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Aspartame dipeptide analogues: effect of number of side-chain methylene group spacers and C^{α}-methylation in the second position

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Abstract: Our model of the active site of the sweet taste receptor is shown to be consistent with the aspartame analogues in which the L-Phe^2 residue is replaced by L-(αMe)Phg, L-(αMe)Phe or L-(αMe)Hph. The analogues containing either the first or the third C^{α}-methylated, phenyl-containing residue in the second position of the dipeptide were synthesized and found to be approximately as sweet as aspartame itself and its L-(αMe)Phe^2 analogue. © 1997 Elsevier Science Ltd

Since the serendipitous discovery of aspartame (H-L-Asp-L-Phe-OMe) **5** in 1965,[1] there has been a huge body of experimental and theoretical work to examine the potential of this class of low-calorie sweeteners. *Inter alia*, it has been shown that many changes are well tolerated in the second position of the dipeptide, but it is the combination of the substituents at the α-carbon and its stereochemical properties that determines the sweetness potency.[2–7] Our interest in aspartame and its analogues dates back to the early '70s.[8,9] More recent efforts from our laboratories have involved synthesis, 3D-structural analysis, and stability tests of conformationally restricted analogues of aspartame, super-aspartame, and anti-aspartame.[10–15]

Our current aim is the development of new low-calorie, heat and pH-stable aspartame analogues and the investigation of the structural properties required to exhibit sweet perception. In this study we checked the reliability of our proposed model for the sweet receptor,[12,16,17] that can discriminate among sweet, bitter and tasteless compounds of very similar chemical constitution but slightly different shape, by the fit of the three conformationally constrained L,L-aspartame analogues **3**, **7**, and **11** C^{α}-methylated in the second position.

The synthesis and taste properties of the L-(αMe)Phe^2 analogue **7**[11] and its D-(αMe)Phe^2 diastereomer **8**[4,5,11] have already been reported. Here we also describe the preparation, characterization, and taste determination of the L-(αMe)Phg^2 **3**, D-(αMe)Phg^2 **4**, L-(αMe)Hph^2 **11**, and D-(αMe)Hph^2 **12** analogues. A comparison is made with the known taste sensations elicited by the non-C^{α}-methylated L-Phg^2 **1**,[2,3] D-Phe^2 **6**,[2,6] and L-Hph^2 **9**[2] analogues. To our knowledge, the organoleptic properties of the D-Phg^2 **2** and D-Hph^2 **10** analogues have never been published. The chemical formulae of all the compounds discussed in this article are shown in Scheme 1.

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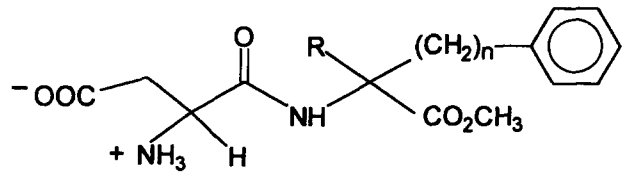

1: n = 0	R = H	(L, L)	**7**: n = 1	R = CH_3	(L, L)
2: n = 0	R = H	(L, D)	**8**: n = 1	R = CH_3	(L, D)
3: n = 0	R = CH_3	(L, L)	**9**: n = 2	R = H	(L, L)
4: n = 0	R = CH_3	(L, D)	**10**: n = 2	R = H	(L, D)
5: n = 1	R = H	(L, L)	**11**: n = 2	R = CH_3	(L, L)
6: n = 1	R = H	(L, D)	**12**: n = 2	R = CH_3	(L, D)

Scheme 1.

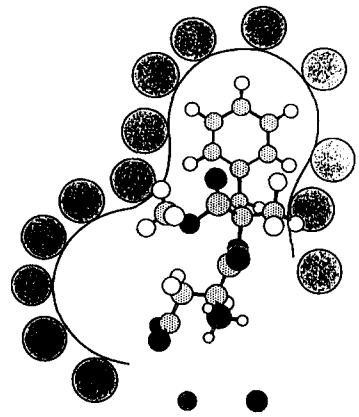

Figure 1. Fit of the X-ray diffraction molecular structure of the aspartame analogue **7**[11] in the model of the active site of the sweet receptor.[12,16,17] The conformation is F_ID_{II}, identical to the proposed bioactive conformation of aspartame. As for aspartame, the ester group sticks out of the main plane of the active site. The donor and acceptor moieties of the AH–B entity of the receptor are represented as a nitrogen and an oxygen atom, respectively.

Results and discussion

As previously described,[12] our model of the active site of the sweet taste receptor was derived from a combination of the mapping with saccharins[16] and 3-anilino-2-styryl-3*H*-naphth(1,2-*d*)imidazolesulphonate.[17] The shape of the receptor active site in the main plane of the flat cavity corresponds to the convolution of the van der Waals radii of the outer atoms of the moulds. Figure 1 shows the fit of the X-ray diffraction structure of L-(αMe)Phe^2-aspartame **7** in the bidimensional contour of the active site. The crystal-state model is virtually identical to conformer F_ID_{II} of aspartame we previously proposed as the bioactive conformation of the sweetener,[16,17] but different from the crystal-state conformation of aspartame itself.[18]

Starting from the crystal-state structure of L-(αMe)Phe^2-aspartame **7** we built corresponding low-energy models for the L-(αMe)Phg^2 **3** and L-(αMe)Hph^2 **11** analogues with the only initial restraint of maintaining the same Asp^1 conformation. Owing to the flexibility of **3** and **11**, it is not possible to pinpoint a meaningful absolute energy minimum. We found several slightly different conformers of comparable energy and selected the lowest energy ones whose shape is close to that of the crystal-

Table 1. Relevant backbone and side-chain torsion angles for the final molecular models of the aspartame analogues **3**, **7**, and **11**

Torsion angle		Analogue **3**	Analogue **7**	Analogue **11**
Asp	ψ	160	160	160
	χ^1	-64	-64	-64
Xxx	φ	-80	-42	-52
	ψ	-29	-29	-30
	$\chi^{1,1}$	-90	172	-109
	$\chi^{1,2}$	-90	----	----
	$\chi^{2,1}$	----	-95	-99
	$\chi^{2,2}$	----	85	----
	$\chi^{3,1}$	----	----	117
	$\chi^{3,2}$	----	----	-64

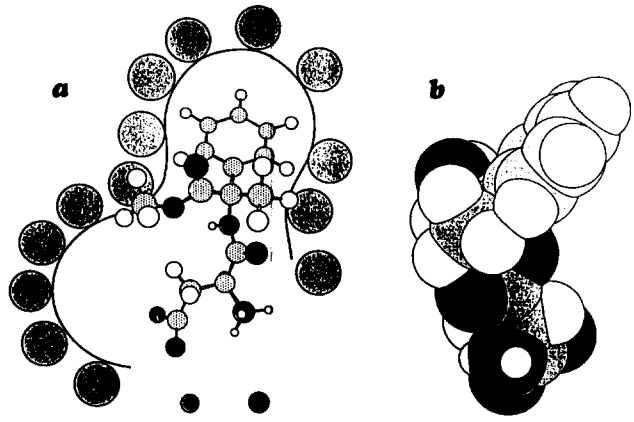

Figure 2. (a) Fit of the minimum energy conformer of the aspartame analogue **3** in the model of the active site of the sweet receptor.[12,16,17] Both the ester and the C^{α}-methyl groups stick out of the main plane of the active site. The donor and acceptor moieties of the AH–B entity of the receptor are represented as a nitrogen and an oxygen atom, respectively. (b) The same conformer of **3** represented as a space-filling model in a direction parallel to the plane of the bidimensional contour.

state structure of **7**. These conformers were subjected to a further unrestrained minimization. Table 1 summarizes the relevant backbone and side-chain torsion angles for the final molecular models of the three analogues. Figures 2 and 3 show the fit of the two calculated models of **3** and **11** in the bidimensional contour of the active site.

It can be appreciated that neither addition nor deletion of a methylene group prevents an interaction comparable to those of aspartame[16,17] and the L-(αMe)Phe^2 analogue **7** (Figure 1). This is due to the fact that it is possible to obtain a fairly flat (nearly bidimensional) conformation for both **3** and **11**, as illustrated in Figure 2b and Figure 3b that show the two molecules in a direction parallel to the plane of the bidimensional contour. This finding is not too surprising in the case of the L-(αMe)Hph^2 analogue **11**, since addition of an extra methylene group increases the conformational freedom with respect to the L-(αMe)Phe^2 analogue **7**. However, it is remarkable that it is still possible to achieve a low-energy flat conformation for the much more constrained L-(αMe)Phg^2 analogue **3**.

For the production of the enantiomerically pure L- and D-(αMe)Phg, and L- and D-(αMe)Hph we

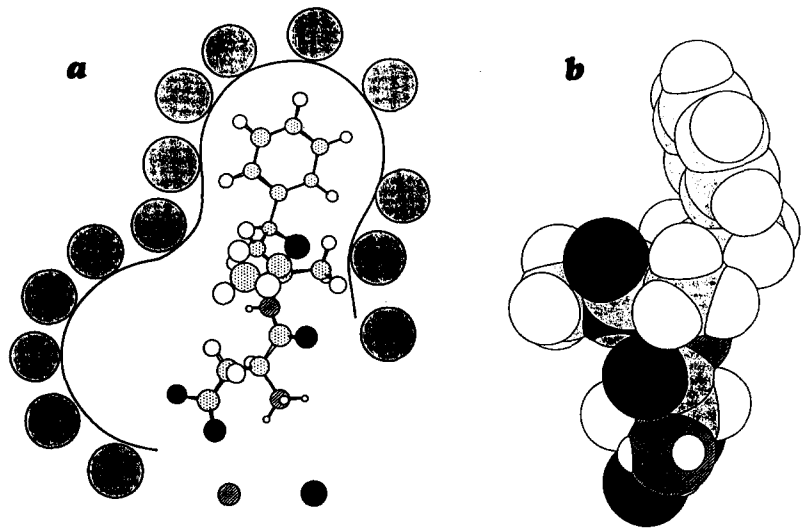

Figure 3. (a) Fit of the minimum energy conformer of the aspartame analogue **11** in the model of the active site of the sweet receptor.[12,16,17] Both the ester and the C^{α}-methyl groups stick out of the main plane of the active site. The donor and acceptor moieties of the AH–B entity of the receptor are represented as a nitrogen and an oxygen atom, respectively. (b) The same conformer of **11** represented as a space-filling model in a direction parallel to the plane of the bidimensional contour.

exploited an economically attractive and generally applicable chemo-enzymatic synthesis developed by the DSM Research group.[19–21] It involves a combination of organic synthesis for the preparation of the racemic amino acid amide, followed by the use of a broadly specific aminopeptidase to achieve resolution on a large scale.

Dipeptides **3**, **4**, **11**, and **12** were prepared from Z-L-Asp(O*t*Bu)-OH and the pertinent α-aminoacyl methylester hydrochloride. Z-L-Asp(O*t*Bu)-OH was synthesized according to the standard literature procedure.[22] The C^{α}-tetrasubstituted α-aminoacyl methylester hydrochlorides were prepared using the thionyl chloride/methanol method,[23] for high yields further addition of fresh thionyl chloride being required.[19] Peptide bond formation was obtained in CH_2Cl_2 solution using the EDC·HCl/HOAt procedure[24] in the presence of a base (NMM). Asp side-chain deprotection was achieved by acidolysis with diluted TFA. The N^{α}-benzyloxycarbonyl group was removed by catalytic hydrogenation (10% Pd/C).

The taste of all these compouds was checked (Table 2). The L-(αMe)Phg[2] **3** and L-(αMe)Hph[2] **11** analogues are approximately as sweet as aspartame itself **5**[1,2] and its L-(αMe)Phe[2] **7** analogue.[11] Noteworthy is the dramatic difference in sweetness of the L–L dipeptides **3** and **11** compared to their L–D diastereomeric counterparts **4** and **12**. The same effect was observed for the L–D diastereomers of aspartame **6**[2,6] and its (αMe)Phe[2] **8** analogue.[4,5,11] Interestingly, the non-C^{α}-methylated L-Phg[2] **1**[2,3] and L-Hph[2] **9**[2] analogues are also sweet.

In summary, this study confirmed that the C^{α}-hydrogen of the C-terminal residue of aspartame is not essential for sweetness,[11] as it can be replaced by a methyl group without loss of the favourable organoleptic properties. Further, it is established that the phenyl ring position (whether linked to the C^{α}-, C^{β}- or C^{γ}-atom) in the side chain of the C-terminal amino acid of the dipeptide is not critical for the sweetness potency. As for the C^{α}-methylated series, where detailed 3D-structural studies have been performed, the present results (see φ, ψ torsion angles in Table 1) are not unexpected, in that the (αMe)Phg,[25,26] (αMe)Phe,[25–27] and (αMe)Hph[25,26,28,29] residues, characterized by an increasing number of side-chain methylene group spacers between the phenyl moiety and the C^{α}-atom, are all known to preferentially adopt backbone torsion angles falling in the helical region of the conformational map.

Table 2. Taste of aspartame analogues

Analogue	Entry	Taste [a]	Reference
H-L-Asp-L-Phg-OMe	1	sweet (140)	2,3
H-L-Asp-D-Phg-OMe	2	-----[b]	-----
H-L-Asp-L-(αMe)Phg-OMe	3	sweet (50-150)	this work
H-L-Asp-D-(αMe)Phg-OMe	4	tasteless	this work
H-L-Asp-L-Phe-OMe	5	sweet (150-200)	1, 2
H-L-Asp-D-Phe-OMe	6	bitter	2, 6
H-L-Asp-L-(αMe)Phe-OMe	7	sweet (200)	11
H-L-Asp-D-(αMe)Phe-OMe	8	bitter	4, 5, 11
H-L-Asp-L-Hph-OMe	9	sweet (100)	2
H-L-Asp-D-Hph-OMe	10	-----[b]	-----
H-L-Asp-L-(αMe)Hph-OMe	11	sweet (50-150)	this work
H-L-Asp-D-(αMe)Hph-OMe	12	bitter	this work

[a] Potencies refer to sucrose as 1. [b] Not known.

Experimental section

Peptide synthesis

Melting points were determined using a Leitz (Wetzlar, Germany) model Laborlux 12 apparatus and are not corrected. Optical rotations were measured using a Perkin–Elmer (Norwalk, CT) model 241 polarimeter equipped with a Haake (Karlsruhe, Germany) model D thermostat. Thin-layer chromatography was performed on Merck (Darmstadt, Germany) Kieselgel $60F_{254}$ precoated plates using the following solvent systems: 1 ($CHCl_3$–EtOH, 9:1); 2 (1-BuOH–AcOH–H_2O, 3:1:1); 3 (toluene–EtOH, 7:1). The chromatograms were examined by UV fluorescence or developed by chlorine–starch–potassium iodide or ninhydrin chromatic reaction as appropriate. All the compounds were obtained in a chromatographically homogeneous state. The solid-state IR absorption spectra (KBr disk or film technique) were recorded with a Perkin–Elmer model 580B spectrophotometer equipped with a Perkin–Elmer model 3600 data station. The ^1H NMR spectra were recorded with a Bruker model AC 250 spectrometer. Measurements were carried out in deuterochloroform (99.96% *d*; Acros Organics, Geel, Belgium), dimethyl-d_6 sulfoxide (99.96% *d*; Acros Organics), deuterium oxide (99.75% *d*; Merck) with tetramethylsilane or 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as internal standards.

HCl·H-L-(αMe)Phg-OMe

MeOH (15 ml) was cooled to −30°C and $SOCl_2$ (0.4 ml, 5.1 mmol) was added dropwise. Then, H-L-(αMe)Phg-OH (0.675 g, 4.08 mmol) was added, and the solution was refluxed for 6.5 h. Then, again $SOCl_2$ (0.4 ml, 5.1 mmol) was added at −30°C and refluxed for 7 h. The MeOH and the excess $SOCl_2$ were evaporated under reduced pressure. The oily product was dissolved in 5% $NaHCO_3$ and extracted with Et_2O. The Et_2O phase was dried over Na_2SO_4 and, after filtration, evaporated under reduced pressure until a 40 ml solution was obtained. Then, a solution of HCl in EtOAc (2.5 ml, 5.2 mmol) was added. The precipitate was isolated by filtration. Yield 77%. M.p. 178–179°C (from Et_2O/EtOAc, HCl); Rf_1=0.80, Rf_2=0.40, Rf_3=0.65; $[\alpha]_D^{20}$=+51.8 (c 0.5, MeOH); IR (KBr): 1729, 1537 cm^{-1}; ^1H NMR (D_2O): δ 7.39 (m, 5H, phenyl CH), 3.71 (s, 3H, OMe CH_3), 1.90 (s, 3H, βCH_3).

HCl·H-D-(αMe)Phg-OMe

This compound was prepared using an identical procedure. Analytical and physico-chemical data are the same as those characterizing the L-enantiomer (with the inversion of the sign of the optical rotation).

HCl·H-L-(αMe)Hph-OMe and its D-enantiomer

These compounds were synthesized as described above for HCl·H-L-(αMe)Phg-OMe. Yield 80%. M.p. 169–170°C (from Et_2O/EtOAc, HCl); Rf_1=0.65, Rf_2=0.35, Rf_3=0.45; $[\alpha]_D^{20}$=+31.1 (c 0.5, MeOH) for the L-enantiomer; IR (KBr): 1745, 1513 cm^{-1}; ^1H NMR (D_2O): δ 7.18 (m, 5H, phenyl **CH**), 3.60 (s, 3H, OMe $\mathbf{CH_3}$), 2.54 (m, 2H, γ$\mathbf{CH_2}$), 2.09 (m, 2H, β$\mathbf{CH_2}$), 1.47 (s, 3H, β$\mathbf{CH_3}$).

Z-L-Asp(OtBu)-L-(αMe)Phg-OMe

Z-L-Asp(O*t*Bu)-OH (0.900 g, 2.78 mmol) was dissolved in anhydrous CH_2Cl_2 (7.5 ml) and cooled to 0°C. Then, HOAt (0.382 g, 2.81 mmol) and EDC·HCl (0.660 g, 3.44 mmol) were added, and stirred until a clear solution was obtained. Then, HCl·H-L-(αMe)Phg-OMe (0.513 g, 2.38 mmol) was added, followed by NMM (310 µl, 2.81 mmol). After stirring the solution at room temperature for 96 h, the reaction mixture was dissolved in EtOAc and washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, H_2O, dried over Na_2SO_4, and evaporated to dryness. The oily product was purified by column chromatography [ICN silica 32–63, 60A; eluent: EtOAc/petroleum ether/ethanol (10: 10: 1)]. Oil. Yield 91%. Rf_1=0.90, Rf_2=0.95, Rf_3=0.55; $[\alpha]_D^{20}$=−1.1 (c 0.5, MeOH); IR (film): 3327, 1729 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.76 (s, 1H, Phg **NH**), 7.41 (m, 5H, Z-phenyl **CH**), 7.28 (m, 5H, Phg-phenyl **CH**), 6.00 (d, 1H, Asp **NH**), 5.15 (s, 2H, Z $\mathbf{CH_2}$), 4.57 (m, 1H, Asp α**CH**), 3.66 (s, 3H, OMe $\mathbf{CH_3}$), 2.75 (m, 2H, Asp β$\mathbf{CH_2}$), 2.00 (s, 3H, Phg β$\mathbf{CH_3}$), 1.40 (s, 9H, O*t*Bu $\mathbf{CH_3}$).

Z-L-Asp-L-(αMe)Phg-OMe

Z-L-Asp(O*t*Bu)-L-(αMe)Phg-OMe (0.612 g, 1.26 mmol) was dissolved in CH_2Cl_2 (10 ml). Then, TFA (10 ml) was added. The solution was stirred for 1.5 h. TFA and CH_2Cl_2 were evaporated under reduced pressure. The residue was dissolved in EtOAc (100 ml) and the product was extracted with a 5% $NaHCO_3$ solution. This solution was washed with Et_2O and acidified with $KHSO_4$ (to pH 3). Then, the product was extracted with EtOAc, washed with water, dried over Na_2SO_4, and evaporated to dryness. Oil. Yield 91%. Rf_1=0.30, Rf_2=0.95, Rf_3=0.15; $[\alpha]_D^{20}$=−10.6 (c 0.5, MeOH); IR (film): 3321, 1727, 1521 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.78 (s, 1H, Phg **NH**), 7.35 (m, 5H, Z-phenyl **CH**), 7.28 (m, 5H, Phg-phenyl **CH**), 5.96 (d, 1H, Asp **NH**), 5.14 (s, 2H, Z $\mathbf{CH_2}$), 4.63 (m, 1H, Asp α**CH**), 3.65 (s, 3H, OMe $\mathbf{CH_3}$), 2.85 (m, 2H, Asp β$\mathbf{CH_2}$), 1.98 (s, 3H, Phg β$\mathbf{CH_3}$).

H-L-Asp-L-(αMe)Phg-OMe 3

Z-L-Asp-L-(αMe)Phg-OMe (0.290 g, 0.68 mmol) was dissolved in MeOH (25 ml) and Z-deprotected by a Pd-catalyzed hydrogenation reaction in 1.5 h. Yield 79%. M.p. 120–122°C (from MeOH/Et_2O); Rf_1=0.00, Rf_2=0.60, Rf_3=0.00; $[\alpha]_D^{20}$=+19.7 (c 0.5, MeOH); IR (KBr): 3426, 1734, 1687, 1534 cm^{-1}; ^1H NMR (DMSO, d_6): δ 9.05 (s, 1H, Phg **NH**), 7.42 (m, 5H, Phg-phenyl **CH**), 4.11 (m, 1H, Asp α**CH**), 3.56 (s, 3H, OMe $\mathbf{CH_3}$), 2.71 (m, 2H, Asp β$\mathbf{CH_2}$), 1.83 (s, 3H, Phg β$\mathbf{CH_3}$).

Z-L-Asp(OtBu)-D-(αMe)Phg-OMe

This compound was prepared according to the procedure described above for Z-L-Asp(O*t*Bu)-L-(αMe)Phg-OMe: Z-L-Asp(O*t*Bu)-OH (0.900 g, 2.78 mmol); CH_2Cl_2 (7 ml); HOAt (0.380 g, 2.78 mmol); EDC·HCl (0.660 g, 3.44 mmol); H-D-(αMe)Phg-OMe (0.427 g, 2.38 mmol); and NMM (310 µl, 2.81 mmol). The oily product was purified by column chromatography [ICN silica 32–63, 60A; eluent: EtOAc/petroleum ether/ethanol (10: 10: 1)]. Oil. Yield 94%. Rf_1=0.95, Rf_2=0.95, Rf_3=0.60; $[\alpha]_D^{20}$=−21.5 (c 0.5, MeOH); IR (film): 3334, 1729, 1683 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.75 (s, 1H,

Phg NH), 7.37 (m, 5H, Z-phenyl CH), 7.27 (m, 5H, Phg-phenyl CH), 5.98 (d, 1H, Asp NH), 5.15 (s, 2H, Z CH_2), 4.55 (m, 1H, Asp αCH), 3.66 (s, 3H, OMe CH_3), 2.75 (m, 2H, Asp βCH_2), 1.98 (s, 3H, Phg βCH_3), 1.43 (s, 9H, O*t*Bu CH_3).

Z-L-Asp-D-(αMe)Phg-OMe

This compound was prepared according to the procedure described above for Z-L-Asp-L-(αMe)Phg-OMe: Z-L-Asp(O*t*Bu)-D-(αMe)Phg-OMe (0.620 g, 1.28 mmol); CH_2Cl_2 (10 ml); TFA (10 ml). Yield 77%. M.p. 124–125°C (from Et_2O); Rf_1=0.25, Rf_2=0.95, Rf_3=0.15; $[\alpha]_D^{20}$=−24.5 (c 0.5, MeOH); IR (KBr): 3310, 3291, 1748, 1715, 1683, 1545 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.80 (s, 1H, Phg NH), 7.35 (m, 5H, Z-phenyl CH), 7.30 (m, 5H, Phg-phenyl CH), 5.95 (d, 1H, Asp NH), 5.16 (s, 2H, Z CH_2), 4.59 (m, 1H, Asp αCH), 3.65 (s, 3H, OMe CH_3), 2.86 (m, 2H, Asp βCH_2), 1.98 (s, 3H, Phg βCH_3).

H-L-Asp-D-(αMe)Phg-OMe ***4***

This compound was prepared according to the procedure described above for H-L-Asp-L-(αMe)Phg-OMe: Z-L-Asp-D-(αMe)Phg-OMe (0.235 g, 0.54 mmol); MeOH (25 ml). Yield 83%. M.p. 127–129°C (from MeOH/petroleum ether); Rf_1=0.00, Rf_2=0.55, Rf_3=0.00; $[\alpha]_D^{20}$=−10.5 (c 0.5, MeOH); IR (KBr): 3418, 1718, 1694, 1551 cm^{-1}; ^1H NMR (DMSO, d_6): δ 8.90 (s, 1H, Phg NH), 7.39 (m, 5H, Phg-phenyl CH), 3.73 (m, 1H, Asp αCH), 3.58 (s, 3H, OMe CH_3), 2.49 (m, 2H, Asp βCH_2), 1.78 (s, 3H, Phg βCH_3).

Z-L-Asp(OtBu)-L-(αMe)Hph-OMe

This compound was prepared according to the procedure described above for Z-L-Asp(O*t*Bu)-L-(αMe)Phg-OMe: Z-L-Asp(O*t*Bu)-OH (0.925 g, 2.86 mmol); CH_2Cl_2 (10 ml); HOAt (0.390 g, 2.86 mmol); EDC·HCl (0.660 g, 3.44 mmol); HCl·H-L-(αMe)Hph-OMe (0.580 g, 2.38 mmol); and NMM (315 µl, 2.87 mmol). The oily product was purified by column chromatography [ICN silica 32–63, 60A; eluent: EtOAc/petroleum ether (3: 7)]. Oil. Yield 95%. Rf_1=0.95, Rf_2=0.95, Rf_3=0.60; $[\alpha]_D^{20}$=−5.5 (c 0.5, MeOH); IR (film): 3337, 1729, 1678 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36 (s, 1H, Hph NH), 7.27 (m, 5H, Z-phenyl CH), 7.20 (m, 5H, Hph-phenyl CH), 6.00 (d, 1H, Asp NH), 5.15 (s, 2H, Z CH_2), 4.53 (m, 1H, Asp αCH), 3.68 (s, 3H, OMe CH_3), 2.75 (m, 2H, Asp βCH_2), 2.57 (m, 2H, Hph γCH_2), 2.21 (m, 2H, Hph βCH_2), 1.58 (s, 3H, Hph βCH_3), 1.42 (s, 9H, O*t*Bu CH_3).

Z-L-Asp-L-(αMe)Hph-OMe

This compound was prepared according to the procedure described above for Z-L-Asp-L-(αMe)Phg-OMe: Z-L-Asp(O*t*Bu)-L-(αMe)Hph-OMe (0.637 g, 1.24 mmol); CH_2Cl_2 (10 ml); TFA (10 ml). Yield 89%. M.p. 128–129°C (from Et_2O/petroleum ether); Rf_1=0.30, Rf_2=0.95, Rf_3=0.15; $[\alpha]_D^{20}$=+11.9 (c 0.5, MeOH); IR (KBr): 3310, 3291, 1737, 1713, 1690, 1544 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.37 (s, 1H, Hph NH), 7.35 (m, 5H, Z-phenyl CH), 7.16 (m, 5H, Hph-phenyl CH), 5.83 (d, 1H, Asp NH), 5.15 (s, 2H, Z CH_2), 4.56 (m, 1H, Asp αCH), 3.69 (s, 3H, OMe CH_3), 2.86 (m, 2H, Asp βCH_2), 2.60 (m, 2H, Hph γCH_2), 2.15 (m, 2H, Hph βCH_2), 1.59 (s, 3H, Hph βCH_3).

H-L-Asp-L-(αMe)Hph-OMe ***11***

This compound was prepared according to the procedure described above for H-L-Asp-L-(αMe)Phg-OMe: Z-L-Asp-L-(αMe)Hph-OMe (0.380 g, 0.83 mmol); MeOH (25 ml). Yield 84%. M.p. 83–85°C (from MeOH/Et_2O); Rf_1=0.00, Rf_2=0.60, Rf_3=0.00; $[\alpha]_D^{20}$=+7.1 (c 0.5, MeOH); IR (KBr): 3364, 1731, 1715, 1678, 1569 cm^{-1}; ^1H NMR (DMSO, d_6): δ 8.85 (s, 1H, Hph NH), 7.20 (m, 5H, Hph-phenyl CH), 3.68 (m, 1H, Asp αCH), 3.56 (s, 3H, OMe CH_3), 2.49 (m, 2H, Hph γCH_2), 2.35 (m, 2H, Asp βCH_2), 2.00 (m, 2H, Hph βCH_2), 1.43 (s, 3H, Hph βCH_3).

Z-L-Asp(OtBu)-D-(αMe)Hph-OMe

This compound was prepared according to the procedure described above for Z-L-Asp(O*t*Bu)-L-(αMe)Phg-OMe: Z-L-Asp(O*t*Bu)-OH (0.926 g, 2.86 mmol); CH_2Cl_2 (10 ml); HOAt (0.391 g, 2.87 mmol); EDC·HCl (0.660 g, 3.44 mmol); H-D-(αMe)Hph-OMe (0.490 g, 2.36 mmol); and NMM (315 µl, 2.86 mmol). The oily product was purified by column chromatography [ICN silica 32–63, 60 A; eluent: EtOAc/petroleum ether (3: 7)]. Oil. Yield 94%. Rf_1=0.95, Rf_2=0.95, Rf_3=0.65; $[\alpha]_D^{20}$=−7.9 (c 0.5, MeOH); IR (film): 3334, 1729, 1678 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.35 (s, 1H, Hph N**H**), 7.30 (m, 5H, Z-phenyl C**H**), 7.24 (m, 5H, Hph-phenyl C**H**), 5.94 (d, 1H, Asp N**H**), 5.15 (s, 2H, Z $C\mathbf{H}_2$), 4.52 (m, 1H, Asp α**CH**), 3.68 (s, 3H, OMe $C\mathbf{H}_3$), 2.75 (m, 2H, Asp β$C\mathbf{H}_2$), 2.55 (m, 2H, Hph γ$C\mathbf{H}_2$), 2.26 (m, 2H, Hph β$C\mathbf{H}_2$), 1.56 (s, 3H, Hph β$C\mathbf{H}_3$), 1.42 (s, 9H, O*t*Bu $C\mathbf{H}_3$).

Z-L-Asp-D-(αMe)Hph-OMe

Z-L-Asp(O*t*Bu)-D-(αMe)Hph-OMe (0.631 g, 1.23 mmol) was dissolved in CH_2Cl_2 (10 ml). Then, TFA (10 ml) was added. The solution was stirred for 1.5 h. TFA and CH_2Cl_2 were evaporated under reduced pressure. A solid product was obtained upon treatment with Et_2O (25 ml). Yield 80%. M.p. 145–146°C (from Et_2O); Rf_1=0.35, Rf_2=0.95, Rf_3=0.20; $[\alpha]_D^{20}$=−18.3 (c 0.5, MeOH); IR (KBr): 3312, 1737, 1713, 1690, 1639, 1544 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.37 (s, 1H, Hph N**H**), 7.25 (m, 5H, Z-phenyl C**H**), 7.14 (m, 5H, Hph-phenyl C**H**), 5.87 (d, 1H, Asp N**H**), 5.16 (s, 2H, Z $C\mathbf{H}_2$), 4.55 (m, 1H, Asp α**CH**), 3.67 (s, 3H, OMe $C\mathbf{H}_3$), 2.85 (m, 2H, Asp β$C\mathbf{H}_2$), 2.50 (m, 2H, Hph γ$C\mathbf{H}_2$), 2.24 (m, 2H, Hph β$C\mathbf{H}_2$), 1.56 (s, 3H, Hph β$C\mathbf{H}_3$).

H-L-Asp-D-(αMe)Hph-OMe ***12***

This compound was prepared according to the procedure described above for H-L-Asp-L-(αMe)Phg-OMe: Z-L-Asp-D-(αMe)Hph-OMe (0.312 g, 0.68 mmol); MeOH (20 ml). Yield 68%. M.p. 134–135°C (from Et_2O/petroleum ether); Rf_1=0.00, Rf_2=0.65, Rf_3=0.05; $[\alpha]_D^{20}$=+8.6 (c 0.5, MeOH); IR (KBr): 3386, 1735, 1681, 1553 cm^{-1}; ^1H NMR (DMSO, d_6): δ 8.61 (s, 1H, Hph N**H**), 7.20 (m, 5H, Hph-phenyl C**H**), 3.66 (m, 1H, Asp α**CH**), 3.58 (s, 3H, OMe $C\mathbf{H}_3$), 2.47 (m, 2H, Hph γ$C\mathbf{H}_2$), 2.35 (m, 2H, Asp β$C\mathbf{H}_2$), 2.09 (m, 2H, Hph β$C\mathbf{H}_2$), 1.41 (s, 3H, Hph β$C\mathbf{H}_3$).

Taste determination

Dipeptides **3**, **4**, **11** and **12** were taste checked by three volunteers from the DSM Research laboratory. The panel was able to achieve reproducible taste intensities involving solutions of sucrose and of these compounds. Sweetness intensities were determined by a ranking test, with aqueous sucrose solutions of 0.5, 2.0, 4.0 and 8.0% (*w/v*) as references. At least three double-blind tests were performed by the panel on each compound.

Stereochemical receptor model for sweet taste

The bidimensional contour of the active site model, used to compare the fit of the aspartame analogues **3**, **7** and **11**, was derived from a combination of the mapping with saccharins and with 3-anilino-2-styryl-3*H*-naphth[1,2-*d*]imidazolesulphonate, as described in refs[16,17]. The shape of the receptor active site corresponds to the convolution of the van der Waals radii of the outer atoms of the moulds. It was obtained by positioning around the moulds fictitious 'monoatomic apolar molecules'. The main features of the receptor 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 part of the cavity contains the main 'electronic features', the most important of which is the AH–B entity (this part is essential for binding). (iii) The upper part of the cavity is hydrophobic and plays an important role in the modulation of sweetness intensity.

Molecular mechanics calculations

MM calculations were based on the all atoms parametrization of the AMBER force field[30,31] as implemented in the SYBYL package. EM calculations were performed with a distance-dependent

dielectric constant (ε=r) and no distance cut-off for the non-bonded interactions. The search of the conformational space accessible to the L-(αMe)Xxx^2 moieties of the aspartame analogues **3**, **7** and **11** was facilitated by the initial restraint of maintaining the same L-Asp^1 conformation as in the crystal state structure of L-(αMe)Phe^2-aspartame **7**. The conformers selected by the search were subjected to energy minimization up to a rms (root mean square) gradient of 10^{-2}. Only conformers within 3 kcal/mol from the absolute minimum were retained. These conformers were further energy minimized up to a rms gradient of 10^{-4}.

Abbreviations

Phg, phenylglycine; (αMe)Phg, C^{α}-methyl phenylglycine; (αMe)Phe, C^{α}-methyl phenylalanine; Hph, homo-phenylalanine; (αMe)Hph, C^{α}-methyl homo-phenylalanine; Z, benzyloxycarbonyl; OMe, methoxy; O*t*Bu, *tert*-butoxy; MeOH, methanol; EtOH, ethanol; 1-BuOH, 1-butanol; AcOH, acetic acid; Et_2O, diethyl ether; EtOAc, ethyl acetate; EDC, N-[3-(dimethylaminopropyl)], N′-ethyl-carbodiimide; HOAt, 1-hydroxy-7-aza-benzotriazole; NMM, N-methyl morpholine; TFA, trifluoroacetic acid.

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