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Structure-taste Relationships of Aspartyl Tripeptide Esters

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A series of twenty four analogues of L-α-Asp-Gly-Gly-OMe has been synthesized in relation to structural features of sweet peptides. The rule in the structure-taste relationships of dipeptides is held in the sweet aspartyl tripeptide esters. In order for the aspartyl tripeptide esters to be sweet, the second amino acid must have a D-configuration and a small, compact alkyl group (Me, Et, or *i*-Pr) at R^2. An L-configuration of the third amino acid is required for a potent sweet taste. It has been concluded that the small alkyl group at R^2 participates in binding with the receptor through a hydrophobic interaction and increases the sweetness potency.

A wide variety of structural features have been known in sweet-tasting compounds. Attempts to elucidate the structural features necessary to elicit a sweet taste have been made, and several molecular theories have been proposed to relate the various structural features[1].

Since the discovery[2] of a sweet taste in L-α-Asp-L-Phe-OMe, a large number of analogues have been synthesized in an attempt to elucidate structure-taste relationships, and to obtain more potent and stable sweet peptides. It has been demonstrated that the L-Asp moiety in the original L-α-Asp-L-Phe-OMe molecule is restricted to L-aspartic acid[2] or aminomalonic acid[3] but that considerable modification can be made to the L-Phe-OMe moiety. The L-Phe-OMe has been successfully replaced by alkyl- and arylamines,[4] D-and L-amino acid esters,[2,5] D-amino acid alkylamides,[6] D-amino acid derivatives,[7] and aminomalonic acid diesters.[8] The importance of the peptide bond has also been demonstrated, since any modification in its structure results in a complete loss of sweetness.[9]

The sweetness potency in L-α-aspartyl dipeptide analogues has been quantitatively analyzed in relation to structural, electronic, and hydrophobic parameters.[10] A possible sweet conformation of L-α-Asp-L-Phe-OMe has also been determined by an NMR study, a potential energy calculation,[11] and an examination of L-α-Asp-Δ^z-Phe-OMe.[12]

In a previous paper,[13] the tastes of aspartyl dipeptides were relationalized through Fischer projection formulas. In this paper, these formulas have been translated into the structure shown in Fig. 1.

The structural features of sweet aspartyl dipeptide esters have been successfully explained on the basis of the general structure (Ia),[13a] in which the carboxyl and amino groups serve as a proton acceptor and a proton donor, respectively, in the hydrogen bonding with the receptor. The small hydrophobic side chain (S) is considered to participate in the hydrophobic interaction

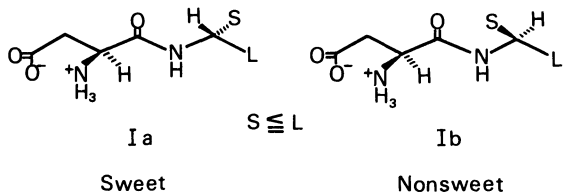


Fig. 1. General structure for sweet aspartyl dipeptide esters: S=small hydrophobic group (1—4 atoms); L=larger hydrophobic group (3—6 atoms).[13a]

with the receptor and to increase the sweetness potency. The larger hydrophobic group (L) seems to be associated with the conformation and hydrophilic-hydrophobic balance of the whole molecule. The hydrophilic-hydrophobic balance in a molecule is another important factor. Space-filling properties are also important, since the receptor site seems to be in the shape of a deep narrow cleft,[11] a pocket,[13b] or a hydrophobic tube[14] with the binding sites inside it.

In an attempt to extend the general structure (Ia) for sweet dipeptides to tripeptides, several aspartyl tripeptides have been synthesized and tested.[13b] The sweetness potencies were weak, 1—3 times sweeter than sucrose.

In order to confirm our previous conclusion on the roles of the S and L groups in the structure (Ia) and to extend the general structure (Ia) to tripeptides, various aspartyl tripeptide esters were synthesized, and several intensely-sweet tripeptides were obtained after an examination of the sweet structure (Ia) and a consideration of the hydrophilic-hydrophobic balance in a sweet molecule.

Synthesis

The protected dipeptides in Table 1 were prepared by the dicyclohexylcarbodiimide (DCC) method.[15] When the dipeptides (**1**, **3**, **5**) were obtained as oily residues, a *t*-butoxycarbonyl (Boc) group of these peptides was deprotected with hydrogen chloride in methanol and the resulting hydrochlorides (**2**, **4**, **6**) were crystallized. Compound **7** was left over from a previous experiment. The Boc group of the protected dipeptides in Table 1 was removed with *p*-toluenesulfonic acid monohydrate[16] in methanol, and the resulting dipeptide ester *p*-toluenesulfonates were used for the next step without further purification. The protected tripeptides in Table 2 were prepared by condensation of β-benzyl *N*-benzyloxycarbonyl-L-aspartate with the appropriate dipeptide ester using DCC. Compounds **42—44**, **47—49**, and **51** were prepared by the active ester method,[17] in which *N*-benzyloxycarbonyl-L-aspartic acid β-benzyl α-succinimido ester was coupled with the appropriate dipeptide ester. The desired tripeptide esters in Table 3 were obtained by deprotection of the benzyloxycarbonyl and benzyl groups from the protected tripeptides in Table 2 by hydrogenation over Pd/C.

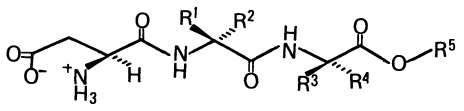
Results and Discussion

In order to evaluate contributions of the side chains

Table 1. Protected dipeptides

No.	Compound[a]	Yield %	Mp θ/°C	Crystn solvent[b]	Appearance[c]	$[\alpha]_D$ Degree[d]	Temp °C
1	Boc-Gly-L-Ala-OMe[e]	89.7	Oil				
2	HCl·H-Gly-L-Ala-OMe[f]	86.4	158.5—159.5	M—E	P	−49.8	23
3	Boc-Gly-D-Ala-OMe[e]	67.3	Oil				
4	HCl·H-Gly-D-Ala-OMe	93.8	158.5—159.5	M—E	P	+50.4	23
5	Boc-Gly-L-Val-OMe[e]	97.8	Oil				
6	HCl·H-Gly-L-Val-OMe	75.5	139.5—140.5	M—E	N	−18.8	22
7	HCl·H-Gly-L-Leu-OMe	—	163—165	M—E	P	−32.0	23
8	Boc-D-Ala-L-Ala-OMe	75.4	65.5—66.5	EA—H	N	−3.2	25
9	Boc-D-Ala-D-Ala-OMe	80.9	109—110	EA—H	N	+60.8	25
10	Boc-D-Ala-L-Ala-OEt	68.4	81—82	H	Pr	−1.8	23
11	Boc-D-Ala-L-Val-OMe	61.1	101.5—102.5	EA—H	P	+15.5	25
12	Boc-D-Ala-L-Leu-OMe	66.4	82—83.5	EA—H	N	−2.2	25
13	Boc-D-Ala-L-Phe-OMe	73.8	97.5—98.5	EA—H	N	+24.9	25
14	Boc-D-Val-L-Ala-OMe	72.9	98—99	EA—H	N	−8.1	25
15	Boc-D-Val-D-Ala-OMe	65.3	141—142	EA—H	N	+52.3	25
16	Boc-D-Val-L-Val-OMe	77.3	109—110	EA—H	N	+3.6	25
17	Boc-D-Val-L-Leu-OMe	60.9	87—88	H	Pr	−7.7	16
18	Boc-D-Val-L-Phe-OMe	72.3	105.5—106.5	EA—H	N	+4.5	23
19	Boc-D-Leu-L-Ala-OMe[g]	66.1	85—86	H	Pr	+3.0	21
20	Boc-L-Ala-Gly-OMe[e]	79.8	Oil				
21	Boc-L-Ala-L-Ala-OMe[h]	77.0	109—110	EA—H	N	−61.2	25
22	Boc-L-Abu-L-Val-OMe	75.0	110—111	EA—H	N	−44.5	23
23	Boc-L-Ala-L-Val-OMe[i]	67.5	65—66	EA—H	N	−50.0	30
24	Boc-L-Val-L-Ala-OMe[j]	62.8	141—142	EA—H	N	−53.2	27
25	Boc-L-Val-D-Ala-OMe[k]	57.4	97—98	EA—H	N	+8.7	21
26	Boc-L-Val-L-Val-OMe[l]	64.2	167—168	EA—H	N	−44.2	29
27	Boc-L-Val-L-Leu-OMe[m]	67.1	133—134	EA—H	Pr	−51.9	21

a) Abbreviations follow the recommendations of the IUPAC-IUB Commision on Biochemical Nomenclature in *J. Biol. Chem.*, **247**, 977 (1972). All compounds were analyzed for C, H, and N (Compounds **2**, **4**, **6**, and **7** were analyzed for C, H, N, and Cl), and results were within ±0.3% of the theoretical values. Complete analytical data for all compounds have been deposited at the office of the Chemical Society of Japan (Document No. 8447). b) Crystallization solvent: M, Methanol; E, Ether; EA, Ethyl Acetate; H, Hexane. c) Appearance: P, plates; N, needles; Pr, Prisms. d) In methanol, $c=1.0\%$. e) Not analyzed. f) G. Weitzel, F.-U. Bauer, and K. Eisele, Hoppe-Seyler's *Z. Physiol. Chem.*, **357**, 187 (1976); mp 161—162 °C; $[\alpha]_{578}^{20}$ −30.0° (*c* 1, DMF). g) E. N. Shepel, S. Iordanov, I. D. Ryabova, A. I. Miroshnikov, V. T. Ivanov, and Y. Ovachinnikov, *Bioorg. Khim.*, **2**, 581 (1976); $[\alpha]_D$ +3.1° (*c* 0.13, MeOH). h) H. Kinoshita, K. Inomata, O. Miyano, and H. Kotake, *Bull. Chem. Soc. Jpn.*, **52**, 2619 (1979); mp 105—106 °C; $[\alpha]_D$ −63.6° (*c* 0.66, MeOH). i) See reference h); mp 63—64 °C; $[\alpha]_D$ −49.5° (*c* 0.31, MeOH). j) P. G. Katsoyannis, Y. Okada, and C. Zault, *Biochemistry*, **12**, 2516 (1973); mp 141—142 °C; $[\alpha]_D^{26}$ −52.9° (*c* 1, MeOH). k) D. E. Nitecki, B. Halpern, and J. Westley, *J. Org. Chem.*, **33**, 864 (1968); mp 91—92 °C; $[\alpha]_D^{25}$ +8.0° (*c* 1, MeOH). l) D. J. Shafer, *J. Chem. Soc., Perkin Trans. 1*, **1972**, 1452; mp 167—168 °C. m) See reference k); mp 126—128 °C, $[\alpha]_D$ −54.2 (*c* 1, MeOH).



II

Fig. 2. L-α-Aspartyl tripeptide esters.

of each amino acid, the substituents R^1—R^5 of II were systematically modified. The results are summarized in Table 4. In the relationships between the sweet dipeptides (Ia) and tripeptides (II), S (a small hydrophobic group) of Ia corresponds to R^2 (a small alkyl group) of II, and L (a larger hydrophobic group) corresponds to $-CONHCR^3(R^4)COOR^5$. In systematic structural variation of the tripeptides (II), it was assumed that R^2 (a small alkyl group) in II was one of the groups responsible for sweetness, through a hydrophobic interaction with the receptor.

As reported previously,[13b] L-α-Asp-Gly-Gly-OMe (**76**), which is tasteless (Table 4), was selected as a standard of tripeptides because it has the simplest structure and a correlation with other tripeptides is easy.

As the first step, the contribution of the third amino acid to sweetness was evaluated. The introduction of a methyl group at R^4 of **76** gave a weakly sweet peptide (**52**). When the methyl group of **52** was introduced on the opposite side (R^3), the resulting peptide (**53**) was also weakly sweet. Replacement of the methyl group in

Table 2. Protected tripeptides, Z-L-Asp(OBzl)-X

No.	Compound[a] X	Yield %	TLC R_f[b]	Mp θ_m/°C	Crystn Solvent[c]	Appearance[d]	$[\alpha]_D$ Degree[e]	Temp °C
28	Gly-L-Ala-OMe[f]	89.3		Oil				
29	Gly-D-Ala-OMe[f]	89.3		Oil				
30	Gly-L-Val-OMe[f]	75.0		Oil				
31	Gly-L-Leu-OMe[f]	99.7		Oil				
32	D-Ala-L-Ala-OMe	68.6	0.81	145—146	EA—H	N	+3.3	22
33	D-Ala-D-Ala-OMe	52.6	0.73	141—142	EA—H	N	+27.7	22
34	D-Ala-L-Ala-OEt	58.6	0.80	139—140	EA	N	+4.2	22
35	D-Ala-L-Val-OMe	60.7	0.80	112—113	EA—H	N	+7.0	23
36	D-Ala-L-Leu-OMe	75.3	0.89	87—89	EA—H	N	−0.8	25
37	D-Ala-L-Phe-OMe	70.3	0.85	108—110	EA—H	A	+16.7	19
38	D-Val-L-Ala-OMe	67.0	0.86	168.5—169.5	EA	N	+4.7	22
39	D-Val-D-Ala-OMe	65.6	0.85	190—191	EA	N	+32.2	22
40	D-Val-L-Val-OMe	57.9	0.91	145—146 (125—126)[g]	EA—H	N	+11.2	19
41	D-Val-L-Leu-OMe	54.4	0.90	161.5—162.5	EA—H	N	+2.2	16
42	D-Val-L-Phe-OMe	67.8	0.90	203—205	D—EA	A	−28.8[h]	25
43	D-Leu-L-Ala-OMe	71.3	0.86	123—124	EA—H	N	+7.1	18
44	L-Ala-Gly-OMe	68.3	0.90	113.5—114.5	EA—H	N	−16.1	20
45	L-Ala-L-Ala-OMe	70.6	0.76	144—145 (114—115)[g]	EA—H	A	−26.0	19
46	L-Ala-L-Val-OMe	55.4	0.88	145—146	EA	N	−18.5	30
47	L-Abu-L-Val-OMe	71.3	0.89	149—150	EA	A	−17.7	18
48	L-Val-L-Ala-OMe	75.2	0.90	202—203	EA	N	−33.1	27
49	L-Val-D-Ala-OMe	89.0	0.95	164—165	EA	A	−10.0	18
50	L-Val-L-Val-OMe	63.9	0.92	145—146	EA	N	−24.6	29
51	L-Val-L-Leu-OMe	87.9	0.96	145—146	EA—H	N	−35.2	18

a) All compounds were analyzed for C, H, and N, and results were within ±0.3% of the theoretical values. Complete analytical data for all compounds have been deposited at the office of the Chemical Society of Japan (Document No. 8447). b) TLC: $CHCl_3$:MeOH:AcOH=45:4:1 (v/v), detected with I_2 vapor. c) Crystallization solvent: EA, Ethyl acetate; H, Hexane; D, *N*,*N*-Dimethylformamide. d) Appearance: N, Needles; A, Amorphous powder. e) In acetic acid, c=1.0%. f) Not analyzed. g) Softened. h) In *N*,*N*-dimethylformamide, c=0.5%.

52 by an isopropyl or an isobutyl group gave **54** and **55**, respectively, which were bitter. These results show that a modification of the third amino acid alone does not effect elicitation of sweetness.

Secondly, the contribution of the second amino acid to sweetness was evaluated. As mentioned above, it seems that a small alkyl group (Me, Et or *i*-Pr) at R^2 is required for sweetness. Therefore, in this experiment, a small alkyl group was introduced at R^2 so as to meet the sweet structure (Ia), in which S corresponds to R^2 of II in the configuration. As reported previously, replacement of a methyl group at R^2 of **76** gave **77**,[13b] which was 3 times sweeter than sucrose. Replacement of the methyl group by an ethyl or an isopropyl group gave **78** and **79**,[13b] which were sweet and tasteless, respectively. The ethyl ester analogue (**80**)[13b] of **79** was also tasteless. As described above, the modification of the second amino acid alone gave a similar result in the case of the modification of the third amino acid.

Thirdly, the combined contribution of the second and third amino acids was evaluated. In this experiment, a small alkyl group of the second amino acid was also introduced at R^2 by the reason mentioned above. Introduction of an alkyl group to the α-carbon of Gly in L-α-Asp-D-Ala-Gly-OMe (**77**) significantly increased the sweetness potency (**56—60**). The potent sweetness seems to result from an increased hydrophobicity as compared with **77**. The sweet taste changed to a bitter taste (**61**) with increasing hydrophobicity of the group (R^4). In the same way, when a small alkyl group was introduced to the α-carbon of Gly in L-α-Asp-D-Val-Gly-OMe (**79**), the resulting peptides (**62—65**) became intensely sweet. Compound **66** may be too hydrophobic to taste sweet. A substituent at R^2 is considered to be a small, compact alkyl group, such as Me, Et or *i*-Pr, from the previous work on the dipeptide sweeteners, especially in L-α-Asp-D-amino acid esters. A need for the small alkyl group (R^2) was confirmed by the fact that L-α-Asp-D-Leu-L-Ala-OMe (**67**) was not sweet but bitter. The *i*-Bu group in **67** seems to be too bulky to interact effectively at the sweet receptor site. These observations suggest that the hydrophilic-hydrophobic balance in a molecule and the space-filling properties are important. It is interesting that the sweetness potencies were significantly changed by altering the configuration of the third amino acid (**52** and **53**; **56** and **57**; **62** and **63**). The observation shows that the L-configuration of the third amino acid is

Table 3. Tripeptide esters, L-α-Asp-X

No.	Compound[a] X	Yield %	TLC R_f[b]	Mp θ_m/°C	Crystn Solvent[c]	Appearance[d]	$[\alpha]_D$ Degree[e]	Temp °C
52	Gly-L-Ala-OMe	57.3	0.12	162—163	W—A	N	−21.1	25
53	Gly-D-Ala-OMe	53.2	0.18	189—190 dec	W—A	N	+83.9	23
54	Gly-L-Val-OMe	40.1	0.25	167—168 (83)[f]	E—H	A	−9.2	20
55	Gly-L-Leu-OMe	64.6	0.21	84—85 dec	A—EA	A	−16.0	30
56	D-Ala-L-Ala-OMe	78.1	0.19	164—165	W—A	N	+16.9	25
57	D-Ala-D-Ala-OMe	87.2	0.16	182—183 dec	W—A	N	+112.9	30
58	D-Ala-L-Ala-OEt	62.2	0.19	166—167	W—A	N	+16.9	22
59	D-Ala-L-Val-OMe	63.3	0.24	182.5—183.5 dec	W	N	+22.7	30
60	D-Ala-L-Leu-OMe	65.3	0.38	189—190 dec	W—A	N	+16.5	26
61	D-Ala-L-Phe-OMe	73.6	0.29	181—182 dec	W—A	N	+40.1	24
62	D-Val-L-Ala-OMe	70.9	0.26	207—208 dec	W	N	+16.0	26
63	D-Val-D-Ala-OMe	66.7	0.24	221—222 dec	W—A	N	+94.7	22
64	D-Val-L-Val-OMe	62.1	0.30	202—203 dec	W	N	+15.7	22
65	D-Val-L-Leu-OMe	52.7	0.44	200—201	W	N	+9.9	23
66	D-Val-L-Phe-OMe	38.6	0.40	209—210	W	N	+16.2[g]	25
67	D-Leu-L-Ala-OMe	58.8	0.35	204—204.5 dec	W	N	+16.3	21
68	L-Ala-Gly-OMe	71.8	0.19	200—201 dec	W—A	N	−23	19
69	L-Ala-L-Ala-OMe	91.0	0.11	210—211 dec	W—A	A	−67.1	24
70	L-Ala-L-Val-OMe	59.2	0.34	185—186 dec	W	N	−53.9	29
71	L-Abu-L-Val-OMe	60.2	0.39	194—195 dec	W	N	−46.9	22
72	L-Val-L-Ala-OMe	74.7	0.32	210—211 dec	W	N	−58.5	25
73	L-Val-D-Ala-OMe	86.3	0.38	209—210 dec	W	N	+27.0	19
74	L-Val-L-Val-OMe	81.2	0.50	225.5—226.5 dec	W	N	−46.6	28
75	L-Val-L-Leu-OMe	72.6	0.38	222.5—223.6 dec	W	N	−36.0[g]	20

a) All compounds were analyzed for C, H, and N, and results were within ±0.3% of the theoretical values. Complete analytical data for all compounds have been deposited at the office of the Chemical Society of Japan (Document No. 8447). b) TLC: $CHCl_3$:MeOH:AcOH:H_2O=32:15:1:3 (v/v), detected with ninhydrin. c) Crystallization solvent: W, Water; A, Acetone. d) Appearance: N, Needles; A, Amorphous powder. e) In water, c=1.0%. f) Softened. g) In acetic acid, c=0.5%.

required for a potent sweet taste. The importance of the spacial orientation of the side chain may suggest another hydrophobic interaction at R^4 with the receptor site. The sweet receptor is considered to be a protein,[18] therefore, such an interaction appears to occur possibly at the receptor site.

Finally, in order to reconfirm the required configurations (R^2=small alkyl group; R^1=H) at the second amino acid, several tripeptides having opposite configurations at this chiral center were synthesized. All of the peptides (**68—75**) were essentially tasteless or bitter. Among them, however, compounds **71**, **73**, **74**, and **75** were bitter-sweet. The sweetness of these compounds did not come from contamination of their sweet isomers. Compound **74** was found by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to be free from **64**. TLC analyses of **71**, **73**, and **75** showed no other compounds to be detectable. The weak sweet taste observed in compounds **71**, **73**, **74**, and **75** with all L-configurations may show that sweetness cannot necessarily be correlated with D-isomerism at the second amino acid. These four compounds, however, were essentially bitter. It is therefore concluded that the general structure (Ia) for sweet dipeptides is extended to that for sweet tripeptides (II), in which R^1 is H and R^2 is a small alkyl group. Exceptions that **71**, **73**, **74**, and **75** with the L-configuration at the second amino acid showed a weak sweet taste along with a bitter taste may be interpreted in terms of their conformations, in which a certain conformer may possibly fit the narrow receptor pocket.

Summary

A series of twenty four analogues of L-α-Asp-Gly-Gly-OMe (**76**) has been synthesized in relation to our previous conclusion that the small alkyl group (S) of the sweet dipeptides (Ia) increases the sweetness potency through a hydrophobic interaction with the receptor. It has been concluded that the small alkyl group at R^2 in the tripeptides (II) participates in binding with the receptor through the hydrophobic interaction and increases the sweetness potency. In order for the tripeptides to be sweet, the second amino acid must have the D-configuration and a small, compact alkyl group (Me, Et or *i*-Pr) at R^2. The L-configuration of the third amino acid is required for a potent sweet taste. This suggests another hydrophobic interaction at R^4. The rule in the general structure (Ia) for the sweet dipeptides is held in the sweet tripeptides (II). The sweetness potencies of the tripeptides obtained here, however, were lower than those of aspar-

Table 4. Taste of tripeptide esters (L-α-Asp-X)[a]

No.	X	R^1	R^2	R^3	R^4	R^5	Taste[b]
76	Gly-Gly-OMe	H	H	H	H	Me	0
52	Gly-L-Ala-OMe	H	H	H	Me	Me	2
53	Gly-D-Ala-OMe	H	H	Me	H	Me	1
54	Gly-L-Val-OMe	H	H	H	*i*-Pr	Me	—
55	Gly-L-Leu-OMe	H	H	H	*i*-Bu	Me	—
77	D-Ala-Gly-OMe	H	Me	H	H	Me	3
78	D-Abu-Gly-OMe	H	Et	H	H	Me	1
79	D-Val-Gly-OMe	H	*i*-Pr	H	H	Me	0
80	D-Val-Gly-OEt	H	*i*-Pr	H	H	Et	0
56	D-Ala-L-Ala-OMe	H	Me	H	Me	Me	50
57	D-Ala-D-Ala-OMe	H	Me	Me	H	Me	5
58	D-Ala-L-Ala-OEt	H	Me	H	Me	Et	15
59	D-Ala-L-Val-OMe	H	Me	H	*i*-Pr	Me	50
60	D-Ala-L-Leu-OMe	H	Me	H	*i*-Bu	Me	15
61	D-Ala-L-Phe-OMe	H	Me	H	Bzl	Me	—
62	D-Val-L-Ala-OMe	H	*i*-Pr	H	Me	Me	25
63	D-Val-D-Ala-OMe	H	*i*-Pr	Me	H	Me	4
64	D-Val-L-Val-OMe	H	*i*-Pr	H	*i*-Pr	Me	30
65	D-Val-L-Leu-OMe	H	*i*-Pr	H	*i*-Bu	Me	40
66	D-Val-L-Phe-OMe	H	*i*-Pr	H	Bzl	Me	—
67	D-Leu-L-Ala-OMe	H	*i*-Bu	H	Me	Me	—
68	L-Ala-Gly-OMe	Me	H	H	H	Me	0
69	L-Ala-L-Ala-OMe	Me	H	H	Me	Me	—
70	L-Ala-L-Val-OMe	Me	H	H	*i*-Pr	Me	0
71	L-Abu-L-Val-OMe	Et	H	H	*i*-Pr	Me	−(2)
72	L-Val-L-Ala-OMe	*i*-Pr	H	H	Me	Me	0
73	L-Val-D-Ala-OMe	*i*-Pr	H	Me	H	Me	−(1)
74	L-Val-L-Val-OMe	*i*-Pr	H	H	*i*-Pr	Me	−(4)
75	L-Val-L-Leu-OMe	*i*-Pr	H	H	*i*-Bu	Me	−(4)

a) For structures, see Figure 2. b) Times sucrose (weight basis, sucrose=1). 0=tasteless; −=bitter; −( )= bitter-sweet, the number in parentheses is sweetness value.

tyl dipeptides. This result suggests the importance of the hydrophilic-hydrophobic balance and space-filling properties (shape, size, length, and conformation) in a sweet peptide. The tripeptides are generally more hydrophilic than potent sweet dipeptides and the hydrophilic property will cause the decreased sweetness potency. The receptor site for sweet amino acids and peptides seems to be not a plane but the shape of a pocket[13b] or a similar one as mentioned before. With increasing length of a peptide, it becomes difficult to fit such a narrow site. The decreased binding of the sweet molecule to the receptor reduces the potency.

Experimental

All melting points were taken on a Yanagimoto capillary melting point apparatus Model MP-21 and are uncorrected. Optical rotations were measured on a JASCO DIP-140 digital polarimeter with a 10-cm cell at room temperature and a 1% concentration. TLC was performed on precoated silica gel $60F_{254}$ plates (E. Merck) and spots were detected with ninhydrin or I_2 vapor. All compounds were essentially homogeneous on TLC. HPLC was carried out on a Hitachi 635A instrument with a Unisil Q C-18 reverse-phase column (4 mm ×25 cm) eluting with 23% methanol in pH 3.5 NaH_2PO_4 buffer at a flow rate of 0.8 ml/min. Sweetness potency was not evaluated by the panel method due to quantities available. In general, a panel evaluation needs a large quantity of sample. The yield, after purification, of each sweet peptide (Table 3) was less than 0.5 g. The quantity of tripeptides obtained here was insufficient for the panel evaluation. Therefore, sweetness evaluation was carried out by the author by matching a threshold concentration of each compound with that of sucrose. Test solutions of the tripeptides were made up at several concentrations. A series of the solutions were tasted up and down. Thus, it was possible to reproducibly determine the concentration which matched a 0.6% aqueous solution of sucrose. In order to confirm the sweetness values, compounds **56**, and **62—65** were also evaluated by a well trained sensory panelist, and the potencies were in fair agreement with those listed in Table 4. Therefore, it has been considered that the sweetness potencies in Table 4 are reproducible and reliable enough to discuss the functions of a small hydrophobic group.

Materials: Boc-amino acids were purchased from Peptide Institute Inc., except for Boc-L-Abu (Kokusan Chemical Works Ltd.). Esters of Gly, D-Ala, L-Phe, and L-Val were synthesized in our laboratory. Z-L-Asp(OBzl)-OH and esters of L-Ala and L-Leu were purchased from Kokusan Chemical Works Ltd.

Boc-Dipeptide Esters (1, 3, 5, 8—27). A typical run (**11** in Table 1) was as follows: To an ice-cooled stirred solution of H-L-Val-OMe·HCl (2.77 g, 16.5 mmol) and triethylamine (Et_3N) (1.67 g, 16.5 mmol) in 40 ml of chloroform was added Boc-D-Ala-OH (2.84 g, 15 mmol), followed by DCC (3.09 g,

15 mmol). The resulting mixture was stirred in an ice-bath for 1 h and then kept standing overnight, after which a few drops of acetic acid were added to the reaction mixture. The mixture was then stirred for 15 min and filtered. The filtrate was washed successively with water, a 10% citric acid solution, a 5% sodium hydrogencarbonate solution and water and then concentrated under reduced pressure to give an oily product. The oil was crystallized from ethyl acetate–hexane to give 2.77 g (61.1%) of Boc-D-Ala-L-Val-OMe (**11**) as plates. Recrystallization was effected from the same solvent. The data are given in Table 1.

Protected Tripeptide Esters (28—51). a) The DCC Method. A typical run (**35** in Table 2) was as follows: To a solution of Boc-D-Ala-L-Val-OMe (**11**, 2.23 g, 7.38 mmol) in 20 ml of methanol was added *p*-toluenesulfonic acid monohydrate (TosOH·H_2O) (1.78 g, 9.4 mmol). The mixture was stirred at 30 °C for 5 h. The solvent was evaporated under reduced pressure at a bath temperature of 50 °C to give H-D-Ala-L-Val-OMe·TosOH as an oily residue. The residue was used for the next step without further purification. H-D-Ala-L-Val-OMe·TosOH thus obtained was dissolved in 40 ml of chloroform and neutralized with Et_3N (0.95 g, 9.4 mmol) in an ice-bath. To the stirred ice-cooled solution was added Z-L-Asp(OBzl)-OH (2.50 g, 7 mmol), followed by DCC (1.44 g, 7 mmol). The mixture was stirred while cooling for 1 h and at room temperature for 3 h and then kept standing overnight. A few drops of acetic acid were then added to the reaction mixture. The mixture was stirred at room temperature for 15 min and then filtered. The filtrate was washed successively with water, 1M HCl (twice), a 5% sodium hydrogencarbonate solution (twice) and water, and then concentrated under reduced pressure to leave an oily product. The oil was crystallized from ethyl acetate–hexane to give Z-L-Asp(OBzl)-D-Ala-L-Val-OMe (**35**) as needles. Recrystallization was carried out from the same solvent. The data are given in Table 2.

b) The active ester method. A typical run (**51** in Table 2) was as follows: DCC (3.10 g, 15 mmol) was added to an ice-cooled mixture of Z-L-Asp(OBzl)-OH (5.36 g, 15 mmol) and *N*-hydroxysuccinimide (2.07 g, 18 mmol) in 50 ml of chloroform with stirring. The mixture was stirred in an ice-bath for 1 h and at room temperature for 3 h and then kept standing overnight. A few drops of acetic acid were then added to the reaction mixture, which was stirred at room temperature for 15 min and filtered. The filtrate was washed successively with water, a 5% sodium hydrogencarbonate solution and water, and then concentrated under reduced pressure to leave an oily product, which turned to crystals upon standing. Recrystallization from ethyl acetate–hexane gave Z-L-Asp(OBzl)-ONSu as needles; yield, 4.75 g (69.7%); mp 83—84 °C; $[\alpha]_D^{24}$ −19.1° (*c* 1, ethyl acetate). To a solution of Boc-L-Val-L-Leu-OMe (**27**, 3.10 g, 9 mmol) in 20 ml of methanol was added TosOH·H_2O (2.05 g, 10.8 mmol). The mixture was stirred at 30 °C for 5 h. The solvent was evaporated under reduced pressure at a bath temperature of 50 °C to give H-L-Val-L-Leu-OMe·TosOH as an oily residue. The oil was dissolved in 40 ml of chloroform and cooled in an ice-bath. To this solution was added Et_3N (1.10 g, 10.8 mmol), followed by Z-L-Asp(OBzl)-ONSu (3.79 g, 8.34 mmol) with stirring. The mixture was stirred at room temperature for 3 h and then kept standing overnight. The reaction mixture was worked up as a) to give Z-L-Asp(OBzl)-L-Val-L-Leu-OMe (**51**) as crystals, which were recrystallized from ethyl acetate—hexane to give **51** as needles. Recrystallization was carried out from the same solvent. The data are given in Table 2.

Tripeptide Esters (52—75). A typical run (**59** in Table 3) was as follows: Z-L-Asp(OBzl)-D-Ala-L-Val-OMe (**35**, 1.85 g) was dissolved in a mixture of acetic acid (40 ml) and water (10 ml), and hydrogenated in the presence of 5% Pd/C (0.6 g) with stirring at atmospheric pressure and room temperature for 6 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to dryness. The residue was dissolved in water and the solvent was evaporated under reduced pressure to remove remaining acetic acid. The procedure was repeated three times. The crystalline residue, thus obtained, was recrystallized from water–acetone to give 0.76 g (63.3%) of L-α-Asp-D-Ala-L-Val-OMe·$2H_2O$ (**59**) as needles. Recrystallization from water afforded 0.26 g of pure **59** dihydrate as needles. The data are given in Table 3. In another experiment, the peptide (**59**) was recrystallized from water to give **59** monohydrate as needles; mp 160—161 °C.

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