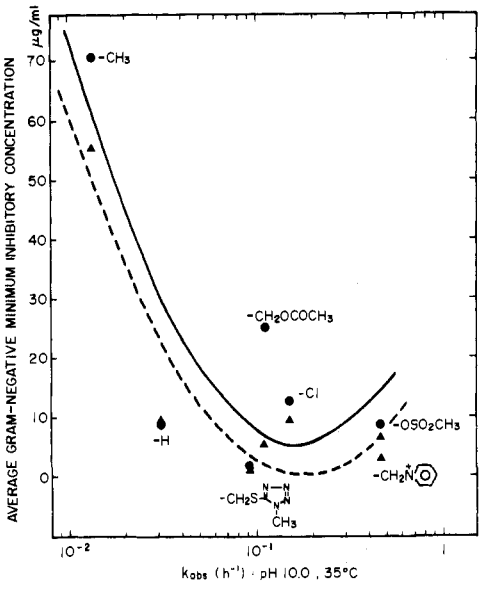

Figure 6. Relationship between log k_{obsd} and MICs for various 3-substituted cephalosporins: (▲) the plot of arithmetic average of MIC of 7-[(2-thienylacetyl)amino]cephalosporin against five Gram-negative test organisms reported by Boyd et al.[11] against log k_{obsd}, (●) the plot of geometrical mean of MIC (see Table II) of 7-[(phenylacetyl)amino]cephalosporin against log k_{obsd}.

Conclusion

We identified parameters for predicting of the β-lactam reactivity: (i) the β-lactam $\nu_{C=O}$ value is a good index for 3-substituted cephalosporins, (ii) ^{13}C NMR chemical shifts δ(C-3) and δ(COO) are rough indices for 3-substituted cephalosporins when cephalosporins are classified into the two groups of those with an OR substituent and those with a H, Cl, or CH_2R substituent, (iii) the Δδ(4–3) value is a good index for the 3-methylene-substituted cephalosporins. After analyzing these parameters, we concluded that the β-lactam reactivity of 3-substituted cephalosporins can be explained by the linear combination of inductive and resonance effects of substituents at C-3 and that of 3-methylene-substituted cephalosporins by the inductive effect of the substituents at C-3′.

The antibacterial activity of cephalosporin may be predictable from the σ_I and $\sigma_R°$ of the substituent at C-3 after prediction of the β-lactam reactivity when the parabolic relationship between the antibacterial activity and the β-lactam chemical reactivity is taken into account. We consider that a similar prediction may be applicable to cephem derivatives, when the substituents at 7α-, 7β-, and 1-positions are fixed. However, the minimal point and the curvature of the parabola may vary depending upon the nature of the substituents at C-1 and C-7 because the atom at the 1-position strongly affects the β-lactam reactivity,[37,39] the 7β-substituent may mainly affect the binding affinity to the target enzymes,[40] and the 7α-methoxy substituent sterically protects from an attack of β-lactamase and affects on another factor of the antibacterial activity of 3-cephem derivatives.[37]

Acknowledgment. We thank Drs. W. Nagata, H. Matsumura, M. Yoshioka, M. Narisada, and S. Yamamoto of these laboratories for providing us with the samples used, Dr. Y. Terui for his helpful discussions, Dr. M. Takasuka for the IR spectral measurements, and Dr. T. Yoshida and K. Motokawa for the MIC measurements.

Registry No. **1a**, 92096-29-2; **1b**, 36923-19-0; **2a**, 39647-23-9; **2b**, 29126-12-3; **3a**, 92096-34-9; **3b**, 85659-59-2; **4a**, 92096-30-5; **4b**, 92096-35-0; **5a**, 92096-31-6; **6a**, 51761-82-1; **6b**, 35246-64-1; **7a**, 26382-85-4; **7b**, 41128-81-8; **8a**, 92096-38-3; **9a**, 70688-01-6; **9b**, 67366-01-2; **10b**, 35246-65-2; **11b**, 54639-48-4; **12a**, 92096-36-1; **12b**, 51761-93-4; **13b**, 51761-94-5; **14a**, 92096-32-7; **14b**, 92096-37-2; **15a**, 92096-33-8; **15b**, 63821-56-7.

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Peptide Sweeteners. 6. Structural Studies on the C-Terminal Amino Acid of L-Aspartyl Dipeptide Sweeteners

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Stereochemical and structural aspects of the variations in the C-terminal residue of L-aspartyl-L-phenylalanine methyl ester have been investigated. Novel configurational analogues such as L-aspartyl-D-alanine benzyl ester and L-aspartyl-D-α-aminobutyric acid benzyl ester were found to be sweet. In addition, chiral and achiral α,α-dialkylglycine and α-aminocycloalkanecarboxylic acids were incorporated into the dipeptides. The L-aspartic acid based dipeptide derivatives of α-aminoisobutyric acid methyl ester, α-aminocyclopropanecarboxylic acid methyl ester, α-aminocyclobutanecarboxylic acid methyl ester, and α-aminocyclopentanecarboxylic acid methyl ester are sweet. Dipeptides with α-aminocyclohexanecarboxylic acid methyl ester and α-aminocycloheptanecarboxylic acid methyl ester are bitter, whereas the analogues with α-aminocyclooctanecarboxylic acid methyl ester, α,α-diethylglycine methyl ester, and α-aminoisobutyric acid benzyl ester are tasteless. Aspects on chirality and effective volume of the C-terminal residue are discussed and correlated with taste.

Since the discovery of L-aspartyl-L-phenylalanine methyl ester,[1] structure–taste studies have shown that the aspartic acid residue is essential for the sweet taste and can be substituted only by an aminomalonate residue.[2] However,

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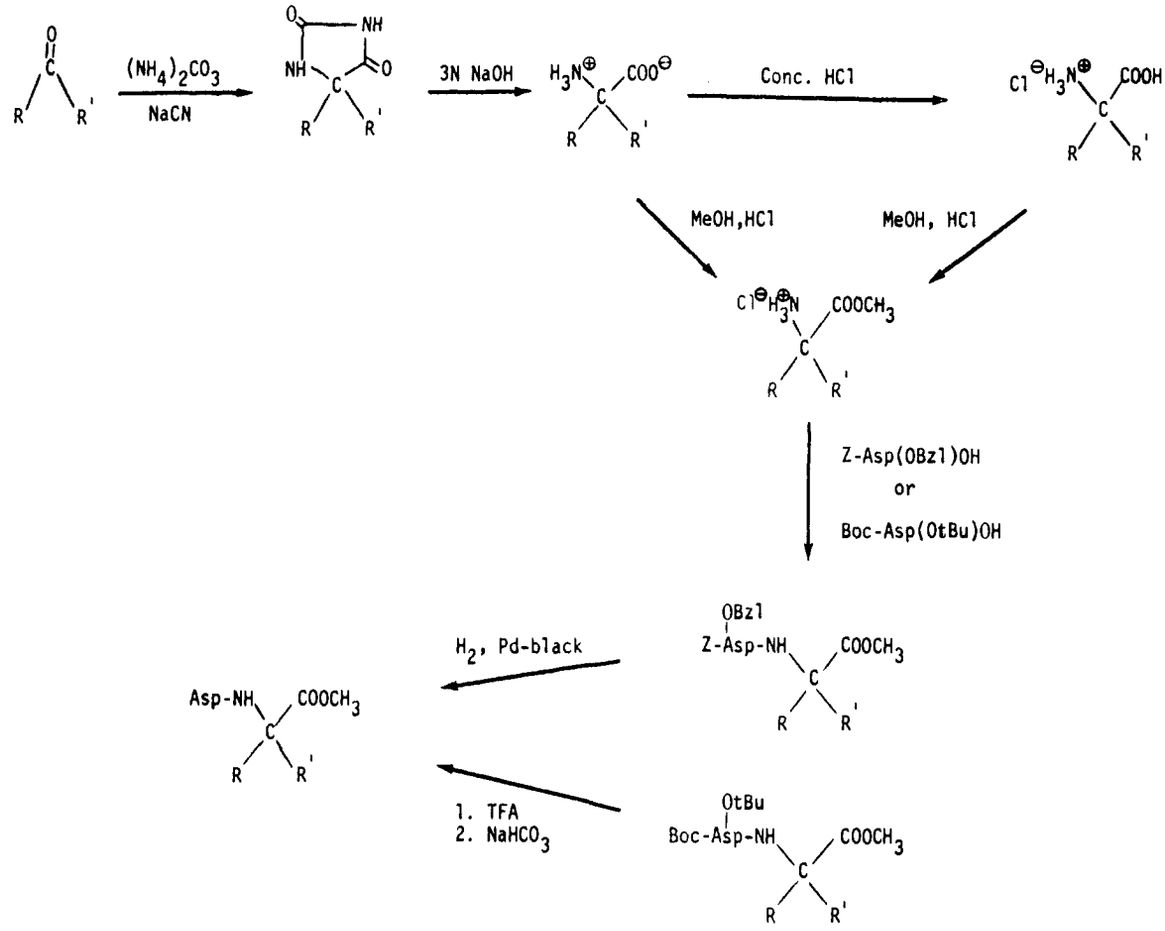
Scheme I. General Synthetic Route for the Synthesis of α,α'-Dialkylglycines and α-Aminocycloalkanecarboxylic Acids and the L-Aspartyl Dipeptide Derivatives



the C-terminal residue has considerable lattitude with respect to modification.[3-8] As suggested in a unified model by Ariyoshi,[4] the C-terminal moieties of sweet peptides consist of two hydrophobic groups, the side chain and the ester, which are different in size. The sweet and nonsweet formulas in this model were represented in Fischer projections as in Figure 1. Furthermore, Mazur et al.[9] showed that the size of the hydrophobic groups is associated with the configurations of the C-terminal residues. In the L-configuration, the side chain, R_2, is required to be considerably larger than the ester group, R_1. Conversely, the relationship is reversed in the D configuration, where the side chain is small and the ester is large.

In this paper, we investigate configurational aspects of the C-terminal residue of L-aspartyl dipeptide sweeteners. L-Aspartyl-D-alanine benzyl ester was prepared and found to be sweet. We then undertook to prepare a series of L-aspartyl dipeptide derivatives with disubstitution on the

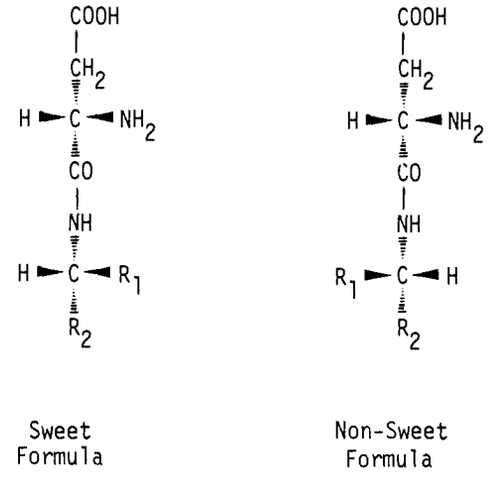


Figure 1. Sweet and nonsweet formulas in Fischer projections.

α-carbon of the C-terminal residues. These structures include chiral and achiral α,α-disubstituted glycines and α-aminocycloalkanecarboxylic acids.

Synthesis. The α,α-disubstituted amino acids and the α-aminocycloalkane amino acids were prepared in good yields according to the Bucherer–Lieb method.[10] This method involves the formation of a hydantoin from a ketone, followed by alkaline hydrolysis to the amino acid. The methyl esters were prepared from the corresponding amino acids by Fischer esterification.[11] The benzyl esters

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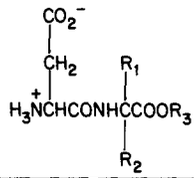
Table I. Structure–Taste Relationships of L-Aspartyl-D-alkylglycine Esters and L-Aspartyl-α,α-dialkylglycine Esters



no.	R_1	R_2	R_3	taste[a]
1	CH_3	H	CH_2Ph	sweet
2	CH_2CH_3	H	CH_2Ph	sweet
3	CH_3	CH_3	CH_2Ph	tasteless
4	CH_3	CH_3	CH_3	sweet
5	CH_2CH_3	CH_2CH_3	CH_3	tasteless

[a] The taste described here is not quantitatively established.

were obtained by refluxing a solution of the amino acid in benzyl alcohol in the presence of *p*-toluenesulfonic acid.[12]

Amino acid esters were coupled to suitably protected aspartic acid by either the *N,N'*-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole method[13] or the mixed anhydride method using isobutyl chloroformate.[14] Protecting groups were removed by conventional methods. A general synthetic route is provided in Scheme I.

In the coupling of α-aminocyclopropanecarboxylic acid methyl ester, *N*-(*tert*-butyloxycarbonyl)aspartic acid β-*tert*-butyl ester was used, since it was reported that the cyclopropane ring is susceptible to cleavage by catalytic hydrogenation.[15]

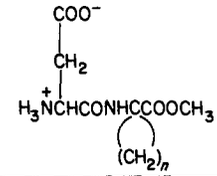
Results and Discussion

Two configurational analogues of L-aspartyl-L-phenylalanine methyl ester, namely, L-aspartyl-D-alanine benzyl ester (1) and L-aspartyl-D-α-aminobutyric acid benzyl ester (2), were prepared. Both compounds were found to be sweet (Table I). In terms of the spatial arrangement of the two hydrophobic moieties and their size, these groups resemble L-aspartyl-L-phenylalanine methyl ester. Since the C-terminal residues are in D-configuration, the benzyl ester replaces the benzyl group of the side chain, while the methyl (compound 1) or the ethyl (compound 2) group subsitutes for the ester. The size relationship among the hydrophobic groups is thus preserved. These results are consistent with the findings by Mazur et al.[9]

In the case of compound 2, which derives from 1 by the addition of a methylene on the side chain, the taste was not altered. On the other hand, if a methyl group is substituted at the α-carbon of the C-terminal amino acid of compound 1, the resulting compound, L-aspartyl-α-aminoisobutyric acid benzyl ester (3), is tasteless (Table I). At this point, we believed that the loss of sweet taste in compound 3 was due to the lack of chirality of the C-terminal amino acids.

This observation led us to prepare the compound L-aspartyl-α-aminoisobutyric acid methyl ester (4), which was found to be quite sweet (Table I). Therefore, the lack of sweet taste of L-aspartyl-α-aminoisobutyric acid benzyl ester (3) cannot be due to the achirality of the C-terminal amino acid residue but must be attributed to a conformational effect. Several α,α-dialkylglycine peptides have also been prepared and found to be sweet. The compounds[20] include L-aspartyl-DL-α,α-methyl-*n*-propylglycine methyl ester, L-aspartyl-DL-α,α-methylphenylglycine methyl ester, and L-aspartyl-DL-α,α-methylbenzylglycine methyl ester.[3] Studies involving NMR and computer simulations are now in progress to determine the preferred conformations for these molecules.

Table II. Structure–Taste Relationships of L-Aspartyl-α-aminocycloalkanecarboxylic Acid Methyl Esters



no.	ring size	taste[a]
6	cyclopropane	sweet
7	cyclobutane	sweet
8	cyclopentane	sweet
9	cyclohexane	bitter
10	cycloheptane	very bitter
11	cyclooctane	tasteless

[a] The taste described here is not quantitatively established.

We then synthesized L-aspartyl-α,α-diethylglycine methyl ester (5), which we found to be tasteless (Table I). In this compound, since the C–C bonds of the side chains are free to rotate, the ethyl groups occupy a much larger volume than the methyl groups in compound 4. It is likely that the overall shape and extensions of the side chains of the C-terminal of 5 cannot be accommodated by the taste receptor.

As a result of the above observations, we embarked on the synthesis of a new series of compounds in which the C-terminal residues consisted of α-aminocycloalkanecarboxylic acid methyl esters. We expected that a cyclic side chain would introduce constraints on the conformational flexibility of the C-terminal residue. Therefore, these cyclic amino acids residues are limited to fewer possible conformations, which in turn allows us to observe the effects of size, hydrophobicity, and rigidity of the cycloalkane ring on taste.

The taste properties of L-aspartyl-α-aminocycloalkanecarboxylic acid methyl esters 6–11 are listed in Table II.

The peptides 6 and 7, with cyclopropane and cyclobutane α-amino acids, respectively, are quite sweet, whereas the next higher homologue 8, is sweet but with reduced potency. As the cycloalkane ring of the dipeptide derivative is expanded to cyclohexane and cycloheptane, both peptides 9 and 10 are bitter. The compound with a cycloheptane α-amino acid is more intensely bitter than the peptide with a cyclohexane ring. The cyclooctane containing homologue 11 is tasteless.

These results showed the close relationship between the effective volume of the side chain and the taste properties. In this homologous series, the peptides containing amino acids with small cycloalkane rings are sweet, whereas those with larger rings are not sweet. Compounds 6–8 can accommodate the hydrophobic requirements of the sweet receptor. Compounds 9 and 10 can have a larger effective interaction with the hydrophobic zone of taste receptor and thereby trigger bitter taste in agreement with a model proposed by Temussi.[16] The cyclooctane derivative 11 is too large to affect the taste receptor.

As expected, L-aspartyl-α-aminocyclopropanecarboxylic acid methyl ester (6) has the same taste property as L-

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aspartyl-α-aminoisobutyric acid methyl ester (4), since the cyclization of the side chains does not involve dramatic changes in molecular dimensions. On the other hand, it is interesting to compare the effective volume of the side chain of L-aspartyl-α-aminocyclopentanecarboxylic acid methyl ester (5). Without the constraint of a ring, the side chains of α,α-diethylglycine are free to rotate and occupy much greater volume than the cyclopentane in compound 8. This difference in size and shape can account for the difference in taste for these two compounds.

In this paper, we have shown that the chirality of the C-terminal amino acid on L-aspartyl dipeptide is not essential for the sweet taste. We have also noted a key factor for the sweet taste, namely, the effective volume of the side chain of the C-terminal residue. This should not be misconstrued to imply that the achiral residue is symmetrically arrayed about the aspartic acid residue. In fact, the NMR spectra of compound 4 showed that the two methyls of the α-aminoisobutyric acid residue are magnetically nonequivalent, whereas in the amino acid itself, the methyls are magnetically equivalent. The same relationships also exist in aminocyclopropane amino acid and its analogous dipeptide derivative.

Since the compounds in the series of L-aspartyl-α-aminocycloalkanecarboxylic acid methyl esters vary only in the number of methylenes in the cycloalkane ring, we believe that all taste responses (sweet and bitter) for these molecules occur at the same receptor. Future work will focus on the influence on taste of selected substitutions on the small rings of cycloalkane amino acid derivatives and on conformational studies of these compounds by NMR and computer simulations.

Experimental Section

Infrared spectra were recorded on a Pye-Unicam SP1025 spectrometer: absorptions are reported in reciprocal centimeters (cm^{-1}), with polystyrene as a calibration standard. Nuclear magnetic resonance spectra were recorded with a Varian EM 360 (60 MHz) or a 360-MHz spectrometer in Fourier-transform mode. Peak positions are reported in parts per million from tetramethylsilane as internal reference. Multiplet (m), quartet (q), triplet (t), doublet (d), or singlet (s) describe the multiplicity of resonances. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 141 polarimeter with a 10-cm water-jacketed cell. Microanalysis were performed by Galbraith Laboratories, Knoxville, TN. The analytical values are within 0.4% of the theoretical values.

All chemicals and reagents were purchased from Bachem, Inc. and Aldrich Chemical Co. Analytical TLC plates were purchased from E. Merck: silica gel 60F-254, aluminum backed. Preparative TLC plates were purchased from Analtech; silica gel GF, 2000 μm, glass backed. The plates were developed with ninhydrin, Cl_2–KI–starch or UV light (254 nm). The following chromatography systems were used: (1) EtOAc/hexane, 5:4; (2) EtOAc/cyclohexane, 1:1; (3) $CHCl_3$/MeOH, 9:1; (4) $CHCl_3$/MeOH/AcOH, 19:2:1; (5) *n*-BuOH/AcOH/H_2O, 3:1:1; (6) *n*-BuOH/AcOH/pyridine/H_2O, 4:1:1:2; (7) *n*-BuOH/AcOH/pyridine/H_2O, 30:10:3:12.

The new compounds have been qualitatively taste tested by four volunteers from our laboratories as solids (equal amounts of each, including standards) or in solution. Tasting of solutions was carried out with a standard 8% sucrose solution and the new compounds dissolved in doubly distilled water. At least three double-blind tests were performed by the panel on each compound to achieve reproducible results.

Preparation of Amino Acid Benzyl Esters. Benzyl D-alaninate Tosylate (1a). Compound 1a was prepared according to literature[12] from benzyl alcohol and p-toluenesulfonic acid as catalyst: 16.4 g (94%); mp 111–112°C (Lit.[12] mp 113–114°C); R_f (3) 0.19.

Benzyl D-α-Aminobutyrate Tosylate (2a). Compound 2a was prepared according to the above method and crystallized from methanol/ether to provide colorless crystals: 16.3 g (83%); mp 116–118 °C; $[\alpha]^{25}_D$ +4.9° (*c* 1.3, MeOH); R_f (4) 0.31. Anal. ($C_{18}H_{23}NO_5S$) C, H, N, S.

Benzyl α-Aminoisobutyrate Hydrochloride (3a). The tosylate salt of benzyl α-aminoisobutyrate was prepared as in the above method and converted to the hydrochloride salt. It was dissolved in a saturated solution of Na_2CO_3 and extracted with ether. The combined ethereal solutions were dried over anhydrous $MgSO_4$ and precipitated from the solution as the hydrochloride salt by the addition of dry HCl gas. The crude product was collected, washed with ether, and crystallized from methanol/ether to yield pure 3a: 26.0 g (58%); mp 164.5–165 °C; R_f (4) 0.34. Anal. ($C_{11}H_{16}NO_2Cl$) C, H, N.

Preparation of Spiro-5′-hydantoins by Bucherer–Lieb Synthesis. 3-Pentanespiro-5′-hydantoin (5a). To a solution of 3-pentanone (25.0 g, 290 mmol) in ethanol (225 mL) and water (200 mL) were added sodium cyanide (28.5 g, 438 mmol) and ammonium carbonate (119 g, 1.13 mol). The mixture was refluxed for 6 h with stirring. After dilution with water, the cooled mixture was acidified with concentrated hydrochloric acid. The crude hydantoin precipitated overnight upon cooling at 5 °C. Pure 8a was crystallized from water: 26.3 g (58%); mp 158–160 °C; R_f (4) 0.68. Anal. ($C_7H_{12}N_2O_2$) C, H, N.

Cyclobutanespiro-5′-hydantoin (7a). The compound was obtained according to the above method as colorless crystals: 6.05 g (67%); mp 223–223.5 °C; R_f (3) 0.51. Anal. ($C_6H_8N_2O_2$) C, H, N.

Cyclopentanespiro-5′-hydantoin (8a). The compound was obtained according to the above method as colorless crystals: 33.9 g (73%); mp 205–205.5 °C (lit.[17] mp 204–205 °C); R_f (3) 0.48.

Cyclohexanespiro-5′-hydantoin (9a). The compound was obtained according to the above method as colorless needles: 13.8 (82%); mp 216–218 °C (lit.[17] mp 221–225 °C); R_f (4) 0.68.

Cycloheptanespiro-5′-hydantoin (10a). The compound was obtained according to the above method as colorless crystals: 31.6 g (76%); mp 213–215 °C (lit.[17] mp 214–216 °C); R_f (3) 0.59.

Cyclooctanespiro-5′-hydantoin (11a). The compound was obtained according to the above method as colorless crystals: 19.32 g (44%); mp 240.5–241 °C; R_f (3) 0.64. Anal. ($C_{10}H_{16}N_2O_5$) C, H, N.

Alkaline Hydrolysis of Spirohydantoins to Amino Acids. α-Aminocyclobutanecarboxylic Acid (7b). Cyclobutanespiro-5′-hydantoin (4a; 5.97 g, 43 mmol) was suspended in 3 N sodium hydroxide (100 mL) and heated to reflux for 21 h. Compounds 5a–10a required 40–60 h. The reaction mixture was cooled and acidified to pH 6 with concentrated hydrochloric acid. All amino acids 5b–10b, except 4b, precipitated from this solution and were filtered, washed with cold water and acetone, and dried. The reaction mixture of 4b was evaporated to dryness. The solid was thoroughly dried under high vacuum and successively washed with warm anhydrous ethanol to obtain crude product. It was crystallized from water/acetone: 1.22 g (25%); mp >300 °C; R_f (5) 0.14.

α-Aminocyclopentanecarboxylic Acid (8b). The compound was obtained according to the above method as a white solid: 11.3 g (75%); mp >300 °C (lit.[17] mp 320 °C); R_f (5) 0.28.

α-Aminocyclohexanecarboxylic Acid (9b). The compound was obtained according to the above method as a white solid: 5.8 g (68%); mp >300 °C (lit.[18] mp 334–335 °C); R_f (5) 0.40.

α-Aminocycloheptanecarboxylic Acid (10b). The compound was obtained according to the above method as a white solid: 18.3 g (100%); mp >300 °C (lit.[17] mp 320 °C); R_f (5) 0.50.

α-Aminocyclooctanecarboxylic Acid (11b). The compound was obtained according to the above method as a white solid: 116.8 g (100%); mp >300 °C (lit.[19] mp 310–316 °C); R_f (5) 0.36.

α,α-Diethylglycine Hydrochloride (5b). 3-Pentanespiro-5′-hydantoin (8a; 24.3 g, 156 mmol) was hydrolyzed with 1.25 N sodium hydroxide under pressure according to the literature.[19]

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(20) The synthesis and taste properties of these compounds will be reported elsewhere.

The reaction mixture was diluted with water, acidified to pH 1 with concentrated hydrochloric acid, and evaporated to dryness. The residue was thoroughly dried under high vacuum and washed with anhydrous ethanol. The combined ethanol washes were treated with decolorizing charcoal, filtered, and evaporated to provide crude amino acid hydrochloride. Compound **5b** was crystallized from methanol/ether to yield a colorless solid: 17.2 g (66%); mp 283–284 °C; R_f (5) 0.20. Anal. ($C_6H_{14}NO_2Cl$) C, H, N.

Conversion of the Zwitterionic Amino Acids to the Hydrochloride Salts. α-Aminocyclopentanecarboxylic Acid Hydrochloride (8c). The amino acid α-aminocyclopentanecarboxylic acid (**8b**; 6.65 g, 51 mmol) was suspended in hot water (50 mL). Concentrated hydrochloric acid (65 mL) was added and the majority of solid dissolved. The solution was filtered, treated with decolorizing charcoal, filtered, and evaporated to dryness under reduced pressure to provide a white solid: 8.61 g (96%); mp >300 °C; R_f (5) 0.28.

α-Aminocyclohexanecarboxylic Acid Hydrochloride (9c). The compound was obtained according to the above method as a white solid: 3.20 g (86%); mp >300 °C; R_f (5) 0.38.

α-Aminocycloheptanecarboxylic Acid Hydrochloride (10c). This compound was obtained according to the above method as a white solid: 7.05 g (44%); mp >300 °C; R_f (5) 0.49.

α-Aminocyclooctanecarboxylic Acid Hydrochloride (11c). This compound was obtained according to the above method as a white solid: 12.3 g (60%); mp >300 °C; R_f (5) 0.32.

Preparation of Amino Acid Methyl Esters by Fischer Esterification. Methyl α-Aminoisobutyrate Hydrochloride (4a). The amino acid α-aminoisobutyric acid (2.36 g, 22.9 mmol) was dissolved in freshly distilled methanol (35 mL). Under anhydrous conditions, dry HCl gas was bubbled into the solution at 0 °C, and the solution was then refluxed for 4 h. After evaporation of the solvent under reduced pressure, the residue was thoroughly dried under high vacuum. The residue was dissolved in ice-cold saturated Na_2CO_3 solution and extracted successively with ether (four times). The combined ethereal extracts were washed with dilute Na_2CO_3 solution (one time) and brine, and dried over anhydrous $MgSO_4$.

The amino acid ester was precipitated from the ether solution as the hydrochloride salt upon the addition of anhydrous HCl at 0 °C. The solid was collected and crystallized from methanol/ether to provide pure **4a**: 1.91 g (54%); mp 181 °C dec; R_f (5) 0.29; IR (KBr) 3480 (NH), 1760 (C=O, ester); NMR (CD_3OD) δ 1.60 (s, 6 H, CH_3), 3.88 (s, 3 H, OCH_3). Anal. ($C_5H_{12}NO_2Cl$) C, H, N.

Methyl α,α-Diethylglycinate Hydrochloride (5c). The compound was obtained according to the above method as a white solid: 1.91 g (35%); mp 194 °C dec; R_f (5) 0.41. Anal. ($C_7H_{16}NO_2Cl$) C, H, N.

Methyl α-Aminocyclopropanecarboxylate Hydrochloride (6a). The compound was obtained according to the above method as colorless crystals: 0.28 g (18%); mp 184–184.5 °C; R_f (5) 0.28; NMR (CD_3OD) δ 1.37 and 1.57 (2 dd, J_{gem} = 6 Hz, J_{trans} = 9 Hz, 4 H, CH_2), 3.82 (s, 3 H, OCH_3). Anal. ($C_5H_{10}NO_2Cl \cdot {}^1/_2H_2O$) C, H, N.

Methyl α-Aminocyclobutanecarboxylate Hydrochloride (7c). The compound was obained according to the above method as a white solid: 0.37 g (65%); mp 197–197.5 °C dec; R_f (5) 0.25. Anal. ($C_6H_{12}NO_2Cl \cdot {}^1/_4H_2O$) C, H, N.

Methyl α-Aminocyclopentanecarboxylate Hydrochloride (8d). The compound was obtained according to the above method as a white solid: 2.64 g (45%); mp 200–200.5 °C dec; R_f (5) 0.46. Anal. ($C_7H_{14}NO_2Cl$) C, H, N.

Methyl α-Aminocyclohexanecarboxylate Hydrochloride (9d). The compound was obtained according to the above method as a white solid: 2.73 g (63%); mp 203.5–204.5 °C dec; R_f (5) 0.49. Anal. ($C_8H_{16}NO_2Cl$) C, H, N.

Methyl α-Aminocycloheptanecarboxylate Hydrochloride (10d). The compound was obtained according to the above method as a white solid: 2.66 g (83%); mp 222–223 °C; R_f (5) 0.49. Anal. ($C_9H_{18}NO_2Cl$) C, H, N.

Methyl α-Aminocyclooctanecarboxylate Hydrochloride (11d). The compound was obtained according to the above method as a white solid: 2.16 g (67%); mp 235–239 °C dec; R_f (5) 0.42. Anal. ($C_{10}H_{20}NO_2Cl$) C, H, N.

Preparation of Protected Dipeptides. *N*-(*tert*-Butyloxycarbonyl)-β-*tert*-butyl-L-aspartyl-D-alanine Benzyl Ester (1b). The dicyclohexylamine salt of *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid β-butyl ester (5.74 g, 12.2 mmol) was liberated with a 1 N $KHSO_4$ solution, extracted into ethyl acetate, dried over $MgSO_4$ and evaporated under reduced pressure. A mixture of benzyl D-alaninate tosylate (**1a**; 4.48 g, 12.8 mmol), *N*-methylmorpholine (14.3 mL, 12.8 mmol), and 1-hydroxybenzotriazole (1.65 g, 12.2 mmol) was dissolved in CH_2Cl_2 (50 mL) at −10 °C and then *N,N'*-dicyclohexylcarbodiimide (2.52 g, 12.2 mmol) was added. The reaction was allowed to come to room temperature overnight. The reaction mixture was filtered and the solvent was evaporated under reduced pressure. The residue was taken up in ethyl acetate, washed with water (one time), 5% $NaHSO_4$ (three times), water (one time), 1% $NaHCO_3$ (three times), water (one time), and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield **1b** as a colorless oil: 5.0 g (91%); R_f (1) 0.47.

***N*-(*tert*-Butyloxycarbonyl)-β-*tert*-butyl-L-aspartyl-D-α-aminobutyric Acid Benzyl Ester (2b).** The compound was obtained according to the above method as a colorless oil: 6.80 g (87%); R_f (4) 0.81.

***N*-(*tert*-Butyloxycarbonyl)-β-*tert*-butyl-L-aspartyl-α-aminoisobutyric Acid Benzyl Ester (3b).** To a solution of *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid β-*tert*-butyl ester (0.81 g, 2.78 mmol) in anhydrous tetrahydrofuran (80 mL) was added *N*-methylmorpholine (0.31 mL, 2.78 mmol). The solution was cooled to −15 °C and isobutyl chloroformate (0.36 mL, 2.78 mmol) was added. After 5 min, a solution of benzyl α-aminoisobutyrate hydrochloride (**3a**; 0.63 g, 2.76 mmol) and *N*-methylmorpholine (0.30 mL, 2.76 mmol) in tetrahydrofuran/water (20 mL) was added. The reaction was allowed to proceed at −15 °C for 1 h and then at 5 °C for 24 h. The solvent was removed under reduced pressure. The residue was dissolved in ether, washed with water (one time), 5% $NaHSO_4$ (three times), water (one time), 1% $NaHCO_3$ (six times), water (one time), brine (one time), and dried over $MgSO_4$. Ether was removed under reduced pressure to yield the crude product, which was crystallized from hexanes as a white solid: 1.15 g (90%); mp 117–119 °C; R_f (2) 0.57; $[\alpha]^{25}_D$ +16.9° (c 1.9, MeOH). Anal. ($C_{24}H_{36}N_2O_7$) C, H, N.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α-aminoisobutyric Acid Methyl Ester (4b).** The compound was obtained according to the above method as a white solid: 0.839 g (87%); mp 44–47 °C; R_f (2) 0.37; $[\alpha]^{25}_D$ −11.9° (c 2.4, MeOH); IR (film) 3400 (NH, amide), 1750 (C=O ester), 1690 (NH, amide I), 1550 (NH, amide II); NMR ($CDCl_3$) δ 1.47 and 1.50 (2 s, 3 H each, CH_3), 2.71 and 3.06 (2 dd, ABX, $^1J_{AB}$ = 17 Hz, $^3J_{AX}$ = 7 Hz, $^3J_{BX}$ = 4 Hz, 2 H, Asp $C_\beta H_2$), 3.64 (s, 3 H, OCH_3), 4.57 (m, 1 H, Asp C_α H), 5.13 (m, 4 H, CH_2Ph) 5.91 (d, ^3J = 5 Hz, 1 H, NH Asp), 6.98 (s, 1 H, $NHCMe_2$), 7.36 (2 s, 5 H each, Ph). Anal. ($C_{24}H_{28}N_2O_7$) C, H, N.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α,α-diethylglycine Methyl Ester (5d).** The compound was obtained according to the above method as an oil: 1.57 g (79%); R_f (2) 0.42; $[\alpha]^{25}_D$ −14.9° (c 2.0, MeOH).

***N*-(*tert*-Butyloxycarbonyl)-β-*tert*-butyl-L-aspartyl-α-aminocyclopropanecarboxylic Acid Methyl Ester (6b).** The compound was obtained according to the above method as fine white needles: 0.11 g (44%); mp 85–86 °C; R_f (4) 0.48; $[\alpha]^{25}_D$ −6.2° (c 1.0, MeOH). Anal. ($C_{18}H_{30}N_2O_7$) C, H, N.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α-aminocyclobutanecarboxylic Acid Methyl Ester (7d).** The compound was obtained according to the above method as fine white needles: 0.23 g (74%); mp 95.5–96 °C; R_f (2) 0.36; $[\alpha]^{25}_D$ −6.8° (c 1.0, MeOH). Anal. ($C_{25}H_{28}N_2O_7$) C, H, N.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α-aminocyclopentanecarboxylic Acid Methyl Ester (8e).** The compound was obtained according to the above method as a white solid: 1.16 g (88%); mp 87.5–88 °C; R_f (3) 0.80; $[\alpha]^{25}_D$ −8.9° (c 1.1, MeOH). Anal. ($C_{26}H_{30}N_2O_7$) C, H, N.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α-aminocyclohexanecarboxylic Acid Methyl Ester (9e).** The compound was obtained according to the above method as a colorless oil: 0.51 g (85%); R_f (3) 0.84; $[\alpha]^{25}_D$ −13.3° (c 1.5, MeOH).

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α-aminocycloheptanecarboxylic Acid Methyl Ester (10e).** The com-

pound was obtained according to the above method as a colorless oil: 1.31 g (98%); R_f (3) 0.81; $[\alpha]^{25}_D$ −12.3° (c 1.4, MeOH).

N-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α-aminocyclooctanecarboxylic Acid Methyl Ester (11e). The compound was obtained according to the above method as a colorless oil: 0.659 (90%); R_f (2) 0.53; $[\alpha]^{25}_D$ −9.9° (c 1.1, MeOH).

Deprotection by Trifluoroacetic Acid. L-Aspartyl-D-alanine Benzyl Ester (1). Ice-cold trifluoroacetic acid (50 mL) was added to *N*-(*tert*-butyloxycarbonyl)-L-aspartyl-*tert*-butyl-D-alanine benzyl ester (**1b**; 5.0 g, 11.1 mmol). The reaction was allowed to proceed for 90 min at room temperature. The solvent was evaporated under reduced pressure. The residue was triturated with isopropyl ether to give a white solid, which was collected by filtration. The trifluoroacetate salt of 1 was dissolved in a mixture of water (30 mL) and methanol (10 mL). The pH of the solution was adjusted to 5 with an aqueous $NaHCO_3$ solution, where 1 precipitated from the solution. It was collected and recrystallized from water: 2.55 g (78%); mp 158–159 °C; R_f (6) 0.60; $[\alpha]^{23}_D$ +26.5° (c 1.0, MeOH). Anal. ($C_{14}H_{18}N_2O_5$) C, H, N.

L-Aspartyl-D-α-aminobutyric Acid Benzyl Ester (2). The compound was obtained according to the above method as colorless crystals: 2.90 g (67%); mp 158.5–159 °C; R_f (6) 0.57; $[\alpha]^{25}_D$ +32.3° (c 1.1, MeOH). Anal. ($C_{15}H_{20}N_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

L-Aspartyl-α-aminoisobutyric Acid Benzyl Ester (3). The compound was obtained according to the above method as a white solid: 0.066 g (43%); mp 173.5–174 °C; R_f (5) 0.39; $[\alpha]^{25}_D$ +20.3° (c 0.5, MeOH). Anal. ($C_{15}H_{20}N_2O_5$) C, H, N.

L-Aspartyl-α-aminocyclopropanecarboxylic Acid Methyl Ester (6). The compound was obtained according to the above method: 0.024 g (79%); mp 110–111 °C dec; R_f (5) 0.18; $[\alpha]^{25}_D$ +35.5° (c 1.0, MeOH). Anal. ($C_9H_{14}N_2O_5$) C, H, N.

Deprotection by Catalytic Hydrogenolysis. L-Aspartyl-α-aminoisobutyric Acid Methyl Ester (4). *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α-aminobutyric acid methyl ester (**4b**; 1.73 g, 3.79 mmol) was dissolved in methanol (50 mL). Nitrogen was bubbled into the solution for 10 min. Palladium-black (ca. 5% by weight) was added at once. Nitrogen addition was continued for 5 min and then a slow stream of hydrogen was bubbled through the solution for 4 h. After filtration and evaporation of the solvent, the solid was precipitated from methanol/ether as a white solid: 0.825 g (94%); mp 100–103 °C; R_f (7) 0.33; $[\alpha]^{25}_D$ +24.1° (c 0.8, MeOH); NMR (CD_3OD) δ 1.46 and 1.50 (2 s, 6 H, CH_3), 2.53 and 2.71 (2 dd, ABX, $^1J_{AB}$ = 17 Hz, $^3J_{AX}$ = 9 Hz, $^3J_{BX}$ = 5 Hz, 2 H, Asp $C_\beta H_2$), 3.65 (s, 3 H, OCH_3), 4.02 (dd, $^3J_{XA}$ = 9 Hz, $^3J_{XB}$ = 5 Hz, 1 H, Asp C_α H). Anal. ($C_9H_{16}N_2O_5$) C, H, N.

L-Aspartyl-α,α-diethylglycine Methyl Ester (5). The compound was obtained according to the above method as a white solid: 0.055 g (96%); mp 104–105 °C; R_f (7) 0.44; $[\alpha]^{25}_D$ +18.6° (c 1.2, MeOH). Anal. ($C_{11}H_{20}N_2O_5$) C, H, N.

L-Aspartyl-α-aminocyclobutanecarboxylic Acid Methyl Ester (7). The compound was obtained according to the above method as a white solid: 0.048 g (86%); mp 112–113 °C dec; R_f (5) 0.30; $[\alpha]^{25}_D$ +34.2° (c 0.86, MeOH). Anal. ($C_{10}H_{16}N_2O_5$) C, H, N.

L-Aspartyl-α-aminocyclopentanecarboxylic Acid Methyl Ester (8). The compound was obtained according to the above method as a white solid: 0.068 g (86%); mp 114–117 °C dec; R_f (5) 0.33; $[\alpha]^{25}_D$ +26.5° (c 1.1, MeOH). Anal. ($C_{11}H_{18}N_2O_5 \cdot {}^2/_3H_2O$) C, H, N.

L-Aspartyl-α-aminocyclohexanecarboxylic Acid Methyl Ester (9). The compound was obtained according to the above method as a white solid: 0.046 g (90%); mp 114–115 °C dec; R_f (5) 0.47; $[\alpha]^{25}_D$ +27.3° (c 1.0, MeOH). Anal. ($C_{12}H_{20}N_2O_5 \cdot {}^1/_2H_2O$) C, H, N.

L-Aspartyl-α-aminocycloheptanecarboxylic Acid Methyl Ester (10). The compound was obtained according to the above method as a white solid: 0.13 g (80%); mp 125–126 °C dec; R_f (5) 0.49; $[\alpha]^{25}_D$ +26.5° (c 1.0, MeOH). Anal. ($C_{13}H_{22}N_2O_5 \cdot {}^2/_3H_2O$) C, H, N.

L-Aspartyl-α-aminocyclooctanecarboxylic Acid Methyl Ester (11). The compound was obtained according to the above method as a white solid: 0.11 g (82%); mp 129–132 °C dec; R_f (5) 0.23; $[\alpha]^{25}_D$ +23.3° (c 1.0, MeOH). Anal. ($C_{14}H_{24}N_2O_5$) C, H, N.

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Registry No. 1, 92398-37-3; **1a**, 41036-32-2; **1b**, 92398-55-5; **2**, 92398-38-4; **2a**, 92470-56-9; **2b**, 92398-56-6; **3**, 92398-39-5; **3a**, 60421-20-7; **3b**, 92398-57-7; **4**, 92398-40-8; **4a**, 15028-41-8; **4b**, 92398-58-8; **5**, 92398-41-9; **5a**, 5455-34-5; **5b**, 92398-53-3; **5c**, 92398-54-4; **5d**, 92398-59-9; **6**, 92398-42-0; **6a**, 72784-42-0; **6b**, 92398-60-2; **7**, 92398-43-1; **7a**, 89691-88-3; **7b**, 22264-50-2; **7c**, 92398-47-5; **7d**, 92398-61-3; **8**, 92420-21-8; **8a**, 699-51-4; **8b**, 52-52-8; **8c**, 92398-48-6; **8d**, 60421-23-0; **8e**, 92398-62-4; **9**, 92398-44-2; **9a**, 702-62-5; **9b**, 2756-85-6; **9c**, 39692-17-6; **9d**, 37993-32-1; **9e**, 92398-63-5; **10**, 92398-45-3; **10a**, 707-16-4; **10b**, 6949-77-5; **10c**, 92398-49-7; **10d**, 92398-50-0; **10e**, 92398-64-6; **11**, 92398-46-4; **11a**, 710-94-1; **11b**, 28248-38-6; **11c**, 92398-51-1; **11d**, 92398-52-2; **11e**, 92398-65-7; benzyl α-aminoisobutyrate tosylate, 79118-16-4; 3-pentanone, 96-22-0; α-aminoisobutyric acid, 62-57-7; *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid-β-*tert*-butyl ester dicyclohexylamine, 1913-12-8; *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid-β-*tert*-butyl ester, 1676-90-0; cyclobutanone, 1191-95-3; cyclopentanone, 120-92-3; cyclohexanone, 108-94-1; cycloheptanone, 502-42-1; cyclooctanone, 502-49-8; α-aminocyclopropanecarboxylic acid, 22059-21-8.

Peptide Sweeteners. 7. Taste Relationships of Trifluoroacetyl-L-aspartylanilides

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A series of analogues of trifluoroacetyl-α-L-aspartylanilides substituted at various positions on the aromatic ring was synthesized and tasted. The position of the substitution is essential for the nature of the taste response. The results clearly establish the close relationship between sweet and bitter taste for these compounds. Combined electronic and topochemical contributions are discussed.

The relationship between structure and taste has been extensively studied for L-aspartyl dipeptide analogues.[1-9] Similar studies have been carried out for a related class of trifluoroacetyl-L-aspartylamides.[10-13] In this paper, our efforts focus on taste–structure relationships for compounds with selected substitutions on the aromatic ring

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