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Synthesis of hexose-related imidazolidinones: Novel glycation products in the Maillard reaction

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Carbohydrate-peptide esters which mimic the reactivity of sugar 6-phosphates in nonenzymatic glycations were used as model compounds for the study of the Maillard reaction *in vitro*. We found that intramolecular cyclization of the monosaccharide ester in which the sugar moiety (D-glucose or D-galactose) is linked, through the C-6 hydroxy group, to the C-terminal carboxy group of the endogenous opioid pentapeptide leucine-enkephalin, in methanol as the solvent, resulted in the formation of imidazolidinone diastereoisomers having *cis* or *trans* relative geometry of the substituents at the imidazolidinone ring moiety. The diastereoisomeric imidazolidinones were separated and each transformed by hydrolysis into the corresponding D-*gluco*- and D-*galacto*-related imidazolidinone products of leucine-enkephalin. Along with the previous evidence that, from the same sugar-peptide esters by changing the reaction conditions Amadori rearrangement products could be obtained [Horvat et al. (1998) *J Chem Soc Perkin Trans 1:*909–13], the presented results point to the possibility that similar carbohydrate-related imidazolidinones may also be generated in the early stage of the Maillard reaction *in vivo.*

Keywords: **Maillard, glycation, imidazolidinone, carbohydrate, opioid**

Introduction

Reducing sugars, such as glucose, can react nonenzymatically with proteins, glycoproteins, lipids and nucleic acids to produce a variety of carbohydrate adducts known as advanced glycation end products (AGEs) [1,2]. AGEs arise by a succesion of chemical steps that begin with the spontaneous reaction of reducing sugars with amino groups, forming reversible Schiff bases. Initially, these adducts rearrange to form keto-sugar derivatives, or Amadori products, but over time further rearrangements occur to produce irreversibly bound moieties that exhibit cross-linking properties, fluorescence and a yellow-brown color [3,4]. This complex reaction, also known as the Maillard reaction, contributes to the pathophysiological changes associated with diabetes and ageing processes [5–7]. Advanced glycation has also been implicated in the pathology of the Alzheimer's disease [8]. In addition to hexoses and pentoses that may be precursors of the glycation products, some intermediate metabolites of the glycolytic pathway, such as sugar 6-phosphate esters, have been shown to be potent glycating agents [9–11]. Although the occurrence of the Maillard reaction with a variety of proteins *in vivo* and *in vitro* has been demonstrated, the reactivity of individual sugar and peptide components as well as the sequence of reactions that Amadori adducts undergo to generate AGEs is still poorly understood.

In order to gain better insight into mechanisms and products of the Maillard reaction, we chose to examine the reactivity of sugar-peptide esters in which different monosaccharide moieties are linked through their C-6 hydroxy groups to the C-terminal carboxy group of the endogenous opioid peptide leucine-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH). Since these molecules contain a free amino group at the N-terminus of the peptide moiety as well as a reducing sugar epitope, we assumed that such carbohydrate esters represent an ideal model system for the study of the Maillard reaction *in vitro*. They will mimic the reactivity of the sugar 6-phosphate esters in the glycation reaction and, for steric reasons, the attached monosaccharides should be more likely to form carbohydrate-peptide adducts than the parent saccharides alone. In the course of such studies we recently demonstrated that, in pyridine-acetic acid as the solvent, these esters readily undergo intramolecular rearrangement leading to Amadori adducts [12]. The obtained results showed that esterification of the primary hydroxy group of the monosaccharide moieties greatly accelerates the formation

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of the Amadori products, presumably due to the increased abundance of the acyclic *aldehydo* sugar form in solution, similarly to glucose 6-phosphate [13]. In a more recent preliminary publication [14], we have reported that the above mentioned monosaccharide esters, in methanol as the solvent, are transformed to imidazolidinone compounds.

In the present paper, we describe in detail the conversion of the esters **1** and **2,** in which either a D-glucose (**1**) or a D-galactose (**2**) moiety has been covalently attached to leucine-enkephalin pentapeptide, into the corresponding imidazolidinone derivatives **5** and **6**, and the subsequent hydrolysis to carbohydrate-substituted imidazolidinones **7** and **8.** Structural assignment for the imidazolidinone compounds **5–8,** established on the basis of NMR experiments, is presented. Finally, the equilibria and species involved in the imidazolidinone **5** ring formation from the glucose-related ester **1** are investigated in the presence and absence of added base.

Materials and methods

Melting points were determined on a Tottoli (Büchi) apparatus and are uncorrected. Optical rotations were measured at room temperature using an Optical Activity LTD automatic AA-10 Polarimeter. Reactions were monitored by TLC on Silica Gel 60 F_{254} plates (Merck; Darmstadt, Germany) using detection with ninhydrin, the chlorine-iodine reagent, or heating with H_2SO_4. RP HPLC was performed on a Varian 9010 HPLC system with a Eurospher 100 reversed-phase C-18 semipreparative column (250 × 8 mm I.D., 5 μm) under isocratic conditions by using different concentrations of MeOH in 0.1% trifluoroacetic acid (TFA), flow rate 1.1 mlmin^{-1}. UV detection (Varian Model 9050 variable-wavelength UV-Vis detector) was performed at 280 nm. NMR spectra were recorded on a Varian Gemini spectrometer, operating at 300.1 MHz for ^{1}H and 75.5 MHz for ^{13}C nuclei. All samples were measured in DMSO-d_6 solution at 25 °C in 5 mm NMR tubes. Chemical shifts, in ppm, are referred to TMS. Elemental analyses were carried out at the Microanalytical Laboratory, Ruđer Bošković Institute.

6-O-*(*L-*Tyrosylglycylglycyl*-L-*phenylalanyl*-L-*leucyl)*-D-*glucopyranose* (**1**) *and 6*-O-*(*L- *tyrosylglycylglycyl*-L-*phenylalanyl*-L-*leucyl)*-D-*galactopyranose* (**2**)

Compounds **1** and **2** were obtained under the conditions described by Horvat et al. [15]. The crude esters obtained were purified by semipreparative RP HPLC using 58% MeOH/0.1% TFA as the eluent. Desalting on a Dowex 1X2, 200 (Ac) column (10 × 0.8 cm) and lyophilization gave pure **1** and **2** which were used in the subsequent experiments.

*cyclo-{*N-*[-6)-1-Deoxy-α,β*-D-*fructofuranos-1-yl]*-L-*tyrosylglycylglycyl*-L-*phenylalanyl*-L-*leucyl-(1→*O*}* (**3**) *and cyclo-{*N-*[-6)-1-deoxy-α,β*-D-*tagatofuranos-1-yl]*-L-*tyrosylglycylglycyl*-L-*phenylalanyl*-L-*leucyl-(1→*O*}* (**4**)

Compounds **3** and **4** were obtained by intramolecular rearrangements of the monosaccharide esters **1** and **2,** respectively, in pyridine-acetic acid (1:1) as previously described by Horvat et al. [12].

*cyclo-{*N-*{[2-[-5)*-D-gluco-*Pentitol-1-yl]-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl-(1→O]}acetylglycyl*-L-*phenylalanyl*-L-*leucyl-}* (**5**)

6-*O*-(L-Tyrosylglycylglycyl-L-phenylalanyl-L-leucyl)-D-glucopyranose (**1**) (50 mg, 0.07 mmol) was dissolved in dry MeOH (83 ml) and the solution was kept in a well-closed round-bottom flask for 3 days at 60 °C. The solvent was evaporated and the residue was purified by semipreparative RP HPLC using 47% MeOH/0.1% TFA as the eluent to give the pure imidazolidinones **5a** (retention time: 25.7 min) and **5b** (retention time: 24.4 min) which were crystallized from MeOH/diisopropyl ether. Major isomer **5a:** (17 mg, 35%); mp 157–162 °C (decomp.); $[\alpha]_D^{22}$ −53° (c = 1.0, MeOH). For ^{13}C NMR data see Table 1. Anal calcd. for $C_{34}H_{45}N_5O_{11}$: C, 58.36; H, 6.48; N, 10.01. Found: C, 58.46; H, 6.67; N, 9.87. Minor isomer **5b:** (5 mg, 10%); mp 171–176 °C (decomp.); $[\alpha]_D^{22}$ −68° (c = 1.0, MeOH). For ^{13}C NMR data see Table 1. Anal calcd. for $C_{34}H_{45}N_5O_{11}$: C, 58.36; H, 6.48; N, 10.01. Found: C, 58.32; H, 6.61; N, 10.09.

*cyclo-{*N-*{[2-[-5)*-D-galacto-*Pentitol-1-yl]-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl-(1→O]}acetylglycyl*-L-*phenylalanyl*-L-*leucyl-}* (**6**)

Ester **2** (204 mg, 0.28 mmol) was dissolved in dry MeOH (320 ml) and kept in a closed round-bottom flask for 2 days at 60 °C. The solvent was evaporated and the residue was purified by semipreparative RP HPLC using 47% MeOH/0.1% TFA as the eluent to give the pure imidazolidinones **6a** (retention time: 27.1 min) and **6b** (retention time: 27.5 min) which were crystallized from MeOH/diisopropyl ether. Major isomer **6a:** (25 mg, 13%); mp 145–150 °C (decomp.); $[\alpha]_D^{22}$ −32° (c = 1.0, MeOH). For ^{13}C NMR data see Table 1. Anal calcd. for $C_{34}H_{45}N_5O_{11}$: C, 58.36; H, 6.48; N, 10.01. Found: C, 58.45; H, 6.71; N, 9.95. Minor isomer **6b** (trifluoroacetate salt): (21 mg, 10%); mp 161–166 °C (decomp.); $[\alpha]_D^{22}$ −29° (c = 1.0, MeOH). For ^{13}C NMR data see Table 1. Anal calcd. for $C_{34}H_{45}N_5O_{11} \cdot CF_3COOH$: C, 53.13; H, 5.70; N, 8.61. Found: C, 53.21; H, 5.92; N, 8.67.

N-*{[2-(*D-gluco-*Pentitol-1-yl)-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl]}acetylglycyl*-L-*phenylalanyl*-L-*leucine (***7a***)*

Compound **5a** (40 mg, 0.057 mmol) was dissolved in 0.1 M NaOH (4 ml) and the solution was stirred at room temperature for 5 min. The pH was adjusted to 5.5 by addition of 0.1 M HCl and the solution was applied to a Sephadex

Table 1. ^{13}C chemical shifts (δ, ppm) of imidazolidinone compounds **5, 6, 7, 8** and **10**[a]

Residue	*Carbon atom*[b]	**5a**	**5b**	**6a**	**6b**[c]	**7a**	**7b**	**8a**	**8b**	**10**
Imidazolidinone ring	2	73.7	74.0	73.6	73.5	72.6	73.4	74.9	73.2	72.7
	4	59.3	58.3	59.4	59.4	59.1	58.4	59.1	58.0	59.0
p-hydroxybenzyl	a	34.3	34.8	34.5	35.6	34.2	35.3	34.3	34.4	34.4
	b	126.3	125.9	126.4	127.2	126.3	—[d]	126.4	125.9	126.3
	c	130.5	130.5	130.5	130.3	130.4	130.5	130.4	130.5	130.4
	d	115.4	115.7	115.5	115.4	115.5	115.6	115.5	115.4	115.5
	e	156.6	156.8	156.6	156.5	156.6	156.7	156.6	156.9	156.6
N^1-CH_2	f	43.2	44.7	43.7	45.7	42.8	44.3	42.8	43.6	42.7
Sugar moiety	1′	67.2	71.0	66.9	65.5[e]	68.3	70.1	65.3	—[d]	68.3
	2′,3′,4′	69.4	68.4	68.5	68.7	69.1	70.6	69.1	68.5	69.2
		71.7	68.7	69.3	69.1	70.9	70.9	69.5	69.1	70.8
		72.3		71.0	71.3	71.1	71.2	70.9	69.2	71.1
	5′	66.8	67.1	64.1	65.0	63.3	63.2	63.0	62.9	63.2
Gly	α	42.2	41.7	42.4	42.1	41.8	41.7	41.8	41.7	41.8
Phe	α	54.9	54.6	54.0	53.6	53.8	53.7	53.8	53.8	53.7
	β	37.2	37.4	37.3	38.5	37.8	37.8	37.8	37.9	37.8
	γ	137.9	138.5	138.1	138.2	138.0	138.0	138.1	138.1	138.0
	δ	129.3	129.3	129.3	129.7	129.5	129.5	129.5	129.6	129.5
	ε	128.5	128.4	128.4	128.2	128.3	128.3	128.3	128.4	128.3
	ζ	126.7	126.6	126.6	126.5	126.5	126.6	126.6	126.6	126.6
Leu	α	50.8	51.2	51.4	51.7	50.5	50.5	50.5	50.5	50.5
	β	39.6	39.6	38.7	38.8	39.6	39.6	39.9	38.8	39.7
	γ	24.2	24.2	24.2	24.2	24.4	24.4	24.4	24.4	24.3
	δ	21.4	21.3	21.4	21.2	21.5	21.4	21.5	21.5	21.4
	δ′	23.1	23.0	23.0	22.8	22.9	22.9	22.9	23.0	22.8
$COOCH_3$										52.0

[a]In DMSO-d_6 at 25 °C; [b]See Scheme 1 for carbon atom numbering; [c]Trifluoroacetate salt; [d]Not assigned; [e]Uncertain assignation.

G-15 column (30 × 1.5 cm) and eluted with aq. 1% acetic acid. Fractions containing compound **7a** were pooled, evaporated and purified by semipreparative RP HPLC using 47% MeOH/0.1% TFA as the eluent to give imidazolidinone compound **7a** (retention time:18.3 min) (34 mg, 83%) which was crystallized from MeOH/diethyl ether; mp 133–138 °C (decomp.); $[\alpha]_D^{22}$ −32° (c = 1.0, MeOH). For ^{13}C NMR data see Table 1. Anal calcd. for $C_{34}H_{47}N_5O_{12}$: C, 56.89; H, 6.60; N, 9.76. Found: C, 56.82; H, 6.81; N, 9.80.

N-*{[2-(*D-gluco-*Pentitol-1-yl)-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl]}acetylglycyl*-L-*phenylalanyl*-L-*leucine (***7b***)*

Compound **5b** (30 mg, 0.043 mmol) was treated in the same way as described for compound **7a** to give pure imidazolidinone **7b** (retention time: 17.6 min) (24 mg, 77%) which was crystallized from MeOH/diethyl ether; mp 125–135 °C (decomp.); $[\alpha]_D^{22}$ −42° (c = 1.0, MeOH). For ^{13}C NMR data see Table 1. Anal calcd. for $C_{34}H_{47}N_5O_{12}$: C, 56.89; H, 6.60; N, 9.76. Found: C, 56.70; H, 6.55; N, 9.62.

N-*{[2-(*D-galacto-*Pentitol-1-yl)-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl]}acetylglycyl*-L-*phenylalanyl*-L-*leucine (***8a***)*

Compound **6a** (27 mg, 0.038 mmol) was treated in the same way as described for compound **7a** to give pure imidazolidinone **8a** (retention time: 17.4 min) (26 mg, 96%) which was crystallized from MeOH/diethyl ether; mp 102–108 °C (decomp.); $[\alpha]_D^{22}$ −33° (c = 1.0, MeOH). For ^{13}C NMR data see Table 1. Anal calcd. for $C_{34}H_{47}N_5O_{12}$: C, 56.89; H, 6.60; N, 9.76. Found: C, 56.91; H, 6.63; N, 9.86.

N-*{[2-(*D-galacto-*Pentitol-1-yl)-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl]}acetylglycyl*-L-*phenylalanyl*-L-*leucine (***8b***)*

Compound **6b** (24 mg, 0.034 mmol) was treated in the same way as described for compound **7a** to give pure imidazolidinone **8b** (retention time: 18.1 min) (23 mg, 96%) which was crystallized from MeOH/diethyl ether; mp 142–147 °C (decomp.); $[\alpha]_D^{22}$ −50° (c = 1.0, MeOH). For ^{13}C NMR data see Table 1. Anal calcd. for $C_{34}H_{47}N_5O_{12}$: C, 56.89; H, 6.60; N, 9.76. Found: C, 56.75; H, 6.80; N, 9.69.

Intramolecular cyclization/transesterification of monosaccharide ester **1** *in the presence of base*

To a solution of compound **1** (50 mg, 0.07 mmol) in MeOH (83 ml), *N*-ethylmorpholine (NEM) (88 µl, 0.70 mmol) was added. The solution was incubated at 60 °C in a round-bottom flask for 8 days. The solvent was evaporated and the residue was purified by semipreparative RP HPLC by using 44% MeOH/0.1% TFA as the eluent. Purification afforded imidazolidinones **5a/5b** (6 mg, 12%), leucine-enkephalin methyl ester (**9**) (retention time: 30 min) (5 mg, 13%) and *N*-{[2-(D-*gluco*-pentitol-1-yl)-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl]}acetylglycyl-L-phenylalanyl-L-leucine methyl ester (**10**) (retention time: 37 min) (8 mg, 14%). Compounds **9** and **10** were crystallized from MeOH/diisopropyl ether. Compound **9:** mp 125–140 °C (decomp.); $[\alpha]_D^{22}$ +10° (c = 1.0, MeOH). ^{1}H and ^{13}C NMR spectra were identical with those previously reported in the literature [16]. Compound **10** (trifluoroacetate salt): mp 75–95 °C (decomp.); $[\alpha]_D^{22}$ −48° (c = 1.0, MeOH). For ^{13}C NMR data see Table 1. Anal calcd. for $C_{35}H_{49}N_5O_{12} \cdot CF_3COOH$: C, 52.54; H, 5.96; N, 8.28. Found: C, 52.42; H, 5.88; N, 8.49.

RP HPLC analysis of the incubation mixtures of compounds **1** *and* **5a** *in the absence and presence of base*

Compound **1** (1.5 mg, 2.09 µmol) dissolved in dry MeOH (2.5 ml) was incubated at 60 °C in the absence or in the presence of NEM. The molar ratio of compound **1** to NEM was 1:1 or 1:10. For comparison, imidazolidinone compound **5a** (1.5 mg, 2.14 µmol) dissolved in dry MeOH (2.5 ml) was also incubated under the identical reaction conditions. The progress of the reaction was monitored on the aliquots removed every 24 h from the incubation mixtures over the period of 8 days. The respective samples were directly analysed by RP HPLC by using 44% MeOH/0.1% TFA as the eluent. The obtained results are presented in Figure 3.

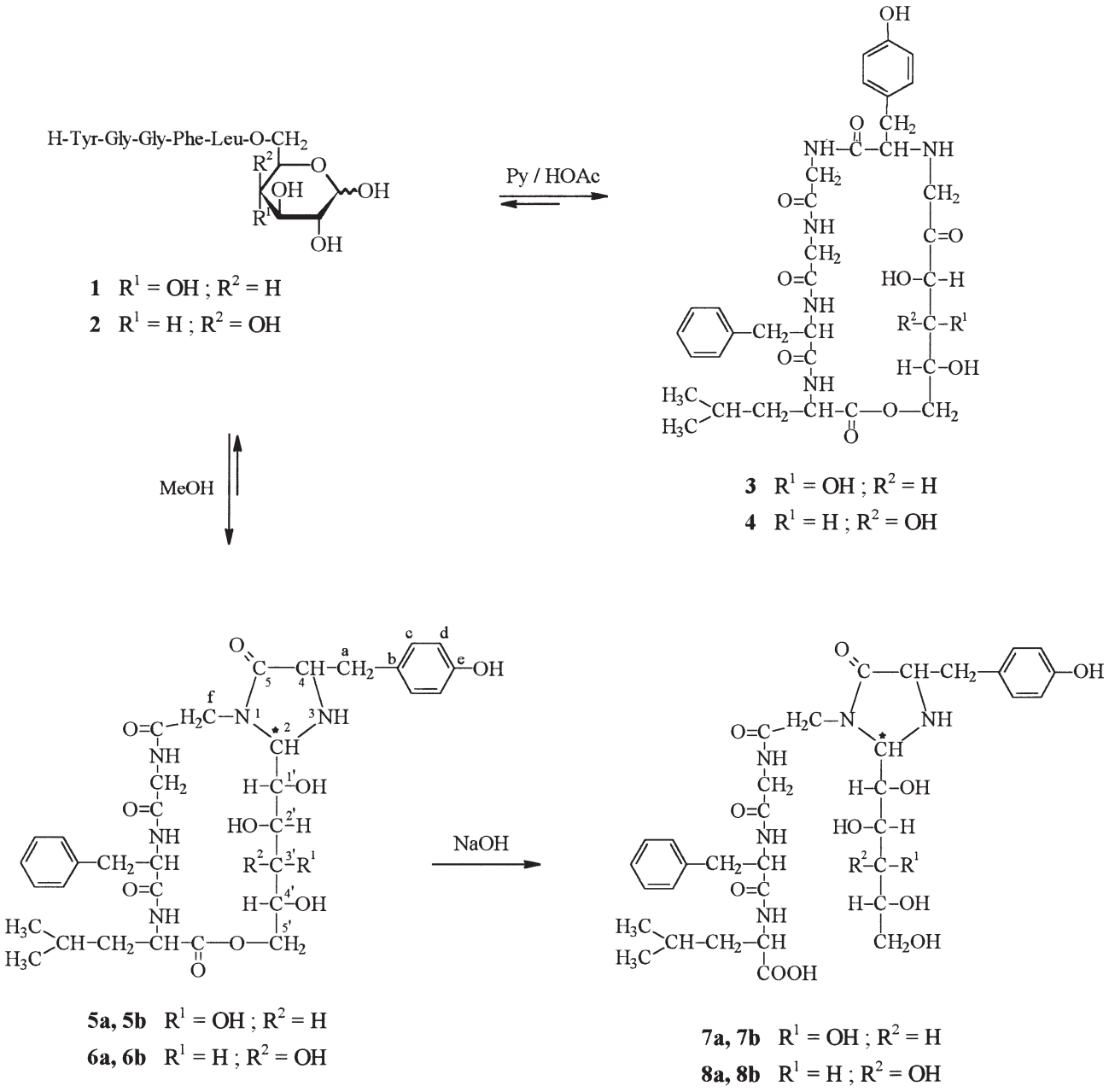
Results and discussion

The monosaccharide esters of the opioid pentapeptide leucine-enkephalin related to D-glucose (**1**) and D-galactose (**2**) were obtained from the corresponding free sugars through the stepwise elongation of the peptide chain and the subsequent RP HPLC purification [15].

When dissolved in dry methanol and incubated at 60 °C for several days esters **1** and **2** were transformed to the imidazolidinone derivatives **5** and **6,** respectively (Scheme 1). By the intramolecular cyclization of compound **1** a mixture of imidazolidinone isomers was obtained in 3 days which required RP HPLC semipreparative chromatography to be separated. The major product isolable in the yield of 35% is the bicyclic imidazolidinone **5a,** with the respective **5b** (10%) as the minor component. Under identical reaction conditions D-galactose-related ester **2** after 2 days gave following RP HPLC isolation imidazolidinones **6a** (13%) and **6b** (10%).

The four isomers of imidazolidinones **5** and **6** were characterized by NMR spectroscopy. The ^{13}C NMR assignments are summarized in Table 1. Designations of the particular atoms are given on Scheme 1. The chemical shifts of the C-2 atoms found in the region of 73–74 ppm in all spectra are typical for a carbon atom bonded to two nitrogen atoms, as in the imidazolidinone structure. Similar ^{13}C resonances were observed for the imidazolidinone adducts of hemoglobin and related peptides with acetaldehyde [17]. The resonances in the carbohydrate region (65–72 ppm) are in agreement with data for the acyclic sugar pentitol derivatives with D-*gluco* and D-*galacto* structures [18]. Considering the peptide parts of imidazolidinone isomers **5** and **6,** the ^{13}C resonances for identical amino acid residues (Gly, Phe, Leu) are generally very similar and in accord with those reported for enkephalin-related glycoconjugates [12, 15]. The full ^{13}C NMR data firmly establish the structures of compounds **5** and **6** as imidazolidinone derivatives, however, the small differences in chemical shifts observed between C-2 and C-4 imidazolidinone ring carbon atoms as well as for the C-f and C-1′ atoms, provide support for the assumption that each pair of isomers studied (**5a/5b, 6a/6b**) are diastereomers having the *cis* and *trans* relative geometry of the carbon substituents at the imidazolidinone ring moiety (Figure 1). Further NMR investigations are needed to determine the relative configurations of imidazolidinones **5a,5b** and **6a,6b** from nuclear *Overhauser* effects.

Considering the mechanism of the formation of imidazolidinones **5** and **6,** a diastereoisomeric mixture is expected, since the course of **1**→**5** and **2**→**6** transformation is rationalized as follows. In the first step, similarly to the formation of Amadori products **3** and **4** from esters **1** and **2** [12], the aldehyde group of the open-chain form of the carbohydrate moiety is attacked by the free amino terminus of the peptide moiety. The cyclic Schiff base formed, instead of Amadori rearrangement to the corresponding keto-sugar, undergoes nucleophilic attack by the Gly^2 nitrogen to yield imidazolidinones **5** and **6,** in which a new N,N′-acetal centre is formed. The conversion of D-glucose-related ester **1** to the corresponding imidazolidinone isomers **5a** and **5b** exhibited a moderate degree of stereocontrol. As evidenced by RP HPLC, after equilibrium was reached, imidazolidinones **5a** and **5b** were obtained in a 2.7:1 diastereoisomeric ratio. On the contrary, the equilibrated solution of D-galactose-related ester **2** contained a 1.3:1 diastereomeric mixture of imidazolidinones **6a** and **6b,** indicating that the ring closure occured with almost equal facility to either the *Re* or the *Si* face of the initially formed Schiff base. The higher diastereoselectivity observed in imidazolidinone ring formation from ester **1,** as compared to the cyclization of compound **2,** is difficult to rationalize at this moment. We can hypothesize that the



Scheme 1. Asterisk indicates either *(S)*- or *(R)*-configuration at the N,N′-acetal centre.

configuration of the HO-4 group in the parent monosaccharide ester **1** should impose some limits to the conformational freedom in the reactive D-*gluco*-related species leading to more efficient stereochemical control.

Incubation of either **1** or **2** in methanol yields only ~5% of the corresponding Amadori products **3** or **4,** while the yields of **3** and **4** in incubations carried out in pyridine-acetic acid as the solvent are relatively high (**1**→**3,** 58%; **2**→**4,** 50%) [12]. It is generally believed that the Amadori rearrangement is a complex acid-base catalysed reaction in which the balance of the acidity and basicity in the reaction system controls the simultaneous and consecutive reactions [3]. The Schiff base formation was assumed to be the rate-determining step for the Amadori rearrangement, mainly determined by the molecular structures of the reactants and some environmental conditions that affect the structures of the reactants such as pH [19]. Thus, pyridine-acetic acid appears to be an ideal solvent for the Amadori rearrangement catalysing all transformation steps to the keto-sugar derivatives **3** and **4**. Results obtained from the rearrangements of compounds **1** and **2** carried out in methanol as the solvent, suggest the participation of metha-

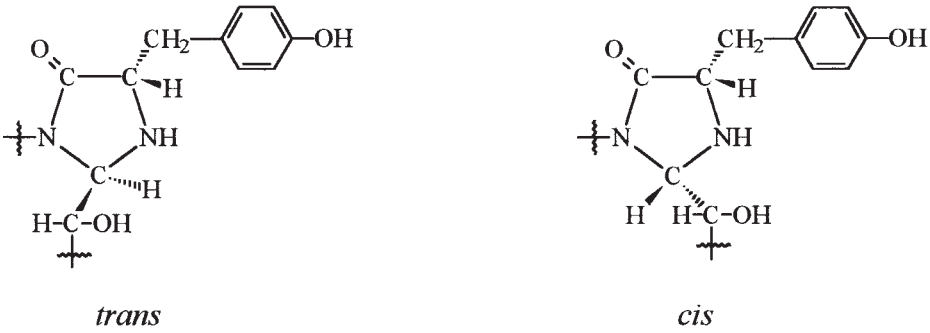


Figure 1. Relative geometry of the substituents at the imidazolidinone ring moiety.

nol in the imidazolidinone ring-closing reaction by formation of a proton-transfering (both donation and abstraction) transition state. It is to be emphasized that the results obtained with the leucine-enkephalin-related monosaccharide esters **1** and **2** demonstrate for the first time that, from the same substrate, in addition to Amadori rearrangement, an alternative pathway for carbohydrate-induced modification of peptides is possible yielding imidazolidinones from the initially formed Schiff bases.

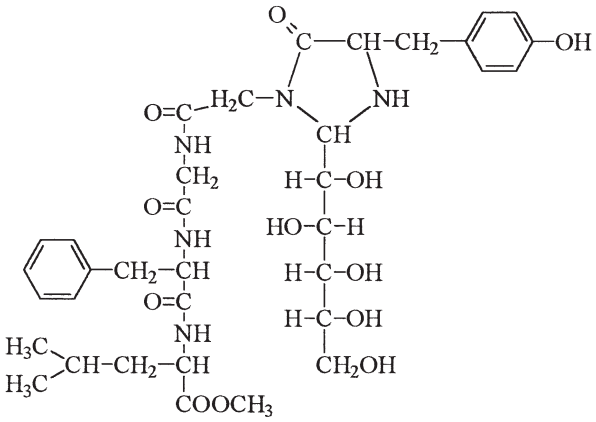
Cleavage of the ester bonds in both the major and minor isomers of compounds **5** and **6** was carried out in 0.1 M NaOH at room temperature and led to the corresponding D-*gluco*- (**7a, 7b**) and D-*galacto*-related imidazolidinones (**8a, 8b**) (Scheme 1) in 77–96% yield after RP HPLC chromatography.

The ^{13}C NMR assignments of the four imidazolidinone isomers **7a, 7b, 8a** and **8b** are summarized in Table 1. In the NMR spectra of the deesterified imidazolidinones the resonances for the primary carbon atoms (C-5′) of the sugar residues displayed characteristic upfield shifts owing to the loss of the deshielding effect of the acyl (peptidyl) residue. All other ^{13}C resonances were in agreement with a monocyclic structure composited of a trisubstituted imidazolidinone ring core.

The imidazolidinone compounds **7** and **8** described in this report, demonstrated similar stability during isolation and characterization as Amadori rearrangement products obtained from the reaction of leucine-enkephalin with either D-glucose or D-galactose [12]. This observation, and the fact that the process of the nonenzymatic glycation begins by the reaction of reducing sugar with protein amino group(s) to form Schiff bases, points to the possibility that, depending on the physiological environment, similar carbohydrate-related imidazolidinones may be also generated in the early stage of the Maillard reaction *in vivo*.

As the basicity of the N-terminus in peptides can be considered to be a measure for its nucleophilicity, we next examined the chemistry of **1**→**5** rearrangement in the presence of added base. Thus, D-glucose-related ester **1** was incubated in methanol at 60 °C in the presence of *N*-ethylmorpholine (NEM) (10 eqv) for a period of 8 days. As evidenced by RP HPLC, incubation mixture contained, in addition to a diastereoisomeric mixture of imidazolidinones **5a**/**5b,** two newly formed products which were isolated and characterized as leucine-enkephalin methyl ester H-Tyr-Gly-Gly-Phe-Leu-OCH_3, **9** (13%) and as imidazolidinone methyl ester derivative **10** (14%) (Figure 2). The ^1H and ^{13}C NMR data obtained for compound **9** were indistinguishable from the chemical shift data for leucine-enkephalin methyl ester reported in the literature [16]. The ^{13}C chemical shifts of the imidazolidinone-related methyl ester **10,** summarized in Table 1, are almost identical to those of the imidazolidinone compound **7a,** thus clearly indicating bicyclic compound **5a** as the precursor of the methyl ester **10** in the transesterification reaction.

In order to gain a better understanding of the different parameters influencing the conversion of the ester **1** to the imidazolidinones **5** as well as to evaluate the effects of NEM on the formation of transesterification products **9** and **10,** compound **1** was incubated in methanol at 60 °C in the absence and presence of either one or ten equivalents of added NEM. For comparison, to examine the pos-



10

Figure 2. Structure of the transesterification product **10.**

sible effects of NEM on the stability of the imidazolidinone ring, compound **5a** was also incubated in methanol under the same reaction conditions. Figure 3 depicts the graphical representation of the RP HPLC concentration profiles of the incubation mixtures obtained after different intervals of time in the absence and presence of base, for both the monosaccharide ester **1** and imidazolidinone compound **5a.**

The data presented in Figure 3 show that addition of one equivalent of the base to the incubation mixture of the ester **1** did not significantly influence the formation of imidazolidinone **5a** whereas the relative concentration of **5b** was decreased. With ten equivalents of NEM added, the relative concentrations of both **5a** and **5b** had slightly decreased after 3 days, however, after 8 days of incubation the amount of the imidazolidinones **5** had markedly decreased

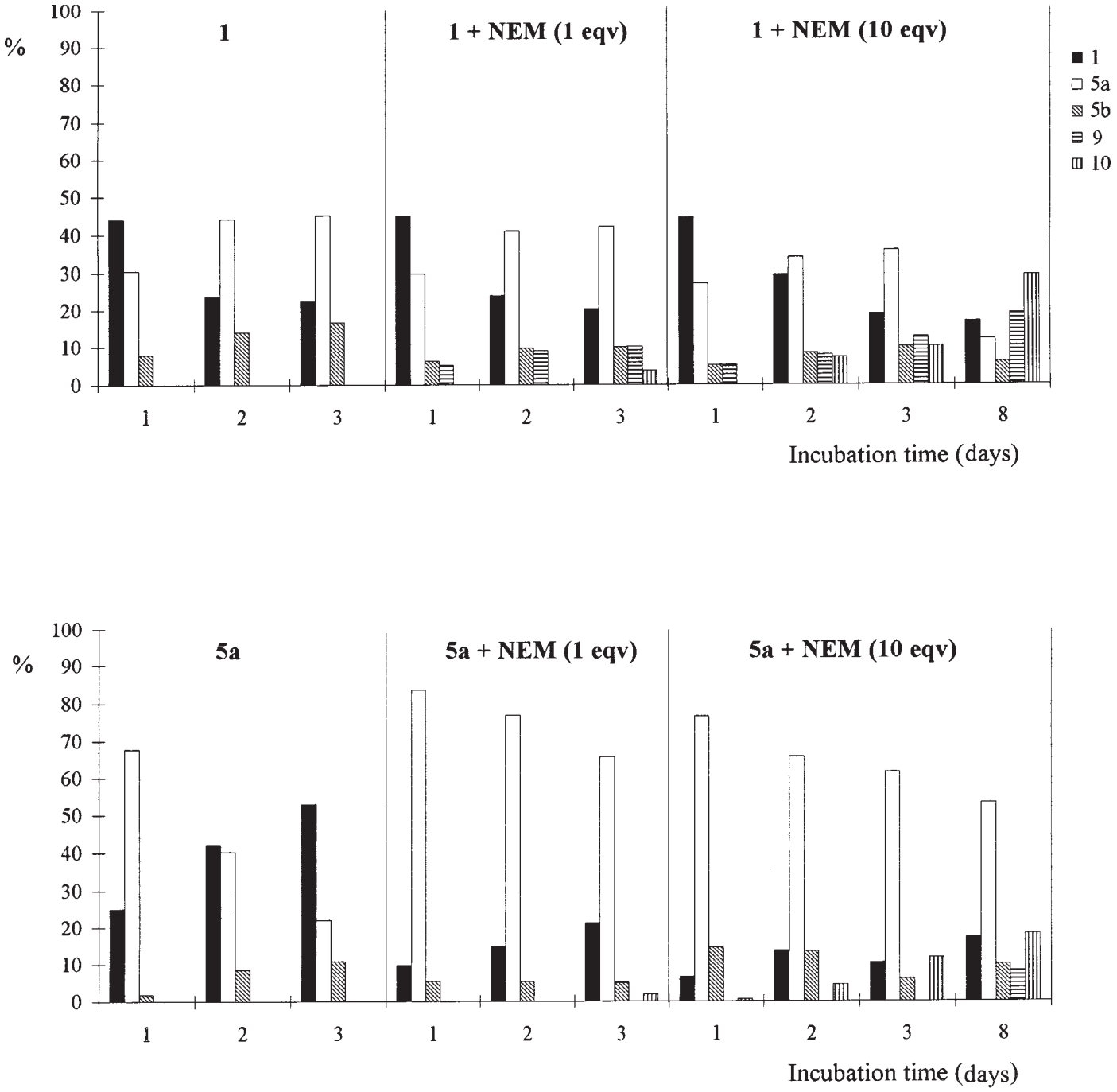


Figure 3. Relative distribution of the products formed vs time by the incubation of compounds **1** and **5a** in MeOH at 60 °C in the absence and presence of *N*-ethylmorpholine (NEM). The relative concentrations were determined by RP HPLC (see Materials and methods).

due to the extensive formation of the transesterification products **9** and **10.**

Imidazolidinone **5a** when incubated in methanol without added base shows, after 3 days at 60 °C, 53% of the starting compound to be converted to the glucose-related ester **1** and 11% to the corresponding imidazolidinone isomer **5b.** This finding confirms the reversibility of the **5a** to **1** transformation reaction and indicates that the Schiff base is the reactive intermediate in the solution which either dissociates into monosaccharide ester **1** or the ring closure to imidazolidinone occurs resulting in the compounds **5a** and **5b,** respectively. As shown in Figure 3, the stability of the imidazolidinone compound **5a** was significantly greater when one or ten equivalents of the base were added to the incubation mixture. It has been reported that the hydrolysis of imidazolidinones show a sigmoidal pH-rate profile [20,21]. The mechanism proposed involves as a rate-determining step imidazolidinone ring N-C bond cleavage with formation of an amide anion and an immonium ion. In subsequent steps, a water molecule transfers a proton to the amide ion and a hydroxide ion to the immonium ion, giving a carbinolamine which decomposes to the corresponding carbonyl compound and amine. Based on these results, it is conceivable to assume that the presence of the proton-abstracting base (NEM) in the incubation mixture significantly inhibits the proton-transfer step(s) thus increasing the stability of the imidazolidinone ring core in **5a.** Comparison of the results obtained for ester **1** and imidazolidinone **5a,** when incubated in the presence of base, firmly establish monosaccharide ester **1** as the precursor of leucine-enkephalin methyl ester (**9**) in the transesterification reaction.

Taken together, the reported results on the intramolecular rearrangements of the endogenous opioid pentapeptide-related monosaccharide esters strongly suggest that hexose-related imidazolidinones should be considered, in addition to Amadori rearrangement products, as intermediates in the early stages of the Maillard reaction between reducing sugars and the amino groups of peptides or proteins.

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