Structures of Sweet and Bitter Peptide Diastereomers by NMR, Computer Simulations, and X-ray Crystallography

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Abstract: New dipeptide taste ligands L-aspartyl-(*S*)-cyclohexylglycine methyl ester [Asp-(*S*)-Chg-OCH_3: 50-times sweeter than sucrose] and L-aspartyl-(*R*)-cyclohexylglycine methyl ester [Asp-(*R*)-Chg-OCH_3: bitter] were synthesized. Preferred conformations of these diastereomers were studied by ^{1}H-NMR spectroscopy, computer simulations, and X-ray crystallography. The sweet-tasting analog, Asp-(*S*)-Chg-OCH_3, preferentially assumes an "L" shaped structure, with the AH (hydrogen bond donor) and B (hydrogen bond acceptor) containing aspartyl moiety forming the stem of the "L" and the hydrophobic cyclohexyl side chain (X) as the base of the "L", coplanar with and nearly perpendicular to the aspartyl zwitterionic ring. In contrast, a reversed "L" shaped structure is accessible to the corresponding diastereomer Asp-(*R*)-Chg-OCH_3, and the cyclohexyl group preferentially orients behind the stem of the "L", resulting in a large $-z$ component. Therefore, Asp-(*R*)-Chg-OCH_3 produces a bitter taste. The structural studies of the taste ligands Asp-(*S*)-Chg-OCH_3 and Asp-(*R*)-Chg-OCH_3 provide strong support for the previously developed "L" shape model for sweet and bitter tastes.

Introduction

Many attempts have been made to generalize structural features among sweet molecules. The results of these efforts have led to the agreement that sweet molecules contain a hydrogen bond donor (AH) and a hydrogen bond acceptor (B)[1] and that highly potent sweet molecules have a hydrophobic site (X) along with the AH/B functions.[2-5] In the case of aspartyl-based sweeteners, the α-amino and β-carboxyl groups of the N-terminal aspartyl residue are assigned as the AH and B elements, respectively. The distances between the AH and B elements are in agreement with AH to B distances identified in numerous non-peptide sweet-tasting molecules (2.5-4.0 Å).[1] A large hydrophobic group among the side chain or the ester or amide substituent on the C-terminal residue serves as the third binding site X.

The relative orientation of the X group of the C-terminal residue to the AH/B groups of the aspartyl moiety determines the particular taste response that a molecule elicits. This orientation could be conformationally fixed when molecules bind to taste receptors. From the results of conformational studies on a number of aspartyl dipeptides and peptidomimetic analogs by NMR spectroscopy, X-ray crystallography, and computer simulations, we have proposed a three-dimensional model describing molecular structures required for sweet and bitter tastes.[6-11] The overall conformations of the various sweet-tasting analogs can be described as possessing an "L" shape, with the AH and B zwitterionic aspartyl moiety as the stem of the "L" in the $+y$ axis and the hydrophobic moiety X in the $+x$ axis as the base of the "L", coplanar with and nearly perpendicular to the aspartyl zwitterionic ring. Bitter-tasting analogs assume characteristic conformations with significant extension of the X moiety into the $-z$ axis. In tasteless analogs, the X moiety orients in the other directions ($-x$, $-y$, and $+z$). The model has been probed using the structure–taste relationships observed for a new class of L-aspartyl dipeptide taste ligands containing the peptidomimetic 2-aminocyclopentanecarboxylic acid methyl ester (Asp-2-Ac^{5}c-OCH_3) as the C-terminal residue.[10] Although the model has been developed with analogs in which the ester or amide on the C-terminal residue serves as the hydrophobic moiety X, it fits the X-ray crystal structure of L-aspartyl-L-phenylalanine methyl ester (aspartame),[12] in which the benzyl side chain functions as the X element, with only a minor modification of the conformation. The side chain conformation about the C^α–C^β bond (χ_1) of phenylalanine is switched from g^+ ($\sim +60°$) to g^- ($\sim -60°$), which is most preferred in solution.[13]

Table I. Crystal Data for L-Aspartyl-(*S* and *R*)-cyclohexlglycine Methyl Ester

	Asp-(*S*)-Chg-OCH_3	Asp-(*R*)-Chg-OCH_3
experimental formula	$C_{13}H_{22}N_2O_5 \cdot 2H_2O$	$C_{13}H_{22}N_2O_5 \cdot H_2O$
color, habit	colorless, plate-like	colorless, plate-like
crystal size	0.15 × 0.25 × 0.30	0.07 × 0.20 × 0.25 mm
crystal system	monoclinic	monoclinic
space group	$P2_1$	$P2_1$
unit cell dimensions	a = 10.0134 (15) Å	a = 6.367 (3) Å
	b = 4.7822 (12) Å	b = 6.412 (3) Å
	c = 18.215 (9) Å	c = 18.952 (8) Å
	β = 98.70 (2)°	β = 95.97 (3)°
volume	862.2 (5) Å^3	769.5 (6) Å^3
Z	2	2
formula weight	322.3	304.3
density (calc)	1.242 g cm^{-3}	1.313 g cm^{-3}
absorption coefficient	0.941 mm^{-1}	0.832 mm^{-1}
F(000)	348	328
no. independent reflections	1270 (R_{int} = 2.14%)	1260 (R_{int} = 2.68%)
unique reflections used (m)	895 [$F_o < 3.0\sigma(F_o)$]	868 [$F_o < 4.0\sigma(F_o)$]
no. parameters refined (n)	135	179
final R value	0.1085	0.0625
final weighted R value	0.1217	0.0738

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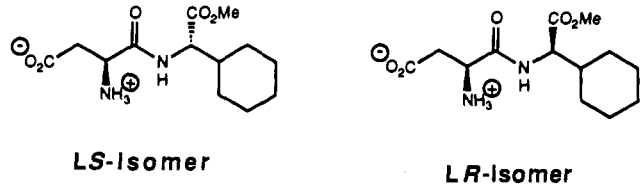

Figure 1. Structures of L-aspartyl-(*S*)-cyclohexylglycine methyl ester (L-*S*-isomer) and L-aspartyl-(*R*)-cyclohexylglycine methyl ester (L-*R*-isomer).

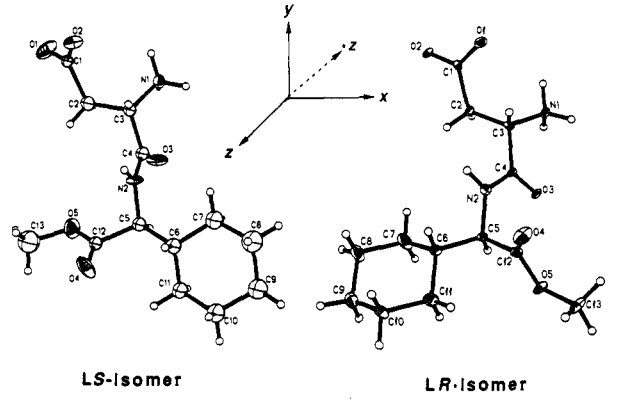

Figure 2. X-ray structures of L-aspartyl-(*S*)-cyclohexylglycine methyl ester (L-*S*-isomer) and L-aspartyl-(*R*)-cyclohexylglycine methyl ester (L-*R*-isomer).

In this paper we report on the structures of two diastereomeric compounds, L-aspartyl-(*S*)-cyclohexylglycine methyl ester [Asp-(*S*)-Chg-OCH_3] and L-aspartyl-(*R*)-cyclohexylglycine methyl ester [Asp-(*R*)-Chg-OCH_3]; the former is sweet and the latter is bitter. The inclusion of Asp-Chg-OCH_3 into the family of peptide-based taste ligands studied thus far serves to strengthen our model. In addition, we include the X-ray crystal structures for both molecules and note that this is the first reported crystal structure of a bitter peptide-based taste ligand.

Results and Discussion

Figure 1 illustrates the structures of the diastereomeric taste ligands Asp-(*S*)-Chg-OCH_3 (L-*S*-isomer) and Asp-(*R*)-Chg-OCH_3 (L-*R*-isomer), which were synthesized by established procedures. The colorless crystals for both diastereomers were grown from a solution of 2-propanol and water by slow evaporation. The molecule Asp-(*S*)-Chg-OCH_3 crystallized with two molecules of water while the molecule Asp-(*R*)-Chg-OCH_3 crystallized with one molecule of water. Both diastereomers crystallized in the monoclinic $P2_1$ space group with two molecules in the unit cell. Crystallographic data are reported in Table I. The X-ray crystal structures of the two diastereomers are shown in Figure 2. It is readily seen that Asp-(*S*)-Chg-OCH_3 adopts an "L" shaped structure with the zwitterionic aspartyl moiety as the stem of the "L" in the $+y$ axis and the hydrophobic cyclohexyl side chain as the base of the "L" in the $+x$ axis, while Asp-(*R*)-Chg-OCH_3 adopts a "reversed L" structure where the cyclohexyl side chain orients towards the $-x$ axis. Despite the presence of a different number of water molecules in the two crystals, the packing is governed primarily by the electrostatic interactions between charged groups of the zwitterionic moiety of the Asp residue. This gives rise to a hydrophilic core stabilized by a complicated H-bonding pattern between charged groups in which the water molecules also participate. The resulting hydrophilic cores in both crystals are surrounded by hydrophobic surfaces represented by the stacked cyclohexyl or methyl groups.

Table II. Selected Torsion Angles (deg) for L-Aspartyl-(*S* and *R*)-cyclohexylglycne Methyl Esters Determined by X-Ray Diffraction Studies

torsion	Asp-(*S*)-Chg-OCH_3	Asp-(*R*)-Chg-OCH_3
Asp ψ	144.9	169.0
ω	172.9	177.4
χ_1	-66.9	-81.0
χ_2	151.0	174.8
Chg ϕ	-118.2	88.3
ψ	63.4	-134.1
ω	-172.5	178.6
χ_1[a]	-48.9/-173.2	71.8/-168.9

[a] Since the cyclohexylglycine residue possesses two γ-carbons, two values of χ_1 are reported.

Table III. ^{1}H-NMR Parameters[a] Observed for L-Aspartyl-(*S* and *R*)-cyclohexylglycine Methyl Esters

^{1}H-NMR parameters	Asp-(*S*)-Chg-OCH_3	Asp-(*R*)-Chg-OCH_3
$J_{\alpha-\beta h}$(Asp)/Hz	8.1	8.0
$J_{\alpha-\beta l}$(Asp)/Hz	5.5	5.4
$J_{NH-\alpha}$(Chg)/Hz	9.3	9.2
$J_{\alpha-\beta}$(Chg)/Hz	4.5	4.3
NOE(Asp H^{α}–Chg NH)	strong	strong
NOE(Asp $H^{\beta l}$–Chg NH)	weak	weak
NOE(Asp $H^{\beta h}$–Chg NH)	none	none
NOE(Chg H^{α}–Chg NH)	weak	weak
NOE(Chg H^{β}–Chg NH)	medium	medium
NOE(Chg H^{α}–Chg N^{β})	medium	medium

[a] The observed NOEs are qualitatively assigned according to their intensities.

The aspartyl residue in both diastereomers is very similar in conformation: (ψ, χ_1) = (144.9°, -66.9°) for the L-*S*-isomer and (167.1°, -77.0°) for the L-*R*-isomer (Table II). The similar conformation of the aspartyl residue has also been observed in the crystal structures of aspartame [(ψ, χ_1) = (149.6°, -60°)],[12] L-aspartyl-*N*'-[(2,2,5,5-tetramethylcyclopentyl)carbonyl]-(*R*/*S*)-1,1-diaminoethane [(ψ, χ_1) = (165.2°, -75.4°) for the *S*-isomer and (172.0°, -82.6°) for the *R*-isomer],[8] L-aspartyl-D-alanine *N*-(2,2,4,4-tetramethylthietanyl)amide [four independent molecules cocrystallized in the unit cell: (ψ, χ_1) = (158.5°, -63.6°), (144.1°, -66.8°), (153.3°, -64.0°), and (167.4°, -63.7°)];[9] all of these molecules are sweet. The opposite chiralities existing in the the second amino acid residue of Asp-Chg-OCH_3 lead to changes in the torsion angles of the cyclohexylglycine residue with resulting differences in their overall structures. The shapes of the Asp-(*S*)-Chg-OCH_3 and Asp-(*R*)-Chg-OCH_3 molecule are consistent with the structure we proposed for sweet and bitter tastes; the L-*S*-isomer is sweet (50-times sweeter than sucrose) and the L-*R*-isomer is bitter.

The two diastereomers were also studied by high-resolution ^{1}H-NMR spectroscopy in DMSO-d_6 solution. The results are summarized in Table III. The nuclear Overhauser effects (NOEs) observed in the ROESY spectra were assigned as strong, medium, or weak relative to one another according to their intensities. For both diastereomers, a strong NOE was observed between the α-proton of the Asp residue and the NH proton of the Chg residue, indicating that a torsion angle ψ for the N–C^{α}–C(O)–N moiety of the Asp residue is restricted to values from 60° to 180°. Investigation of the NOEs and the vicinal ^{1}H–^{1}H coupling constants $J_{\alpha-\beta}$'s observed for the two β-protons of the aspartyl residue allowed assignment of the prochiralties of these β-protons. The resonance at higher field (βh) was assigned to the *pro-R* proton, and the lower field resonance (βl), to the *pro-S* proton. The same assignments have also been obtained for the L-aspartyl dipeptides previously studied.[10,11] Fractions of three conformers (g^-, t, g^+) about the C^{α}–C^{β} bond (χ_1) of the Asp residue were estimated from the $J_{\alpha-\beta h}$ and $J_{\alpha-\beta l}$ values by using rotational isomeric-state approximation. The trans and gauche couplings necessary for this treatment were set to 13.56 and 2.60 Hz, respectively, following

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Table IV. Minimum Energy Conformations

	torsion angles/deg								
	Asp					Chg			
no.	ψ	ω	χ_1	χ_2	ϕ	ψ	ω	χ_1[a]	ΔE/(kcal mol^{-1})
	a. L-Aspartyl-(*S*)-cyclohexylglycine Methyl Ester								
S1	164.9	178.1	-63.4	63.5	-77.0	-45.9	179.9	-163.7, 69.3	0.000
S2	164.5	-179.9	-63.2	63.5	-138.0	-48.9	179.6	-165.4, 67.3	0.383
S3	157.8	179.6	-62.0	62.8	-144.6	135.0	179.9	-165.7, 67.1	0.473
S4	166.3	179.0	-63.0	64.0	-79.5	134.7	179.8	-164.0, 69.1	0.596
S5	164.5	178.8	-63.2	63.4	-77.2	-43.7	179.6	61.6, -66.4	0.801
S6	166.1	179.3	-63.4	63.5	-91.0	-63.5	179.3	-59.1, 177.2	0.969
S7	162.6	-179.9	-62.5	63.7	-96.5	105.8	-179.1	-58.1, 177.2	1.344
S8	166.0	179.6	-62.8	63.9	-80.2	136.4	-179.9	61.1, -66.8	1.466
S9	158.1	-180.0	-62.0	63.0	-131.4	134.9	-179.7	58.5, -69.3	1.529
	b. L-Aspartyl-(*R*)-cyclohexylglycine Methyl Ester								
R1	155.9	-178.7	-62.7	62.8	77.0	-139.8	-179.2	163.6, -69.5	0.000
R2	162.6	179.7	-63.3	63.4	81.1	47.1	178.4	164.0, -69.0	0.343
R3	161.8	177.7	-63.1	63.2	141.1	51.5	178.9	166.2, -66.3	0.405
R4	158.3	177.0	-62.3	62.5	140.2	-137.8	-178.7	165.7, -67.0	0.642
R5	155.4	-179.8	-62.6	62.6	77.8	-145.3	-179.6	-62.1, 66.0	0.673
R6	161.2	177.9	-63.2	63.2	101.5	68.0	178.9	59.4, -177.0	0.881
R7	157.7	177.6	-62.6	62.6	88.2	-120.5	-179.2	58.7, -177.6	0.915
R8	162.2	178.0	-63.1	63.2	81.9	44.7	178.8	-61.8, 66.1	0.982

[a] Since the cyclohexylglycine residue possesses two γ-carbons, two values of χ_1 are reported.

Pachler.[14] The results revealed that the most preferred conformer for the Asp side chain χ_1 was g^- in both diastereomers. The fractions of g^- were 0.50 for the L-*S*-isomer and 0.49 for the L-*R*-isomer. The fractions of the remaining t and g^+ states were respectively estimated to be 0.27 and 0.23 for the L-*S*-isomer and 0.16 and 0.24 for the L-*R*-isomer. A similar treatment of the $J_{\alpha-\beta}$ values of the Chg residue provided a fraction of the conformer in which the α- and β-protons orient trans to each other, 0.26 for (*S*)-Chg and 0.25 for (*R*)-Chg. A torsion angle (ϕ) about the N–C$^\alpha$ bond of the Chg residue can be deduced from a vicinal ^{1}H–^{1}H coupling constant $J_{NH-\alpha}$ for the H–N–C$^\alpha$–H moiety. By use of a Karplus-type relationship reported by (i) Bystrov[15] and (ii) Cung,[16] two values were calculated for the ϕ angle of each diastereomer: (i) -92°, -148° or (ii) -104°, -136° for (*S*)-Chg and (i) 92°, 148° or (ii) 103°, 137° for (*R*)-Chg. In these conformations, the NH and C$^\alpha$H protons orient in a nearly trans arrangement, which is consistent with a weak NOE(Chg NH–Chg C$^\alpha$H) observed for both Asp-(*S*)-Chg-OCH$_3$ and Asp-(*R*)-Chg-OCH$_3$. Since both diastereomers assume essentially the same conformation with the exception of the signs of the ϕ angles because of the stereochemistry of the Chg residue, the crucial difference in taste must arise from the conformation about the N–C$^\alpha$ bond of the Chg residue. This conformational angle determines the orientation of the hydrophobic functional group X (the cyclohexyl side chain) with respect to the AH/B functionalities in the aspartyl moiety.

The preferred geometries of the two diastereomers were examined using the flexible geometry program DISCOVER. An extensive search for minimum energy conformations was accomplished in a stepwise fashion, starting with small model compounds such as L-aspartyl-*N*-methylamide (Asp-NHCH$_3$) and *N*-acetylcyclohexylglycine methyl ester (Ac-Chg-OCH$_3$) and working toward the structure of Asp-Chg-OCH$_3$ (see Experimental Section). The results are shown in Table IV. The distances (2.5–4.0 Å) between the AH (α-amino) and B (β-carboxyl) groups required for sweet-taste response are observed only when the aspartyl side chain assumes either $\chi_1 = g^-$ or g^+ state. Although both the $\chi_1 = g^-$ and g^+ containing conformations were calculated as energy minima, only the conformers with $\chi_1 = g^-$ are reported in Table IV, as both Asp-(*S*)-Chg-OCH$_3$ and Asp-(*R*)-Chg-OCH$_3$ prefer the g^- state about the aspartyl side chain in solution. In addition, both diastereomers adopt the g^- state in the crystalline state. The

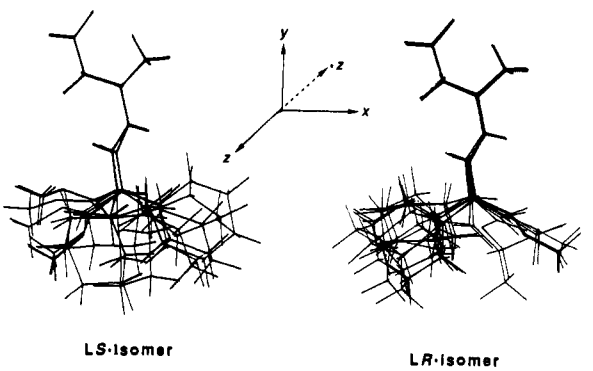

Figure 3. Superimposed minimized structures of L-aspartyl-(*S*)-cyclohexylglycine methyl ester (L-*S*-isomer) and L-aspartyl-(*R*)-cyclohexylglycine methyl ester (L-*R*-isomer).

substituent (CH(NH-)COO-) on the cyclohexyl side chain assumes both the axial and equatorial positions; the former conformation is calculated to be at least 7 kcal mol^{-1} higher in energy than the latter. Therefore, only the conformers in which the substituent is on the equatorial position are reported.

The accessible conformational space for the hydrophobic cyclohexyl side chain with respect to the zwitterionic aspartyl moiety is best illustrated by superposition of the preferred structures as shown in Figure 3. It can be seen that the cyclohexylglycine residue shows flexibility. The facile rotation about the N–C$^\alpha$ bond (ϕ) of the Chg residue is primarily responsible for such flexibility. A rotation of the cyclohexyl side chain about the C$^\alpha$–C$^\beta$ bond (χ_1) causes less change in the overall structure of the molecule, since the constrained six-membered cyclohexane ring is directly attached to the α-carbon. For each diastereomer, the calculated ϕ angles are restricted to two regimes: -145° to -131° and -97° to -77° for the L-*S*-isomer and 77° to 102° and ca. 140° for the L-*R*-isomer (Table IV). These angles are in agreement with the experimental values deduced from the ^{1}H-NMR studies. In the molecule Asp-(*S*)-Chg-OCH$_3$, the hydrophobic cyclohexyl side chain orients along the +*x* axis (the base of the "L" structure), when the (*S*)-Chg residue adopts the angle ϕ in the range of -145° to -131°. This structure causes the molecule to be sweet. With ϕ in the range -97° to -77° for the (*S*)-Chg residue, the cyclohexyl side chain orients in the +*z* dimension (in front of the stem of the "L" structure) and this causes the molecule to be tasteless. In contrast, the cyclohexyl side chain of the molecule Asp-(*R*)-Chg-OCH$_3$ orients along the -*x* axis (reversed "L" shape) with ϕ = 77–102° for the (*R*)-Chg residue and to the -*z* axis

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(behind the stem of the "L") with $\phi \sim 140°$ for the same residue. In the latter structures the molecule possesses a large $-z$ component. Therefore, the molecule Asp-(*R*)-Chg-OCH_3 is found to be bitter.

Several molecular models have been reported for sweet taste in addition to our model.[17-19] Among these, an extended structure proposed by Temussi et al.[17] from conformational analysis of the flexible molecule aspartame is different from our "L" shape model. The major difference between the Temussi model and ours is caused by the side-chain χ_1 conformation for the second residue [(Phe) of aspartame]. This residue assumes the $\chi_1 = t$ ($\sim 180°$) state in Temussi's extended model and the g^- ($\sim -60°$) state in our "L" shape model. In solution the Phe residue of aspartame adopts both χ_1 states with a preference of g^- over t,[13] and thus the aspartame molecule adopts both extended and "L" shaped structures. Unlike the case of aspartame, the χ_1 conformation of the second residue of Asp-Chg-OCH_3 does not affect the overall structure of the molecule. The sweet-tasting Asp-(*S*)-Chg-OCH_3 assumes the "L" shape structure as mentioned above. However, extended structures which fit the Temussi model are not accessible for this analog because the conformationally constrained cyclohexyl ring is directly bonded to the α-carbon. These results provide evidence in favor of the "L" shape over the extended structure proposed by Temussi et al.[17]

Conclusions

We have recently proposed a three-dimensional molecular model to explain the taste properties of L-aspartyl dipeptide derivatives. A molecule can elicit a sweet taste when it assumes an "L" shape with the hydrogen bond donor (AH) and hydrogen bond acceptor (B) containing zwitterionic ring of the aspartyl moiety forming the stem of the "L" in the $+y$ axis and the hydrophobic X group projecting out along the base of the "L" in the $+x$ axis, almost coplanar with the zwitterionic ring. Substantial projection of the X moiety into the $-z$ dimension results in bitter taste ligands. Although the model has been developed with analogs containing the ester or amide as the hydrophobic X group on the C-terminal residue, it can also explain sweetness of aspartame, which has a benzyl side chain functional group as the X group.

In order to probe the capability of our model to predict taste properties of L-aspartyl dipeptide analogs in which the hydrophobic X group is the side chain of the second residue, we undertook the synthesis of two diastereomeric taste ligands L-aspartyl-(*S*)-cyclohexylglycine methyl ester [Asp-(*S*)-Chg-OCH_3] and L-aspartyl-(*R*)-cyclohexylglycine methyl ester [Asp-(*R*)-Chg-OCH_3]. Taste assays revealed that Asp-(*S*)-Chg-OCH_3 is 50-times sweeter than sucrose while Asp-(*R*)-Chg-OCH_3 is bitter. In these analogs the hydrophobic X group (the cyclohexyl side chain) sweeps out an arc nearly coplanar with the xz plane, since the conformationally constrained cyclohexyl group is directly bonded to the α-carbon of the second residue. Therefore, these analogs provide a unique test for the model regarding the orientation of the X group relative to the AH and B groups within the aspartyl moiety which is conformationally fixed when taste ligands bind to taste receptors.

The X-ray analyses demonstrated that the sweet Asp-(*S*)-Chg-OCH_3 molecule adopts an "L" shape structure while the Asp-(*R*)-Chg-OCH_3 molecule adopts a "reversed L" shape structure which is consistent with the structures we proposed for sweet and bitter tastes. These structures turned out to be one of the preferred conformations for each diastereomer in solution estimated by the ^{1}H-NMR and molecular modeling studies. Conformational analyses in solution indicate that the molecule Asp-(*S*)-Chg-OCH_3 occupies the accessible space along the $+x$ axis ("L" shape: sweet) to the $+z$ axis (in front of the stem of the "L": tasteless) for the cyclohexyl side chain and does not possess a $-z$ component. Therefore, it is sweet. In contrast, the cyclohexyl side chain of the molecule Asp-(*R*)-Chg-OCH_3 orients in a space from the $-x$ axis (reversed "L" shape: tasteless) to the $-z$ axis (behind the stem of the "L"). It possesses a large $-z$ component. Therefore the molecule is bitter. The taste properties of L-aspartylcyclohexylglycine methyl esters whose cyclohexyl side chain functions as the hydrophobic group (X) are correctly explained by our "L" shape model for sweet and bitter tastes developed from studies of L-aspartyl-based peptide taste ligands with the ester or amide serving as the X group in the C-terminal.

Experimental Section

Materials. The optically pure cyclohexylglycine was prepared by hydrogenolysis of the corresponding phenylglycine. Cyclohexylglycine methyl ester hydrochloride (H-Chg-OCH_3·HCl) was obtained by refluxing the cyclohexylglycine with thionyl chloride in dry methanol under N_2. The protected dipeptide derivative was synthesized by coupling of Z-Asp(OBzl)-OH (*Z* = (benzyloxy)carbonyl) with H-Chg-OCH_3 using ethyl diisopropylcarbodiimide hydrochloride as a condensing reagent. Hydrogenolysis of this derivative in the presence of Pd-C produced the target compound Asp-Chg-OCH_3. The crude product was purified by HPLC.

***N*-((Benzyloxy)carbonyl)-β-benzyl-L-aspartyl-(*S*)-cyclohexylglycine Methyl Ester**: 55%; mp 95–98 °C; $[\alpha]_D$ +23.8° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.40 (m, 10 H, phenyls of Z and Bzl), 6.98 (d, *J* = 8.4 Hz, 1 H, NH of Chg), 5.99 (d, *J* = 7.9 Hz, 1 H, NH of Asp), 5.17 and 5.12 (d, *J* = 12.2 Hz, d, *J* = 12.2 Hz, 2 H, CH_2Ph of Z), 5.14 (s, 2 H, CH_2Ph of Bzl), 4.65 (br, 1 H, C^αH of Asp), 4.47 (dd, *J* = 5.0 and 8.4 Hz, 1 H, C^αH of Chg), 3.71 (s, 3 H, OCH_3), 3.09 and 2.74 (dd, *J* = 4.0 and 17.3 Hz, dd, *J* = 6.8 and 17.3 Hz, 2 H, $C^\beta H_2$ of Asp), 1.80–1.50 and 1.30–0.95 (m, m, 11 H, cyclohexyl of Chg); MS *m/e* 512 (31), 511 (100), 467 (21), 377 (18). HRMS calcd for $C_{28}H_{35}N_2O_7$: 511.2444. Found: 511.2469.

L-Aspartyl-(*S*)-cyclohexylglycine Methyl Ester: 84%; mp 169–170 °C (dec.); $[\alpha]_D$ +3.3° (*c* 0.9, MeOH); ^{1}H-NMR (DMSO-d_6) δ 8.70 (br, 1 H, NH of Chg), 4.17 (br, 1 H, C^αH of Asp), 3.71 (dd, *J* = 4.5 and 9.3 Hz, 1 H, C^αH of Chg), 3.62 (s, 3 H, OCH_3), 2.42 and 2.18 (dd, *J* = 5.5 and 16.0 Hz, dd, *J* = 8.1 and 16.0 Hz, 2 H, $C^\beta H_2$ of Asp), 1.76–1.47 and 1.30–0.90 (m, 11 H, cyclohexyl of Chg); MS *m/e* 287 (100), 227 (11), 172 (11). Anal. calcd for $C_{13}H_{22}N_2O_5 \cdot H_2O$: C, 51.30; H, 7.95; N, 9.20. Found: C, 50.95; H, 7.61; N, 9.36.

***N*-((Benzyloxy)carbonyl)-β-benzyl-L-aspartyl-(*R*)-cyclohexylglycine Methyl Ester**: 85%; mp 128–129 °C; $[\alpha]_D$ −13.0° (*c* 2.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.60–7.25 (m, 10 H, phenyls of Z and Bzl), 6.92 (d, *J* = 7.6 Hz, 1 H, NH of Chg), 5.99 (d, *J* = 7.9 Hz, 1 H, NH of Asp), 5.20–5.10 (m, 4 H, $2CH_2$Ph of Z and Bzl), 4.67 (br, 1 H, C^αH of Asp), 4.48 (dd, *J* = 5.0 and 7.6 Hz, 1 H, C^αH of Chg), 3.70 (s, 3 H, OCH_3), 3.04 and 2.77 (dd, *J* = 3.9 and 16.9 Hz, dd, *J* = 6.3 and 16.9 Hz, 2 H, $C^\beta H_2$ of Asp), 2.00–1.50 and 1.45–1.25 (m, m, 11 H, cyclohexyl of Chg); MS *m/e* 512 (35), 511 (100), 467 (13). HRMS calcd for $C_{28}H_{35}N_2O_7$: 511.2444. Found: 511.2433.

L-Aspartyl-(*R*)-cyclohexylglycine Methyl Ester: 61%; mp 108 °C (dec.); $[\alpha]_D$ 0° (*c* 1.0, MeOH); ^{1}H-NMR (DMSO-d_6) δ 8.75 (br, 1 H,NH of Chg), 4.18 (br, 1 H, C^αH of Asp), 3.73 (dd, *J* = 4.3 and 9.2 Hz, 1 H, C^αH of Chg), 3.64 (s, 3 H, OCH_3), 2.41 and 2.18 (dd, *J* = 5.4 and 16.0 Hz, dd, *J* = 8.0 and 16.0 Hz, 2 H, $C^\beta H_2$ of Asp), 1.65–0.90 (m, m, m, 11 H, cyclohexyl of Chg); MS *m/e* 287 (100), 227 (18), 172 (16). Anal. calcd for $C_{13}H_{22}N_2O_5 \cdot H_2O$: C, 51.30; H, 7.95; N, 9.20. Found: C, 51.66; H, 7.77; N, 9.37.

Taste Assessment. Taste tests were carried out by a "sip and spit" taste assessment of solutions of the molecules using a three-member panel. All the compounds were tested in water at room temperature without any pH adjustment, starting at 2000 ppm concentration. The test solutions were diluted as necessary in order to match a 200 ppm aspartame solution used as our standard. Conversion to sucrose values was made on the basis of the sweetness potency of aspartame (150 × sucrose). The compound Asp-(*S*)-Chg-OCH_3 produces sweet taste (50 × sucrose) while the corresponding diastereomer Asp-(*R*)-Chg-OCH_3 is bitter.

X-ray Structure Determinations. The X-ray diffraction studies were carried out on a Rigaku AFC6R diffractometer equipped with a copper rotating anode and a highly oriented graphite monochromator. The data for Asp-(*S*)-Chg-OCH_3 were collected in a range 4.0–120.0° of 2θ employing a 2θ–θ scan mode with a constant scan speed of 16.0° min^{-1} in ω. The weak reflections [$I > 5\,\sigma(I)$] were rescanned to a maximum of four times and the counts accumulated to assure good counting statistics.

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The h, k, l ranges were 0–11, 0–5, and −20 to +20, respectively. A total of 1270 independent reflections (R_{int} = 0.0214) were measured, 895 of which had $F_o > 3.0\sigma(F_o)$ and thus were considered "observed" and used for refinement. The structure was solved by direct methods with the SHELXS program. The full-matrix least-squares method was used to minimize the quantities $\Sigma w(F_o - F_c)^2$ with weight $w = [\sigma^2(F_o) + gF_o^2]^{-1}$ (g = 0.001). Nitrogen and oxygen atoms were refined anisotropically, while carbon atoms were refined isotropically. All hydrogen atoms were included in the final cycles of refinement with fixed thermal parameters of 0.08 Å^2. The final R indices, $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ and $R_w = [\Sigma(|F_o| - |F_c|)^2/\Sigma|F_o|^2]^{1/2}$, and goodness to fit, $S = [\Sigma w(|F_o| - |F_c|)^2/(m - n)^2]^{1/2}$ where m and n respectively represent numbers of observed reflections and parameters refined, were 10.85%, 12.12%, and 2.24, respectively.

The data for Asp-(R)-Chg-OCH_3 were collected employing the same conditions as used for the S-isomer. The h, k, l ranges were 0–7, 0–7, and −21 to +21, respectively. In total 1422 reflections were processed using profile analyses to give 1260 unique reflections (R_{int} = 0.0268); 868 reflections with $F_o > 4.0\sigma(F_o)$ were considered "observed" and used in the refinement. The structure was solved and refined adopting g = 0.0005 by SHELXS. All non-hydrogen atoms were refined anisotropically. The refinement including all hydrogen atoms in ideal positions with fixed thermal parameters of 0.08 Å^2 converted at R = 6.25%, R_w = 7.38%, and S = 2.13.

^{1}H-NMR Measurements. The ^{1}H-NMR spectra were recorded on a General Electric GN-500 spectrometer operating at 500 MHz. All experiments were carried out in DMSO-d_6 (MSD Isotopes). The peak assignments were made using two-dimensional homonuclear Hartman-Hahn (HOHAHA)[20a] and the rotating frame nuclear Overhauser enhancement (ROESY)[21] experiments. The HOHAHA experiments employed the MLEV 17 spin-locking sequence suggested by Bax and Davis.[20b] The time proportional phase increment[22] was used to obtain the absolute phase. A mixing time of 100 ms with a spin locking field of 10.2 kHz was employed. The ROESY experiments were carried out using mixing times of 50–250 ms with a spin-locking field of 2 kHz. All of the two-dimensional spectra were obtained using 2K data points in the f_2 domain and 256 points in the f_1 domain. Applying zero filling procedure to the f_1 domain resulted in a final matrix of 2K × 2K data points. Gaussian multiplication was used to enhance the spectra. Vicinal coupling constants were obtained from the one-dimensional spectra containing 16K data points in 5000 Hz.

Computer Simulations. Conformational energy calculations were carried out with a quasi Newton-Raphson algorithm until all derivatives were smaller than 0.001 kcal mol^{-1} Å^{-1} by employing the DISCOVER program.[23] Conventional values of the bond lengths and bond angles for cyclohexylglycine (Chg) were taken from the crystallographic data for Asp-(S)-Chg-OCH_3 and Asp-(R)-Chg-OCH_3. Conformational energies were expressed by the valence force field provided in the DISCOVER program. The default dielectric constant of 1.0 was used for all calculations in vacuo.

Flexible geometry energy minimizations were carried out for a model compound, L-aspartyl-N-methylamide (Asp-$NHCH_3$). While the torsion angles ψ and χ_2 were varied in increments of 30°, 144 structures were examined for each of three χ_1 states, g^- (∼ −60°), t (∼180°), and g^+ (∼60°). As a result, two minimum energy conformers were calculated: ψ = −104.6°, χ_1 = 57.7°, χ_2 = −67.5° and ψ = 159.5°, χ_1 = −63.8°, χ_2 = 64.3°. The latter conformation is in agreement with the ^{1}H-NMR results observed for diastereomeric taste ligands Asp-(S)-Chg-OCH_3 and Asp-(R)-Chg-OCH_3 [ψ = 120–180°, $f(g^-)$ = 0.6], although the energy is 0.920 kcal mol^{-1} higher than that of the former. Minimum energy conformations were calculated for a model compound N-acetyl-(S)-cyclohexylglycine methyl ester [Ac-(S)-Chg-OCH_3]. Initial structures were generated by varying torsion angles ϕ, ψ, and χ_1. Incerements of 30° were used for ϕ and ψ. For χ_1 three values of −60° (g^-), 180° (t), and 60° (g^+) were examined. The axial and equatorial substituted chair structures were assumed for the cyclohexyl side chain as starting structures. The compound Ac-(R)-Chg-OCH_3 shows mirror-image behavior of Ac-(S)-Chg-OCH_3. After the above treatment, flexible geometry energy minimizations were carried out for two diastereomers of Asp-Chg-OCH_3. Initial structures were generated using the values of torsion angles estimated for the model compounds Asp-$NHCH_3$ and Ac-Chg-OCH_3.

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Supplementary Material Available: Tables of crystal data, data collection and structural refinement information, atomic coordinates, equivalent isotropic displacement coefficients, bond lengths, bond angles, anisotropic displacement coefficients, H-atom coordinates, and anisotropic displacement coefficients and figures showing unit cell packing diagrams for L-aspartyl-(S)-cyclohexylglycine methyl ester and L-aspartyl-(R)-cyclohexylglycine methyl ester (10 pages); listings of observed and calculated structure factors for L-aspartyl-(S)-cyclohexylglycine methyl ester and L-aspartyl-(R)-cyclohexylglycine methyl ester (6 pages). Ordering information is given on any current masthead page.

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