Probing a Molecular Model of Taste Utilizing Peptidomimetic Stereoisomers of 2-Aminocyclopentanecarboxylic Acid Methyl Ester

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On the basis of the preferred conformations of L-aspartyl dipeptide derivatives containing α-amino acids at the second position and their retro-inverso analogues deduced by a combination of X-ray crystallography, ^{1}H NMR spectroscopy, and molecular mechanics calculations, we have proposed a model describing the molecular array required for the sweet taste. The conformation of a sweet molecule is described as possessing an "L shape", with the AH (proton donor) and B (proton acceptor) zwitterionic ring of the aspartyl moiety forming the stem, and the hydrophobic group X forming the base of the "L". Planarity of the molecule in the *x* and *y* dimensions is critical for sweet taste. Substantial deviation from this plane into negative *z* dimension is correlated with bitter taste while other deviations lead to tasteless molecules. To examine the model, the preferred conformations for a series of L-aspartyl dipeptides containing a 2-aminocyclopentanecarboxylic acid (2-Ac^{5}c) residue at the second position were calculated using molecular mechanics. The peptidomimetic 2-Ac^{5}c residue is a β-amino acid with two chiral centers, resulting in four isomers [*trans*-(1*S*,2*S*)-2-Ac^{5}c, *trans*-(1*R*,2*R*)-2-Ac^{5}c, *cis*-(1*R*,2*S*)-2-Ac^{5}c, and *cis*-(1*S*,2*R*)-2-Ac^{5}c]. Two stereoisomers, L-aspartyl-*trans*-(1*R*,2*R*)-2-aminocyclopentanecarboxylic acid methyl ester [Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe] and L-aspartyl-*cis*-(1*S*,2*R*)-2-aminocyclopentanecarboxylic acid methyl ester [Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe], prefer the L-shape conformations and are thus predicted to be sweet. For L-aspartyl-*trans*-(1*S*,2*S*)-2-aminocyclopentanecarboxylic acid methyl ester [Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe], the methyl ester group projects behind the stem of the L shape, producing a large negative *z* component and is predicted to exhibit a bitter taste. The calculations predict that L-aspartyl-*cis*-(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid methyl ester [Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe] will be tasteless. In this investigation, in addition to the calculations, we report the synthesis and experimental conformational analysis of the four stereoisomers of Asp-2-Ac^{5}c-OMe. The absolute configurations of the 2-Ac^{5}c residues were assigned by X-ray diffraction studies and by correlating optical rotation and enantiomeric excess values. These studies fully confirm our configurational assignments of the stereoisomers of Asp-2-Ac^{5}c-OMe. Thus, the structure–taste relationships observed for the new class of L-aspartyl taste ligands containing the 2-Ac^{5}c β-amino acid methyl esters in the second position agree with and strengthen our model for the sweet and bitter taste responses.

Introduction

The relationship between the structure and taste of sweet molecules has been studied by many groups. Shallenberger and Acree[1] have proposed the proper spatial arrangement of a proton donor (AH) and a proton acceptor (B) which are separated by 2.5–4.0 Å (AH/B model) by examining structures of unrelated sweet molecules such as sugars, saccharin, chloroform, unsaturated alcohols, and 1-propoxy-2-amino-4-nitrobenzene. These AH and B groups are required for the formation of intermolecular hydrogen bonds between the molecule and the receptor. From the examinatioan of 2-amino-4-nitrobenzene derivatives Deutsch and Hansch[2] have suggested that a hydrophobic binding area is necessary in a series of potent sweet compounds in addition to the AH/B requirement. By employing molecular orbital calculations for sweet amino acids and a series of substituted 2-amino-4-nitrobenzenes, Kier et al.[3] have proposed the presence and approximate location of a third structural hydrophobic feature, denoted as X, relative to the postulated AH/B features in glucophores.

Since the accidental discovery that the dipeptide L-aspartyl-L-phenylalanine methyl ester (aspartame, Asp-Phe-OMe) is about 200 times sweeter than sucrose,[4] a large number of L-aspartyl di- and tripeptide derivatives have been synthesized and found to be as sweet or sweeter than Asp-Phe-OMe. The AH/B moiety in this type of sweetener can unmistakably be attributed to the N-terminal NH_3^+ and the side-chain $C^\beta OO^-$ groups in the zwitterionic ring structure of the L-aspartyl residue. From the analysis of the preferred conformations for Asp-Phe-OMe by a combined use of ^{1}H NMR and potential energy calculations, Temussi et al.[5] proposed an extended structure with the zwitterionic ring of the Asp residue and the side chain of the Phe residue 180° apart in a flat parallel array as an active conformation.

During their work on L-aspartyl dipeptide esters, Brussel et al.[6a] and van der Heijden et al.[6b,c] observed that the lengths of side chains with respect to the AH/B moiety are extremely important for the L-aspartyl dipeptide esters to taste sweet. Utillizing the conformations of Asp-Phe-OMe reported by Temussi and his associates,[5a] van der Heijden et al.[6b] developed a model for the sweet taste that was different from that of Temussi but that allowed for differentiation of molecules by the size of the side chain and the C-terminal ester group.

From conformational studies of L-aspartyl dipeptide derivatives containing a 2,2,5,5-tetramethylcyclopentanyl substituent as the terminal end group and their retro-inverso analogues by use of X-ray crystallography, ^{1}H NMR, and molecular mechanics calculations, we have developed models describing the molecular arrays required for the sweet and bitter taste.[7-9] The overall conformation of the

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(1) Shallenberger, R. S.; Acree, T. E. *Nature* **1967**, *216*, 480–2.
(2) Deutsch, E.W.; Hansch, C. *Nature* **1966**, *211*, 75.
(3) (a) Kier, L. B. *J. Pharm. Sci.* **1972**, *61*, 1394–7. (b) Holtje, H. D.; Kier, L. B. *J. Pharm. Sci.* **1974**, *63*, 1722–5.
(4) Mazur, R. H.; Schlatter, J. M.; Goldkamp, A. H. *J. Am. Chem. Soc.* **1969**, *91*, 2684–91.
(5) (a) Lelj, F.; Tancredi, T.; Temussi, P. A.; Toniolo, C. *J. Am. Chem. Soc.* **1976**, *98*, 6669–75. (b) Temussi, P. A.; Lelj, F.; Tancredi, T. *J. Med. Chem.* **1978**, *21*, 1154–8. (c) Temussi, P. A.; Lelj, F.; Tancredi, T.; Morelli, M. A. C.; Pastore, A. *Int. J. Quantum Chem.* **1984**, *26*, 889–906.
(6) (a) Brussel, L. B. P.; peer, H. G.; van der Heijden, A. *Z. Lebensm. Untersuch-Forsch.* **1975**, *159*, 337–43. (b) van der heijden, A.; Brussel, L. B. P.; Peer, H. G. *Food Chem.* **1978**, *3*, 207–11. (c) van der Heijden, A.; Brussel, L. B. P.; Peer, H. G. *Chem. Senses Flav.* **1979**, *4*, 141–52.
(7) Goodman, M.; Coddington, J.; Mierke, D. F.; Fuller, W. D. *J. Am. Chem. Soc.* **1987**, *109*, 4712–4.
(8) 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.

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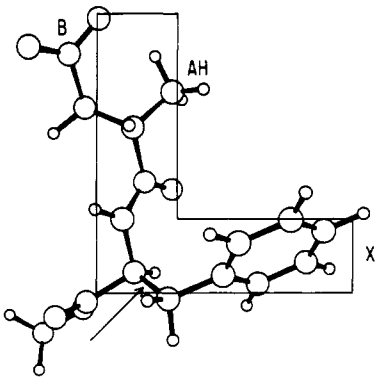

Figure 1. Model of the sweet taste with L-aspartyl-L-phenylalanine methyl ester superimposed. The side-chain conformation abou the C^{α}–C^{β} bond of the phenylalanine residue, shown by the arrow, has been rotated from a g^+ state observed in the X-ray diffraction strucure[11] to a g^- state which is predominant in solution.[14] The AH/B and X groups of the molecule are illustrated according to Kier et al.'s suggestions.[3]

sweet tasting analogues can be described as possessing an "L-shape" structure with the aspartyl moiety as the stem of the "L" and the tetramethylcyclopentanyl group, considered to be a hydrophobic site X, as the base of the L. The zwitterionic ring of the aspartyl residue is coplanar and essentially perpendicular to the tetramethylcyclopentanyl ring. The conformation of the bitter tasting analogue L-aspartyl-L-alanyl-2,2,5,5-tetramethylcyclopentanylamide, on the other hand, has the zwitterionic ring and tetramethylcyclopentanyl rings nearly aligned rather than the 90° necessary for the "L shape" and the rings are twisted out of plane from each other by more than 60°. Recently, it has been reported that the above models correctly predict the tastes of L-aspartyl-D-alanyl-2,2,4,4-tetramethylthiethane (alitame) and the related L,L stereoisomer, which are sweet and bitter, respectively.[10] The model for the sweet taste fits the structure of Asp-Phe-OMe with only a minor modification of the conformation reported from X-ray crystallographic studies;[11] the side-chain conformation about the C^{α}–C^{β} bond of the Phe residue is changed from g^+ to g^- state (Figure 1).[12] Considering the vicinal ^1H–^1H coupling constants for a moiety H–C^{α}–C^{β}–H of the Phe residue given by Lelj et al.[5a] and the assignment of the two protons on the β-carbon atom determined for *N*-acetyl-glycylphenylalanine methyl ester by use of stereospecifically β-monodeuterated phenylalanine,[13] the g^- conformation about the C^{α}–C^{β} bond of the Phe residue may be predominant in solution over the other two conformations, the g^+ and t states. Recently, the preference of the g^- conformation for the Phe residue was proven by Castiglione-Morelli et al. using aspartame with β-monodeuterated phenylalanine, (2*S*,3*S*)-3-[^{2}H]-Phe.[14]

The models for the sweet taste by Temussi et al.,[5] van der Heijden et al.,[6b] and from our laboratories[7-10] have been developed on the basis of the preferred conformations of the L-aspartyl dipeptide esters containing α-amino acids

(9) Benedetti, E.; Blasio, B. D.; Pavone, V.; Pedone, C.; Fuller, W. D.; Mierke, D. F.; Goodman, M. *J. Am. Chem. Soc.* **1990**, *112*, 8909–12.
(10) Feinstein, R. D.; Polinsky, A.; Douglas, A. J.; Beijer, C. M. G. F.; Chadha, R. K.; Benedetti, E., Goodman, M. *J. Am. Chem. Soc.* **1991**, *113*, 3467–73.
(11) Hatada, H.; Jancarik, J.; Graves, B.; Kim, S. H. *J. Am. Chem. Soc.* -1985, *107*, 4279–82.
(12) This structure is different from that previously shown in ref 7. A rotation of 40° about the ϕ bond leads to a high-energy conformation from the X-ray diffraction structure.[10]
(13) Kobayashi, J.; Nagai, U. *Biopolymers* **1978**, *17*, 2265–77.
(14) Castiglione-Morelli, M. A.; Lelj, F.; Naider, F.; Tallon, M.; Tancredi, M.; Temussi, P. A. *J. Med. Chem.* **1990**, *33*, 514–20.

Asp-(*trans and cis*)-2-Ac^{5}c-OMe

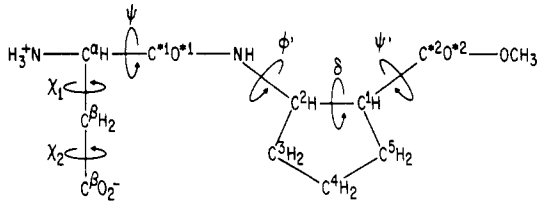

Figure 2. Schematic illustration of L-aspartyl-2-aminocyclopentanecarboxylic acid methyl ester.

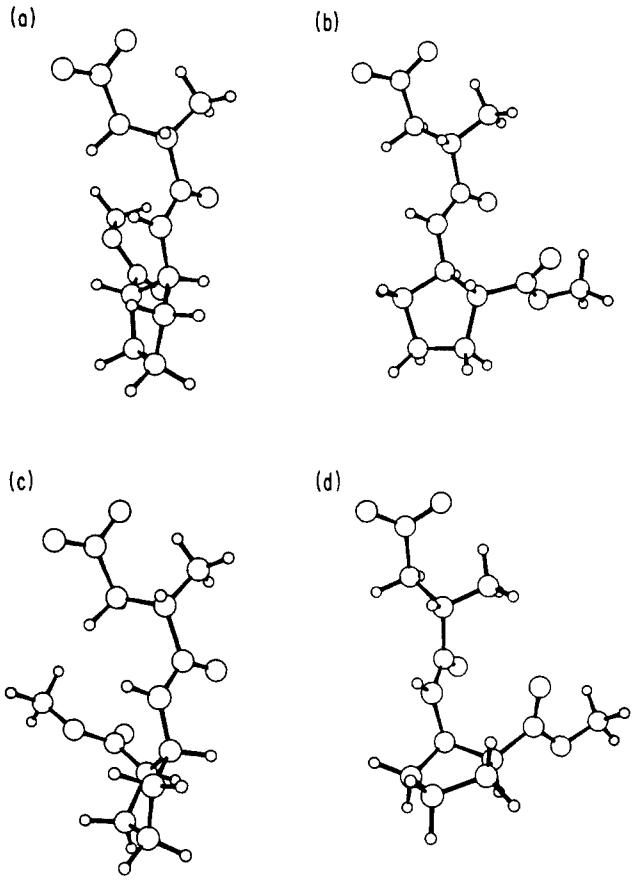

Figure 3. Low-energy conformations calculated for (a) Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe, (b) Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe, (c) Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe, and (d) Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe. Structures are projected in the *xy* plane: the *y* axis is taken to be parallel to the direction from C^{β} of the 2-Ac^{5}c residue to C^{α} to the Asp residue, the *x* axis in the plane formed by the zwitterionic ring of the aspartyl moiety, and the *z* axis perpendicular to the plane. From comparison of the overall topology with our models describing the molecular arrays required for the sweet and bitter tastes,[7-10] (b) Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe and (d) Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe both assuming the L-shape conformations are predicted to taste sweet while (a) Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe assuming the conformation with a large negative *z* component is predicted to be bitter. The overall topology of (c) Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe does not match the models for both the sweet and bitter tastes, and thus this analogue is predicted to be tasteless.

at the second position. In an attempt to test these models, we designed a series of L-aspartyl dipeptides containing 2-aminocyclopentanecarboxylic acid methyl ester as the second residue (Asp-2-Ac^{5}c-OMe) by using molecular mechanics calculations. A schematic representation of Asp-2-Ac^{5}c-OMe is shown in Figure 2: the location of atoms and rotation angles are identified by the suffixes.

The peptidomimetic 2-Ac^{5}c is a β-amino acid with two chiral centers, thus four configurational isomers exist, i.e., two trans [(1*S*,2*S*)-2-Ac^{5}c and (1*R*,2*R*)-2-Ac^{5}c] and two cis [(1*R*,2*S*)-2-Ac^{5}c and (1*S*,2*R*)-2-Ac^{5}c].

Low-energy conformations calculated for the four stereoisomers of Asp-2-Ac^{5}c-OMe are shown in Figure 3-(a)-(d), where the *y* axis is taken to be parallel to the direction from C$^\beta$ of the 2-Ac^{5}c residue to C$^\alpha$ of the Asp residue, the *x* axis in the plane formed from the zwitterionic ring of the aspartyl moiety, and the *z* axis perpendicular to the plane. The overall structures of Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe (Figure 3(b)) and Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe (Figure 3(d)) are topologically defined by the L-shape conformation which we have previously proposed for the sweet taste. The zwitterionic ring of the aspartyl moiety and the part of cyclopentane ring plus the methyl ester group are essentially in the *xy* plane and almost 90° from each other leading to molecules with small *z* components. Therefore, our model predicts a sweet taste for Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe and Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe. For Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe, on the other hand, the methyl ester group is out of the *xy* plane with respect to the aspartyl zwitterionic ring (Figure 3(a)). This analogue has a large negative *z* component and is predicted by our model to be bitter. The conformation calculated for Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe (Figure 3(d)) has only a small *z* component. However, the methyl ester group projects toward the −60° direction in the *xy* plane relative to the stem of the "L" formed by the aspartyl moiety. The overall topology of this analogue is different from our model for the sweet and bitter tastes. The *cis*-(1*R*,2*S*)-2-Ac^{5}c-containing analogue should be tasteless.

In order to examine the above taste predictions for the four configurational isomers of Asp-2-Ac^{5}c-OMe, we synthesized the four dipeptide stereoisomers and assigned the absolute configurations of the 2-Ac^{5}c residues. We also carried out conformational studies on these L-aspartyl peptidomimetic dipeptide derivatives. For the conformational analysis, the ^{1}H NMR measurements were carried out in dimethyl sulfoxide (DMSO). The vicinal ^{1}H–^{1}H coupling constant for the H–C$^\alpha$–C$^\beta$–H moiety of the Asp residue and the qualitative intraresidue and sequential NOEs were studied to obtain the information regarding the preferred conformations of these molecules. Conformational energy calculations were also undertaken in conjunction with these experimental observations. From the resulting preferred conformations, we compared the geometries of these petidomimetic dipeptide analogues with the models for sweet and bitter tastes.

Results and Discussion

Synthesis. The synthesis of 2-aminocyclopentanecarboxylic acid (2-Ac^{5}c) was undertaken employing two methodologies which specifically generated either cis [(1*R*,2*S*)-2-Ac^{5}c and (1*S*,2*R*)-2-Ac^{5}c] or trans [(1*S*,2*S*)-2-Ac^{5}c and (1*R*,2*R*)-2-Ac^{5}c] configurational isomers. These routes were utilized in separate syntheses in order to obtain the all-cis or -trans isomers (Figure 4). The cis isomers were obtained as a racemic mixture according to the method of Plieninger and Schneider by Hofmann degradation of *cis*-2-carbamoylcyclopentanecarboxylic acid.[15,16] The trans isomers were prepared as a racemic mixture by the Michael addition of ammonia to 1-cyclopentenecarboxylic acid.[15-17] Therefore, the synthesis of the peptidomimetic dipeptide derivatives (Asp-2-Ac^{5}c-OMe) was carried out as a diastereomeric mixture with either cis or trans isomers. The methyl esters of the cis and trans isomers were prepared and coupled to *N*-(benzyloxycarbonyl)-β-benzyl-L-aspartate [*Z*-Asp(OBzl)OH] using a *N*-hydroxybenzotriazole (HOBt) ester coupling procedure. Deprotection by hydrogenolysis yielded the two diastereomeric mixtures which were separated by reversed-phase HPLC. The identity of each of the four configurational isomers was determined by using 500-MHz ^{1}H NMR spectroscopy.

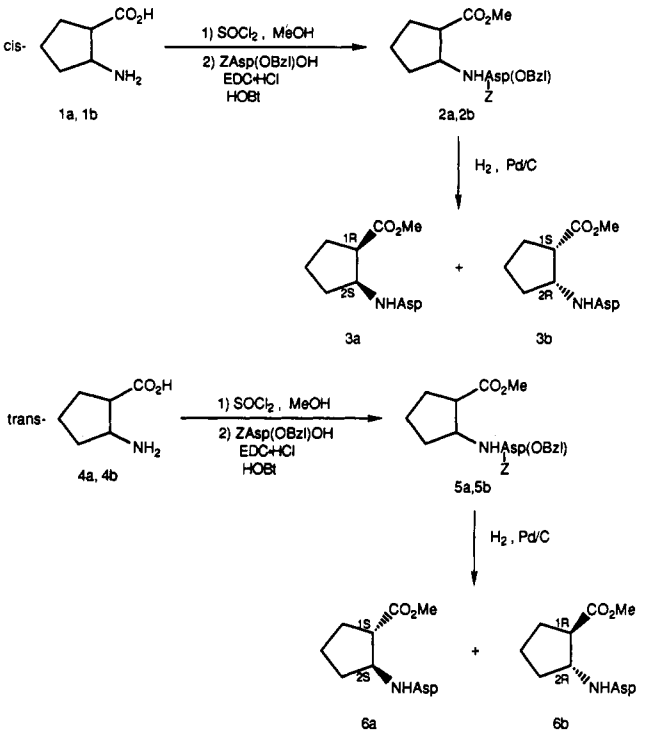

Figure 4. Schemes for the syntheses of the peptidomimetic derivatives (Asp-2-Ac^{5}c-OMe). The synthesis was carried out as a diastereomeric mixture with either cis (**1a,1b**) or trans (**4a,4b**) isomers of 2-Ac^{5}c. The diastereomers were separated at the final stage by reversed-phase HPLC. The absolute configurations of the 2-Ac^{5}c residues were assigned by use of optical resolution methods described in Figure 5 (*cis*-2-Ac^{5}c) and Figure 6 (*trans*-2-Ac^{5}c).

Table I. Taste Properties of Asp-2-Ac^{5}c-OMe

compd	abs config of 2-Ac^{5}c	taste[a]
trans-fast **6a**[b]	*trans*-(1*S*,2*S*)[c]	bitter
trans-slow **6b**[b]	*trans*-(1*R*,2*R*)[c]	very sweet
cis-fast **3a**[d]	*cis*-(1*R*,2*S*)[c]	tasteless
cis-slow **3b**[d]	*cis*-(1*S*,2*R*)[e]	sweet

[a] The taste was determined by a qualitative "sip and spit" taste assessment of dilute solution of the molecule. [b] Trans-fast and trans-slow are the fast- and slow-moving isomers of Asp-*trans*-2-Ac^{5}c-OMe on reversed-phase HPLC. [c] The absolute configuration of the *trans*-2-Ac^{5}c residue was assigned by the X-ray diffraction study (see Figure 6). [d] Cis-fast and cis-slow are the fast- and slow-moving isomers of Asp-*cis*-2-Ac^{5}c-OMe on reversed-phase HPLC. [e] The absolute configuration of the *cis*-2-Ac^{5}c residue was assigned based on the optical rotation (see Figure 5).

Taste properties of the four configurational isomers of Asp-2-Ac^{5}c-OMe are listed in Table I. The tastes of the compounds were tested by a qualitative "sip and spit" taste assessment of dilute solutions of the molecules. The absolute configurations for the 2-Ac^{5}c residues in the derivatives are assigned as described below.

Absolute Configuration of 2-Aminocyclopentanecarboxylic Acid. Asp-*cis*-2-Ac^{5}c-OMe. The absolute configuration of the *cis*-2-Ac^{5}c residue in Asp-*cis*-2-Ac^{5}c-

(15) Plieninger, H.; Schneider, K. *Chem. Ber.* **1959**, *92*, 1594–9.
(16) Conners, T. A.; Ross, W. C. J. *J. Chem. Soc.* **1960**, 2119–32.
(17) Bernath, G.; Lang, K. L.; Gondos, G.; Marai, P.; Kovacs, K. *Acta Chim. Acad. Sci. Hung.* **1972**, *74*, 479–97.

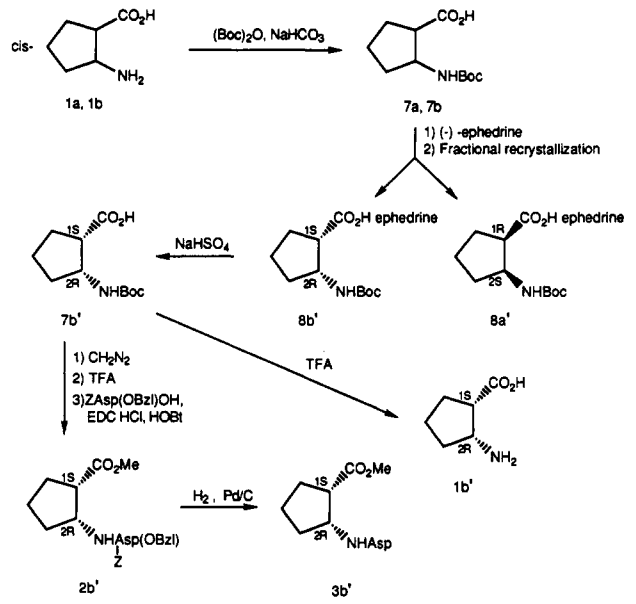

Figure 5. Scheme for the synthesis of the optically active Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe (**3b'**). Optical resolution with (−)-ephedrine was applied to a racemic mixture of Boc-*cis*-2-Ac^{5}c (**7a,7b**). For a given enantiomer illustrated for the *cis*-(1*R*,2*S*)-2-Ac^{5}c isomer **7b'**, the Boc protecting group was removed to give the optically active free amino acid **1b'** with the value of $[\alpha]^{20}_D$ +5.9° (*c* 1.0, H_2O) [lit.[22] $[\alpha]^{20}_D$ −8.9° (*c* 1.0, H_2O)]. The L-aspartyl dipeptide methyl ester **3b** was prepared as shown. The optical rotation value for the amino acid **1b'** indicated that this compound contained *cis*-(1*R*,2*S*)-2-Ac^{5}c and *cis*-(1*S*,2*R*)-2-Ac^{5}c in a ratio of 17:83. The reversed-phase HPLC analysis showed that the dipeptide methyl **3b'** was a mixture of the compounds 3a (cis-fast) and 3b (cis-slow) with a ratio of 15:85, which is consistent with the ratio for the amino acid **1b'**. Therefore, the tasteless cis-fast (**3a**) and sweet tasting cis-slow (**3b**) analogues were assigned as Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe and Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe, respectively.

OMe was assigned by use of the optical resolution method with (−)-ephedrine according to the scheme in Figure 5. The amino acid derivative Boc-*cis*-2-Ac^{5}c-OH (**7a,7b**), which was obtained by treatment of racemic mixture of *cis*-2-Ac^{5}c (**1a,1b**) with di-*tert*-butyl dicarbonate [$(Boc)_2O$], was mixed with (−)-ephedrine. The resulting salt was fractionally recrystallized from an ethyl acetate/ether solvent system. The fractional recrystallization procedure was repeated until the value of optical rotation became constant. Finally, there was obtained the partially resolved salt **8b'** with $[\alpha]^{20}_D$ −7.9° (*c* 1.0, MeOH). Treatment of **8b'** with $NaHSO_4$ afforded partially resolved Boc-*cis*-2-Ac^{5}c-OH (**7b'**): $[\alpha]^{20}_D$ +47.7° (*c* 1.4, MeOH). The final L-aspartyl dipeptide methyl ester **3b** was obtained by the usual procedures as shown in Figure 5. The ratio of cis-"fast" **3a** to cis-"slow" **3b** was found to be 15:85 by reversed-phase HPLC adopting the same conditions used for the separation of diastereomers.

Recently, Kawabata et al.[18] assigned the absolute configuration of a new antifungal antibiotic, FR109615, which is identical with (−)-*cis*-2-Ac^{5}c. The structure of (−)-*cis*-2-Ac^{5}c was proven to be (1*R*,2*S*)-2-Ac^{5}c with $[\alpha]^{20}_D$ −8.9° (*c* 1.0, H_2O) by an X-ray diffraction study. On the basis of this optical rotation value, we estimated the optical purity of the partially resolved Boc-*cis*-2-Ac^{5}c-OH (**7b'**). The Boc protecting group of **7b'** was cleaved by trifluoroacetic acid to give *cis*-2-Ac^{5}c (**1b'**) with plus optical rotation $[\alpha]^{20}_D$ +5.9° (*c* 1.0, H_2O), indicating that the ratio

(18) Kawabata, K.; Inamoto, Y.; Sakane, K. *J. Antibiotics* **1990**, *43*, 513–518.

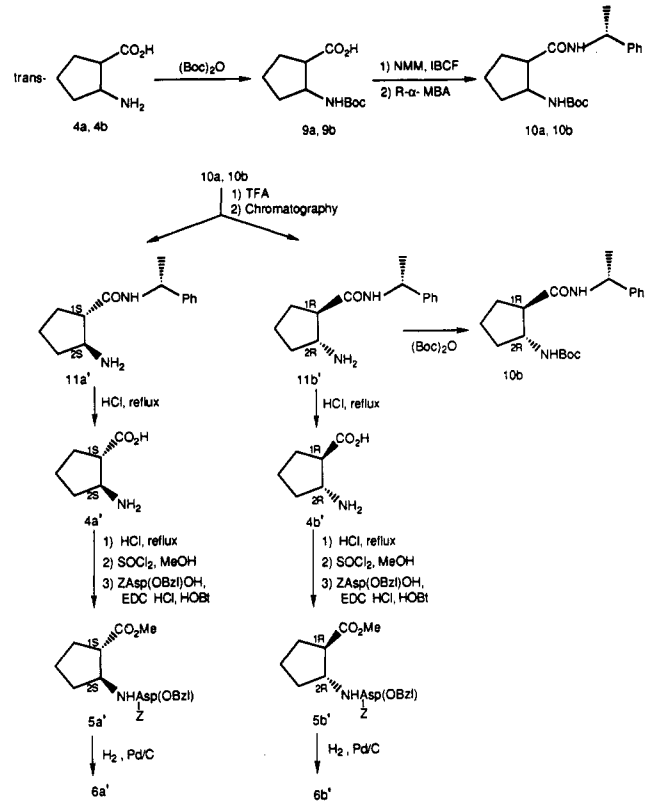

Figure 6. Scheme for the synthesis of Asp-*trans*-2-Ac^{5}c-OMe using the optically active *trans*-2-Ac^{5}c. The separation of diastereomers of *trans*-2-Ac^{5}c-(*R*)-αMBA **11a'** and **11b'** was carried out using silica gel column chromatography with $CHCl_3$:MeOH:AcOH = 19:1:1 solvent system. The absolute configuration of the *trans*-2-Ac^{5}c residue of the compound **11b'** was determined to be 1*R*,2*R* by the X-ray crystallographic analysis of the Boc-protected compound **10b** prepared from **11b'**. The reversed-phase HPLC analysis showed that the final dipeptides **6a'** with the purity of 90% and **6b'** with the purity of 95% correspond to the analogues **6a** (trans-fast) and **6b** (trans-fast) in Figure 4. Therefore, the bitter-tasting trans-fast and sweet-tasting trans-slow were assigned as Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe and Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe, respectively.

of (1*R*,2*S*)-2-Ac^{5}c to (1*S*,2*R*)-2-Ac^{5}c wass 17:83. This ratio was consistent with the ratio for the final compound Asp-*cis*-2-Ac^{5}c-OMe (**3b'**) determined by HPLC (cis-fast:cis-slow = 15:85) noted above. Therefore, the tasteless cis-fast and the sweet-tasting cis-slow analogue in Table I were assigned as Asp-(1*R*,2*S*)-2-Ac^{5}c-OMe and Asp-(1*S*,2*R*)-2-Ac^{5}c-OMe, respectively, which are in agreement with the predictions of our model for the sweet and bitter tastes.

Asp-*trans*-2-Ac^{5}c-OMe. In an attempt to determine the absolute configuration of the *trans*-2-Ac^{5}c residue in Asp-*trans*-2-Ac^{5}c-OMe, we synthesized both the optically active (1*S*,2*S*)-2-Ac^{5}c (**4a'**) and (1*R*,2*R*)-2-Ac^{5}c (**4b'**) by optical resolution of a racemate, *trans*-2-Ac^{5}c (**4a,4b**), according to the route as shown in Figure 6. The tert-butyloxycarbonylation of a racemic mixture of *trans*-2-Ac^{5}c (**4a, 4b**) with $(Boc)_2O$ provided Boc-*trans*-2-Ac^{5}c-OH (**9a, 9b**), which was acylated with *R*-(+)-α-methylbenzylamine ((*R*)-αMBA) by use of a mixed anhydride to yield the acylated compound **10a,10b** as a mixture of diastereomers. The separation of diastereomers could be performed using silica gel column chromatography with $CHCl_3$:MeOH:AcOH = 19:1:1 solvent system to give the optically active amines **11a'** and **11b'** after the acid labile Boc protecting group of **10a,10b** was removed by trifluoroacetic acid. The optical purities of **11a'** and **11b'** were determined to be 90% and 95%, respectively, by the reversed-phase HPLC

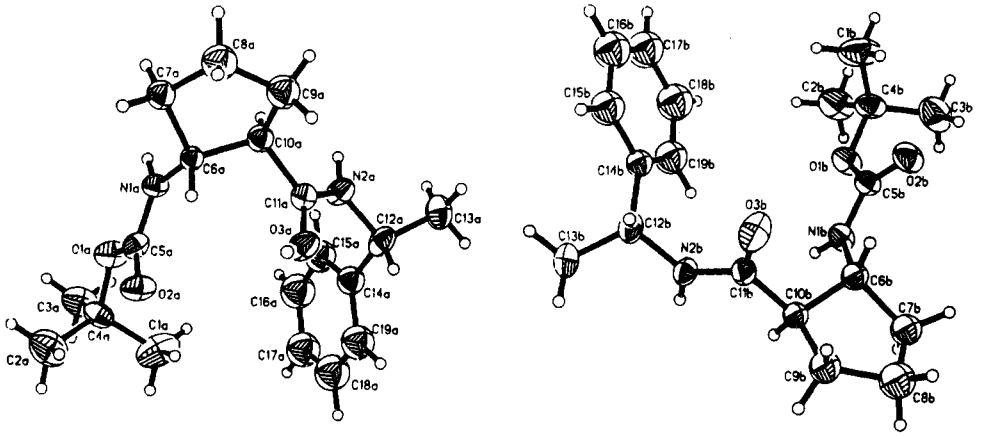

Figure 7. Molecular structures of [*N*-(*tert*-butyloxycarbonyl)-*trans*-(1*R*,2*R*)-2-aminocyclopentanecarbonyl]-(*R*)-(+)-α-methylbenzylamine. Two different structures (a) and (b) coexist in the crystalline state. The conformational differences between the molecules (a) and (b) were observed in orientations of the two peptide groups relative to the cyclopentane ring, i.e., torsion angles for the C6–C10–C11–N2 and C5–N1–C6–C10 moieties (see text). In both of the molecules, the cyclopentane ring of the *trans*-(1*R*,2*R*)-2-Ac^{5}c residue assumes a conformation in which the N1H and C11O3 groups are as nearly equatorial as possible.

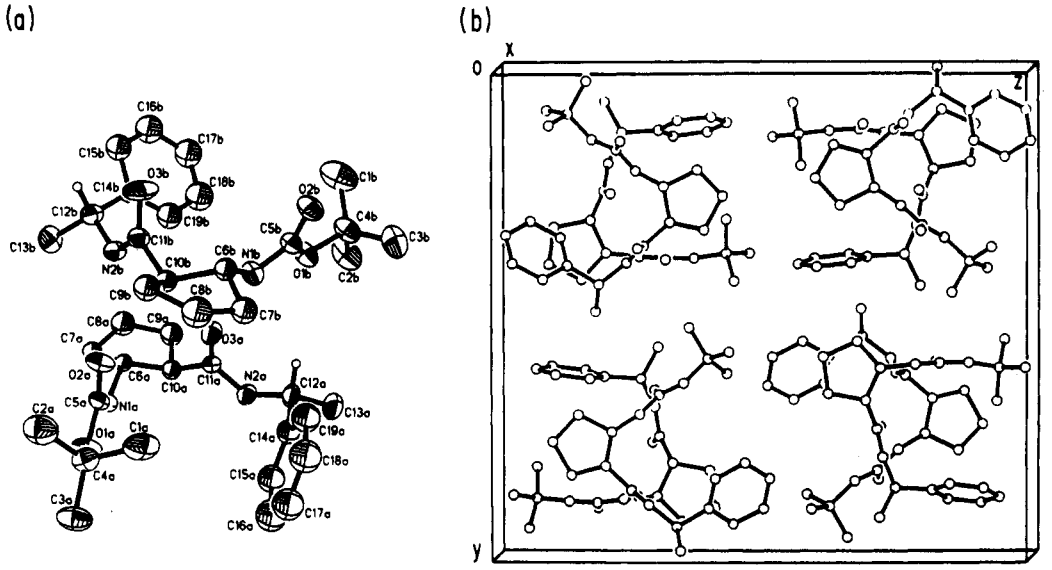

Figure 8. Unit cell-packing diagram for the crystals of [*N*-(*tert*-butyloxycarbonyl)-*trans*-(1*R*,2*R*)-2-aminocyclopentanecarbonyl]-(*R*)-(+)-α-methylbenzylamine. Hydrogen atoms are omitted for clarity. (a) Among the eight molecules within the unit cell, two molecules a and b, whose structures are slightly different from each other (see Figure 7), are closely packed together. (b) Four pairs of the molecules a and b are located within the unit cell in different orientations.

analysis. The absolute configuration of the *trans*-2-Ac^{5}c residue of the compound **11b′** was determined to be 1*R*,2*R* by X-ray crystallographic analysis of the optically pure **10b** obtained from **11b′**. The structure of Boc-(1*R*,2*R*)-2-Ac^{5}c-R-αMBA (**10b**) determined by X-ray diffraction analysis is reported below.

Optically active (1*S*,2*S*)-2-Ac^{5}c (**4a′**) and (1*R*,2*R*)-2-Ac^{5}c (**4b′**) were prepared by acidic hydrolysis of **11a′** and **11b′** in concentrated hydrogen chloride solution and desalting with an anion exchange resin. The optically active dipeptides Asp-(1*S*,2*S*)-2-Ac^{5}c-OMe (**6a′**) and Asp-(1*R*,2*R*)-2-Ac^{5}c-OMe (**6b′**) were synthesized from **4a′** and **4b′**, respectively, according to the same procedures as described above for the diastereomeric mixture (Figure 4). The reversed-phase HPLC analysis indicated that the diasteroemers **6a′** and **6b′** corresponded to the trans-fast and trans-slow isomers in Table I, respectively. Therefore, the trans-fast with a bitter taste and the trans-slow with a sweet taste were assigned as Asp-(1*S*,2*S*)-2-Ac^{5}c-OMe and Asp-(1*R*,2*R*)-2-Ac^{5}c-OMe, respectively. These experimental assignments of the absolute configurations are once again in agreement with the predictions of our model.

Crystal Structure of Boc-*trans*-(1*R*,2*R*)-2-Ac^{5}c-R-αMBA. [*N*-(*tert*-Butyloxycarbonyl)-*trans*-(1*R*,2*R*)-2-aminocyclopentanecarbonyl]-(*R*)-(+)-α-methylbenzylamine [Boc-*trans*-(1*R*,2*R*)-2-Ac^{5}c-(*R*)-αMBA] (**10b**) crystallized in the orthorhombic $P2_12_12_1$ space group with eight molecules in the independent unit. The cell dimensions and experimental parameters are given in Table II. Among the eight molecules, two molecules a and b (Figure 7), whose structures are slightly different from each other, are packed closely to each other (Figure 8(a)). Then four pairs of the molecules a and b are located within the one unit cell in different orientations. The molecular packing within the independent unit is depicted in Figure 8(b).

In both of the molecules a and b, the (1*R*,2*R*)-2-Ac^{5}c residue adopts a conformation in which the NH and CO groups are as nearly equatorial as possible. The torsion angles for N1–C6–C10–C11 moieties, which correspond to the δ angle in Figure 2, were found to be −74.3 and −71.2° for the molecules and b, respectively. Similar values have been observed for the racemic (±) and the optically active (+) forms of 1,2-*trans*-cyclopentanedicarboxylic acid by X-ray diffraction studies.[19] The conformational differ-

Table II. Cell Dimensions and Experimental Parameters of Boc-*trans*-(1*R*,2*R*)-2-Ac^{5}c-R-αMBA[a]

experimental mol formula	$C_{19}H_{28}N_2O_3$
mol weight	332.4
color; habit	colorless, platelike
crystal size	0.13 × 0.50 × 0.75 mm
crystal system	orthorhombic
space group	$P2_12_12_1$ (no. 19, D_2^4)
unit cell dimensions	a = 9.356 (2) Å
	b = 19.678 (4) Å
	c = 21.632 (5) Å
unit cell volume	3982.7 (14) Å^3
Z	8 molecules/unit cell
density (calcd)	1.109 g cm^{-3}
radiation	Mo Kα
independent reflections	2975 (2θ–θ)
observed reflections (m)	2092 ($F > 4.0\sigma(F)$)
parameters refined (n)	323
final R	0.0667
final wR	0.0897
largest and mean Δ/σ	0.136, 0.020

[a] The estimated standard deviation of the least significant figure is given in parentheses.

ences between the molecules a and b were observed in the orientations of the two peptide groups relative to the cyclopentane ring. The observed values of the torsion angles for the C6–C10–C11–N2 moieties, which correspond to the ψ' angle in Figure 2, were 140.9 and 122.3° for the molecules a and b, respectively. The deviation of the observed torsion angles for the C5–N1–C6–C10 moieties (113.7 and 145.1° for the molecules a and b, respectively), which correspond to the ϕ' angle in Figure 2, are slightly larger than that for the C6–C10–C11–N2 moieties. However, it should be noted that the N1H and C6H protons lie in nearly trans orientations in both the molecules.

Structure–Taste Relationships. All of the proton resonances observed in the ^{1}H NMR spectra of Asp-2-Ac^{5}c-OMe were assigned using two-dimensional homonuclear Hartman–Hahn (HOHAHA) and rotating frame nuclear Overhauser (ROESY) experiments. The vicinal ^{1}H–^{1}H coupling constants of the moieties H–C$^\alpha$–C$^\beta$–H ($J_{\alpha-\beta}$) for the Asp residue were estimated from ABX spin analysis of the spectra observed for the C$^\alpha$HC$^\beta$H$_2$ fragment. The values of $J_{\alpha-\beta}$'s determined in this manner and NOEs observed at 20 °C for the four configurational isomers of Asp-2-Ac^{5}c-OMe are summarized in Table III, where the two β-protons are distinguished by appending an additional superscript h for the higher field and l for the lower field resonances, respectively. The NOEs observed in ROESY spectra are qualitatively classified according to their intensities, i.e., s, strong; m, medium; and w, weak. The temperature coefficient $d\delta/dT$ of the amide proton (NH) chemical shift determined over the range of 20–80 °C is also included in the table.

For all of the configurational isomers, the observed values of $J_{\alpha-\beta l}$ and $J_{\alpha-\beta h}$ are, respectively, found to be 4.3–4.5 and 9.3–9.7 Hz. Similar values ($J_{\alpha-\beta l}$ = 4.2–5.1 and $J_{\alpha-\beta h}$ = 8.2–10.3 Hz) have been reported for Asp-Phe-OMe in various solvents by Lelj et al.[5a]

Fractions of three conformers g$^-$, t, g$^+$ around the C$^\alpha$–C$^\beta$ bond (χ_1) of the Asp residue can be estimated by using the conventional expressions:

$$f(g^-) = (J_{\alpha-\beta R} - J_G)/(J_T - J_G)$$

$$f(t) = (J_{\alpha-\beta S} - J_G)/(J_T - J_G)$$

$$f(g^+) = 1 - [f(g^-) + f(t)] \quad (1)$$

Table III. Experimental Values of Vicinal ^{1}H–^{1}H Coupling Constant for a Moiety H–C$^\alpha$–C$^\beta$–H, NOE[a] Observed in DMSO-d_6 at 20 °C, and Temperature Coefficient of Amide Proton (NH) Chemical Shift

	Asp-*trans*-2-Ac^{5}c-OMe		Asp-*cis*-2-Ac^{5}c-OMe	
^{1}H NMR parameter	(1*S*,2*S*)-2-Ac^{5}c	(1*R*,2*R*)-2-Ac^{5}c	(1*R*,2*S*)-2-Ac^{5}c	(1*S*,2*R*)-2-Ac^{5}c
$J_{\alpha-\beta l}$, Hz	4.38	4.54	3.71	4.41
$J_{\alpha-\beta h}$, Hz	10.12	9.74	10.56	9.34
NOE(H$^\alpha$–H$^{\beta l}$)	s	s	m	s
NOE(H$^\alpha$–H$^{\beta h}$)	–	–	–	–
NOE(H$^\alpha$–NH)	s	s	s	s
NOE(H$^{\beta l}$–NH)	w	m	w	w
NOE(H$^{\beta h}$–NH)	–	–	–	–
NOE(NH–C^{2}H)	w	w	–	–
NOE(NH–C^{1}H)	s	s	–	–
NOE(C^{1}H–C^{2}H)	–	–	s	s
$d\delta/dT$, –ppb/deg	8.6	9.2	8.6	8.6

[a] The observed NOE's are qualitatively classified according to their intensities: s, strong; m, medium; w, weak; –, absent.

Following Pachler,[20] values of J_T = 13.56 Hz and J_G = 2.60 Hz were used for the trans and gauche couplings, respectively. The prochiralities of the β-protons could be assigned by using sequential NOE(H$^\alpha$–NH) between H$^\alpha$ of the Asp residue and NH of the 2-Ac^{5}c residue and NOE(H$^\beta$–NH) between H$^\beta$ of the Asp residue and NH of the 2-Ac^{5}c residue. The observed strong NOE(H$^\alpha$–NH) indicates that a torsion angle ψ for the N–C$^\alpha$–C*^{1}–N moiety of the Asp residue is restricted to values from 60°–180°. When the ψ angle of the Asp residue is within this range, the observed NOE(H$^{\beta l}$–NH) and the lack of NOE(H$^{\beta h}$–NH) allow us to assign H$^{\beta l}$ and H$^{\beta h}$ to H$^{\beta S}$ and H$^{\beta R}$, respectively, thus $J_{\alpha-\beta R} - J_{\alpha-\beta h}$ and $J_{\alpha-\beta S} = J_{\alpha-\beta l}$. Therefore, the values of $f(\chi_1)$ were estimated from the observed $J_{\alpha-\beta l}$ and $J_{\alpha-\beta h}$ values by using eq (1) as follows: $f(g^-)$ = 0.62–0.69, $f(t)$ = 0.10–0.18, and $f(g^+)$ = 0.15–0.22. The conformations, similar to those estimated for the Asp residue of Asp-2-Ac^{5}c-OMe in this work, have been reported from X-ray diffraction studies on Asp-Phe-OMe (ψ = 149.6° and $\chi_1 = g^-$),[11] *N*-(L-aspartyl)-*N'*-(2,2,5,5-tetramethylcyclopentanecarbonyl)-(*R* or *S*)-1,1-diaminoethane (ψ = 174.4° and $\chi_1 = g^-$ for the *R* isomer, ψ = 165.2° and $\chi_1 = g^-$ for the *S* isomer),[9] and L-aspartyl-(2,2,4,4-tetramethyl-3-thietanyl)-D-alaninamide (alitame) (ψ = 158.5° and χ_1 = –63.6° for molecule A, ψ = 144.1° and χ_1 = –66.8° for molecule B, ψ = 153.3° and χ_1 = –64.0° for molecule C, and ψ = 167.4° and χ_1 = –63.7° for molecule D).[10]

A torsion angle ϕ' for the C*^{1}–N–C^2–C^1 moiety of the 2-Ac^{5}c residue (Figure 2) may be deduced from an intraresidue NOE(NH–C^{2}H). A weak NOE(NH–C^{2}H) observed for all configurational isomers is a strong indication of a nearly trans orientation for the NH and C^{2}H protons, which is achieved when $\phi' \sim 120°$ for an *R* configuration at the C^2 carbon and $\phi' \sim -120°$ for an *S* configuration. A strong NOE(NH–C^{1}H) observed for the trans configurational isomers also supports this trans arrangement of the H–N–C^2–H moiety. Although the vicinal ^{1}H–^{1}H coupling constant J_{NH-C^2H} provides the same information regarding the torsion angle ϕ', we do not use these NMR data since the NH proton resonance is relatively broad and the value of J_{NH-C^2H} is somewhat obscured by the quadrupolar interaction with the ^{14}N nucleus to which it is bonded.

For both the trans isomers, the lack of NOE(C^{1}H–C^{2}H) indicates that the C^{1}H and C^{2}H protons are oriented in a nearly trans arrangement and that the internal rotation angle about the C^1–C^2 bond in the cyclopentane ring takes

(19) Benedetti, E.; Corradini, P.; Pedone, C. *J. Phys. Chem.* **1972**, *76*, 790–7.

(20) Pachler, K. G. P. *Spectrochim. Acta* **1964**, *20*, 581–7.

Table IV. Minimum Energy Conformations of *N*-Acetyl-2-aminocyclopentanecarboxylic Acid Methyl Esters: (a) Ac-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe and (b) Ac-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe

	torsion angle (deg)			orientation		ΔE
	ϕ'	δ	ψ'	NH	C*^{2}O*^{2}	(kcal mol^{-1})
	(a) Ac-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe					
t1	97.5	−75.6	−58.5	eq	eq	0.000
t2	94.6	−80.6	97.5	eq	eq	0.316
t3	145.4	−73.9	−61.2	eq	eq	0.438
t4	146.2	−75.0	100.7	eq	eq	0.962
t5	−56.7	−68.2	131.4	eq	eq	3.708
t6	−57.8	−66.8	−55.5	eq	eq	4.812
	(b) Ac-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe					
c1	82.0	39.1	−143.7	ax	eq	0.000
c2	101.3	−50.1	−77.4	eq	ax	0.467
c3	101.4	−52.6	76.2	ax	eq	0.683
c4	84.1	35.8	52.8	eq	ax	0.710
c5	151.4	37.3	67.8	eq	ax	1.313
c6	−61.4	−19.8	99.8	ax	eq	5.576
c7	−79.5	46.8	−74.3	eq	ax	6.300
c8	−59.7	−15.9	55.8	ax	eq	6.659
c9	−78.0	50.1	−84.9	eq	ax	7.091

the highest possible value in order to bring the NH and C*^{2}O*^{2} groups to the most equatorial position possible. Similar conformations with both the NH and C*^{2}O*^{2} groups in equatorial positions have also been observed for the crystal structures of the *trans*-(1*R*,2*R*)-2-Ac^{5}c residue of Boc-*trans*-(1*R*,2*R*)-2-Ac^{5}c-R-αMBA (see above).

Conformational energy calculations were carried out for the four stereoisomers of Asp-2-Ac^{5}c-OMe using the DISCOVER program to obtain molecular geometries of preferred conformations. Initial structures were generated by taking account of minimum energy conformations calculated for model compounds L-aspartyl methylamide (Asp-NHMe) and *N*-acetyl-2-aminocyclopentanecarboxylic acid methyl esters (Ac-*trans*-2-Ac^{5}c-OMe and Ac-*cis*-2-Ac^{5}c-OMe). This treatment is based on an assumption that interresidue interactions are trivial for the determination of preferred conformations, which is supported by the independence of the NMR data from the chiralities of the 2-Ac^{5}c residue obtained in both cases of Asp-*trans*-2-Ac^{5}c-OMe and Asp-cis-2-Ac^{5}c-OMe.

Two minimum energy conformations were calculated for Asp-NHMe: ψ = −104.6°, χ_1 = 57.7° (g$^+$), χ_2 = −67.5° (Asp-1) and ψ = 159.5°, χ_1 = −63.8° (g$^-$), χ_2 = 64.3° (Asp-2). The latter conformation is in agreement with the experimental results (ψ = 120° − 180°, f(g$^-$) = 0.62 − 0.69), although the energy is 0.920 kcal mol^{-1} higher than that of the former conformation (Asp-1).

Molecular mechanics calculations were carried out for Ac-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe and Ac-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe. In conformity with the rotational isomeric-state approximation, energy minimizations were achieved by starting from regularly staggered conformations. With regard to rotations around the N–C^2 (ϕ') and C^1–C*^{2} (ψ')[21,22] bonds, six angles (0°, 60°, 120°, 180°, 240°, and 300°) were examined. The half-chair conformation was assumed for a starting structure of the cyclopentane ring of the 2-Ac^{5}c residue. The conformational energy minima thus derived are summarized in Table IV(a) and (b). The values of ΔE represent the total energies of the conformers with respect to the lowest energy conformation of the indivual isomers in the table.

Six conformations with different combinations of (ϕ', ψ') angles, resulting from three values of ϕ' (96°, 146°, and −57°) and two values of ψ' (−58° and 110°), were calculated as the energy minima of Ac-*trans*-2-(1*R*,2*R*)-2-Ac^{5}c-OMe. In all the six conformations, the *trans*-2-Ac^{5}c residue adopts the half-chair structure with the NH and C*^{2}O*^{2} groups diequatorially disposed about the C^2–C^1 bond (−80.6° < δ < −66.8°, see Figure 2). Among the above six minimum energy conformations, the four lowest energy conformations (t1–t4) with energies over a range of 1.0 kcal mol^{-1} above the global minimum value were consistent with the observed NMR data (Table III). They exhibit a weak NOE(NH–C^{2}H), a strong NOE(NH–C^{1}H), and a lack of NOE(C^{1}H–C^{2}H) while the other two (t5 and t6) with higher energies of 3.7 and 4.8 kcal mol^{-1} above the global minimum do not agree with a weak NOE(NH–C^2). It is worthwhile mentioning that the four lowest energy conformations (t1–t4) are similar to the crystal structures of the *trans*-(1*R*,2*R*)-2-Ac^{5}c residue of Boc-*trans*-(1*R*,2*R*)-2-Ac^{5}c-(*R*)-αMBA (ϕ' = 113.7° and 145.1° and δ = −74.2° for the molecules a and b, respectively).

For Ac-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe, nine minimum energy conformations were derived (Table IV(b)). Conformers with ϕ' = −60° to −80° (c6–c9) which are inconsistent with the observed weak NOE(NH–C^{2}H) are energetically less favored (ΔE > 5.5 kcal mol^{-1}).

After the above treatment, energy minimizations were carried out for the four configurational isomers of Asp-2-Ac^{5}c-OMe. The initial structures were generated by adopting the values of ψ, χ_1, χ_2 estimated for the Asp-2 conformation of Asp-NHMe and ϕ', δ, ψ' estimated for t1–t4 of Ac-*trans*-2-Ac^{5}c-OMe and for c1–c5 of Ac-*cis*-2-Ac^{5}c-OMe. The results of calculations are summarized in Table V(a)–(d). The preferred conformations of each configurational isomer are consistent with the NMR data observed in solution. For each configurational isomer, the minimum energy conformations listed in TAble V assume topologically similar structures. The only difference between them is observed in the rotation around the C^1–C*^{2} bond (ψ'). Two preferred states are calculated for each molecular system. Since the Asp moieties in all the conformers assume essentially identical structures, the energy differences are attributed to differences in steric interaction energies caused from conformational changes in the 2-Ac^{5}c residue.

The most preferred conformations of the individual configurational isomers in Table V(a)–(d) correspond to the structures shown in Figure 3(a)–(d), respectively. In spite of the similarity of the observed ^{1}H NMR data, the overall structures of these four configurational isomers are quite different from one another. The major difference is the orientation of the terminal methyl ester group (C*^{2}O*^{2}OMe), considered to be a hydrophobic site X, relative to the plane formed from the zwitterionic group of the Asp residue containing a proton donor AH (N-terminal NH_3^+) and a proton acceptor B (side chain C$^\beta$COO$^-$). The preferred conformations of the sweet-tasting analogue Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe shown in Figure 3(b) are topologically very similar to the L-shape conformation previously developed for the sweet taste.[7-10] The zwitterionic ring of the aspartyl moiety and the methyl ester group are essentially in the plane and almost 90° from each other leading to a molecule with a small *z*-component.

For the bitter-tasting analogue Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe, on the other hand, the methyl ester group is out of the plane with respect to the aspartyl zwitterionic ring. This analogue has a large negative z-component (Figure 3(a)): the separation of the terminal methyl group

(21) Abe and co-workers have proposed a three-state scheme for the rotation around the C*–C bond, in which the C*–C/C–C eclipsed form is more stable than the C*–C/C–H eclipsed form.[22]

(22) (a) Abe, A. *J. Am. Chem. Soc.* **1984**, *106*, 14–9. (b) Abe, A.; Miura, I.; Furuya, H. *J. Phys. Chem.* **1987**, *91*, 6496–502.

Table V. Minimum-Energy Conformations of L-Aspartyl-2-aminocyclopentanecarboxylic Acid Methyl Esters: (a) Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe, (b) Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe, (c) Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe, and (d) Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe

	torsion angle (deg)						orientation		ΔE
	ψ	χ_1	χ_2	ϕ'	δ	ψ'	NH	C*^{2}O*^{2}	(kcal mol^{-1})
				(a) Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe					
SS1	161.0	−63.1	69.5	−95.7	76.2	−94.4	eq	eq	0.000
SS2	170.1	−63.8	69.9	−90.7	78.3	64.2	eq	eq	0.461
				(b) Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe					
RR1	153.7	−63.0	68.9	92.3	−76.3	−70.5	eq	eq	0.000
RR2	162.7	−63.3	69.5	96.4	−78.5	96.7	eq	eq	1.097
RR3	161.2	−63.3	69.4	148.9	−74.8	125.4	eq	eq	1.542
				(c) Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe					
RS1	167.5	−63.2	66.8	−83.2	−1.7	−62.7	eq	ax	0.000
RS2	161.0	−62.8	68.2	−100.0	52.4	−78.2	ax	eq	0.014
RS3	160.6	−62.3	67.3	−77.5	−38.1	93.2	eq	ax	0.418
RS4	171.0	−63.3	70.0	−92.2	51.3	96.5	ax	eq	1.283
				(d) Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe					
SR1	119.8	−63.3	66.6	89.4	−49.9	−112.2	ax	eq	0.000
SR2	149.8	−63.1	68.9	71.9	34.6	81.8	eq	ax	0.923
SR3	162.1	−63.1	69.1	100.6	−63.1	76.8	ax	eq	1.630
SR4	154.5	−62.0	67.8	75.0	38.7	−157.6	eq	ax	1.967

from the *xy* plane is estimated to be 4 Å. The overall topology of Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe resembles the low-energy conformations found for the bitter-tasting L-aspartyl-L-alanyl-2,2,5,5-tetramethylcyclopentanylamide[7] and L-aspartyl-L-alanyl-2,2,4,4-tetramethylthiethane.[10]

From Figure 3(d), it can be seen that the sweet-tasting cis analogue Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe assumes a similar conformation to that estimated for the trans analogue Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe. The methyl ester group is in the *xy* plane and essentially perpendicular to the zwitterionic ring of the Asp residue leading to the L-shape conformation.

The preferred conformations estimated for the tasteless isomer Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe are different from those of the other stereoisomers. Planarity of the molecule in the *x* and *y* dimensions is reasonably similar to the *trans*-(1*R*,2*R*)-2-Ac^{5}c- and *cis*-(1*S*,2*R*)-2-Ac^{5}c-containing isomers with sweet tastes. However, the methyl ester group projects −60° in the *xy* plane relative to the stem of the L formed by the aspartyl moiety (Figure 3(c)). The correlation between the structures and tastes observed for the four stereoisomers Asp-2-Ac^{5}c-OMe is in agreement with our model for the sweet and bitter tastes.[7-10]

Temussi et al.[5] have proposed an extended structure, in which the AH/B moiety in the zwitterionic ring of the Asp 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, as a sweet conformation from the conformational analysis of Asp-Phe-OMe. As shown in Figure 1, however, Asp-Phe-OMe can easily adopt the L-shape conformation which fits our model.

Recently, Taylor et al.[23] reported molecular mechanics calculations on L-aspartyl dipeptide analogues with a conformationally constrained 1-aminocyclopropanecarboxylic acid (1-Ac^{3}c). For a sweet tasting *n*-propyl ester analogue (Asp-1-Ac^{3}c-OPr), an extended conformation and an L-shape conformation, which respectively correspond to the Temussi model and ours, are calculated to be essentially isoenergetic at the global energy minimum. They concluded that the active conformation of Asp-1-Ac^{3}c-OPr msut be either one of these two conformations. However, as the authors have mentioned, it is not possible on the basis of this compound alone to discriminate between the extended and L-shape structures for the active conformation of a sweet molecule.

The sweet-tasting analogues Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe and Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe assume the L-shape conformations as mention above. However, extended conformations which fit to the Temussi model are not accessible for these analogues because of the constrained nature of the 2-Ac^{5}c residue. These results provide evidence in favor of the L-shape over the extended structure proposed by Temussi et al.[5]

Figure 9, parts (a) and (b), respectively, show the *yz* projections of the most preferred conformations found for the bitter-tasting Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe (SS1) and the sweet tasting Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe (RR1) listed in Table V(a) and (b). Shallenberger et al.[24] have proposed the existence of a barrier (Shallenberger barrier) at $z \sim 3$ Å to explain the differences in taste between D and L configurations observed for amino acids having side chains larger than an ethyl group. The bitter taste of L-amino acids was attributed to the interference of the barrier with the side chain. The experimental observations reported in our previous works[7-10] and the present studies show that the molecules with a large negative *z* component exhibit a bitter taste. These results suggest the presence of a similar kind of barrier in the negative *z* region for our compounds. Our model does not indicate the existence of the barrier in the positive *z* region.

Conclusion

We have recently proposed a model for the sweet and bitter tastes from the conformational analysis of a series of L-aspartyl dipeptide derivatives containing α-amino acids at the second position and their retro-inverso analogues.[7-10] The overall structure of a sweet molecule can be described as possessing an L shape, with the proton donor (AH) and proton acceptor (B) group 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. The zwitterionic ring is coplanar with the cyclopentane ring system. Planarily of the molecule in the *xy* plane is critical

(23) Taylor, E. W.; Wilson, S.; Stammer, C. H. *Sweeteners: Discovery, Molecular Design, and Chemoreceptor*; Walters, D. E., Orthoefer, F. T., DuBois, G. E., Eds.; American Chemical Society: Washington, DC, 1991; pp 162-75.

(24) Shallenberger, R. S.; Acree, T. E.; Lee, C. Y. *Nature* **1969**, *221*, 555-6.

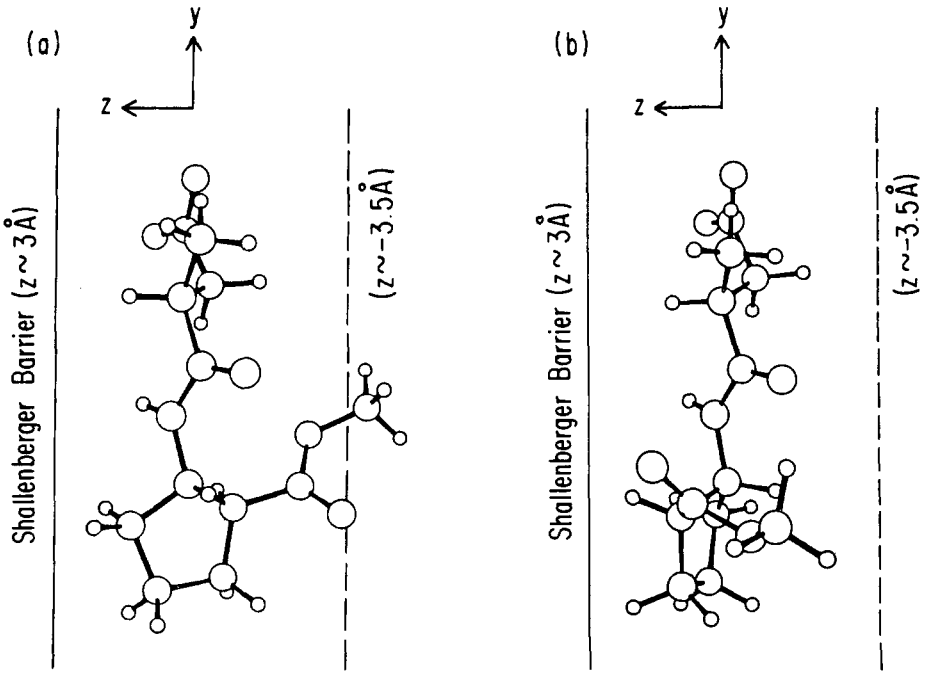

Figure 9. Projections along the *yz* axis of the most preferred conformations for (a) Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe (SS1) and (b) Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe (RR1) shown in Table VIII(a) and (b), respectively.

for the sweet taste, while substantial projection of the X moiety into the negative *z* axis results in a bitter taste.

In order to extend our model to other families of taste ligands, we designed a series of L-aspartyl dipeptide derivatives containing 2-aminocyclopentanecarboxylic acid methyl ester as a second residue (Asp-2-Ac^{5}c-OMe). The peptidomimetic 2-Ac^{5}c is a constrained β-amino acid residue, which displays strong conformational preferences. Preliminary molecular mechanics calculations have shown that among the four stereoisomers, Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe and Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OME with an *R* configuration at the C^2 carbon, to which the NH group is bonded, tend to assume the L-shape conformations and are predicted to be sweet by our model. The conformation with a large negative *z* component has been calculated for Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe, suggesting that this analogue is bitter. The remaining analogue Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe assumes a planar conformation. However, the methyl ester group projects ca. −60° relative to the stem of the L in the *xy* plane. The overall topology of this analogue is different from our model for the sweet and bitter tastes. Therefore, the *cis*-(1*R*,2*S*)-2-Ac^{5}c containing analogue was predicted to be tasteless.

The above preliminary results for the structures and the proposed taste relationships for the four stereoisomers of Asp-2-Ac^{5}c-OMe led us to synthesize these analogues to study the conoformational preferences and carry out qualitative taste assessment. The synthesis of Asp-2-Ac^{5}c-OMe was originally carried out as a diastereomeric mixture from a racemate of either *cis*- or *trans*-2-Ac^{5}c, followed by the separation using reversed-phase HPLC. In order to assign the absolute configuration of the 2-Ac^{5}c residue, we also carried out the synthesis using the optically active 2-aminocyclopentanecarboxylic acid. The optically active *cis*-2-Ac^{5}c (**1b′**), which contained 17% of *cis*-(1*R*,2*S*)-2Ac^{5}c and 83% of *cis*-(1*S*,2*R*)-2-Ac^{5}c, was obtained by optical resolution of a racemate of Boc-*cis*-2-Ac^{5}c (**7a,b**) with (−)-ephedrine. The final L-aspartyl dipeptide methyl ester **3b′** prepared from **1b′** was found to be a diastereomeric mixture of the tasteless cis-fast analogue and the sweet cis-slow analogue with a ratio of 15:85 by the reversed-phase HPLC analysis. Therefore, the tasteless cis-fast and the sweet cis-slow analogues were proven to be L-aspartyl-*cis*-(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid methyl ester and L-aspartyl-*cis*-(1*S*,2*R*)-2-aminocyclopentanecarboxylic acid methyl ester, respectively. These assignments of the absolute configurations of the *cis*-2-Ac^{5}c residues are in agreement with predictions made using our model.

The optically active *trans*-(1*S*,2*S*)-2-Ac^{5}c and *trans*-(1*R*,2*R*)-2-Ac^{5}c were prepared by the separation of diastereomers of *trans*-2-Ac^{5}c-R-αMBA, followed by acidic hydrolysis in concentrated hydrogen chloride. The X-ray diffraction study proved that the bitter-tasting trans-fast and the sweet-tasting trans-slow analogues were L-aspartyl-*trans*-(1*S*,2*S*)-2-aminocyclopentanecarboxylic acid methyl ester and L-aspartyl-*trans*-(1*R*,2*R*)-aminocyclopentanecarboxylic acid methyl ester, respectively. These assignments of the absolute configurations of the *trans*-2-Ac^{5}c residues are again in agreement with the predictions based on our model.

By analyzing the preferred conformations of aspartame (Asp-Phe-OMe) by a combined use of ^{1}H NMR and energy minimizations, Temussi et al.[5] proposed an extended structure, in which the zwitterionic ring of the Asp residue and the aromatic ring (X) of the Phe residue are 180° apart from each other in a flat array, as an active conformation of the sweet molecule. The root of the difference between Temussi et al.'s model and ours is the conformational flexibility of Asp-Phe-OMe, which adopts both the extended and the L-shape conformations. In contrast, the 2-Ac^{5}c containing dipeptide derivatives studied here display strong conformational preferences because of the constrained nature of the 5-membered ring in the 2-Ac^{5}c residue and therefore provide a unique judgement for the models. The conformational analyses of the four stereoisomers of Asp-2-Ac^{5}c-OMe using ^{1}H NMR and molecular mechanics calculations indicated that the two sweet-tasting analogues Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe and Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe assume the L-shape conformations but cannot assume extended conformations. The inclusion of the series of Asp-2-Ac^{5}c-OMe into the family of peptide-based taste ligands studied thus far serves as strong support for our model.

A large negative z component of the conformation derived for the bitter-tasting compounds suggests the existence of a limit similar to the Shallenberger barrier[24] in the negative z region. An estimation of the relative position of this barrier will be essential to explain the structure–taste relationships of a series of L-aspartyl-D-alanyl-1-aminocycloalkanecarboxylic acid methyl ester tripeptides.[25] The tastes of these molecules goes from sweet to bitter as the ring size of the C-terminal amino acid increases. Conformational studies on this series of tripeptides will be reported in a forthcoming paper.[26]

Experimental Section

X-ray Diffraction. Colorless, plate-like crystals of *N*-(*tert*-butyloxycarbonyl)-*trans*-(1*R*,2*R*)-2-Ac^{5}c-(*R*)-(+)-α-methylbenzylamine [Boc-trans-(1*R*,2*R*)-2-Ac^{5}c-R-α-MBA] were grown from ethyl acetate by slow evaporation. Determination of the cell constants, crystal system, and symmetry and collection of the X-ray intensity data were carried at 25 °C out on a Siemens R3m/V four circle diffractometer with use of Mo Kα radiation, using a highly oriented graphite crystal in the range 4.0–45.0° of 2θ. A $2\theta - \theta$ scan mode with variable speed (1.50–3.61° min^{-1}) and a scan range of 0.6° Kα separation was selected. Three standard reflections were monitored every 200 reflections to detect the crystal decay; intensities were decreased by ca. 30% during 68 h of X-ray exposure. An appropriate scale factor was applied to account for the decay. The h, k, and l ranges were 0–10, 0–21, and 0–23, respectively. A total of 2975 reflections were measured, 2092 (m) of which had $F_o > 4.0s(F_o)$ and thus were considered "observed".

Unit cell dimensions were obtained by a least-squares procedure on the angular parameters of 20 reflections in the 2θ range of 15–30°. The structure was solved by direct methods and refined with the SHELXTL PLUS program on a Micro VAX II computer. The full-matrix least-squares procedure was used minimizing the quantity $\sum w(F_o - F_c)^2$ with weight $w = (\sigma^2(F_o) + 0.0021F_o^2)^{-1}$. All nitrogen and oxygen atoms and some carbon atoms (C1 – C5 and C11 – C13) were refined with anisotropic temperature factors, while the remaining carbon atoms were refined with isotropic temperature factors. Hydrogen atoms were introduced in the final structure refinement with isotropic temperature factors of 0.08 Å^2. The total number of parameters (n) refined was 323. The final R indeces, where $R = \sum||F_o| - |F_c||/\sum|F_o|$ and $wR = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$, and goodness to fit, $S = [\sum w(|F_o| - |F_c|)^2/(m - n)]^{1/2}$, were 0.0667, 0.0897, and 1.54, respectively, with largest and mean Δ/σ of 0.136 and 0.020.

^{1}H NMR Measurements. Temperatures were maintained at given values within ±1 °C during measurements. The samples were prepared in DMSO-d_6 purchased from Merck Sharp and Dohme Canada Ltd. at a concentration of 2 w/v%. Tetramethylsilane was used as an internal reference for the determination of chemical shifts.

The one-dimensional spectra contain 16 K data points in 5000 Hz. The two dimensional homonuclear Hartman–Hahn (HOHAHA) experiments[27a] were performed using the MLEV17 suggested by Bax et al.[27b] and the time proportional phase increment (TPPI).[28] A mixing time of 100 ms (48 cycles of MLEV sequence) with a spin-locking field of 10.2 kHz was employed. The rotating frame nuclear Overhauser (ROESY) experiments[29] were carried out with varying mixing times from 50 to 250 ms with a spin-locking field of 2.5 kHz. All of the two-dimensional spectra were obtained using 2 K data points in the f_2 domain and 256 points in the f_1 domain. Applying the zero-filing procedure to the f_1 domain resulted in a final matrix of 2 K × 2 K data points. Multiplication with either a phase-shifted sine or Gaussian function was used to enhance the spectra.

Energy Calculations. Conformational energy minimizations were carried out with a quasi-Newton–Raphson method until the maximum derivative was less than 0.001 kcal mol^{-1} Å^{-1} by employing the DISCOVER force field program.[30] Conventional values of the bond lengths and bond angles for 2-Ac^{5}c residues were taken from the crystallographic data observed for [*N*-(*tert*-butyloxycarbonyl)*trans*-(1*R*,2*R*)-2-aminocyclopentanecarbonyl]-(*R*)-(+)-α-methylbenzylamine [Boc-*trans*-(1*R*,2*R*)-2-Ac^{5}c-(*R*)-αMBA]. Conformational energies were estimated as the sum of nonbonded van der Waals interactions, Coulombic interactions, intrinsic torsional potentials, and energies of deformation of bond lengths and bond angles. Parameters required for the description of the torsional potentials for the internal bond rotation are provided in the DISCOVER program and used without modification. Various force constants defined in the force field scheme were also adopted as specified in the program, except for the *trans*-2-Ac^{5}c and *cis*-2-Ac^{5}c residues. The force constants for the *trans*-2-Ac^{5}c and *cis*-2-Ac^{5}c residues were created based on the values for the β-alanine residue provided in the DISCOVER program.

Synthesis. General Procedures. Melting points are uncorrected.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-*cis*-2-aminocyclopentanecarboxylic Acid Methyl Ester (2a,2b).** To a solution of the racemic mixture of H-*cis*-2-Ac^{5}c-OH (**1a,1b**)[15,16] (1.290 g, 10 mmol) in 6.5 mL of MeOH was added distilled thionyl chloride ($SOCl_2$, 0.88 mL, 12 mmol) dropwise at −50 °C. The reaction mixture was stirred overnight at room temperature and refluxed for 4 h. The MeOH was removed under reduced pressure, and the slightly brown oil was treated with 6.5 mL of 4 N HCl/1,4-dioxane. The solvent was removed and the resulting oil triturated with ether to yield the racemic mixture of HCl*H-2-Ac^{5}c-OMe as a white crystalline solid (980 mg, 56%).

A solution of *N*-(benzyloxycarbonyl)-β-benzyl-L-aspartate (1.070 g, 3.0 mmol) and HCl*H-*cis*-2-Ac^{5}c-OMe (0.540 g, 3.0 mmol) in 11 mL of *N*,*N*-dimethylformamide (DMF) was cooled to −30 °C. The reactants *N*-hydroxybenzotriazole (HOBt, 0.588 g, 3.6 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC HCl: 0.690 g, 3.6 mmol) were added, and the apparent pH was adjusted to 6 with triethylamine. The reaction mixture was stirred for 1 h at −30 °C and 2 h at room temperature. The DMF was removed under reduced pressure. The yellow residue was dissolved in ethyl acetate and successively washed with 1 N NaOH, 0.5 M citric acid, saturated $NaHCO_3$ aqueous solution, brine, and water. After drying over $MgSO_4$, the solvent was removed under the reduced pressure. The product Z-Asp(OBzl)-*cis*-2-Ac^{5}c-OMe (**2a,2b**) was obtained by column chromatography of silica gel eluting with a mixed solvent of ethyl acetate and hexane (1.10 g, 82%). ^{1}H NMR: see below for the chemical shifts for the partially resolved compounds **2b′**. FAB-MS m/e: 483 (100), 451 (5).

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-*trans*-2-aminocyclopentanecarboxylic Acid Methyl Ester (5a,5b).** The diastereomeric mixture of Z-Asp(OBzl)-*trans*-2-Ac^{5}c-OMe (**5a,5b**) was prepared (1.07 g, 80%) using exactly the same procedure mentioned above, coupling Z-Asp(OBzl)-OH (1.070 g, 3.0 mmol) and HCl*H-*trans*-2-Ac^{5}c-OMe (0.540 g, 3.0 mmol). ^{1}H NMR: see below for the chemical shifts for the optically active compounds **5a′** and **5b′**. FAB-MS m/e: 483 (100), 451 (8).

L-Aspartyl-*cis*-2-aminocyclopentanecarboxylic Acid Methyl Ester (3a,3b). The protected peptidomimetic dipeptide derivatives Z-Asp(OBzl)-*cis*-2-Ac^{5}c-OMe (**2a,2b**) (0.800 g, 1.66 mmol) were dissolved in 50 mL of MeOH and hydrogenated over palladium-black (10%) for 24 h at room temperature under atmospheric hydrogen pressure. After the catalyst was removed by filtration and the solvent removed under reduced pressure, the solid was precipitated from MeOH/ether. The white product was filtered and dried in vacuo over P_2O_5 (416 mg, 97%).

The diastereomeric mixture of Asp-*cis*-2-Ac^{5}c-OMe (**3a,3b**) (50 mg) was separated using a reversed-phase HPLC system consisting of a Waters automated gradient controller, two Waters model 510 pumps, a Katos SF 757 UV detector operating at 213 nm, and

(25) Rodriguez, M.; Bland, J. M.; Tsang, J. W.; Goodman, M. *J. Med. Chem.* **1985**, *28*, 1527–9.
(26) Yamazaki, T.; Goodman, M. To be published.
(27) (a) Bax, A.; Davis, D. G. *J. Am. Chem. Soc.* **1985**, *107*, 2820–1; (b) *J. Magn. Reson.* **1985**, *65*, 355–60.
(28) Bodenhausen, G.; Vold, R. L.; Vold, R. R. *J. Magn. Reson.* **1980**, *37*, 93–106.
(29) Bothner-By, A. A.; Steppens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–3.
(30) Hagler, A. T. In *The Peptides*; Udenfriend, S., Meienhofer, J., Hruby, V. J., Eds.; Academic Press: Orlando, 1985; Vol. 7, pp 214–96.

a Perkin-Elmer LCI-100 laboratory computing integrator. A VYDAC Protein & Peptide C18 3 cm × 25 cm column was used for preparative separations, using an isocratic mixed solvent of water and acetonitrile. The flow rate was set to 10 mL min^{-1}.

The fast diastereomer (cis-fast, **3a**): 20.1 mg was a white fluffy powder after lyophilization. This isomer was tasteless. Mp 107–110 °C. $[\alpha]^{20}_D$: –67.5° (*c* 1.0, H_2O). ^1H NMR (DMSO-*d36*) for Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe: δ 8.27 (bd, 1 H, NH of 2-Ac^{5}c), 4.38 (bm, 1 H, C^{2}HN of 2-Ac^{5}c), 3.64 (dd, 1 H, *J* = 3.7 and 10.6 Hz, CH of ASP), 3.54 (s, 3 H, OMe), 2.94 (m, 1 H, C^{1}HCOO of 2-Ac^{5}c), 2.32, 2.10 (dd, 1 H, *J* = 3.7 and 16 Hz, dd, 1 H, *J* = 10.6 and 16 Hz, CH_2 of Asp), 1.95–1.50 (m, 6 H, $C^3H_2C^4H_2C^5H_2$ of 2-Ac^{5}c). FAB-MS, *m/e*: 259 (100), 260 (17), 227 (14). HRMS (+FAB): calcd 259.1294, found 259.1282. Anal. Calcd for $C_{11}H_{18}N_2O_5$: C, 51.16; H, 7.03; N, 10.85. Found: C, 50.90; H, 6.63; N, 11.01.

The slow diastereomer (cis-slow, **3b**): 23.1 mg was a white fluffy powder after lyophilization. This isomer was sweet. Mp 110–113 °C. $[\alpha]^{20}_D$ +80.7° (c 1.0, H_2O). ^1H NMR (DMSO-d_6) for Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe: δ 8.19 (bd, 1 H, NH of 2-Ac^{5}c), 4.32 (bm, 1 H, C^{2}HN of 2-Ac^{5}c), 3.59 (dd, 1 H, *J* = 4.4 and 9.3 Hz, CH of ASP), 3.57 (s, 3 H, OMe), 2.93 (m, 1 H, C^{1}HCOO of 2-Ac^{5}c), 2.48, 2.22 (dd, 1 H, *J* = 4.4 and 16 Hz, dd, 1 H, *J* = 9.3 and 16 Hz, CH_2 of Asp), 1.95–1.50 (m, 6 H, $C^3H_2C^4H_2C^5H_2$ of 2-Ac^{5}c). FAB-MS *m/e*: 259 (100), 260 (14), 227 (14). HRMS (+FAB): calcd 259.1294, found 259.1292. Anal. Calcd for $C_{11}H_{18}N_2O_5$: C, 51.16; H, 7.03; H, 10.85. Found: C, 51.03; H, 6.94; N, 10.49.

L-Aspartyl-*trans*-2-aminocyclopentanecarboxylic Acid Methyl Ester (6a,6b). The desired compounds **6a,6b** (312 mg, 96%) were obtained as colorless powders fom the hydrogenation of Z-Asp(OBzl)-*trans*-2-Ac^{5}c-OMe (**5a,5b**) (0.610 g, 1.26 mmol) using the same procedure used for the cis isomers **3a,3b**.

The diastereomeric mixture of Asp-*trans*-2-Ac^{5}c-OMe (**6a,6b**) (50 mg) was separated by reversed-phase HPLC with the same conditions as described for Asp-*cis*-2-Ac^{5}c-OMe (**3a,3b**).

The fast diastereomer (trans-fast, **6a**): 35.0 mg was a white fluffy powder after lyophilization. This isomer was bitter. Mp 183–184 °C dec. $[\alpha]^{20}_D$: +44.7° (*c* 1.0, H_2O). ^1H NMR (DMSO-d_6) for Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe: δ 8.34 (bd, 1 H, NH of 2-Ac^{5}c), 4.17 (bm, 1 H, C^{2}HN of 2-Ac^{5}c), 3.57 (dd, 1 H, *J* = 4.4 and 10.1 Hz, CH of ASP), 3.55 (s, 3 H, OMe), 2.57 (m, 1 H, C^{1}HCOO of 2-Ac^{5}c), 2.38, 2.16 (dd, 1 H, *J* = 4.4 and 16 Hz, dd, 1 H, *J* = 10.1 and 16 Hz, CH_2 of Asp), 1.98–1.42 (m, 6 H, $C^3H_2C^4H_2C^5H_2$ of 2-Ac^{5}c). FAB-MS *m/e*: 259 (82), 260 (12), 227 (10). Anal. Calcd for $C_{11}H_{18}N_2O_5$ with 1.5H_2O: C, 46.31; H, 7.42; N, 9.82. Found: C, 46.10; H, 7.07; N, 9.82.

The slow diastereomer (trans-slow, **6b**): 36.2 mg was a white powder after lyophilization. This isomer was intensely sweet. Mp 151–153 °C dec. $[\alpha]^{20}_D$: –8.1° (*c* 1.0, H_2O). ^1H NMR (DMSO-d_6) for Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe: δ 8.33 (bd, 1 H, NH of 2-Ac^{5}c), 4.18 (bm, 1 H, C^{2}HN of 2-Ac^{5}c), 3.57 (s, 3 H, OMe), 3.56 (dd, 1 H, *J* = 4.5 and 9.7 Hz, CH of ASP), 2.63 (m, 1 H, C^{1}HCOO of 2-Ac^{5}c), 2.39, 2.17 (dd, 1 H, *J* = 4.5 and 16 Hz, dd, 1 H, *J* = 9.7 and 16 Hz, CH_2 of Asp), 2.00–1.41 (m, 6 H, $C^3H_2C^4H_2C^5H_2$ of 2-Ac^{5}c). FAB-MS *m/e*: 259 (54), 260 (8), 227 (11). HRMS (+FAB): calcd 259.1294, found 259.1290. Anal. Calcd for $C_{11}H_{18}N_2O_5$: C, 51.16; H, 7.03; N, 10.85. Found: C, 51.22; H, 6.77; N, 10.50.

Optical Resolution of *N*-(*tert*-Butyloxycarbonyl)-*cis*-2-aminocyclopentanecarboxylic Acids (7a,7b). The protected amino acids Boc-*cis*-2-Ac^{5}c-OH (**7a,7b**)[31] (1.7 g, 7.4 mmol) and (–)-ephedrine (930 mg, 5.6 mmol) were dissolved in 10 mL of hot ethyl acetate. The solution was diluted with 100 mL of ether and allowed to stand for 24 h at 0 °C. The white precipitate was collected by filtration and then redissolved in hot acetonitrile. These procedures were repeated until optical rotation of the salt became constant. Finally, the precipitated salt **8b′** was obtained (880 mg). $[\alpha]^{20}_D$: –7.9° (*c* 1.0, MeOH). Mp 144–145 °C.

To a solution of the salt **8b′** in 20 mL of ethyl acetate was added 1 N $NaHSO_4$ with stirring until the pH of the water layer became 3. The ethyl acetate layer was dried over $MgSO_4$ and taken to dryness under reduced pressure. The desired free acid **7b′** was obtained (500 mg, 29%). $[\alpha]^{20}_D$: +46.7° (*c* 1.47, MeOH).

***cis*-(1*S*,2*R*)-2-Aminocyclopentanecarboxylic Acid (1b′).** The partially resolved Boc-(1*S*,2*R*)-2-Ac^{5}c (**7b′**) (115 mg, 0.5 mmol) was treated with 2 mL of trifluoroacetic acid (TFA) at 0 °C. After 2 h, the TFA was removed under reduced pressure. The residue was dissolved in 2 mL of water and neutralized with 30% NH_3 solution. The solution was passed through cation-exchange resin (Dowex-50W) and washed with H_2O to remove salts. The solution was then washed with 10% NH_3 solution. The NH_3 solution was collected and concentrated to yield the free amino acid (**1b′**) (51 mg, 79%) with 66% enaniomeric excess. $[\alpha]^{20}_D$: +5.9° (*c* 1.0, H_2O) [lit.[18] +8.9° (*c* 1.0, H_2O)].

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-*cis*-(1*S*,2*R*)-2-aminocyclopentanecarboxylic Acid Methyl Ester (2b′).** To a solution of the partially resolved Boc-*cis*-(1*S*,2*R*)-2-Ac^{5}c (**7b′**) (90 mg, 0.39 mmol) in 5 mL of ether was added CH_2N_2 solution in ether at 0 °C until the solution maintained yellow color for 0.5 h. After the ether solvent was removed, the residue was treated with 4 N HCl/1,4-dioxane for 2 h at 0 °C. The removal of the solvent gave a white powder (68 mg, 96%). The crude product was immediately coupled to Z-Asp(OBzl)-OH without further purification as in the procedure for the diastereomeric mixture **2a,2b**. The desired compound **2b′** was obtained by chromatography (162 mg, 89%). ^1H NMR (DMSO-d_6) for Z-Asp(OBzl)-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe: δ 7.87 (d, 1 H, *J* = 8.2 Hz, NH of Asp), 7.54 (d, 1 H, *J* = 8.3 Hz, NH of 2-Ac^{5}c), 7.40–7.24 (m, 10 H, Ph of Z and Ph of Bzl), 5.07 (s, 2 H, CH_2 of Bzl), 5.02 (d, 2 H, CH_2 of Z), 4.39 (m, 1 H, CH of Asp), 4.28 (m, 1 H, C^{2}HN of 2-Ac^{5}c), 3.48 (s, 3 H, OMe), 2.88 (m, 1 H, C^{1}HCOO of 2-Ac^{5}c), 2.66, 2.56 (dd, 1 H, *J* = 3.4 and 16 Hz, dd, 1 H, *J* = 9.0 and 16 Hz, CH_2 of Asp), 1.94–1.42 (m, 6 H, $C^3H_2C^4H_2C^5H_2$ of 2-Ac^{5}c). ^1H NMR (DMSO-d_6) for Z-Asp(OBzl)-*cis*-(1S,2R)-2-Ac^{5}c-OMe: δ 7.77 (d, 1 H, *J* = 8.4 Hz, NH of Asp), 7.51 (d, 1 H, *J* = 8.6 Hz, NH of 2-Ac^{5}c), 7.40–7.24 (m, 10 H, Ph of Z and Ph of Bzl), 5.07 (s, 2 H, CH_2 of Bzl), 5.02 (d, 2 H, CH_2 of Z), 4.39 (m, 1 H, CH of Asp), 4.28 (m, 1 H, C^{2}HN of 2-Ac^{5}c), 3.48 (s, 3 H, OMe), 2.93 (m, 1 H, C^{1}HCOO of 2-Ac^{5}c), 2.73, 2.57 (dd, 1 H, *J* = 4.9 and 16 Hz, dd, 1 H, *J* = 9.4 and 16 Hz, CH_2 of Asp), 1.94–1.42 (m, 6 H, $C^3H_2C^4H_2C^5H_2$ of 2-Ac^{5}c). FAB-MS *m/e*: 483 (100). Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.71; H, 6.27; N, 5.81. Found: C, 64.48; H, 6.34; N, 5.83.

L-Aspartyl-*cis*-(1*S*,2*R*)-2-aminocyclopentanecarboxylic Acid Methyl Ester (3b′). The procted dipeptide **2b′** (160 mg, 0.33 mmol) was hydrogenated following the same procedure described for the diastereomeric mixture **3a,3b**. The desired compound **3b′** was obtained as a white solid in a yield of 73 mg (85%). The reversed-phase HPLC analysis showed that the ratio of the fast-moving diasteremers Asp-*cis*-(1*R*,2*S*)-2-Ac^{5}c-OMe (**3a**) to the slow-moving one Asp-*cis*-(1*S*,2*R*)-2-Ac^{5}c-OMe (**3b**) was 15:85. This ratio was consistent with the enantiomeric excess value (66%) obtained for the amino acid **1b′**, which was derived from the Boc-*cis*-(1*S*,2*R*)-2-Ac^{5}c (**7b′**).

[*N*-(*tert*-Butyloxycarbonyl)-*trans*-2-aminocyclopentanecarbonyl]-(*R*)-(+)-α-methylbenzylamine (10a,10b). To a solution of the racemic mixture of Boc-*trans*-2-Ac^{5}c-OH (**9a,9b**)[31] (2.4 g, 10.5 mmol) in 100 mL of dry tetrahydrofuran were added *N*-methylmorpholine (NMM) (1.2 g, 11.5 mmol) and isobutyl chloroformate (IBCF) (1.57 g, 11.5 mmol) under N_2 at –15 °C. After being stirred for 10 min, the solution was treated with (*R*)-(+)-α-methylbenzylamine in 10 mL of THF. The mixture was stirred for 1 h at –15 °C and for 4 h at room temperature. After 20 mL of water was added, the mixture was extracted with 100 mL of ethyl acetate three times. The extract was succesively washed with saturated $NaHCO_3$, brine, and water and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the residue was purified by chromatography to yield the compounds **10a,10b** (3.21 g, 92%). The two diastereomers could not be separated at this stage. ^1H NMR (diastereomers, $CDCl_3$): δ 8.26, 7.92 (bs, bd, 1 H, NH), 7.42–7.20 (m, 5 H, Ph), 5.08 (m, 1 H, PhCHN), 4.67 (bs, 1 H, NH), 4.05, 3.06 (b, b, 1 H, CHN), 2.63–2.57 (m, 6 H, $C^3H_2C^4H_2C^5H_2$), 1.45, 1.44 (d, s, 9 H, Boc). FAB-MS *m/e*: 333 (100). Anal. Calcd for $C_{19}H_{28}N_2O_3$: C, 68.64; H, 8.49; N, 8.43. Found: C, 68.58; H, 8.48; N, 8.51.

(*trans*-(1*R*,2*R*)-2-Aminocyclopentanecarbonyl)-(*R*)-(+)-α-methylbenzylamine (11a′ and 11b′). The diastereomeric compounds **10a,10b** (4.58 g, 14.6 mmol) were dissolved in 20 mL

(31) Mierke, D. F.; Noßner, G.; Schiller, P. W.; Goodman, M. *Int. J. Peptide Protein Res.* **1990**, *35*, 35–45.

of TFA at 0 °C. The solution was stirred for 2 h at room temperature. After TFA was removed under reduced pressure, the residue salts were dissolved in 100 mL of ethyl acetate, washed with saturated $NaHCO_3$, and dried over $MgSO_4$. The removal of the solvent provided a yellow oil (3.2 g, 94%). The two diastereomers were separated by chromatography (40 μL of silica gel, $CHCl_3$:MeOH:AcOH = 19:1:1) to give the fast-moving isomer **11b′** (1.3 g) and the slow isomer **11a′** (0.2 g). Repeating the chromatography with the mixture gave another 0.9 g of the slow-moving isomer.

The fast isomer **11b′**. ^1H NMR (DMSO-d_6): δ 8.41 (d, 1 H, J = 7.8 Hz, NH), 7.40–7.20 (m, 5 H, Ph), 4.96 (m, 1 H, PhCHN), 3.71 (m, 1 H, C^2HN), 2.70 (m, 1 H, C^1HCOO), 2.15–1.55 (m, 6 H, $C^3H_2C^4H_2C^5H_2$), 1.34 (d, 3 H, J = 6.8 Hz, Me). FAB-MS m/e: 233 (100). HRMS (+FAB): calcd for $C_{14}H_{20}N_2O$ 233.1654, found 233.1661.

The slow isomer **11a′**. ^1H NMR (DMSO-D_6): δ 8.53 (d, 1 H, J = 7.6 Hz, NH), 7.31–7.16 (m, 5 H, Ph), 4.89 (m, 1 H, PhCHN), 3.63 (m, 1 H, C^2HN), 2.77 (m, 1 H, C^1HCOO), 2.06–1.56 (m, 6 H, $C^3H_2C^4H_2C^5H_2$), 1.33 (d, 3 H, J = 6.9 Hz, Me). FAB-MS m/e: 233 (100). HRMS (+FAB): calcd for $C_{14}H_{20}N_2O$ 233.1654, found 233.1657.

[*N*-(*tert*-Butyloxycarbonyl)-*trans*-(1*R*,2*R*)-2-aminocyclopentanecarbonyl]-(*R*)-(+)-α-methylbenzylamine (10b). To a solution of the fast-moving isomer, *trans*-(1*R*,2*R*)-2-Ac^{5}c (**11b′**) (232 mg, 1.0 mmol) in 5 ml of dry tetrahydrofuran was added *tert*-butylpyrocarbonate (250 mg, 1.17 mmol). After the reaction mixture was stirred for 30 min at room temperature, the solvent was removed under reduced pressure. The residue was purified by chromatography and evaporated to dryness. The residue was dissolved in 10 mL of hot ethyl acetate. The solution was allowed to stand for 2 days at room temperature to yield colorless, platelike crystals of Boc-*trans*-(1*R*,2*R*)-2-Ac^{5}c-(*R*)-αMBA (**10b**) used for the X-ray diffraction study. Mp 172–173 °C. $[\alpha]^{20}_D$ + 19.5° (*c* 1.0, MeOH). ^1H NMR ($CDCl_3$) δ 8.04 (bs, 1 H, NH), 7.39–7.21 (m, 5 H, Ph), 5.10 (m, 1 H, PhCHN), 4.68 (bs, 1 H, NH), 3.95 (m, 1 H, C^2HN), 2.68 (m, 1 H, C^1HCOO), 2.10–1.85 (m, 2 H, CH_2), 1.70–1.62 (m, 2 H, CH_2), 1.45–1.37 (m, 2 H, CH_2), 1.46 (d, 3 H, J = 6.8 Hz, Me), 1.46 (s, 9 H, Boc).

***trans*-(1*S*,2*S*)-2-Aminocyclopentanecarboxylic Acid (4a′).** The amide **11a′** (356 mg, 1.5 mmol) was refluxed in 10 mL of concentrated HCl for 8 h. After most of the water was removed, concentrated NH_3 (2 mL) was added. The (*R*)-(+)-α-methylbenzylamine was removed by extraction with ethyl acetate (3 × 20 mL). The free amino acid **4a′** was obtained by purification using an ion-exchange resin (Dowex-50W) (140 mg, 71%). Mp 221 °C dec. $[\alpha]^{20}_D$ +51.8° (*c* 1.0, H_2O).

***trans*-(1*R*,2*R*)-2-Aminocyclopentanecarboxylic Acid (4b′).** The free amino acid **4b′** was obtained (550 mg, 90%) by applying the above procedure to compound **11b′** (1.1 g, 4.7 mmol). Mp 212–214 °C dec. $[\alpha]^{20}_D$ −56.4° (*c* 1.0, H_2O).

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-*trans*-(1*S*,2*S*)-2-aminocyclopentanecarboxylic Acid Methyl Ester (5a′).** The protected dipeptide ester **5a′** was obtained (201 mg, 67%) from *trans*-(1*S*,2*S*)-2-Ac^{5}c (**4a′**) (80 mg) as in the preparation of the diastereomers **2a,2b**. $[\alpha]^{20}_D$ +39° (*c* 1.0, MeOH). ^1H NMR ($CDCl_3$): δ 7.38–7.29 (m, 10 H, Ph of Z and Ph of Bzl), 6.52 (bd, 1 H, NH), 5.91 (bd, 1 H, NH), 5.16–5.08 (m, 4 H, CH_2 of Bzl and CH_2 of Z), 4.53 (m, 1 H, CH of Asp), 4.32 (m, 1 H, C^2HN of 2-Ac^{5}c), 3.64 (s, 3 H, OMe), 3.05, 2.70 (dd, 1 H, J = 4.0 and 17 Hz, dd, 1 H, J = 7.4 and 17 Hz, CH_2 of Asp), 2.50 (m, 1 H, C^1HCOO of 2-Ac^{5}c), 2.10–1.40 (m, 6 H, $C^3H_2C^4H_2C^5H_2$ of 2-Ac^{5}c). FAB-MS m/e: 483 (100). Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.71; H, 6.27; N, 5.81. Found: C, 64.90; H, 6.23; N, 5.79.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-*trans*-(1*R*,2*R*)-2-aminocyclopentanecarboxylic Acid Methyl Ester (5b′).** The protected dipeptide ester **5b′** was prepared (200 mg, 53%) from *trans*-(1*R*,2*R*)-2-Ac^{5}c (**4b′**) (100 mg) as in the preparation of the diastereomers **2a,2b**. Mp 138 °C. $[\alpha]^{20}_D$: −36.6° (*c* 1.0, MeOH). ^1H NMR (DMSO-d_6): δ 8.07 (d, 1 H, J = 7.5 Hz, NH of Asp), 7.53 (d, 1 H, J = 8.4 Hz, NH of 2-Ac^{5}c), 7.36–7.26 (m, 10 H, Ph of Z and Ph of Bzl), 5.06 (s, 2 H, CH_2 of Bzl), 5.02 (d, 2 H, CH_2 of Z), 4.36 (m, 1 H, CH of Asp), 4.15 (m, 1 H, C^2HN of 2-Ac^{5}c), 3.53 (s, 3 H, OMe), 2.73, 2.59 (dd, 1 H, J = 5.3 and 16 Hz, dd, 1 H, J = 8.8 and 16 Hz, CH_2 of Asp), 2.63 (m, 1 H, C^1HCOO of 2-Ac^{5}c), 1.97–1.40 (m, 6 H, $C^3H_2C^4H_2C^5H_2$ of 2-Ac^{5}c). FAB-MS m/e: 483 (100). Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.71; H, 6.27; N, 5.81. Found: C, 64.74; H, 6.24; N, 5.54.

L-Aspartyl-*trans*-(1*S*,2*S*)-2-aminocyclopentanecarboxylic Acid Methyl Ester (6a′). The peptidomimetic dipeptide ester **6a′** was prepared (81 mg, 84%) by hydrogenation of the fully protected compound **5a′** (180 mg) as described for the compounds **3a,3b**. The reversed-phase HPLC analysis showed that the ratio of the fast-moving diastereomer Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe (â) to the slow Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe (**6b**) was 90:10. $[\alpha]^{20}_D$: +36.3° (*c* 1.0, H_2O).

L-Aspartyl-*trans*-(1*R*,2*R*)-2-aminocyclopentanecarboxylic Acid Methyl Ester (6b′). The dipeptide ester **6b′** was obtained (78 mg, 97%) by hydrogenation of the fully protected compound 5b′ (150 mg). The reversed-phase HPLC analysis showed that the ratio of the fast-moving diastereomer Asp-*trans*-(1*S*,2*S*)-2-Ac^{5}c-OMe (**6a**) to the slow Asp-*trans*-(1*R*,2*R*)-2-Ac^{5}c-OMe (**6b**) was 5:95. $[\alpha]^{20}_D$: −8.4° (*c* 1.0, H_2O).

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Registry No. (±)-*cis*-1, 57043-00-2; (±)-*cis*-1·HCl (methyl ester), 129823-60-5; **1b′**, 64191-14-6; *N*-Ac-**1b′** (methyl ester), 136315-78-1; **2a′**, 136237-68-8; **2b′**, 136315-65-6; **3a**, 136237-69-9; **3b**, 136315-67-8; (±)-*trans*-4, 57043-01-3; (±)-*trans*-4·HCl (methyl ester), 136237-67-7; **4a′**, 64191-13-5; **4b′**, 136315-77-0; *N*-Ac-**4b′** (methyl ester), 136237-72-4; **5a′**, 136315-66-7; **5b′**, 136237-68-8; **6a**, 136315-68-9; **6b**, 136315-69-0; (±)-*cis*-7, 136315-70-3; **7b′**, 130981-12-3; **8a′**, 136315-74-7; **8b′**, 136315-72-5; (±)-*trans*-9, 136315-71-4; **10a**, 136237-70-2; **10b**, 136315-75-8; **11a′**, 136237-71-3; **11b′**, 136315-76-9; R-α-MBA, 3886-69-9; Z-Asp(OBZl)-OH, 3479-47-8; (−)-ephedrine, 299-42-3.

Supplementary Material Available: Tables of atomic coordinates and isotropic coefficients, bond lengths and bond angles, anisotropic coefficients, H-atom coordinates and isotropic coefficients, torsion angles, solution and refinement data, and experimental data for *N*-(*tert*-butyloxycarbonyl)-*trans*-(1*R*,2*R*)-2-Ac^{5}c-(*R*)-(+)-α-methylbenzylamine (**10b**) (9 pages); table of observed and calculated structure factors (11 pages). Ordering information is given on any current masthead page.