[Chem. Pharm. Bull. 24(9)2112—2117(1976)]

UDC 547.466.63'466.62.04 : 615.216.87.015.11

Structure-Taste Relationships of L-Aspartyl-Aminomalonic Acid Diesters[1]

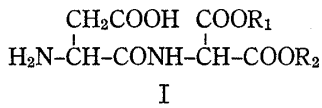
Masahiko Fujino, Mitsuhiro Wakimasu, Mitsuhiko Mano, Kuniyoshi Tanaka,[2a,c] Nobuo Nakajima, and Hisashi Aoki[2b]

Central Research Division[2a] and Food Products Division,[2b] Takeda Chemical Industries, Ltd.

(Received December 5, 1975)

L-Aspartyl-aminomalonic acid diesters are representatives of new group of sweet compounds. Chemical synthesis of the dipeptide esters was effected by coupling carbobenzoxy-β-benzylaspartic acid with an aminomalonic acid diester by the conventional activated ester method, followed by catalytic hydrogenation to remove the protecting groups. Among the 21 products, *trans*-2-methyl-cyclohexyl, methyl diester was over 5000 times sweeter than sucrose, 2,6-dimethyl-cyclohexyl, methyldiester was about 5000 times sweeter and fenchyl, methyl diester was over 20000 times sweeter. These compounds appear to be the most potent sweeteners known of either natural or synthetic origin. On the basis of the potencies of the products, structure-taste relations in the C-terminal part of this molecule were discussed in detail.

In 1969, Mazur, *et al.*[3] reported that L-aspartyl-L-phenylalanine methyl ester was 100—200 times sweeter than sucrose and the taste quality was relatively close to that of sucrose. Mazur, *et al.*[4] and Ariyoshi, *et al.*[5] have also reported many analogs of this dipeptide ester and discussed actual roles of the structural feature which causes the sweet taste. However, none of the analogs were found to be superior to the original dipeptide ester in the potency of sweetness. We have also reported in a brief communication,[6] some intense sweeteners related to the alternative dipeptide ester, L-aspartyl-aminomalonic acid diester [I]. Among them, trans-2-methyl-cyclohexyl, methyl diester was 5000 to 7000 times sweeter, and fenchyl, methyl diester was 22000 to 33000 times sweeter than sucrose. These dipeptide esters appear to be the most intense sweeteners known of either natural or synthetic origin. This discovery is very important for the investigation of the structure-taste relationship in dipeptides of this type, because a variety of ester moieties could be chosen from a variety of the corresponding available alcohols.



I

This paper deals with the synthesis and sweetness intensities of a series of analogs in which the ester groups of the C-terminal aminomalonic acid were systematically replaced.

1) Abbreviations used: Asp=aspartic acid, Ama=aminomalonic acid, OMe=methyl ester, OEt=ethyl ester, OBzl=benzyl ester, Z=carbobenzoxy, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, DCC=N,N′-dicyclohexylcarbodi-imide, DC-urea=N,N′-dicyclohexylurea.

2) Location: *a,b*) *Juso-Honmachi, Yodogawa-ku, Osaka*; *c*) Present address: Prof. K. Tanaka, *Faculty of Pharmaceutical Sciences, Kinki University, Kowakae 3-4-1, Higashi-osaka, Osaka.*

3) R.H. Mazur, J.M. Schlatter, and A.H. Goldkamp, *J. Am. Chem. Soc.*, **91**, 2684 (1969).

4) R.H. Mazur, A.H. Goldkamp, D.A. James, and J.M. Schlatter, *J. Med. Chem.*, **13**, 1217 (1970); R.H. Mazur, J.A. Reuter, K.A. Swiatek, and J.M. Schlatter, *ibid.*, **16**, 1284 (1973).

5) Y. Ariyoshi, N. Yasuda, and T. Yamatani, *Bull. Chem. Soc. Japan*, **47**, 326 (1974).

6) M. Fujino, M. Wakimasu, K. Tanaka, H. Aoki, and N. Nakajima, *Naturwissenschaften*, **60**, 351 (1973); Proc. the 11th Symposium on Peptide Chemistry in Japan, ed. by H. Kotake, 1973, p. 103.

TABLE I. Yields of Protected Carbobenzoxy-Aminomalonic Acid Diesters

$$\begin{array}{l} \text{OR}_1 \\ | \\ \text{Z-Ama-OR}_2 \end{array}$$

Compound R_1	Compound R_2	Yield %	Compound R_1	Compound R_2	Yield %
Methyl	*t*-butyl	*a*)	ethyl	ethyl	*a*)
Methyl	diethylcarbinyl	82	ethyl	*t*-butyl	95
Methyl	diisopropylcarbinyl	71.7	ethyl	diethylcarbinyl	54
Methyl	cyclopentyl	97	ethyl	cyclopentyl	99
Methyl	cyclohexyl	99	ethyl	cyclohexyl	100
Methyl	*cis*-2-methyl-cyclohexyl	48*b*)	Methyl	*trans*-2-methyl-cyclohexyl	84
Methyl	*trans*-2-methyl-cyclohexyl	74*c*)			
Methyl	3-methyl-cyclohexyl	81	ethyl	fenchyl	84
Methyl	4-methy-cyclohexyl	74			
Methyl	2,6-dimethyl-cyclohexyl	51			
Methyl	*cis*-3,3,5-trimethyl-cyclohexyl	48			
Methyl	*dl*-menthyl	74			
Methyl	*l*-isobornyl	97			
Methyl	fenchyl	74			

a) See experimental section.
b) mp 38—40°. *Anal.* Calcd. for $C_{19}H_{25}O_6N$: C, 62.79; H, 6.93; N, 3.85. Found: C, 62.85; H, 6.87; N, 3.81
c) mp 57—59°. *Anal.* Calcd. for $C_{19}H_{25}O_6N$: C, 62.79; H, 6.93; N, 5.85. Found. C, 62.87; H, 7.14; N, 3.77

TABLE II. Physicochemical Properties of Carbobenzoxy-β-benzyl-L-aspartyl-aminomalonic Acid Diesters

$$\begin{array}{l} \text{OBzl OR}_1 \\ | \quad\quad | \\ \text{Z-L-Asp-Ama-OR}_2 \end{array}$$

Compound R_1	Compound R_2	Yield %	mp °C	$[\alpha]_D^{23}$ $c=1.0$	Analysis (%) Calcd. (Found) C	H	N
Methyl	*t*-butyl	95	oil				
Methyl	diethylcarbinyl	90	oil				
Methyl	diisopropylcarbinyl	54.9	oil				
Methyl	cyclopentyl	73	74—78	−7.8°*a*)	62.21 (62.24)	5.97 (5.93)	5.18 (5.09)
Methyl	cyclohexyl	86	61—63	−7.0°*a*)	62.80 (62.80)	6.18 (6.09)	5.05 (4.89)
Methyl	*cis*-2-methyl-cyclohexyl	97	oil				
Methyl	*trans*-2-methyl-cyclohexyl	68	79—81	−10.6°*a*)	63.37 (62.80)	6.38 (6.43)	4.93 (5.10)
Methyl	3-methyl-cyclohexyl	90	oil				
Methyl	4-methyl-cyclohexyl	67	oil				
Methyl	2,6-dimethyl-cyclohexyl	92	oil				
Methyl	*cis*-3,3,5-trimethyl-cyclohexyl	87	oil				
Methyl	*dl*-menthyl	30	95—96	−32.8°*a*)	64.90 (64.82)	6.93 (6.93)	4.59 (4.58)
Methyl	*l*-isobornyl	99	oil				
Methyl	fenchyl	81	oil				
Ethyl	ethyl	99	97—98	−8.5°*a*)	60.69 (60.51)	5.88 (5.85)	5.45 (5.47)
Ethyl	*t*-butyl	90	oil				
Ethyl	diethylcarbinyl	98	oil				
Ethyl	cyclopentyl	80	84—88	−10.8°*b*)	62.80 (63.18)	6.18 (6.10)	5.05 (5.09)
Ethyl	cyclohexyl	71	74—79	−11.4°*b*)	63.37 (63.04)	6.38 (6.26)	4.93 (4.87)
Ethyl	*trans*-2-methyl-cyclohexyl	71	76—79	−10.8°*b*)	63.90 (63.79)	6.57 (6.51)	4.81 (4.94)
Ethyl	fenchyl	72	oil				

a) in methanol *b*) in ethanol

Synthesis of the starting materials, the various diesters of aminomalonic acid, was achieved by catalytic hydrogenation of the carbobenzoxy-aminomalonic acid diesters which were prepared by means of esterification of mono-methyl or mono-ethyl carbobenzoxy-aminomalonate with the corresponding alcohols by the acid-chloride method.[7] Most of the carbobenzoxy-aminomalonic acid diesters were obtained as oils, with the exception of the methyl, *cis*-2-methyl-cyclohexyl derivative and methyl, *trans*-2-methyl-cyclohexyl derivative which

Table III. Physicochemical Properties of L-Aspartyl-Aminomalonic Acid Diesters

$$\text{L-Asp-}\overset{\overset{OR_1}{|}}{\text{Ama}}\text{-}OR_2$$

Compound R_1	Compound R_2	Yield %	mp (°C)	$[\alpha]_D^{23}$ $c=1.0$ in AcOH	Analysis (%) Calcd (Found) C	H	N
Methyl	*t*-butyl	92	127—128	−3.0°[a]	46.00 (45.83)	6.76 (6.70)	8.94[b] (8.85)
Methyl	diethylcarbinyl	90	115—116	−2.5°[a]	47.70 (47.68)	7.08 (6.99)	8.56[b] (8.42)
Methyl	diisopropylcarbinyl	97	86— 87	−4.5°	49.44 (49.27)	7.75 (7.75)	7.69[c] (7.26)
Methyl	cyclopentyl	97	81— 90	−33.1°	47.99 (48.08)	6.51 (6.40)	8.61[b] (8.32)
Methyl	cyclohexyl	96	76— 77	−40.0°	49.55 (49.85)	6.83 (6.92)	8.26[b] (8.02)
Methyl	*cis*-2-methylcyclohexyl	86	88— 91	−29.0°	50.98 (51.58)	7.13 (7.33)	7.93[b] (7.72)
Methyl	*trans*-2-methylcyclohexyl	86	100—102	−41.2°	50.98 (50.86)	7.13 (7.23)	7.93[b] (7.65)
Methyl	3-methyl-cyclohexyl	92	94— 99	−5.5°	49.71 (49.14)	7.23 (6.94)	7.73[c] (7.54)
Methyl	4-methyl-cyclohexyl	91	90— 93	−26.1°	49.71 (50.11)	7.23 (6.95)	7.73[c] (7.30)
Methyl	2,6-dimethylcyclohexyl	91	94— 97	−26.0°	53.62 (53.61)	7.31 (7.54)	7.82 (7.50)
Methyl	*cis*-3,3,5-trimethylcyclohexyl	83	116—117	−24.7°	53.53 (53.44)	7.66 (7.77)	7.35[b] (7.03)
Methyl	*dl*-menthyl	80	96—100	−36.6°	51.17 (51.25)	8.11 (7.29)	6.63[d] (6.51)
Methyl	*l*-isobornyl	89	90— 92	−63.3°	56.24 (56.34)	7.34 (7.59)	7.29 (7.04)
Methyl	fenchyl	75	85— 88	+5.9°	51.42 (51.52)	7.67 (7.29)	6.66[d] (6.30)
Ethyl	ethyl	95	131—132	−0.9°[a]	45.51 (45.41)	6.25 (6.36)	9.65 (9.39)
Ethyl	*t*-butyl	71	84— 86	+5.8°	45.22 (45.40)	7.30 (7.28)	8.11[d] (8.17)
Ethyl	diethylcarbinyl	89	103—106	+5.4°	49.23 (49.28)	7.38 (7.04)	8.21[b] (7.80)
Ethyl	cyclopentyl	78	115—118	+5.7°	49.55 (49.65)	6.83 (6.73)	8.26[b] (8.05)
Ethyl	cyclohexyl	79	112—115	−38.0°	50.98 (50.93)	7.13 (7.08)	7.93[b] (7.87)
Ethyl	*trans*-2-methylcyclohexyl	78	80— 83	−28.2°	53.62 (53.85)	7.31 (7.15)	7.82 (7.56)
Ethyl	fenchyl	84	132—135	+4.8°	53.80 (54.03)	7.68 (7.80)	6.27[b] (6.02)

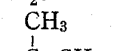
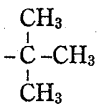
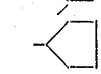
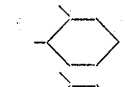
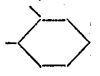
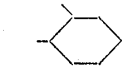
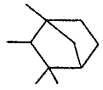
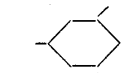
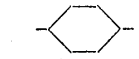
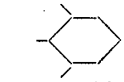
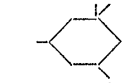
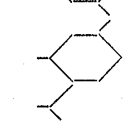
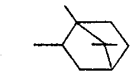
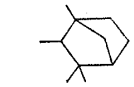
a) in methanol *b*) hemihydrate *c*) monohydrate *d*) dihydrate

7) J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley & Sons, Inc., New York, 1961, p. 965.

were obtained in crystalline form. The yields and properties of the carbobenzoxy-aminomalonic acid diesters prepared are listed in Table I.

Chemical synthesis of L-aspartyl-aminomalonic acid diesters was effected by coupling carbobenzoxy-β-benzyl-L-aspartic acid with an aminomalonic acid diester by the conventional HONB-activated ester method,[8] followed by catalytic hydrogenation to remove the protecting groups. The physical properties of the protected and unprotected dipeptide diesters are listed in Tables II and III, respectively.

TABLE IV. Relative Sweetness of L-Aspartyl-Aminomalonic Acid Diesters

Compound L-Asp-Ama(OR$_2$)-OMe	Potency[a] sucrose=1.0	Compound L-Asp-Ama(OR$_2$)-OEt	Potency[a] sucrose=1.0
R$_2$:		R$_2$:	
	68(6.8)—52(10.4)		16(3.2)—10.3(4.1)
	364(9.1)—302(15.1)		weak
	680(6.8)—470(9.4)		116(2.9)—101(13.2)
	600(7.5)—500(12.5)		156(3.9)—128(6.4)
	880(11.0)—356(17.8)		284(7.1)—192(9.1)
 (*cis*)	4600(4.6)—3700(7.4)	 (*trans*)	648(8.1)—524(13.1)
 (*trans*)	7300(7.4)—5450(10.9)		5400(5.4)—4200(8.4)
	608(7.3)—444(11.1)		
	44(4.4)—29.5(5.9)		
	5300(5.3)—4650(9.3)		
	324(8.1)—206(10.3)		
	no sweetness		
	760(9.5)—528(13.2)		
	33200(8.3)—22200(11.1)		

a) Figures in parentheses show the matched concentration of sucrose solution as a percentage of the compound solution.

8) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa, and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **22**, 1857 (1974).

Evaluation of the relative sweetness of these synthetic dipeptide esters was carried out by a 7-member panel by matching a solution of known concentration of each compound with a sucrose solution of equipotent sweetness. The results are summarized in Table IV.

Of structure-taste relations in natural and synthetic sweeteners, Shallenberger, *et al.*[9] and more recently Kier[10] have suggested the conception of a receptor site which consists of hydrophobic binding between the receptor and the hydrophobic function of a sweetener, together with a hydrogen bond (A–H/B theory) between the receptor on the taste buds and a "sweet unit" of a sweetener. The attempt to place much importance upon charge properties and steric requirements of the sweet unit has been challenged by a number of researchers but has also been given some satisfactory support.[4,5] The other important problem of the space-filling properties of hydrophobic function, however, has yet no completely satisfactory solution.

In regard to this, we are interested to know what structure requirements of the alcohol part of the basic structure I are necessary to cause a highly potent sweet taste.

As can be seen in Table IV, among the mono-methyl substituted cyclohexanol derivatives, 2-methyl substitution gave the sweetest material which was approximately 5000—7000 times sweeter than sucrose, whereas the 4-methyl derivative was only 40 times sweeter. The 3-methyl derivative was as sweet as the non-substituted derivative which was about 600—800 times sweeter than sucrose. Moreover, such an α-methyl effect was also confirmed even in the case of bicyclic alcohol. These results indicate that the methyl group at the α-position makes a major contribution to the sweetness of this type of the compound, and space-filling properties of hydrophobic function are strikingly important for highly potent sweetness. The results also indicate that the size of the hydrophobic binding site of the receptor may be approximately as shown in Fig. 1.

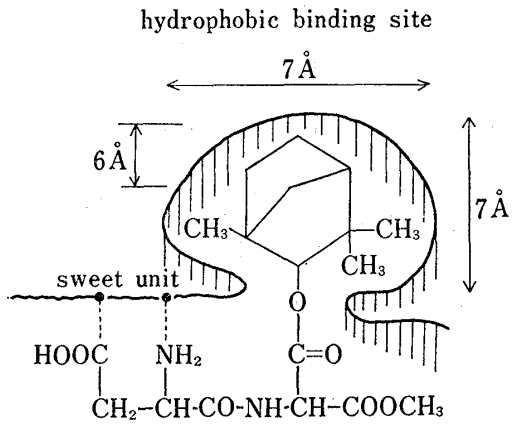


Fig. 1. Gross Model of the Hydrophobic Binding Site of a "Taste Receptor"

It is well known in the case of aspartyl-phenylalanine methyl ester that only the L-form of the phenylalanine residue has a sweet taste; the D-form tastes bitter.[4] Although the compounds described in this paper were racemates at the position of the α-carbon of the aminomalonic acid residue, none of the compounds taste bitter. It can be, therefore, assumed that the D-isomer of these compounds has no particular interaction with the receptor of bitterness.

Experimental

Melting points were taken in open capillaries and are uncorrected. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. Evaporations were carried out in rotary evaporators under reduced pressure at 35—40°. Catalytic hydrogenations were performed at room temperature with palladium black as catalyst. The purity of all intermediates and products was checked by thin layer chromatography on silica gel. Protected compounds were run in $CHCl_3$–MeOH, 9: 1, and $CHCl_3$–MeOH–AcOH, 9: 1: 0.5. Deprotected compounds were run in $CHCl_3$–MeOH–AcOH, 9:1: 0.5, *n*-BuOH–AcOEt–AcOH–H_2O, 1:1:1:1, and AcOEt–pyridine–AcOH–H_2O, 60: 20: 6: 11. All compounds reported here were essentially homogeneous on thin-layer chromatography.

Dimethyl Carbobenzoxy-aminomalonate (1)——To a cold solution of dimethylaminomalonate hydrochloride (95.5 g) in a mixture of $CHCl_3$ (1000 ml) and H_2O (600 ml) were added MgO (46 g) and carbobenzoxy chroride (95 g) with stirring. The mixture was stirred for 2 hr at 0° and for an additional 2 hr at room temperature.

9) R.S. Shallenberger, T.E. Acree, and C.Y. Lee, *Nature*, **221**, 555 (1969).
10) L.B. Kier, *J. Pharm. Sci.*, **61**, 1394 (1972).

The solution was acidified with 1 N HCl, and the $CHCl_3$ layer was collected, washed with 4% aqueous $NaHCO_3$ (500 ml × 2) and 10% aqueous citric acid (400 ml × 2), and dried over anhydr. $MgSO_4$. Evaporation of the solvent yielded a crystal which was recrystallized from ethyl ether and pet. ether: 137.5 g (94%), mp 60—61.5°. *Anal.* Calcd. for $C_{13}H_{15}O_6N$: C, 55.51; H, 5.38; N, 4.98. Found: C, 55.57; H, 5.33; N, 5.13.

Diethyl carbobenzoxy-aminomalonate was prepared from diethyl aminomalonate hydrochloride by the method described above, and the physicochemical properties are listed in Table I: yield, 92%.

Monomethyl Carbobenzoxy-aminomalonate (2)——To a solution of compound **1** (50.6 g) in a mixture of acetone (200 ml), MeOH (180 ml) and H_2O (90 ml) was added 2 N aqueous NaOH (90 ml) with stirring at 0°. The mixture was stirred for 60 min at room temperature. The organic solvents were removed by evaporation and the resulting aqueous solution was washed with ethyl ether (50 ml × 2) and then acidified with 6 N HCl. The product was extracted with ethyl ether (300 ml × 3). The extracts were combined, washed with H_2O and dried over anhydr. Na_2SO_4, then evaporated to dryness to give a crystal which was recrystallized from ethyl ether and pet. ether: 40.8 g (85%), mp 77.5—81°. *Anal.* Calcd. for $C_{12}H_{13}O_6N$: C, 53.93; H, 4.90; N, 5.24. Found: C, 53.76; H, 4.82; N, 5.32.

Monoethyl carbobenzoxy-aminomalonate was prepared by the same procedure: yield, 81%, mp 64—65°. *Anal.* Calcd. for $C_{13}H_{15}O_6N$: C, 55.51; H, 5.38; N, 4.98. Found: C, 55.53; H, 5.48; N, 5.24.

Carbobenzoxy-aminomalonic Acid Methyl, *t*-Butyl Diester (3)——Compound **2** (2.7 g) was dissolved in CH_2Cl_2 (100 ml) and the solution was cooled to −10°. To this, conc. H_2SO_4 (0.3 ml) was added and isobutene gas was bubbled for 10 hr with stirring. The solution was evaporated to dryness and the residue was dissolved in ethyl ether (200 ml), washed with 4% aqueous $NaHCO_3$ and H_2O, dried over anhydr. Na_2SO_4, and evaporated to dryness to give an oily residue which was purified by column chromatography on silica gel, $CHCl_3$–MeOH (9: 1): 3.2 g (99%). *Anal.* Calcd. for $C_{16}H_{21}O_6N$: C, 59.43; H, 6.55; N, 4.33. Found: C, 59-71; H, 6.28; N, 4.07.

Carbobenzoxy-aminomalonic Acid Methyl, Cyclopentyl Diester (4)——To a solution of compound **2** (5.35 g) in dried ethyl ether was added PCl_5 (4.58 g) at 0°. The mixture was stirred for 30 min at room temperature to give a clear solution. To a solution of cyclopentyl alcohol (9.0 g) in a mixture of pyridine (30 ml) and ethyl ether (20 ml), the above solution was added dropwise with stirring at 0°. The pyridine hydrochloride formed was removed by filtration and the filtrate was washed with 10% aqueous citric acid (50 ml × 2), 4% aqueous $NaHCO_3$ (60 ml × 3) and H_2O (50 ml × 2). The ethyl ether layer was dried over anhydr. Na_2SO_4 and evaporated to give an oily residue which was crystallized from ethyl ether and pet. ether: 4.97 g (74%), mp 57.59°. *Anal.* Calcd. for $C_{19}H_{25}O_6N$: C, 62.77; H, 6.93; N, 3.85. Found: C, 62.87; H, 7.14; N, 3.77.

The other diesters were prepared by a method similar to that described above, and the yield and properties are shown in Table I.

Carbobenzoxy-β-benzyl-L-aspartyl-aminomalonic Acid Methyl, Cyclopentyl Diester (5)——Compound **4** (6.04 g) was hydrogenated in MeOH (100 ml) to give the free base which was dissolved in dioxane (100 ml). To this were added carbobenzoxy-β-benzyl-L-aspartic acid (5.79 g), HONB (2.0 g) and DCC (3.71 g) with stirring at 0°, and the mixture was allowed to react for 8 hr at room temperature. The reaction mixture was filtered to remove the formed DC-urea and the filtrate was evaporated to dryness. The resulting residue was dissolved in ethyl acetate (200 ml), washed with 10% aqueous citric acid and 4% aqueous $NaHCO_3$, and dried over anhydr. Na_2SO_4, then evaporated to dryness to give an oily residue which crystallized upon addition of ethyl acetate and pet. ether: 6.36 g (73%), mp 74—78°. *Anal.* Calcd. for $C_{28}H_{32}O_9N_2$: C, 62.21; H, 5.97; N, 5.18. Found: C, 62.24; H, 5.93; N, 5.09.

The other protected dipeptide diesters were prepared from the compounds listed in Table I and carbobenzoxy-β-benzyl-L-aspartic acid by the same method described above. The physicochemical properties are listed in Table II.

L-Aspartyl-Aminomalonic Acid Methyl, Cyclopentyl Diester (6)——Compound **5** (6.0 g) was hydrogenated in MeOH (100 ml) for 5 hr. The mixture was filtered to remove the catalyst and the filtrate was evaporated to dryness to give an oily residue which was triturated with ethyl ether to give a white powder: 3.49 g (97%), mp 81—90°, $[\alpha]_D^{23}$ −33.1° (c=1.0 in AcOH). *Anal.* Calcd. for $C_{13}H_{20}O_7N_2 \cdot 1/2H_2O$: C, 47.99; H, 6.51; N, 8.61. Found: C, 48.08; H, 6.40; N, 8.32.

The other dipeptide diesters were prepared from the corresponding protected dipeptide diester by the procedure described above. The physicochemical properties of the prepared dipeptide esters are listed in Table III, and sweetness potencies of the peptides are shown in Table IV.

Acknowledgement The authors express their gratitude to Drs. S. Tatsuoka, E. Ohmura, and K. Morita of the Central Research Division and Dr. S. Wada of the Food Products Division for their interest and helpful discussion.