CROATICA CHEMICA ACTA
CCACAA **81** (4) 647–656 (2008)

ISSN-0011-1643
CCA-3286
*Original Scientific Paper*

# CD and FTIR Spectroscopic Studies of Carbohydrate-modified Opioid Peptides

**Elemér Vass,[a] Miklós Hollósi,[a] Štefica Horvat,[b] and Maja Roščić[b,*]**

[a]*Department of Organic Chemistry, Institute of Chemistry, Eötvös Loránd University, P. O. Box 32, H-1518 Budapest 112, Hungary*

[b]*Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Laboratory for Carbohydrate, Peptide and Glycopeptide Research, P. O. Box 180, 10002 Zagreb, Croatia*

RECEIVED OCTOBER 23, 2007; REVISED MARCH 20, 2008; ACCEPTED APRIL 29, 2008

The variety of vital functions played by glycoproteins in many physiological and pathological processes inspired the design of glycopeptides and the study of the effect of glycosylation on conformation. This work reports comparative circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopic studies on linear and cyclic Amadori and imidazolidinone-type glycopeptides **3**–**8** in comparison with spectroscopic data of the non-modified flexible parent peptides, Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH, **1**) and its amide (H-Tyr-Gly-Gly-Phe-Leu-$NH_2$, **2**). The CD and FTIR measurements were performed in different solvents in order to expose the structural and conformational differences caused by a keto-sugar, a rigid 5-membered imidazolidinone ring and/or cyclization. The combined application of CD and FTIR spectroscopy allowed to evaluate the relative amount of the extended and folded conformers of Amadori as well as imidazolidinone compounds and proved to be an effective tool for characterization of their structural features leading to a deeper understanding of their conformational behaviour.

*Keywords*
glycopeptides
Amadori
imidazolidinone
conformational analysis
circular dichroism
IR spectroscopy

## INTRODUCTION

Opioid peptides are responsible for a variety of processes in organisms, the best characterized of which are analgesia, sedation, euphoria, respiratory depression, and a peripheral action as an antidiarrheic.[1] From medicinal chemical viewpoint, a very broad spectrum of biological activities render endogenous opioid peptides, Leu- and Met-enkephalin (Tyr-Gly-Gly-Phe-Leu/Met),[2,3] as important targets with a good potential for further development as therapeutic agents. Because of the very poor metabolic stability of biologically active peptides, numerous analogs have been designed to overcome pharmaceutical and pharmacokinetic barriers in clinical drug application.[4–7]

The carbohydrate moieties of glycoproteins and glycopeptides are believed to have important roles in their biological activity.[8,9] Among these, the structural effect on a peptide backbone represents a major function.[10,11] However, the issue of how glycosylation affects protein or peptide structure is unclear. It was reported that carbohydrate moiety aids in the formation of a secondary structure of a peptide backbone, especially the β-turn,[12,13] or stabilizes the peptide backbone conformation.[14,15] In

* Author to whom correspondence should be addressed. (E-mail: roscic@irb.hr)

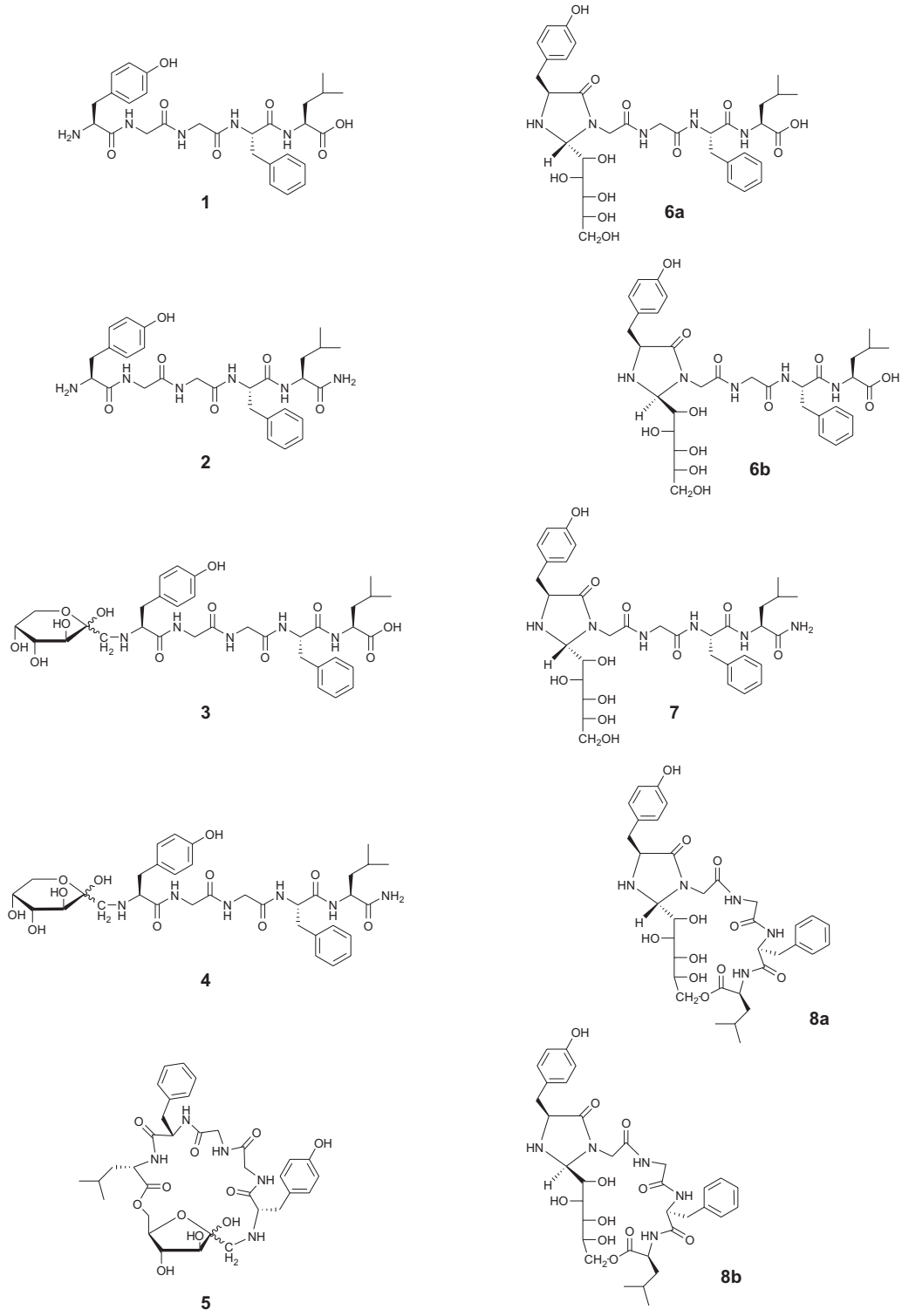



Figure 1. Structures of compounds **1**–**8**: Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu, **1**) and its amide (Tyr-Gly-Gly-Phe-Leu-$NH_2$, **2**) as well as their glycoconjugates, Amadori compounds **3**–**5** and imidazolidinones **6**–**8**.

some cases no structural change is evident,[16] and in some cases the original α-helical structure was found to be broken,[17] or perturbed.[18]

This work reports the comparative circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopic properties of glycopeptides **3**–**8** in comparison with spectroscopic data obtained for the non-modified parent opioid peptides, Leu-enkephalin (**1**) and Leu-enkephalin amide (**2**) (Figure 1). One group of investigated compounds are Amadori compounds **3** and **4** in which the

TABLE I. CD spectral parameters, molar CD ($\Delta\varepsilon$/dm$^3$mol$^{-1}$cm$^{-1}$) and mean residue ellipticity ($[\theta]_{MR}$ / deg cm$^2$ dmol$^{-1}$) of compounds **1**–**5** in TFE, TFE-water (1:1) mixture and water.

| Compound | TFE | | | TFE/$H_2O$ = 1/1 | | | $H_2O$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\lambda$ / nm | Mol. CD | $[\theta]_{MR}$ | $\lambda$ / nm | Mol. CD | $[\theta]_{MR}$ | $\lambda$ / nm | Mol. CD | $[\theta]_{MR}$ |
| **1** | 191.5 | 7.37 | 4860 | 194.0 | 11.90 | 7850 | 196.5 | 7.05 | 4650 |
| | 199.5 | 6.31 | 4160 | | | | | | |
| | 222.0 | 11.09 | 7320 | 224.0 | 7.90 | 5210 | 223.0 | 6.99 | 4610 |
| **2** | 187.5 | 6.69 | 4420 | 190.5 | 5.44 | 3590 | 192.5 | –3.20 | –2110 |
| | 201.0 | –3.68 | –2430 | 200.0 | –4.24 | –2800 | 208.5 | 2.74 | 1810 |
| | 212.0sh | –2.06 | –1360 | 211.0sh | –1.37 | –900 | | | |
| | 227.0 | 4.15 | 2740 | 227.0 | 4.04 | 2670 | 224.0 | 4.76 | 3140 |
| **3** | 190.5 | 13.60 | | 193.0 | 7.75 | | 195.0 | 4.76 | |
| | 223.5 | 7.26 | | 220.5 | 5.73 | | 219.0 | 5.23 | |
| **4** | 188.0 | 10.07 | | 190.0 | 4.58 | | 196.5 | –2.39 | |
| | 198.5 | –3.63 | | 199.5 | –6.75 | | 209.5 | 2.44 | |
| | 212.5sh | –0.87 | | 212.5sh | –1.90 | | | | |
| | 226.0 | 3.10 | | 227.0 | 1.31 | | 219.5 | 3.63 | |
| **5** | 194.5 | 18.31 | | 193.0 | 14.98 | | 194.0 | 11.04 | |
| | 219.0 | 13.05 | | 218.5 | 11.25 | | 220.0 | 8.06 | |

linear peptide is alkylated at the N-terminal position by a 1-deoxy-D-fructose unit. Adduct **5**, the cyclic analog of compound **3**, possesses an ester bond between the C-6 hydroxy group of the sugar moiety and the C-terminal carboxy group of the peptide, 1-deoxy-D-fructofuranose acting as a bridge between the Leu-enkephalin terminal parts. The rigid 5-membered imidazolidinone ring is characteristic of the second class of compounds. In these adducts an imidazolidinone moiety connects the acyclic sugar residue with the linear peptide chain (**6**, **7**). The imidazolidinone compounds **6** with D-*gluco* sugar pentitol structures are diastereoisomers with *trans* (**6a**) and *cis* (**6b**) relative geometry of the carbon substituents at the imidazolidinone ring moiety. In the corresponding bicyclic imidazolidinone analogs **8a** and **8b**, a 19-membered ring is formed through an ester bond between the primary hydroxyl group of the D-*gluco*-pentitolyl residue and the C-terminus of the peptide.

The measurements were performed in different solvents in order to expose the structural and conformational differences caused by a keto-sugar, a rigid 5-membered imidazolidinone ring and/or cyclization.

## EXPERIMENTAL

### *Glycopeptide Preparation*

Leu-enkephalin (**1**) and Leu-enkephalin amide (**2**) were purchased from Bachem. Amadori compounds, *N*-(1-deoxy-D-fructos-1-yl)-L-tyrosylglycylglycyl-L-phenylalanyl-L-leucine (**3**) and cyclo-{*N*-[-6)-1-deoxy-α,β-D-fructofuranos-1-yl]-L-tyrosylglycylglycyl-L-phenylalanyl-L-leucyl-(1→*O*} (**5**) were synthesized under the conditions described by Horvat *et al*.[19] Imidazolidinone derivatives, *N*-{[2-(D-*gluco*-pentitol-1-yl)-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl]} acetylglycyl-L-phenylalanyl-L-leucine (**6a**, *trans* isomer; **6b**, *cis* isomer) and cyclo-{*N*-{[2-[-5)-D-*gluco*-pentitol-1-yl]-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl-(1→*O*]}acetylglycyl-L-phenylalanyl-L-leucyl-} (**8a**, *trans* isomer; **8b**, *cis* isomer) were prepared according to the reported procedure.[20] *N*-(1-deoxy-D-fructos-1-yl)-L-tyrosylglycylglycyl-L-phenylalanyl-L-leucine amide (**4**) and *N*-{[2-(D-*gluco*-pentitol-1-yl)-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl]}acetylglycyl-L-phenylalanyl-L-leucine amide (**7**, *trans* isomer) were obtained as described by Roščić *et al*.[21] All compounds with the exception of **4** and **5** were desalted using an octadecylsilica solid-phase extraction (SPE) cartridge (500 mg, 2.8 mL).

### *CD Measurements*

Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectrometer at room temperature. A quartz cuvette with 0.2 mm path length was used for far-UV (250–185 nm) measurements and a quartz cuvette with 10.0 mm path length for near-UV (310–250 nm) measurements. CD spectra were taken in 2,2,2-trifluoroethanol (TFE; Aldrich, NMR grade), water and in 1:1 mixture of these solvents. The sample concentrations ranged between 0.2–0.4 mg/mL.

### *FTIR Measurements*

FTIR spectra were recorded on a Bruker Equinox 55 spectrometer at room temperature in a $CaF_2$ cell of 0.2 mm path length. The sample concentration in TFE was 2 mg/mL in each measurement.

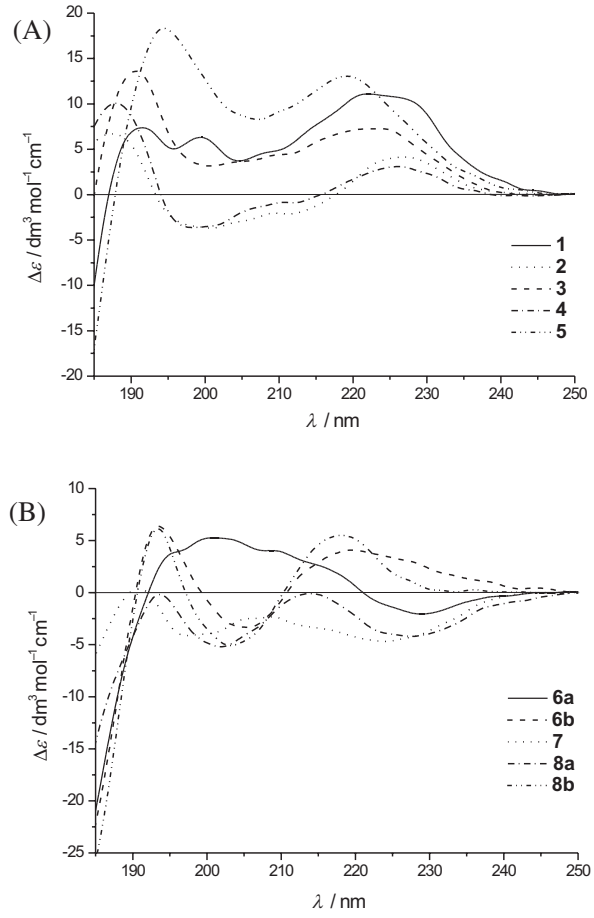



Figure 2. The far-UV region circular dichroism spectra in TFE of compounds **1**–**5** (A) and **6**–**8** (B). The spectra were obtained at room temperature and expressed as molar CD ($\Delta\varepsilon$/dm$^3$mol$^{-1}$cm$^{-1}$) from 185 to 250 nm.

## RESULTS

### *CD Studies of Compounds 1–8*

CD spectral parameters in TFE, water and TFE-water (1:1) mixture of Leu-enkephalin (**1**), its amide **2** and their Amadori derivatives **3**–**5** are summarized in Table I.

As indicated by previous CD studies, the parent unmodified peptide Leu-enkephalin (**1**) is present in TFE as an ensemble of conformers rather than one or two prevailing ones.[22,23] The far-UV spectral region is contributed by the CD of the backbone amide groups and the $^1L_a$ and $^1B_b$ bands of Tyr and Phe side-chains. As shown in Figure 2A the spectrum of Leu-enkephalin (**1**) differs significantly from that of its amide (**2**)" indicating different population of folded (β- and γ-turns) and extended conformers. In TFE-water (1:1) mixture and water the sign pattern of the spectrum of **1** remains unchanged but the band positions and intensities are changed. The CD spectra of **2** are rather similar in TFE and in the solvent mixture. The spectra of **2** measured in TFE and water differ most significantly. The negative band observed at 192.5 nm in water reflects definite contribution of unordered conformation.

The CD spectra of Amadori derivatives **3** and **4** in TFE resemble those of their parent peptides **1** and **2**. In the spectrum of **3** the positive band of **1** at 199.5 nm is missing, the spectrum between ≈200 and ≈220 nm is positive but unstructured (Figure 2A). The solvent dependence of the CD of **3** and **4** also differs from that of **1** and **2** (Table I).

The CD spectrum in TFE of the bicyclic Amadori derivative **5** is marked by two intense positive bands (Figure 2A). The positive band near 220 nm is contributed by the aromatic residues as in the case of the open-chain Amadori derivatives. The band positions do not change significantly, but the intensities are decreased in the solvent mixture and water. These spectral features are compatible with high population of a type II β-turn showing class C' CD spectrum.[24]

The CD spectral parameters of imidazolidinone compounds **6**–**8** are summarized in Table II. The CD curves in TFE generally show not too much similarity. The only exception is *cis* isomer of the »free hands« compound **6b** and bicyclic one **8b** the CD spectra of which, with minor alterations, feature bands with the same sign pattern and intensity (Figure 2B). This suggests the foldedness of the open »free hands« compound **6b**. The corresponding *trans* isomers **6a** and **8a** have very different CD spectra in TFE (Figure 2B). The CD spectra of the *trans* »free hands« compounds **6a** (acid) and **7** (amide) are also different (Table II and Figure 2B). The spectrum of **6a** shows a broad positive band with fine structure between ≈190 and ≈210 nm. All the other imidazolidinone derivatives show negative CD in this region regardless of the *trans*- or *cis* relative orientation of the aromatic chromophore and the sugar antennae. This likely reflects difference of the foldedness of the overall structure. Beacuse of the presence of the macroring **8a** and **8b** are necessarily folded. As shown in Figure 2B, the *cis*-isomers **6b** and **8b** have strong positive bands at 193.5 nm and near 220 nm. This may be due to the same *cis* steric orientation of the aromatic chromophore and sugar antennae. Apparently, the CD spectra of imidazolidinones **6**–**8** are rather complicated: they are determined by the foldedness of the peptide backbone, the environment of the aromatic chromophores and the CD contribution of the imidazolidinone.

Further, it is noteworthy to observe that in the far-UV region the long-wavelength band has opposite sign in *trans* and *cis* imidazolidinone diastereoisomers (Figure 2B). The sign of this band appears to be dependent on the relative orientation of the aromatic chromophore and sugar antennae but its position and intensity are strongly affected by the polarity of the solvent.[25] In the spectra of the *cis* isomers (**6b** and **8b**) in TFE the positive bands are present only as shoulders.

TABLE II. Molar CD ($\Delta\varepsilon$/dm$^3$mol$^{-1}$cm$^{-1}$) of compounds **6**–**8** in TFE, TFE-water (1:1) mixture and water

| Compound | TFE | | TFE/$H_2O$ = 1/1 | | $H_2O$ | |
|---|---|---|---|---|---|---|
| | $\lambda$ / nm | Mol. CD | $\lambda$ / nm | Mol. CD | $\lambda$ / nm | Mol. CD |
| **6a** | 195.5 | 3.84 | 194.5 | 3.96 | 196.5 | 5.54 |
| | 200.5 | 5.26 | 204.0 | 4.23 | 202.0 | 6.48 |
| | 210.0 | 3.92 | 209.0 | 4.05 | 212.0 | 5.07 |
| | 217.0 | 1.98 | 215.5 | 2.72 | 219.5 | 3.50 |
| | 229.0 | –2.06 | 229.5 | –1.15 | 234.0 | <0.03 |
| **6b** | 193.5 | 6.37 | 194.5 | 0.94 | | |
| | 205.5 | –3.32 | 201.5 | –2.17 | 202.0 | –2.20 |
| | 219.5 | 4.10 | 217.0 | 4.56 | 218.0 | 3.44 |
| **7** | 191.0 | 0.38 | 191.0 | 0.50 | | |
| | 197.0 | –4.12 | 198.0 | –8.03 | 197.5sh | –6.11 |
| | 224.0 | –4.68 | 228.0 | –3.00 | 216.0 | 0.86 |
| **8a** | 193.5 | 0 | | | | |
| | 202.0 | –5.24 | | | | |
| | 214.0 | 0 | 214.0 | 0.51 | 205–245 | ca. 0.8 |
| | 227.5 | –4.25 | 225.5 | –2.30 | | |
| **8b** | 193.5 | 6.14 | 194.0 | 4.90 | | |
| | 203.0 | –5.08 | 202.0 | –7.00 | 201.0 | –5.91 |
| | 218.0 | 5.49 | 218.5 | 3.41 | 219.5 | 2.56 |

As reported earlier for Amadori compound **3**,[22] the attachment of free sugar influences the side chain conformer distribution of Tyr and likely Phe. In agreement with this, CD spectra of **3**–**5** in the $^1L_b$ region change sign relative to the band of parent Leu-enkephalins **1** and **2**. The most intense vibrational fine structure is observed in the spectrum of the cyclic Amadori derivative **5** (Figure 3A).

The imidazolidinone moiety incorporated into the structure of compounds **6**–**8** does not influence the positive sign of the broad $^1L_b$ band relative to parent peptides **1** and **2**, but have higher intensity in the spectra of »free hands« derivatives **6a**, **6b** and **7** regardless of the relative orientation (*trans* or *cis*) of the ring substituents (Figures 3A and 3B).

### *FTIR Spectroscopic Characterization of Compounds 1–8*

The infrared spectra of Leu-enkephalin (**1**), Leu-enkephalin amide (**2**), their Amadori (**3**–**5**) and imidazolidinone derivatives (**6**–**8**) were taken in TFE in the 1800–1500 cm$^{-1}$ spectral region, which is mainly composed of bands due to $\nu_{CO}$ (COOH), amide I, amide II, and $\nu_{as}$ ($COO^-$) vibrations (Figure 4; spectra of compounds **2** and **6b** not shown). Absorptions by the aromatic Tyr and Phe side chains also give contribution in this spectral region. Amadori compounds **4** and **5** are present as trifluoroacetate ($TFA^-$) salts. The strong $\nu_{as}$ ($COO^-$) band of $TFA^-$ appears at ≈1675 cm$^{-1}$. Band assignment is based on the data in Scheme 1.[22,26] The position of the weak bands

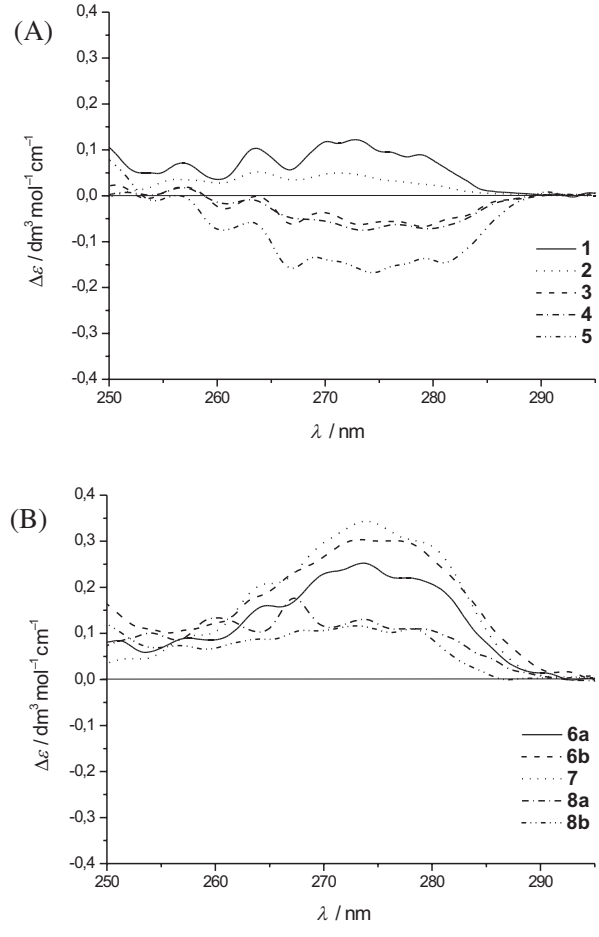
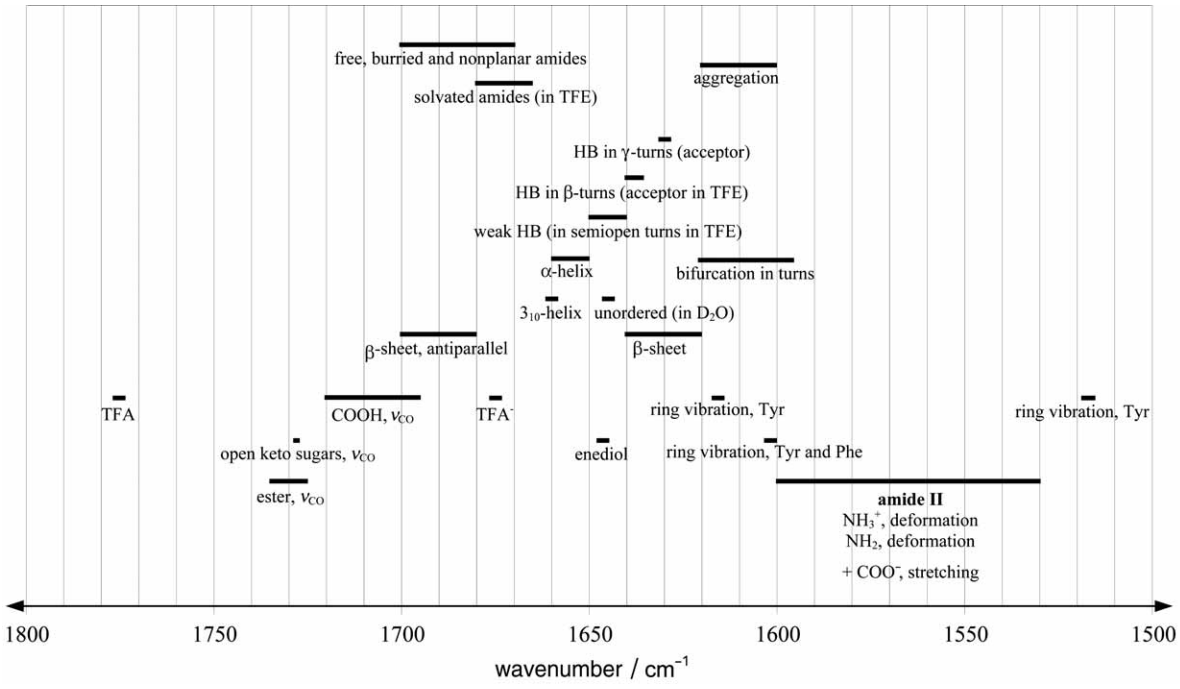



Figure 3. The near-UV region circular dichroism spectra in TFE of compounds **1**–**5** (A) and **6**–**8** (B). The spectra were obtained at room temperature and expressed as molar CD ($\Delta\varepsilon$/dm$^3$mol$^{-1}$cm$^{-1}$) from 250 to 295 nm.




Scheme 1. Assignment of bands in the amide I and amide II infrared region. Data taken from Refs. 22 and 26.

and shoulders have been confirmed or corrected by Fourier self-deconvolution (FSD).

The FTIR spectra of Leu-enkephalin (**1**) and its Amadori derivative (**3**) have been reported earlier.[22] The data measured herein are in agreement with the published ones. The sharp component bands near ≈1519, ≈1600 and ≈1616 $cm^{-1}$ in the infrared spectra of all analyzed compounds are assigned to the ring vibration of Tyr and Phe (see Scheme 1). Concerning the ionization state of compounds **1**–**8**, it was observed that spectra show differences due to the $NH_2$-peptide-COOH $\rightleftharpoons$ $NH_3^+$-peptide-$COO^-$ equilibrium in TFE. In the case of compounds **1** and **3** the rather low intensity of $\nu_{CO}$ COOH and the increase and broadening of the band below ≈1600 $cm^{-1}$ are an indication of the predominance of increased population of the zwitterionic form with $COO^-$. Furthermore, it appears that imidazolidinones **6a**, **6b** possess higher amount of the protonated species. The carboxylic $\nu_{CO}$ stretching vibration at ≈1720 $cm^{-1}$ increases in both diastereoisomers while the carboxylate antisymmetric stretching band below ≈1600 $cm^{-1}$ vanishes. As expected, no contributions have been observed in these two diagnostic domains in the FTIR spectra of amide compounds **2**, **4** and **7**. The macro-ring of the bicyclic Amadori compound **5** as well as of diastereoisomeric bicyclic imidazolidinones **8a**, **8b** is closed by an ester bond. In their spectra, the corresponding $\nu_{CO}$ ester band with medium intensity turns up around 1730 $cm^{-1}$. A slightly lower frequency observed for the latter two compounds may be due to the formation of H-bonds between the carbonyl oxygen and the neighboring hydroxyl groups.

As known from previous FTIR studies, Leu-enkephalin exhibits in TFE an ensemble of conformations, the main components of the amide I band were assigned to β-sheet, γ-turn, semi-open and open conformers.[22] The shape and deconvolution of the amide I component bands of compounds **2**–**8** gave us some information about their backbone conformation. In general, the FTIR spectra of the zwitterionic compounds **1**, **3** and **6a** reflect in TFE solution the presence of more folded conformers (β- and/or γ-turns) than their corresponding amide analogs **2**, **4** and **7**. In the case of cyclic Amadori compound **5** the $\nu_{as}$ stretching band of trifluoroacetate ($TFA^-$) at ≈1672 $cm^{-1}$ is overlapped with the amide I band and dominates the spectrum. However, shoulders in its FSD spectrum at 1646, 1638 and 1629 $cm^{-1}$ are present, which indicate the presence of turns. These findings are in agreement with the results of CD spectroscopy measurements. Evidence for the presence of the acyclic form of reducing sugars in solution has recently been proved by FTIR spectroscopy, the $\nu_{CO}$ band of the open-keto-chain as well as enediol absorption being found near ≈1728 $cm^{-1}$ and ≈1647 $cm^{-1}$, respectively.[27,28] In the case of Amadori compounds **3** and **5** these spectral regions are obscured by $\nu_{CO}$ bands of COOH or ester group, respectively, and by the weak hydrogen bonds in the semi-open turns in the low-wavenumber amide I region (see Scheme 1). Therefore we can not reliably estimate the contribution of the open sugar ring. No absorption band has been observed in the »open-keto« $\nu_{CO}$ spectral domain of Amadori compound **4**. We presume that the ring oxygen protonation as promoter step for carbohydrate ring-opening

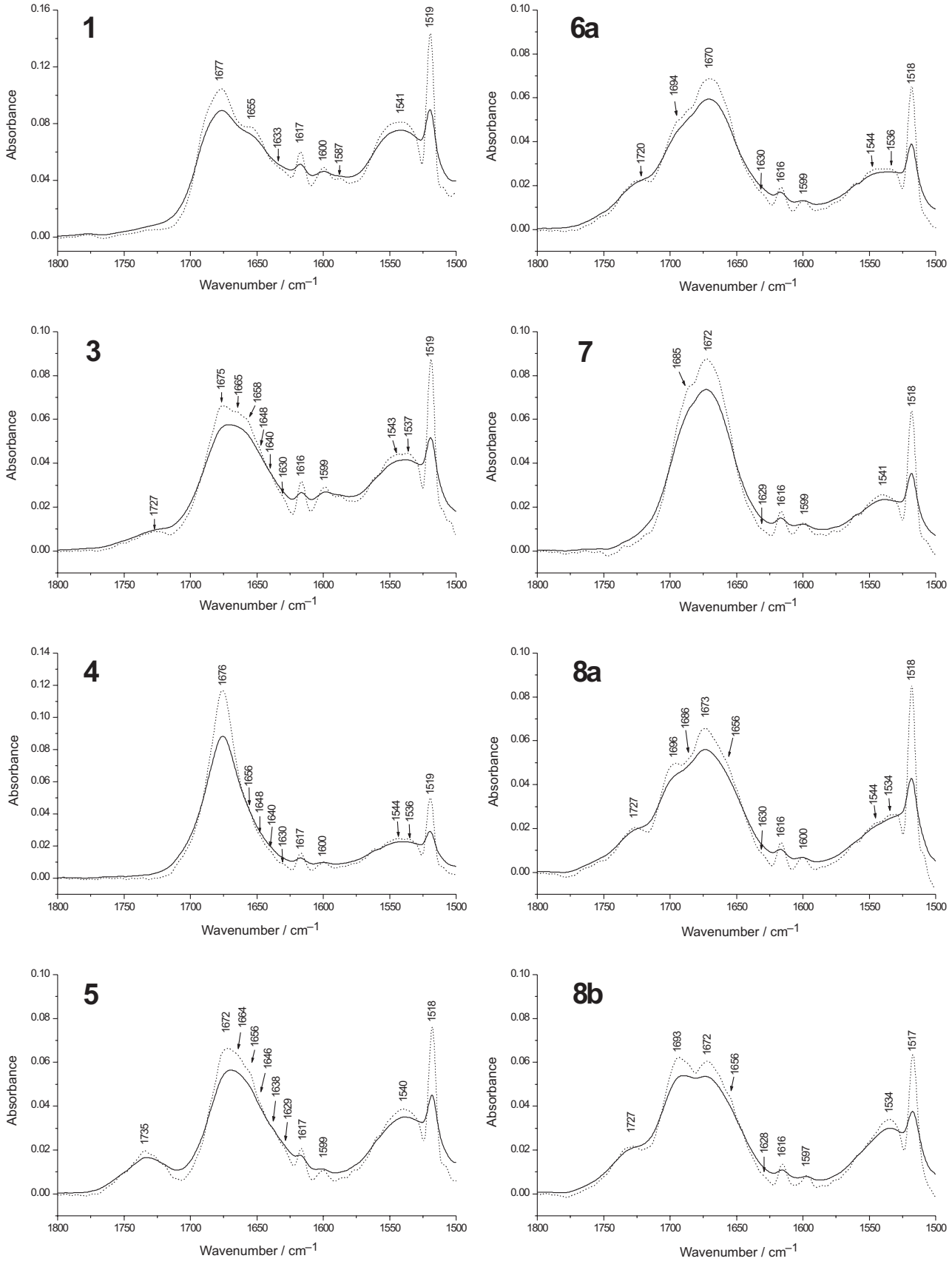



Figure 4. Experimental FTIR spectra of compounds **1**, **3**–**5**, **6a**, **7**, **8a** and **8b** (solid line) and their Fourier self-deconvolution (FSD) traces (dashed line) assuming Lorentzian band shape and values of 30 $cm^{-1}$ and 0.2 for the bandwidth and noise reduction parameters, respectively.

reaction is suppressed in compound **4** due to the absence of the COOH group.

The »free-hands« type imidazolidinone compounds **6a**, **6b** and **7** comprise a heterocyclic unit as well as sugar and peptide chains. The open sugar chain does not influence the spectrum between 1500 and 1800 $cm^{-1}$ but the N-substituted lactam subunite of all imidazolidinone compounds (**6–8**) has a characteristic spectral contribution below 1700 $cm^{-1}$ (Figure 4).

The main spectral difference in the FTIR spectra of the *trans* and *cis* »free-hands« imidazolidinones **6a** and **6b** is the increased relative intensity of the shoulder at ≈1690 $cm^{-1}$ in the spectrum of the *cis* form. Furthermore, the *trans* and *cis* isomers of the ester-bonded bicyclic imidazolidinone compounds **8a** and **8b** show a great difference in the intensity and the position of the band assigned to the imidazolidinone *cis* lactam bond. In the spectrum of the *cis* form (**8b**) there is a separated band at ≈1690 $cm^{-1}$ (appearing at 1693 $cm^{-1}$ in the FSD trace), while the corresponding band of the *trans* form (**8a**) appears only as a shoulder near 1696 $cm^{-1}$. This observation may be a sign of the presence of more folded conformers in the less crowded *trans* isomers in which, due to formation of turn(s), the $\nu_{C=O\cdots HNR}$ band of the intramolecularly hydrogen bonded exocyclic carbonyl group is shifted to the lower spectral region.

## DISCUSSION

The majority of small linear peptides of biological importance, like Leu-enkephalin, in aqueous solution are present as mixtures of rapidly interconverting conformers. Traditionally, CD experiments in organic solvents such as halogenated alcohols are used for exploring their tendency to adopt a detectable or even predominant amount of intra-H-bonded folded structures.[29] Besides, halogenated alcohols have been suggested to mimic the microenvironment established by apolar proteins or membranes.[30]

In general, the CD and FTIR spectra of the zwitterionic peptide Leu-enkephalin **1**, Amadori derivative **3** and *trans*-isomer of the »free hands« imidazolidinone **6a** reflect in TFE solution more expressed foldedness than their corresponding amide analogs **2**, **4** and **7**, respectively. According to the literature,[26] based on information obtained by CD, FTIR and/or NMR spectroscopy, there are no general rules on which glycosylation influences structural propensities of parent peptides, so it can alter backbone conformation to different degrees. Based on the far-UV CD spectra (190–250 nm), the keto-sugar attached to the N-terminus of enkephalins in compounds **3** and **4** does not have a significant effect upon the backbone conformer equilibrium of the parent peptides. While in the far-UV range information related to the secondary structure of the peptide backbone can be obtained, in the near-UV spectral region, between 250 and 300 nm, the CD of most peptides/proteins is dominated by the contributions of the aromatic amino acids Phe, Tyr and Trp, and by the disulfide chromophore of cystine.[31,32] Tyr has lower symmetry than Phe and therefore has more intense absorption band in the 270–290 nm UV-range. The opposite, negative sign of the $^1L_b$ band in the CD spectrum of the Amadori compounds can be explained by the difference in the orientation of the Tyr side chain. $^1L_b$ transition has been found to be extremely sensitive in both sign and magnitude to rotation about the $C^\beta$-$C^\gamma$ bond.[33]

It is an intriguing question whether or not the imidazolidinone ring, acting as a bridge between the carbohydrate and peptide antennas in the studied compounds **6–8**, has a significant contribution to their conformer distribution. Correlation between the conformational geometry and the sign of Cotton effect (CE) of simple α-amino acid 4-imidazolidinones and 5-oxazolidinones, respectively, have been studied earlier on the basis of their CD spectra.[25,34] It was concluded that the sign of the n→π* lactam band depends on the envelope conformation of this five-membered heterocyclic ring and the position and type of the attached bulky substituents. Because of the complexity of compounds **6–8** and overlapping of n→π* transition bands with the $^1L_a$ bands of Tyr and Phe side-chains, it is hard to draw a clear-cut conclusion. However, since in the long-wavelength far-UV region of imidazolidinones CD spectra, all *trans* derivatives possess negative CE while the corresponding *cis* isomers have positive CE, it is reasonable to presume that these findings allow for stereochemical correlation and makes possible to distinguish between *trans*- and *cis*-substituted imidazolidinones. The imidazolidinone moiety incorporated into the structure of compounds **6–8** does not change the positive sign of the broad $^1L_b$ band of the parent peptides **1** and **2** in the near-UV CD spectra.

In FTIR spectra, the N-substituted lactam subunite of all imidazolidinone compounds (**6–8**) has a characteristic contribution below 1700 $cm^{-1}$. Based on literature data, angular ring strain of the 5-membered heterocyclic imidazolidinone and oxazolidinone molecules, shifts the carbonyl stretching frequency into the higher spectral region by the order of 20–30 $cm^{-1}$, compared to their acyclic analogs.[35,36] With a decrease in the bond angle, the carbonyl stretching mode should appear at higher frequency, since it is more difficult for the carbon atom of an exocyclic carbonyl group to move into the ring during a cycle of $\nu_{CO}$ due to the restriction of the movement of the two adjacent atoms. Besides, the molecular orbital calculations led to the conclusion that in conjugated cyclic π-electronic molecular systems increase of angular strain is accompanied by bond hybridization changes which are reflected in higher stretching force constants and frequencies.[35] The *cis* lactam band between 1690–1700 $cm^{-1}$ in the spectrum of the *trans* and *cis* »free hands« and

bicyclic imidazolidinone diastereoisomers, differs in position and intensity indicating different population of folded and extended conformers.

Due to their limited ability to form secondary structures such as α-helix, β-sheet, or random coil, cyclic peptides are very good models for turn(s).[37,38] The most intense band in the IR spectrum, the amide I band (carbonyl stretching coupled with in-plane NH bending and CN stretching modes, around 1695–1610 $cm^{-1}$), consists of a series of overlapped component bands which occur as a result of the secondary structures present in peptides. The analysis of the amide I band permits to determine the peptide secondary structures. The low wavenumber shoulder of the amide I band may be a sign of the spectral contribution of turn(s). The amide I component band of the acceptor CO of intramolecular H-bond(s) of β-turns [1←4 ($C_{10}$) H-bonding] and γ-turns [1←3 ($C_7$) H-bonding] was suggested to appear near 1640 and 1620 $cm^{-1}$, respectively.[24] Accordingly, FTIR spectroscopy has a unique power to detect turns in small and midsize peptides. Moreover, the CD/FTIR approach was already applied for detection of turns in glycosylated peptides.[22,26,38,39] Accordingly, the CD and FTIR spectral features of the bicyclic Amadori **5** derivative are compatible with high population of a type II β-turn.

It is shown in this paper that CD and FTIR spectroscopy represent an effective tool for studying the influence of the sugar residue, a rigid 5-membered imidazolidinone ring and cyclization on the structural and conformational features of parent unmodified peptides. The combined application of these two techniques allowed to evaluate the relative amount of the extended and folded conformers of Amadori compounds **3–5** as well as imidazolidinones **6–8**.

*Acknowledgements***.** – This work was supported by grant from the Hungarian Government (Scholarship to M.R. established on the basis of bilateral Hungarian/Croatian agreement), the Hungarian Scientific Research Fund OTKA (grants T 047186 to E.V. and NI 68466 to M.H.), the Economic Competitiveness Operational Program GVOP-KMA (GVOP-3.2.1-2004-04-0345/3.0), the Ministry of Science, Education and Sports of Croatia grant No. 098-0982933- 2936, and by financial support from the bilateral Hungarian/ Croatian project No. CRO-5/2006.

## REFERENCES

1. R. J. Bodnar and G. E. Klein, *Peptides* **26** (2005) 2629–2711.
2. J. Hughes, *Brain Res.* **88** (1975) 295–308.
3. J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, and H. R. Morris, *Nature* **258** (1975) 577–579.
4. J. Z. Yang, K. C. Bastian, R. D. Moore, J. F. Stobaugh, and R. T. Borchardt, *J. Chromatogr., B* **780** (2002) 269–281.
5. R. T. Borchardt, *J. Controlled Release* **62** (1999) 231–238.
6. M. Rinnová, A. Nefzi, and R. A. Houghten, *Tetrahedron Lett.* **43** (2002) 2343–2346.
7. L. Lund, A. Bak, G. J. Friis, L. Hovgaard, and L. L. Christrup, *Int. J. Pharm.* **172** (1998) 97–101.
8. A. Varki, *Glycobiology* **3** (1993) 97–130.
9. H. Lis and N. Sharon, *Eur. J. Biochem*. **218** (1993) 1–27.
10. R. A. Dwek, *Chem. Rev.* **96** (1996) 683–720.
11. B. Imperiali, *Acc. Chem. Res*. **30** (1997) 452–459.
12. A. H. Andreotti and D. Kahne, *J. Am. Chem. Soc*. **115** (1993) 3352–3353.
13. E. E. Simanek, D.-H. Huang, L. Pasternack, T. D. Machajewski, O. Seitz, D. S. Millar, H. J. Dyson, and C.-H. Wong, *J. Am. Chem. Soc*. **120** (1998) 11567–11575.
14. G. Mer, H. Hietter, and J.-F. Lefevre, *Nat. Struct. Biol*. **3** (1996) 45–53.
15. D. F. Wyss, J. S. Choi, J. Li, M. H. Knoppers, and K. J. Willis, *Science* **269** (1995) 1273–1277.
16. H. Kessler, M. Matter, G. Gemmecker, M. Kottenhahn, and J. W. Bats, *J. Am. Chem. Soc*. **114** (1992) 4805–4818.
17. H. Otvos, Jr., J. Thurin, E. Kollat, L. Urge, H. H. Mantsch, and M. Hollosi, *Int. J. Pept. Protein Res*. **38** (1991) 476–482.
18. M. Tagashira, H. Iijima, and K. Toma, *Glycoconjugate J*. **19** (2002) 43–52.
19. Š. Horvat, M. Roščić, L. Varga-Defterdarović, and J. Horvat, *J. Chem. Soc., Perkin Trans. 1* (1998) 909–913.
20. Š. Horvat, M. Roščić, and J. Horvat, *Glycoconjugate J.* **16** (1999) 391–398.
21. M. Roščić and Š. Horvat, *Bioorg. Med. Chem.* **4** (2006) 4933–4943.
22. Š. Horvat, A. Jakas, E. Vass, J. Samu, and M. Hollósi, *J. Chem. Soc., Perkin Trans. 2* (1997) 1523–1528.
23. Š. Horvat, L. Otvos, Jr., L. Urge, J. Horvat, M. Čudić, and L. Varga-Defterdarović, *Spectrochim. Acta, Part A* **55** (1999) 2347–2352.
24. E. Vass, M. Hollósi, F. Besson, and R. Buchet, *Chem. Rev.* **103** (2003) 1917–1954.
25. T. Polonski, *Tetrahedron* **41** (1985) 611–616.
26. E. Vass, M. Hollósi, M. Kveder, B. Kojić-Prodić, M. Čudić, and Š. Horvat, *Spectrochim. Acta, Part A* **56** (2000) 2479–2489.
27. V. A. Yaylayan and A. A. Ismail, *Carbohydr. Res.* **276** (1995) 253–265.
28. V. A. Yaylayan, A. A. Ismail, and S. Mandeville, *Carbohydr. Res.* **248** (1993) 355–360.
29. M. Jackson and H. H. Mantsch, *Biochim. Biophys. Acta* **1118** (1992) 139–143.
30. D. W. Urry, L. Masotti, and J. R. Krivacic, *Biochim. Biophys. Acta* **241** (1971) 600–612.
31. S. M. Kelly and N. C. Price, *Curr. Protein Pept. Sci.* **1** (2000) 349–384.
32. N. Sreerama, M. C. Manning, M. E. Powers, J. X. Zhang, D. P. Goldenberg, and R. W. Woody, *Biochemistry* **38** (1999) 10814–10822.
33. J.W. Snow and T.M. Hooker, *J. Am. Chem. Soc*. **97** (1975) 3506–3511.
34. T. Polonski, *Tetrahedron* **41** (1985) 603–609.
35. B. Galabov, D. Simov, and K. Krustev, *J. Mol. Struct.* **34** (1976) 235–244.
36. R. A. Nyquist, C. L. Putzig, and T. D. Clark, *Vib. Spectrosc.* **12** (1996) 81–91.

37. D. F. Mierke, M. Kurz, and H. Kessler, *J. Am. Chem. Soc.* **116** (1994) 1042–1049.
38. E. Vass, M. Kurz, R. K. Konat, and M. Hollósi, *Spectrochim. Acta, Part A* **54** (1998) 773–786.
39. I. Laczko, M. Hollósi, L. Urge, K. E. Ugen, D. B. Weiner, H. H. Mantsch, J. Thurin, and L. Otvos, Jr., *Biochemistry* **31** (1992) 4282–4288.

## SAŽETAK

### CD i FTIR spektroskopska istraživanja ugljikohidratnih derivata opioidnih peptida

**Elemér Vass, Miklós Hollósi, Štefica Horvat i Maja Roščić**

Važnost i raznolikost funkcija glikoproteina u mnogim fiziološkim i patološkim procesima potakla nas je na dizajn novih tipova glikopeptidnih molekula i na istraživanje utjecaja glikacije na konformacijska obilježja ishodnih peptida. U ovom su radu prikazani rezultati CD i FTIR spektroskopskih istraživanja strukturno različitih linearnih i cikličkih glikopeptida, Amadorijevih i imidazolidinonskih spojeva **3**–**8**, te uspoređeni sa spektroskopskim svojstvima ishodnih peptida, Leu-enkefalina (H-Tyr-Gly-Gly-Phe-Leu-OH, **1**) i Leu-enkefalin amida (**2**). CD i FTIR mjerenja provedena su u različitim otapalima u nastojanju da se utvrde utjecaji keto-šećera, rigidnog 5-članog imidazolidinonskog prstena i/ili ciklizacije na strukturna i konformacijska obilježja peptida. Primjenom CD i FTIR spektroskopije uspjeli smo odrediti relativnu zastupljenost istegnutih i savijenih konformera Amadorijevih i imidazolidinonskih spojeva i dokazali da uporaba ovih tehnika predstavlja dobar alat za strukturnu karakterizaciju i dublje razumijevanje njihovih konformacijskih obilježja.