Articles

Synthesis and Taste Properties of L-Aspartyl-Methylated 1-Aminocyclopropanecarboxylic Acid Methyl Esters

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Several isomers of L-aspartyl-1-aminocyclopropanecarboxylic acid methyl ester with methyl group substitutions on the cyclopropane ring were synthesized. Conformational analyses were carried out on these molecules using ^{1}H NMR and molecular modeling studies. Their taste properties are explained on the basis of our previously reported topochemical model for taste response.

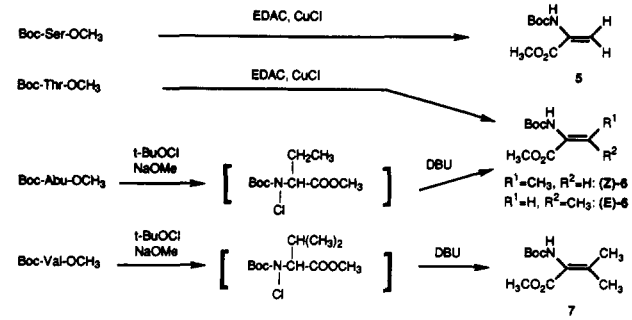
Introduction

Since the discovery of L-aspartyl-L-phenylalanine methyl ester (aspartame),[1] a variety of dipeptide analogues have been subjected to taste tests. Replacement of the L-aspartyl moiety invariably led to bitter or tasteless analogues, with the exception of aminomalonyl, ureido-L-aspartyl, and *N*-trifluoro- or *N*-(trichloroacetyl)-L-aspartyl residues. In all other cases the zwitterionic α-amino and β-carboxylic acidic functions of the aspartyl residue must remain unsubstituted to elicit a sweet taste. The charged amino group and the carboxylate represent the hydrogen bond donor (AH) and acceptor (B) of the Shallenberger and Acree glucophore hypothesis.[2] Kier[3] proposed the existence of an additional interacting site, hydrophobic group (X), which can be widely varied. As suggested by Mazur[4] and Ariyoshi,[5] the C-terminal residue of sweet dipeptides consists of two hydrophobic groups with different sizes, the amino acid side chain and the ester function. Studies of the variation in the C-terminal residue have determined the structural features of these groupings for the sweet taste.[5] In the L configuration of the C-terminal residue, the amino acid side chain is required to be considerably larger than the ester group. Conversely, the relationship is reversed in the D configuration, where the side chain is small and the ester is large. A larger hydrophobic group among the two serves as the third binding site, X.

In spite of the above considerations on chiralities, it does not appear that the chirality of the C-terminal residue in and of itself is necessary for taste. This can be seen by the fact that L-aspartyl-1-aminocyclopropanecarboxylic acid methyl ester (Asp-Ac^{3}c-OCH$_3$) is sweet.[6] Therefore, the molecular basis of taste is defined by the spatial arrangements of the three functions AH, B, and X. The relative orientation of the X group of the C-terminal residue to the AH/B groups of the aspartyl moiety, which is conformationally fixed, is determined by conformations about the C-terminal residue. These conformations are often but not always dependent upon chirality.

From conformational studies of various L-aspartyl peptide sweeteners utilizing ^{1}H NMR spectroscopy, X-ray crystallography, and molecular mechanics calculations, we have developed a model describing the molecular arrays required for the sweet taste.[7-11] A molecule can elicit a sweet taste when it assumes an "L" shape with the AH and B 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 plane of the zwitterionic ring is almost identical to the plane of the "L" shape. Substantial projection of the X moiety into the $-z$ dimension results in bitter molecules.

Scheme I. Synthetic Routes to α,β-Dehydro Amino Acid Methyl Esters



To probe the topochemical basis of taste for L-aspartyl dipeptides, we undertook the synthesis of L-aspartyl dipeptides where the C-terminal residue consists of methyl-substituted 1-aminocyclopropanecarboxylic acid methyl esters [Asp-(CH$_3$)$_n$Ac^{3}c-OCH$_3$, n = 1-3]. We chose the methyl-substituted cyclopropanes because the parent compound Asp-Ac^{3}c-OCH$_3$ is sweet[6] and because the positions, the extent, and stereochemistry of substitution

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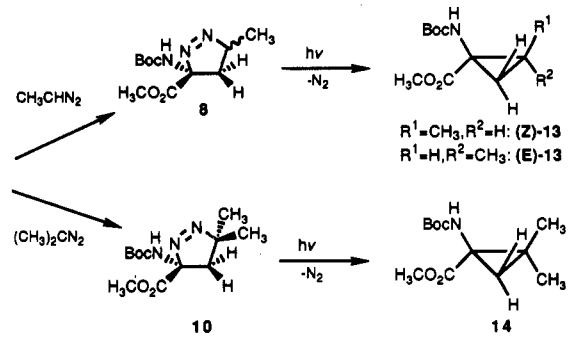
Scheme II. [1,3]-Dipolar Cycloaddition of Diazoalkanes with Boc-DhAla-OCH_3 and Photolysis of 1-Dehydropyrazolines



of the methyl groups can be varied. In addition, the methyl groups on the cyclopropane ring provide further constraints to the conformation about the Ac^3c residue. Furthermore, the $(CH_3)_nAc^3c$ residues display different conformational preferences dependent upon the number and positions of the methyl substituents. In this paper, we also include the conformational analysis of the dipeptide derivatives by ^1H-NMR spectroscopy and molecular mechanics calculations. By assessing the preferred conformations, we are able to relate the stereochemical effects of the methyl substitutions to taste.

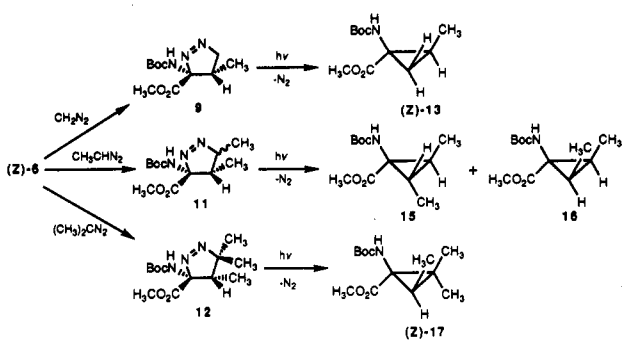
Results and Discussion

Synthesis. Our goal was to synthesize stereospecifically substituted methyl 1-aminocyclopropanecarboxylic acids. A variety of cyclopropane amino acid derivatives ranging from the monosubstituted to the trisubstituted compounds were prepared by allowing appropriate diazoalkanes to react with specific α,β-dehydro amino acid derivatives.

The fully protected α,β-dehydro amino acids were prepared by two different routes (Scheme I): The first route involved the treatment of a protected β-hydroxy amino acid with carbodiimide in the presence of cuprous chloride according to Miller[12] to afford the dehydroamino acid. The *N*-(*tert*-butyloxycarbonyl)dehydroalanine methyl ester, Boc-DhAla-OCH_3 (**5**), was prepared from *N*-(*tert*-butyloxycarbonyl)serine methyl ester, Boc-Ser-OCH_3, in good yields. However, the synthesis of (*Z*)-*N*-(*tert*-butyloxycarbonyl)-2-aminodehydrobutyric acid methyl ester, Boc-(*Z*)-DhAbu-OCH_3 [(*Z*)-**6**], and its stereoisomer, Boc-(*E*)-DhAbu-OCH_3 [(*E*)-**6**], from *N*-(*tert*-butyloxycarbonyl)threonine methyl ester, Boc-Thr-OCH_3, resulted in significantly lower yields (31%). The *Z*:*E* ratio of the isomeric products was found to favor the more thermodynamically stable *Z* isomer by a ratio of approximately 11:1.[13,14] The assignment of the *Z* and *E* configuration of two products was based on comparison of ^1H NMR signals to well-defined olefinic systems reported in the literature.[15]

For comparison purposes, the *Z* and *E* isomers of Boc-DhAbu-OCH_3 (**6**) were also prepared from *N*-(*tert*-butyloxycarbonyl)-2-aminobutyric acid methyl ester, Boc-Abu-OCH_3, using the second route, i.e. the dehydrohalogenation route with a moderate yield (63%).[16] The *Z*:*E* product ratio (15:1) was found to be comparable to that

Scheme III. [1,3]-Dipolar Cycloaddition of Diazoalkanes with Boc-DhAbu-OCH_3 and Photolysis of 1-Dehydropyrazolines



obtained by the first route. The dehydro amino acid residue with dimethyl substitution, *N*-(*tert*-butyloxycarbonyl)dehydrovaline methyl ester, Boc-DhVal-OCH_3 (**7**), was prepared through the dehydrohalogenation route.

The diazoalkanes were generated according to literature procedures.[17-19] The cycloaddition reactions are shown in Schemes II and III. [1,3]-Dipolar cycloadditions of the diazoalkanes with Boc-DhAla-OCH_3 (**5**) and with Boc-(*Z*)-DhAbu-OCH_3 [(*Z*)-**6**] afforded the 1-dehydropyrazolines in high yields. However, no reactions took place between Boc-DhVal-OCH_3 (**7**) and any of the diazoalkanes, including the most reactive of the three, 2-diazopropane. This is most likely due to electronic and steric factors which have been reviewed by Huisgen.[20]

The resulting 1-dehydropyrazolines can be decomposed to afford the cyclopropane derivatives through either thermolysis or photolysis. Based on our experience, the photolysis method resulted in higher yield and purity. In the photolytic decomposition of the 1-dehydropyrazolines derived from Boc-(*Z*)-DhAbu-OCH_3 [(*Z*)-**6**], stereoselectivity was observed in the formation of isomeric cyclopropane products (Scheme III). Our findings are consistent to those of Van Auken and Rinehart who reported that stereoselectivity was observed for the photolysis of 3-carboxymethoxy-*cis*(or *trans*)-3,4-dimethyl-1-dehydropyrazoline.[21] The photolysis of the resulting 1-dehydropyrazoline (**9**) led to one product, Boc-(*Z*)-$(CH_3)Ac^3c$-OCH_3 [(*Z*)-**13**]. Similarly, the photolysis of the 1-dehydropyrazoline (**12**) also led to one compound Boc-[2,2,3(*Z*)-$(CH_3)_3Ac^3c$-OCH_3 [(*Z*)-**17**]. Their relative configurations were assigned on the basis of the mechanism of the photolysis and confirmed by NMR analyses.

The relative stereochemistry of 1-amino-2,3-dimethylcyclopropanecarboxylic acid methyl esters (**15** and **16**) was deduced from ^1H NMR data. Four signals corresponding to the CH and the CH_3 of the cyclopropane ring were observed for compound **15** whereas only two signals were observed for compound **16**. Since the cis isomer is a symmetrical molecule, it was assigned to the compound **16**. Thus, the relative stereochemistry of the methyl substituents on the cyclopropane ring was deduced to be trans for compound **15** and cis for compound **16** (Scheme III) with a ratio of 14:1 in favor of the trans product.

The *N*-(*tert*-butyloxycarbonyl)-protected cyclopropane amino acids were deprotected with hydrogen chloride in

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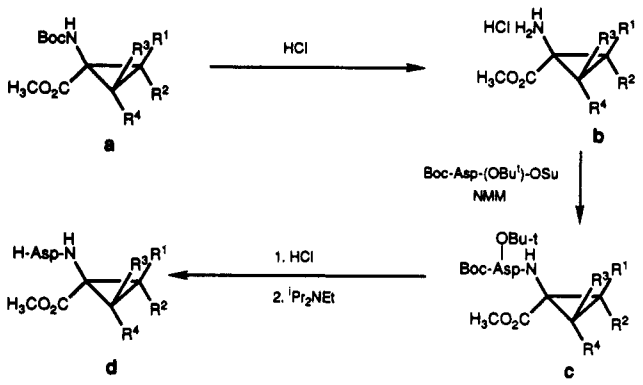
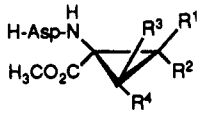
Scheme IV. Coupling Reactions for Asp-$(CH_3)_n$Ac^{3}c-OCH$_3$



a	b	c	d	R^1, R^2, R^3, R^4
(E)-13	(E)-18	(E)-23	(E)-1	R^1=R^2=R^3=H, R^4=CH_3 and R^1=R^3=R^4=H, R^2=CH_3
(Z)-13	(Z)-18	(Z)-23	(Z)-1	R^1=R^2=R^4=H, R^3=CH_3 and R^3=R^4=R^2=H, R^1=CH_3
14	19	24	2	R^3=R^4=H, R^1=R^2=CH_3 and R^1=R^2=H, R^3=R^4=CH_3
15	20	25	3	R^2=R^3=H, R^1=R^4=CH_3 and R^1=R^4=H, R^2=R^3=CH_3
(Z)-17	(Z)-21	(Z)-26	(Z)-4	R^4=H, R^1=R^2=R^3=CH_3 and R^2=H, R^1=R^3=R^4=CH_3

Table I. Taste Properties of Asp-$(CH_3)_n$Ac^{3}c-OCH$_3$



compound	R^1	R^2	R^3	R^4	taste
Asp-Ac^{3}c-OCH$_3$	H	H	H	H	75–100[a]
(*E*)-1	H	CH_3	H	H	sweet[b]
	H	H	H	CH_3	
(1*R*,2*R*)-(*E*)-1	H	CH_3	H	H	50–80[a]
(1*S*,2*S*)-(*E*)-1	H	H	H	CH_3	tasteless
(*Z*)-1	CH_3	H	H	H	tasteless[b]
	H	H	CH_3	H	
2	CH_3	CH_3	H	H	bitter[b]
	H	H	CH_3	CH_3	
3	CH_3	H	H	CH_3	tasteless[b]
	H	CH_3	CH_3	H	
(*Z*)-4	CH_3	CH_3	CH_3	H	bitter[b]
	CH_3	H	CH_3	CH_3	

[a] The taste potencies relative to a 10% sucrose solution were determined by a semiquantitative assessment by members of our research group. [b] Taste of a mixture of diastereomers.

dioxane to give the amino acid hydrochloride salts in high yields. The syntheses of L-aspartyl peptides containing methyl-substituted cyclopropane amino acids are shown in Scheme IV. Coupling reactions proceeded smoothly to afford the protected peptides in high yields. The protecting groups of the dipeptide were removed simultaneously by treatment with hydrogen chloride in dioxane without affecting the cyclopropane ring.

Taste results are shown in Table I. A single methyl group on the cyclopropane ring with the *E* configuration results in a sweet analogue L-aspartyl-(*E*)-1-amino-2-methylcyclopropanecarboxylic acid methyl ester [(*E*)-1] and a tasteless analogue (*Z*)-1. The *gem*-dimethyl analogue **2** is bitter whereas the *trans*-dimethyl analogue **3** is tasteless. The trimethyl-substituted dipeptide (*Z*)-4 is bitter. It is noted that all compounds were tasted as diastereomeric mixtures.

To elucidate the origin of a sweet taste of the isomers of Asp-(*E*)-(CH_3)Ac^{3}c-OCH$_3$, (*E*)-1, we carried out the synthesis using optically pure enantiomers of (*E*)-1-amino-2-methylcyclopropanecarboxylic acids. According to the method of Baldwin,[22] the optically pure amino acid (+)-(1*S*,2*S*)-(CH_3)Ac^{3}c-OH [(+)-(*E*)-**22**] and its enantiomer (−)-(1*R*,2*R*)-(CH_3)Ac^{3}c-OH [(−)-(*E*)-**22**] were prepared. Treatment of this pair of amino acids with di-*tert*-butyl dicarbonate and followed by diazomethane provided Boc-protected amino acid methyl esters (+)-(*E*)-**13** and (−)-(*E*)-**13** (Scheme V). The final L-aspartyl dipeptide methyl esters were obtained by established procedures. It has been found that the dipeptide [(−)-(*E*)-1] containing (−)-(1*R*,2*R*)-(CH_3)Ac^{3}c residue is sweet whereas the peptide [(+)-(*E*)-1] containing the amino acid with 1*S*,2*S* configurations is tasteless. The ^{1}H NMR spectrum of the compound (−)-(*E*)-**13** indicated that it was contaminated by 14% of racemic *Z* isomer (*Z*)-**13**, which was originally produced during the synthetic process of racemic amino acid (±)-**22**. This led to the same contamination of the compound (*Z*)-1 in the dipeptide (−)-(*E*)-1. The compound (+)-(*E*)-1 was found to be optically pure by ^{1}H NMR spectroscopy. Since the dipeptide [(*Z*)-1] was shown to be tasteless, only the analogue Asp-(−)-(*E*)-(1*R*,2*R*)-(CH_3)Ac^{3}c-OCH$_3$ [(−)-(*E*)-1] exhibits a sweet taste (Table I).

Scheme V. Preparation of Optically Pure Boc-2-(CH_3)Ac^{3}c-OCH$_3$

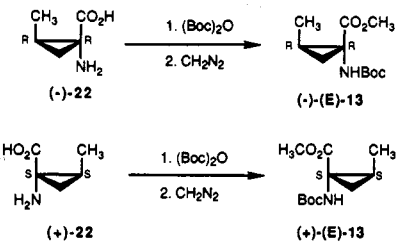


Table II. Experimental Values of Vicinal ^{1}H–^{1}H Coupling Constants and NOEs for Asp-2-(CH_3)Ac^{3}c-OMe Observed in DMSO-d_6 at 25 °C

	Asp-(*E*)-2-(CH_3)-Ac^{3}c-OMe		Asp-(*Z*)-2-(CH_3)-Ac^{3}c-OMe[a]	
^{1}H-NMR parameter	1*R*,2*R*	1*S*,2*S*	I	II
$J_{\alpha\text{-}\beta l}$, Hz	4.30	4.26	4.57	4.62
$J_{\alpha\text{-}\beta h}$, Hz	9.32	8.27	9.44	9.42
$J_{H^2\text{-}H^{3Z}}$,[b] Hz	9.11	9.38	7.53	7.53
$J_{H^2\text{-}H^{3E}}$,[b] Hz	7.84	8.01	9.31	9.53
NOE(Asp H$^\alpha$-Ac$_3$c NH)	strong	strong	strong	strong
NOE(Asp H$^{\beta l}$-Ac^{3}c NH)	medium	medium	medium	medium
NOE(Asp H$^{\beta h}$-Ac^{3}c NH)	–	–	–	–
NOE(Ac^{3}c H^2-Ac^{3}c NH)	medium	medium	–	–
NOE(Ac^{3}c H^{3Z}-Ac^{3}c NH)[b]	–	–	medium	medium
NOE(Ac^{3}c CH_3-Ac^{3}c NH)	–	–	–	–

[a] The ^{1}H NMR measurements were carried out for the diastereomeric mixture. The configuration of the (*Z*)-2-(CH_3)-Ac^{3}c residues in isomer I and II are unknown. [b] H^{3Z} and H^{3E} denote the C^{3}H protons to be located in the syn and anti positions to the NH group, respectively.

The Molecular Basis of Taste. To understand the difference in tastes of the series of L-aspartyl-1-aminocyclopropanecarboxylic acid methyl esters with methyl group substitutions on the cyclopropane ring [Asp-$(CH_3)_n$Ac^{3}c-OCH$_3$, n = 1–3], we undertook conformational analyses of the dipeptides using ^{1}H NMR spectroscopy and molecular mechanics calculations. The vicinal ^{1}H–^{1}H coupling constants for the H–C$^\alpha$–C$^\beta$–H groupings of the aspartyl residue and nuclear Overhauser effects (NOE) used in defining the conformations of the four monomethyl

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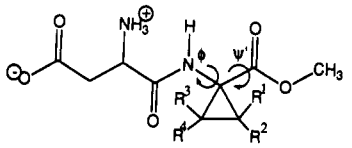

Figure 1. Schematic illustration of L-aspartyl-methylated 1-aminocyclopropanecarboxylic acid methyl esters (R^1, R^2, R^3, R^4 = H or CH_3). The values for ϕ, ψ represent torsion angles about C(O)–N–C–C(O) and N–C–C(O)–O of the C-terminal residue as shown in the figure.

analogues [Asp-2-$(CH_3)Ac^3c$-OCH_3] are shown in Table II. The two β-protons of the aspartyl residue are distinguished by appending an additional superscript h for the higher field and l for the lower field resonances. The two geminal protons of the C^3H_2 group of the cyclopropane ring were assigned by comparing chemical shifts and vicinal coupling constants for the H–C^2–C^3–H groupings with those reported for the stereospecifically C^2-alkylated and C^3-monodeuterated 1-aminocyclopropanecarboxylic acids.[22]

For the monomethyl derivatives, all of the four isomers display similar conformational preferences for the aspartyl group independent of the configurations of the 2-$(CH_3)Ac^3c$ residue. Using NOE data and coupling constants according to Pachler,[23] we established the amino and carboxyl groups of the aspartyl residue are arranged in the correct geometry to act as the AH/B functions of the Shallenberger and Acree model for taste.[2] The difference in taste properties for the Asp-2-$(CH_3)Ac^3c$-OCH_3 isomers must arise from differences in the (ϕ, ψ) conformations of the 2-$(CH_3)Ac^3c$ residue. The definition of the (ϕ, ψ) angles is shown in Figure 1. These angles determine the orientation of the hydrophobic function X (the methyl ester group) with respect to the AH/B functions in the aspartyl moiety.

The NMR spectra for the (*E*)-2-$(CH_3)Ac^3c$ isomers exhibit a high preference for the ϕ angle. A medium NOE(Ac^3c H^2-Ac^3c NH) and a lack of NOE(Ac^3c H^{3Z}-Ac^3c NH) indicate Ac^3c NH points toward the methine group, not the methylene group. This structure requires the ϕ angle of the (*E*)-(1*R*,2*R*)-$(CH_3)Ac^3c$ analogue is from 50° to 90° while that of the (*E*)-(1*S*,2*S*)-$(CH_3)Ac^3c$ analogue is from −90° to −50°.

Minimum energy conformations of Asp-(*E*)-(1*R*,2*R*)-$(CH_3)Ac^3c$-OCH_3 and Asp-(*E*)-(1*S*,2*S*)-$(CH_3)Ac^3c$-OCH_3, consistent with the ^1H NMR observations, are shown in Figure 2, parts a and b, respectively. In both structures, the aspartyl residue assumes the conformation with $\phi \sim$ 165° and $\chi_1 \sim$−64° (g^-). The rest of the molecule, containing two chiral carbon atoms with opposite absolute configurations, exhibits torsion angles with opposite signs which is the origin of the difference in the overall structures. The (ϕ, ψ) angles of the Ac^3c residue in the (*E*)-(1*R*,2*R*)-$(CH_3)Ac^3c$ analogue (Figure 2a) are (73°, 59°), which are close to the values (68.1°, 26.1°) reported for the crystal structure of (*E*)-*N*-(chloroacetyl)-(1*R*,2*R*)-1-amino-2-methylcyclopropanecarboxylic acid.[22] The (*E*)-(1*S*,2*S*)-2-$(CH_3)Ac^3c$ residue in Figure 2b adopts the (ϕ, ψ) = (−68°, −56°). Similar values (−62.5°, −33.0°) have been observed for the 1*S*,2*S* isomer in a racemic mixture of (*E*)-*N*-(phenylacetyl)-1-amino-2-chlorocyclopropanecarboxylic acid methyl ester by X-ray crystallography.[24]

The overall structure of the sweet Asp-(*E*)-(1*R*,2*R*)-$(CH_3)Ac^3c$-OCH_3 (Figure 2a) is topologically defined by the "L"-shaped conformation, where the methyl substituent projects in the +*z* direction. This result is consistent with our model previously proposed for sweet taste.[7-11] On the other hand, the structure of the tasteless diastereomer Asp-(*E*)-(1*S*,2*S*)-$(CH_3)Ac^3c$-OCH_3 is defined by the "reversed L"-shaped conformation (Figure 2b). The placement of the methyl ester group is such that it interferes with binding of the taste ligand to the receptor. Thus the molecule is tasteless. Since the aspartyl residue adopts the same conformation in both diastereomers, the crucial difference in taste must be due to the stereochemistry of the cyclopropane ring which projects the hydrophobic methyl ester group in appropriate or inappropriate areas of the space for recognition at the sweet receptor.

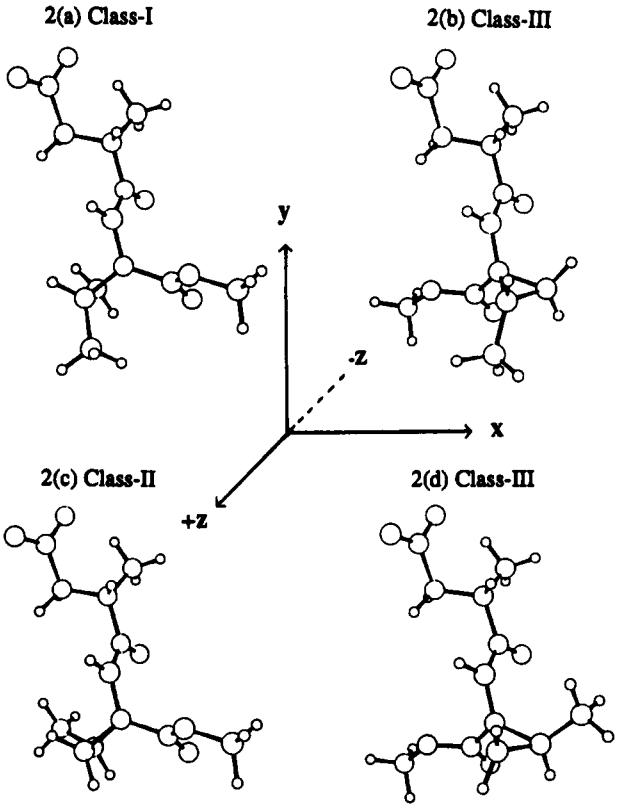

Figure 2. Calculated preferred conformations for four diastereomers of L-aspartyl-1-amino-2-methylcyclopropanecarboxylic acid methyl esters: (a) Asp-(*E*)-(1*R*,2*R*)-$(CH_3)Ac^3c$-OCH_3 (sweet, class I), (b) Asp-(*E*)-(1*S*,2*S*)-$(CH_3)Ac^3c$-OCH_3 (tasteless, class III), (c) Asp-(*Z*)-(1*S*,2*R*)-$(CH_3)Ac^3c$-OCH_3 (tasteless, class II), (d) Asp-(*Z*)-(1*R*,2*S*)-$(CH_3)Ac^3c$-OCH_3 (tasteless, class III). Structures are projected in *xy* plane, where the *y* axis is taken to be parallel to the direction from C-1 of the $(CH_3)Ac^3c$ residue to C^α of the Asp residue, the *x* axis is in the plane formed by zwitterionic ring of the aspartyl residue, and the *z* axis is perpendicular to that plane. The structure of class I is defined by an "L" shape with zwitterionic aspartyl moiety forming the stem of the "L" in the *z* axis and the hydrophic methyl ester group projecting out along the base of the "L" in the *x* axis. In this structure the methyl substituent on the cyclopropane ring projects toward the +*z* domain and therefore the molecule is sweet. The class II structure is also defined by the "L" shape. However, the methyl group in the −*z* domain. Unfavorable interactions between the methyl group and the sweet receptor result in a lack of sweet taste for the molecule. The class III structure is defined by a "reversed L" conformation and thus the molecule is tasteless.

For the (*Z*)-2-$(CH_3)Ac^3c$ isomers, a medium NOE was observed between Ac^3c NH and H^{3Z} but no NOE was observed between Ac^3c NH and CH_3 protons (Table II). These two observations indicate that the ϕ angle is restricted from −90° to −50° for the (*Z*)-(1*R*,2*S*)-$(CH_3)Ac^3c$ analogue and from 50° to 90° for the (*Z*)-(1*S*,2*R*)-(CH_3)-Ac^3c analogue.

Minimum energy conformations of the diastereomers of Asp-(*Z*)-2-$(CH_3)Ac^3c$-OCH_3 are shown in Figure 2, parts

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(24) Varughese, K. I.; Srinivasan, A. R.; Stammer, C. H. *Int. J. Peptide Protein Res.* **1985**, *26*, 242–251.

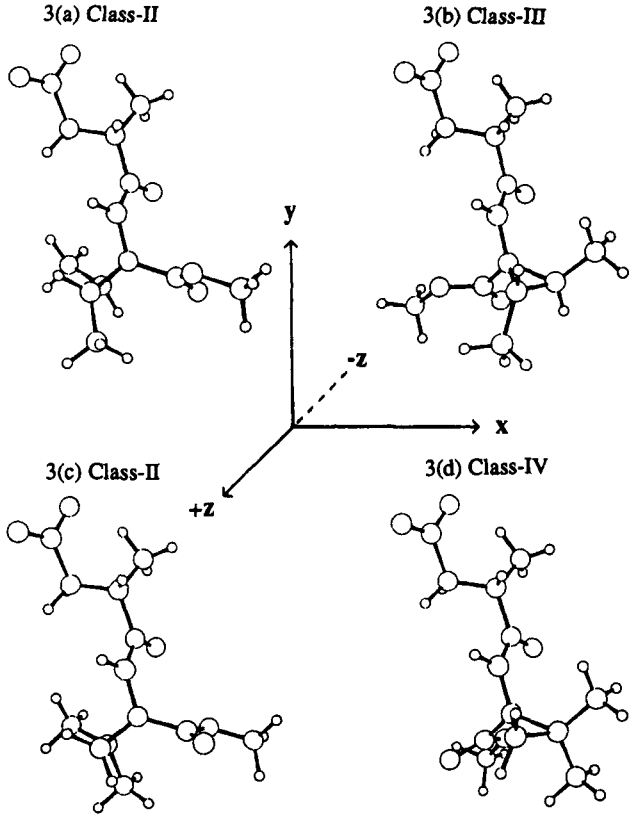

Figure 3. Calculated preferred conformations for (a) Asp-*trans*-(2*R*,3*R*)-$(CH_3)_2Ac^3c$-OCH_3 (tasteless, class II), (b) Asp-*trans*-(2*S*,3*S*)-$(CH_3)_2Ac^3c$-OCH_3 (tasteless, class III), (c) Asp-(*S*)-2,2-$(CH_3)_2Ac^3c$-OCH_3 (tasteless, class II), (d) Asp-(*R*)-2,2-$(CH_3)_2Ac^3c$-OCH_3 (bitter, class IV). The axes and class II, class III are defined as described in the caption for Figure 2. The class IV structure has a large $-z$ component, and our model predicts a bitter taste for the molecule.

c and d. The values of $(\phi, \psi) = (77°, 59°)$ and $(-73°, -56°)$ were calculated for the Asp-(*Z*)-(1*S*,2*R*)-$(CH_3)Ac^3c$-OCH_3 (Figure 2c) and Asp-(*Z*)-(1*R*,2*S*)-$(CH_3)Ac^3c$-OCH_3 (Figure 2d), respectively. For the crystal structure of (*Z*)-*N*-[(*S*)-*O*-(ethoxycarbonyl)mandeloyl]-(1*R*,2*S*)-1-amino-2-methylcyclopropanecarboxylic acid methyl ester, the values for $(\phi, \psi) = (-64.9°, -11.2°)$ have been reported.[22] These angles are in agreement with our calculations.

The analogue Asp-(*Z*)-(1*S*,2*R*)-$(CH_3)Ac^3c$-OCH_3 (Figure 2c) assumes a similar conformation as proposed for the sweet analogue (*E*)-(1*R*,2*R*)-$(CH_3)Ac^3c$ (Figure 2a). The lack of sweet taste must result from the small but unfavorable interactions between the *pro-S* methyl side chain located on a $-z$ domain and the sweet receptor (Figure 2c). The conformation of Asp-(*Z*)-(1*R*,2*S*)-$(CH_3)Ac^3c$-OCH_3 (Figure 2d) can be described by the "reversed L" shape, similar to that of the tasteless (*E*)-(1*S*,2*S*)-$(CH_3)Ac^3c$ analogue (Figure 2b). Therefore a mixture of the two diastereomers is tasteless.

Preferred conformations estimated for the diastereomers of L-aspartyl-1-amino-*trans*-dimethylcyclopropanecarboxylic acid methyl ester are shown in parts a and b of Figure 3. The lack of sweet taste for the analogue Asp-(2*R*,3*R*)-$(CH_3)_2Ac^3c$-OCH_3 shown in Figure 3a, which possesses the "L"-shaped conformation necessary for sweet taste, results from the same reason to the (*Z*)-(1*S*,2*R*)-$(CH_3)Ac^3c$ analogue (Figure 2c). On the other hand, the analogue Asp-(2*S*,3*S*)-$(CH_3)_2Ac^3c$-OCH_3 shown in Figure 3b adopts the "reversed L" conformation, and thus is tasteless simply by conformational effects described above for the (*E*)-(1*S*,2*S*)-$(CH_3)Ac^3c$ analogue (Figure 2b) and (*Z*)-(1*R*,2*S*)-$(CH_3)Ac^3c$ analogue (Figure 2d).

The analogues containing (2,2)-$(CH_3)_2Ac^3c$ display somewhat different conformational preferences for the cyclopropane residues from the other analogues mentioned above. Because of steric repulsive interactions between the NH proton and the methyl group at a Z position and between the C=O oxygen and the methyl group at an *E* position, the 2,2-$(CH_3)_2Ac^3c$ residues prefer the $(\phi, \psi') = (78°, -97°)$ conformation for Asp-(*S*)-2,2-$(CH_3)_2Ac^3c$-OCH_3 (Figure 3c) and the $(-78°, 93°)$ conformation for Asp-(*R*)-2,2-$(CH_3)Ac^3c$-OCH_3 (Figure 3d). The *S* isomer adopts the "L"-shaped conformation, but it is tasteless because the two methyl groups interfere with the binding to the receptor. The *R* isomer orients the methyl ester group toward $-z$ direction resulting in the molecule with a bitter taste as predicted by our model. Thus we conclude that a bitter taste observed for Asp-(*S* and *R*)-2,2-$(CH_3)_2Ac^3c$-OCH_3 diastereomers originates from the *R* isomer.

Preferred conformations elucidated for the L-aspartyl-(*Z*)-1-amino-2,2,3-trimethylcyclopropanecarboxylic acid methyl esters, Asp-(*Z*)-(1*S*,3*S*)-2,2,3-$(CH_3)_3Ac^3c$-OCH_3 and Asp-(*Z*)-(1*R*,3*R*)-2,2,3-$(CH_3)_3Ac^3c$-OCH_3, are almost the same as those for the *S*- and *R*-2,2-dimethyl analogues, respectively. Therefore, the taste properties of the trimethyl analogues can be similarly explained. The bitter taste arises from the Asp-(*Z*)-(1*R*,3*R*)-2,2,3-trimethyl Ac^3c-OCH_3.

Conclusions

A series of L-aspartyl-1-aminocyclopropanecarboxylic acid methyl esters with methyl substitutions on the cyclopropane ring were synthesized to investigate the structural requirements of the C-terminal amino acid needed to elicit a taste response.

The taste properties of Asp-$(CH_3)_nAc^3c$-OCH_3 strongly depend on the number and the positions of the methyl substituents. Among the four stereoisomers of the monomethyl analogues, only L-aspartyl-(*E*)-1*R*,2*R*-1-amino-2-methylcyclopropanecarboxylic acid methyl ester displays a sweet taste while the other isomers are tasteless. The dimethyl analogues with two methyl substituents in a trans position, L-aspartyl-1-amino-*trans*-2,3-dimethylcyclopropanecarboxylic acid methyl esters, are tasteless. On the other hand, the analogue containing two methyl groups at the same carbon of the cyclopropane ring, L-aspartyl-1-amino-(2,2)-dimethylcyclopropanecarboxylic acid methyl ester, exhibits a bitter taste. The trimethyl analogue L-aspartyl-(*Z*)-1-amino-2,2,3-trimethylcyclopropanecarboxylic acid methyl ester is also bitter.

The conformational studies by ^1H NMR spectroscopy and molecular mechanics calculations indicate that preferred conformations of the series of Asp-$(CH_3)_nAc^3c$-OCH_3 are divided into four classes: (I) an "L"-shaped conformation with the methyl substituent on the *pro-R* β-carbon of the cyclopropane ring projecting towards $+z$ direction (sweet), (II) an "L"-shaped conformation with the methyl substituents on the *pro-S* β-carbon projecting toward $-z$ direction (tasteless), (III) a "reversed L"-shaped conformation with no $-z$ component (tasteless), and (IV) a conformation with a large $-z$ component (bitter). In all four classes, the aspartyl residue adopts essentially the same conformation where the amino and carboxylic groups are arranged in the correct geometry to act as the AH/B functions. The difference in the overall structures and thus in tastes arise from differences in the (ϕ, ψ') conformation of $(CH_3)_nAc^3c$ residues. These angles determine the orientation of the hydrophobic function X (the methyl ester group) relative to the AH/B functions. The $(CH_3)_nAc^3c$ residues exhibit different (ϕ, ψ') preference depending upon the number and the positions of the methyl sub-

stitutions on the cyclopropane ring.

The sweet monomethyl analogue Asp-(*E*)-(1*R*,2*R*)-$(CH_3)Ac^3c$-OCH_3 (Figure 2a) assumes the "L" shaped conformation of class I. The analogues Asp-(*R*)-2,2-$(CH_3)_2Ac^3c$-OCH_3 (Figure 3d) and Asp-(*Z*)-(1*R*,3*R*)-2,2,3-$(CH_3)_3Ac^3c$-OCH_3 adopt class IV conformations and thus exhibit bitter tastes. Among the remaining tasteless analogues, Asp-(*E*)-(1*S*,2*S*)-$(CH_3)Ac^3c$-OCH_3 (Figure 2b), Asp-(*Z*)-(1*R*,2*S*)-$(CH_3)Ac^3c$-OCH_3 (Figure 2d) and Asp-*trans*-(2*S*,3*S*)-$(CH_3)_2Ac^3c$-OCH_3 (Figure 3b), prefer the "reversed L" shaped conformations of class III. The preferred conformations of the other tasteless analogues, Asp-(*Z*)-(1*S*,2*R*)-$(CH_3)Ac^3c$-OCH_3 (Figure 2c), Asp-*trans*-(2*R*,3*R*)-$(CH_3)_2Ac^3c$-OCH_3 (Figure 3a), Asp-(*S*)-2,2-$(CH_3)_2Ac^3c$-OCH_3 (Figure 3c), and Asp-(*Z*)-(1*S*,3*S*)-2,2,3-$(CH_3)_3Ac^3c$-OCH_3, belong to class II. The structure–taste relationships for the entire series of L-aspartyl-1-aminocyclopropanecarboxylic acid dipeptide ester analogues observed in this investigation agree with our previously reported model for the sweet and bitter tastes.

Experimental Section

Synthesis. Mass spectra and high-resolution mass spectra were obtained in the +FAB mode. The values in parentheses represent the relative intensities. For safety considerations, the syntheses of diazoalkanes were carried out in a Diazald Kit (glassware without ground glass joints). Photochemical reactions were carried out in a Rayonet photochemical reactor equipped with UV lamps at 254 nm. The workup of light-sensitive products was conducted under amber safelights.

General Procedures for the Dehydro Amino Acid Derivatives. (1) By Carbodiimide and Cuprous Chloride. *N*-(*tert*-Butyloxycarbonyl)dehydroalanine Methyl Ester (5). Under a nitrogen atmosphere, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.13 g, 5.90 mmol) was added to a solution containing Boc-Ser-OCH_3 (1.23 g, 5.62 mmol) and freshly prepared CuCl (0.58 g, 5.90 mmol) in dry $CHCl_3$ (40 mL) at 0 °C in a vessel covered with foil since α,β-dehydroamino acids are light sensitive. The reaction mixture was thoroughly flushed with N_2 and allowed to proceed at room temperature for 32 h. Under safelights, the reaction mixture was concentrated under reduced pressure. The oily residue was taken up in ethyl acetate (200 mL), washed with water, and brine, and then dried over Na_2SO_4. After removing the solvent under reduced pressure, the crude product was purified by flash chromatography on a column (silica gel, CH_2Cl) (0.91 g, 81%): ^1H NMR ($CDCl_3$) δ 7.07 (b, 1 H, NH), 6.17 and 5.73 (2 s, 2 H, vinyl H), 3.83 (s, 3 H, OCH_3), 1.50 (s, 9 H, Boc).

2-*N*-(*tert*-Butyloxycarbonyl)dehydroaminobutyric acid methyl esters [(*Z*)- and (*E*)-6] were prepared as described above starting with Boc-Thr-OCH_3 (2.00 g, 8.57 mmol). The isomers were separated by flash chromatography (ethyl acetate/hexanes, 2:8). The isomer (*Z*)-6 was obtained as a white solid (0.62 g, 31%) whereas the isomer (*E*)-6 was obtained as a colorless oil (0.06 g, 3%). (*Z*)-6: mp 71–72 °C; ^1H NMR ($CDCl_3$) δ 6.71 (q, J = 8.0 Hz, 1 H, vinyl H), 6.23 (b, 1 H, NH), 3.77 (s, 3 H, OCH_3), 1.80 (d, J = 8.0 Hz, 3 H, β-CH_3), 1.48 (s, 9 H, Boc). (*E*)-6: ^1H NMR ($CDCl_3$) δ 6.80 (q, J = 8.0 Hz, 1 H, vinyl H), 6.67 (b, 1 H, NH), 3.85 (s, 3 H, OCH_3), 2.07 (d, J = 8.0 Hz, 3 H, β-CH_3), 1.53 (s, 9 H, Boc).

(2) By N-Chlorination and β-Elimination. (*Z*)-6 and (*E*)-6. Following the procedures of Olsen and Kolar,[16] *tert*-butyl hypochlorite (1.14 mL, 9.56 mmol) was added to a solution of Boc-Abu-OCH_3 (1.30 g, 5.97 mmol) in dry methanol (6 mL) at 0 °C. After 5 min, a catalytic amount of 1% $NaOCH_3$ (0.69 mL, 0.30 mmol) was added slowly. The flask was covered with foil, and the reaction was kept at 4 °C for 23 h. Under safelights, the mixture was concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (150 mL), washed with ice-cold water and brine, and dried over $MgSO_4$. After filtering, the solution was treated with DBU (0.93 mL, 6.15 mmol) at room temperature for 15 min and refluxed for 5 h. It was washed with water, 0.5 N HCl, 0.1 N $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated. Crude products were separated by flash chromatography (ethyl acetate–hexanes, 2:8): 0.26 g (63%); *Z*:*E* ratio 15:1, based on isolated yield.

***N*-(*tert*-Butyloxycarbonyl)dehydrovaline methyl ester (7)** was prepared as described above (yield 70%): mp 70–71 °C (lit.[25] mp 70–72 °C); ^1H NMR ($CDCl_3$) δ 5.70 (b, 1 H, NH), 3.73 (s, 3 H, OCH_3), 2.13 and 1.87 (2 s, 3 H each, CH_3), 1.50 (s, 9 H, Boc).

General Procedures for [1,3]-Dipolar Cycloaddition Reactions and Decomposition of 1-Dehydropyrazolines. 1. Cycloaddition. To a foil-covered flask containing a solution of the protected dehydro amino ester (typically 5–10 mmol) in anhydrous THF (2–10 mL) at 0 °C was added an ethereal solution of diazoalkane (2–3 mL) slowly. Upon completion, excess diazoalkane was destroyed by the addition of Na_2SO_4. After filtration, the solution was concentrated to yield the crude 1-dehydropyrazoline which was used in the next step without further purification.

2. Decomposition by Photolysis. *N*-(*tert*-Butyloxycarbonyl)-1-amino-(*Z*)-2-methylcyclopropanecarboxylic Acid Methyl Ester [(*Z*)-13] and Its *E* Isomer [(*E*)-13]. A solution of the 1-dehydropyrazoline 8 (1.91 g, 7.41 mmol) in anhydrous THF was purged with N_2 for 15 min in a 100-mL round-bottom quartz flask. The solution was then irradiated at 254 nm in a Rayonet photoreactor. The crude product was separated by flash chromatography (ether/hexanes) to provide compounds (*Z*)-13 and (*E*)-13 (1.46 g, E/Z = 1.9/1, 95 %). (*Z*)-13 was also obtained from the photolysis of compound 9: mp 55–57 °C; ^1H NMR ($CDCl_3$) δ 4.90 (s, 1 H, NH), 3.69 (s, 3 H, OCH_3), 1.67–1.55 and 1.23 (2 m, 3 H, 2-H, 3-H), 1.46 (s, 9 H, Boc), 1.18 (d, J = 7.0 Hz, 3 H, 2-CH_3); MS m/z 230 (M + 1, 15), 174 (100). Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.93; H, 8.42; N, 6.06. (*E*)-13: mp 58–60 °C; ^1H NMR ($CDCl_3$) δ 5.20 (b, 1 H, NH), 3.72 (s, 3 H, OCH_3), 1.65–1.52 and 1.23 (2 m, 3 H, 2-H, 3-H), 1.45 (s, 9 H, Boc), 1.24 (d, J = 7.0 Hz, 3 H, 2-CH_3); MS m/z 230 (M + 1, 13); 174 (100). Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.82; H, 8.11; N, 6.07.

***N*-(*tert*-Butyloxycarbonyl)-1-amino-2,2-dimethylcyclopropanecarboxylic acid methyl ester (14)**: ^1H NMR ($CDCl_3$) δ 5.69 (s, 1 H, NH), 3.74 (s, 3 H, OCH_3), 1.80–1.48 (m, 2 H, 3-H), 1.47 (s, 9 H, Boc), 1.29 and 1.26 (2 s, 3 H each, 2-CH_3); MS m/z 244 (M + 1, 18), 243 (14), 188 (100). Anal. Calcd for $C_{12}H_{21}NO_4$: C, 59.24; H, 8.70; N, 5.57. Found: C, 59.55; H, 8.83; N, 5.61.

***N*-(*tert*-Butyloxycarbonyl)-1-amino-*trans*-2,3-dimethylcyclopropanecarboxylic acid methyl ester (15)**: ^1H NMR ($CDCl_3$) δ 5.21 (s, 1 H, NH), 3.72 (s, 3 H, OCH_3), 1.79 (m, 2 H, 2-H, 3-H), 1.50 (s, 9 H, Boc), 1.19 and 1.10 (2 d, J = 7.0 Hz, 3 H each, 2-CH_3, 3-CH_3); MS m/z 244 (M + 1, 16), 243 (9), 188 (100). Anal. Calcd for $C_{12}H_{21}NO_4$: C, 59.24; H, 8.70; N, 5.56. Found C, 59.51; H, 8.53; N, 5.78.

***N*-(*tert*-Butyloxycarbonyl)-1-amino-*cis*-2,3-dimethylcyclopropanecarboxylic acid methyl ester (16)**: ^1H NMR ($CDCl_3$) δ 5.25 (s, 1 H, NH), 3.70 (s, 3 H, OCH_3), 1.75 (q, J = 7.0 Hz, 2 H, 2-H), 1.52 (s, 9 H, Boc), 1.15 (d, J = 7.0 Hz, 6 H, 2-CH_3, 3-CH_3); MS m/z 244 (M + 1, 14), 243 (11), 188 (100). Anal. Calcd for $C_{12}H_{21}NO_4$: C, 59.24; H, 8.70; N, 5.56. Found: C, 59.18; H, 8.93; N, 5.34.

***N*-(*tert*-Butyloxycarbonyl)-1-amino-(*Z*)-2,2,3-trimethylcyclopropanecarboxylic Acid Methyl Ester [(*Z*)-17].** The *E* isomer of the product was not observed using HPTLC: ^1H NMR ($CDCl_3$) δ 5.20 (s, 1 H, NH), 3.70 (s, 3 H, OCH_3), 1.80 (m, 1 H, 3-H), 1.50 (s, 9 H, Boc), 1.20 and 1.12 (2 s, 6 H, 2-CH_3), 0.98 (d, J = 7.5 Hz, 3 H, 3-CH_3); MS m/z 258 (M + 1, 18), 257 (11), 202 (100). Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.93; H, 8.97; N, 5.43.

Procedures for Preparation of Optically Pure *N*-(*tert*-Butyloxycarbonyl)-1-amino-(*E*)-2-methylcyclopropanecarboxylic Acid Methyl Esters. (+)-(*E*)-13. To the amino acid (+)-22 (120 mg, 1.04 mmol) in the mixed solvent of 1 N $NaHCO_3$ (4 mL) and THF (4 mL) was added di-*tert*-butyl bicarbonate (262 mg, 1.2 mmol) while stirring at rt for 8 h. The

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mixture was acidified cautiously by 1 N $NaHSO_4$ at 0 °C, the product was extracted with ethyl acetate, and the extract was washed with brine and then dried over $MgSO_4$. After removal of the solvent, the resulting oil was dissolved in anhydrous CH_2Cl_2 (10 mL) at 0 °C. An ethereal solution of diazomethane was added until saturation was reached. After 30 min, the solution was concentrated under reduced pressure to provide the ester (+)-(*E*)-13 (0.21 g, 94%): $[\alpha]^{25}_D$ +21.3° (*c* 1, MeOH); ^1H NMR and MS spectral data are the same as for the racemic one. Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.39; H, 8.54; N, 6.09.

(−)-(*E*)-13. This compound was obtained as the same method as described above. Yield is 99%: $[\alpha]^{25}_D$ −19.0° (*c* 1, MeOH); ^1H NMR and MS spectral data are the same as for the racemic one. Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.35; H, 8.58; N, 6.01.

General Procedures for the Deprotection of the Methyl-Substituted 1-Aminocyclopropanecarboxylic Acid Esters. (*E*)-1-Amino-2-methylcyclopropanecarboxylic Acid Methyl Ester Hydrochloride [(*E*)-18]. A cold solution of 4 N HCl in dioxane (5 mL) was added to a solution of [(*E*)-13] in anhydrous CH_2Cl_2 at 0 °C. The reaction was maintained at 0 °C for 1 h and then was allowed to proceed at room temperature until completion. The solvent was then removed under reduced pressure. Anhydrous ether was added to the residue and removed under reduced pressure to yield an oily residue which solidified upon standing. The crude product was recrystallized from methanol/ether. Compound (*E*)-18 was obtained as a white solid (0.08 g, 95%): mp 186.0–186.5 °C; ^1H NMR (DMSO-d_6) δ 8.82 (b, 3 H, NH_3), 3.75 (s, 3 H, OCH_3), 1.74 (m, 1 H, 2-H), 1.54 and 1.29 (2 m, 2 H, 3-H), 1.16 (d, *J* = 9.0 Hz, 3 H, 2-CH_3); MS *m/z* 130 (M + 1, 100). Anal. Calcd for $C_6H_{12}NO_2Cl$: C, 43.51; H, 7.30; N, 8.46. Found: C, 43.48; H, 7.46; N, 8.41.

The optically pure compounds (+)-(*E*)-18 were prepared by the same procedure as used for the racemic (*E*)-18: $[\alpha]^{25}_D$ −0.87° (*c* 1.0, MeOH) for the compound (−)-(*E*)-18 and $[\alpha]^{25}_D$ +1.0° (*c* 1.0, MeOH) for the compound (+)-(*E*)-18.

(*Z*)-1-Amino-2-methylcyclopropanecarboxylic acid methyl ester hydrochloride [(*Z*)-18]: mp 174.0–175.5 °C dec; ^1H NMR (DMSO-d_6) δ 8.88 (b, 3 H, NH_3), 3.71 (s, 3 H, OCH_3), 1.69 (m, 1 H, 2-H), 1.55 and 1.23 (2 m, 2 H, 3-H), 1.26 (d, *J* = 6.5 Hz, 3 H, 2-CH_3); MS *m/z* 130 (M + 1, 100). Anal. Calcd for $C_6H_{12}NO_2Cl$: C, 43.51; H, 7.30; N, 8.40. Found: C, 43.29; H, 7.28; N, 8.37.

1-Amino-2,2-dimethylcyclopropanecarboxylic acid methyl ester hydrochloride (19): mp 224–225 °C dec; ^1H NMR (DMSO-d_6) δ 9.10 (b, 3 H, NH_3), 3.75 (s, 3 H, OCH_3), 1.45 and 1.43 (2 d, *J* = 6.1 Hz, 1 H each 3-H), 1.35 and 1.13 (2 s, 3 H each, 2-CH_3); MS *m/z* 144 (M + 1, 100). Anal. Calcd for $C_7H_{14}NO_2Cl$: C, 46.80; H, 7.85; N, 7.80. Found: C, 46.45; H, 7.65; N, 7.90.

1-Amino-*trans*-2,3-dimethylcyclopropanecarboxylic acid methyl ester hydrochloride (20): mp 164–167 °C; ^1H NMR (DMSO-d_6) δ 8.86 (b, 3 H, NH_3), 3.75 (s, 3 H, OCH_3), 1.60 and 1.61 (2 overlapping m, 1 H each, 2-H, 3-H), 1.25 & 1.16 (2 d, *J* = 5.4 Hz, 3 H, each, 2-CH_3, 3-CH_3); MS *m/e* 144 (M + 1, 100). Anal. Calcd for $C_7H_{14}NO_2Cl$: C, 46.80; H, 7.85; N, 7.80. Found: C, 46.94; H, 7.98; N, 7.74.

1-Amino-(*Z*)-2,2,3-trimethylcyclopropanecarboxylic acid methyl ester hydrochloride [(*Z*)-21]: mp 207–208 °C dec; ^1H NMR (DMSO-d_6) δ 8.82 (b, 3 H, NH_3), 3.74 (s, 3 H, OCH_3), 1.73 (q, *J* = 6.5 Hz, 1 H, 3-H), 1.19 and 1.15 (2 s, 3 H each, 2-CH_3), 1.14 (d, *J* = 6.5 Hz, 3 H, 3-CH_3); MS *m/e* 158 (M + 1, 100). Anal. Calcd for $C_8H_{16}NO_2Cl$: C, 49.61; H, 8.33; N, 7.23. Found: C, 49.39; H, 8.48; N, 7.10.

General Procedures for the Preparation of the Protected Dipeptides by Coupling Reactions. *N*-(*tert*-Butyloxycarbonyl)-(β-*tert*-butyl ester)-L-aspartyl-(*E*)-1-amino-2-methylcyclopropanecarboxylic Acid Methyl Ester [(*E*)-23]. *N*-Methylmorpholine (0.05 g, 0.42 mmol) was added to a stirred solution of (*E*)-18 (0.07 g, 0.42 mmol) in anhydrous DMF (2 mL) and then cooled to 0 °C. The *N*-hydroxysuccinimide ester of *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid β-*tert*-butyl ester (0.16 g, 0.40 mmol) was added, and the reaction mixture was allowed to warm to room temperature. The reaction mixture was concentrated under reduced pressure. The residue was taken up in ethyl acetate, washed with 0.4 N $NaHSO_4$, 0.1 N $NaHCO_3$, water, and brine, and dried. Ethyl acetate was removed under reduced pressure, and the residue was purified on column (ethyl acetate/hexanes) to provide 0.154 g (99%) of (*E*)-23: ^1H NMR ($CDCl_3$) δ 7.10 (b, 1 H, Asp-NH), 5.64 (b, 1 H, NH), 4.45 (b, 1 H, Asp-C$^\alpha$-H) 3.68 (s, 3 H, OCH_3), 2.85 and 2.60 (2 m, 2 H, Asp-C$^\beta$-H), 1.53–1.49, 1.25 (2 m, 3 H, 2-H, 3-H), 1.45 (s, 18 H, Boc and t-Bu), 1.25 (m, 3 H, 2-CH_3); MS *m/z* 402 (M + 1, 9), 401 (M, 43), 345 (22), 289 (100). Anal. Calcd for $C_{19}H_{32}N_2O_7$: C, 56.98; H, 8.06; N, 7.00. Found: C, 57.88; H, 7.98; N, 6.78.

***N*-(*tert*-Butyloxycarbonyl)-(β-*tert*-butyl ester)-L-aspartyl-(*Z*)-1-amino-2-methylcyclopropanecarboxylic acid methyl ester [(*Z*)-23]:** ^1H NMR ($CDCl_3$) δ 7.00 (b, 1 H, Asp-NH), 5.76 (b, 1 H, NH), 4.45 (b, 1 H, Asp-C$^\alpha$-H), 3.66 (s, 3 H, OCH_3), 2.88 and 2.62 (2 m, 2 H, Asp-C$^\beta$-H), 1.90–1.65 (m, 3 H, 2-H, 3-H), 1.45 (s, 18 H, Boc and t-Bu), 1.14, 1.12 (2 d, 3 H, *J* = 6.0 Hz, *J'* = 5.0 Hz, 2-CH_3); MS *m/z* 402 (M + 1, 9), 401 (M, 34), 345 (22), 289 (90), 245 (100). Anal. Calcd for $C_{19}H_{32}N_2O_7$: C, 56.98; H, 8.06; N, 7.00. Found: C, 57.07; H, 7.87; N, 6.95.

***N*-(*tert*-Butyloxycarbonyl)-(β-*tert*-butyl ester)-L-aspartyl-1-amino-2,2-dimethylcyclopropanecarboxylic acid methyl ester (24):** ^1H NMR ($CDCl_3$) δ 7.10 (b, 1 H, Asp-NH), 5.70 (b, 1 H, NH), 4.50 (m, 1 H, Asp-C$^\alpha$-H), 3.67 (s, 3 H, OCH_3), 2.90 and 2.70 (2 m, 2 H, Asp-C$^\beta$-H), 1.78 (m, 2 H, 3-H), 1.45 and 1.44 (2 s, 18 H, Boc, t-Bu), 1.15–1.12 (m, 6 H, 2-CH_3); MS *m/z* 416 (M + 1, 9), 415 (M, 32), 414 (14), 359 (20), 303 (100). Anal. Calcd for $C_{20}H_{34}N_2O_7$: C, 57.95; H, 8.27; N, 6.76. Found: C, 58.07; H, 8.06; N, 6.92.

***N*-(*tert*-Butyloxycarbonyl)-(β-*tert*-butyl ester)-L-aspartyl-1-amino-*trans*-2,3-dimethylcyclopropanecarboxylic acid methyl ester (25):** ^1H NMR ($CDCl_3$) δ 6.97 (b, 1 H, Asp-NH), 5.74 (m, 1 H, NH), 4.50 (b, 1 H, Asp-C$^\alpha$-H), 3.88 (s, 3 H, OCH_3), 3.29 and 3.70 (2 m, 2 H, Asp-C$^\beta$-H), 1.90–1.82 (m, 2 H, 2-H, 3-H), 1.45 (s, 18 H, Boc and t-Bu), 1.23, 1.15 (2 m, 2 × 3 H, 2-CH_3, 3-CH_3); MS *m/z* 415 (M + 1, 75), 414 (M, 15), 359 (29), 303 (100), 271 (96). Anal. Calcd for $C_{20}H_{34}N_2O_7$: C, 59.95; H, 8.27; N, 6.76. Found: C, 58.14; H, 8.11; N, 7.04.

***N*-(*tert*-Butyloxycarbonyl)-(β-*tert*-butyl ester)-L-aspartyl-(*Z*)-1-amino-2,2,3-trimethylcyclopropanecarboxylic acid methyl ester [(*Z*)-26]:** ^1H NMR ($CDCl_3$) δ 6.90 (b, 1 H, Asp-NH), 5.85 (s, 1 H, NH), 4.50 (m, 1 H, Asp-C$^\alpha$-H), 3.67 (s, 3 H, OCH_3), 2.95 and 2.78 (2 m, 2 H, Asp-C$^\beta$-H), 1.88 (m, 1 H, 3-H), 1.53 (s, 18 H, Boc and t-Bu), 1.18 and 1.08 (2 s, 3 H each, 2-CH_3), 0.93 (d, *J* = 6.8 Hz, 3 H, 3-CH_3); HRMS calcd for $C_{21}H_{37}N_2O_7$ 429.2607, found 429.2601.

Procedures for Preparation of the Optically Pure Protected Dipeptides. (+)-(*E*)-23. To a solution of compound (+)-(*E*)-18 (170 mg, 1.12 mmol) in dry DMF (10 mL) were added Boc-Asp(OBu-t)-OH·DCHA (527 mg, 1.12 mmol), HOBt (155 mg, 1.12 mmol), and EDC·HCl (288 mg, 1.5 mmmol), respectively, at −30 °C with stirring. After 30 min, the mixture was stirred at room temperature overnight. Chromatography provided the pure protected dipeptide (+)-(*E*)-23 (300 mg, 73%): $[\alpha]^{25}_D$ +22.8° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.14 (s, 1 H, Asp-NH), 5.68 (b, 1 H, NH), 4.45 (b, 1 H, Asp-C$^\alpha$-H), 3.69 (s, 3 H, OCH_3), 2.84 (dd, 1 H, *J* = 18.4, 4.5 Hz, Asp-C$^\beta$-H^1), 2.60 (dd, 1 H, *J* = 18.4, 7.2 Hz, Asp-C$^\beta$-H^2), 1.64, 1.51, and 1.20 (3 m, 3 H, 2-H, 3-H), 1.45 (s, 18 H, Boc and t-Bu), 1.24 (d, *J* = 5.0 Hz, 3 H, 2-CH_3); MS *m/e* 402 (M + 1, 11), 401 (M, 51); HRMS calcd for $C_{19}H_{32}N_2O_7$ + H 401.2288, found 401.2275.

The compound (−)-(*E*)-23 was obtained by using the above procedure (320 mg, 44%): $[\alpha]^{25}_D$ −10.7° (*c* 2.0, MeOH); ^1H NMR ($CDCl_3$) δ 7.13 (b, 1 H, Asp-NH), 5.66 (b, 1 H, NH), 4.43 (b, 1 H, Asp-C$^\alpha$-H), 3.68 (s, 3 H, OCH_3), 2.82 (dd, *J* = 16.8, 5.0 Hz, 1 H, Asp-C$^\beta$-H^1), 2.61 (dd, *J* = 16.8, 6.8 Hz, 1 H, Asp-C$^\beta$-H^2), 1.60, 1.46 and 1.25 (3 m, 3 H, 2-H, 3-H), 1.45 (s, 18 H, Boc and t-Bu), 1.24 (d, *J* = 5.5 Hz, 3 H, 2-CH_3); MS *m/e* 402 (M + 1, 11), 401 (M, 52); HRMS calcd for $C_{19}H_{32}N_2O_7$ + H 401.2288, found 401.2290.

General Procedures for the Deprotection of the Dipeptides. L-Aspartyl-(*E*)-1-amino-2-methylcyclopropanecarboxylic Acid Methyl Ester [(*E*)-1]. A solution of 4 N HCl in dioxane (8 mL) was added to a stirred solution of (*E*)-23 (0.16 g, 0.40 mmol) in anhydrous CH_2Cl_2 at 0 °C. The reaction was maintained at 0 °C for 4 h and then was allowed to proceed at room temperature until completion. The reaction mixture was concentrated under reduced pressure and then dried under high

vacuum. The hydrochloride salt of the deprotected peptide was dissolved in a minimum amount of methanol. The solution was diluted with ethyl acetate until a slight cloudiness appeared and neutralized with *N,N*-diisopropylethylamine as determined on prewetted Alkacid paper. After standing at 5 °C overnight, the precipitated dipeptide was collected, washed with ethyl acetate and ether, and dried. The crude product was recrystallized to afford the pure product (*E*)-1 as a mixture of diastereomers (0.05 g, 53%): mp 157–162 °C dec; ^{1}H NMR (DMSO-d_6), see below for the optically active analogue; MS *m/z* 245 (M + 1, 49), 213 (9). Anal. Calcd for $C_{10}H_{16}N_2O_5 \cdot 1.25H_2O$: C, 45.02; H, 6.99; N, 10.50. Found: C, 44.75; H, 7.02; N, 10.42.

(−)-***E***-1: mp 190–191 °C; $[\alpha]^{25}_D$ −21.4° (*c* 1.0, CH_3OH); 500-MHz ^{1}H NMR (DMSO-d_6) δ 8.86 (s, 1 H, Ac^{3}c-NH), 3.57 (s, 3 H, OCH_3), 3.47 (dd, *J* = 4.3, 9.3 Hz, 1 H, Asp-C$^\alpha$-H), 2.41 (dd, *J* = 16.1, 4.3 Hz, 1 H, Asp-C$^\beta$-H^1), 2.19 (dd, *J* = 16.1, 9.3 Hz, 1 H, Asp-C$^\beta$-H^2), 1.47 (m, 1 H, 2-H), 1.29 (dd, *J* = 7.8, 4.9 Hz, 1 H, 3-H at the *E* position), 1.13 (d, *J* = 6.1 Hz, 3 H, CH_3), 1.05 (dd, *J* = 9.1, 4.9 Hz, 1 H, 3-H at the *Z* position). Anal. Calcd for $C_{10}H_{16}N_2O_5 0.85H_2O$: C, 46.27; H, 6.87; N, 10.79. Found: C, 46.15; H, 6.99; N, 10.66. [(+)-(***E***)-1]: mp 182–183 °C dec; $[\alpha]^{25}_D$ +58.7° (*c* 1.0, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 9.07 (s, 1 H, Ac^{3}c-NH), 3.86 (dd, *J* = 4.3, 8.3 Hz, 1 H, Asp-C$^\alpha$-H), 3.57 (s, 3 H, OCH_3), 2.73 (dd, *J* = 4.3, 17.3 Hz, 1 H, Asp-C$^\beta$-H^1), 2.61 (dd, *J* = 8.3, 17.3, Hz, 1 H, Asp-C$^\beta$-H^2), 1.49 (m, 1 H, 2-H), 1.32 (dd, *J* = 8.0, 4.9 Hz, 1 H, 3-H at the *E* position), 1.10 (d, *J* = 6.1 Hz, 3 H, CH_3), 1.09 (dd, *J* = 9.4, 4.9 Hz, 1 H, 3-H at the *Z* position). Anal. Calcd for $C_{10}H_{16}N_2O_5 \cdot 0.6H_2O$: C, 47.09; H, 6.80; N, 10.98. Found C: 47.11; H, 6.98; N, 10.80.

L-Aspartyl-(*Z*)-1-amino-2-methylcyclopropanecarboxylic acid methyl ester [(*Z*)-1]: mp 160–168 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.78 (s, 1 H, Ac^{3}c-NH), 3.73 (dd, *J* = 4.6, 9.4 Hz, 1 H, Asp-C$^\alpha$-H), 3.56 (s, 3 H, OCH_3), 2.51 (dd, *J* = 16.3, 4.6 Hz, 1 H, Asp-C$^\beta$-H^1), 2.27 (dd, *J* = 16.3, 9.4 Hz, 1 H, Asp-C$^\beta$-H^2), 1.69 (m, 1 H, 2-H), 1.45 (dd, *J* = 9.3, 4.8 Hz, 1 H, 3-H at the *E* position), 1.01 (d, *J* = 6.3 Hz, 3 H, 2-CH_3), 0.75 (dd, *J* = 7.5, 4.8 Hz, 1 H, 3-H at the *Z* position) for isomer I; 8.74 (s, 1 H, Ac^{3}c-NH), 3.73 (dd, *J* = 4.6, 9.4 Hz, 1 H, Asp-C$^\alpha$-H), 3.55 (s, 3 H, OCH_3), 2.49 (dd, *J* = 16.3, 4.6 Hz, 1 H, Asp-C$^\beta$-H^1), 2.29 (dd, *J* = 16.3, 9.4 Hz, 1 H, Asp-C$^\beta$-H^2), 1.69 (m, 1 H, 2-H), 1.45 (dd, *J* = 9.5, 4.9 Hz, 1 H, 3-H at the *E* position), 1.02 (d, *J* = 6.3 Hz, 3 H, 2-CH_3), 0.72 (dd, *J* = 7.5, 4.9 Hz, 1 H, 3-H at the *Z* position) for isomer II; MS *m/e* 245 (M + 1, 72), 213 (12). Anal. Calcd for $C_{10}H_{16}N_2O_5 \cdot 1.25H_2O$: C, 45.02; H, 6.99; N, 10.50. Found: C, 45.31; H, 6.81; N, 10.45.

L-Aspartyl-1-amino-2,2-dimethylcyclopropanecarboxylic acid methyl ester (2): mp 171–176 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.03 (s, 1 H, NH), 3.93 (m, 1 H, Asp-NH), 3.58 (s, 3 H, OCH_3), 2.70 and 2.62 (2 m, 2 H, Asp-C$^\beta$-H), 1.54 and 0.86 (2 m, 1 H each, 3-H), 1.21 and 1.15 (2 s, 3 H each, 2-CH_3); MS *m/e* 259 (M + 1, 100). Anal. Calcd for $C_{11}H_{18}N_2O_5 \cdot 0.5H_2O$: C, 47.05; H, 6.82; N, 9.98. Found: C, 47.01; H, 6.95; N, 9.80.

L-Aspartyl-1-amino-*trans*-2,3-dimethylcyclopropanecarboxylic acid methyl ester (3): mp 162–169 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.79 and 8.73 (2 s, 1 H, NH), 3.70 (m, 1 H, Asp-C$^\alpha$-H), 3.58 and 3.57 (2 s, 3 H, OCH_3), 2.47 and 2.24 (2 m, 2 H, Asp-C$^\beta$-H), 1.58 (m, 2 H, 2-H, 3-H), 1.09 (b, 3 H, 2-CH_3 or 3-CH_3), 0.96 (2 d, *J* = 7.6 Hz, 3 H, 2-CH_3 or 3-CH_3); MS *m/e* 273 (M + 1, 100). Anal. Calcd for $C_{11}H_{18}N_2O_5 \cdot H_2O$: C, 47.82; H, 7.30; N, 10.14. Found: C, 47.46; H, 7.21; N, 9.87.

L-Aspartyl-(*Z*)-1-amino-2,2,3-trimethylcyclopropanecarboxylic acid methyl ester [(*Z*)-4]: mp 146–155 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.68 and 8.61 (2 s, 1 H, NH), 3.76 (m, 1 H, Asp-C$^\alpha$-H), 3.56 and 3.55 (2 s, 3 H, OCH_3), 2.41 (dd, *J* = 17.0, 5.0 Hz, 1 H, Asp-C$^\beta$-H^1), 2.20 (dd, *J* = 17.0, 7.0 Hz, 1 H, Asp-C$^\beta$-H^2), 1.69 (m, 1 H, 3-H), 1.08 and 1.04 (2 s, 3 H each, 2-CH_3), 0.85 and 0.81 (2 d, *J* = 6.5 Hz, 3 H, 3-CH_3); MS *m/e* 273 (M + 1, 100). Anal. Calcd for $C_{12}H_{20}N_2O_5 \cdot 1.25H_2O$: C, 48.89; H, 7.69; N, 9.50. Found: C, 49.12; H, 7.50; N, 9.51.

^{1}H NMR Measurements. The ^{1}H NMR spectra of the final L-aspartyl dipeptide esters were recorded on a General Electric GN-500 spectrometer operating at 500 MHz. Temperatures were maintained at given values within ±1 °C during measurements. The samples were prepared in DMSO-d_6. The 1D spectra contains 16K points in 5000 Hz. The 2D homonuclear Hartman–Hahn experiments[26] were carried out using the MLEV 17 suggested by Bax et al.[27] and the time proportional phase increment.[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 experiments[29] were carried out by varying mixing time from 50 to 250 ms with a spin locking field of 2.5 kHz. All of the 2D spectra were obtained using 2K data points in the f_2 domain and 256 points in the f_1 domain. Applying the zero filling procedure to the f_1 domain resulted in a final matrix of 2K × 2K data points. Multiplication with either a phase-shifted sine or Gaussian function was used to enhance the spectra.

Molecular Mechanics Calculations. Conformational energy minimizations were carried out with the Newton–Raphson method until the maximum derivative was less than 0.001 kcal mol^{-1} Å^{-1} by employing the DISCOVER program.[30] Conventional values of the bond lengths and bond angles for methyl-substituted 1-aminocyclopropanecarboxylic acid residues were taken from the crystallographic data reported in the literature.[22,24] 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 Ac^{3}c residues. The force constants for the Ac^{3}c residues were created based on the values for aminoisobutyric acid provided in the DISCOVER program.

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Supplementary Material Available: The reaction conditions for (1) the 1,3-dipolar cycloadditions, (2) the photolytic and thermal decompositions of 1-dehydropyrazolines, (3) the deprotection of Boc-$(CH_3)_n$Ac^{3}c-OCH_3, (4) the coupling of Boc-Asp(O-t-Bu)-OSu with HCl·$(CH_3)_n$Ac^{3}c-OCH_3, and (5) the acidolyses of Boc-Asp(O-t-Bu)-$(CH_3)_n$Ac^{3}c-OCH_3 (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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