Journal of Peptide Science, Vol. 1, 349–359 (1995)

# Sweet and Bitter Taste: Structure and Conformations of Two Aspartame Dipeptide Analogues

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*Received 24 February 1995*
*Accepted 5 May 1995*

Abstract: The synthesis and X-ray diffraction analysis of two dipeptide taste ligands have been carried out as part of our study of the molecular basis of taste. The compounds L-aspartyl-D-α-methylphenylalanine methyl ester [L-Asp-D-(αMe)Phe-OMe] and L-aspartyl-D-alanyl-2,2,5,5-tetramethylcyclopentanyl ester [L-Asp-D-Ala-OTMCP] elicit bitter and sweet taste, respectively. The C-terminal residues of the two analogues adopt distinctly different conformations in the solid state. The aspartyl moiety assumes the same conformation found in other dipeptide taste ligands with the side-chain carboxylate and the amino groups forming a zwitterionic ring with a conformation defined by $\psi,\chi_1 = 157.7°, -61.5°$ for L-Asp-D-Ala-OTMCP and 151.0°, −68.8° for L-Asp-D-(αMe)Phe-OMe. In the second residue, a left-handed helical conformation is observed for the (αMe)Phe residue of L-Asp-D-(αMe)Phe-OMe with $\phi_2 = 49.0°$ and $\psi_2 = 47.9°$, while the Ala residue of L-Asp-D-Ala-OTMCP adopts a semi-extended conformation characterized by dihedral angles $\phi_2 = 62.8°$ and $\psi_2 = -139.9°$. The solid-state structure of the bitter L-Asp-D-(αMe)Phe-OMe is extended; while the crystal structure of the sweet L-Asp-D-OTMCP roughly adopts the typical L-shaped structure shown by other sweeteners. The data of L-Asp-D-(αMe)Phe-OMe are compared with those of its diastereoisomer L-Asp-L-(αMe)Phe-OMe. Conformational analysis of the two taste ligands in solution by NMR and computer simulations agrees well with our model for sweet and bitter tastes.

Keywords: X-ray structures; conformational analysis; taste ligands; peptidomimetics; sweetener

## INTRODUCTION

The transduction of taste is believed to be initiated by receptor proteins located on the surface of the taste cell. The tastant and the taste receptor protein form a complex which produces a sense of taste. The taste ligand must assume a specific three-dimensional structure in the formation of the complex. The structure of the taste receptors is presently unknown.

A wide variety of unrelated compounds is known to elicit a sweet taste and there have been numerous attempts [1–10] to generalize the molecular features that are required for a sweet taste. There is general agreement that a sweet molecule must contain a hydrogen bond donor (AH) and a hydrogen bond acceptor (B) that are approximately 2.5–4 Å from one another [1]. Most potently sweet molecules also have

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CCC 1075-2617/95/060349-11

a hydrophobic site (X) that is positioned 3–6 Å from the AH and B functionalities [3]. In the case of aspartame [L-Asp-L-Phe-OMe], which is a typical example of a dipeptide sweetener, the protonated α-amino and the β-carboxylate groups of the N-terminal aspartyl moiety are assigned as the AH and B elements, respectively. The hydrophobic X group is represented by the benzyl side chain of the Phe residue.

The C-terminal residue of sweet dipeptides consists of two hydrophobic groups of differing sizes, the amino acid side chain and the ester or amide group. In the L-configuration of the C-terminal residue, the amino side chain is required to be larger than the ester or amino group. This relationship is reversed in the D-configuration, where the side chain is small and the ester or amide group is large.

Extensive NMR, computer modelling and X-ray crystallography studies on aspartame and various analogues containing peptidomimetic replacements of the phenylalanine residue led us to propose a different model for the required molecular structure of a sweet molecule [11] (Figure 1). This model suggests that a sweet-tasting molecule possesses

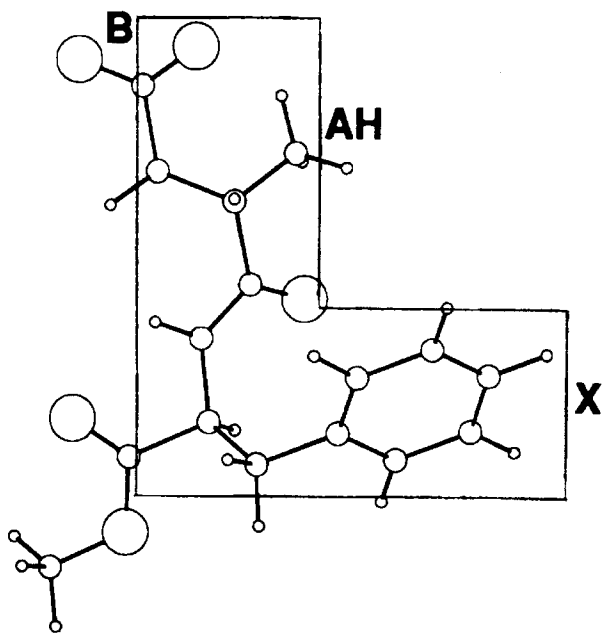


Figure 1 Pictorial representation of the Goodman model for taste properties of dipeptide taste ligands showing one of the minimum energy structures of L-Asp-L-Phe-OMe. The molecule is seen to adopt an L-shape with the stem of the L formed from the AH/B containing zwitterionic ring of the L-Asp residue and the base of the L formed from the hydrophobic benzyl side chain (X) of the L-Phe residue. The groups are perpendicular and coplanar.

an L-shape. The stem of the L is formed from the zwitterionic (AH and B) ring of the aspartyl residue and the base of the L is formed from the hydrophobic group X. The zwitterionic ring and hydrophobic group must be coplanar in the $x$ and $y$ dimensions. Extension of the hydrophobic group into the $+z$ dimension is thought to produce a tasteless molecule, while projection into the $-z$ dimension causes a molecule to be bitter.

In all aspartyl dipeptide taste ligands investigated, the AH and B groups are represented by the protonated amino and the side-chain carboxylate groups of the N-terminal residue, respectively. The compounds fall into two classes. The first (type A) are dipeptide derivatives containing the hydrophobic group X in the side chain of the C-terminal residue, the second (type B) contain the hydrophobic group X as either an amide or an ester function at the C-terminus.

Through the series of taste ligands investigated, the structure and conformation of the N-terminal aspartyl residue remains essentially unchanged. The orientations of the hydrophobic X groups with respect to the AH/B elements are substantially different from one analogue to the next. It was also noted that the conformations adopted by the molecules in the solid state are not necessarily identical to the taste-determining conformations of the molecules in solution. Even if the conformation assumed by each molecule in the crystal is mainly the result of packing forces, valuable information can always be gained from the solid-state structures of these dipeptides, since the observed conformations are close to the global minimum.

In the present paper we report on the structure of the two dipeptide taste ligands, L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP. The first peptide elicits a bitter taste; the second is intensely sweet. Both compounds have a D-residue at the C-terminal residue with side chains ester groups of differing size.

## MATERIALS AND METHODS

### General

All materials were obtained from commercial suppliers and used without further purification unless otherwise indicated. Cbz-L-Asp(OBzl)-OH and Cbz-D-Ala-OH were purchased from Bachem, CA. 4-Dimethylaminopyridine and 1-hydroxybenzotriazole

were purchased from Aldrich Chemical Company. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide and *N,N'*-dicyclohexylcarbodiimide were purchased from Chem-Impex International. 2,2,5,5-Tetramethylcyclopentanone was a generous gift from William D. Fuller of Bioresearch, San Diego. Solvents were dried using known methods and distilled under nitrogen immediately prior to use. Tetrahydrofuran was distilled from sodium/benzophenone. Dichloromethane was distilled from calcium hydride. *N,N*-Dimethylformamide was distilled from ninhydrin.

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin layer chromatography was carried out using EM Reagents 0.25 mm silica gel 60-$F_{254}$ plates. $^1$H-NMR spectra were recorded on a General Electric QE-300 spectrometer, a homebuilt 360 MHz spectrometer consisting of a Tecmag pulse programmer and an Oxford magnet, and a Bruker AMX 500 spectrometer. Optical rotations were measured using Perkin-Elmer 141 and Perkin-Elmer 241 polarimeters. Microanalyses were performed by Desert Analytics, Tucson, Arizona. Fast atom bombardment mass spectra were obtained from the University of California, Riverside Mass Spectroscopy Facility. High-pressure liquid chromatography was carried out using a Vydac C18 1″ prep. column on Waters HPLC systems equipped with two Waters 510 solvent pumps, a Waters 484 multiwavelength detector or a Waters 996 photodiode array detector and a Perkin-Elmer LC-100 integrator.

## Synthesis

### *Synthesis of L-Aspartyl-D-α-methylphenylalanine methyl ester (L-Asp-D-(αMe)Phe-OMe).*

*N-Benzyloxycarbonyl-L-aspartyl-(β-benzyl ester)-D-α-methylphenylalanine methyl ester.* The D-α-methylphenylalanine residue was prepared using the method of Stein *et al.* [12] and Turk *et al.* [13] and is described in the literature. D-α-Me-phenylalanine (0.36 g, 2.0 mmol) was dissolved in dry methanol and cooled to 0 °C. Thionyl chloride was added dropwise to the stirring solution and the mixture was refluxed under $N_2$ for 12 h. The solution was concentrated *in vacuo* and the residue was suspended in ether. The solution was concentrated *in vacuo* again to remove as much HCl as possible, yielding an oily residue. The oil was dissolved in 10 ml dry *N,N*-dimethylformamide and *N*-benzyloxycarbonyl-L-aspartic acid-(β-benzyl ester) (0.71 g, 2.00 mmol) and 1-hydroxybenzotriazole (0.27 g, 2.0 mmol) was added. The solution was cooled to − 40 °C and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (0.38 g, 2.0 mmol) was added. The pH of the solution was adjusted to 6.0 with triethylamine. The mixture was allowed to stir at − 40 °C for 2 h. The solution was allowed to warm to room temperature and stirred overnight.

The solution was concentrated under reduced pressure to yield an oily residue. The residue was dissolved in ethyl acetate and the resulting solution was washed with 2 × 50 ml portions of water. The solution was dried with $MgSO_4$, filtered and concentrated under reduced pressure to yield a clear oil. The oil was chromatographed on silica gel with an ethyl acetate/hexanes 7:3 solvent system yielding the desired protected dipeptide *N*-Cbz-L-Asp-D-(αMe)-Phe-OMe (0.90 g, 85%) as a clear colourless oil. $[\alpha]_D = 6.3°$ ($c = 1.0$, chloroform); $^1$H-NMR ($CDCl_3$, TMS) $\delta$ 7.65–6.95 (m, 16H), 5.93 (d, $J = 7.8$ Hz, 1H), 5.10 (m, 4H), 4.59 (m, 1H), 3.76 (s, 3H), 3.41 (d, $J = 13.4$ Hz, 1H), 3.24 (d, $J = 13.4$ Hz, 1H), 3.06 (dd, $J = 16.9$, 3.4 Hz, 1H), 2.69 (dd, $J = 16.9$, 6.6 Hz, 1H), 1.57 (s, 3H); HRMS calculated for $C_{30}H_{33}N_2O_7$: 533.2288; found: 533.2291.

*L-aspartyl-D-α-methylphenylalanine methyl ester.* *N*-Cbz-L-Asp(OBzl)-D-(αMe)-Phe-OMe (0.60 g, 1.1 mmol) was dissolved in methanol and a catalytic amount of 10% Pd/C was added under a $N_2$ atmosphere. The solution was stirred 4 h under an atmosphere of hydrogen gas, filtered and concentrated under reduced pressure to yield the crude dipeptide as a white solid. The product was purified by RPHPLC (C18, 25 × 10 cm) and lyophilized yielding L-Asp-D-(αMe)Phe-OMe (0.04 g, 12%) as a white solid. Melting point: 128–129 °C; $[\alpha]_D = 68.2°$ ($c = 1.0$, methanol); $^1$H-NMR ($d_6$-DMSO, TMS) $\delta$ 8.55 (br s, 1H), 7.40–7.05 (m, 5H), 3.65–3.55 (m, 1H), 3.58 (s, 3H), 3.22 (d, $J = 13.0$ Hz, 1H), 3.04 (d, $J = 13.0$ Hz, 1H), 2.43 (dd, $J = 15.9$, 5.0 Hz, 1H), 2.18 (dd, $J = 15.9$, 9.0 Hz, 1H), 1.23 (s, 3H); HRMS calculated for $C_{15}H_{21}N_2O_5$: 309.1450; found 309.1454. Anal. calculated for $C_{15}H_{20}N_2O_5 \cdot H_2O$: C, 55.21; H, 6.79; N, 8.58; found: C, 55.29; H, 6.68; N, 8.63.

### *Synthesis of L-Aspartyl-D-alanyl-2,2,5,5-tetramethylcyclopentanyl ester (L-Asp-D-Ala-OTMCP).*

*2,2,5,5-Tetramethylcyclopentanol:* CAUTION! Extreme care must be taken when performing this procedure to prevent fire and/or explosion. This reaction should not be performed by inexperienced

personnel. This reaction was carried out under argon using techniques for handling air sensitive reagents. Lithium aluminium hydride (4.06 g, 107.0 mmol) was suspended in 80 ml dry diethyl ether with stirring. The suspension was heated at reflux for 30 min. The mixture was allowed to cool slightly and 2,2,5,5-tetramethylcyclopentanone (10.00 g, 71.3 mmol), dissolved in 100 ml dry diethyl ether, was added dropwise to the stirring mixture over a period of 2 h. The resulting mixture was heated at reflux for 48 h.

The mixture was cooled to 0 °C and methanol was *cautiously* added until gas evolution ceased. The reaction mixture was diluted with 100 ml deionized water and 100 ml ether and 50 ml 50% $H_2SO_4$ was added. The mixture was poured into a separatory funnel and vigorously shaken. The phases were separated and the aqueous phase was washed with an additional 2 × 100 ml ether. The combined organic extracts were dried with $Na_2SO_4$, filtered and evaporated under reduced pressure yielding 13.40 g (quantitative) of the desired alcohol as a white solid. The material was used without further purification. $^1$H-NMR ($CDCl_3$, TMS) $\delta$ 3.25 (s, 1H), 1.55 (br s, 1H), 1.44 (m, 4H), 1.03 (s, 6H), 0.92 (s, 6H).

*N-Benzyloxycarbonyl-D-alanine-2,2,5,5-tetramethylcyclopentanyl ester.* *N*-Benzyloxycarbonyl-D-alanine (3.79 g, 17.0 mmol), 2,2,5,5-tetramethylcyclopentanol (2.42 g, 17.0 mmol) and 4-dimethylaminopyridine (0.42 g, 3.4 mmol) were stirred at 0 °C in 200 ml dry dichloromethane. *N,N'*-Dicyclohexylcarbodiimide (3.86 g, 18.7 mmol) was added and the mixture was allowed to warm to room temperature and stirring continued for 12 h. The solution was filtered and concentrated under reduced pressure to yield an oily residue. The residue was suspended in 400 ml ethyl acetate and filtered through a pad of silica gel. The filtrate was washed with 2 × 50 ml portions of saturated $NaHCO_3$, 4N $NaHSO_4$ and brine, respectively. The solution was dried with $MgSO_4$, filtered and concentrated under reduced pressure to yield a light yellow solid. The residue was purified by chromatography on silica gel using an ethyl acetate/hexanes 1:5 solvent system yielding 4.12 g (70%) of the desired ester as a white solid. Melting point: 55–56 °C; $R_f$ = 0.25 (ethyl acetate/hexanes 1:5); $^1$H-NMR ($CDCl_3$, TMS) $\delta$ 7.35–7.27 (m, 5H), 5.33 (d, J = 7.0 Hz, 1H), 5.07 (dd, J = 14.6, 12.8 Hz, 1H), 4.48 (s, 1H), d 4.39 (m, 1H), 1.6–1.35 (m, 4H), 1.43 (d, J = 7.0 Hz, 3H), 1.03 (s, 3H), 1.02 (s, 3H), 0.88 (s, 6H).

*N-Benzyloxycarbonyl-L-aspartyl-(β-benzyl ester)-D-alanine-2,2,5,5-tetramethylcyclopentanyl ester.* *N*-Benzyloxycarbonyl-D-alanine-2,2,5,5-tetramethylcyclopentyl ester (3.12 g, 9.0 mmol) was dissolved in 75 ml glacial acetic acid. A catalytic amount of 10% Pd/C was added and the mixture was stirred under vacuum for 15 min. The solution was stirred overnight under an atmosphere of hydrogen gas. The solution was filtered and 2 ml concentrated HCl was added. The solution was concentrated under reduced pressure to yield a clear oil.

The oil was combined with *N*-benzyloxycarbonyl-L-aspartic acid-(β-benzyl ester) (3.21 g, 9.0 mmol), 1-hydroxybenzotriazole (1.82 g, 13.5 mmol) and dissolved in 150 ml dry *N,N*-dimethylformamide. The mixture was stirred and cooled to 0 °C. 4-Methylmorpholine (1.48 ml, 13.5 mmol) was added to the stirring solution. After 5 min 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (2.58 g, 13.5 mmol) was added. The flask was purged with argon and stoppered. The mixture was allowed to warm to room temperature and stirring was continued overnight. The solution was concentrated under reduced pressure to yield a clear light yellow oil. The oil was suspended in 300 ml ethyl acetate and the resulting slurry was filtered through a pad of silica gel. The filtrate was washed with 2 × 50 ml portions of saturated $NaHCO_3$, 4N $NaHSO_4$ and brine, respectively. The solution was dried with $MgSO_4$, filtered and concentrated under reduced pressure to yield a clear oil. The product was chromatographed on silica gel with an ethyl acetate/hexanes 20:55 solvent system yielding 2.91 g (59%) of the protected dipeptide as a clear oil. $R_f$ = 0.50 (ethyl acetate/hexanes 1:1); $^1$H-NMR ($CDCl_3$, TMS) $\delta$ 7.33 (m, 10H), 7.00 (d, J = 5.9 Hz, 1H), 5.90 (d, J = 8.4 Hz, 1H), 5.12 (m, 4H), 4.61 (m, 1H), 4.56 (m, 1H), 4.52 (s, 1H), 3.07 (dd, J = 17.6, 3.3 Hz, 1H), 2.75 (dd, J = 17.2, 6.2 Hz, 1H), 1.6–1.45 (m, 4H), 1.38 (d, J = 7.0 Hz, 3H), 1.06 (s, 3H), 1.05 (s, 3H), 0.91 (s, 6H).

*L-aspartyl-D-alanyl-2,2,5,5-tetramethylcyclopentanyl ester.* *N*-Benzyloxycarbonyl-L-aspartyl-(β-benzyl ester)-D-alanine-2,2,5,5-tetramethylcyclopentyl ester (1.02 g, 1.8 mmol) was dissolved in 60 ml glacial acetic acid. A catalytic amount of 10% Pd/C was added and the mixture was stirred under vacuum for 15 min. The mixture was stirred for 8 h under an atmosphere of hydrogen gas. The solution was filtered, diluted with 30 ml deionized water and lyophilized yielding a white solid. The product was purified by RPHPLC (C18, 25 × 10 cm acetonitrile/water 40:60, isocratic). The product containing fractions were pooled and lyophilized yielding 0.10 g

(17%) of the desired peptide as a fluffy white solid. Melting point: 139–141 °C; $[\alpha]_D = 25.9°$ ($c = 1.00$, acetic acid); $R_f = 0.20$ (chloroform/methanol 4 : 1), $R_f = 0.57$ (*n*-butanol/acetic acid/water 4 : 1 : 1); $^1$H-NMR ($d_6$-DMSO, TMS) $\delta$ 8.59 (s, 1H), 4.35 (s, 1H), 4.29 (m, 1H), 3.60 (m, 1H), 2.43 (d, $J = 3.6$ Hz, 1H), 2.18 (dd, $J = 16.9$, 8.6 Hz, 1H), 1.47 (m, 4H), 1.30 (d, $J = 7.2$ Hz, 3H), 0.99 (s, 6H), 0.88 (s, 3H), 0.86 (s, 3H); FAB-MS M/H$^+$: 329.909; Anal. calculated for $C_{16}H_{28}N_2O_5$; H, 8.59; N, 8.53; found: C, 58.60; H, 8.33; N, 8.50.

### X-ray Diffraction Analyses

Colourless single crystals were obtained by slow evaporation of ethanol/$H_2O$ and of a 2-propanol/$H_2O$ solution for L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP, respectively. Unit cell parameters were determined by least-squares refinement of the setting angles of 25 high-angle reflections ($16 < \theta < 30°$). A Siemens R3m/V automated diffractometer of the Chemistry Department of the University of California at San Diego was employed for the data collection of L-Asp-D-(αMe)Phe-OMe with graphite-monochromated MoKα radiation ($\lambda = 0.71073$ Å), while in the case of the smaller crystals of L-Asp-D-Ala-OTMCP of rather poor quality the Rigaku AFC5R automated diffractometer with the 12 kW rotating anode and graphite monochromated CuKα radiation ($\lambda = 1.54178$ Å) of the Istituto di Strutturistica Chimica of the CNR in Monterotondo (Italy) was used. Crystallographic data are reported in Table 1. Totals of 1966 and 1842 unique reflections were measured at room temperature in the range $0° < 2\theta < 50°$ for L-Asp-D-(αMe)Phe-OMe and $0° < 2\theta < 140°$ for L-Asp-D-Ala-OTMCP, respectively. A total of 1619 reflections with $I \geq 4.0\ \sigma(I)$ was classified as observed and used for structure determination and refinement of L-Asp-D-(αMe)Phe-OMe, while in the case of L-Asp-D-Ala-OTMCP a total of 682 reflections with $I \geq 2.0\ \sigma(I)$ was considered 'observed' and used in the successive calculations.

The structures were solved by direct methods using SHELX PLUS (PC version) [14] and SIR 92 packages [15] for L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP, respectively. The best E maps in both cases revealed most of the non-H atoms. The remaining atoms and the O atom of the co-crystallized water molecules were found from subsequent Fourier syntheses. At the end of the isotropic refinement hydrogen atoms were in part located on successive Fourier maps, and in part calculated in their stereochemically expected positions. Refinements of the structures were performed by a full matrix least-squares procedure minimizing the quantity $\Sigma\omega(F_o - F_c)^2$, with $\omega = 1/\sigma(F_o)^2 + 0.0010F^2$

Table 1 Crystal Data for L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP

| | L-Asp-D-(αMe)Phe-OMe | L-Asp-D-Ala-OTMCP |
|---|---|---|
| Molecular formula | $C_{15}H_{20}N_2O_5 \cdot H_2O$ | $C_{16}H_{28}N_2O_5 \cdot 2H_2O$ |
| Colour, habit | Colourless prism | Colourless needle |
| Formula weight (Da) | 326.3 | 364.4 |
| Crystal system | Orthorhombic | Monoclinic |
| Space group | $p2_12_12_1$ | $p2_1$ |
| $Z$ (molecules/cell) | 4 | 2 |
| $a$ (Å) | 6.205(10) | 7.361(4) |
| $b$ (Å) | 8.508(2) | 6.277(1) |
| $c$ (Å) | 30.828(5) | 22.022(1) |
| $\beta$ (deg) | 90.00 | 98.97 (6) |
| $V$ (Å$^3$) | 1627.5(6) | 1005.2(8) |
| $d$ (calc.) (g/cm$^3$) | 1.322 | 1.210 |
| Radiation used | MoKα ($\lambda = 0.710,73$ Å) | CuKα ($\lambda = 1.541,78$ Å) |
| Absorption coefficient (mm$^{-1}$) | 0.097 | 0.0792 |
| Reflections collected | 1966 | 1842 |
| Reflections used | 1619 [$I > 4\sigma(I)$] | 682 [$I > 2\sigma(I)$] |
| $R$ | 0.038 | 0.086 |
| $R_w$ | 0.050 | 0.089 |
| G.O.F. | 1.120 | 1.102 |
| Temperature (K) | 296 | 295 |
| Residue max./min. $\Delta F$ (e/Å$^3$) | +0.31/−0.19 | +0.30/−0.30 |

for L-Asp-D-(αMe)Phe-OMe and with $\omega = F_o/p$ if $F_o \geq p$ or $\omega = p/F_o$ if $F_o < p$ (with $p = 15$) for L-Asp-D-Ala-OTMCP. All non-H atoms were refined anisotropically. H atoms were introduced in the calculations with isotropic thermal factors equal to the Beq of the carrier atom and their parameters were not refined. Final $R$ and $R_w$ values were 0.038 and 0.050 for L-Asp-D-(αMe)Phe-OMe and 0.086 and 0.089 for L-Asp-D-Ala-OTMCP. In the final difference Fourier synthesis the maximum and minimum electron densities were 0.31 and $-0.19$ eÅ$^3$ or 0.30 and $-0.30$ eÅ$^3$ for L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP, respectively. The scattering factors for all atomic species were calculated using the method of Cromer and Waber [16]. Final atomic parameters and equivalent thermal factors for non-hydrogen atoms with their standard deviations are reported in Tables 2 and 3. Tables of anisotropic thermal factors, hydrogen positional and thermal parameters, bond lengths and bond angles have been deposited as supplementary material with the Cambridge Crystallographic Data Bank.

Table 2 Final Atomic and Thermal Parameters for L-Asp-D-Ala-OTMCP

| | *x/a* | *y/b* | *z/c* | Ueq. ( × $10^3$) |
|---|---|---|---|---|
| $O_1^{\delta 1}$ | 0.1930(3) | 0.9510(4) | 0.4890(8) | 0.10(1) |
| $O_1^{\delta 2}$ | 0.2880(2) | 1.2720(4) | 0.5187(7) | 0.07(1) |
| $C_1^{\lambda}$ | 0.2750(4) | 1.0640(4) | 0.5300(1) | 0.08(1) |
| $C_1^{\alpha}$ | 0.3990(3) | 0.7500(4) | 0.5950(1) | 0.05(1) |
| $C_1^{\beta}$ | 0.3580(3) | 0.9980(4) | 0.5940(1) | 0.07(1) |
| $N_1$ | 0.2170(2) | 0.6410(4) | 0.5770(8) | 0.07(1) |
| $C_1'$ | 0.4580(3) | 0.6870(4) | 0.6596(9) | 0.04(1) |
| $O_1$ | 0.3570(2) | 0.6300(4) | 0.6960(6) | 0.07(1) |
| $N_2$ | 0.6430(3) | 0.7050(4) | 0.6763(7) | 0.05(1) |
| $C_2^{\alpha}$ | 0.7280(3) | 0.6770(4) | 0.7421(9) | 0.04(1) |
| $C_2^{\beta}$ | 0.9300(2) | 0.7210(4) | 0.7497(9) | 0.07(1) |
| $C_2'$ | 0.6920(3) | 0.4450(4) | 0.7630(1) | 0.05(1) |
| $O_2$ | 0.7120(3) | 0.2940(4) | 0.7279(7) | 0.09(1) |
| OT | 0.6530(2) | 0.4360(3) | 0.8175(6) | 0.05(1) |
| C(1) | 0.6080(3) | 0.2310(4) | 0.8420(1) | 0.05(1) |
| C(2) | 0.4080(4) | 0.2160(5) | 0.8430(1) | 0.07(1) |
| C(3) | 0.4080(4) | 0.0580(6) | 0.8950(2) | 0.13(2) |
| C(4) | 0.5820(5) | 0.0930(6) | 0.9410(1) | 0.12(2) |
| C(5) | 0.7160(4) | 0.1880(5) | 0.9048(9) | 0.07(1) |
| C(6) | 0.3290(3) | 0.4240(4) | 0.8620(2) | 0.12(2) |
| C(7) | 0.2930(7) | 0.1570(6) | 0.7820(1) | 0.11(1) |
| C(8) | 0.8720(4) | 0.330(7) | 0.9030(1) | 0.13(2) |
| C(9) | 0.7930(4) | 0.3850(5) | 0.9400(1) | 0.13(2) |
| $Ow^1$ | 0.9120(2) | 0.2990(3) | 0.6231(6) | 0.07(1) |
| $Ow^2$ | 0.8780(2) | 0.8620(3) | 0.5958(7) | 0.06(1) |

The anisotropic displacement exponent takes the form: $-2\pi^2(h^2a^{*2}U_{11} + \ldots + 2hka^*b^*U_{12})$.

## NMR Spectroscopy and Computer Simulations

The $^1$H-NMR spectra were recorded with a Bruker AMX500 spectrometer at 300 K. Samples were prepared in DMSO-$d_6$ at concentrations between 30 and 40 mM. The resonance of DMSO-$d_6$ ($\delta = 2.49$ p.p.m.) was used as an internal standard. The one-dimensional spectra were collected with 8 K data points and with spectral widths of ±2500 Hz. The two-dimensional homonuclear Hartmann–Hahn (HoHaHa) [17] experiments were performed using the MLEV-17 sequence and the time-proportional phase increment. Mixing time of 75 ms with a spin lock field of 10 kHz were employed. The rotating fame nuclear Overhauser (ROESY) [18] experiments were carried out with mixing time of 150 ms and a spin lock field of 2.5 kHz. All of the two-dimensional spectra were obtained using 2 K data points in the t2 domain and 256 data points in the t1 domain. Multiplication with phase-shifted sine functions were employed.

All calculations were performed on a personal Iris 40–25 work station. Energy minimizations were carried out with the DISCOVER force field program [19]. The distance-dependent dielectric constant was

Table 3 Final Atomic and Thermal Parameters for L-Asp-D-(αMe)Phe-OMe

| | *x/a* | *y/b* | *z/c* | Ueq. ( × $10^3$) |
|---|---|---|---|---|
| $O_1^{\delta 1}$ | 0.7936(4) | 1.0528(3) | 0.2433(1) | 0.047(1) |
| $O_1^{\delta 2}$ | 0.1188(4) | 1.0113(3) | 0.2153(1) | 0.038(1) |
| $C_1^{\lambda}$ | 0.9219(6) | 0.9780(4) | 0.2209(1) | 0.028(1) |
| $C_1^{\beta}$ | 0.8430(6) | 0.8288(4) | 0.1980(1) | 0.034(1) |
| $C_1^{\alpha}$ | 0.6093(5) | 0.7840(3) | 0.2064(1) | 0.027(1) |
| $N_1$ | 0.5813(4) | 0.7322(3) | 0.2523(1) | 0.031(1) |
| $C_1'$ | 0.5453(6) | 0.6467(3) | 0.1770(1) | 0.027(1) |
| $O_1$ | 0.5725(4) | 0.5100(2) | 0.1883(1) | 0.034(1) |
| $N_2$ | 0.4678(4) | 0.6897(3) | 0.1383(1) | 0.029(1) |
| $C_2^{\alpha}$ | 0.4382(6) | 0.5773(3) | 0.1028(1) | 0.027(1) |
| $C_2^{2\beta}$ | 0.6556(6) | 0.5325(4) | 0.0843(1) | 0.038(1) |
| $C_2^{1\beta}$ | 0.2983(6) | 0.6603(3) | 0.0677(1) | 0.032(1) |
| $C_2^{\lambda}$ | 0.2719(5) | 0.5693(4) | 0.0260(1) | 0.031(1) |
| $C_2^{1\delta}$ | 0.4021(7) | 0.6004(4) | −0.0089(1) | 0.043(1) |
| $C_2^{2\delta}$ | 0.1125(6) | 0.4559(4) | 0.0210(1) | 0.042(1) |
| $C_2^{1\epsilon}$ | 0.3794(8) | 0.5200(4) | −0.0477(1) | 0.055(1) |
| $C_2^{2\epsilon}$ | 0.0888(7) | 0.3776(4) | −0.0179(1) | 0.047(1) |
| $C_2^{\zeta}$ | 0.2181(7) | 0.4099(4) | −0.0523(1) | 0.048(1) |
| $C_2'$ | 0.3178(5) | 0.4331(4) | 0.1194(1) | 0.028(1) |
| $O_2$ | 0.3699(5) | 0.3003(3) | 0.1107(1) | 0.048(1) |
| OT | 0.1452(4) | 0.4711(2) | 0.1423(1) | 0.035(1) |
| CT | 0.0256(6) | 0.3427(4) | 0.1611(1) | 0.044(1) |
| Ow | 0.7114(5) | 0.4873(3) | 0.3660(1) | 0.062(1) |

The anisotropic displacement exponent takes the form: $-2\pi^2(h^2a^{*2}U_{11} + \ldots + 2hka^*b^*U_{12})$.

used for all calculations. The grid scan method was used in the conformational search. Structures generated from the grid scan were minimized by the VA09A algorithm until all the derivatives were smaller than 0.001 kcal/mol Å.

## RESULTS AND DISCUSSION

Figures 2(a) and (b) show the ORTEP drawings of the structures of L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP. In Table 4 the relevant dihedral angles defining the conformation of the structures are listed. The molecules L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP crystallize as a monohydrate and a dihydrate, respectively. As usually observed in dipeptide taste ligands, the N-terminal aspartyl moiety in both structures exists as a zwitterion. The L-aspartyl residue in both compounds assumes a conformation very similar to that observed in other dipeptide taste analogues [11], where on the average the dihedral angles $\phi$, $\omega$ $\chi_1^1$ and $\chi_1^{2,1}$ show values of +156°, +175°, −69° and −173° (or +7° for $\chi_1^{2,2}$), respectively. These conformational angles have values of +151.0°, +167.7°, −68.8° and 177.2° or +157.7°, +171.8°, −61.5° and −159.9° for L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP, respectively. The conformation about the Asp $C^\alpha$–$C^\beta$ bond ($\chi_1$) is gauche − ($g^-$). The carboxylate of the aspartyl side chain is nearly coplanar with the $C^\alpha$–$C^\beta$ bond: the $\chi_1^{2,1}$ and $\chi_1^{2,2}$ dihedral angles are close to 180° and 0°, respectively.

Table 4 Relevant Conformational Parameters

| | | L-Asp-D-Ala-O-TMCP | L-Asp-D-(αMe)-Phe-OMe |
|---|---|---|---|
| $N_1$-$C_1^\alpha$-$C_1'$-$N_2$ | $\psi^1$ | 157.7° | 151.0° |
| $N_1$-$C_1^\alpha$-$C_1^\beta$-$C_1^\gamma$ | $\chi_1^1$ | −61.5° | −68.8° |
| $C_1^\alpha$-$C_1^\beta$-$C_1^\gamma$-$O^{\delta 2}$ | $\chi_1^{2,1}$ | −159.9° | 177.2° |
| $C_1^\alpha$-$C_1^\beta$-$C_1^\gamma$-$O^{\delta 1}$ | $\chi_1^{2,2}$ | 22.9° | −1.7° |
| $C_1^\alpha$-$C_1'$-$N_2$-$C_2^\alpha$ | $\omega_2$ | 171.8° | 167.7° |
| $C_1'$-$N_2$-$C_2^\alpha$-$C_2'$ | $\phi_2$ | 62.8° | 49.0° |
| $N_2$-$C_2^\alpha$-$C_2'$-OT | $\psi_2$ | −139.9° | 47.9° |
| $C_2^\alpha$-$C_2'$-OT-C(1) | | 177.2° | −177.3° |
| $N_2$-$C_2^\alpha$-$C_2^\beta$-$C_2^\gamma$ | $\chi_2^1$ | — | 173.2° |
| $C_2^\alpha$-$C_2^\beta$-$C_2^\gamma$-$C_2^{\delta 1}$ | $\chi_2^{2,1}$ | — | −96.8° |
| $C_2^\alpha$-$C_2^\beta$-$C_2^\gamma$-$C_2^{\delta 2}$ | $\chi_2^{2,2}$ | — | 85.4° |

Dipeptide taste ligands exhibit varying degrees of conformational freedom in the C-terminal amino acid residue that depend upon the configuration and the conformational constraints of the molecules. In the

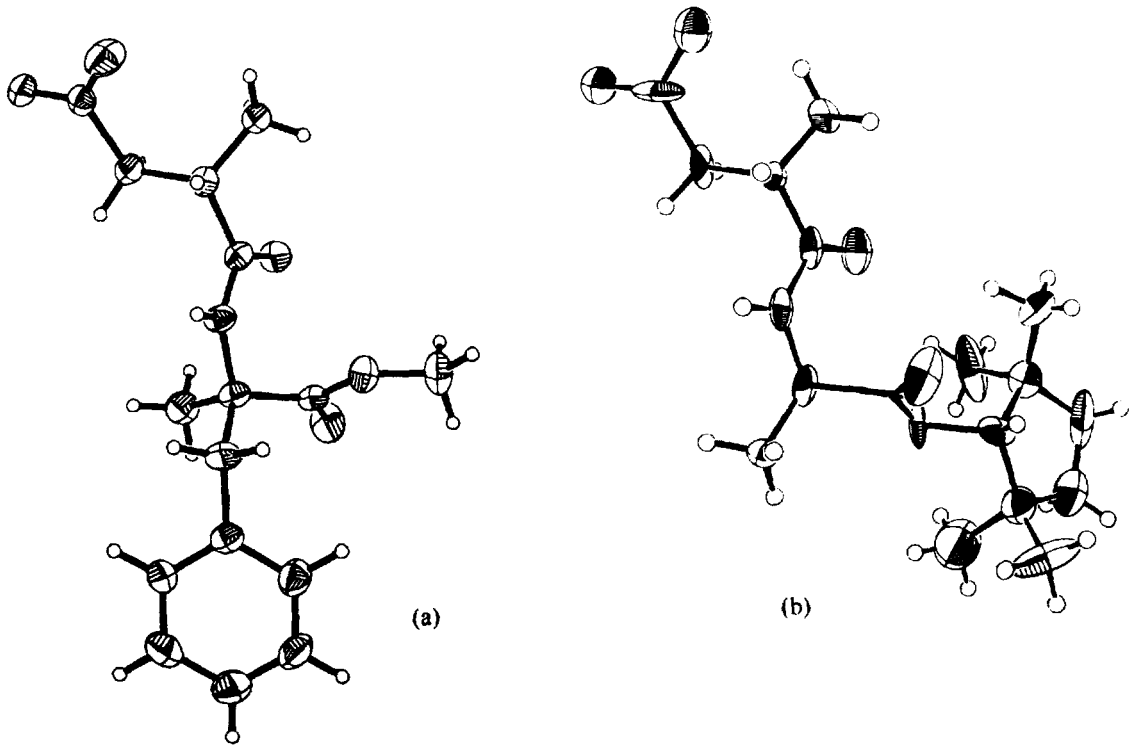


Figure 2 ORTEP drawings of (a) L-Asp-D-(αMe)Phe-OMe and (b) L-Asp-D-Ala-OTMCP. Thermal ellipsoids are drawn at the 50% probability level.

present studies, both molecules have a second residue with the D-configuration, but quite different structural constraints: the $C^{\alpha,\alpha}$-dialkylated (αMe)Phe residue has definite conformational preferences while the D-Ala residue is more flexible. In fact, a left-handed helical conformation is observed for the (αMe)Phe residue of L-Asp-D-(αMe)Phe-OMe with $\phi_2 = 49.0°$ and $\psi_2 = 47.9°$: folded helical structures are highly preferred in peptides containing α,α-dialkylamino acid residues [20, 21]. In contrast, the Ala residue of L-Asp-D-Ala-OTMCP adopts a conformation characterized by dihedral angles $\phi_2 = 62.8°$ and $\psi_2 = -139.9°$.

The modes of packing of L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP are shown in Figures 3 and 4, respectively. In both cases the hydrophilic zwitterionic rings of the aspartyl moieties of symmetry related molecules face each other in the unit cell interacting through electrostatic forces and hydrogen bonds. In these interactions the co-crystallized water molecules also take part. Hydrophobic interactions between aromatic benzyl moieties occur at the other end of the molecules.

Nuclear magnetic resonance-(NMR) and computer simulation studies were carried out on the two analogues. For the sweet analogue L-Asp-D-Ala-OTMCP, two possible conformations were found with the lowest energies, as shown in Figure 5. Conformer I is L-shaped as defined by our model for sweetness, conformer II is almost L-shaped with the C-terminal group pointing in the $+z$ direction. The X-ray structure is similar to conformer I where the dihedral angles of the second residue are $\phi_2 = 62.8$ and $\psi_2 = -139.9$ for the X-ray structure and $\phi_2 = 84$

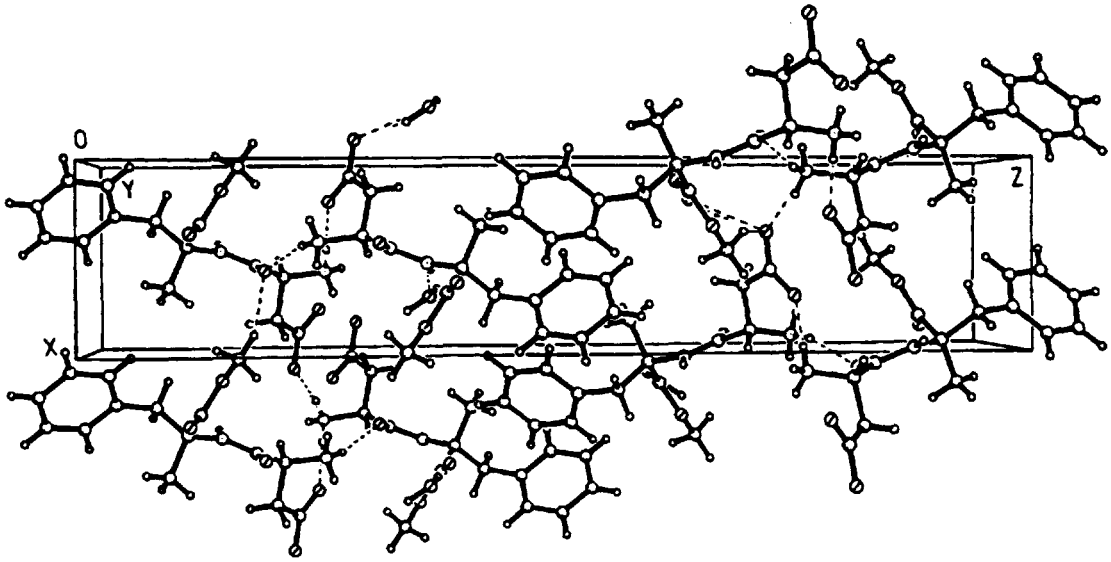


Figure 3 Molecular packing of the L-Asp-D-(αMe)Phe-OMe molecules. Hydrogen bonds are indicated as dashed lines.

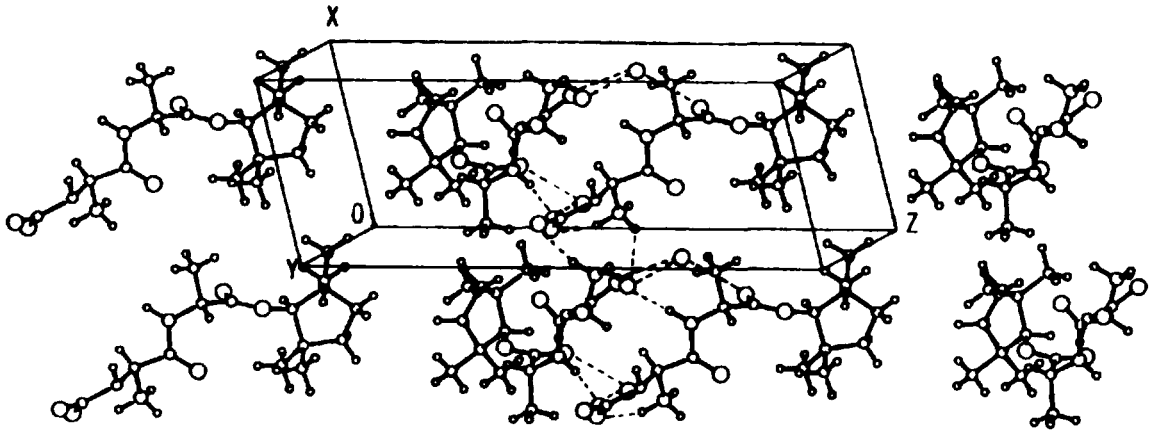


Figure 4 Molecular packing of the L-Asp-D-Ala-OTMCP molecules. Hydrogen bonds are indicated as dashed lines.

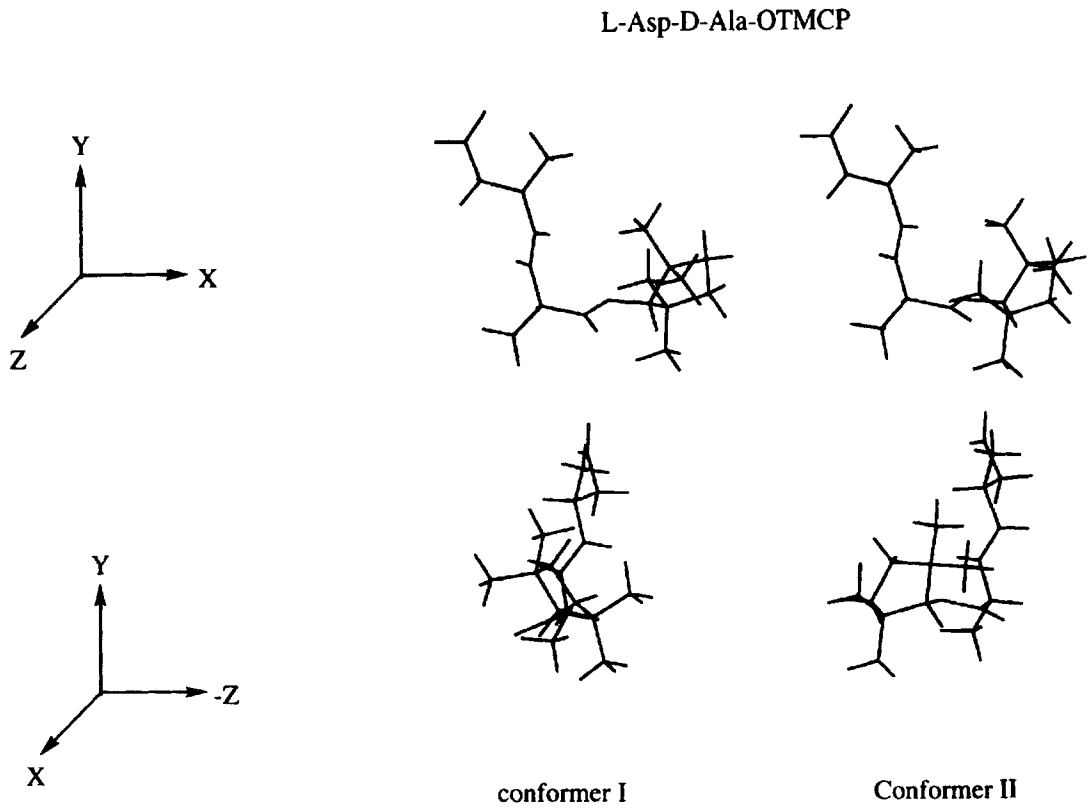


Figure 5 Preferred conformations of L-Asp-D-Ala-OTMCP in solution.

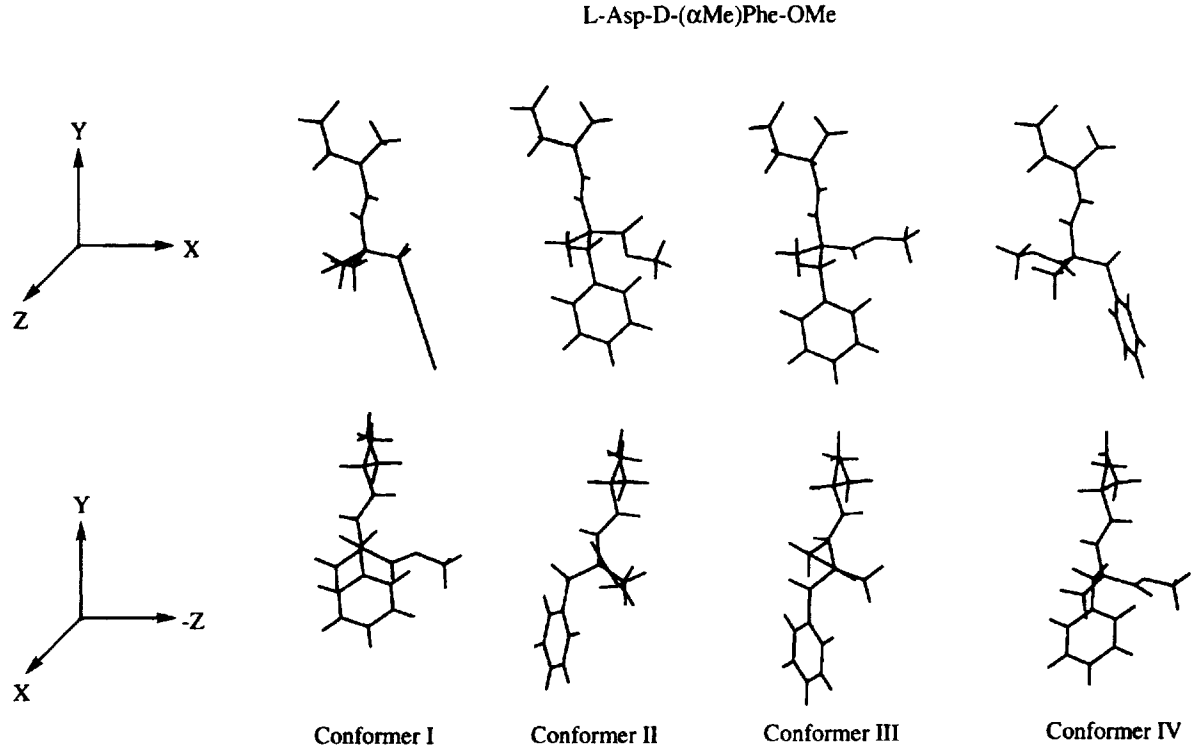


Figure 6 Preferred conformations of L-Asp-D-(αMe)Phe-OMe in solution.

and $\psi_2 = -84$ for conformer I. As shown in Figure 6, the analogue L-Asp-D-(αMe)Phe-OMe displays four comparable low-energy conformations. In both conformer I and IV, the hydrophobic X group extends into the $-z$ direction. According to our model this should lead to a bitter compound. In the second and third conformation, the phenyl ring and the zwitterionic ring are in an extended conformation, which should be tasteless according to our model. The X-ray structure corresponds to conformer III where the dihedral angles of the second residue are $\phi_2 = 49.0$ and $\psi_2 = 47.9$ for the X-ray structure and $\phi_2 = 53$ and $\psi_2 = 49$ for conformer III.

A comparison of the X-ray structures of L-Asp-D-(αMe)Phe-OMe, a bitter analogue, and that of its diastereoisomer L-Asp-L-(αMe)Phe-OMe [22], a sweet analogue, shows that they are essentially the same. In fact, the (αMe)Phe side chain in both diastereoisomers assumes the *trans* conformation about the $C^{\alpha}$–$C^{\beta}$ bond: the relative orientation of the zwitterionic ring of the aspartyl moiety and the benzyl side chain, which are major groups for hydrophilic and hydrophobic interactions, respectively, is exactly the same.

The conformational preferences of the two Asp-(αMe)-Phe-OMe diastereoisomers in solution, estimated by NMR spectroscopy and computer simulations [11], indicated that the molecules exist as equilibrium mixtures of various preferred conformers. The sweet diastereoisomer L-Asp-D-(αMe)Phe-OMe can assume four different topochemical arrays of the AH/B and X functions very similar to those shown by aspartame. One of these array corresponds to the L-shaped conformation. The bitter diastereoisomer L-Asp-D-(αMe)Phe-OMe can also assume four topochemical arrays, but cannot adopt the L-shaped conformation required for sweetness. The structures of L-Asp-D-(αMe)Phe-OMe in solution display significant extension into the $-z$ dimension which explains the bitter taste of this molecule.

## CONCLUSIONS

In this paper the synthesis, X-ray crystallography and conformational studies in solution of the dipeptide taste ligands L-Asp-D-(αMe)Phe-OMe and L-Asp-D-Ala-OTMCP are reported. These analogues are similar to a large number of dipeptide taste ligands whose structure–taste properties are known and have been extensively studied [11]. These studies demonstrate that the dipeptide taste ligands we studied are very similar in terms of overall electronic properties and that the basic taste properties (i.e. sweet, bitter and tasteless) of these analogues are governed entirely by their conformational preferences in solution. The cause of differences in intensity of a given taste response however, are not presently known. These differences in taste potency could be manifestation of subtle electronic effects (induced dipole, dispersion forces, etc.).

The crystal structure of the bitter L-Asp-D-(αMe)Phe-OMe was compared with the crystal structure of its sweet diastereomer L-Asp-L-(αMe)Phe-OMe [22]. Both compounds were found to assume extended conformations in the solid state. These extended structures are inconsistent with the structural requirements of our model for sweet or bitter taste. The conformational analysis of these two analogues in solution by NMR and computer simulations shows that in addition to the extended structures seen in the solid state, the molecules can assume conformations that are consistent with their taste properties as predicted by our model. The peptide L-Asp-D-(αMe)Phe-OMe can assume conformations that have significant extension into the $-z$ dimension which leads to a bitter taste, while L-Asp-L-(αMe)Phe-OMe can readily assume L-shaped structures consistent with a sweet taste.

The sweet compound L-Asp-D-Ala-OTMCP assumes L-shaped structures in the solid state and in solution. These results agree with our L-shaped model for sweet taste. These results indicate that one must use caution when making taste predictions that are based on X-ray structures when working with such small flexible molecules.

### Acknowledgements

The authors gratefully acknowledge the financial support of the Ministry of Education in Italy, the Progetto Finalizzato 'Chimica Fine II' of the CNR (grant PF 91.1657), the Human Capital and Mobility Program of the European Community (contract ERBCHRXCT930286) and the National Institutes of Health (DE05476).

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