CD and FTIR spectroscopic studies of Amadori compounds related to the opioid peptides

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Circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy have been used to investigate conformational effects of glycation on the secondary structure of opioid peptide Leu-enkephalin and on structurally related peptides in 2,2,2-trifluoroethanol (TFE) solution. CD spectral analysis of Leu-enkephalin-related Amadori compounds revealed that attachment of the protected or free sugar may influence not only the distribution of the backbone but also the side-chain conformation of the Tyr moiety. The amide I region of the FTIR spectra analysed by self-deconvolution and curve-fitting methods revealed that Leu-enkephalin is present as a mixture of β-sheet and γ-turn conformers in TFE solution, while its methyl ester likely adopts a β-turn conformation. FTIR spectroscopy has shown that no major spectral changes occur in the peptide part of glycated (Amadori) compounds as compared to parent peptides. The structurally related Tyr-Gly-Gly tripeptide derivatives contain amide I components at *ca.* 1630 and *ca.* 1645 cm^{-1} consistent with the presence of γ-turns with strong and weak 1 ⟵ 3 H-bondings, respectively. The attachment of the protected or free sugar moiety to pentapeptides appears to destabilize β-turns but not to affect H-bonded γ-turns. In the spectra of Amadori compounds containing a free sugar moiety, the component band at *ca.* 1730 cm^{-1} suggests the presence of the open-chain sugar form. Based on the studies presented herein, FTIR spectroscopy is shown to be a powerful tool for the structural analysis of glycated peptides, in particular for the detection of the keto form of the sugar and turn conformations of the peptide part of the molecule.

Introduction

The process involving the nonenzymatic modification of various proteins by physiological sugars (Maillard reaction) *in vivo* has been associated with the progression of many long term complications of diabetes, renal disorders and normal ageing.[1,2] This modification involves covalent binding of reducing sugars, such as glucose, to primary amino groups of proteins forming 1-amino-1-deoxy-ketose derivatives by Amadori rearrangement. Amadori adducts then slowly undergo further intramolecular rearrangement to produce advanced glycation end products (AGEs) which are responsible for the modification of circulating and tissue proteins that lead to structural and functional changes.[3,4]

The chemistry of Maillard transformations in biological systems is extremely complex and yet poorly understood; thus synthetic Amadori compounds are considered to be effective tools for investigating the impact of glycation on the biological, physical and chemical properties of protein fragments. Although many studies dealing with the synthesis and properties of Amadori derivatives of amino acids have been published,[5] there are only a few reports referring to the synthesis of peptide-related Amadori compounds.[6–8]

In an effort to better understand the role that Amadori compounds may play in biologically relevant interactions we have recently reported[9] the synthesis, analytical characterization and ^{13}C NMR analysis of Amadori compounds related to the endogenous opioid peptide Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu), involved in numerous physiological functions.[10] It is reasonable to suppose that glycation of the *N*-terminal amino group of the tyrosine residue will affect the biological activity of Leu-enkephalin since it was shown that after *N*-alkylation of enkephalins, depending on substitution pattern, activity may be enhanced, reduced, eliminated or even reversed to give antagonists.[11] Although there have been many contributions in the last few years which demonstrate that nonenzymatic glycation leads to functional changes in the affectedproteins,[1–4] little is known of how the attached carbohydrate molecule influences the conformational properties of the *N*-alkylated protein.

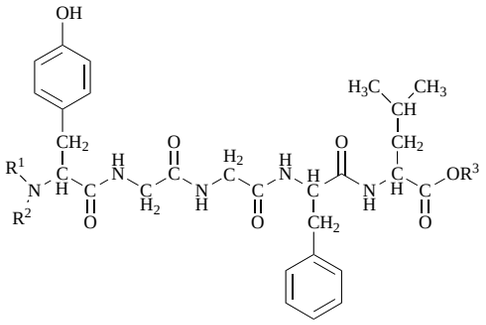
To address this question, circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopic studies were performed on Leu-enkephalin-related Amadori compounds. FTIR spectroscopy has been shown to be a sensitive tool for providing information on the secondary structure of proteins in solution.[12–14] This method has also been used for acquiring information about the structure of linear[15,16] and cyclic peptides.[17] An important advantage of FTIR spectroscopy is that it provides information on both the side-chain functionalities[18] and backbone conformation in terms of changes of pH, solvation or addition of metal ions, detergents *etc.*[14,19,20,23–25]

This is the first report on the chiroptical and vibrational spectroscopic characterization of well-defined Amadori compounds related to biologically active opioid peptides. In order to detect differences caused by the presence of sugar, we investigated protected (**3**–**5**,**9**,**10**) and unprotected (**6**,**7**,**11**) *N*-glycated derivatives in comparison with the corresponding parent peptides (**1**,**2**,**8**). *N*,*N*-Dialkylated Amadori compounds **4** and **10** were selected on the basis of the observation[21] that reactive aldehydes formed *in vivo* by oxidation and decomposition of Amadori products may further react with amino acid residues in proteins to form cross-linkages.

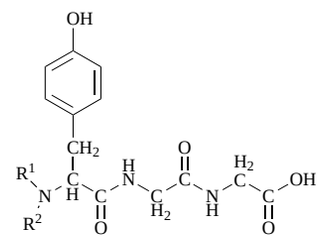
Results and discussion

CD studies

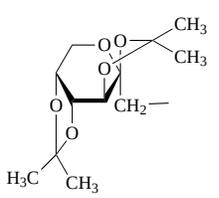
The dependence on solvent, pH and cations of the CD spectra



		R^1	R^2	R^3
1	YGGFL	H	H	H
2	YGGFL-OMe	H	H	Me
3	P-YGGFL	P	H	H
4	P-MeYGGFL	P	Me	H
5	P-YGGFL-OMe	P	H	Me
6	A-YGGFL	A	H	H
7	A-YGGFL-OMe	A	H	Me

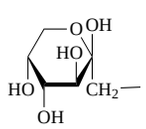


		R^1	R^2
8	YGG	H	H
9	P-YGG	P	H
10	P-MeYGG	P	Me
11	A-YGG	A	H



P

1-deoxy-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranos-1-yl



A

1-deoxy-β-D-fructopyranos-1-yl

of enkephalins has been studied (for a review see ref. 22). In aqueous solution small peptides are expected to be present as a mixture of conformers. Fig. 1 shows the effect of Amadori glycation on the CD spectra in 2,2,2-trifluoroethanol (TFE) solution. TFE is well-known for shifting the conformational equilibrium of peptides towards the adoption of folded conformers stabilized by intramolecular H-bondings.[29,33] The far-UV region of the spectra is contributed to by both the backbone amides and the side-chain chromophores of Tyr and Phe. The CD spectra of YGGFL (**1**) and its methyl ester **2** are marked by two positive bands (the high noise level obscures an analysis of the spectra below *ca.* 200 nm). The increased intensity of the band between 200–210 nm in the CD spectrum of **2** may be the sign of a shift of the conformational equilibrium towards folded structures. Linkage of the protected sugar (**3**) has a marked spectral effect suggesting a significantly different conformer distribution. The Amadori compound **6** shows a CD spectrum intermediate between those of **1** and **3**. Apparently, the attachment of the protected or free sugar may influence not only the distribution of backbone conformers but also the side-chain conformer distribution of Tyr. This effect is clearly reflected in the aromatic ^1L_b region (Fig. 1, inset) which, upon attachment of the sugar, changes sign. The CD spectra (not

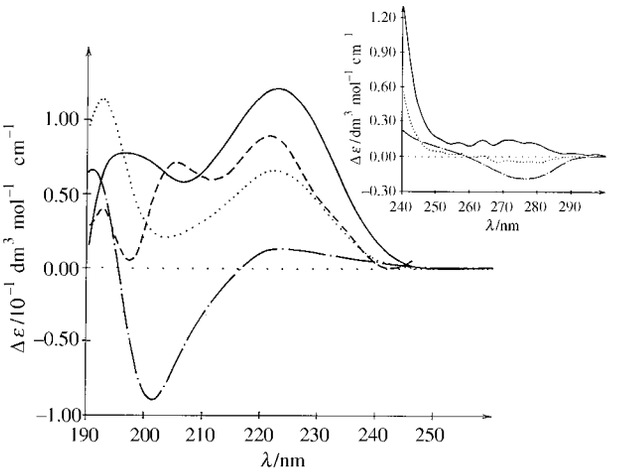


Fig. 1 Circular dichroism spectra in TFE of YGGFL (**1**, $c = 0.49$ mM) (—); YGGFL-OMe (**2**, $c = 0.29$ mM) (----); P-YGGFL (**3**, $c = 0.50$ mM) (—·—); and A-YGGFL (**6**, $c = 0.88$ mM) (……). Inset: The near-UV region of the CD spectra of **1** (—), **3** (—·—) and **6** (……); concentration as above.

shown) of the tripeptides are dominated by the side-chain contribution of Tyr. Briefly, in the case of short-chain aromatic peptides such as enkephalins CD spectroscopy is diagnostic of conformational changes but cannot discriminate between aromatic and conformational effects.

FTIR studies

The FTIR absorption and corresponding Fourier self-deconvolution (FSD) spectra of **5** and **11** in TFE solution are presented in Fig. 2. Table 1 lists component band frequencies and intensities in the 1500–1730 cm^{-1} region as determined by curve-fitting analysis and FSD. Band assignment is summarized in Table 2.

In the curve-fitted FTIR spectra of compounds **1**–**11** the strongest amide I component band appears at 1668–1678 cm^{-1}. Another strong band appears at 1655–1665 cm^{-1}. The latter component is of exceedingly high intensity in the spectra of compounds **1**–**5**. As previously observed for short peptides,[15,29] bands appearing in TFE at *ca.* 1675 cm^{-1} can be attributed to free or weakly solvated amide carbonyls. However, in the case of trifluoroacetate (TFA^-) salts this band is strongly contributed to by the ν_{as} stretching of the carboxylate of TFA^-. The peptide chain of the studied compounds is rather short and comprises two helix-breaker Gly residues. Thus, the adoption of an α-helix can be excluded but the occurrence of 3_{10} helical conformers is possible in pentapeptides **1**–**7** (*cf.* Tables 1 and 2). However, the most probable explanation for the appearance of two intense high-frequency (>1655 cm^{-1}) amide I components in the spectra of TFA^- free compounds is that the majority of amide groups are exposed to the TFE solvent but the solvational state of the peptide backbone is different in folded and open conformers.

A strong low-frequency amide I component between 1620–1640 cm^{-1} is diagnostic of a β-sheet (β-strand) conformation of polypeptides in D_2O solution.[13,14,23–25] An accompanying band at *ca.* 1690 cm^{-1}, suggesting amide I splitting, is a sign of antiparallel orientation of β-strands.[12] For Leu-enkephalin (**1**) the concentration dependence of its CD spectrum also supports the assignment of the band near 1630 cm^{-1} to a β-sheet conformation which, in this compound, can be stabilized by attractive electrostatic head-to-tail interactions.

In TFE the 1640–1600 cm^{-1} region features amide I components which can also be associated with the acceptor C=O group of $1 \longleftarrow 4$ (C_{10}) H-bonded β-turns or $1 \longleftarrow 3$ (C_7) H-bonded γ-turns. The assignment given in Table 2 is based on comparative NMR and FTIR spectroscopic studies on a great number of cyclic[17,26–28] and linear peptides[15,16] (for a recent

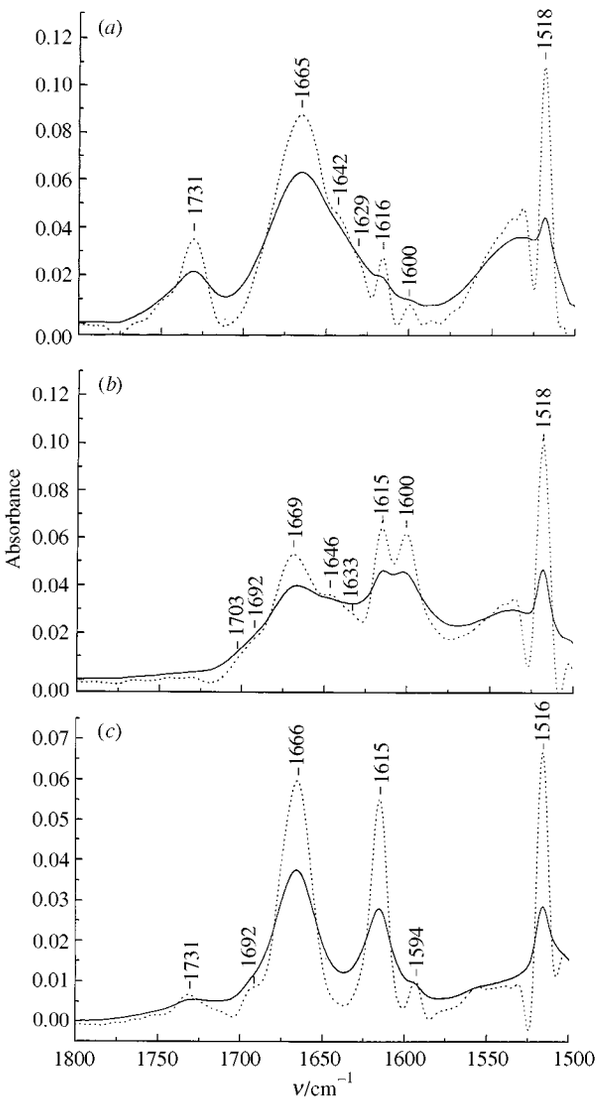


Fig. 2 The 1500–1800 cm^{-1} region of the FTIR spectra of (*a*) P-YGGFL-OMe (**5**) in TFE; (*b*) A-YGG (**11**) in TFE; and (*c*) A-YGG (**11**) in DMSO. Experimental spectra (—); FSD spectra (.....) (see also Table 1).

review see ref. 29). The 'non-acceptor' amide groups of turns also absorb in the high-frequency (>1650 cm^{-1}) amide I region. However, the occurrence and position of one or more high-frequency components is diagnostic of neither the presence nor type of turn, because absorption between 1660 and 1690 cm^{-1} is also influenced by nonconformational factors such as transition dipole coupling, solvation (see earlier) or nonplanarity of the amide groups.[23–25] Thus, Leu-enkephalin **1** is present as a mixture of β-sheet, γ-turn, semi-open and open conformers in TFE solution, while its methyl ester **2** probably adopts a β-turn conformation (strong acceptor band at 1636 cm^{-1}).[15] Structural analysis has demonstrated the flexibility of Leu-enkephalin (for recent reviews see refs. 30, 31), thought to be responsible for nonspecific binding to opioid receptor sites.

The FTIR spectra of the protected (**3**–**5**,**9**,**10**) and unprotected Amadori compounds (**6**,**7**,**11**) show possible turn-acceptor amide I component bands near 1630, 1640 and 1645 cm^{-1} in TFE. (The protected sugar moiety does not have a vibrational contribution in this region.) In the case of pentapeptide derivatives with a free C-terminus (**3**,**4**,**6**) the decreased relative intensity of the component at *ca.* 1630 cm^{-1} may indicate the breakage of the β-sheet conformation of **1** but the preservation of the H-bonded γ-turn structures (Table 1).

In TFE the β-turn acceptor band in a series of linear *N*-protected opioid peptides with NMR-based β-turn structure was found between 1635 and 1640 cm^{-1}.[15] This allows us to assign component bands at 1635 (**6**), 1636 (**2**) and 1641 cm^{-1} (**5**) to 1 ⟵ 4 H-bonded β-turns. The assignment of the component at 1633 cm^{-1} in **7** is uncertain. Due to poor separation of the frequency regions (Table 2), an amide I component band between *ca.* 1644 and 1650 cm^{-1} is taken as a sign of the presence of semi-open conformers with a weak 1 ⟵ 4 or 1 ⟵ 3 H-bonding. The tripeptide derivatives **9**–**11** are not long enough to adopt a β-turn conformation. In agreement with this, their spectra contain components at *ca.* 1630 and *ca.* 1645 cm^{-1} which are consistent with the presence of γ-turns with strong and weak 1 ⟵ 3 H-bondings, respectively.[26–28] In the case of unprotected Amadori compounds **6**, **7** and **11** the latter band may also be contributed to by the enediol form, which, for D-fructose, gives a band at 1647 cm^{-1} in D_2O solution.[32] The turn assignment given in Table 2 is also supported by the FTIR spectra measured in dimethyl sulfoxide (DMSO) (Fig. 2) which show no or only low-intensity amide I component bands below *ca.* 1645 cm^{-1}. DMSO is known to be a solvent which destroys weak H-bondings.[33] The sharp component band at 1615 cm^{-1} is due to ring vibration of Tyr (Table 2). The NMR study of Tyr-Gly-Gly **8** suggested at least two conformations (random coil, γ-turn) for this tripeptide in H_2O–D_2O solution.[34]

The 1750–1700 cm^{-1} range of the IR spectra of compounds **1**–**11** features $\nu_{C=O}$ bands. The spectra of the ester derivatives **2**, **5** and **7** contain a strong or medium intensity component at 1729–1730 cm^{-1}. The position of this band is confirmed by FSD (Fig. 2). This component is assigned to the stretching mode of the ester group. The ν_{as} carboxylate band turns up in the amide II region, below *ca.* 1600 cm^{-1}. As reported earlier,[35] the protonation equilibrium in TFE is not shifted completely towards the zwitterionic form: NH_2-peptide-COOH ⇌ $^+NH_3$-peptide-COO^- ⇌ $^+NH_3$-peptide-COOH. In the case of trifluoroacetate salts **6**, **7** and **11**, the IR spectra of which are dominated by the strong ν_{as} band of CF_3COO^- at 1675 cm^{-1}, a positively charged species with a free COOH function also appears. The $\nu_{C=O}$ band of the COOH group is seen between *ca.* 1720 and 1690 cm^{-1}, depending on the average strength of H-bondings.[36] The intensity of the COOH $\nu_{C=O}$ band is rather low in the spectra of all non-ester derivatives. This is an indication of the predominance of conformers with COO^- at the C-terminus. In the spectra of unprotected Amadori compounds **6** and **11** the components at 1727 and 1728 cm^{-1}, respectively, suggest the presence of the open chain sugar form. Evidence for the presence of the keto form of D-fructose in solution has recently been provided by FTIR spectroscopy, the $\nu_{C=O}$ band of the open-chain form being found at 1728 cm^{-1} in aqueous solution.[32] In the spectrum of A-YGGFL-OMe **7** the weak keto band is covered by the $\nu_{C=O}$ band of the ester group at 1730 cm^{-1} but the band at 1647 cm^{-1} (enediol[32]) suggests a contribution of the open sugar ring. In H_2O or D_2O solution the ratio of the keto and enediol forms, relative to the ring structure(s), was found to depend on temperature and concentration in addition to the structure of the sugar.[32,37] The highest equilibrium keto concentration (3% of the total concentration) was measured in DMSO by using ^{13}C NMR.[37] This finding is in good agreement with the FTIR spectrum of A-YGG (**11**) showing a relatively strong component band at 1729 cm^{-1} [Fig. 2(*c*)].

In summary, FTIR spectroscopy can be applied to the structural analysis of small-sized glycated linear peptides. The main power of this method is that it allows us to follow glycation-induced shifts of the conformer distribution of peptide backbone and to detect the presence of the keto form of the sugar moiety.

Experimental

General methods

Column chromatography was performed on Silica Gel (Merck, 0.040–0.063 mm). Melting points were determined in capillaries

Table 1 1500–1730 cm^{-1} region of the FTIR spectra (ν/cm^{-1}) of Amadori compounds and related peptides in TFE solution[a]

YGGFL **1**	YGGFL-OMe **2**	P-YGGFL **3**	P-MeYGGFL **4**	P-YGGFL-OMe **5**	P-YGG **9**	P-MeYGG **10**	A-YGGFL **6**	A-YGGFL-OMe **7**	A-YGG **11**
1519 (50)	1518 (47)	1518 (36)	1518 (32)	1518 (36)	1518 (21)	1518 (26)	1519 (39)	1519 (36)	1518 (42)
1519	1518	1518	1518	1518	1518	1518	1518	1519	1518
1531 (28)	1532 (29)	1532 (22)	1530 (17)	1530 (22)	1532 (8)	1532 (9)	1532 (20)	1532 (21)	1531 (17)
1533	1534	1532	1533	1531	1533		1535	1535	1534
1542 (21)	1544 (26)	1543 (21)	1542 (20)	1541 (17)	1544 (10)	1543 (10)	1544 (22)	1544 (19)	1542 (18)
	1544	1540	1540		1547		1545	1544	1543
1554 (23)	1554 (21)	1552 (17)	1552 (13)	1551 (15)	1554 (8)	1552 (10)	1555 (15)	1554 (15)	1553 (14)
			1549					1554	
1561 (21)	1566 (19)	1561 (17)	1561 (12)	1561 (10)	1565 (10)	1562 (11)	1568 (16)	1565 (16)	1567 (16)
			1560		1563				
		1572 (14)	1573 (13)	1575 (4)		1574 (11)			
			1575						
1586 (25)	1582 (10)	1585 (14)	1586 (11)	1586 (3)	1583 (14)	1586 (15)	1586 (11)	1583 (8)	1584 (16)
1588		1587	1588	1586	1582				
1602 (24)	1600 (10)	1600 (19)	1600 (17)	1600 (6)	1600 (15)	1600 (21)	1601 (11)	1601 (7)	1600 (30)
1600	1600	1600	1600	1599	1600	1600	1600	1600	1600
1616 (28)	1616 (12)	1615 (19)	1615 (20)	1615 (13)	1616 (20)	1616 (28)	1616 (11)	1616 (13)	1616 (34)
1617	1617	1616	1616	1616	1616	1616	1616	1616	1615
1630 (30)	1636 (27)	1632 (17)	1629 (12)	1629 (10)	1631 (13)	1630 (13)	1635 (18)	1633 (17)	1630 (9)
1632	1636	1631	1630	1629	1630	1631	1635	1634	1633
1646 (31)		1646 (21)	1644 (14)	1641 (14)	1645 (14)	1644 (31)		1647 (15)	1644 (19)
1650		1646	1643	1642	1647	1644		1646	1646
1659 (34)	1661 (36)	1658 (34)	1658 (34)	1665 (58)	1657 (15)	1655 (12)	1656 (32)	1658 (20)	
1658	1662	1660	1659	1665	1659		1658	1658	
1677 (42)	1678 (43)	1674 (48)	1672 (23)		1675 (59)	1674 (56)	1676 (62)[b]	1675 (94)[b]	1668 (33)[b]
1681	1675	1672	1671		1675	1674	1676	1675	1669
1691 (31)		1690 (5)	1691 (12)	1690 (11)	1693 (15)	1693 (13)	1690 (4)		1692 (10)
1687		1691	1692		1691	1690			1692
	1701 (7)	1701 (6)	1707 (3)	1709 (2)		1705 (4)	1702 (8)	1699 (13)	1701 (2)
	1703	1702	1706			1706	1702	1701	1703
1712 (9)					1717 (17)				
1716					1717				
	1729 (20)			1730 (21)			1727 (7)	1730 (17)	1728 (1)
	1730			1730			1728	1729	1731

[a] Component bands in the curve-fitted spectra which do not have a well-defined counterpart in the FSD spectra are not listed. Relative band intensities are given in parentheses and FSD band positions are underlined. [b] Contributed by the ν_{CO} band of TFA^-.

and are uncorrected. ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer operating at 75.5 MHz (^{13}C) and 300.1 MHz (^{1}H); δ values are given in ppm relative to an internal dioxane standard; J values are given in Hz. HPLC was performed on a Varian 9010 HPLC system with Eurospher 100 reversed-phase C-18, 5 μm, analytical (250 × 4 mm) and semipreparative (250 × 8 mm) columns.

Materials

Leu-enkephalin (acetate salt) **1** and Tyr-Gly-Gly **8** were purchased from Sigma (St. Louis, USA). Leu-enkephalin methyl ester **2** was prepared as described previously.[38] The synthesis and analytical characterization of Amadori compounds **3**, **5**, **6**, **7**, **9** and **11** have been described.[9] The unprotected Amadori compounds **6**, **7** and **11** were finally purified (see ref. 9 for details) by semipreparative reversed-phase HPLC under isocratic conditions using 60% methanol in 0.1% trifluoroacetic acid (TFA). The strong band at *ca.* 1675 cm^{-1} in the FTIR spectra clearly indicated the presence of trifluoroacetate (TFA^-) in compounds **6**, **7** and **11**.

N-(1-Deoxy-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranos-1-yl)-*N*-methyl-L-tyrosylglycylglycyl-L-phenylalanyl-L-leucine 4

Protected Amadori pentapeptide **3** (100 mg, 0.13 mmol) was dissolved in methanol (10 cm^3) and hydrogenated in the presence of formaldehyde solution (36%; 0.1 cm^3) and 10% Pd/C (100 mg) for 24 h. The catalyst was filtered off and the filtrate evaporated. Chromatography of the residue on a silica gel column with EtOAc–EtOH–AcOH–H_2O (70:10:2:2) afforded after crystallization from methanol–diethyl ether 12 mg (11%) of title compound **4**, mp 54–60 °C; $\delta_C(CD_3OD)$ 22.3, 23.9 (Leu, $C^{\delta,\delta'}$), 24.5, 25.8 (isopropylidene, Me), 26.3 (Leu, C^{γ}), 26.7, 27.1 (isopropylidene, Me), 34.9 (Tyr, C^{β}), 39.0 (Phe, C^{β}), 42.2 (Leu, C^{β}), 43.4 (N-Me), 43.9 (Gly, C^{α}), 44.0 (Gly, C^{α}), 56.4 (Phe, C^{α}), 61.7 (C-1), 62.6 (C-6), 71.4 (C-5), 71.8 (Tyr, C^{α}), 72.5 (C-3), 73.4 (C-4), 104.3 (C-2), 110.4, 110.5 (isopropylidene, C), 116.8 (Tyr, C^{ε}), 128.1 (Phe, C^{ζ}), 129.7 (Tyr, C^{γ}), 129.8 (Phe, C^{ε}), 130.7 (Phe, C^{δ}), 131.8 (Tyr, C^{δ}), 138.8 (Phe, C^{γ}), 157.6 (Tyr, C^{ζ}), 171.5, 171.6, 171.8, 173.8, 173.9 (Tyr, Gly, Gly, Phe, Leu, C=O).

N-(1-Deoxy-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranos-1-yl)-*N*-methyl-L-tyrosylglycylglycine 10

Compound **9** (100 mg, 0.19 mmol) was hydrogenated in the presence of formaldehyde as described in the preparation of **4** to yield after column purification and crystallization (chloroform–diethyl ether) 68 mg (67%) of *N*-methyl derivative **10**, mp 155–156 °C; $\delta_C(CD_3OD)$ 24.5, 25.9, 26.6, 27.1 (isopropylidene, Me), 35.4 (Tyr, C^{β}), 43.3 (N-Me), 43.9 (Gly, C^{α}), 44.5 (Gly, C^{α}), 61.2 (C-1), 62.4 (C-6), 71.91 (Tyr, C^{α}), 71.94 (C-5), 72.7 (C-3), 73.0 (C-4), 105.4 (C-2), 109.7, 110.4 (isopropylidene, C), 116.6 (Tyr, C^{ε}), 131.0 (Tyr, C^{γ}), 131.6 (Tyr, C^{δ}), 157.3 (Tyr, C^{ζ}), 171.8, 175.5 (Tyr, Gly, Gly, C=O).

CD measurements

CD spectra were recorded on a Jobin-Yvon Mark VI dichrograph (calibrated with epiandrosterone) at room temperature in 0.02 or 1.0 cm cells. TFE (Aldrich, NMR grade) was used as solvent, the sample concentrations ranging between 0.3–1.0 mg ml^{-1}. The spectra were smoothed by the Savitzky–Golay algorithm.

FTIR measurements

IR spectra at a resolution of 2 cm^{-1} were obtained in TFE solution with a Bruker IFS-55 FTIR spectrometer using a 0.025

Table 2 Assignment for 1500–1750 cm^{-1} region of the FTIR spectra[a,b] (ν/cm^{-1})

Functionalities, amide II mode[18]	Conformation-dependent bands	Solvation, aggregation
1518–1519		
ring vibration, Tyr		
1530–1600	1595–1620	1600–1620
amide II	bifurcation in turns	aggregation
COO^-, stretching		
NH_3^+, deformation		
NH_2, deformation		
1600–1602		
ring vibration, Tyr and Phe		
1615–1616		
ring vibration, Tyr[33]		
	1620–1640	
	β-sheet	
	ca. 1630	
	HB in γ-turns (acceptor)[26–28]	
	1635–1640	
	HB in β-turns (acceptor in TFE)[15,17,29]	
	ca. 1645	
	unordered (in D_2O)	
ca. 1647	1640–1650	
enediol[32,37]	weak HB (in semiopen turns in TFE)[26–29]	
	1650–1660	
	α-helix	
	ca. 1660	1665–1680
	3_{10} helix	solvated amides (in TFE)[15,28,29]
1675		1670–1700
TFA^-[15,29]		free, burried and nonplanar amides
	1680–1700	
	β-sheet, antiparallel	
1690–1720		
COOH, ν_{co}[36]		
ca. 1728		
ν_{co} in open keto sugars[32,37]		
1725–1735		
ν_{co}, ester[18]		

[a] For the assignment of amide I component bands to polypeptide secondary structures in D_2O see refs. 12–14 and 23–25. TFE was found to shift conformational equilibria without having significant influence upon the position of bands due to helices and β-sheet conformation.[15,29,33] [b] Abbreviations: HB, H-bonding.

cm cell with CaF_2 windows. The sample concentrations were the same as in the case of CD studies. Solvent spectra obtained under identical conditions were subtracted from the sample spectra. The contribution of the H–O–H deformation appearing at *ca.* 1633 cm^{-1}, due to traces of water, was removed on the basis of the combination band of O–H stretching and H–O–H deformation vibrations at 5293 cm^{-1}.[19] The amide I region of the spectra was decomposed into component bands by the Levenberg–Marquardt nonlinear curve-fitting method using weighted sums of Lorentz and Gaussian functions. The choice of the starting parameters was assisted by Fourier self-deconvolution (FSD).[39] Both curve-fitting and FSD procedures were part of the instrument's software package (OPUS, version 2.0). Fig. 2 serves as an example to demonstrate that FSD is superior to curve-fitting in determining component band positions while the power of the latter method is that it gives rough estimates of the number of amide groups in a certain conformational or solvational environment.

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