Molecular Design of Inverted-Aspartame-Type Sweeteners

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In the series of studies of salty peptides, some basic dipeptides were found to have a sweet taste instead of a salty taste. On the basis of the findings of these sweet peptides, we tried to develop a new type of sweetener according to the theory of the well-known AH-B-X system in aspartame. The compounds, which we call inverted-aspartame-type sweeteners, were successfully designed. For one of them, a sweetness potency of 50 times that of sucrose was reached. Since these inverted-aspartame-type sweeteners have no ester functions, they are considered to be very useful because of their stability in solution and lack of toxicity.

Since the discovery of aspartame (Asp-Phe-OMe) by Mazur et al. (1969), peptide sweeteners have entered the spotlight as sugar substitutes in beverages and low-calorie foods. To date, much research has been done to establish molecular requirements for the sweet taste (Ariyoshi, 1976, 1980; Ariyoshi et al., 1974; Mazur et al., 1970, 1973; Miyoshi et al., 1978; Shallenberger et al., 1967, 1969). It is considered most likely, as reported by many workers (Fujino et al., 1973, 1976; Mapelli et al., 1987), that the sweet taste of aspartame is elicited by the trifunctional unit AH-B-X, where AH is an acidic proton, B is an electronegative atom or center, and X is a hydrophobic group, as indicated in Figure 1. In further detail, Shinoda et al. (1985) reported the contributions of these trifunctional units for sweetness as well as bitterness.

Aspartame is now sold under the brand name NutraSweet by NutraSweet Co. However, it has some problems. The most serious one is the instability of the molecule in solution, because the methyl ester at the C terminus makes it easy to convert the molecule to the tasteless cyclic form in solution. Also, after aspartame is consumed, the methyl ester is hydrolyzed by an esterase to give methanol.

Recently, during the study of salty peptides, we found the basic dipeptides Gly-Lys and γ-Abu-Lys produced no saltiness but a sweet taste, which was of the same level as that of sucrose (Tada et al., 1984). In comparison of the structure of these basic dipeptides with that of aspartame, it is predictable that the ε-NH_2 group and the α-COOH group in the C-terminal Lys and the N-terminal amino acid residue corresponded to AH, B, and X in aspartame, respectively (see Figure 1). It should be possible by adding suitable hydrophobic groups (X) to the N terminus of Gly-Lys and γ-Abu-Lys to enhance remarkably the sweetness of the peptides. It can be realized that, for example, Bz-Gly-Lys (1), in which a benzoyl group is introduced to the N terminus of Gly-Lys, has an opposite triangle from that made by the trifunctional units of aspartame (see Figure 1). We named these kinds of dipeptides "inverted-aspartame-type sweeteners" and synthesized a series of such dipeptides to investigate their utility. First, we report the sweet taste of Bz-Gly-Lys compared with that of Gly-Lys. Then, we discuss the molecular design of more effective sweeteners based on the theory described above.

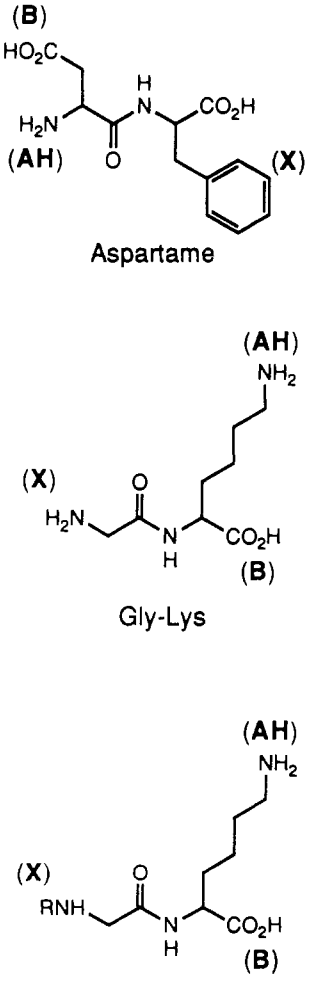

Figure 1. Structure of aspartame and our inverted-aspartame-type sweetener.

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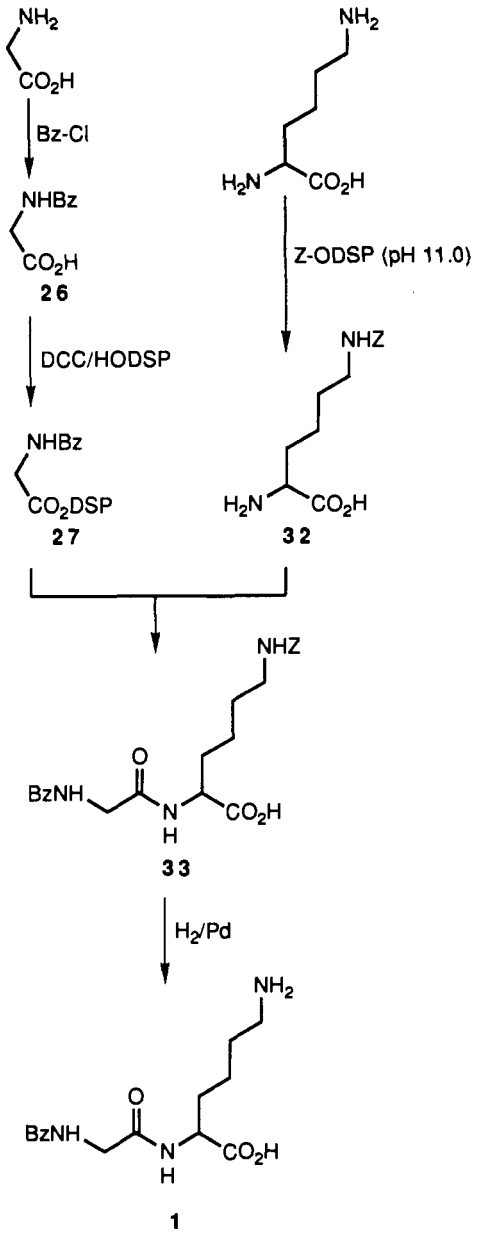


Figure 2. Synthetic route to Bz-Gly-Lys-OH.

Table I. Result of Sensory Test

compound	T.V.,[a] mM	R_{suc}[b]	taste
1, Bz-Gly-Lys-OH	0.67	7.5	sweet > sour
H-Gly-Lys-OH·HCl	5.48	0.9	sour > sweet
γ-Abu-Lys-OH·HCl	1.56	3.2	sweet > sour
sucrose	5.00	1.0	sweet

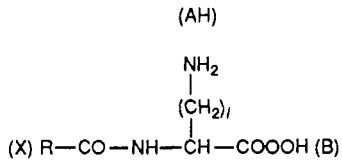
[a] Threshold value. [b] A sweetness potency of compounds comparing with that of sucrose, a standard compound.

EXPERIMENTAL PROCEDURES

General Procedures. All the melting points are uncorrected. The thin-layer chromatography was carried out on Merck precoated aluminum sheets of silica gel 60 with the solvent systems: R_f1, 1-butanol–acetic acid–pyridine–water (4:1:1:2 v/v); R_f2, chloroform–methanol (5:1 v/v). Spots of materials with a free amino group were detected on a thin-layer plate by spraying with ninhydrin and those of amino group blocked materials by spraying with 25% hydrogen bromide in acetic acid and then ninhydrin. The optical rotations were measured on a Union PM-101 polarimeter. Prior to analyses including sensory tests, the compounds were dried over phosphorus pentaoxide at 66 °C (2 mmHg, 1 mmHg = 133 PPa) for 4–6 h.

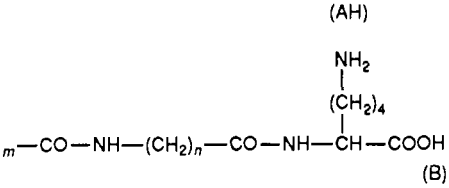
Sensory Test. The taste of all synthetic peptide derivatives was organoleptically evaluated by a panel of four or five

Table II. Result of Sensory Test



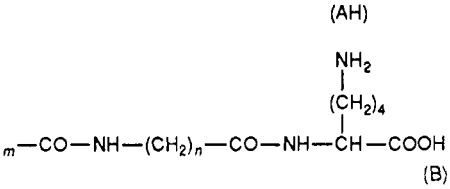
compound	T.V., mM	R_{suc}	taste
2, Bz-Lys-OH	2.37	2.1	sweet
3, Bz-Orn-OH	6.33		bitter > sweet
4, Bz-Dap-OH	6.63		bitter > sweet
1, Bz-Gly-Lys-OH	0.67	7.5	sweet > sour
5, Bz-Gly-Orn-OH	1.97		sweet > bitter
6, Bz-Gly-Dab-OH	0.88		bitter > sour > sweet
H-Gly-Lys-OH·HCl	5.48	0.9	sour > sweet
γ-Abu-Lys-OH·HCl	1.56	3.2	sweet > sour
sucrose	5.00	1.0	sweet

Table III. Result of Sensory Test



compound	T.V., mM	R_{suc}	taste
2, Bz-Lys-OH	2.37	2.1	sweet
1, Bz-Lys-OH	0.67	7.5	sweet > sour
7, Bz-β-Ala-Lys-OH	0.24	20.8	sweet
8 Bz-γ-Abu-Lys-OH	0.22	22.7	sweet
9, Ph-Ac-Lys-OH	1.20		sweet > bitter
10, Ph-Ac-Gly-Lys-OH	0.10	50.0	sweet > sour
11, Ph-Ac-β-Ala-Lys-OH	0.53		sweet > sour > bitter
12, Ph-Pr-Lys-OH	0.55		sweet > bitter
13, Ph-Pr-Gly-Lys-OH	0.34	14.7	sweet > sour
14, Ph-Pr-β-Ala-Lys-OH	0.66		sweet > bitter > sour
15, H-L-Phe-Lys-OH·HCl	0.26		sweet > bitter > sour
16, H-D-Phe-Lys-OH·HCl	0.52		sweet > bitter
17, α-naphthoyl-Lys-OH	1.02		sweet > bitter
sucrose	5.00	1.0	sweet

Table IV. Result of Sensory Test



compound	$(m+n)$	T.V., mM	R_{suc}	taste
7, Bz-β-Ala-Lys-OH	(0 + 2)	0.24	20.8	sweet
8, Bz-γ-Abu-Lys-OH	(0 + 3)	0.22	22.7	sweet
10, Ph-Ac-Gly-Lys-OH	(1 + 1)	0.10	50.0	sweet > sour
11, Ph-Ac-β-Ala-Lys-OH	(1 + 2)	0.53		sweet > sour > bitter
13, Ph-Pr-Gly-Lys-OH	(2 + 1)	0.34	14.7	sweet > sour
14, Ph-Pr-β-Ala-Lys-OH	(2 + 2)	0.66		sweet > sour > bitter
sucrose		5.00	1.0	sweet

Table V. Result of Sensory Test

compound	T.V., mM	R_{suc}	taste
15, H-L-Phe-Lys-OH·HCl	0.26		sweet > bitter > sour
16, H-D-Phe-Lys-OH·HCl	0.52		sweet > bitter
18, Bz-Ala-Lys-OH	0.25		sweet > sour > bitter
19, Ph-Ac-Ala-Lys-OH	0.24		sweet > bitter > astringent
20, Ph-Pr-Ala-Lys-OH	0.19		sweet > bitter > astringent
21, Bz-α-Abu-Lys-OH	0.19		sweet > astringent > sour
22, Ph-Ac-α-Abu-Lys-OH	0.24		sweet > sour > bitter
23, Ph-Pr-α-Abu-Lys-OH	0.27		sweet > bitter
24, Ac-L-Phe-Lys-OH	0.22	22.7	sweet > sour
25, Ac-D-Phe-Lys-OH	0.32		sweet > sour > bitter
sucrose	5.00	1.0	sweet

Table VI. Yields of and Analytical Data for Intermediates

compound	yield, %	mp, °C	$[\alpha]^{25}_D$, deg (solvent)	R_{f1}	R_{f2}
26, Bz-Gly-OH	75	186–188		0.70	0.19
27, Bz-Gly-ODSP	92	114–118	+1.98 (*c* 1.0, DMF)	0.48	0.12
28, H-Dab(*Z*)-OH	55	225–227	+9.0 (*c* 1.0, DMF)	0.56	0.04
29, Bz-Gly-Dab(*Z*)-OH	47	157–160.5	−12.87 (*c* 1.0, DMF)	0.69	0.12
30, H-Orn(*Z*)-OH	65	254–255 (dec)	+17.3 (*c* 0.7, 6 M HCl)	0.59	0.05
31, Bz-Gly-Orn(*Z*)-OH	45	120–125	+11.0 (*c* 1.0, DMF)	0.80	0.18
32, H-Lys(*Z*)-OH	69	250–253 (dec)	+16.0 (*c* 1.0, 6 M HCl)	0.56	0.06
33, Bz-Gly-Lys(*Z*)-OH	47	123–127.5	−2.99 (*c* 1.0, DMF)	0.75	0.13
34, Bz-Dab(*Z*)-OH	76	94–99	−17.64 (*c* 0.5, DMF)	0.81	0.26
35, Bz-Orn(*Z*)-OH	77	144.5–147	+0.99 (*c* 1.0, DMF)	0.82	0.17
36, Bz-Lys(*Z*)-OH	75	101.5–105	+0.99 (*c* 1.0, DMF)	0.81	0.30
37, Ph-Ac-Lys(*Z*)-OH	70	100.5–102	−3.97 (*c* 1.0, DMF)	0.85	0.30
38, Ph-Pr-ODSP	93	71–77		0.52	0.20
39, Ph-Pr-Lys(*Z*)-OH	50	99–104	−2.00 (*c* 1.0, DMF)	0.83	0.19
40, α-Naph-Lys(*Z*)-OH	49	121–126	−19.94 (*c* 1.0, DMF)	0.88	0.35
41, Bz-β-Ala-OH	62	107–109		0.81	0.11
42, Bz-β-Ala-ODSP	97	140–142.5	+1.98 (*c* 1, DMF)	0.53	0.06
43, Bz-β-Ala-Lys(*Z*)-OH	40	117–121	+9.0 (*c* 1.0, DMF)	0.78	0.22
44, Bz-γ-Abu-OH	71	67–72		0.84	0.12
45, Bz-γ-Abu-ODSP	95	oil		0.56	0.07
46, Bz-γ-Abu-Lys(*Z*)-OH	54	100–105	−1.0 (*c* 1.0, DMF)	0.78	0.23
47, Ph-Ac-Gly-OH	62	138.5–140		0.63	0.30
48, Ph-Ac-Gly-ODSP	81	99–103		0.47	0.03
49, Ph-Ac-Gly-Lys(*Z*)-OH	51	113–116	−3.0 (*c* 1.0, DMF)	0.76	0.32
50, Ph-Pr-Gly-OH	66	99–102		0.68	0.39
51, Ph-Pr-Gly-ODSP	74	61–64		0.65	0.05
52, Ph-Pr-Gly-Lys(*Z*)-OH	44	127–130	−1.0 (*c* 1.0, DMF)	0.71	0.33
53, Ph-Ac-β-Ala-OH	59	87.5–90		0.78	0.25
54, Ph-Ac-β-Ala-ODSP	98	59–62		0.59	0.10
55, Ph-Ac-β-Ala-Lys(*Z*)-OH	52	79–83	+1.0 (*c* 1.0, DMF)	0.69	0.42
56, Ph-Pr-β-Ala-OH	58	98.5–101		0.79	0.20
57, Ph-Pr-β-Ala-ODSP	94	80–85		0.59	0.10
58, Ph-Pr-β-Ala-Lys(*Z*)-OH	52	104–107	+2.0 (*c* 1.0, DMF)	0.83	0.20
59, *Z*-Phe-OH	90	84–88	+3.99 (*c* 1.0, EtOH)	0.67	
60, *Z*-Phe-ODSP	98	oil	−11.98 (*c* 1.0, DMF)	0.77	0.41
61, *Z*-Phe-Lys(*Z*)-OH	56	117–121	−6.90 (*c* 1.0, DMF)	0.85	0.49
62, *Z*-D-Phe-OH	90	84–87	+10.0 (*c* 1.0, MeOH)	0.88	
63, *Z*-Phe-ODSP	95	oil	+13.93 (*c* 1.0, DMF)	0.77	0.41
64, *Z*-D-Phe-Lys(*Z*)-OH	55	136–139.5	+2.98 (*c* 1.0, DMF)	0.82	0.42
65, Bz-Ala-OH	66	141–143	+6.0 (*c* 1.0, MeOH)	0.62	0.51
66, Bz-Ala-ODSP	97	oil		0.61	0.33
67, Bz-Ala-Lys(*Z*)-OH	58	oil		0.80	0.12
68, Bz-α-Abu-OH	77	105–108	+2.0 (*c* 1.0, MeOH)	0.69	0.37
69, Bz-α-Abu-ODSP	91	oil		0.53	0.25
70, Bz-α-Abu-Lys(*Z*)-OH	49	oil		0.85	0.19
71, Ph-Ac-Ala-OH	68	98–99.5	−28.0 (*c* 1.0, MeOH)	0.69	0.50
72, Ph-Ac-Ala-ODSP	96	oil		0.52	0.25
73, Ph-Ac-Ala-Lys(*Z*)-OH	42	96–100	−3.0 (*c* 1.0, DMF)	0.78	0.26
74, Ph-Ac-α-Abu-OH	70	100–104.5	−24.0 (*c* 1.0, MeOH)	0.69	0.56
75, Ph-Ac-α-Abu-ODSP	96	oil		0.57	0.34
76, Ph-Ac-α-Abu-Lys(*Z*)-OH	54	oil		0.82	0.25
77, Ph-Pr-Ala-OH	63	75–78	−28.0 (*c* 1.0, MeOH)	0.83	0.43
78, Ph-Pr-Ala-ODSP	94	oil		0.64	0.18
79, Ph-Pr-Ala-Lys(*Z*)-OH	50	72–75	+3.0 (*c* 1.0, DMF)	0.79	0.34
80, Ph-Pr-α-Abu-OH	65	109.5–112	−25.5 (*c* 1.0, MeOH)	0.77	0.51
81, Ph-Pr-α-Abu-ODSP	95	oil		0.67	0.22
82, Ph-Pr-α-Abu-Lys(*Z*)-OH	58	39–45	+1.0 (*c* 1.0, DMF)	0.75	0.38
83, Ac-Phe-OH	66	166–167.5	+45.0 (*c* 1.0, MeOH)	0.77	0.21
84, Ac-Phe-ODSP	96	oil		0.57	0.23
85, Ac-Phe-Lys(*Z*)-OH	49	122–125	+1.0 (*c* 1.0, DMF)	0.75	0.38
86, Ac-D-Phe-OH	52	166–167	−39.0 (*c* 1.0, MeOH)	0.78	0.34
87, Ac-D-Phe-ODSP	95	oil		0.60	0.22
88, Ac-D-Phe-Lys(*Z*)-OH	56	119–123	+7.0 (*c* 1.0, DMF)	0.83	0.16

people to determine the taste character and threshold value (T.V.). For each test compound, a series of solutions, differing in dilution by half in sequence, was prepared, followed by testing by the panel. After rinsing of the mouth, each solution was kept in the mouth for about 10 s. The character and intensity of taste were evaluated. All the panel members had the same evaluation for the taste character, while there were some personal differences in the T.V. evaluation, owing to personal and experimental conditions. However, this personal deviation in T.V. was eliminated by tasting standard substances and comparing the results at the same time. Sucrose was selected as a standard sweet substance. Analytical deviation in T.V. was markedly lessened by revision process as described, the range being ±20–25%. Therefore, a difference in T.V. of greater than 2 and 2.5 times was considered the significance level.

Synthesis of Peptides. The peptides synthesized are listed in Tables I–IV. The synthetic route to Bz-Gly-Lys (1) is shown in Figure 2, for example. All the other dipeptide derivatives were fundamentally synthesized by the same method.

Acylation of amino acids which had no functional group in their side chain or in which the functional group was blocked was performed by the conventional method using acyl chloride. The functional group in the side chain of some amino acids was acylated selectively in one step using HODSP (Kouge et al., 1987; Azuse et al., 1989). Acyl amino acids were prepared like this after catalytic hydrogenation if necessary. Acyl amino acids

Table VII. Physical Constants of the Final Products

compound	yield, %	mp, °C	$[\alpha]^{25}_D$, deg (concn, solvent)	R_{f1}	R_{f2}	formula	calcd C	calcd H	calcd N	found C	found H	found N
1, Bz-Gly-Lys-OH	52	69–73	+5.0 (c 1.0, MeOH)	0.43	0.00	$C_{15}H_{21}N_3O_4 \cdot {}^1/_2H_2O$	56.95	7.01	13.28	56.88	7.07	13.22
2, Bz-Lys-OH	81	209–211 (dec)	+12.0 (c 1.0, AcOH)	0.46	0.00	$C_{13}H_{18}N_2O_3 \cdot {}^1/_{10}H_2O$	61.93	7.28	11.11	61.90	7.30	11.10
3, Bz-Om-OH	80	221–224 (dec)	+19.0 (c 1.0, AcOH)	0.50	0.00	$C_{12}H_{16}N_2O_3$	61.00	6.83	11.86	60.93	6.87	11.83
4, Bz-Dab-OH	71	187.5–190.5 (dec)	+7.0 (c 1.0, AcOH)	0.55	0.00	$C_{11}H_{14}N_2O_3$	59.45	6.35	12.60	59.39	6.36	12.56
5, Bz-Gly-Om-OH	69	78–79.5	+6.0 (c 1.0, MeOH)	0.41	0.00	$C_{14}H_{19}N_3O_4 \cdot {}^1/_4H_2O$	56.46	6.60	14.11	56.44	6.63	14.09
6, Bz-Gly-Dab-OH	65	56.5–61	+3.0 (c 1.0, MeOH)	0.45	0.00	$C_{13}H_{17}N_3O_4 \cdot {}^1/_5H_2O$	55.19	6.20	14.86	55.16	6.23	14.85
7, Bz-β-Ala-Lys-OH	66	183.5–185	+7.0 (c 1.0, MeOH)	0.47	0.00	$C_{16}H_{23}N_3O_4 \cdot {}^1/_4H_2O$	58.97	7.27	12.90	58.95	7.29	12.88
8, Bz-γ-Abu-Lys-OH	63	180–183	+18.0 (c 1.0, MeOH)	0.50	0.00	$C_{17}H_{24}N_3O_4 \cdot {}^1/_8H_2O$	60.47	7.54	12.90	60.45	7.55	12.90
9, Ph-Ac-Lys-OH	75	227.5–230 (dec)	−15.0 (c 1.0, DMF)	0.51	0.00	$C_{14}H_{20}N_2O_3$	63.61	7.63	10.60	63.56	7.66	10.56
10, Ph-Ac-Gly-Lys-OH	53	118–122	+16.0 (c 1.0, MeOH)	0.45	0.00	$C_{16}H_{23}N_3O_4 \cdot {}^1/_3H_2O$	58.70	7.29	12.84	58.67	7.30	12.81
11, Ph-Ac-β-Ala-Lys-OH	73	165–170 (dec)	+17.0 (c 1.0, MeOH)	0.46	0.00	$C_{17}H_{25}N_3O_4 \cdot {}^1/_4H_2O$	60.07	7.56	12.36	60.02	7.59	12.31
12, Ph-Pr-Lys-OH	74	198–200 (dec)	0.00 (c 1.0, DMF)	0.61	0.00	$C_{15}H_{22}N_2O_3$	64.72	7.97	10.07	64.67	7.99	10.01
13, Ph-Pr-Gly-Lys-OH	65	202–206 (dec)	+19.0 (c 1.0, MeOH)	0.45	0.00	$C_{17}H_{25}N_3O_4 \cdot {}^3/_8H_2O$	59.67	7.59	12.28	59.65	7.59	12.28
14, Ph-Pr-β-Lys-OH	88	197–200 (dec)	+20.0 (c 1.0, MeOH)	0.46	0.00	$C_{18}H_{27}N_3O_4 \cdot {}^1/_5H_2O$	61.24	7.82	11.90	61.22	7.84	11.86
15, H-L-Phe-Lys-OH·HCl	98	hygroscopic	+31.0 (c 4.3, MeOH)	0.33	0.00	$C_{15}H_{24}N_3O_3Cl \cdot {}^1/_3H_2O$	53.64	7.40	12.51	53.60	7.45	12.53
16, H-D-Phe-Lys-OH·HCl	98	hygroscopic	−181.0 (c 4.7, MeOH)	0.38	0.00	$C_{15}H_{24}N_3O_3Cl \cdot {}^1/_2H_2O$	53.17	7.44	12.40	53.11	7.47	12.36
17, α-napthoyl-Lys-OH	89		−41.0 (c 4.0, MeOH)	0.55	0.00	$C_{16}H_{20}N_2O_3$	66.64	6.99	9.72	66.60	7.04	9.70
18, Bz-Ala-Lys-OH	77		+17.0 (c 7.7, MeOH)	0.47	0.07	$C_{16}H_{23}N_3O_4 \cdot {}^1/_8H_2O$	59.38	7.24	13.00	59.35	7.26	13.01
19, Ph-Ac-Ala-Lys-OH	76	149–152.5 (dec)	−9.0 (c 1.0, MeOH)	0.57	0.00	$C_{17}H_{25}N_3O_4 \cdot {}^1/_4H_2O$	60.07	7.56	12.36	60.00	7.56	12.33
20, Ph-Pr-Ala-Lys-OH	66	142.5–143 (dec)	+6.0 (c 1.0, MeOH)	0.59	0.00	$C_{18}H_{27}N_3O_4 \cdot {}^1/_4H_2O$	61.05	7.83	11.87	61.01	7.87	11.81
21, Bz-α-Abu-Lys-OH	75	171–172 (dec)	+4.0 (c 1.0, MeOH)	0.57	0.00	$C_{17}H_{25}N_3O_4 \cdot {}^1/_5H_2O$	60.23	7.56	12.40	60.18	7.57	12.42
22, Ph-Ac-α-Abu-Lys-OH	67	190–193	+7.0 (c 1.0, MeOH)	0.58	0.00	$C_{18}H_{27}N_3O_4 \cdot {}^1/_4H_2O$	61.05	7.83	11.87	61.03	7.87	11.85
23, Ph-Pr-α-Abu-Lys-OH	67	198.5–201.5	+2.0 (c 1.0, MeOH)	0.56	0.00	$C_{19}H_{29}N_3O_4 \cdot {}^1/_5H_2O$	62.17	8.07	11.45	62.13	8.06	11.43
24, Ac-L-Phe-Lys-OH	82	71–74 (dec)	+12.0 (c 1.0, MeOH)	0.50	0.00	$C_{17}H_{25}N_3O_4 \cdot {}^1/_5H_2O$	60.23	7.56	12.40	60.16	7.55	12.38
25, Ac-D-Phe-Lys-OH	71	130–134 (dec)	+11.0 (c 1.0, MeOH)	0.51	0.00	$C_{17}H_{25}N_3O_4 \cdot {}^1/_4H_2O$	60.07	7.56	12.36	60.05	7.58	12.35

were converted to active esters by use of DCC and HODSP. These amino acid active esters were reacted with H-Lys(Z)-OH (**32**) to yield protected acyldipeptide esters. In the case of the preparation of **5** and **6**, H-Orn(Z)-OH (**30**) and H-Dab(Z)-OH (**34**) were used instead of **32**. Catalytic hydrogenations gave the final products. The procedures of each step are described in detail.

Acylation of Amino Acid, Bz-Gly-OH (**26**). To a solution of Gly (0.75 g, 10 mmol) in water (10 mL) were added Bz-Cl (1.40 mL, 12 mmol) and Na_2CO_3 (1.40 g, 10 mmol) at 0 °C with stirring. The reaction mixture was stirred in an ice bath for 2 h. The mixture was washed with ether, and the aqueous layer was acidified to pH 2 with 0.5 M HCl. The precipitate was obtained by filtration.

Synthesis of Active Ester, Bz-Gly-ODSP (**27**). Bz-Gly-OH (**26**; 0.90 g, 5 mmol) and HODSP (1.60 g, 6 mmol) were dissolved in CH_3CN (20 mL), and DCC (1.03 g, 5 mmol) was added to the solution below 0 °C with stirring. After stirring, the reaction mixture was held overnight at 0 °C DCurea was filtered off, and the filtrate was evaporated in vacuo. The oily residue was crystallized from acetone.

Blocking of Side Chain in Amino Acid, H-Lys(Z)-OH (**32**). H-Lys-OH·HCl (1.83 g, 10 mmol) and Et_3N (1.4 mL, 10 mmol) were dissolved in water, and Z-ODSP (4.0 g, 10 mmol) was added to the solution at room temperature with stirring. The pH was automatically maintained at 10.0 with 1 M Na_2CO_3. After 3 h, 20% citric acid was added to the solution, and the mixture was kept overnight in a refrigerator. The precipitate was collected by filtration and washed out with water and ethyl acetate. The purity of the product was confirmed by comparison with the authentic compound (Kuwata and Watanabe, 1965).

Coupling Reaction, Bz-Gly-Lys(Z)-OH (**33**). H-Lys(Z)-OH (**32**; 1.40 g, 5 mmol) and 2 M NaOH (2.5 mL) were dissolved in water (30 mL), and Bz-Gly-ODSP (**27**; 2.14 g, 5 mmol) was added to the solution at room temperature with stirring. After the solution was stirred overnight, 0.5 M HCl was added to the solution, and the pH of the solution was adjusted to 3.0. The mixture was dissolved in ethyl acetate and was washed successively with 0.5 M HCl and water and then dried over anhydrous sodium sulfate. The filtrate was evaporated in vacuo, and the oily residue was crystallized from ether.

Catalytic Hydrogenation, Bz-Gly-Lys-OH (**1**). A solution of Bz-Gly-Lys(Z)-OH (**33**; 0.44 g, 1 mmol) in acetic acid (2 mL) was hydrogenated in the presence of palladium black at room temperature for 24 h. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo. The residue was solidified with ether.

The purity of synthetic peptides and their intermediates was confirmed by thin-layer examination with two solvent systems, together with elemental analysis. The yields of and analytical data for the synthetic compounds and their intermediate are summarized in Tables VI and VII.

The abbreviations recommended by the IUPAC–IUB Commission on Biochemical Nomenclature (1972) were used. All amino acids used except glycine were of the L configuration, unless otherwise noted. Additional abbreviations: DCC, dicyclohexylcarbodiimide; DCurea *N,N'*-dicyclohexylurea; Ph-AC, phenylacetyl; Ph-Pr, phenylpropionyl; β-Ala, β-alanine, γ-Abu, γ-aminobutyric acid; α-Abu, α-aminobutyric acid; Dab, α,γ-diaminobutyric acid; HODSP, (*p*-hydroxyphenyl)dimethylsulfonium methyl sulfate.

RESULTS AND DISCUSSION

Sweet Taste of By-Gly-Lys. The taste of synthesized Bz-Gly-Lys (**1**) was measured by the method mentioned under Experimental Procedures. Results of sensory tests are presented in Table I. The sweet taste of Bz-Gly-Lys (**1**) was about 8 times as strong as that of Gly-Lys. Introducing the Bz group, as a hydrophobic group (X), to Gly-Lys increased the sweet taste. This means that our theory on the structure–taste relationship for sweet taste, the AH–B–X system, is reasonable. This compound is free at the C terminus and is thought to avoid some of the problems of aspartame. Thus, the usefulness of our inverted-aspartame-type sweetener was confirmed.

However, Bz-Gly-Lys (**1**) is not satisfactory for practical use because of its low sweetness potency. Thus, the next step is to increase the sweetness potency of our inverted-aspartame-type sweetners. Our synthetic plan considered especially the following three points: (1) determine the distance between AH and B in the C-terminal basic amino acid; (2) choose the optimal hydrophobic group as X; investigate the optimal distance between X and AH–B.

Optimization of the Distance between AH and B. We investigated the optimal distance between AH and B, which is represented as *l* in Table II. We synthesized benzoyl amino acids and dipeptide derivatives that varied at the portion of the C-terminal amino acid from Lys

to Dab, as listed in Table II. The syntheses of these compounds were fundamentally the same method as described under Experimental Procedures. For both benzoyl amino acids and benzoylglycyl amino acids, the more the side chain of the C-terminal amino acid was shortened, the more the potency of bitter taste was increased. Lysine derivatives were superior in both taste qualities and potencies to Orn and Dab derivatives. Consequently, the optimal side-chain length (l) was determined as 4, that is of Lys.

Determination of Hydrophobic Group X. As for the hydrophobic group X, some varied phenyl groups were introduced to Lys, summarized in Table III. Among Bz, Ph-Ac, and Phe-Pr groups, there were no obvious differences. Further, the α-napthoyl group instead of the phenyl group also gave an unsatisfactory result; the sweetness was accompanied with a bitter taste. From theese results, it was suggested that the phenyl group was sufficient for the hydrophobic group X.

Optimization of the Distance between X and AH–B. The most important problem is how to position the phenyl group efficiently in the compounds with AH–B groups. The data were rearranged from the aspect of the difference of distance between AH–B (Lys) and the phenyl group (fixed as X). The number of methylene groups in the introduced acyl group was represented as m, and that in the N-terminal amino acid was represented as n. There were no significant taste differences on varying the numbers of m and n in the sweeteners, as shown in Table IV. A certain distance between X and the AH–B group is required for the good sweet taste. Bz-β-Ala-Lys (**7**) and Bz-γ-Abu-Lys (**8**) were found to have good sweet taste without bitterness, and the potency was about 20 times stronger than that of sucrose. Further, Ph-Ac-Gly-Lys (**10**) was found to have a good sweet taste which was about 50 times as strong as that of sucrose. Ph-Pr-Gly-Lys (**13**) was also found to have a good taste without bitterness, but the potency was slightly weaker than that of Ph-Ac-Gly-Lys (**10**). However, when the total carbon number was too high, good sweet taste with no bitterness was not maintained. For both Ph-Ac-β-Ala-Lys (**11**) and Ph-Pr-β-Ala-Lys (**14**) bitterness was detected in addition to the sweet taste. Consequently, the optimal total carbon number between AH–B and X was determined to be 6 or 7. Under these conditions, the compound 50 times sweeter than sucrose was obtained.

Sweet Taste of the Branched Molecule. It is believed that aspartame produces the strong sweet taste because it has a branched structure at the Phe moiety so that the flexibility of the X functional group (phenyl group of Phe) is limited. Therefore, we introduced a branched structure into the N-terminal amino acid of our inverted-aspartame-type system to reduce the flexibility of the X functional group and increase the sweetness potency. Among the compounds prepared, Ac-L-Phe-Lys (**24**) was found to be useful with a strong sweet taste and high taste quality (see Table V). On the other hand, Ac-D-Phe-Lys (**25**) was not so good because of the coexistence of a bitter taste. Each of the other compounds possessed a bitter or an astringent taste that decreased the taste quality.

Though the conformational analyses have not yet been completed, AH, B, and X functional groups of Ac-L-Phe-Lys (**24**) seem to locate at the proper position for sweetness as do those of aspartame. Therefore, the diastereomers of Ac-L-Phe-Lys (**24**), whose three functional groups probably are not able to attach the sweet taste receptor, are thought to produce a bitter taste like those of aspartame.

It is well-known that the methyl ester in aspartame is inevitable for the strong sweet taste; however, it has not been clarified as to how the methyl ester in aspartame works for the sweet taste. The methyl ester in aspartame is considered to merely stabilize the whole molecule. In addition, the role of the methyl ester in aspartame is thought to block the α-COOH group in the C terminus so the β-COOH group in the Asp moiety can function effectively. Our inverted-aspartame-type sweeteners do not require the branched structure like aspartame. From these results, the methyl ester itself was not thought to work directly as a functional unit for the sweet taste receptor.

CONCLUSION

We attempted to develop new types of sweeteners according to the AH–B–X theory and successfully designed the inverted-aspartame-type sweeteners. In one of them, the sweetness potency reached a level 50 times stronger than that of sucrose.

All the synthetic compounds reported in this paper do not contain a methyl ester in their molecules. This is one of the most remarkable features and is thought to be a great advantage when compared with aspartame from the aspects of stability in solution and nutritious problems. In addition, by use of the newly developed water-soluble active ester, p-dimethylsulfoniophenyl ester (HODSP; Kouge et al., 1987), ω-protected basic amino acids were prepared without formation of the cupric complex, and the synthetic procedure was reduced to five steps, as indicated in Figure 2. Therefore, it is expected that this series of sweeteners can be synthesized at lower cost than aspartame, and almost all reactions can be performed in water. Furthermore, Ac-L-Phe-Lys (**24**) has the possibility of being synthesized by an enzyme. These new types of sweeteners are throught to be very promising both in the field of elemental study and in practical use.

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Novel Monoterpene Disaccharide Glycosides of *Vitis vinifera* Grapes

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Acuminoside (geranyl 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside) has been characterized in *Vitis vinifera* vars. Muscat of Frontignan, Muscat of Alexandria, Muscat Ottonel, Muscat of Hambourg, and Gewürztraminer. Moreover, similar disaccharide glycosides of monoterpenols, of 2-phenylethanol, and of benzyl alcohol were tentatively identified on the basis of the GC–EIMS and GC–CIMS of their trimethylsilyl and trifluoroacetyl derivatives.

1. INTRODUCTION

Since Cordonnier and Bayonove (1974) first suggested the occurrence of monoterpene glycosides in *Vitis vinifera* var. Muscat of Alexandria, extensive research has been carried out on their chemical structure. These compounds, although odorless, can be hydrolyzed under certain conditions to yield increases in the concentrations of volatile flavorants with quite low aroma threshold values (Günata et al., 1989). Williams et al. (1982, 1983) first identified them as 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosides (β-rutinosides) (I), 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides (II), and β-D-glucopyranosides (III) with monoterpenyl aglycons at the linalool oxidation state and benzyl and 2-phenylethyl aglycons (Figure 1); the same group described later such glycosides with other aglycons, monoterpenoids, C13 norisoprenoids, phenols, and other compounds (Strauss et al., 1987a,b, 1988), and presented data substantiating the importance of these tasteless compounds as a reserve of grape flavor (Strauss et al., 1985; Noble et al., 1987, 1988).

To further progress with the knowledge of their structural, chemical, and biochemical properties, we focused on their chemical synthesis (Voirin et al., 1989; Günata et al., 1989a), their enzymatic hydrolysis (Günata et al.,

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