Absolute Configurations and Conformations of Sweet and Tasteless Aminomalonyl (Ama) Dipeptide Esters: Ama-Phe-OMe and Ama-Phe-OEt

Toshihiko Ando,[†,‡] Masafumi Ota,[‡] Tatsuki Kashiwagi,[‡] Nobuya Nagashima,[‡] Yasuo Ariyoshi,[‡] Raj K. Chadha,[†] Toshimasa Yamazaki,[†] and Murray Goodman*[,†]

Contribution from the Department of Chemistry, University of California, San Diego, La Jolla, California 92093-0343, and Central Research Laboratories, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki 210, Japan. *Received May 26, 1992*

Abstract: Absolute configurations of sweet aminomalonyl-(*S*)-phenylalanine methyl ester [Ama-(*S*)-Phe-OMe] and its ethyl ester analogue [Ama-(*S*)-Phe-OEt] were experimentally determined. The X-ray crystallographic analyses revealed that the isomer of the methyl ester analogue which is sweet is (*R*)-Ama-(*S*)-Phe-OMe while the tasteless isomer of the ethyl ester analogue is (*S*)-Ama-(*S*)-Phe-OEt. These results clearly establish that an (*R*) configuration of the aminomalonyl moiety is necessary for the sweet taste. In the crystalline sate, both the sweet (*R*)-Ama-(*S*)-Phe-OMe and tasteless (*S*)-Ama-(*S*)-Phe-OEt adopt extended structures under the influence of molecular packing forces mainly because of stacking between adjacent aromatic rings. The X-ray structures of these molecules are topochemically equivalent and thus cannot explain differences in the observed taste properties. The ^{1}H-NMR and molecular modeling studies indicate that the molecules studied are flexible and exist as mixtures of various preferred conformers in solution. The preferred conformations of the sweet-tasting analogues (*R*)-Ama-(*S*)-Phe-OMe and (*R*)-Ama-(*S*)-Phe-OEt in solution can be described as possessing an "L" shape, with the AH (hydrogen bond donor) and B (hydrogen bond acceptor) containing aminomalonyl moiety as the stem of the "L" and the hydrophobic phenylalanine side chain as the base of the "L". The plane defined by the zwitterionic ring of the aminomalonyl moiety is almost coplanar with the plane of the aromatic side chain of the phenylalanine residue forming an "L" shaped structure. In contrast, no such "L" shape conformation is accessible to the corresponding diastereomers (*S*)-Ama-(*S*)-Phe-OMe and (*S*)-Ama-(*S*)-Phe-OEt. These molecules are tasteless. The tastes of the aminomalonyl-(*S*)-phenylalanine esters studied are correctly predicted by the "L" shape model for the sweet taste previously developed with L-aspartyl-based peptide sweeteners.

Introduction

It has been demonstrated that the L-aspartyl moiety of a sweet-tasting peptide, L-aspartyl-L-phenylalanine methyl ester (L-Asp-L-Phe-OMe),[1] is restricted to L-Asp or aminomalonic acid (Ama) which is a shorter homologue of L-Asp. A higher homologue L-Glu-L-Phe-OMe is bitter.[1] The L-Asp residue has been successfully replaced by Ama, retaining sweet potency (300–400 × sucrose).[2,3] It was originally supposed that (*S*)-Ama-(*S*)-Phe-OMe[4] would be sweet by analogy to the parent sweet-tasting aspartyl peptide containing an L-L sequence. On the contrary, Ariyoshi[5] and Goodman et al.[6] independently predicted that (*R*)-Ama-(*S*)-Phe-OMe would be sweet from investigations of the sweet molecules (Figure 1) and the topochemical structures of various sweet and nonsweet peptide derivatives. However, it has not been definitely established which diastereometer (*R*)-Ama-(*S*)-Phe-OMe or (*S*)-Ama-(*S*)-Phe-OMe is sweet, since the Ama residue is susceptible to facile racemization and thus the sweet peptide has been obtained as a mixture of the two diastereomers designated as "*ambo*-Ama-(*S*)-Phe-OMe" following the IUPAC-IUB recommendation.[7]

In the present investigation, the absolute configuration of the sweet-tasting isomer of Ama-(*S*)-Phe-OMe was determined by X-ray crystallographic analysis. We also confirmed the configuration for the Ama residue of the tasteless ethyl ester analogue Ama-(*S*)-Phe-OEt by X-ray analysis. In addition, conformational analyses were carried out for these derivatives by ^{1}H-NMR spectroscopy and molecular mechanics calculations. By assessing the preferred conformations, we are able to relate the configurational and conformational effects of the aminomalonyl dipeptide esters to their tastes.

Results and Discussion

Absolute Configurations of Aminomalonyl-(*S*)-phenylalanine Esters. The taste ligands aminomalonyl-(*S*)-phenylalanine methyl ester [Ama-(*S*)-Phe-OMe] and its ethyl ester analogue [Ama-(*S*)-Phe-OEt] were synthesized by coupling of Z-(*R*,*S*)-Ama-(OBzl)-OH and the corresponding L-phenylalanine esters with dicyclohexylcarbodiimide followed by hydrogenolysis. In both cases, the final compound was obtained as a diastereomeric mixture in a ratio of approximately 1:1. It was very difficult to prepare crystals of either (*R*)-Ama-(*S*)-Phe-OMe or (*S*)-Ama-(*S*)-Phe-OMe suitable for X-ray analysis since the two diastereomers always existed as an equilibrated mixture in solution because of rapid racemization of the Ama residue. The less soluble isomer precipitated from the mixture as an amorphous powder or small crystals. We were able to use the crystals of the less soluble isomer thus obtained for X-ray analysis without further purification. Recrystallization was ineffective because of rapid racemization of the Ama residue in solution. After extensive efforts, the sweet isomer of Ama-(*S*)-Phe-OMe [late-eluting isomer on reversed-phase high-pressure liquid chromatography (RP-HPLC); 800 × sucrose] was crystallized from a 0.1 M $CaCl_2$ solution as small, thin triclinic crystals. Four independent molecules were contained in the unit cell. In contrast to the case of the methyl ester analogue, the tasteless isomer of Ama-(*S*)-Phe-OEt (early-eluting isomer on RP-HPLC) readily crystallized from aqueous solution in a monoclinic space with two molecules in the unit cell. Attempts to crystallize the sweet isomer of Ama-(*S*)-Phe-OEt (late-eluting isomer on RP-HPLC; 50 × sucrose) did not succeed because the late-eluting isomer was more soluble than the early-eluting compound and the two diastereomers equilibrated in solution.

* To whom correspondence should be addressed.
† University of California, San Diego.
‡ Ajinomoto Co., Inc.

(1) Mazur, R. H.; Schlatter, J. M.; Goldkamp, A. H. *J. Am. Chem. Soc.* **1969**, *91*, 2684–2691.
(2) Briggs, M. T.; Morley, J. S. Brit. Patent 1299265, 1972.
(3) Fujino, M.; Wakimatsu, M.; Tanaka, K.; Aoki, H.; Nakajima, N. *Naturwissenschaften* **1973**, *60*, 351.
(4) The DL system cannot be used for designating configuration at the α-carbon of the Ama residue of Ama-containing peptides. Thus, the *RS* system is used for all Ama-containing peptides throughout this paper to avoid using two different designating systems for the one compound.
(5) (a) Ariyoshi, Y. In *Chemistry of Taste and Smell*; The Chemical Society of Japan: Gakkai Shuppan Center, Tokyo, 1976; pp 85–128. (b) Ariyoshi, Y. *Agr. Biol. Chem.* **1976**, *40*, 983–992.
(6) Kawai, M.; Nyfeler, R.; Berman, J. M.; Goodman, M. *J. Med. Chem.* **1982**, *25*, 397–402.
(7) IUPAC-IUB Joint Commission on Biochemical Nomenclature. *Eur. J. Biochem.* **1984**, *138*, 5–7.

0002-7863/93/1515-397$04.00/0 © 1993 American Chemical Society

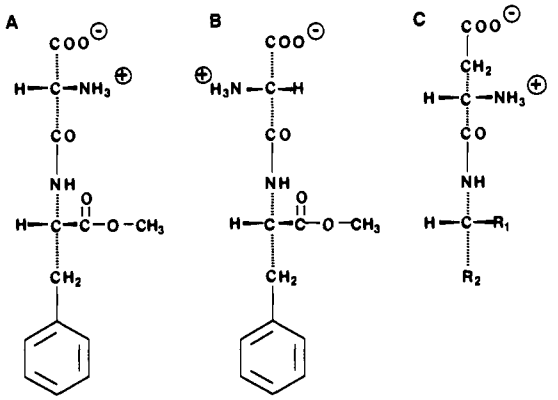

Figure 1. The Fischer projection formulas of (A) (*R*)-aminomalonyl-(*S*)-phenylalanine methyl ester and (B) (*S*)-aminomalonyl-(*S*)-phenylalanine methyl ester. The (*R*)-(*S*) isomer (A) is compatible with the sweet formula for the L-aspartyl-based dipeptide (C) where R_1 and R_2 are the small and large hydrophobic substituents, respectively.

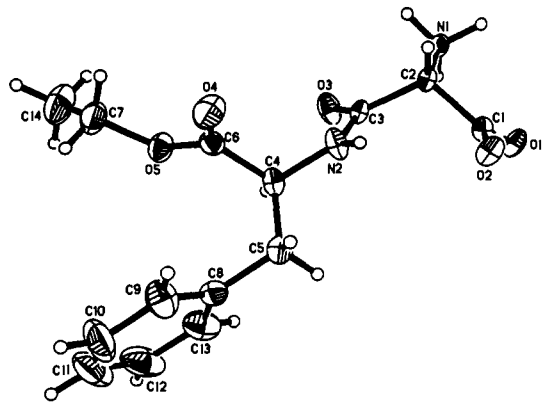

Figure 2. An ORTEP drawing of the tasteless diastereomer (*S*)-aminomalonyl-(*S*)-phenylalanine ethyl ester in the crystalline state.

At first, a single-crystal X-ray analysis was carried out on a monoclinic crystal of the tasteless isomer of Ama-(*S*)-Phe-OEt. The compound crystallized in the space group $P2_1$ with unit cell dimensions of a = 5.295 (4) Å, b = 8.520 (7) Å, c = 16.713 (13) Å, and β = 96.75 (7)°. The two molecules in the unit cell result in a calculated density of 1.305 g cm^{-3}. Three-dimensional X-ray diffraction data were collected on a colorless plate-shape crystal 0.14 × 0.42 × 0.75 mm in size, using graphite monochromatized Mo Kα radiation. The structure was solved by direct methods and refined by full-matrix least-squares to R = 5.32% (wR = 7.25%). Figure 2 shows an ORTEP drawing of the tasteless molecule. Selected torsion angles defined by non-hydrogen atoms are given in Table I. The X-ray analysis revealed that the tasteless isomer of Ama-(*S*)-Phe-OEt contains an (*S*) configuration on the α-carbon of the aminomalonyl moiety. Therefore, the corresponding sweet-tasting isomer must be (*R*)-Ama-(*S*)-Phe-OEt.

As noted above, the sweet-tasting isomer of Ama-(*S*)-Phe-OMe crystallized in the triclinic space group *P*1 with unit cell dimensions of a = 8.297 (2) Å, b = 22.528 (2) Å, b = 22.528 (2) Å, c = 8.225 (2) Å, α = 93.13 (1)°, β = 103.36 (2)°, and γ = 91.53 (1)°. The four molecules in the unit cell result in a calculated density of 1.333 g cm^{-3}. Three-dimensional X-ray diffraction data were collected on a colorless thin plate-shape crystal 0.20 × 0.02 × 0.50 mm in size, using graphite monochromatized Cu Kα radiation. Because there are four independent molecules in the asymmetric unit, the crystal structure was difficult to determine by the general direct methods procedure with the MITHRIL program.[8] As a result, the crystal structure was solved by the DIRDIF program[9,10] which is useful when a part of the structure is known. The non-hydrogen atoms of (*S*)-aminomalonyl-(*S*)-phenylalanine ethyl ester including the amino, carboxyl, and benzyl groups were used as the known partial structure. Since the known fragment includes the asymmetric carbon atom in the (*S*)-phenylalanine moiety, the absolute configuration of the solved structure was fixed. The structure was refined by block-matrix least-squares to R = 7.3% (wR = 11.2 %). Figure 3 shows ORTEP drawings of the four

Table I. Selected Torsion Angles for (*R*)-Aminomalonyl-(*S*)-phenylalanine Methyl Ester and (*S*)-Aminomalonyl-(*S*)-phenylalanine Ethyl Ester Determined by X-ray Diffraction Studies

		(*R*)-Ama-(*S*)-Phe-OMe[a]				
torsion		1	2	3	4	(*S*)-Ama-(*S*)-Phe-OEt
Ama	ψ	171	174	175	168	-177
	ω	179	-179	179	-177	-175
	χ_1	-160	-161	-164	-160	162
Phe	ϕ	-93	-82	-97	-84	-97
	ψ	130	131	113	140	135
	ω	-177	175	-179	177	174
	χ_1	-176	-172	-177	-177	179
	χ_2	-131	-81	-155	-80	-92
OEt	τ[b]					156

[a] The compound (*R*)-Ama-(*S*)-Phe-OMe crystallized in the triclinic *P*1 space with four independent molecules in the asymmetric unit. [b] The angle τ is defined as C(O)–O–C–C in the C-terminal ethyl ester group.

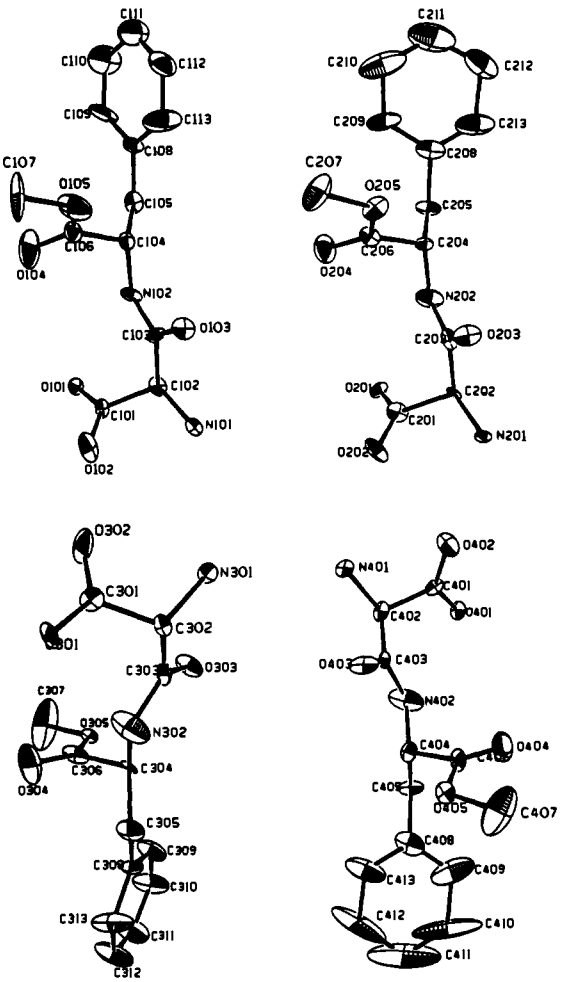

Figure 3. ORTEP drawings of the four independent molecules included in the unit cell of the crystal of the sweet-tasting isomer (*R*)-aminomalonyl-(*S*)-phenylalanine methyl ester. The major conformational differences in the four structures were observed in orientations for the C-terminal methyl ester group and the phenyl ring, i.e., torsion angles for the N*02-C*04-C*06-O*06 (ψ) and N*02-C*04-C*05-C*08 (χ^2) moieties of the (*S*)-Phe residue.

(8) Gilmore, C. J. *J. Appl. Crystallogr.* **1984**, *17*, 42–46.
(9) Beurskens, P. T. In *Technical Report 1984/1 Crystallography Laboratory, Toermooiceld, 6525*; Nijmegen Ed., Netherland.
(10) Karle, J. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1968**, *B24*, 182–186.

Table II. Taste Properties of Aminomalonyl-(*S*)-phenylalanine Dipeptide Esters

compd	HPLC profile	taste
(*S*)-Ama-(*S*)-Phe-OMe	early-eluting isomer	tasteless
(*R*)-Ama-(*S*)-Phe-OMe[a]	late-eluting isomer	sweet (800 × sucrose)
(*S*)-Ama-(*S*)-Phe-OEt[a]	early-eluting isomer	tasteless
(*R*)-Ama-(*S*)-Phe-OEt	late-eluting isomer	sweet (50 × sucrose)

[a] The absolute configurations of the aminomalonyl residues were determined by X-ray diffraction studies.

molecules included in the unit cell of the crystal of the sweet-tasting isomer of Ama-(*S*)-Phe-OMe. These structures clearly reveal that the absolute configuration on the α-carbon of the aminomalonyl moiety is (*R*). The four independent (*R*)-Ama-(*S*)-Phe-OMe molecules assume essentially the same overall structures. The conformation of the (*R*)-Ama residue is highly conserved, with no torsion varying by more than 7° (Table I). The greatest variabilities were observed for torsions ψ and χ_2 of the (*S*)-Phe residue with approximate deviations of 25°. These torsions allow rotations of the C-terminal methyl ester group and the phenyl ring, respectively, but permit less change in the overall structure of the molecule.

The results of the above experiments are summarized in Table II. The sweet-tasting isomers of Ama-(*S*)-Phe-OMe and Ama-(*S*)-Phe-OEt have been determined as (*R*)-Ama-(*S*)-Phe-OMe and (*R*)-Ama-(*S*)-Phe-OEt, respectively. As a result, it is confirmed that an (*R*) configuration of the aminomalonyl moiety is necessary for the ligand to produce a sweet taste. This observation is in agreement with our previous predictions.[5,6]

The Molecular Basis of Taste. There have been numerous attempts to generalize structural features among sweet molecules. Investigations into the molecular basis of sweet taste resulted in a model in which the components of the glucophore were shown to involve a hydrogen bond donor (AH), a hydrogen bond acceptor (B), and a hydrophobic site (X).[11-13] The aspartyl peptide sweeteners contain the AH and B elements in the zwitterionic N-terminal aspartyl residue as the NH_3^+ and β-carboxyl groups. This distances from one another when participating in the formation of a six-membered zwitterionic ring are in agreement with AH to B distances identified in numerous non-peptide sweet-tasting molecules (2.5–4.0 Å).

In the case of aminomalonyl dipeptides, the α-amino and α-carboxyl groups of the aminomalonyl moiety are respectively assigned as the AH and B elements of the Shallenberger–Acree glucophore. In the crystalline state, both the sweet (*R*)-Ama-(*S*)-Phe-OMe and tasteless (*S*)-Ama-(*S*)-Phe-OEt molecules adopt very much the same extended conformations in which the AH/B moiety in the Ama residue and the hydrophobic site X in the aromatic ring of the Phe residue are 180° apart from each other in a flat parallel array. Therefore, the X-ray structures by themselves cannot explain taste properties of these molecules. This observation is also verified by a comparison of the X-ray structure of the sweet (*R*)-Ama-(*S*)-Phe-OMe with that of the corresponding sweet L-aspartyl analogue L-Asp-L-Phe-OMe reported by Kim et al.[14] Significant differences have been observed for the ϕ and χ_1 angles of the Phe residue; ϕ = −157.7° and χ_1 = 58.7° (g^+) for L-Asp-L-Phe-OMe while $\langle\phi\rangle$ = −89° and $\langle\chi_1\rangle$ = −176° (t) for (*R*)-Ama-(*S*)-Phe-OMe where $\langle\ \rangle$ represents the average of the four structures. Thus the overall structures of these two sweet compounds are different from each other in the crystalline state.

From analysis of a number of aspartyl dipeptides by NMR spectroscopy, X-ray crystallography, and computer simulations, we have arrived at a three-dimensional model which describes the conformational preferences of the sweet receptor.[15-20] The overall

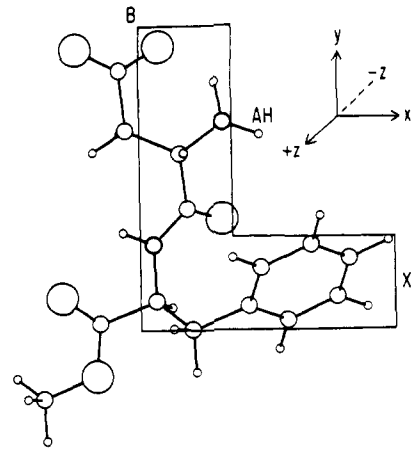

Figure 4. The "L" shape model for the sweet taste with L-aspartyl-L-phenylalanine methyl ester, where AH, B, and X represent a hydrogen bond donor, a hydrogen bond acceptor, and a hydrophobic site, respectively.

conformations of the various sweet-tasting analogues can be described as possessing an "L" shape with the AH and B containing aspartyl moiety as the stem of the "L" in the *y* axis and a hydrophobic moiety X as the base of the "L" in the *x* axis. The plane defined by the zwitterionic ring of the aspartyl moiety is almost coplanar with the plane of the aromatic ring of the phenylalanine residue forming an "L" shaped structure. Planarity of the molecule in the *x* and *y* dimensions is critical for sweet taste. Substantial deviation from this plane into the *z* dimension is correlated with tasteless (+*z*) and bitter (−*z*) molecules. The model has been probed by the structure–taste relationships observed for a new class of L-aspartyl taste ligands containing 2-aminocyclopentanecarboxylic acid methyl esters (L-Asp-2-Ac^{5}c-OMe).[20] These analogues display strong conformational preferences because of the constrained nature of the 5-membered ring in the 2-Ac^{5}c residue, and thus provide a unique test for the model. Our model fits the structure of L-Asp-L-Phe-OMe (X = phenyl ring) with only a minor modification of the conformation reported from X-ray crystallographic studies.[14] The side-chain conformation about the C^α–C^β bond (χ_1) of the Phe residue is changed from g^+ (∼ 60°) to g^- (∼ −60°) which is the most preferred in solution (Figure 4).

The X-ray structures of (*R*)-Ama-(*S*)-Phe-OMe do not fit our model without modification as in the case of L-Asp-L-Phe-OMe. However, it should be noted that molecular packing forces are major factors determining the crystal structures of small molecules. Stacking of adjacent aromatic rings is particularly important. Such aromatic ring stacking is quite obvious in molecular-packing diagrams of (*S*)-Ama-(*S*)-Phe-OEt and (*R*)-Ama-(*S*)-Phe-OMe shown in parts A and B of Figures 5, respectively. Of cource, in solution the Ama-(*S*)-Phe-OMe and L-Asp-L-Phe-OMe molecules are solvated and devoid of packing forces, and thus exist as equilibrium mixtures of various preferred conformers.

Preferred conformations of the two diastereomeres (*R*)-Ama-(*S*)-Phe-OMe (sweet) and (*S*)-Ama-(*S*)-Phe-OMe (tasteless) were studied by ^{1}H-NMR spectroscopy and molecular mechanics calculations. The ^{1}H-NMR parameters for (*R*)-Ama-(*S*)-Phe-OMe were obtained from the spectra measured for the

(11) Shallenberger, R. S.; Acree, T. E. *Nature* **1967**, *216*, 480–482.
(12) Deutsch, E. W.; Hansch, C. *Nature* **1966**, *211*, 75.
(13) (a) Kier, L. B. *J. Pharm. Sci.* **1972**, *61*, 1394–1397. (b) Holtje, H. D.; Kier, L. B. *J. Pharm. Sci.* **1974**, *63*, 1722–1725.
(14) Hatada, H.; Jancarik, J.; Graves, B., Kim, S. H. *J. Am. Chem. Soc.* **1985**, *107*, 4279–4282.
(15) Goodman, M.; Coddington, J.; Mierke, D. F.; Fuller, W. D. *J. Am. Chem. Soc.* **1987**, *109*, 4712–4714.
(16) Goodman, M.; Mierke, D. F.; Fuller, W. D. In *Peptide Chemistry 1987*; Shiba, T., Sakakibara, S., Eds.; Peptide Research Foundation: Japan, 1988, pp 699–704.
(17) Benedetti, E.; Blasio, B. D.; Pavone, V.; Pedone, C.; Fuller, W. D.; Mierke, D. F.; Goodman, M. *J. Am. Chem. Soc.* **1990**, *112*, 8909–8912.
(18) Feinstein, R. D.; Polinsky, A.; Douglas, A. J.; Beijer, C. M. G. F.; Chadha, R. K.; Goodman, M. *J. Am. Chem. Soc.* **1991**, *113*, 3467–3473.
(19) Zhu, Y.-F.; Yamazaki, T.; Tsang, J. W.; Lok, S.; Goodman, M. *J. Org. Chem.* **1992**, *57*, 1074–1081.
(20) Yamazaki, T.; Zhu, Y.-F.; Probstl, A.; Chadha, R. K.; Goodman, M. *J. Org. Chem.* **1991**, *56*, 6644–6655.

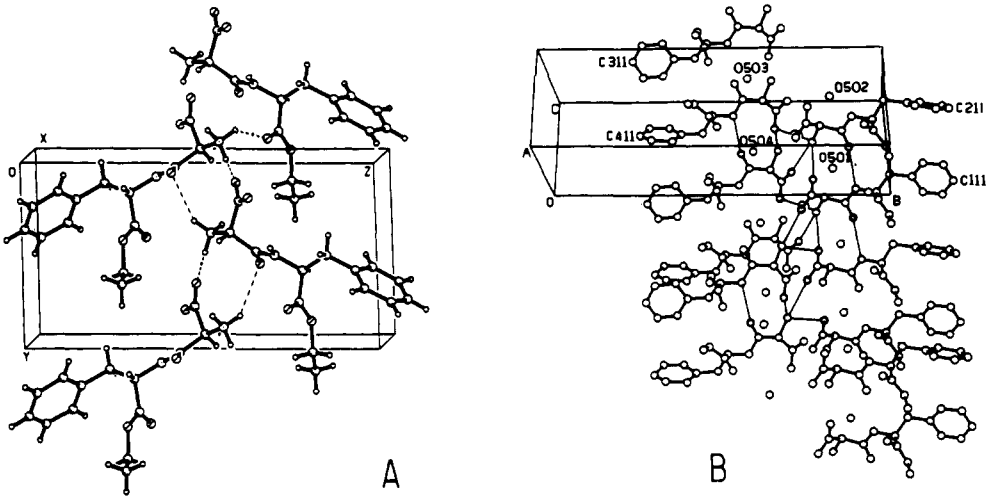

Figure 5. Molecular packing for the crystal of (A) (*S*)-aminomalonyl-(*S*)-phenylalanine ethyl ester and (B) (*R*)-aminomalonyl-(*S*)-phenylalanine methyl ester.

Table III. Experimental Values of Vicinal ^{1}H-^{1}H Coupling Constants and NOEs[a] for Two Diastereomers of Ama-(*S*)-Phe-OMe Obtained from Spectra Measured in DMSO-d_6 at 25 °C

^{1}H-NMR parameters	(*R*)-Ama-(*S*)-Phe-OMe	(*S*)-Ama-(*S*)-Phe-OMe
$J_{\alpha-\beta h}$/Hz	8.1	8.0
$J_{\alpha-\beta l}$/Hz	5.5	5.4
NOE(Ama H$^\alpha$-Phe NH)	medium	medium
NOE(Phe NH-Phe H$^\alpha$)	weak	weak
NOE(Phe NH-Phe H$^{\beta h}$)	medium	medium
NOE(Phe NH-Phe H$^{\beta l}$)	none	none
NOE(Phe H$^\alpha$-Phe H$^{\beta h}$)	weak	weak
NOE(Phe H$^\alpha$-Phe H$^{\beta l}$)	strong	strong

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

optically pure compound while those for the (*S*)-Ama-(*S*)-Phe-OMe were estimated from the spectra measured for the mixture of the two diastereomers. By use of the two-dimensional HOHAHA and ROESY experiments, the two sets of the ^{1}H signals observed on the spectra for the diastereomeric mixture could be assigned to the individual isomers. Comparing the chemical shifts of these two sets of the ^{1}H signals with those of (*R*)-Ama-(*S*)-Phe-OMe determined from the NMR studies for the optically pure component, we were able to obtain the ^{1}H-NMR parameters for (*S*)-Ama-(*S*)-Phe-OMe. The vicinal ^{1}H-^{1}H coupling constants for the H-C$^\alpha$-C$^\beta$-H groupings of the Phe residue ($J_{\alpha-\beta}$'s) and nuclear Overhauser effects (NOEs) used in defining the conformations are shown in Table III. The NOEs observed in the ROESY spectra are assigned as strong, medium, or weak relative to one another according to their intensities. Investigations of the observed values of $J_{\alpha-\beta}$'s and NOEs involving the β-protons of the Phe residue allow us to assign the *pro*chiralities of the two β-protons as the resonance at the higher field (βh) to the *pro-R* proton and the lower field resonance (βl) to the *pro-S* proton. These assignments are in agreement with the results reported for L-Asp-L-Phe-OMe.[21]

Although the configuration on the α-carbon of the aminomalonyl residue is opposite in the two diastereomers, the observed ^{1}H-NMR parameters are quite similar. For both the diastereomers, a medium NOE was observed between the α-proton of the Ama residue and the NH proton of the (*S*)-Phe residue, indicating that the torsion angle for the N-C$^\alpha$-C(O)-N moiety of the (*R*)-Ama residue is restricted to values from 60° to 180° while the same angle of the (*S*)-Ama residue is restricted to values from −180° to −60°. The side-chain conformation of the (*S*)-Phe residue can be analyzed from the vicinal ^{1}H-^{1}H coupling constants $J_{\alpha-\beta}$'s for the H-C$^\alpha$-C$^\beta$-H gropings. The observed values of $J_{\alpha-\beta l}$ and $J_{\alpha-\beta h}$ are found to be 5.4–5.5 and 8.0–8.1 Hz, respectively. Similar values ($J_{\alpha-\beta l}$ = 3.6–5.9 and $J_{\alpha-\beta h}$ = 8.2–11.3 Hz) have been reported for the Phe residue of L-Asp-L-Phe-OMe in various solvents by Lelj et al.[22] Fractions of three conformers g$^-$, t, g$^+$ about the C$^\alpha$-C$^\beta$ bond (χ_1) of the Phe residue were estimated from the $J_{\alpha-\beta l}$ and $J_{\alpha-\beta h}$ values by using rotational isomeric state approximation.[23] The *trans* and *gauche* couplings necessary for this treatment were set to 13.85 and 3.55 Hz, respectively, following Cung and Marraud.[23] The results revealed that the most preferred conformer about the Phe side chain χ_1 was g$^-$ (0.43–0.44), with the remaining t and g$^+$ comprising 0.18–0.19 and 0.37–0.39, respectively, in both of the diastereomers. The margin of error of the estimated side-chain conformer fractions could be ca. 5%. A weak NOE between the Phe NH and H$^\alpha$ protons observed for both diastereometers is an indication of a nearly *trans* orientation for these two protons. This structure requires the ϕ angle of the (*S*)-Phe residue from −180° to −60°.

Conformational energy calculations were carried out for the two diastereomers of Ama-(*S*)-Phe-OMe using the CHARMM program to obtain molecular geometries of preferred conformations. An extensive search for minimum energy conformations was accomplished in a stepwise fashion, starting with small model compounds such as aminomalonyl methylamide (Ama-NHMe) and *N*-acetylphenylalanine methyl ester (Ac-L-Phe-OMe) and working toward the structure of Ama-(*S*)-Phe-OMe (see Experimental Section).

The minimum energy conformations consistent with the ^{1}H-NMR parameters observed in solution are summarized in Table IV. Since the Ama residue adopts essentially the same structure [(ϕ, χ_1) ~ (−171°, −157°) for (*R*)-Ama and (173°, 155°) for (*S*)-Ama], the energy minima are characterized by the conformation of the Phe residue. The backbone conformation is defined in terms of the conventional letter codes introduced by Zimmerman et al.[24] Although the Ama residue is conformationally fixed, a large number of minimum energy conformations have been obtained for each diastereomer, indicating that the Phe residue has great flexibility. For (*R*)-Ama-(*S*)-Phe-OMe, one of the preferred conformations is very similar to the X-ray structure. However, this structure is not the lowest energy conformer but 1.66 kcal mol^{-1} higher in energy. Similarly, the preferred conformation calculated for (*S*)-Ama-(*S*)-Phe-OMe, which is close to the X-ray structure of the corresponding ethyl ester analogue (*S*)-Ama-(*S*)-Phe-OEt, is 2.72 kcal mol^{-1} higher in energy. It is worthwhile

(21) Castiglione-Morelli, M. A.; Leji, F.; Naider, F.; Tallon, M.; Tancredi, M.; Temussi, P. A. *J. Med. Chem.* **1990**, *33*, 514–520.

(22) (a) Leji, F.; Tancredi, T.; Temussi, P. A.; Toniolo, C. *J. Am. Chem. Soc.* **1976**, *98*, 6669–6675. (b) Temussi, P. A.; Leji, F.; Tancredi, T. *J. Med. Chem.* **1978**, *21*, 1154–1158. (c) Temussi, P. A.; Leji, F.; Tancredi, T.; Castiglione-Morelli, M. A.; Pastore, A. *Int. J. Quantum Chem.* **1984**, *26*, 889–906.

(23) Cung, M. T.; Marraud, M. *Biopolymers* **1982**, *21*, 953–967.

(24) Zimmerman, S. S.; Pottle, M. S.; Nemethy, G.; Scheraga, H. A. *Macromolecules* **1977**, *10*, 1–9.

Table IV. Minimum Energy Conformations for Two Diastereomers of Ama-(*S*)-Phe-OMe[a,b]

	backbone	(*R*)-Ama-(*S*)-Phe-OMe side chain χ_1			(*S*)-Ama-(*S*)-Phe-OMe side chain χ_1		
		g^-	t	g^+	g^-	t	g^+
E	$-180° < \phi < -110°$	0.83	1.53	0.32	0.41	2.69	0.00
	$110° < \psi < 220°$	(8)	(13)	(4)	(2)	(15)	(1)
D	$-180° < \phi < -110°$	1.25	2.51		1.03	3.74	0.94
	$-20° < \psi < 110°$	(11)	(15)		(8)	(17)	(5)
G	$-180° < \phi < -110°$	0.56	0.89	0.56	0.48	2.23	0.46
	$-90° < \psi < -40°$	(6)	(9)	(7)	(4)	(13)	(3)
F	$-110° < \phi < -40°$	0.53	1.66[d]	0.00	0.97	2.72	0.99
	$130° < \psi < 220°$	(5)	(14)	(1)	(6)	(16)	(7)
C	$-110° < \phi < -40°$	1.37	2.88		1.60	4.40	2.21
	$50° < \psi < 130°$	(12)	(16)		(11)	(18)	(11)
A	$-110° < \phi < -40°$	0.30	0.93	0.06	1.06	2.25	1.39
	$-90° < \psi < -10°$	(3)	(14)	(2)	(9)	(14)	(10)

[a] The minimum energy structures are characterized by the conformations of the Phe residue since the Ama residue adopts essentially the same conformation in all the structures; $(\phi, \chi_1) \sim (-171°, -157°)$ for (*R*)-Ama and (173°, 155°) for (*S*)-Ama. [b] Values in parentheses represent the orders of relative stabilities. [c] Backbone conformational states are defined according to Zimmerman et al.[24] [d] The conformation is similar to the X-ray structure.

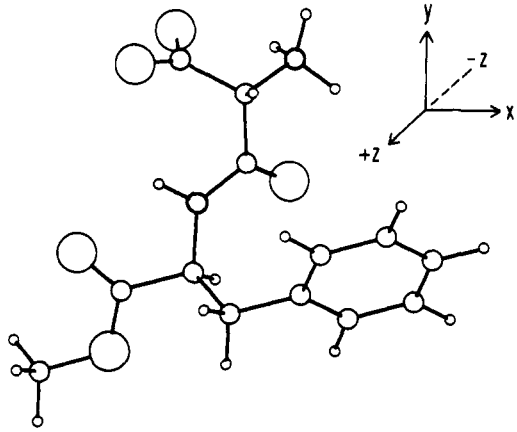

Figure 6. The preferred conformation (Eg^- in Table IV) of the sweet-tasting isomer (*R*)-aminomalonyl-(*S*)-phenylalanine methyl ester possessing an "L" shape required for the sweet taste.

mentioning that similar preferred conformations are also obtained for the (*R*)-Ama-(*S*)-Phe-OEt, (*S*)-Ama-(*S*)-Phe-OEt, and L-Asp-L-Phe-OMe molecular systems (results are not shown).

For sweet-tasting dipeptide derivatives with the hydrophobic site X in the side chain of the second residue such as (*R*)-Ama-(*S*)-Phe-OMe and L-Asp-L-Phe-OMe, the "L" shape of the molecular structure can be maximized when the second residue adopts the angles $\phi = -180°$ to $-110°$ and $\chi_1 \sim -60°$ (g^-). Such requirements are satisfied in the minimum energy conformers Eg^-, Dg^-, and Gg^- for (*R*)-Ama-(*S*)-Phe-OMe in Table III. These are not calculated to be the lowest energy conformers, but they likely exist in substantial amounts in solution because the $\chi_1 = g^-$ state is predominant in solution as revealed by ^{1}H-NMR spectroscopy. The structure of the Eg^- conformer is depicted in Figure 6, where the aminomalonyl moiety containing AH and B groups and the hydrophobic phenylalanine side chain (X) are arrayed in the stem and the base of the "L". This structure is topochemically almost equivalent to that of L-Asp-L-Phe-OMe shown in Figure 4.

None of the preferred conformations estimated for the tasteless isomer (*S*)-Ama-(*S*)-Phe-OMe fit our "L" shape model for sweet taste. In order to examine the possibility that (*S*)-Ama-(*S*)-Phe-OMe assumes "L" shaped structures, additional energy minimizations were carried out by generating initial structures which were described by the "L" shape. When the (*S*)-Ama residue adopts the angle $\psi \sim +60°$ and the (*S*)-Phe residue adopts the angles $\phi = -180°$ to $-110°$ and $\chi_1 \sim -60°$ (g^-), the overall structures of (*S*)-Ama-(*S*)-Phe-OMe can be described as possessing the "L" shape. However, these conformations are at least 6 kcal mol^{-1} higher in energy as compared to those of the corresponding minimum energy conformations with $\psi \sim -172°$ for (*S*)-Ama and $\phi = -180°$ to $-110°$ and $\chi_1 \sim -60°$ for (*S*)-Phe. In addition, these "L" shape structures of (*S*)-Ama-(*S*)-Phe-OMe are not in agreement with the observed medium NOE between the (*S*)-Ama H^α and (*S*)-Phe NH, which indicates the ψ angle is restricted in a range of $-180°$ to $-60°$.

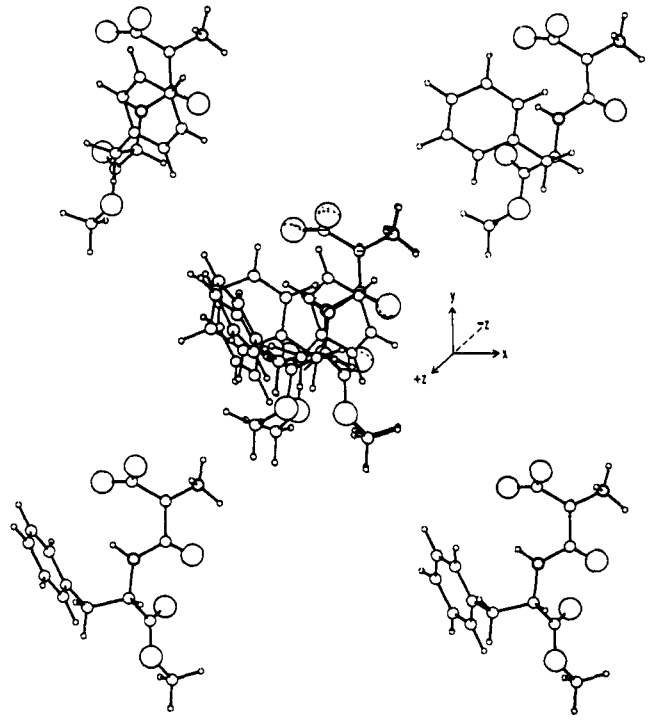

Figure 7. The preferred conformations, Eg^- (upper left), Eg^+ (upper right), Fg^- (lower left), and Fg^+ (lower right), from Table IV, estimated for the tasteless isomer (*S*)-aminomalonyl-(*S*)-phenylalanine methyl ester. The superposition of these four structures is shown in the center. The hydrophobic phenylalanine side chain orients in a space from the $+z$ axis (in front of the "L") to the $-x$ axis (reversed "L" shape).

The minumum energy conformations in which the (*S*)-Phe adopts the angle $\chi_1 \sim 180°$ (t) are described as extended structures. These structures are very similar to the X-ray structure shown in Figure 2. The structures of the minimum energy conformations in which the (*S*)-Phe residue adopts $\chi_1 \sim -60°$ (g^-) and 60° (g^+) are shown in Figure 7. Setting the amine (AH) and carboxyl (B) groups of the (*S*)-Ama residue in the correct zwitterionic form, the hydrophobic Phe side chain mostly projects in a space from the $+z$ axis (in front of the "L") to the $-x$ axis (reversed "L" shape). Thus the molecule is tasteless.

Conclusions

It has been reported that the L-aspartyl residue of the sweet-tasting dipeptide methyl ester (L-Asp-L-Phe-OMe) can be successfully replaced by aminomalonic acid (Ama) without reducing sweetness.[2,3] By analogy to the configurational nature of the parent compound (L-Asp-L-Phe-OMe), (S)-Ama-(*S*)-Phe-OMe was originally proposed to be sweet. On the contrary, the authors claimed that (*R*)-Ama-(*S*)-Phe-OMe would be sweet because the (*R*)–(*S*) isomer is compatible with the sweet formula[5] and could also fit the topochemical model for the sweet response.[6] In order unambiguously to determine which diastereomer is sweet, an X-ray analysis was carried out on the sweet-tasting isomer of Ama-(*S*)-Phe-OMe, which proved to be (*R*)-Ama-(*S*)-Phe-OMe. The tasteless isomer of Ama-(*S*)-Phe-OEt proved to be (*S*)-Ama-(*S*)-Phe-OEt by X-ray analysis. Therefore, we conclude that an (*R*) configuration of the aminomalonyl moiety is necessary for a ligand to produce a sweet taste.

The X-ray crystal structures of the sweet-tasting (*R*)-Ama-(*S*)-Phe-OMe and tasteless (*S*)-Ama-(*S*)-Phe-OEt molecules are very much the same, in which the AH and B containing aminomalonyl moiety and the hydrophobic site X in the aromatic group of the Phe side chain are 180° apart from each other in a flat parallel array. A comparison of the X-ray structures of (*R*)-Ama-(*S*)-Phe-OMe and the corresponding L-aspartyl analogue L-Asp-L-Phe-OMe[14] shows that these two sweet molecules adopt different topochemical structures in the crystalline state. These observations indicate that the X-ray structrues cannot explain taste properties of these dipeptide taste ligands because molecular packing forces primarily determine the crystal structures of small molecules.

The ^{1}H-NMR and molecular modeling studies have demonstrated that both (*R*)-Ama-(*S*)-Phe-OMe and L-Asp-L-Phe-OMe are flexible and show similar conformational preferences in solution. The most preferred state of the Phe side-chain χ_1 conformation in solution has been estimated as g^- ($\sim -60°$) for both compounds, which is different from those observed in the crystalline state. Conformational analyses in solution indicated that the sweet-tasting dipeptide esters (*R*)-Ama-(*S*)-Phe-OMe and L-Asp-L-Phe-OMe assume the "L" shape structures among others with $\chi_1 = g^-$ for the Phe residue. On the other hand, no "L" shape conformation can be attained for the tasteless isomer (*S*)-Ama-(*S*)-Phe-OMe. Similar results were observed for the ethyl ester analogues (*R*)-Ama-(*S*)-Phe-OEt (sweet) and (*S*)-Ama-(*S*)-Phe-OEt (tasteless). The tastes of the aminomalonyl-(*S*)-phenylalanine dipeptide esters [i.e., the (*R*)–(*S*) and (*S*)–(*S*) isomers; sweet and tasteless, respectively] are correctly explained by our "L" shape model for sweet taste developed with L-aspartyl-based peptide sweeteners.

Experimental Section

Materials. Two diastereomers of aminomalonyl-(*S*)-phenylalanine methyl ester, (*R*)-Ama-(*S*)-Phe-OMe and (*S*)-Ama-(*S*)-Phe-OMe, were synthesized by coupling of Z-(*R*,*S*)-Ama(OBzl)-OH and L-Phe-OMe with dicyclohexylcarbodiimide (DCC) followed by hydrogenolysis. The protected compound Z-*ambo*-Ama(OBzl)-(*S*)-Phe-OMe was eluted as two peaks at 8.89 and 10.33 min in a ratio of approximately 1:1 on a normal phase YMC A-012 column using a hexane–ethyl acetate (7:3 (v/v)) solvent system at a flow rate of 1 mL min^{-1}. The elution profile was monitored at 254 nm. The mixture was hydrogenated in the presence of Pd/C and then lyophilized. The lyophilized product was subjected to a reversed-phase Inertsil ODS-2 column with a linear gradient of 10% acetonitrile containing 0.05% trifluoroacetic acid (TFA) to 50% acetonitrile containing 0.05% TFA obtained in 20 min at a flow rate of 1 mL min^{-1}. The elution profile was monitored at 210 nm. The final compound *ambo*-Ama-(*S*)-Phe-OMe was eluted as two peaks at 16.17 and 17.00 min in a ratio of approximately 1:1.

The ethyl ester analogue, aminomalonyl-(*S*)-phenylalanine ethyl ester, was prepared by coupling of Z-(*R*,*S*)-Ama(OBzl)-OH and L-Phe-OEt with DCC and subsequent hydrogenolysis of the resulting protected dipeptide. The protected dipeptide Z-*ambo*-Ama-(*S*)-Phe-OEt was eluted as two peaks at 7.36 and 8.05 min in a ratio of approximately 1:1 on analytical normal phase HPLC under the same conditions as described for the methyl ester analogue. The protected compound was hydrogenated in the presence of Pd/C and then lyophilized. The target compound was analyzed by HPLC using an Inertsil ODS-2 column under a linear gradient condition from 10% acetonitrile containing 0.05% TFA to 30% acetonitrile containing 0.05% TFA obtained in 20 min at a flow rate of 1 mL min^{-1}. The elution profile monitored at 210 nm revealed that *ambo*-Ama-(*S*)-Phe-OEt was eluted as two peaks at 23.96 and 24.64 min in a ratio of approximately 1:1.

X-ray Diffraction. The data collection for (*S*)-Ama-(*S*)-Phe-OEt was carried out on a Siemens R3m/V diffractometer with Mo Kα by using a highly oriented graphite crystal in the range 4.0–50.0° of 2θ. A 2θ–θ scan mode with variable speed (1.00–2.49 deg min^{-1}) and a scan range of 0.6° plus Kα separation was selected. The h, k, l ranges were 0 to 6, 0 to 10, and −19 to 19, respectively. A total of 1426 independent reflections were measured, 1260 of which had $F_o > 4.0\sigma(F_o)$ and thus were considered "observed" and used for refinement. The structure was solved by direct methods with the MITHRIL program.[8] The full-matrix least-squares procedure was used, minimizing the quantities $\sum\omega(F_o - F_c)^2$ with weight $\omega = [\sigma^2(F_o) + 0.0028F_o^2]^{-1}$. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included in the final cycles of refinement with fixed thermal parameters of 0.08 Å^2. The final R indices, $R = \sum||F_o| - |F_c||/\sum|F_o|$ and $wR = [\sum(|F_o| - |F_c|)^2/\sum|F_o|^2]^{1/2}$, and goodness to fit, $S = [\sum\omega(|F_o| - |F_c|)^2/(m - n)^2]^{1/2}$ where m and n respectively represent numbers of observed reflections and parameters refined, were 5.32%, 7.25%, and 1.22, respectively.

The data for (*R*)-Ama-(*S*)-Phe-OMe were collected on a Rigaku AFC5S diffractometer with Cu Kα in the range 6.0–120.1° of 2θ using an ω–2θ scan mode with a scan rate of 32.0 deg min^{-1} in ω and scan width (1.57 + 0.30 tan θ)°. In total 4940 reflections were processed using profile analyses to give 4455 unique reflections (R_{int} = 0.045); 2629 reflections with $F_o > 3.0\sigma(F_o)$ were considered "observed" and used in the refinement. The structure was solved by direct methods with the DIRDIF program.[9,10] Block-matrix least-squares refinement minimizing the quantities $\sum\omega(F_o - F_c)^2$ and least-squares weights $\omega = 4F_o^2/\sigma^2(F_o^2)$ were used. 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 = 7.3%, wR = 11.2%, and S = 0.27.

^{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 Hatman–Hahn (HOHAHA)[25a] and the rotating frame nuclear Overhauser enhancement (ROESY)[26] experiments. The HOHAHA experiments employed the MLEV 17 spin-locking sequence suggested by Bax and Davis.[25b] The time proportional phase increment[27] 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.

Energy Calculations. Conformational energy calculations were carried out with the Adopted Basis Newton-Raphson algorithm until all derivatives were smaller than 0.001 kcal mol^{-1} Å^{-1} employing QUANTA 3.0 (Polygen) and CHARMm.[28,29] Conformational energies were expressed by the valence force field implemented in the CHARMM program with the PARM 30 parameter set. A distance dependent dielectric constant ($\epsilon = 2r$) was used in all calculations.

Computer analysis was carried out for a model compound (*R*)-aminomalonyl methylamide [(*R*)-Ama-NHMe]. Two minimum energy conformations were calculated from 144 initial structures generated by varying the ψ and χ_1 angles in increments of 30°. The lowest energy conformer adopted torsion values of $\psi = -172°$ and $\chi_1 = -174°$, similar to the conformation observed for the (*R*)-Ama residue to (*R*)-Ama-(*S*)-Phe-OMe by X-ray analysis. The remaining minimum conformer with $\psi = -176°$ and $\chi_1 = 160°$ was 1.50 kcal mol^{-1} higher in energy. The compound (*S*)-Ama-NHMe shows mirror-image behavior of (*R*)-Ama-NHMe. Minimum energy conformations were calculated for a model compound *N*-acetyl-L-phenylalanine methyl ester (Ac-L-Phe-OMe). Varying torsion angles ϕ and ψ in increments of 30°, 144 structures were examined for each of three side-chain χ_1 states g^- ($\sim -60°$), t ($\sim 180°$), and g^+ ($\sim 60°$). After the above treatment, energy minimizations were carried out for the two diastereomers of Ama-(*S*)-Phe-OMe. The initial structures were generated by adopting the values of torsion angles estimated for the model compounds Ama-NHMe and Ac-L-Phe-OMe.

Acknowledgment. The authors gratefully acknowledge the support of the National Institute of Dental Research (DE-05476) and the Ajinomoto Co., Inc.

Supplementary Material Available: Tables of atomic coordinates and equivalent temperature factors, bond lengths, bond angles, torsion angles, crystal data, experimental data, and solution and refinement data for (*R*)-aminomalonyl-(*S*)-phenylalanine methyl ester and (*S*)-aminomalonyl-(*S*)-phenylalanine ethyl ester (11 pages); listing of observed and calculated structure factors for $C_{14}H_{18}N_2O_5$ (22 pages). Ordering information is given on any current masthead page.

(25) (a) Bax, A.; Davis, D. G. *J. Am. Chem. Soc.* **1985**, *107*, 2820–2821. (b) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355–360.
(26) Bothner-By, A. A.; Steppens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813.
(27) Bodenhausen, G.; Vold, R. L.; Vold, R. R. *J. Magn. Reson.* **1980**, *37*, 93–106.
(28) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187–217.
(29) Nilsson, L.; Karplus, M. *J. Comput. Chem.* **1986**, *7*, 591–616.