Tetrahedron Vol. 49, No. 17, pp. 3641-3653, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Bioactive and Model Peptides Characterized by the Helicogenic (αMe)Phe Residue

Claudio Toniolo, [a*] Fernando Formaggio, [a] Marco Crisma, [a] Giovanni Valle, [a] Wilhelmus H.J. Boesten, [b] Hans E. Schoemaker, [b] Johan Kamphuis, [b] Piero A. Temussi, [c] Elmer L. Becker, [d] and Gilles Précigoux [e]

[a]Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova, 35131 Padova, Italy;
[b]DSM Research, Bio-organic Chemistry Section, 6160 MD Geleen, The Netherlands;
[c]Department of Chemistry, University of Naples, 80134 Naples, Italy;
[d]Department of Pathology, University of Connecticut Health Center, Farmington, CT 06032, USA
[e]Laboratory of Crystallography and Crystalline Physics, University of Bordeaux I, 33405 Talence, France

(*Received in USA* 19 *February* 1993)

Key Words: aspartame analogues; chemotactic peptide; molecular recognition; sweeteners

Abstract: *We have synthesized and fully characterized the hypersweet super-aspartame analogue pCN-C_6H_4-NHCO-L-Asp-L-(αMe)Phe-OMe 1; the [D-(αMe)Phe]^3-analogue of the formyl-methionyl tripeptide chemoattractant HCO-L-Met-L-Leu--D-(αMe)Phe-OMe 2, the first D-chemotactic peptide being found more active than its L-diastereomer; and the model pentapeptide pBrBz-D-(αMe)Phe-(Aib)$_2$-D-(αMe)Phe-Aib-OtBu 3. The preferred conformation of the three peptides, as determined by X-ray diffraction analyses, is discussed in terms of the proposed receptor models for sweet perception [peptide 1] and neutrophil chemotaxis [peptide 2], and as a promising candidate for molecular recognition studies [peptide 3].*

The increasing interest in the study of α-amino acids methylated at the α-carbon is based on the following factors:

(i) Two of these amino acids, Aib (α-aminoisobutyric acid) and Iva (isovaline) (Scheme 1), characterize an important family of natural antibiotics, the peptaibols, [1,2] which alter the ionic permeability of biological membranes by forming channels. [3-5]

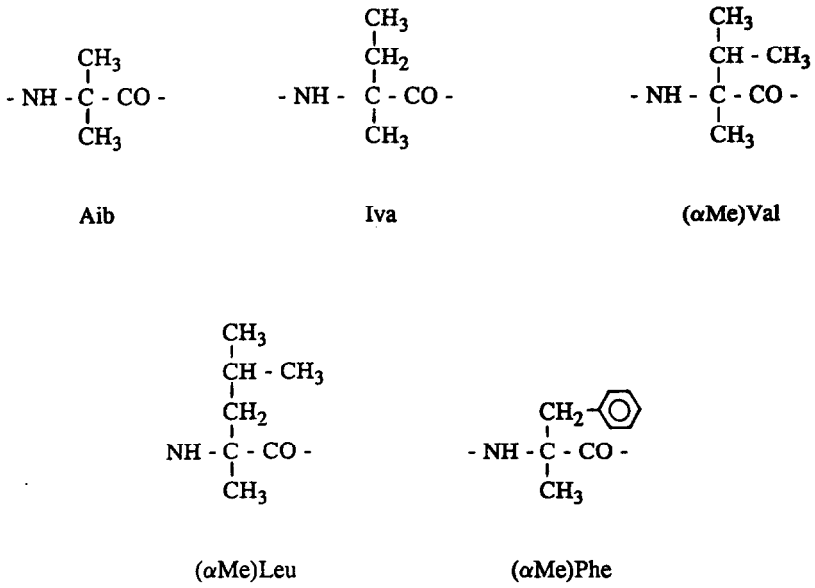
(ii) Tetrasubstitution at the α-carbon in these amino acids results in a severe steric hindrance, the synthesis of the related peptides being greatly complicated by this property. [6-8]

(iii) Derivatives and peptides of these residues show extremely high crystallinity, thus allowing one to perform an X-ray characterization of conformation and electronic structure of the N- and C-

protecting groups, and C-activating groups (including reactive intermediates such as symmetrical anhydrides, [9-11] mixed anhydrides, [12] active esters, [9] 5(4*H*)oxazolones, [13-18] carboxylic acid chlorides [12] and azides, [12] and unprotected and protected N-carboxyanhydrides [19]) of common use in peptide synthesis.

(iv) The stereochemistry of peptides containing these amino acids is rather unique, since they possess significant constraints on their conformational freedom. [20,21] In particular, this point is relevant to (a) the exploitation of these compounds as precise molecular rulers or as scaffolding blocks in the *de novo* design of protein and enzyme mimetics, [22-24] and (b) the 3D structure-activity relationships of backbone-modified, conformationally constrained, enzyme resistant agonists and antagonists of bioactive peptides [25,26].

Scheme 1



The crystal-state and solution structural preferences of Aib, the prototype of the family of α-amino acids methylated at the α-carbon, have extensively been examined by a variety of physico-chemical techniques. Detailed results may be found in many recent review-articles. [27-36] From the experimental data the following conclusions may be drawn: (i) Aib *homo*-peptides, beginning at the trimer level, adopt the 3_{10}-helical structure, irrespective of main-chain length. The α-helical structure has never been observed. (ii) Tripeptides and longer peptides containing Aib residues along with protein amino acids are folded either in the 3_{10}- or in the α-helical structure, depending upon main-

chain length, Aib content, sequence, and environmental conditions. In particular, the minimum peptide main-chain length for α-helix formation in the crystal state corresponds to about seven residues. Since Aib is an achiral residue, the screw sense of the helix that is formed depends upon the chirality of the constituent protein amido acids. (iii) Aib is a very powerful β-bend forming residue, specifically at positions $i+1$ and $i+2$ of type I (I') and type III (III') β-bends and at position $i+2$ of a type II (II') β-bend. However, it has rarely been seen at position $i+1$ of a type II (II') β-bend. (iv) γ-Bends and fully extended structures are extremely uncommon observations for this residue, while β-pleated sheet structures have never been found.

In general, the conclusions listed above for Aib apply also for Iva, (αMe)Val (C^{α}-methyl valine), (αMe)Leu (C^{α}-methyl leucine), and (αMe)Phe (C^{α}-methyl phenylalanine), the other α-amino acids methylated at the α-carbon investigated to date (for a review-article see ref. 37). In addition, the relationship between configuration at the α-carbon of these chiral residues and handedness of the helix that is formed seems to depend upon presence and position of side-chain branching.

In this article we describe the synthesis, characterization, and 3D-structural analysis by X-ray diffraction of three backbone-modified, (αMe)Phe-containing peptides: (i) *p*CN-C_6H_4-NHCO-L-Asp-L-(αMe)Phe-OMe (OMe, methoxy) **1**, a very sweet *super-aspartame* analogue; (ii) HCO-L-Met-L-Leu-D-(αMe)Phe-OMe **2**, the first D-amino acid containing chemoattractant being found more potent than its L-diastereomer; and (iii) *p*BrBz-D-(αMe)Phe-$(Aib)_2$-D-(αMe)Phe-Aib-O*t*Bu (*p*BrBz, *para*-bromobenzoyl; O*t*Bu, tert-butoxy) **3**, a compound with a potential *host* site for molecular recognition studies.

RESULTS AND DISCUSSION

(αMe)Phe Containing Dipeptide Sweeteners

In 1983 Nofre and Tinti [38,39] reported that the *para*-cyanophenylcarbamoyl derivative of the dipeptide aspartame, *p*CN-C_6H_4-NHCO-L-Asp-L-Phe-OMe, has a sweetening potency 14,000 times higher than that of sucrose. Thus, combination of recognition units present in the prototype compounds, cyanosuosan (*p*CN-C_6H_4-NHCONH-CH_2-CH_2-COO^-), 450 times sweeter than sucrose, [40] and aspartame (H-L-Asp-L-Phe-OMe), 200 times sweeter than sucrose, [41] yields a sweetener, referred to as *super-aspartame* by the French authors, with dramatically increased potency relative to members of either class. Replacement of the *para*-cyanophenyl group by *para*-nitrophenyl, also an electron deficient aryl moiety, gave *p*NO_2-C_6H_4-NHCO-L-Asp-L-Phe-OMe, another hypersweet N-carbamoyl dipeptide. [42] More recently, we have shown that the conformationally constrained [L-(αMe)Phe]^2-analogue of aspartame is as sweet as aspartame itself, but more stable at pH 4. [43] Interestingly, our model of the active site of the sweet taste receptor was shown to be consistent with the arylureido dipeptides and with the crystal-state structure of the [L-(αMe)Phe]^2-analogue of aspartame. [44,45]

We have now synthesized the conformationally restricted *super-aspartame* analogues *p*CN-C_6H_4-NHCO-L-Asp-L-(αMe)Phe-OMe **1** and *p*NO_2-C_6H_4-NHCO-L-Asp-L-(αMe)Phe-OMe by treatment of H-L-Asp-L-(αMe)Phe-OMe [43] [or its synthetic precursor H-L-Asp(O*t*Bu)-L-(αMe)Phe-OMe [43]] with the appropriate *para*-substituted arylisocyanate (and removal of the side-chain protecting group of the L-Asp residue by acidolysis). Both compounds are extremely sweet. Crystalline compound **1** was subjected to a X-ray diffraction analysis. The molecular structure is shown in Fig. 1.

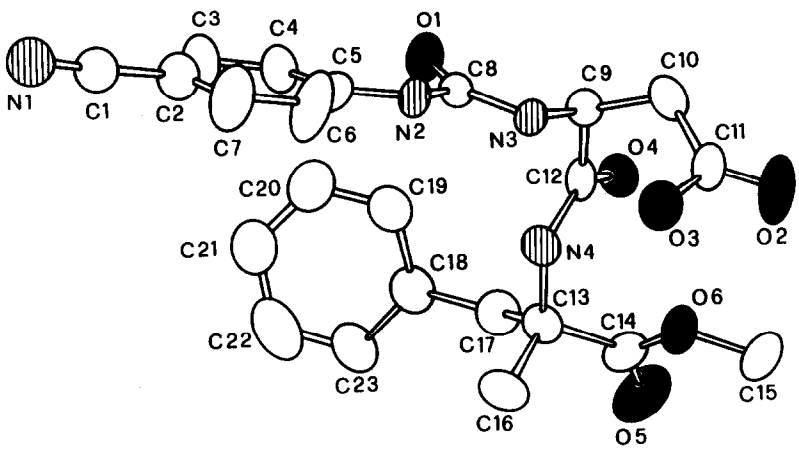


Fig. 1. X-ray diffraction structure of *p*CN-C_6H_4-NHCO-L-Asp-L-(αMe)Phe-OMe **1** with atom numbering.

The sets of ϕ_1, ψ_1 and ϕ_2, ψ_T backbone torsion angles [46] for the L-Asp and L-(αMe)Phe residues are indicative of a right- and left-handed helical structure, respectively [C8-N3-C9-C12 -87.4(4)°; N3-C9-C12-N4 -13.0(4)°; C12-N4-C13-C14 52.2(4)°; N4-C13-C14-O6 29.2(4)°]. The chirality-screw sense relationship exhibited by L-(αMe)Phe is inverse to that commonly shown by protein amino acids, including L-Phe. However, this finding is not surprising in view of our recent crystallographic and conformational energy computation results on (αMe)Phe peptides, which strongly support the view that the stability difference between the right- and left-handed diastereomeric helices formed by the two enantiomers of this C^{α}-methylated amino acid is significantly lower than that of the two diastereomeric helices formed by its unmethylated counterpart (Phe).[37]

The ureine, peptide, and ester groups are all roughly *trans* planar, the only significant deviation from planarity being represented by the ureine C5-N2-C8-N3 torsion angle [-167.1(3)°]. The conformation of the L-Asp and L-(αMe)Phe side chains (χ^1 torsion angle) is that commonly observed [N3-C9-C10-C11 68.6(4)°; N4-C13-C17-C18 -62.1(4)°]. [37,47] The O3 atom of the β-carboxyl moiety of the L-Asp residue is protonated [C11-O2 1.191(5)Å; C11-O3 1.326(4)Å].

The SAR of sweet molecules can be easily tested by means of a simple receptor model we have detailed during the last few years. [44,45] The main features of the model can be summarized as follows: (i) The active site of the receptor is a shallow, flat cavity with the outer side accessible even during interaction with the agonist. (ii) The lower section of the cavity contains the main "electronic features", the essential part of these being the *AH-B* entity proposed by Shallenberger and Acree. [48] (iii) The upper section of the cavity is hydrophobic and plays an important role in the modulation of sweetness intensity.

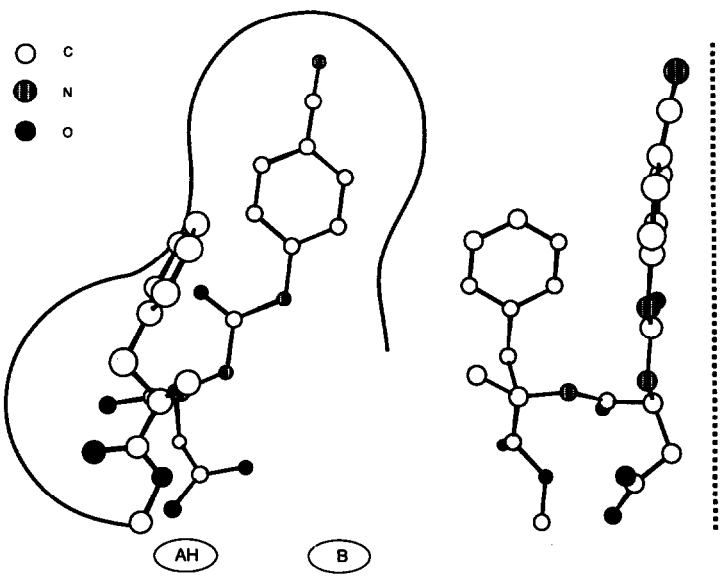


Fig. 2. Fit of the X-ray diffraction structure of *p*CN-C_6H_4-NHCO-L-Asp-L-(αMe)Phe-OMe **1** in the model of the active site of the sweet receptor. The rigth part of the Figure shows a side view that illustrates the flat shape of the molecule facing the "Shallenberger" barrier (dashed line).

The molecular model of compound **1**, resulting from the crystal structure analysis, is slightly different from the minimum energy conformer found for *p*CN-C_6H_4-NHCO-L-Asp-L-Phe-OMe by

means of molecular mechanics calculations. [45] However, as shown in Fig. 2, it fits the model site even better. From a comparison of the relevant torsion angles of the two models it can be appreciated that the only significant difference between the two structures involves the χ^1 angle of the Asp side chain. Hence, the interaction of the *B* moiety (of the *AH-B* entity) of the molecule with the *AH* moiety of the receptor is modified, but, being essentially electrostatic, it can tolerate different orientations (and distances) between the Asp β-carboxylate function and *AH*.

(αMe)Phe Containing Formyl-Methionyl Tripeptide Chemoattractant

The discovery that N$^{\alpha}$-formyl-methionyl tripeptides (*e.g.*, HCO-L-Met-L-Leu-L-Phe-OMe) are potent chemoattractants for neutrophils and capable of inducing lysosomal enzyme release has led to the investigation of the peptide-receptor interaction. SAR studies have determined *inter alia* that there is a close fit between a relatively large hydrophobic pocket of the receptor and the Phe^3 side chain. [49]

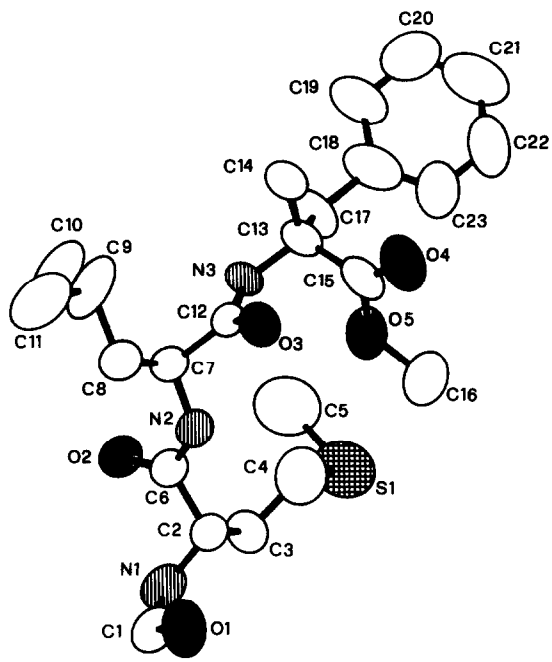


Fig. 3. X-ray diffraction structure of HCO-L-Met-L-Leu-D-(αMe)Phe-OMe **2** with atom numbering.

Recently, we have reported that the conformationally constrained [L-(αMe)Phe]^3 analogue is remarkably less active (*ca.* 800-fold) than its parent compound [ED_{50} for the ability to induce secretion of β-glucosaminidase from rabbit peritoneal leukocytes (7.0 ± 1.3)x10^{-8}M]. [50] In parallel, a crystal-state analysis was performed by X-ray diffraction.

We have now synthesized the diastereomeric tripeptide analogue HCO-L-Met-L-Leu-D-(αMe)Phe-OMe **2** using HCl H-D-(αMe)Phe-OMe [50,51] as the starting material. Compound **2**, although less active than the parent compound, is 12 times *more* active [ED_{50} $(5.4\pm1.3)x10^{-9}M$] than the [L-(αMe)Phe]^3-diastereomer. This is the first instance in this field of a D-peptide being found more active than its L-diastereomer. The X-ray structure of crystalline compound **2** is illustrated in Fig. 3.

The peptide backbone adopts an irregular conformation, partially extended at residues 1 and 2, and *left*-handed helical at residue 3. The ϕ_1 [C1-N1-C2-C6] and ψ_1 [N1-C2-C6-N2], ϕ_2 [C6-N2-C7-C12] and ψ_2 [N2-C7-C12-N3], ϕ_3 [C2-N3-C13-C15] and ψ_T [N3-C13-C15-O5] values are -105.1(11) and 136.3(9)°, -140.5(9) and 111.4(9)°, 45.2(12) and 49.2(11)°, respectively. The peptide and ester groups are *trans*, with ω_1 [C2-C6-N2-C7] deviating markedly from planarity [170.4(8)°]. The L-Met side chain adopts the (*t*, *t*, g^+) conformation [47], the N1-C2-C3-C4, C2-C3-C4-S1, and C3-C4-S1-C5 torsion angles being -172.4(9), -176.2(8), and 87.1(11)°, respectively. The L-Leu side chain is found in the [*t* (g^+, *t*)] conformation, the N2-C7-C8-C9, C7-C8-C9-C10, and C7-C8-C9-C11 torsion angles being -176.8(8), 57.9(14), and -178.8(10)°, respectively. The N3-C13-C17-C18, C13-C17-C18-C19, and C13-C17-C18-C23 torsion angles of the D-(αMe)Phe residue have values of 177.8(9), -104.2(13), and 78.5(14)°, respectively.

Thus, the major differences between compound **2** and the less active [L-(αMe)Phe]^3-diastereomer are seen in the screw sense of the helical conformation of the (αMe)Phe residue and in the conformation about the C4-S1 bond of the Met residue.

(αMe)Phe Containing Model Pentapeptide

A proper understanding of intramolecular interactions depends heavily upon the ability to design and build conformationally constrained structures whose intercomponent geometry is well defined. We chose to focus on structurally restricted helical oligopeptides which fold to bring into close proximity two partners positioned one turn apart. [22-24] In the present study we have crystallized and solved the X-ray diffraction structure of a model pentapeptide [51] containing three Aib and two D-(αMe)Phe residues to induce a strong 3_{10}-helical bias. [27-37] The two aromatic residues are installed within the sequence at positions 1 and 4. The X-ray structure of one of the two independent molecules (molecule I) in the asymmetric unit of *p*BrBz-D-(αMe)Phe-$(Aib)_2$-D-(αMe)Phe-Aib-O*t*Bu **3**, together with a view of the same molecule along the helix axis showing interaction between the two protruding aromatic side chains, is reported in Fig. 4.

Both molecules I and II adopt a regular, *right*-handed 3_{10}-helical structure [52] with average ϕ, ψ torsion angles 56.4, 33.7° for I and 55.8, 33.2° for II. The structure is stabilized by three C=O...H-N intramolecular H-bonds of the β-turn type. [53,54] The range of observed N...O distances is 2.940(8)-3.150(8)A. [55] The C-terminal helical Aib residue of each molecule has an handedness opposite to that of the preceding residues, a common observation for 3_{10}-helix forming peptide esters in the crystal state. [29] Significant deviations ($\Delta_\omega > 10°$) from the amide, peptide or ester *trans* planar conformation

are shown by the C12-C11-N1-C1 (and the corresponding torsion angle of molecule II), C26-C27-N5-C36, and C36-C37-O7-C40 torsion angles of molecule I. The side-chain conformation (χ^1 torsion angle) of each D-(αMe)Phe residue in the two molecules is g^+. Conversely, the only significant conformational difference between molecule I and molecule II is seen in the χ^2 torsion angle of the [D-(αMe)Phe]^1 residue, 86.8(9)° for molecule I, while -80.1(10)° for molecule II. The average distance between corresponding atoms of the two spatially adjacent aromatic rings is 5.40Å for molecule I, while 6.13Å for molecule II. The angle between normals to the average planes of the two phenyl rings is 24.1(3)° for molecule I, while 21.5(3)° for molecule II.

Figure 4 (right part) clearly shows that in the pentapeptide structure a cleft is formed that includes an aromatic section [(αMe)Phe side chains] and an internal polar section characterized by peptide N-H and C=O groups. Thus, conformational constrained chiral peptide templates rich in α-amino acids methylated at the α-carbon may prove to be useful tools for molecular recognition studies.

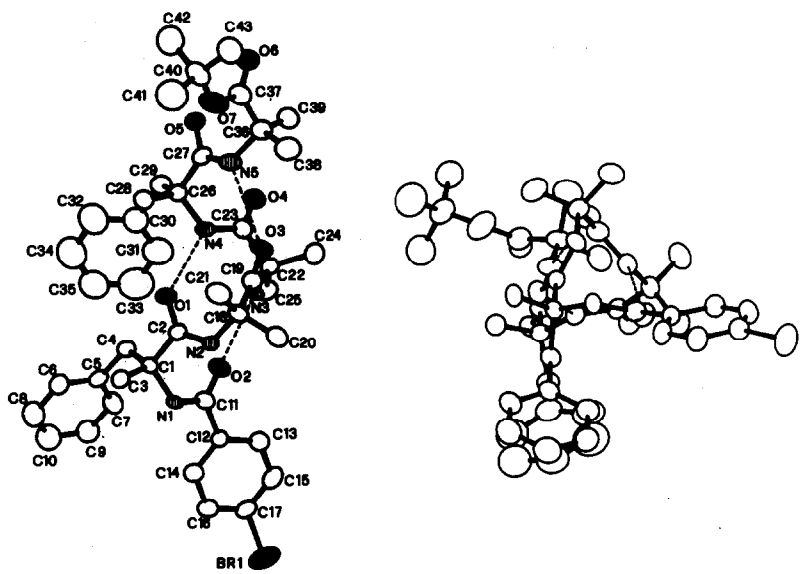


Fig. 4. Left part: X-ray diffraction structure of one of the two independent molecules (I) in the asymmetric unit of *p*BrBz-D-(αMe)Phe-$(Aib)_2$-(D-αMe)Phe-Aib-O*t*Bu **3** with atom numbering. Right part: Projection along the helix axis of the same molecule.

EXPERIMENTAL

para-Cyanophenylcarbamoyl-β-tert-butoxy-L-aspartyl-L-(α-methyl)phenylalanine methylester

This compound was synthesized in 66% yield by treatment of H-L-Asp-(O*t*Bu)-L-(αMe)Phe-OMe (obtained by Pd-catalyzed hydrogenolysis of the corresponding N^{α}-benzyloxycarbonyl protected dipeptide [43]) with *para*-cyanophenylisocyanate [prepared from 4-aminobenzonitrile and triphosgene] in acetonitrile in the presence of N-methylmorpholine. The product was purified by flash-chromatography by eluting the column with a 1:2 isocratic mixture of ethyl acetate-petroleum ether. M.p.167-168°C (diethyl ether-petroleum ether); t.l.c. (silica gel plates 60F-254 Merck) R_{F1} (chloroform-ethanol 9:1) 0.95, R_{F2} (toluene-ethanol 7:1) 0.80, R_{F3} (ethyl acetate-petroleum ether 1:2) 0.30; $[\alpha]_D^{20}$ -50.0° (c=0.5 in methanol). I.r. (KBr) 3345, 2224, 1737, 1727, 1696, 1656, 1595 cm^{-1}. ^1H N.m.r. ($CDCl_3$, 10 mM) δ 7.80 (s, 1H, NH), 7.48 (s, 4H, *para*-cyanophenyl CH); 7.47 (s, 1H, NH); 7.23 and 7.09 [2m, 5H, (αMe)Phe phenyl CH]; 6.56 (d, 1H, Asp NH); 4.78 (m, 1H, Asp αCH); 3.69 (s, 3H, OMe CH_3); 3.20 [m, 2H, (αMe)Phe β-CH_2]; 2.79 (m, 2H, Asp β-CH_2); 1.50 [s, 3H, (αMe)Phe β-CH_3]; 1.45 (s, 9H, O*t*Bu CH_3).

para-Cyanophenylcarbamoyl-L-aspartyl-L-(α-methyl)phenylalanine methyl ester (1)
This compound was prepared in 90% yield by treating *para*-cyanophenylcarbamoyl-β-*tert*-butoxy-L-aspartyl-L-(α-methyl)phenylalanine methylester with a 2:1 trifluoroacetic acid-methylene chloride mixture for 90 min. M.p. 215-216°C (ethyl acetate-petroleum ether); t.l.c. R_{F1} 0.10, R_{F4} (1-butanol-acetic acid-water 6:2:2) 0.80; $[\alpha]_D^{20}$ -63.7° (c= 0.5 in methanol). I.r. (KBr) 3376, 3353, 2226, 1745, 1732, 1684, 1638, 1594, 1544 cm^{-1}. ^1H N.m.r. (dimethylsulphoxide-d_6, 1 mM) δ 9.35 (s, 1H, NH); 8.17 (s, 1H, NH); 7.60 (m, 4H, *para*-cyanophenyl CH); 7.21 and 7.09 [2m, 5H, (αMe)Phe phenyl CH]; 6.68 (d, 1H, Asp NH); 4.54 (m, 1H, Asp α-CH); 3.55 (s, 3H, OMe CH_3); 3.10 [m, 2H, (αMe)Phe β-CH_2]; 2.60 (m, 2H, Asp β-CH_2); 1.22 [s, 3H, (αMe)Phe β-CH_3].

para-Nitrophenylcarbamoyl-L-aspartyl-L-(α-methyl)phenylalanine methyl ester
This compound was synthesized in 72% yield from H-L-Asp-L-(αMe)Phe-OMe and *para*-nitrophenylisocyanate in N, N-dimethylformamide in the presence of triethylamine. The product was purified by flash-chromatography by eluting the column with a 8:2 isocratic mixture of chloroform-methanol. M.p. 213-215°C (methanol-diethyl ether); t.l.c. R_{F1} 0.10, R_{F2} 0.05, R_{F4} 0.90, R_{F5} (chloroform-methanol 8:2) 0.35; $[\alpha]_D^{20}$ 72.6° (c= 0.5 in methanol). I.r. (KBr) 3371, 1728, 1659, 1612, 1559 cm^{-1}. ^1H N.m.r. (CD_3OD, 10 mM) δ 8.11 and 7.65 (2d, 4H, *para*-nitrophenyl CH), 7.19 [m, 5H, (αMe)Phe phenyl CH], 4.77 (m, 1H, Asp α-CH), 3.72 (s, 3H, OMe CH_3), 3.28 [m, 2H, (αMe)Phe, β-CH_2], 2.74 (m, 2H, Asp β-CH_2), 1.47 [s, 3H, (αMe)Phe β-CH_3].

N^{α}-Benzyloxycarbonyl-L-leucyl-D-(α-methyl)phenylalanine methyl ester
This dipeptide was prepared in 50% yield *via* the mixed anhydride method by treating Z-L-Leu-OH with HCl·H-D-(αMe)Phe-OMe [50,51] in a tetrahydrofuran-chloroform 1:1 mixture in the presence of isobutylchloroformate and N-methylmorpholine for 4 h. M.p. 128-129°C (ethyl acetate-petroleum ether); t.l.c. R_{F1} 0.95, R_{F2} 0.65, R_{F4} 0.90; $[\alpha]_D^{20}$ 25.0° (c= 0.5 in methanol). I.r. (KBr) 3332, 1721, 1712, 1665, 1544 cm^{-1}. ^1H N.m.r. ($CDCl_3$, 10 mM) δ 7.34 (m, 5H, Z phenyl CH), 7.20 and 7.01 [2m, 5H, (αMe)Phe phenyl CH], 6.48 [s, 1H, (αMe)Phe NH], 5.10 (d, 1H, Leu NH), 5.08 (s, 2H, Z CH_2), 4.11 (m, 1H, Leu α-CH), 3.76 (s, 3H, OMe CH_3), 3.33 [m, 2H, (αMe)Phe β-CH_2], 1.61 [s, 3H, (αMe)Phe β-CH_3], 1.50 (m, 3H, Leu γ-CH and β-CH_2), 0.89 (m, 6H, Leu δ CH_3). Amino acid analysis (C. Erba Model 3A29 amino acid analyzer): Leu 0.94, (αMe)Phe 1.06.

N^{α}-tert-Butyloxycarbonyl-L-methionyl-L-leucyl-D-(α-methyl)phenylalanine methyl ester
This tripeptide was synthesized in 80% yield *via* the symmetrical anhydride method by treating *t*-Boc-L-Met-OH with H-L-Leu-D-(αMe)Phe-OMe (obtained by Pd-catalyzed hydrogenolysis in methanol of the corresponding Z-protected dipeptide described above) in acetonitrile in the presence of N-ethyl, N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride and N-methylmorpholine for 24 h. M.p. 84-85°C (chloroform-petroleum ether); t.l.c. R_{F1}0.95, R_{F2} 0.70, R_{F4} 0.95; $[\alpha]_D^{20}$ 0.4° (c= 0.5 in methanol); $[\alpha]_{436}^{20}$ 10.1° (c= 0.5 in methanol). I.r.(KBr) 3326, 1744, 1692, 1646, 1523 cm^{-1}. ^1H N.m.r. ($CDCl_3$, 10 mM) δ 7.23 and 7.02 [2m, 5H, (αMe)Phe phenyl CH], 6.57 [s, 1H, (αMe)Phe

NH], 6.50 (d, 1H, Leu NH), 5.12 (d, 1H, Met NH), 4.35 (m, 1H, Leu α-CH), 4.21 (m, 1H, Met α-CH), 3.76 (s, 3H, OMe CH_3), 3.33 [m, 2H, (αMe)Phe β-CH_2], 2.51 (m, 2H, Met γ-CH_2), 2.09 (s, 3H, Met S-CH_3), 1.95 (m, 2H, Met β-CH_2), 1.61 [s, 3H, (αMe)Phe β-CH_3], 1.50 (m, 3H, Leu γ-CH and β-CH_2), 1.44 (s, 9H, *t*-Boc CH_3), 0.88 (m, 6H, Leu δ-CH_3). Amino acid analysis: Met 0.96, Leu 1.00, (αMe)Phe 1.04.

N^{α}-Formyl-L-methionyl-L-leucyl-D-(α-methyl)phenylalanine methyl ester (2)
This tripeptide was prepared in 68% yield by treatment of HCl·H-L-Met-L-Leu-D-(αMe)Phe-OMe (obtained by acidolysis of the corresponding *t*-Boc protected tripeptide described above with a solution of 4N HCl in methanol) with formic acid in acetonitrile in the presence of N-ethyl,N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride and N-methylmorpholine for 45 min. [56] M.p. 190-191°C (ethyl acetate-petroleum ether); t.l.c. R_{F1} 0.90, R_{F2} 0.45, R_{F4} 0.85; $[\alpha]_D^{20}$ 4.1° (c= 0.5 in methanol) I.r. (KBr) 3272, 1744, 1688, 1638, 1546 cm^{-1}. ^1H N.m.r. ($CDCl_3$, 10 mM) δ 8.14 (t, 1H, formyl HCO), 7.23 and 7.01 [2m, 5H, (αMe)Phe phenyl CH], 6.73 (d, 1H, Leu NH), 6.61 [s, 1H, (αMe)Phe NH], 6.58 (d, 1H, Met NH), 4.72 (m, 1H, Met α-CH), 4.39 (m, 1H, Leu α-CH), 3.77 (s, 3H, OMe CH_3), 3.33 [m, 2H, (αMe)Phe β-CH_2], 2.51 (m, 2H, Met γ-CH_2), 2.09 (s, 3H, Met S-CH_3), 2.02 (m, 2H, Met β-CH_2), 1.61 [s, 3H, (αMe)Phe β-CH_3], 1.51 (m, 3H, Leu γ-CH and β-CH_2), 0.89 (m, 6H, Leu δ-CH_3). Amino acid analysis: Met 0.96, Leu 0.99, (αMe)Phe 1.04.
The synthesis and characterization of *N^{α}-para-bromobenzoyl-D-(α-methyl)phenylalanyl-α-aminoisobutyryl-α-aminoisobutyryl-D-(α-methyl)phenylalanyl-α-aminoisobutyric acid methyl ester* (3) have already been described. [51]

Crystal data for pCN-C_6H_4-NHCO-L-Asp-L-(αMe)Phe-OMe (1)
$C_{23}H_{24}N_4O_6$; crystal size 0.6x0.6x0.6 mm; orthorhombic; $P2_12_12_1$; a= 24.018(2), b= 10.691(2), c= 8.898(1)Å; V= 2284.8(5)Å^3; Z= 4; D_c= 1.38 g cm^{-3}; μ= 0.91 cm^{-1}; 3120 independent and 2168 observed [$F \geqslant 7\sigma(F)$] reflections; R value 0.051; R_w value 0.056 with $w=1/[\sigma^2(F) + 0.0035 F^2]$; S 1.12; $\Delta\rho_{min}$ and $\Delta\rho_{max}$ -0.29 and 0.28 e·Å^{-3}, respectively.

Crystal data for HCO-L-Met-L-Leu-D-(αMe)Phe-OMe (2)
$C_{23}H_{35}N_3O_5S$; crystal size 0.16x0.16x1.2 mm; monoclinic; $P2_1$; a= 12.708(2), b= 9.309(2), c= 11.362(2)Å; β= 91.8(2)°; V= 1343.5(5)Å^3; Z=2; D_c= 1.15 g cm^{-3}; μ= 1.47 cm^{-1}; 3309 independent and 1143 observed [$F \geqslant 7\sigma(F)$] reflections; R value 0.063; R_w value 0.070 with $w=1/[\sigma^2(F) + 0.0027 F^2]$; S 1.39; $\Delta\rho_{min}$ and $\Delta\rho_{max}$ -0.18 and 0.24 e·Å^{-3}, respectively.

Crystal data for pBrBz-D-(αMe)Phe-(Aib)$_2$-D-(αMe)Phe-Aib-OtBu (3)
$C_{43}H_{56}N_5O_7Br$; crystal size 0.03x0.2x0.3 mm; monoclinic; $P2_1$; a= 11.904(4), b= 16.201(6), c= 23.752(3)Å; β= 95.74(3)°; V= 4557.8(5)Å^3; Z= 4; D_c= 1.22 g cm^{-3}; μ= 17.7 cm^{-1}; 4022 independent and 3270 observed [$F \geqslant 2\sigma(F)$] reflections; R value 0.046; R_w value 0.053 with $w=1/[\sigma^2(F) + 0.001557 F^2]$; S 1.12; $\Delta\rho_{min}$ and $\Delta\rho_{max}$ -0.14 and 0.14 e·Å^{-3}, respectively.

Single crystals of compounds **1**, **2**, and **3** were grown by slow evaporation from aqueous methanol, 2-butanone, and methanol solutions, respectively. X-ray diffraction data were collected on a Philips PW 1100 four-cicle diffractometer using MoKα radiation (λ= 0.71069 Å) for **1** and **2**, respectively, while on an Enraf-Nonius CAD4 diffractometer using CuKα radiation (λ= 1.5418 Å) for **3**. The scan mode was θ-2θ to θ_{max}= 28° for **1** and **2**, while ω-θ to θ_{max}= 60° for **3**. The structures of **1** and **2** were solved by application of the direct methods and the SHELXS 86 program [57], while that of **3** by Patterson-Fourier synthesis. The structures of **1**-**3** were refined by the blocked least-squares procedure using the SHELX 76 program [58] with anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms for **1** were in part found on a ΔF map and the remaining calculated and all isotropically refined; hydrogen atoms for **2** were in part found on a ΔF map and the remaining calculated, but not refined; hydrogen atoms for **3** were all calculated and not refined. Tables of atomic

coordinates, bond lengths and bond angles, and anisotropic temperature factors have been deposited and are available with the Cambridge Crystallographic Data Centre.

Acknowledgement- The work by one of us (E.L.B.) was partially supported by U.S.P.H.S. grant AI09648.

REFERENCES

1. Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G.M. *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 7951-7954.

2. Brückner, H.; Graf, H. *Experientia* **1983**, *39*, 528-530.

3. Jung, G.; Brückner, H.; Schmitt, H. in *Structure and Activity of Natural Peptides*; Voelter, W.; Weitzel, G. Eds.; de Gruyter: Berlin, 1981; pp. 75-114.

4. Nagaraj, R.; Balaram, P. *Accts. Chem. Res.* **1981**, *14*, 356-362.

5. Mathew, M.K.; Balaram, P. *Mol. Cellul. Biochem.* **1983**, *50*, 47-64.

6. Leplawy, M.T.; Jones, D.S.; Kenner, G.W.; Sheppard, R.C. *Tetrahedron* **1960**, *11*, 39-51.

7. Jones, D.S.; Kenner, G.W.; Preston, J.; Sheppard, R.C. *J. Chem. Soc.* **1965**, 6227-6239.

8. Brückner, H.; Currle, M. in *Second Forum on Peptides*; Aubry, A.; Marraud, M.; Vitoux, B. Eds.; Libbey: London, 1989; pp. 251-255.

9. Valle, G.; Toniolo, C.; Jung, G. *Liebigs Ann. Chem.* **1986**, 1809-1822.

10. Valle, G.; Formaggio, F.; Crisma, M.; Bonora, G.M.; Toniolo, C.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1371-1376.

11. Valle, G.; Crisma, M.; Toniolo C. *J. Chem. Soc., Perkin Trans. 2* **1988**, 393-398.

12. Crisma, M.; Moretto, V.; Valle, G.; Formaggio, F.; Toniolo, C. *Int. J. Pept. Protein Res.*, submitted.

13. Nair, C.M.K.; Vijayan, M. *Acta Cryst. B* **1980**, *36*, 1498-1500.

14. Toniolo, C.; Bonora, G.M.; Crisma, M.; Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C. *Int. J. Pept. Protein Res.* **1983**, *22*, 603-610.

15. Valle, G.; Crisma, M.; Toniolo, C. *Can. J. Chem.* **1988**, *66*, 2575-2582.

16. Valle, G.; Crisma, M.; Toniolo, C.; Holt, E.M.; Tamura, M.; Bland, J.; Stammer, C.H. *Int. J. Pept. Protein Res.* **1989**, *34*, 56-65.

17. Crisma, M.; Valle, G.; Bonora, G.M.; De Menego, E.; Toniolo, C.; Lelj, F.; Barone, V.; Fraternali, F. *Biopolymers*, **1990**, *30*, 1-11.

18. Toniolo, C.; Valle, G.; Formaggio, F.; Crisma, M.; Boesten, W.H.J.; Polinelli, S.; Schoemaker, H.E.; Kamphuis, J. *J. Chem. Soc., Perkin Trans. 1*, **1991**, 3386-3388.

19. Valle, G.; Crisma, M.; Formaggio, F.; Toniolo, C. *Zeit. Kristallogr.* **1992**, *199*, 229-237.

20. Marshall, G. in *Intra-Science Chemistry Report*; Kharasch, N. Ed.; Gordon and Breach: New York, 1971; pp. 305-316.

21. Aleman, C.; Subirana, J.A.; Perez, J.J. *Biopolymers* **1992**, *32*, 621-631.

22. Mutter, M.; Vuilleumier, S. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 535-554.

23. Karle, I.L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747-6756.

24. Balaram, P. *Pure Appl. Chem.* **1992**, *64*, 1061-1066.

25. Spatola, A. in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B. Ed.; Dekker: New York, 1983; *vol. 7*, pp. 267-357.

26. Hruby, V.J.; Al-Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249-262.

27. Venkataram Prasad, B.V.; Balaram, P. *C.R.C. Crit. Rev. Biochem.* **1984**, *16*, 307-348.

28. Uma, K.; Balaram, P. *Indian J. Chem.* **1989**, *28B*, 705-710.

29. Toniolo, C.; Bonora, G.M.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C. *Biopolymers* **1983**, *22*, 205-215.

30. Toniolo, C. *British Polym. J.* **1986**, *18*, 221-225.

31. Toniolo, C.; Benedetti, E.; Pedone, C. *Gazz. Chim. Ital.* **1986**, *116*, 355-359.

32. Toniolo, C.; Benedetti, E. *ISI Atlas of Science: Biochemistry* **1988**, *1*, 225-230.

33. Toniolo, C. *Biopolymers* **1989**, *28*, 247-257.

34. Toniolo, C. *Int. J. Pept. Protein Res.* **1990**, *35*, 287-300.

35. Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004-4009.

36. Aubry, A.; Boussard, G.; Cung, M.T.; Marraud, M.; Vitoux, B. *J. Chim. Phys. Phys.-Chim. Biol.* **1988**, *85*, 345-359.

37. Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Précigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1992**, in press.

38. Nofre, C.; Tinti, J.M.; *Eur. Patent Appl.* **1983**, 0, 107, 597.

39. Tinti, J.M.; Nofre, C. in *Sweeteners: Discovery, Molecular Design, and Chemoreception*; Walters, D.E.; Orthoefer, F.T.; Du Bois, G.E. Eds.; ACS Symposium Series 450; American Chemical Society: Washington, DC, 1991; pp. 88-99.

40. Tinti, J.M.; Durozard, D.; Nofre, C. *Naturwissenschaften* **1980**, *67*, 193-194.

41. Mazur, R.H.; Schlatter, J.M.; Goldkamp, A.H. *J. Am. Chem. Soc.* **1969**, *91*, 2684-2691.

42. Tinti, J.M.; Nofre, C. Fr. Demande FR **1984**, 2, 533, 210; *Chem. Abstr.* **1984**, *101*, 152354k.

43. Polinelli, S.; Broxterman, Q.B.; Schoemaker, H.E.; Boesten, W.H.J.; Crisma, M.; Valle, G.; Toniolo, C.; Kamphuis, J. *Bioorg. Med. Chem. Letters* **1992**, *2*, 453-456.

44. Temussi, P.A.; Lelj, F.; Tancredi, T. in *Sweeteners: Discovery, Molecular Design, and Chemoreception*; Walters, D.E.; Orthoefer, F.T.; Du Bois, G.E. Eds.; ACS Symposium Series 450; American Chemical Society: Washington, DC, 1991; pp. 143-161.

45. Kamphuis, J.; Lelj, F.; Tancredi, T.; Toniolo, C.; Temussi, P.A. *QSAR*, **1992**, in press.

46. IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry* **1970**, *9*, 3471-3479.

47. Benedetti, E.; Morelli, G.; Némethy, G.; Scheraga, H.A. *Int. J. Pept. Protein Res.* **1983**, *22*, 1-15.

48. Shallenberger, R.S.; Acree, T. *Nature* (London) **1967**, *216*, 480-482.

49. Becker, E.L.; Freer, R.J.; Toniolo, C.; Balaram, P. in *Membrane Receptors and Cellular Regulation*; Czech, M.P.; Kahn, C.R. Eds.; Liss: New York, 1985; pp. 129-134.

50. Toniolo, C.; Crisma, M.; Pegoraro, S.; Valle, G.; Bonora, G.M.; Becker, E.L.; Polinelli, S.; Boesten, W.H.J.; Schoemaker, H.E.; Meijer, E.M.; Kamphuis, J.; Freer, R. *Peptide Res.* **1991**, *4*, 66-71.

51. Toniolo, C.; Formaggio, F.; Crisma, M.; Bonora, G.M.; Pegoraro, S.; Polinelli, S.; Boesten, W.H.J.; Schoemaker, H.E.; Broxterman, Q.B.; Kamphuis, J. *Peptide Res.* **1992**, *5*, 56-61.

52. Toniolo, C.; Benedetti, E. *TIBS* **1991**, *24*, 350-353.

53. Toniolo, C. *C.R.C. Crit. Rev. Biochem.* **1980**, *9*, 1-44.

54. Rose, G.D.; Gierasch, L.M.; Smith, J.A. *Adv. Protein Chem.* **1985**, *37*, 1-109.

55. Görbitz, C.H. *Acta Crystallogr. Sect. B* **1984**, *40*, 390-395.

56. Waki, M.; Meienhofer, J. *J. Org. Chem.* **1977**, *42*, 2019-2020.

57. Sheldrick, G.M. *SHELXS 86, Program for Crystal Structure Determination*, University of Göttingen, Germany, 1986.

58. Sheldrick, G.M. *SHELX-76, Program for Crystal Structure Determination*, University of Cambridge, England, 1976.