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Synthesis and conformation of dipeptide taste ligands containing *homo*-β-amino acid residues†

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epoc ABSTRACT: The synthesis and conformational properties of a series of dipeptide taste ligands, differing from the commercial sweetener aspartame by the presence of a methylene group between the C^{α} and the C′ carbon atoms (as in *homo*-β-residues) in either the L-Asp or the L-Phe residues, are described. *Homo*-β-residues such as *homo*-β-aspartic acid, *homo*-β-phenylglycine and *homo*-β-phenylalanine, obtained by homologation of the corresponding proteinogenic α-amino acids, have been used in the solution peptide synthesis of the following aspartame analogues in protected and unprotected forms: NH_2-*homo*-β-(L or D)-Asp-L-Phe-OMe, NH_2-L-Asp-*homo*-β-L-Phg-OMe and NH_2-L-Asp-*homo*-β-L-Phe-OMe. Lengthening of the peptide skeleton at the L-Asp site results in a drastic loss of sweetness with the production of tasteless compounds; on the other hand, lengthening of the skeleton at the *C*-terminal L-Phe site partially mantains the sweet taste in both NH_2-L-Asp-*homo*-β-L-Phe-OMe and NH_2-L-Asp-*homo*-β-L-Phg-OMe.

The solution conformation of the synthesized dipeptide taste ligands was investigated by NMR and circular dichroism techniques. The analysis of NMR data combined with restrained molecular dynamics calculations shows that all peptides are fairly flexible and they do not assume a preferred conformation in DMSO and methanol. The peptides containing *homo*-β-L-Phe and *homo*-β-L-Phg do adopt a discrete number of conformations among which mainly extended and 'L-shaped' conformation are represented. The circular dichroism spectra are consistent with the NMR results, indicating a significant flexibility for these compounds. Copyright © 1999 John Wiley & Sons, Ltd.

KEYWORDS: synthesis; homo-β-amino acids; conformational studies; sweetner

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INTRODUCTION

β-Amino acids, although less abundant in nature than α-amino acids, have become increasingly important during the last decade. They are found in a variety of natural products,[1,2] including peptides.[3] According to Spatola,[4] β-amino acids and *homo*-β-amino acids are $^{+}H_3NCH_2CHRCOO^{-}$ and $^{+}H_3NCHRCH_2COO^{-}$, respectively. The growing interest in this new class of residues rests both on their yet largely unexplored conformational behaviour and on the possibility of inserting them as convenient new molecular tools in bioactive natural products in order to improve both the resistance to biodegradation and the pharmacokinetic properties.

α-Amino acids are the ideal starting material for the preparation of *homo*-β-amino acids using a homologation reaction sequence.[5,6] In a recent paper,[7] a new and efficient procedure to homologate *N*-protected α-amino acids, with the production of the corresponding chirally pure *homo*-β-derivatives, was reported. According to this method, we have prepared in enantiopure form *N*- and *C*-protected *homo*-β-(*S*)-leucine derivatives[8] and several new *homo*-β-amino acids with aliphatic, aromatic and functionalized side-chains in order to use them in the synthesis of analogues of biologically active compounds.

Since the discovery[9] of the potent sweet-tasting α-L-aspartyl-L-phenylalanine methyl ester (aspartame), the special interest in dipeptide sweeteners has increased and a large number of analogues related to aspartame have been synthesized.[10] It has been demonstrated[11] that the sweet-tasting activity of the dipeptide is preserved if the aspartyl moiety is restricted to L-Asp or to the shorter homologue aminomalonic acid (Ama) in which the side-chain methylene group is missing; elongation of the side-chain as in the higher homologue NH_2-L-Glu-L-Phe-OMe led to bitter compounds.[12] For a sweet-tasting ligand the

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'bioactive conformation' was proposed to be an 'L-shaped' structure.[11,12]

The synthesis of several aspartame dipeptide analogues whose backbone was elongated by one methylene group was performed by Miyoshi *et al*.,[13] who analysed the steric effect in relation to the potency of sweetness. The structure–taste relationships accumulated so far prompted us to investigate the effect on taste of the systematic insertion of a methylene group between the C^{α} and C′ carbon atoms of either the L-Asp or the L-Phe residue in dipeptide analogues. For this purpose, using an innovative synthetic procedure[7,8] in the preparation of *homo*-*β*-amino acids, we prepared a series of new taste ligand dipeptide analogues.

In the present paper we report the synthesis and solution characterization by NMR and circular dichroism (CD) techniques of the following *homo*-*β*-residues containing dipeptides: NH_2-*homo*-*β*-(L or D)-Asp-L-Phe-OMe (**9a** and **9b**), NH_2-L-Asp-*homo*-*β*-L-Phg-OMe (**14**) and NH_2-L-Asp-*homo*-*β*-L-Phe-OMe (**19**). Lengthening of the peptide skeleton at the L-Asp site results in a drastic loss of sweetness with the production of tasteless compounds; on the other hand, lengthening of the skeleton at the *C*-terminal L-Phe site partially mantains the sweet taste in both NH_2-L-Asp-*homo*-*β*-L-Phe-OMe and NH_2-L-Asp-*homo*-*β*-L-Phg-OMe.

EXPERIMENTAL

Materials and methods. α-Amino acids and the activating agent benzotriazol-1-yloxytrispyrrolidinophosphonium hexafluorophosphate (PyBop) were purchased from Novabiochem (Switzerland). Solvents were of reagent grade; the other organic reagents, commercially available, were used without further purification. High-performance liquid chromatographic (HPLC) analyses were performed on a Beckman System Gold instrument, equipped with a UV 166 module detector and a Chromatopac R6A recorder. Purifications were carried out on a Millipore-Waters Delta Prep 3000 HPLC system.

NMR characterization. NMR measurements were carried out on Varian Gemini 200, Bruker 250 and Varian UNITY 400 MHz spectrometers. All ^{1}H NMR spectra were recorded at 298 K and referenced to DMSO-d_6 (δ = 2.5 ppm), to CD_3OH (δ = 3.33 ppm) and $CDCl_3$ (δ = 7.26 ppm). For structural characterization, NMR samples were prepared by dissolving about 5 mg of peptide in 0.75 ml of DMSO-d_6 (99.96% ^{2}H atom, Cambridge Isotope Laboratories) or in 0.75 ml of CD_3OH (99% ^{2}H atom, Cambridge Isotope Laboratories). Additional very dilute solutions were prepared by dissolving 0.2 mg of peptides in 0.75 ml of both solvents.

One-dimensional experiments were typically acquired using 32–64 scans with 32K data points. The spectra for the determination of the temperature coefficients were recorded at 298–318 K in steps of 5 K.

Phase-sensitive double quantum filtered correlated spectroscopy (DQFCOSY),[14] homonuclear Hartmann–Hahn (HOHAHA)[15] and nuclear Overhauser effect spectroscopy in the rotating frames (ROESY)[16] were performed according to the States–Haberkorn method and applying standard phase cycling schemes.

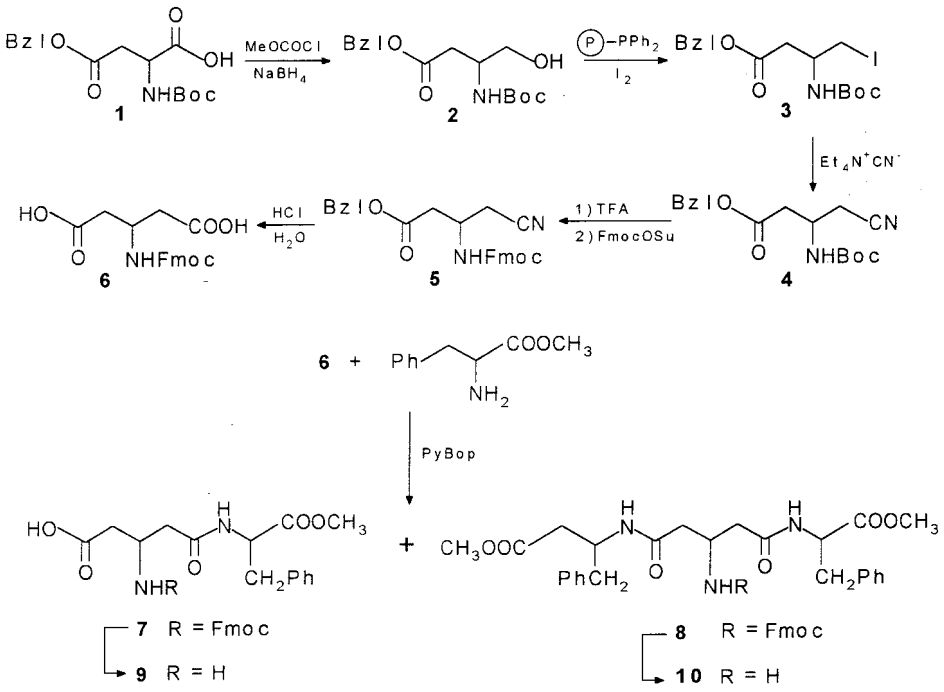
These experiments were typically acquired with 256 increments and 2048 data points in t_2. The FIDs were multiplied by a square shifted sine-bell and zero filled to 1K in F_1 prior to Fourier transformation. The mixing time for TOCSY was 70 ms; a continuous spin lock was used for the compensated[17] ROESY spectrum (mixing time 150 ms). Cross-peak intensities were evaluated by volume integration using the available Varian software.

Restrained molecular dynamics. Restrained molecular dynamics (RMD) simulations were carried out on a Silicon Graphics O2 workstation employing the INSIGHT/DISCOVER program.[18] The consistent valence force field (CVFF) was utilized for all simulations.[19–21] The equations of motion were solved using the so-called Leapfrog integration algorithm.[22]

Starting models of the *N*-protected (**13, 18**) and deprotected dipeptides (**14, 19**) for the RMD were built using standard parameters supplied by the INSIGHT software package. One hundred steps of energy minimizations to eliminate hot spots[23] using the conjugate gradient method[18] were performed for the starting structure. The energy-minimized structure was used as the initial structure for the RMD *in vacuo* at 300 K. All unambiguous NOE effects were used for the RMD calculations. In each RMD simulation, performed with a time step of 0.5 fs, the molecule was equilibrated for 50 ps. After this first step, an additional 100 ps of simulation without rescaling was carried out since energy conservation was observed and the average temperature remained essentially constant around the target value of 300 K. During this step, the structures were extracted and minimized every 1 ps.

The final structure was then checked for consistency with all observable NOE. Coordinates and velocities for the simulation were dumped to a disk every 10 steps during the last 20 ps of the simulations. The dumped data were used for statistical analysis.

Circular dichroism characterization. CD measurements were carried out on a Jasco J715 spectropolarimeter. Peptides **9a, 14** and **19** were dissolved in water–trifluoroethanol (1:1) at concentrations of 2.29×10^{-5}, 3.05×10^{-5} and 1.91×10^{-5} M, respectively. A single Jasco cylindrical cuvette with a pathlength of 1 cm was used for all measurements. The spectra were recorded using a scan speed of 5 nm min^{-1} with a resolution of 0.2 nm, a sensitivity of 50 mdeg, a time constant of 16 s and a spectral window from 260 to 190 nm.



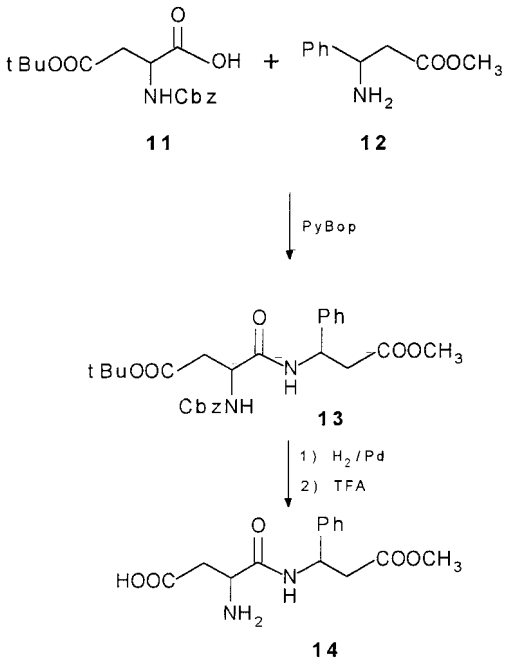
Scheme 1

Syntheses

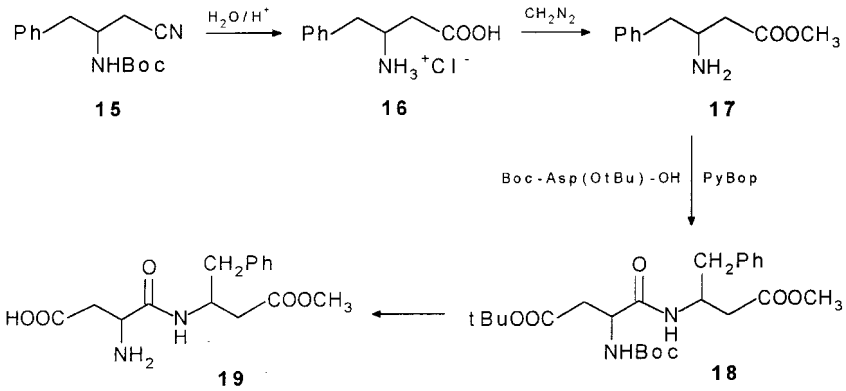
The syntheses of the NH_2-*homo*-β-phenylalanine methyl ester **17** and NH_2-*homo*-β-phenylglycine methyl ester **12** were carried out starting from Boc-L-Phe-OH and Cbz-L-Phg-OH, following a slightly modified literature procedure.[7] In an analogous manner, NH_2-*homo*-β-aspartic acid has been prepared from Boc-L-Asp-(OBzl)-OH.

As depicted in Scheme 1, the starting material was converted into the corresponding *N*-protected *homo*-β-amino acid *via* the β-amino alcohol, the β-amino iodide and the β-amino nitrile followed by hydrolysis of the last compound under acidic conditions. The key step in the preparation of the desired compounds was the conversion of the *N*-alkoxycarbonyl-protected β-amino alcohol into the corresponding iodide by a polymer-bound triarylphosphine–I_2 complex, under very mild, high-yielding and non-racemizing conditions. This is a very convenient procedure to homologate α-amino acids directly; the alternative to this process involves an application of the Arndt–Eistert reaction, under classical[24] or modified[25] conditions, the major drawback of which resides in the use of the extremely hazardous diazomethane, which strongly limits scale-up reactions. We successfully applied the protocol described by Caputo *et al.*[7] on a large scale using inexpensive reagents to obtain the target compounds in the *N*-protected form. The *homo*-β-amino acid derivatives were then coupled with the α-amino acid partner in the preparation of the desired dipeptide taste ligands following standard protocols for peptide synthesis in solution (Schemes 1–3). After the usual work-up, protected and deprotected dipeptides were purified by HPLC and their structures confirmed by ^1H and ^{13}C NMR and fast atom bombardment mass spectrometric (FAB-MS) data.

Synthesis of the dipeptide NH_2-*homo*-β-Asp-Phe-OMe (9a and 9b). *Synthesis of 3-(N-tert-butyloxycarbonylamino)-4-hydroxy benzylbutyrate (**2**).* To a solution of 3.2 g of Boc-Asp(Bzl)-OH (**1**, 10 mmol) in THF



Scheme 2



Scheme 3

(30 ml), 4-methylmorpholine (1.2 ml, 11 mmol) and methyl chloroformate (0.86 ml, 11 mmol) were added at −15 °C under stirring. After 15 min, 1 M $NaBH_4$ aqueous solution (15 ml) was added dropwise and the resulting mixture was left at room temperature (r.t.) until the disappearance of gas evolution, then concentrated under reduced pressure, redissolved in AcOEt and washed repeatedly with water. The organic phase, dried over Na_2SO_4, was taken to dryness to give 2.60 g of pure **2** (8.4 mmol, 84% yield) as a foam: m.p. 59.30 °C; R_f 0.85 in petroleum ether–AcOEt (1:9, v/v). ^{1}H NMR (200 MHz, $CDCl_3$), δ (ppm): 7.34 (s, 5H, phenyl protons); 5.23 (d, 1H, NH); 5.12 (s, 2H, C*H*$_2$Ph); 4.02 (m, 1H, α CH); 3.68 (d, 2H, C*H*$_2$OH); 2.65 (d, 2H, C*H*$_2$COOBzl); 1.44 (s, 9H, 3 CH_3 Boc group). ^{13}C NMR (62 MHz, $CDCl_3$), δ (ppm): 170.44 (COOBzl); 154.58 (OCONH); 135.09, 128.06, 127.92, 127.79 (phenyl ring carbons); 79.70 [$(CH_3)_3C$]; 66.12 (*C*H$_2$Ph); 64.43 (*C*H$_2$OH); 47.71 (α CH); 36.10 (*C*H$_2$COOBzl); 28.19 (3 CH_3 Boc group).

*Synthesis of 3-(N-tert-butyloxycarbonylamino)-4-iodo benzylbutyrate (**3**).* A mixture of 4.0 g of polystyryldiphenylphosphine (12 mmol), swollen in anhydrous CH_2Cl_2 (100 ml), and 2.8 g of I_2 (11 mmol), was left at r.t. for 15 min, then 0.80 g of imidazole (12.5 mmol) and 1.5 g of **2** (5 mmol) were successively added under stirring at r.t. and the resulting mixture was refluxed for 1 h, then cooled, filtered on Celite and washed with CH_2Cl_2 (100 ml). The organic phase, washed with 5 M $Na_2S_2O_3$ (50 ml) and successively with water until neutralization, was dried over Na_2SO_4 and concentrated under reduced pressure, affording 1.5 g of **3** (3.5 mmol, 70% yield); m.p.: 50.40 °C (from CH_2Cl_2–*n*-hexane); R_f 0.9 in petroleum ether–AcOEt (1:9, v/v). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.32 (s, 5H, phenyl protons); 5.20 (d, 1H, NH); 5.10 (s, 2H, C*H*$_2$Ph); 3.91 (m, 1H; α CH); 3.38 (d, 2H, C*H*$_2$I); 2.70 (d, 2H, C*H*$_2$COOBzl); 1.40 (s, 9H, 3 CH_3 Boc group). ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 170.37 (COOBzl); 154.52 (OCONH); 135.27, 128.45, 128.23, 128.06 (phenyl ring carbons); 79.79 [$(CH_3)_3C$]; 66.52 (*C*H$_2$Ph); 47.55 (α CH); 38.61 (*C*H$_2$COOBzl); 28.16 (3 CH_3 Boc group); 11.04 (*C*H$_2$I).

*Synthesis of 3-(N-tert-butyloxycarbonylamino)-4-cyano benzylbutyrate (**4**).* A 1.26 g amount of **3** (3 mmol) was dissolved in CH_2Cl_2 (100 ml) and the solution was treated with 0.97 g of tetrabutylammonium cyanide (6.1 mmol) at reflux for 4 h and then kept at r.t. overnight. The reaction mixture, dried *in vacuo*, was purified by flash chromatography, eluting the column with increasing amounts of AcOEt in petroleum ether. The fractions eluted with 20% AcOEt in petroleum ether gave 530 mg of the pure title compound (1.7 mmol, 56% yield): R_f 0.15 in cyclohexane–AcOEt (8:2, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.34 (s, 5H, phenyl protons); 5.18 (d, 1H, NH); 5.07 (s, 2H, C*H*$_2$Ph); 4.12 (m, 1H, α CH); 2.35 (m, 4H, overlapped signals, C*H*$_2$CN and C*H*$_2$COOBzl); 1.40 (s, 9H, 3 CH_3 Boc group). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 169.84 (COOBzl); 154.45 (OCONH); 134.94, 128.34, 128.19, 127.97 (phenyl ring carbons); 116.78 (CN); 79.98 [$(CH_3)_3C$]; 66.61 (*C*H$_2$Ph); 44.13 (α CH); 37.13 (*C*H$_2$COOBzl); 27.98 (3 CH_3 Boc group); 22.59 (*C*H$_2$CN).

*Synthesis of 3-(N-fluorenylmethoxycarbonylamino)-4-cyano benzylbutyrate (**5**).* A 530 mg amount of **4** (1.7 mmol) was treated with 50% TFA in CH_2Cl_2 (10 ml) under stirring for 1 h at r.t. The reaction mixture was concentrated under reduced pressure and then co-evaporated several times with diethyl ether. The residue, redissolved in THF (10 ml), was taken to pH 9.0 by dropwise addition of 10% Na_2CO_3 aqueous solution and successively treated with 540 mg of FmocOSu (1.7 mmol). The reaction mixture was left at 0 °C for 30 min and at r.t. for 1 h, then filtered and concentrated under reduced pressure. The crude compound was purified by flash chromatography, eluting the column with increasing amounts of AcOEt in petroleum ether. The fractions eluted with petroleum ether–AcOEt (8:2, v/v), collected and taken to dryness afforded 310 mg of pure **5** (0.7 mmol, 41% yield): R_f 0.25 in petroleum

ether–AcOEt (8:2, v/v). ^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm): 7.80–7.22 (complex system, 13H, aromatic Fmoc and phenyl protons); 5.48 (d, 1H, NH); 5.12 (s, 2H, C*H*$_2$Ph); 4.47 (d, 2H, CH_2 Fmoc); 4.35 (t, 1H, CH Fmoc); 4.20 (m, 1H, α CH); 2.75 (d, 4H, overlapped signals, C*H*$_2$CN and C*H*$_2$COOBzl). ^{13}C NMR (62 MHz, $CDCl_3$) δ (ppm): 169.95 (COOBzl); 155.20 (OCONH); 143.45, 141.19, 134.93, 128.48, 128.25, 128.04, 127.94, 126.98, 124.80 and 119.91 (aromatic Fmoc and phenyl ring carbons); 116.67 (CN); 66.97 (CH_2 Fmoc); 66.90 (*C*H$_2$Ph); 46.97 (CH Fmoc); 44.90 (α CH); 36.97 (*C*H$_2$COOBzl); 22.61 (*C*H$_2$CN). MS (70 eV, EI), *m/z*: 440 (M^+).

*Synthesis of 3-(N-fluorenylmethoxycarbonylamino)glutaric acid (**6**).* A 250 mg amount of **5** (0.57 mmol), dissolved in dioxane (5 ml), was treated with 37% HCl (5 ml) and the resulting solution was left at reflux for 4 h, then at r.t. overnight. The reaction mixture was extracted with AcOEt and washed exhaustively with water. The organic phase, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, was purified by HPLC on a preparative RP-18 column (Vydac C_{18}, 25 × 2.2 cm i.d.), eluted with a linear gradient of CH_3CN, containing 0.1% TFA (solvent B), in H_2O, containing 0.1% TFA (solvent A) (from 20% to 80% B in 30 min, flow-rate 12 ml min^{-1}, λ = 220 nm). The peak at retention time (t_R) 15.2 min, taken to dryness, gave 150 mg of pure **6** (0.41 mmol, 71%): R_f 0.2 in petroleum ether–AcOEt (2:3, v/v). ^{1}H NMR (200 MHz, CD_3OD), δ (ppm): 7.79–7.25 (complex system, 8H, aromatic Fmoc protons); 4.34–4.18 (complex system, 4H, overlapped signals, CH_2 and CH Fmoc and α CH); 2.60 (d, 4H, *J* = 7.0 Hz, 2 C*H*$_2$COOH). ^{13}C NMR (50 MHz, CD_3OD), δ (ppm): 174.84 (2 COOH); 158.21 (OCONH); 145.57, 142.83, 129.04, 128.44, 126.55 and 121.19 (aromatic Fmoc carbons); 68.09 (CH_2 Fmoc); 48.67 (α CH); 46.93 (CH Fmoc); 39.78 (2 *C*H$_2$COOH).

*Synthesis of Fmoc-homo-β-(L,D)-Asp-L-Phe-OMe (**7a** and **7b**).* A 50 mg amount of **6** (0.14 mmol) was dissolved in anhydrous THF (1 ml) and treated with 70 mg of PyBOP (0.14 mmol) under stirring at r.t. A solution of 14.6 mg of L-phenylalanine methyl ester chloridate (0.06 mmol) and DIEA (60 μl, 0.24 mmol) in anhydrous THF (2 ml) was added to the reaction vessel in 1 h. The resulting mixture was left at r.t. for 2 h, then concentrated under reduced pressure and purified by HPLC on a preparative RP-18 column (Vydac C_{18}, 25 × 2.2 cm i.d.), eluted with a linear gradient of CH_3CN (0.1% TFA) and H_2O (0.1% TFA) from 20% to 80% B in 60 min (flow-rate 12 ml min^{-1}, λ = 220 nm). The peak at t_R 17.8 min, taken to dryness, gave 10 mg of pure **7** (0.02 mmol, 31% yield); the peak at t_R 15.3 min gave 15 mg of unreacted Fmoc-*homo*-β-Asp-OH (**6**).

^{1}H NMR data for **7** revealed the presence of two different sets of signals, in a 1:1 ratio, attributable to diastereomers **7a** and **7b**, which were successively separated by HPLC on a Baker Chiralcel OD-R analytical column (250 × 0.46 mm i.d., 5 μm), eluted with a linear gradient of CH_3CN in H_2O (from 5 to 100% CH_3CN in 20 min, flow-rate 0.8 ml min^{-1}, λ = 254 nm).

7a: R_f 0.4 in cyclohexane–AcOEt (2:3, v/v); t_R 20.2 min. ^{1}H NMR 1D and 2D (400 MHz, CD_3OH), δ (ppm): 8.33 (d, 1H, NH Phe residue); 7.83–7.18 (complex system, 13H, aromatic Fmoc and phenyl protons); 7.01 (d, H, NH *homo*-β-Asp); 4.70 (m, 1H, α CH Phe); 4.25 (m, 1H, α CH *homo*-β-Asp); 4.39–4.20 (complex system, 3H, CH_2 and CH Fmoc); 3.67 (s, 3H, OCH_3); 3.16 and 2.96 (2 dd, 2H, β CH_2 Phe); 2.58–2.40 (complex signals, 4H, 2 CH_2 *homo*-β-Asp). FAB-MS, *m/z*: 531 ($[M + H]^+$).

7b: R_f 0.4 in cyclohexane–AcOEt (2:3, v/v); t_R 21.2 min. ^{1}H NMR 1D and 2D (400 MHz, CD_3OH), δ (ppm): 8.37 (d, 1H, NH Phe residue); 7.83–7.13 (complex system, 13H, aromatic Fmoc and phenyl protons); 7.06 (d, 1H, NH *homo*-β-Asp); 4.68 (m, 1H, α CH Phe); 4.25 (m, 1H, α CH *homo*-β-Asp); 4.39-4.19 (complex system, 3H, CH_2 and CH Fmoc); 3.68 (s, 3H, OCH_3); 3.14 and 2.96 (2 dd, 2H, β CH_2 Phe); 2.56–2.42 (complex signals, 4H, 2 CH_2 *homo*-β-Asp). FAB-MS, *m/z*: 531 ($[M + H]^+$).

From the crude **7**, the HPLC purification furnished also a peak at t_R 21.6 min, which was collected and identified as the tripeptide **8** (5 mg, 0.0072 mmol, 12% yield): R_f 0.9 in cyclohexane–AcOEt (2:3, v/v). ^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm): 8.02 (d, 1H, NH Phe residue); 7.76–7.19 (complex system, 19H, aromatic Fmoc and phenyl protons); 6.56 (d, 1H, NH *homo*-β-Asp residue); 4.92 (m, 2H, 2 α CH Phe); 4.45–4.16 (m, 4H, CH_2 and CH Fmoc system and α CH *homo*-β-Asp); 3.96 and 3.92 (2 s, 3H each, 2 OCH_3); 3.12–2.35 (complex system, 6H, 2 CH_2 *homo*-β-Asp and CH_2 Phe). FAB-MS, *m/z*: 691 (M^+); 692 ($[M + H]^+$). This compound was the main reaction product when the coupling was performed using stoichiometric amounts of the reagents.

*Synthesis of homo-β-(L,D)-aspartyl-L-phenylalanine methyl ester (**9a** and **9b**).* A 5 mg (0.01 mmol) amount of **7a** was dissolved in CH_3CN (2 ml) and treated with piperidine (2 ml) at r.t. After 1 h the formation of a compound at R_f 0.15 in $CHCl_3$–CH_3OH (9:1, v/v) was observed, with the concomitant disappearance of the starting material. The reaction mixture was then concentrated under reduced pressure and purified by HPLC on an analytical RP-18 column (Varian 90A, 250 × 0.46 mm i.d., 5 μm) eluted with a linear gradient of CH_3CN in H_2O (from 5 to 100% CH_3CN in 25 min, flow-rate 0.8 ml min^{-1}, λ = 254 nm). The peak at t_R 11.43 min gave the desired compound **9a** in almost quantitative yield. ^{1}H NMR (400 MHz, D_2O), δ (ppm): 7.45–7.29 (complex system, 5H, phenyl protons); 4.75 (m, 1H, α CH Phe); 3.79 (s, 3H, OCH_3); 3.61 (t, 1H, α CH *homo*-β-Asp); 3.30–3.03 (AB part of an ABX system, 2H, CH_2 Phe); 2.56 (d, 2H, CH_2 backbone *homo*-β-Asp);

2.32 (d, 2H, CH_2 side-chain *homo*-β-Asp). FAB-MS, *m/z*: 309 ($[M + H]^+$).

The same procedure on compound **7b** afforded **9b**: t_R 11.51 min. ^1H NMR (400 MHz, D_2O), δ (ppm): 7.45–7.28 (complex system, 5H, phenyl protons); 4.85 (m, 1H, α CH Phe); 3.78 (s, 3H, OCH_3); 3.69 (m, 1H, α CH *homo*-β-Asp); 3.39–3.02 (AB part of an ABX system, 2H, CH_2 Phe); 2.54–2.68 (AB part of an ABX system, 2H, CH_2 backbone *homo*-β-Asp); 2.40–2.28 (AB part of an ABX system, 2H, CH_2 side-chain *homo*-β-Asp). FAB-MS, *m/z*: 309 ($[M + H]^+$).

Analogously to **7a** and **7b**, deprotection of tripeptide **8** (5 mg, 0.0072 mmol) was achieved by addition of piperidine (2 ml) in CH_3CN (2 ml) at r.t. After 1 h the formation of a compound at R_f 0.35 in $CHCl_3$–CH_3OH (9:1, v/v) was observed, with the concomitant disappearance of the starting material. The reaction mixture was then concentrated under reduced pressure and purified by HPLC on a preparative RP-18 column (Vydac C_{18}, 25 × 2.2 cm i.d.), eluted with a linear gradient of CH_3CN, containing 0.1% TFA, in H_2O, containing 0.1% TFA (from 20% to 80% B in 60 min, flow-rate 12 ml min^{-1}, λ = 220 nm). The peak at t_R 41.7 min gave the desired compound **10** in almost quantitative yield. ^1H NMR (400 MHz, CD_3OH), δ (ppm): 8.44 and 8.41 (two ds, 2H, 2 NH Phe residues); 7.96 (bs, 1H, NH *homo*-β-Asp residue); 7.33–7.19 (complex system, 10H, phenyl protons), 4.73 (m, 2H, α CH Phe); 3.74 (s, 6H, 2 OCH_3); 3.72 (m, 1H, α CH *homo*-β-Asp); 3.18 and 2.94 (AB parts of two ABX systems, 4H, 2 CH_2 Phe); 2.60–2.44 (complex signals, 4H, side-chain and backbone CH_2 *homo*-β-Asp). FAB-MS, *m/z*: 470 ($[M + H]^+$).

Synthesis of the dipeptide NH_2-L-Asp-*homo*-β-L-Phg-OMe (14). *Synthesis of Cbz-L-Asp(OtBu)-homo-β-L-Phg-OMe (**13**).* The dipeptide ester **13** was prepared from 1.0 g of Cbz-L-Asp(OtBu)-OH (**11**, 3.1 mmol) and 0.56 g of NH_2-*homo*-β-L-Phg-OMe (**12**, 3.1 mmol), prepared according to a literature procedure,[7] in anhydrous THF (10 ml) in the presence of 1.6 g of PyBop (3.1 mmol) and DIEA (3.0 ml, 12 mmol). After 3 h under stirring at r.t., the reaction mixture was taken to dryness, redissolved in AcOEt and washed several times with 10% citric acid, saturated $NaHCO_3$ and water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated to give 1.2 g of pure **13** (2.48 mmol, 80% yield): R_f 0.65 in $CHCl_3$–CH_3OH (95:5); t_R 19.1 min on an RP-18 column (LiChrosorb, LabService, 250 × 4.6 mm i.d., 5 μm) eluted with a linear gradient of CH_3CN in H_2O (from 20 to 80% CH_3CN in 30 min, flow-rate 1.0 ml min^{-1}, λ = 220 nm). ^1H NMR 1D and 2D (400 MHz, CD_3OH), δ (ppm): 8.51 (d, 1H, NH *homo*-β-Phg); 7.40 (d, 1H, NH Asp); 7.38–7.24 (complex system, 10H, aromatic phenyl protons); 5.34 (m, 1H, α CH *homo*-β-Phg); 5.13 and 5.09 (AB part of an ABX system, 2H, CH_2 Cbz); 4.54 (m, 1H, α CH Asp); 3.63 (s, 3H, OCH_3); 2.89 and 2.84 (2 dd, 2H, CH_2 *homo*-β-Phg); 2.76 and 2.56 (2 dd, 2H, CH_2 Asp); 1.43 (s, 9H, OtBu protons).

*Synthesis of NH_2-L-aspartyl-homo-β-L-phenylglycine methyl ester (**14**).* A 126 mg amount of **13** (0.26 mmol), dissolved in CH_3OH (5 ml), was hydrogenated over 0.5 mg of 10% Pd on charcoal under stirring at r.t. After 2 h the catalyst was removed, the resulting solution concentrated under reduced pressure and the residue treated with TFA (10 ml) at r.t. under stirring for 1 h to ensure the removal of the *tert*-butyl group. The mixture was co-evaporated several times with diethyl ether, taken to dryness and then lyophilized, affording 100 mg of pure **14** (0.25 mmol, 95% yield): R_f 0.15 in $CHCl_3$–CH_3OH (95:5 v/v); t_R 13.3 min on an RP-18 column (LiChrosorb, LabService, 250 × 4.6 mm i.d., 5 μm) eluted with a linear gradient of CH_3CN in H_2O (from 20 to 80% CH_3CN in 30 min, flow-rate 1.0 ml min^{-1}, λ = 220 nm). ^1H NMR 1D and 2D (400 MHz, CD_3OH), δ (ppm): 8.86 (d, 1H, NH *homo*-β-Phg); 7.41–7.26 (complex system, 5H, phenyl protons); 5.38 (apparent q, 1H, α CH *homo*-β-Phg); 4.16 (apparent q, 1H, α CH Asp); 3.67 (s, 3H, OCH_3); 2.98 (dd, 1H, H_l of CH_2 Asp); 2.88 (d, 2H, CH_2 *homo*-β-Phg residue); 2.86 (dd, 1H, H_h of CH_2 Asp).

Synthesis of the dipeptide NH_2-L-Asp-*homo*-β-L-Phe-OMe (19). *Synthesis of homo-β-L-phenylalanine methyl ester (**17**).* The title compound[7] was obtained in quantitative yield by treating with an ethereal diazomethane solution *homo*-β-phenylalanine (**16**) dissolved in CH_3OH, which had previously been obtained in 77% yield by acidic hydrolysis with 37% HCl of **15**, prepared according to a literature procedure.[7]

*Synthesis of Boc-L-Asp(OtBu)-homo-β-L-Phe-OMe (**18**).* A 147 mg amount (0.51 mmol) of Boc-L-Asp(OtBu)-OH and 267 mg (0.51 mmol) of PyBop, dissolved in CH_2Cl_2 (5 ml), were treated with 117 mg (0.51 mmol) of **17** and 500 μl (2.0 mmol) of DIEA under stirring at r.t. After 2 h the reaction mixture was concentrated under reduced pressure and worked up as described for **13**. The crude mixture was purified by HPLC on a preparative RP-18 column (Vydac C_{18}, 25 × 2.2 cm i.d.), eluted with a linear gradient of CH_3CN, containing 0.1% TFA, in H_2O, containing 0.1% TFA (from 20% to 80% B in 30 min, flow-rate 12 ml min^{-1}, λ = 220 nm). The peak at t_R 29.2 min, taken to dryness, gave 108 mg (0.23 mmol, 47% yield) of pure **18;** R_f 0.75 in $CHCl_3$–CH_3OH (98:2). 1H NMR 1D and 2D (400 MHz, CD_3OH), δ (ppm): 7.93 (d, 1H, NH *homo*-β-Phe); 7.33–7.17 (complex system, 5H, phenyl protons); 6.83 (d, 1H, NH Asp); 4.37 (m, 1H, α CH Asp); 4.42 (m, 1H, α CH *homo*-β-Phe); 3.65 (s, 3H, OCH_3); 2.81 and 2.87 (2 dd, 2H, β CH_2 side-chain Phe); 2.62 (dd, 1H, H_l of CH_2 Asp); 2.55 and 2.47 (2 dd, 2H, CH_2 backbone *homo*-β-Phe); 2.42 (dd, 1H, H_h of CH_2 Asp); 1.46 and 1.45 (two s, 18H, Boc and OtBu protons). FAB-MS, *m/z*: 465 ($[M + H]^+$).

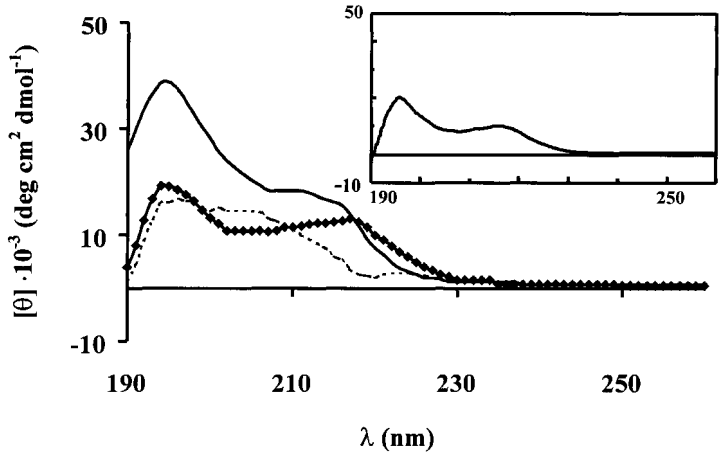


Figure 1. Circular dichroism spectra of peptides **9a** (◆), **14** (——) and **19** (-----). The aspartame CD spectrum is shown for comparison (inset)

*Synthesis of NH_2-L-aspartyl-homo-β-L-phenylalanine methyl ester (**19**).* A 50 mg amount (0.107 mmol) of **18** was treated with 50% TFA in CH_2Cl_2 (5 ml) at r.t. under stirring. After 2 h the solution was concentrated under reduced pressure and then co-evaporated several times with diethyl ether, giving 45 mg (0.102 mmol, 95% yield) of pure **19**, as the trifluoroacetate salt: R_f 0.1 in $CHCl_3$–CH_3OH (9:1, v/v). ^{1}H NMR 1D and 2D (400 MHz, CD_3OH), δ (ppm): 8.37 (d, 1H, NH *homo*-β-Phe); 7.35–7.20 (complex system, 5H, phenyl protons); 4.45 (m, 1H, α CH *homo*-β-Phe); 4.05 (apparent q, 1H, α CH Asp); 3.66 (s, 3H, OCH_3); 2.91 (dd, 1H, H_l of CH_2 side-chain Phe); 2.82–2.89 (partially overlapped signals, 2 dd, 2H, H_h of CH_2 side-chain Phe and H_l of CH_2 Asp); 2.76 (dd, 1H, H_h of CH_2 Asp); 2.60 and 2.47 (2 dd, 2H, CH_2 backbone *homo*-β-Phe). FAB MS, *m/z*: 309 ($[M + H]^+$).

RESULTS AND DISCUSSION

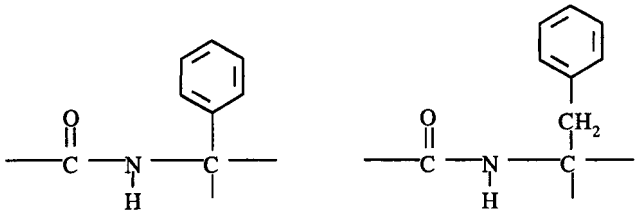
Circular dichroism

In order to investigate the structure–activity relationships (SAR) of the dipeptide taste ligands with respect to the natural dipeptide aspartame, a solution conformational analysis was carried out by means of CD and NMR techniques. For CD experiments all peptides were dissolved in a 1:1 (v/v) TFE–H_2O mixture to minimize differences in solubility between the protected and the deprotected forms and to increase possible conformational differences.

The CD spectra of dipeptides **9a, 14** and **19** are shown in Fig. 1. Gaussian deconvolution shows that all the spectra may be regarded as linear combinations of a medium-intensity CD band with a maximum at about 195 nm and a second positive band with a maximum between 210 and 220 nm. By comparison with the CD spectra of aliphatic *homo*-β-amino acids containing peptides (unpublished data), whose unstructured conformation was achieved by x-ray diffractometry, the CD band between 210 and 230 nm was interpreted as a result of the superposition of a band distinctive of the spectra of *homo*-β-amino acids containing peptides and a positive contribution of the aromatic phenyl side-chain.[26]

The band near 195 nm is a characteristic feature of the spectra of peptides containing a phenyl residue in the side-chain.[26]

The major differences in the spectra may be explained as a consequence of the presence of the following two alternative arrays of chromophores:



Chromophore I **Chromophore II**

The intrinsic chromophores are the same, so that strong changes in intensity between the corresponding CD spectra should arise from a difference in the number of conformers which contribute to the CD. A less intense signal in the peptides containing the chromophore II corresponds to a wider set of conformers, whose CD contributions tend to cancel each other. This is not unexpected, since the CH_2 interposed between the phenyl and the backbone α-carbon in chromophore II contributes to an overall increased flexibility of the corresponding side-chain.

Peptide **14**, showing the more intense CD spectrum, presents a type I chromophore. It almost mantains the aspartame sweetness even if larger differences in its CD spectrum are seen with respect to that of aspartame.

In contrast, all type II peptides share a CD spectrum very reminescent of that of natural aspartame; however,

Table 1. ^{1}H chemical shifts (ppm) of the dipeptides in DMSO-d_6 at 298 K

Compound	AA	NH	αH	βH	hβH	Others
13	Asp	7.53	4.38	2.60/2.42		CH_2(Cbz) 5.01; 2,6H Cbz 7.33; 3,4,5H Cbz 7.30; Boc 1.37;
	Phg	8.46	5.20		2.79	2,6H Phg 7.31; 3,4,5H Phg 7.30; OMe 3.54
14	Asp	8.13	4.05	2.84/2.72		
	Phg	8.95	5.23		2.80	OMe 3.57
18	Asp	6.93	4.21	2.45/2.31		Boc/OtBu 1.38
	Phe	7.80	4.21	2.77/2.70	2.41	OMe 3.54; 2,4,6H 7.19; 3,5H 7.27
19	Asp	n.d.	3.91	2.70/2.62		
	Phe	8.46	4.26	2.81/2.76	2.41/2.51	OMe 3.56; 2,4,6H 7.22; 3,5H 7.31

Table 2. $^3J_{NH\text{-}\alpha CH}$ (Hz), $^3J_{\alpha H\text{-}\beta CH}$ (Hz) and temperature coefficients, $\Delta\delta/\Delta T$ (ppb K^{-1}), for the dipeptides in DMSO and in CD_3OH (in parentheses) at 298 K

Parameter	**13**	**14**	**18**	**19**
$^3J_{NH\text{-}\alpha CH}$ (Asp)	8.5	Broad	8.6	—
	(8.0)	(—)	(7.8)	(—)
$J_{\alpha CH\text{-}\beta CH^{pro\text{-}S}}$ (Asp)	4.8	6.2	4.9	3.8
	(5.4)	(4.2)	(5.2)	(4.2)
$^3J_{\alpha CH\text{-}\beta CH^{pro\text{-}R}}$ (Asp)	9.4	8.2	9.3	8.4
	(8.6)	(8.7)	(8.7)	(8.8)
$^3J_{NH\text{-}\alpha CH}$ (Phe or Phg)	8.5	8.3	8.5	8.2
	(8.3)	(8.6)	(8.6)	(8.2)
$^3J_{\alpha CH\text{-}\beta CH}$ (Phe)	—	—	7.2	$7.0^{pro\text{-}S}$
	(—)	(—)	(7.3)	$(7.6)^{pro\text{-}S}$
$^3J_{\alpha CH\text{-}\beta' CH}$ (Phe)	—	—	6.4	$6.6^{pro\text{-}R}$
	(—)	(—)	(7.1)	$(6.6)^{pro\text{-}R}$
$^3J_{\alpha CH\text{-}h\beta CH}$ (Phe or Phg)	7.4	—	5.7	$5.4^{pro\text{-}S}$
	(7.9)	(7.6)	(5.5)	$(5.0)^{pro\text{-}S}$
$^3J_{\alpha CH\text{-}h\beta' CH}$ (Phe or Phg)	7.4	—	7.1	$8.0^{pro\text{-}R}$
	(6.1)	(7.6)	(7.2)	$(8.4)^{pro\text{-}R}$
$\Delta\delta/\Delta T$ (Asp)	−5.4	−1.5	−6.3	—
	(−8.0)	(—)	(−8.4)	(—)
$\Delta\delta/\Delta T$ (Phe or Phg)	−6.0	−3.7	−5.2	−4.1
	(−8.5)	(−6.0)	(−7.3)	(−5.5)

Table 4. Side-chain populations (%) in DMSO and in CD_3OH (in parentheses)

Amino acid		**13**	**14**	**18**	**19**
Asp	P_I ($g-$)	62	51	61	52
		(55)	(56)	(56)	(57)
	P_{II} (t)	20	33	21	11
		(26)	(14)	(24)	(15)
	P_{III} ($g+$)	18	16	18	37
		(19)	(30)	(20)	(28)
Phe	P_I ($g-$)	—	—	35	37
				(41)	(37)
	P_{II} (t)	—	—	42	40
				(43)	(46)
	P_{III} ($g+$)	—	—	23	23
				(16)	(17)
$h\beta CH_2$	P_I ($g-$)				49
					(53)
	P_{II} (t)				25
					(22)
	P_{III} ($g+$)				26
					(25)

replacement of the L-Asp residue with *homo-β*-Asp causes a loss of taste.

NMR

The conformation of the dipeptides was investigated by 1H NMR spectroscopy in CD_3OH and DMSO-d_6 solutions at 298 K.

Dipeptides **7a, 7b, 9a** and **9b**, studied by high-resolution ^{1}H NMR spectroscopy in DMSO-d_6 and CD_3OH solutions, showed ROESY spectra with very little significant structural information (data not shown) and revealed an enhanced flexibility in solution, probably because of the extra CH_2 group in the *homo-β*-Asp residue. In addition, the observed NOEs cannot be explained in terms of one preferential conformation, but in terms of different conformations in rapid exchange.

The ^{1}H chemical shift assignments in DMSO at 298 K for **13, 14, 18** and **19** are reported in Table 1. The $^3J_{NH\text{-}\alpha\ CH}$ and $^3J_{\alpha H\text{-}\beta CH}$ coupling constants and the temperature coefficients are listed in Table 2. Tables 3 and 4 contain the calculated torsion angles ϕ and the side-

Table 3. Torsion angles ϕ in DMSO and in CD_3OH (in parentheses) determined by $^3J_{NH\text{-}\alpha CH}$ coupling constants

Amino acid	**13**	**14**	**18**	**19**
Asp	−154, −91, 40–80	—	−154, −91, 40–80	—
	(−157, −86, 37–83)	(—)	(−157, −84, 43, 80)	(—)
Phe or Phg	−154, −91, 40–80	−154, −89, 40–80	−154, −91, 40–80	−157, −86, 37–83
	(−154, −89, 40–80)	(−154, −91, 40–80)	(−154, −91, 40–80)	(−157, −86, 37–83)

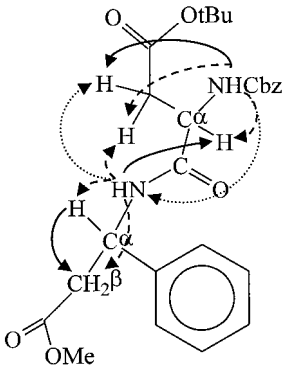


Figure 2. Relevant NOE correlations (strong = solid line, medium = dashed line, weak = dotted line) for Cbz-NH-L-Asp(OtBu)-*homo-β*-L-Phg-OMe recorded in DMSO at 298 K

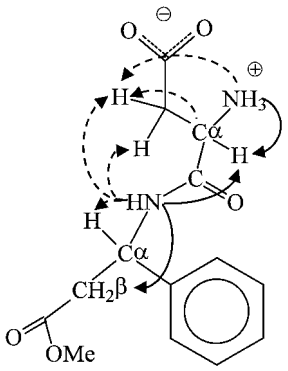


Figure 4. Relevant NOE correlations (strong = solid line, medium = dashed line, weak = dotted line) for NH_2-L-Asp-*homo-β*-L-Phg-OMe recorded in DMSO at 298 K

chain populations. Chemical shifts and temperature coefficients were shown to be independent on the peptide concentration in the range 0.5–22 mM, suggesting that there is no aggregation at the concentrations used for the 2D analysis.

The NOEs were obtained from the ROESY experiments and were classified according to their relative intensities as strong, medium and weak. The main NOE correlations are summarized in Figs 2–5. As expected, coupling constants, temperature coefficients and NOE data, observed for all molecules, do not indicate the existence of a single preferred conformation in solution.

For all compounds a strong inter-residue NOE was observed between the α-proton of the Asp and the NH proton of the following residue, indicating that the torsion angle ψ (Asp) is restricted to values between 60° and 180°.

In **13, 14** and **18**, the prochiralities of the two β-protons of the Asp residue were assigned[27] using the coupling constants $^3J_{\alpha\beta}$ and the NOE connectivities Asp NH–Asp Hβ^1 and Asp NH–Asp Hβ^h. For each compound the proton at higher field (Hβ^h) is pro-*R* and that at lower field (Hβ^1) is pro-*S*. Analysis of populations for the side-chain of Asp residue was carried out using the $^3J_{\alpha\beta^1}$ and $^3J_{\alpha\beta^h}$ experimental values and following the treatment suggested by Jardetzky and Roberts;[28] in each case the g^- conformation is the preferred structure. The populations of peptide **19** side-chains were calculated assuming the Hβ^h as pro-*R* and the Hβ^1 as pro-*S* protons in analogy with peptides **13, 14** and **18**, since NOE correlations NH–Hβ^1 and NH–Hβ^h could not be evaluated. A similar treatment for the Phe side-chain in **18** indicates a preference for the *trans* orientation. Using the same procedure we determined the *homo-β*—CH_2 backbone populations for **19** and a preference for the g^- orientation was found.

The torsion angles ϕ for the Phe residues was evaluated from the $^3J_{\text{NH-}\alpha\text{CH}}$ coupling constants, using a Karplus-type relationship reported by Bystrov;[29] all compounds have similar values which are consistent with the medium NOEs NH Phe–Hα Phe observed.

Medium and weak NOE connectivities, reported in Figs 2–5, were observed for all compounds; the *C*-terminal part of the molecules appears less defined since no correlation with the methyl ester group was detected. An important difference between compounds **14** and **19**

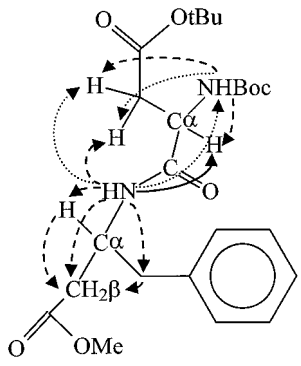


Figure 3. Relevant NOE correlations (strong = solid line, medium = dashed line, weak = dotted line) for Boc-NH-L-Asp(OtBu)-*homo-β*-L-Phe-OMe recorded in DMSO at 298 K

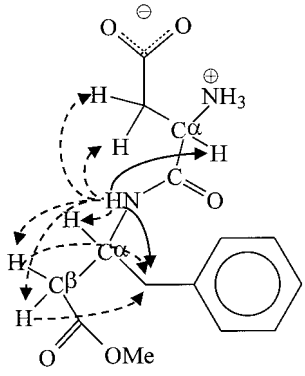


Figure 5. Relevant NOE correlations (strong = solid line, medium = dashed line, weak = dotted line) for NH_2-L-Asp-*homo-β*-L-Phe-OMe recorded in DMSO at 298 K

and compounds **13** and **18** was found. For the deprotected dipeptides (**14** and **19**) the NOEs NH Phe–Hβ^1 Asp and NH Phe–Hβ^h Asp have the same medium intensities, whereas in the protected compounds the NH Phe–Hβ^{proR} Asp effect was found to be weaker than that for NH Phe–Hβ^{proS} Asp. An accurate analysis was conducted also in CD_3OH and the related NMR conformational parameters are reported in Tables 2–4. The structural data obtained are similar to those reported in DMSO.

Molecular dynamics

All the structurally significant NMR parameters were used for RMD simulations *in vacuo* for the various compounds.† The conformational statistical analysis of the RMD simulations at 300 K reveals that a preferred conformation was found for **13**. The average structure of peptide **13** appears consistent with all the observable NMR NOE effects. The conformational analysis underlines that the preferred conformation of these dipeptide taste ligands can be described with an 'L-shape.' This class of conformers also corresponds to a minimum energy conformation. In particular, the aspartyl residue has dihedral angles ϕ and ψ of $-111.1°$ and 134.4°, and a g^- preferred side-chain conformation ($\chi 1$) for the Asp residue. Similar behaviour has also been observed in crystal structures of aspartame and its analogues. The analysis of the *homo*-β-L-Phg residue shows dihedral angles ϕ, μ and ψ of $-97.5°$, 71.2° and $-86.4°$, respectively.

The conformation analysis of RMD simulation for **18** shows that it can adopt mainly extended and 'L-shaped' conformations, among others, with similar energy. The 'L-shaped' conformer presents dihedral angles of the aspartyl residue of ϕ, ψ and $\chi 1$ of $-128°$, 103° and $-58°$, respectively, similar to those observed in several crystal structures of dipeptide taste ligand analogues of aspartame. The analysis of the *homo*-β-L-Phe residue shows dihedral angles ϕ, μ and ψ of $-120.3°$, 80.2° and $-82.1°$, respectively.

The analysis of the RMD results for **14** and **19** indicates that they can adopt mainly extended and 'L-shaped' conformations, among others, and that these conformations have comparable energies. For both compounds, the 'L-shaped' conformer presents average values of dihedral angles ψ and $\chi 1$ (147° and $-70°$, respectively) of the aspartyl residue similar to those found in other dipeptide taste ligands. The analysis of the *homo*-β-L-Phg residue in **14** shows dihedral angles ϕ, μ and ψ of $-137°$, 173° and $-73°$ for the extended conformation and $-145°$, 45° and $-70°$ for the 'L-shaped' structure. The conformational parameters for the *homo*-β-L-Phe residue in **19** shows a similar behaviour with dihedral angles ϕ, μ and ψ of $-134°$, 179° and 90° for the extended conformation and $-134°$, $-95°$ and 112° or $-134°$, $-95°$ and 112° for the two equi-energetic extended and 'L-shaped' conformations and preferred *trans* conformation ($\chi 1$) for the Phe residue side-chain for both the extended and 'L-shaped' conformers.

CONCLUSION

The conformational effect due to the insertion of a methylene group in the backbone skeleton of the dipeptide taste ligand aspartame has been investigated. The solution conformation of the newly synthesized dipeptide taste ligands was investigated by ^{1}H NMR and CD spectroscopy.

The NMR spectra were fully assigned by a combination of TOCSY, DQFCOSY and ROESY maps. Chemical shifts and temperature coefficients were shown to be independent of the peptide concentration in the range 0.5–22 mM. For the dipeptides NH_2-*homo*-β-(L or D)Asp-L-Phe-OMe (**9a** and **9b**), the NMR data were poor with little structural information, revealing an enhanced flexibility in solution certainly due to the extra CH_2 moiety of the *homo*-β-Asp residue. Thus only for the dipeptides NH_2-L-Asp-*homo*-β-L-Phg-OMe (**14**) and NH_2-L-Asp-*homo*-β-L-Phe-OMe (**19**) were computational analyses possible.

The CD spectra are consistent with the NMR results, indicating great flexibility for peptides **9a, 9b** and **19** and a reduced flexibility for peptide **14**. Moreover, the populations of the different families of conformers are the same on comparing analogues **9a, 9b** and **19** with aspartame, even if these new dipeptide taste ligand compounds differ in their sweetness potency with respect to aspartame.

The analysis of the conformational behaviour of the synthesized dipeptides shows that all of the compounds can adopt preferentially extended and 'L-shaped' conformations with different relative populations. Several other conformations are also possible but with smaller relative populations.

The relationship between conformation and sweet taste underlines that the lengthening of the peptide skeleton at the L-Asp site results in a loss of sweetness with the production of tasteless compounds, thus confirming that (i) the *C*-terminal end is the more important function in imparting the sweet taste to the molecule and (ii) the orientation of the amide group linking the Asp and Phe residue of aspartame analogues plays a decisive role in obtaining very sweet compounds. On the other hand, lengthening of the skeleton at the *C*-terminal L-Phe site maintains the sweet character in both NH_2-L-Asp-*homo*-β-L-Phe-OMe and NH_2-L-Asp-*homo*-β-L-Phg-OMe. The decrease in sweetness potency in these compounds with

†The figures of the superimposition obtained for the minimized structures of compounds **13, 14, 18** and **19** during the simulation are deposited as Supplementary Material on the EPOC website (http://www.wiley.com/epoc).

respect to aspartame can be ascribed to an increase of the chain flexibility due to the methylene insertion, where the *C*-terminal ester group is almost comparable in size with the side-chain aromatic ring. This would make more likely the exchange in the position of either group in the interaction with the receptor. In the light of these results, it seems reasonable to confirm that the sweet power of aspartame analogues can only be modulated by modifying the *C*-terminal moiety of the molecule.

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