for identical amino acid residues in compounds **1–4** are generally very similar to **5** (differences within a range of 0.5 p.p.m.) and in accord with those of enkephalins reported in the literature.[9,12,16,17] However, esterification of the C-terminal leucine residue in **1–3** with the sugar molecule, or in **4** with methanol, causes an upfield shift of the Leu^5 carbonyl carbon residues by 4.6 and 6.0 p.p.m., respectively. The same holds for the Leu^5 α-carbon atoms in **1–4**, which were shifted upfield by ~2.5 p.p.m.

Considering the carbohydrate parts of glucopeptides **1–3**, the attachment of the pentapeptidyl group onto O-1 or O-6 influences the shifts of the adjacent carbon atoms. Thus, esterification of C-1 in compound **1** is revealed by an upfield shift for C-1 and C-2 (2.0 and 3.7 p.p.m., respectively) with respect of β-D-glucopyranose.[30] Introduction of the peptide group at the C-6 hydroxyl group of the D-glucopyranose moiety generated a downfield shift of this carbon atom (2.5 p.p.m.) in compound **2** typical of *O*-acylation effects in carbohydrates.[31] Owing to the presence of acetyl groups in compound **3**, only the small downfield shift (1.2 p.p.m.) of C-6 in comparison to 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose[32] is observed.

^{1}H-NMR Study in DMSO-d_6

Resonances belonging to various spin systems from the different amino acid residues and the D-glucosyl moiety were identified by two-dimensional COSY, relayed COSY, and 2D double quantum experiments for protons (Figures 1–3). The corresponding chemical shifts are listed in Table II. It can be seen that most of the chemical shifts are close to the values given in the literature for disordered conformations,[33] suggesting that folded conformations, if present, are not predominant.

In the ^{1}H-NMR spectra of glucoconjugates **1–3**, the resonances of the two α-protons of both Gly^2 and Gly^3 show a small anisochrony up to about 0.15 p.p.m. In spite of the larger extent of non-equivalence (0.5–0.8 p.p.m.) which has been reported for the

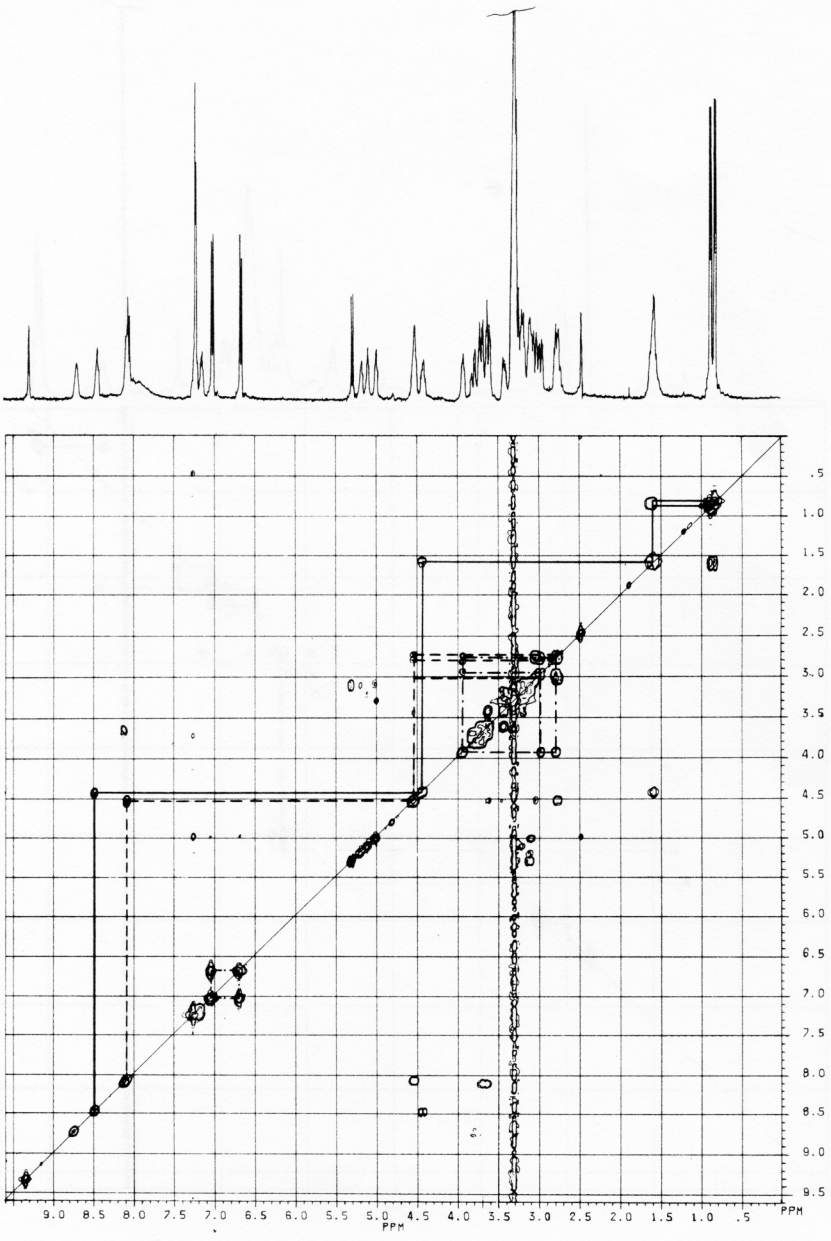

Figure 1. COSY spectrum (magnitude mode) of 1–*O*–([Leu^5]–enkephalyl)–*β*–Glc (**1**) in DMSO–d_6, 25 °C. The lines indicate correlations within Leu^5 (——), Phe^4 (----), and Tyr^1 residues (– · – · –), respectively.

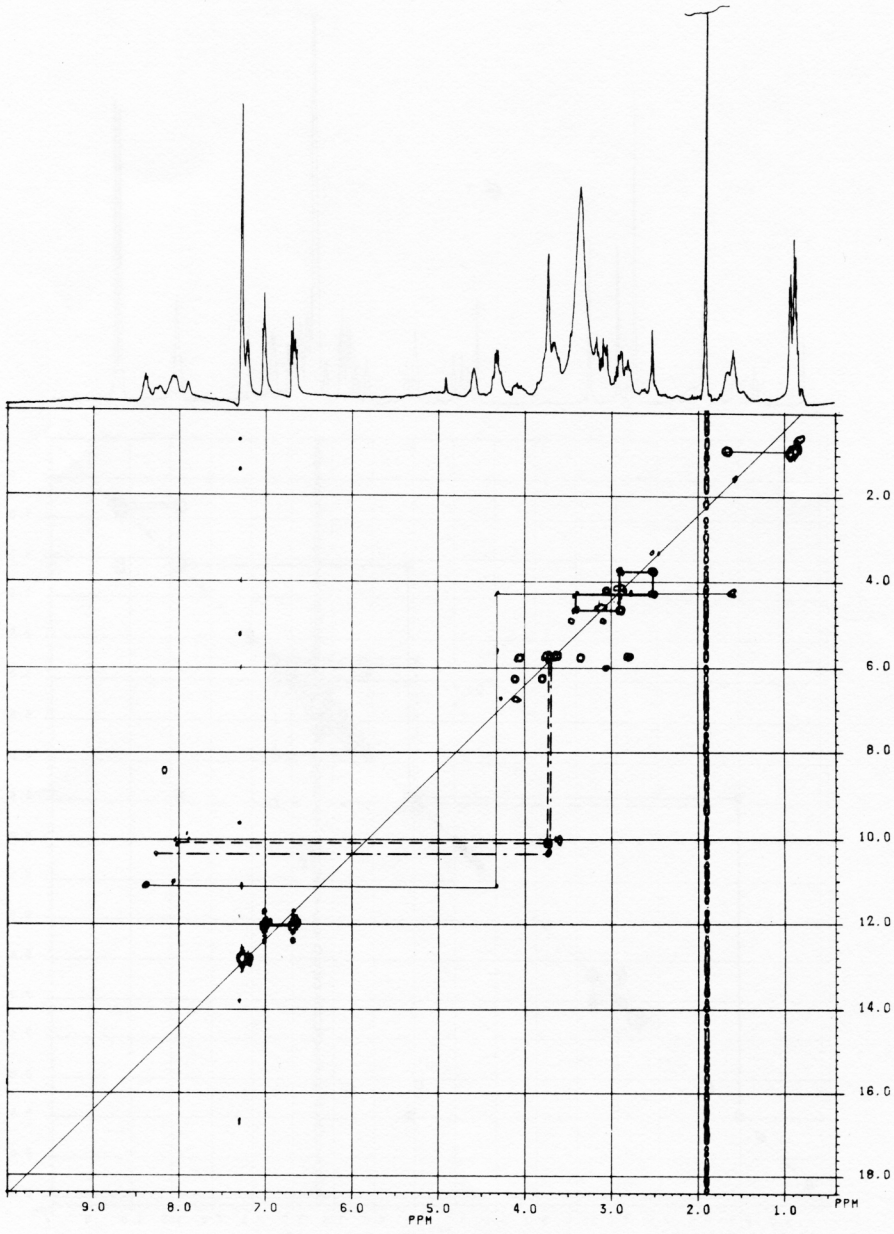

Figure 2. ^{1}H–^{1}H INADEQUATE spectrum of 6–*O*–([Leu^{5}]–enkephalyl)–Glc (**2**) in DMSO–d_6, 25 °C. The lines indicate correlations within Leu^{5} (——), Tyr^{1} (——), Gly^{2} (– · – · –), and Gly^{2} (- - - -), respectively.

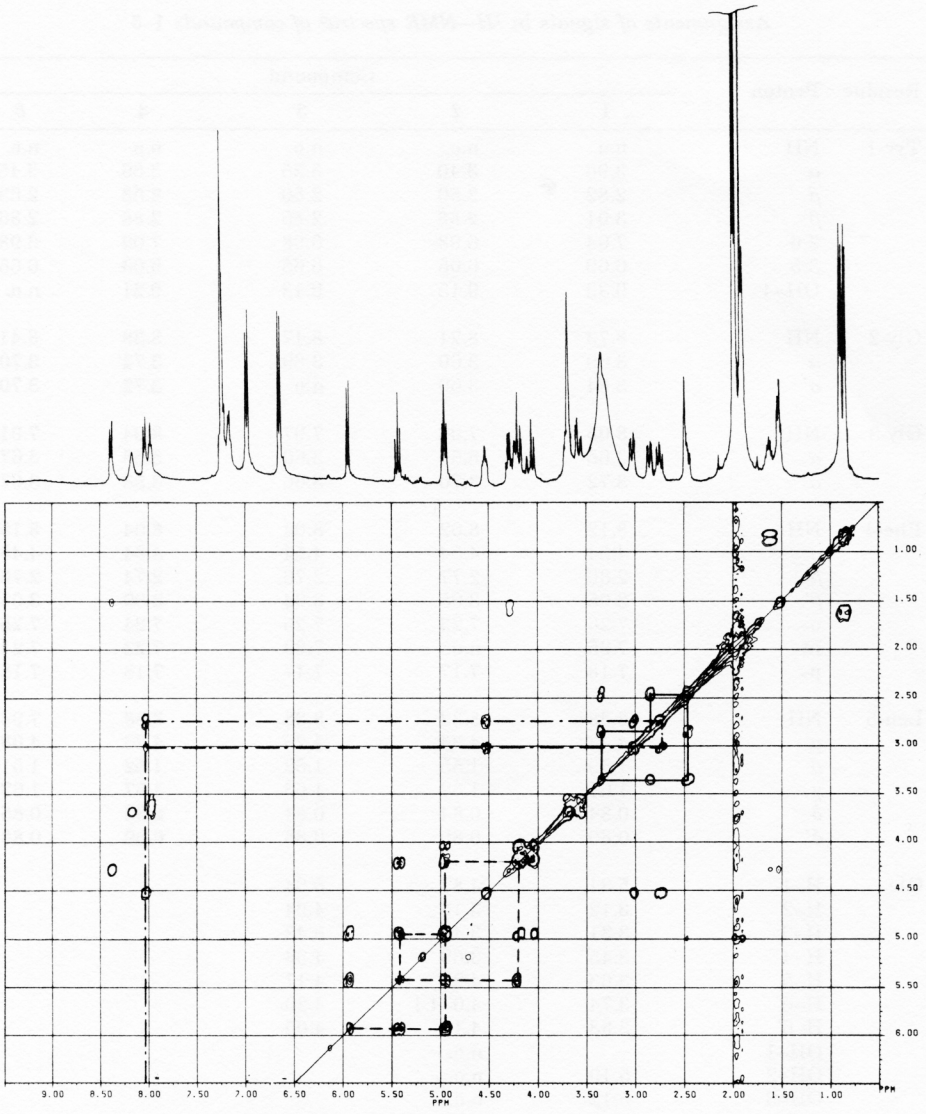

Figure 3. Relayed COSY spectrum (magnitude mode) of 6–*O*–([Leu^{5}]–enkephalyl–β–Ac$_4$ Glc (**3**) in DMSO–d_6 25 °C. The lines indicate correlations within Tyr^{1} (——), Phe^{4} (– · – · –), and D–glucopyranosyl residues (- - - -), respectively.

Gly residues in other peptides, even these small chemical shift differences do not exclude some restrictions of the motion of the Gly residues for all the three molecules studied.[34]

TABLE II

Assignments of signals in ^1H—NMR spectra[a] of compounds **1–5**

Residue	Proton	Compound				
		1	**2**	**3**	**4**	**5**
Tyr-1	NH	n.o.	n.o.	n.o.	n.o.	n.o.
	α	3.96	3.40	3.35	3.56	3.45
	β	2.82	2.50	2.50	2.58	2.63
	β'	3.01	2.88	2.85	2.88	2.86
	2,6	7.04	6.98	6.98	7.00	6.98
	3,5	6.69	6.65	6.65	6.66	6.65
	OH–4	9.33	9.15	9.13	9.21	n.o.
Gly-2	NH	8.73	8.21	8.17	8.38	8.41
	α	3.69	3.60	3.69	3.72	3.70
	α'	3.84	3.69	n.o.	3.72	3.70
Gly-3	NH	8.05	7.99	7.97	8.04	7.91
	α	3.65	3.60	3.60	3.58	3.67
	α'	3.72	3.70	3.66	3.58	3.67
Phe-4	NH	8.12	8.02	8.04	8.04	8.15
	α	4.54	4.54	4.54	4.54	4.45
	β	2.80	2.77	2.76	2.74	2.76
	β'	3.05	3.05	3.04	2.99	3.04
	o–	7.26	7.23	7.25	7.24	7.25
	m–	7.25	n.o.	7.23	7.22	7.24
	p–	7.18	7.17	7.17	7.18	7.18
Leu-5	NH	8.38	8.33	8.38	8.38	7.94
	α	4.43	4.28	4.29	4.26	4.08
	β	1.59	1.55	1.52	1.52	1.51
	γ	1.61	1.62	1.63	1.57	1.62
	δ	0.84	0.84	0.84	0.83	0.85
	δ'	0.89	0.89	0.88	0.89	0.88
Glc	H–1	5.31	4.87	5.94		
	H–2	3.12	3.13	4.94		
	H–3	3.21	3.41	5.43		
	H–4	3.45	3.69	4.98		
	H–5	3.63	4.25	4.17		
	H–6	3.74	4.0–4.1	4.20		
	H–6'	3.83	4.1	4.05		
	OH–1		n.o.			
	OH–2	5.19	n.o.			
	OH–3	5.12	n.o.			
	OH–4	5.01	n.o.			
	OH–6	n.o.				
	OAc–1,2,3,4			1.92 1.96		
				1.98 1.99		
	OMe				3.60	

[a] InDMSO–d_6 at 25 °C. The values are chemical shifts relative to DMSO–d_6 (2.49 p.p.m.).
n.o. = not observed

Although the NH protons of the Gly^{2} residues in **1–5** are well observable, their resonances are relatively broad. These broadenings are due to exchange of these protons with the trace of water present in the samples, but exchange rates are slow on the NMR time scale. The other amide protons also exchange with water but their rates of exchange are much smaller. The resonances due to the OH protons of Tyr^{1} residues are observable in **1–4**, as well.

Temperature Dependence of the NH and OH Chemical Shifts

Interpretation of temperature coefficients, $d\delta/dT$, of the chemical shifts of amide protons in terms of hydrogen bonds is straightforward when they are close to zero, but it is very difficult in all other cases, at least in polar solvents.[35] All [Leu^{5}]enkephalin NH protons share linear behaviour, with fairly large temperature coefficients (Table III). However, none of them is low enough to indicate the presence of stable hydrogen bonds in the temperature range examined and the values are not uniform, suggesting different accessibility to solvent molecules along the sequence. In particular, the $d\delta/dT$ coefficient of Gly^{2} in **2** (–3.0 p.p.b/°C) is rather low, possibly an average of larger values (typical of exposed amide protons) and a very low value, typical of an immobilized NH. No concentration dependence of NH proton chemical shifts have been observed that could support association between molecules as observed for non-conjugated enkephalins.[15,36]

TABLE III

Temperature coefficients for the chemical shifts of amide and hydroxyl proton (p.p.b./°)[a] of [Leu^{5}]enkephalin derivatives **1–5**

Residue	Proton	C(p.p.b./°)				
		1	**2**	**3**	**4**	**5**
Gly-2	NH	–5.4	–3.0	–6.0	–6.8	–2.2
Gly-3	NH	–4.8	–4.3	–4.8	–4.2	–1.6
Phe-4	NH	–7.0	–5.6	–6.6	–5.4	–7.3
Leu-5	NH	–7.1	–7.0	–7.0	–5.8	–7.1
Glc	OH–2	–7.2	n.o.			
	OH–3	–7.6	n.o.			
	OH–4	–6.8	n.o.			

[a] In DMSO-d_6 Temperature range 25–55 °C.

The temperature dependence of OH chemical shifts in **1** (Table III) agrees well with that reported in the literature[37] for β-D-glucopyranosides (~ –7 p.p.b./°C), thus providing additional evidence for the β-configuration of **1**.

Analysis of Coupling Constants

In order to obtain a better insight into the conformations of **1–5**, the coupling constants $^{3}J_{\alpha,NH}$ were determined by using 1D spectra. With the exception of the Gly^{2} and

Gly^3 residues in **1–5**, the values (Table IV) are as high as expected for an extended, disordered or β–sheet conformation.[38]

As seen from Table IV, Phe^4 and Leu^5 show $^3J_{\alpha,NH}$ values of 8 Hz, which correspond to torsion angles, ϕ, around $-90°$ or $-150°$, calculated from Karplus-Bystrow curves.[39] Since ϕ angles of L–amino acids in proteins have been reported[40] to be in the range $-30°$ to $-180°$, both values obtained for Phe^4 and Leu^5 are possible. Following the Bystrow curves for Gly residues,[39], the measured $^3J_{\alpha NH}$ vicinal coupling constants for Gly^2 and Gly^3 around 5.5 Hz, lead to ϕ around $+25°$ or $-25°$ for all the five compounds studied. The angles ϕ around $140°$ can be excluded because coupling constants in all three cases were about 1 Hz lower than predicted by the Bystrow curve. Only minor changes in coupling constants were observed with temperature variation, indicating that, under the present conditions, the conformation is temperature independent. Furthermore, coupling constants show strong similarities through the series of glucopeptides investigated and indicate that there are few, it any, changes of these angles caused by interaction with the nearest neighbors.

The method of Pachler[41] applied to the $C_\alpha H$–$C_\beta H$ coupling constants can provide information about relative amounts of rotamer populations.The conformations quoted g$^+$t, g$^+$g$^-$ and tg$^-$ are those with the C_β–C_{arom} bond anti periplanar to the carbonyl C=O, the peptide NH and $C_\alpha H$ bond, respectively. The side chains of Tyr^1 and Phe^4 residues in **1–3** favor one of the two possible *trans*–gauche orientations similar to those found for other enkephalin derivatives.[16,18,19]

Analysis of ROESY Spectra

Measurement of cross-relaxation rates with 2D NOESY spectroscopy is one of the most important tools for the determination of molecular conformation in solution. However, in medium-sized molecules, like **1–5**, NOE cross-peak intensities in the commonly used proton frequency range of 300–500 MHz, are often close to zero when the correlation time τ_c approaches the inverse of the Larmor frequency of the protons.[26] For these reasons, the conformation of compounds **1–3** in DMSO–d_6 was studied using two–dimensional n.O.e. experiments in the rotating frame (ROESY).[27]

ROE signals have great diagnostic potential, and if we limit our analysis to local folding pattern, such as β–turns, they can be used to discriminate among various types. For turns of type I, the shortest distances are between NH^2 and NH^3 and between NH^3 and NH^4, which are of the order of $\leq$ 2.5 Å.[33] Another diagnostic for type I turns are the effects between β–protons(s) and NH of adjacent residues, whose distances can be as low as 2,9 Å.[33] For turns of type II, the shortest distances are between CαH^i and NH^{i+1} (of the order of 2.2 Å) and between NH^3 and NH^4 (2.4 Å). The distinction between the two types relies on the presence (type I) or absence (type II) of an effect between NH^2 and NH^3, since in type II the distance between these protons increases to 4.5 Å.

In the ROESY spectrum of **1** (Figure 4), the following inter-residual cross-peaks were observed: Tyr^1αH–Gly^{2}NH (A), Phe^4αH–Tyr^{1}OH (B), Phe^4αH–Leu^{5}NH (C), Phe^4 aromatic protons(s)–glucose OH–4 (D), Tyr^{1}OH–glucose OH–2 (E), and Gly^3αH–Phe^{4}NH (F). The presence of sequential ROESY cross-peaks marked as A, C and F indicates an extended backbone, which does not corroborate the presence of a folded structure, *i.e.* a β–bend type structure. On the other hand, the weak Phe^4αH–Tyr^{1}OH (B), Phe^4 aromatic proton(s)–glucose OH–4 (D), and Tyr^{1}OH–glucose OH–2 (E) inter-residual

TABLE IV

Coupling constants $^3J_{(H,H)}$ and dihedral angles (e) for [Leu^5]enkephalin derivatives **1–5** *derived from $^3H_{(\alpha,NH)}$ values*[a]

Residue	Proton (s)	**1** J/Hz	Θ/°	**2** J/Hz	Θ/°	**3** J/Hz	Θ/°	**4** J/Hz	Θ/°	**5** J/Hz	Θ/°
Tyr-1	α–β	n.o.		1.9		n.o.		n.o.		4.0	
	α–β'	4.2		5.0		n.o.		4.0		5.2	
	β–β'	14.4		14.4		13.8		13.9		13.3	
	2,6–3,5	8.2		8.75		8.3		8.3		8.3	
Gly-2	NH–α	5.0	20,–20	6.25	25,–25	5.4	20,–20	5.9	25,–25	5.1	20,–20
	NH–α'	n.o.		n.o.		5.2		n.o.		n.o.	
	α–α'	15.9		17.5		n.o.		17.3		15.7	
Gly-3	NH–α	5.1	20,–20	6.25	25,–25	5.2	20,–20	5.2	20,–20	5.3	20,–20
	NH–α'	5.2		n.o.		5.4		5.5		n.o.	
	α–α'	16.5		17.5		15.1		17.5		16.7	
Phe-4	NH–α	8.5	–90,–155	8.75	–95,–145	8.0	–90,–150	8.3	–90,–155	8.4	–90,–155
	α–β	4.2		3.75		3.3		4.1		3.2	
	α–β'	10.0		10.6		10.4		9.8		10.2	
	β–β'	14.5		14.4		13.6		13.9		13.7	
Leu-5	NH–α	7.8	–85,–155	8.1	–90,–150	7.6	–85,–155	7.7	–85,–155	7.7	–85.–155
	α–β	7.8		7.8		7.3		7.8		n.o	
	β–γ	5.1		n.o		6.8		5.3		6.5	
	γ–δ	6.5		6.25		6.4		6.5		6.7	
	γ–δ'	6.5		6.25		6.4		6.5		6.7	
Glc	H1–H2	8.25		3.4		8.3					

[a] See reference 39
n.o. = not observed

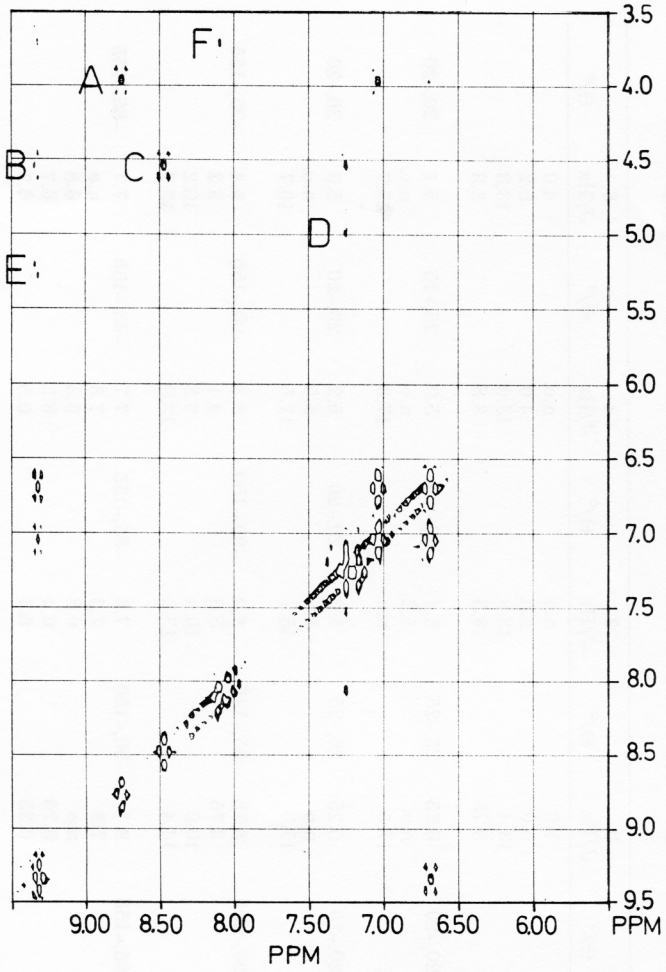

Figure 4. Amide/αH region of the ROESY spectrum of compound **1** in DMSO-d_6, 23 mM, 25 °C, mixing time t_m = 300 ms. Negative levels are shovn. (A) Tyr^1αH–Gly^{2}NH; (B) Phe^4αH–Tyr^{1}OH; (C) Phe^4αH–Leu^{5}NH; (D) Phe arom H-GlcOH–4; (E) Tyr^{1}OH–GlcOH–2, and (F) Gly^3αH–Phe^{4}NH.

ROESY cross-peaks are observed (Figure 4), pointing to the existence of two turns in **1** involving Tyr^1 and Gly^2, and Leu^5 and glucose residues.

For glucoconjugates **2** and **3**, similar interresidual ROESY cross-peaks as in **1** are observed (not shown). In addition, the Leu^5αH–Gly^{2}NH cross-peak is detected in both glucopeptide derivatives, while Tyr^1βH–Phe^{4}NH is observed in **2**, and Tyr^1βH–Phe^4αH cross-peak in **3**, indicating different compact structures of **2** and **3**, involving the main chain of the amino acid residues, while the side chains are on the surface.

CONCLUSION

The results of the proton magnetic resonance study on [Leu^5]enkephalin glycoconjugates in DMSO–d_6 solution suggest that the molecules are represented by a mixture of at least two *quasi* isoenergetic peptide backbone conformations averaged in the NMR time-scale. We assume that the bulk of all three glucoconjugates has essentially an extended conformation, while only a small fraction of them has limited backbone flexibility and possesses a structure with two turns in the case of **1**, and a compact structure involving the main chain of the amino acid residues in **2** and **3**. This indicates that glycosylation affects the characteristic folded β–bend conformation of the enkephalin pentapeptide moiety. Since enkephalin derivatives **1–3** were relatively more δ–receptor selective than native enkephalin, the well known β–bend conformation of the native peptide is not of critical importance for its activity. There are some doubts about the identity of the structures in DMSO and D_2O solution which should be considered in future research.

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SAŽETAK

Studij glukokonjugata leucin-enkefalina pomoću ^1H i ^{13}C nuklearne magnetne rezonancije

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Sintetski priređene glukokonjugate leucin-enkefalina (**1–3**) studirali smo s pomoću nekoliko metoda jedno- i dvo-dimenzijske ^1H i ^{13}C nuklearne magnetne rezonancije (NMR). Iako su sve vrijednosti temperaturnih koeficijenata kemijskog pomaka amidnih protona bile veće od onih koje se očekuju za protone uključene u vodikove veze, u ROESY spektrima bilo je moguće opaziti nekoliko signala nuklearnog Overhauserova efekta unutar istoga i između različitih aminokiselinskih i ugljikohidratnih ostataka. To pokazuje da struktura glukokonjugata **1–3** u otopini DMSO–d_6 nije potpuno nasumična. Pažljiva analiza temperaturnih koeficijenata, konstanti sprege i ROESY spektara ne podupire postojanje samo jedne konformacije. ROESY signali između ostataka u **1** upućuju na postojanja barem jednog konformera s dva zavoja koji uključuju Tyr^1 i Gly^2, te Leu^5 i glukozni ostatak, dok u glukokonjugatima **2** i **3** ROESY signali između ostatka upućuju na konformer s različitom kompaktnom strukturom koja uključuje glavni lanac aminokiselinskih ostataka dok su pobočni lanci na površini.