Structure–Taste Relationships of Some Dipeptides

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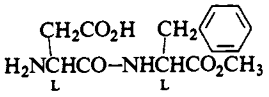
Abstract: The discovery of a dipeptide ester, L-Asp-L-Phe-OMe, having an intense sweet taste is reported. Investigation of structure–taste relationships showed that L-aspartic acid is critical for sweetness but that considerable modification of the phenylalanine portion can be tolerated.

Synthetic sweetening agents have been important substances since the discovery of saccharin in 1879.[1] In recent years, particularly because of emphasis on weight control, they have enjoyed widespread use in the United States. Domestic consumption in 1967 was estimated at 15.2 million pounds for salts of N-cyclohexylsulfamic acid.[2] In sweetening capacity, this is approximately equivalent to 500 million pounds of sucrose. Along with increased consumption of sugar substitutes has come greatly increased concern about possible toxic effects.[3]

The discovery of sweet-tasting compounds has been completely accidental. It is not possible to predict with any certainty whether a new structure will taste sweet or even have a taste at all. There is virtually no knowledge of the detailed chemistry of taste although proteins have been isolated from different areas of the tongue which can complex with sweet and bitter substances, respectively.[4] Structure–taste relationships consist merely of tasting compounds and looking at their structures. Correlations have been attempted but they have no predictive value outside a particular series.[5,6]

In fact, the diversity of structures involved[7,8] suggests that the taste bud protein responsible for initiating a sensation of sweetness has many active receptor sites. This would be possible if, for example, a certain tertiary structure were required for sweetness and this structure could form by association with a small molecule at any one of a number of different sites.

We wish to report another accidental discovery of an organic compound with a pronounced sucrose-like taste. During work on the synthesis of the C-terminal tetrapeptide of gastrin, tryptophylmethionylaspartylphenylalanine amide, one of us (J. M. S.) was crystallizing



aspartylphenylalanine methyl ester[9] and noticed that it was sweet. Preliminary testing showed this compound to have a potency of 100–200 times sucrose depending on concentration and on what other flavors were present and to be devoid of unpleasant aftertaste. In fact, one subject to whom sucrose was objectionable thought that aspartylphenylalanine methyl ester had a very pleasant taste.

Although aspartylphenylalanine methyl ester is derived from amino acids, it in no way reflects the tastes of L-aspartic acid and L-phenylalanine.[10] The former is tasteless while the latter is bitter. In fact, alanine is the only L-amino acid even slightly sweet, being about twice sucrose. The other amino acids with a sweet taste are all D isomers, histidine, leucine, phenylalanine, tryptophan, and tyrosine. Glycine, with no asymmetric center, is also slightly sweet.

The plan of the present work was to vary independently the two amino acids and the C-terminal functional groups and to use these results, if definite structural requirements for a sweet taste were discovered, in the design of additional compounds. Test compounds were made up as a 1% solution, a cotton swab stick was soaked in the test solution, and the compound was sucked off the swab. Successive dilutions were made as required to determine relative potency. This procedure gave satisfactorily consistent results from subject to subject. The 1% concentration was chosen because it is the approximate threshold value for sucrose for untrained tasters. Sweetness potency was estimated as follows: + = sucrose, ++ = 10× sucrose, +++ = 100× sucrose. In addition, 0 = tasteless, − = bitter. No attempt was made to quantitate the latter taste. On this scale, aspartylphenylalanine methyl ester was +++.

The first change studied was the replacement of aspartic acid by other amino acids. C-Terminal phenylalanine dipeptides were obtained commercially;[11] methyl esters were prepared by acid-catalyzed esterification and the dipeptide esters tasted as the hydrochlorides and as the free basic esters. Under both conditions, all the products were bitter. The compounds synthesized were the methyl esters of alanylphenylalanine, glycylphenylalanine, histidylphenylalanine, isoleucylphenylalanine, leucylphenylalanine, lysylphenylalanine, norleucylphenylalanine, norvalylphenylalanine, phenylalanylphenylalanine, prolylphenylalanine, sarcosylphenylalanine, serylphenylalanine, threonylphenylalanine, tryptophylphenylalanine, tyrosylphenylalanine, and valylphenylalanine.

The results of replacement of phenylalanine were of more interest since several of the esters were sweet. In particular, aspartyltyrosine methyl ester and aspartylmethionine methyl ester were similar in potency to aspar-

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[11] Cyclo Chemical Corp., Los Angeles, Calif.

Table I

OBzl
|
Z-Asp-X-OMe[a]

X	Yield, %	Mp, °C	[α]D,[c] deg	Anal., %[d] C	H	N
Ala	100[b]	126.5–127.5	+20	62.43	5.92	6.33
	D	P	CH	62.61	6.03	6.54
Me	72	76–82	+16	59.00	5.78	5.74
|	D	ET	CH	59.05	5.77	5.82
Cys						
Me(O$_2$)	73	96–98	+14	55.37	5.42	5.38
	D	E–CY	CH	55.30	5.35	5.46
Cys						
Gly	79	128.5–131	+ 9	61.67	5.65	6.54
	D	P	CH	61.69	5.75	6.65
Leu	74	66.5–67.5	+14	64.45	6.66	5.78
	D	P	CH	64.27	6.62	6.01
Met	69	78–78.5	+26	59.74	6.02	5.58
	D	P	CH	60.08	6.04	5.85
D-Met	85	102–104	−8.5	59.74	6.02	5.58
	D	P	CH	59.92	5.97	5.94
O$_2$	80	84, 112	+30	56.17	5.66	5.24
|	D	P	CH	56.18	5.71	5.46
Met						
Phe[e]	79	119–120	−10	67.17	5.83	5.40
	D	P	M	67.30	6.09	5.41
Trp	93	115–116	+53	66.77	5.60	7.54
	D	P–CY	CH	66.88	5.89	7.66
D-Trp	100	135.5–137	−21	66.77	5.60	7.54
	D	M–MC	CH	66.85	5.78	7.69
Tyr	57	125–127.5	−5	65.16	5.66	5.24
	EA	EA–ET	M	64.85	5.86	5.30
Val	89	110.5–112	+18	63.81	6.43	5.95
	D	P	CH	63.99	6.55	6.08

[a] All amino acids have the L configuration unless otherwise stated. Abbreviations according to IUPAC-IUB Commission on Biochemical Nomenclature, *Biochim. Biophys. Acta*, **121**, 1 (1966). In addition, O$_2$ = sulfone. [b] Solvents used for coupling and crystallization are shown under yield and melting point, respectively. Solvents are abbreviated as follows: A, acetone; 75AC and 90AC, 75 and 90% acetic acid; C, carbon tetrachloride; CH, chloroform; CY, cyclohexane; D, dimethylformamide; E, ethanol; EA, ethyl acetate; ET, ether; H, 1 *N* HCl; M, methanol; MC, methylene chloride; P, isopropyl alcohol; PA, isopropyl acetate; S, Skellysolve B; W, water. [c] Rotations were measured at room temperature at 1% concentration in the solvent indicated. [d] Calculated values on first line, found on second. [e] Reference 9, mp 116–117°, $[\alpha]^{22}$D −15.3° (*c* 1, DMF).

tylphenylalanine methyl ester. All protected dipeptides were made by the active ester method[12] using protecting groups derived from benzyl alcohol so that hydrogenolysis would give the compounds desired for tasting.

The physical properties of protected dipeptides are shown in Table I and free dipeptide esters in Table II. Further modifications of both the phenylalanine and tyrosine compounds were investigated. Aspartylmethionine methyl ester had a slight sulfury taste which became quite objectionable on standing.

The necessary distance between amino and carboxyl groups was studied by moving the peptide bond to the β-carboxyl of aspartic acid and by replacing aspartic by glutamic acid. The required stereochemistry of the molecule was determined by synthesizing all possible optical isomers of aspartylphenylalanine methyl ester. The protected intermediates are described in Table III and the final products in Table IV. All of the latter compounds were bitter.

Finally, considerable attention was given to the importance of the functional groups. With one exception (aspartylphenylalanine ethanolamide) an ester on the C-terminal carboxyl was required for sweetness. Also, the aspartic acid amino group had to be unsubstituted. The results with phenylalanine are shown in Tables V and VI, with tyrosine in Tables VII and VIII.

The above results, taken together, enable several very interesting conclusions to be drawn. Since we are dealing with a biochemical reaction, possibly complex formation with specific binding sites on a protein surface, it is not surprising that structural requirements for sweet taste are rather rigid. However, certain changes are permitted and a definite pattern can be discerned.

The presence of both the free, unsubstituted amino and one carboxyl group of aspartic acid as well as the distance between them and the absolute configuration of the asymmetric carbon are completely critical. This is strikingly shown by Me$_2$Asp-Phe-OMe, Asp(OMe)-Phe, Asp(Phe-OMe), Glu-Phe-OMe, and D-Asp-L-Phe-OMe. These ionic groups must bind directly to a taste-triggering receptor site in the taste buds.

Two other sites, one or both of which may be primarily hydrophobic, are involved and are slightly less critical although obviously still very important. The requirement of absolute L configuration still holds (L-Asp-D-Phe-OMe is bitter). At first sight, an electron-rich side

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Table II

		Asp-X-OMe			Anal., %[d]		
X	Yield, %	Mp, °C	[α]D, deg	C	H	N	Taste
Ala	66	227.5–241	−26	44.03	6.47	12.84	−
	75AC	M–ET	W	43.72	6.49	13.21	
Me	68	146.5–149	−26	40.90	6.10	10.60	+
|	75AC	M–P	W	40.92	6.18	10.99	
Cys							
Me(O_2)	83	135–136	−9	35.40	5.61	9.18	0
|	75AC	W–P	W	35.18	5.81	9.10	
Cys·$0.5H_2O$							
Gly	69	159.5–161	+38	41.17	5.92	13.72	−
	75AC	W–P	W	41.43	5.86	13.87	
Ile[a]	64	104–108	+2	50.75	7.75	10.76	−
	75AC	M–P	W	50.49	7.68	10.76	
Leu	90	132–133.5	−22	50.75	7.75	10.76	−
	75AC	E–ET	W	51.22	7.90	10.77	
Met	83	136–145, 200–214	−20	43.15	6.52	10.07	+++
	75AC	M–ET	W	43.37	6.35	10.34	
D-Met	90	119–127	+44	43.15	6.52	10.07	0
	75AC	M–ET	W	43.05	6.63	10.51	
O	71	157–158	−2	38.70	5.85	9.03	++
|	75AC	W–P	W	38.91	5.76	8.88	
Met							
Phe[b]	82	190, 245–247	0	57.13	6.17	9.52	+++
	75AC	E–W	W	57.33	6.40	9.45	
Thr·$0.5H_2O$[c]	30	162–163.5	−8	42.10	6.68	10.89	0
	75AC	W–P	W	42.38	7.13	10.65	
Trp·$0.5H_2O$	37	157.5–162	+4	56.15	6.17	12.27	−
	75AC	M–P	W	56.66	6.01	12.12	
D-Trp	84	147–150	+29	57.65	5.75	12.61	−
	75AC	M–ET	W	57.33	5.76	12.59	
Tyr	84	180–185, 230–250	+4	54.19	5.85	9.03	++
	75AC	E–W	W	53.78	6.21	8.88	
Val	85	214–219	−12	48.77	7.36	11.38	−
	75AC	M–P	W	48.41	7.37	11.57	

[a] Z-Asp(OBzl)-Ile-OMe was obtained in 80% yield as an oil and was used directly. [b] Reference 9, mp 246–247°, $[\alpha]^{22}D$ −2.3° (*c* 1, 1 *N* HCl). [c] Z-Asp(OBzl)-Thr-OMe was obtained in 71% yield as a gum and was used directly. [d] Calculated values on first line, found on second.

chain seems to be necessary (Asp-Met-OMe, Asp-Phe-OMe, Asp-Tyr-OMe) but this is belied by the lack of sweetness of Asp-His-OMe and Asp-Trp-OMe. Size may be the most important factor since Asp-Tyr(Me)-OMe and Asp-Tyr(Et)-OMe are less sweet than Asp-Tyr-OMe. Binding also takes place to the relatively nonpolar ester group (Asp-Phe, Asp-Phe-NH_2, and Asp-Phe-$NHNMe_2$ are not sweet). There seems to be a definite size requirement since sweetness falls off rapidly with increasing bulk (Asp-Phe-OMe, Asp-Phe-OEt, Asp-Phe-OPr).

In summary, if retention of sweetness is desired, changes in the aspartic acid part cannot be made but there is room for substantial manipulation of the phenylalanine portion. Work in progress is directed along these lines.

Experimental Section

Elemental analyses were done under the direction of E. Zielinski, rotations under the direction of A. J. Damascus. We would like to thank W. M. Selby, J. D. Choi, and J. Serauskas for many hydrogenations. Initial taste testing was supervised by D. L. Knapp.

All intermediates and products were controlled by thin layer chromatography on silica. Fully protected compounds were run in a suitable mixture of methanol and chloroform. Compounds with at most one free functional group were run in methanol–chloroform or *n*-butyl alcohol–acetic acid–water 7:1:2. When more than one free functional group was present, the latter solvent was used. Spots were detected by the *t*-butyl hypochlorite–starch–iodide method.[13] All compounds reported here were essentially homogeneous on tlc.

Gly-Phe-OMe·HCl. The general procedure used for conversion of commercially available C-terminal phenylalanine dipeptides to methyl esters is illustrated. Thionyl chloride (11.9 g, 0.1 mol) was added to 75 ml of MeOH without cooling and the solution made up to 100 ml with additional MeOH. Gly-Phe (20 mg) was dissolved in 2.0 ml of the above solution and the reaction followed by tlc (*n*-BuOH–HOAc–H_2O system). Esterification was very rapid being about one-half over by the time the starting material had dissolved and complete after 1 hr. The solution was evaporated to dryness under a nitrogen stream and the residue dried overnight in a vacuum desiccator over KOH pellets. The crude product (homogeneous, tlc) was dissolved in 2.0 ml of tap water and the solution (pH 5) tasted. Solid $KHCO_3$ was added to pH 7 and the solution tasted again. No attempt was made to characterize the product other than tlc. The following ester hydrochlorides were prepared similarly: Ala-Phe-OMe·HCl, His-Phe-OMe·HCl, Ile-Phe-OMe·HCl, Leu-Phe-OMe·HCl, Lys-Phe-OMe·HCl, Nle-Phe-OMe·HCl, Nva-Phe-OMe·HCl, Phe-Phe-OMe·HCl, Pro-Phe-OMe·HCl, Sar-Phe-OMe·HCl, Ser-Phe-OMe·HCl, Thr-Phe-

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Table III. Isomers and Homologs. Protected Dipeptides

Compound	Yield, %	Mp, °C	$[\alpha]_D$, deg	Anal., %[b]		
				C	H	N
Phe-OMe | Z-Asp-OBzl	90 D	131–133 P	−2 M	67.17 67.06	5.83 6.05	5.40 5.29
Tyr-OMe | Z-Asp-OBzl	95 D	147–149 P–W	+2 M	65.16 64.86	5.66 5.64	5.24 5.35
OBzl | Z-Glu-Phe-OMe	89 D	78–80 P	−11 M	67.65 67.58	6.06 6.07	5.26 5.54
Phe-OMe | Z-Glu-OBzl	92 D	115–118 P	−13 M	67.65 67.61	6.06 6.30	5.26 5.34
OBzl | Z-Glu-Tyr-OMe	97 D	125–126 P–W	−44 M	65.68 65.69	5.88 5.84	5.11 5.06
OBzl | Z-Asp-Phe-OMe L D	84 D	96–97 P	−5 M	67.17 67.02	5.83 5.72	5.40 5.56
OBzl | Z-Asp-Phe-OMe D L	95 D	98–99 P	+5 M	67.17 67.17	5.83 5.87	5.40 5.60
OBzl | Z-Asp-Phe-OMe D D	80[a] EA	118.5–119.5 EA	+10 M	67.17 67.20	5.83 5.88	5.40 5.53

[a] The product crystallized from the reaction solution. [b] Calculated values on first line, found on second.

Table IV. Isomers and Homologs. Dipeptide Methyl Esters

Compound	Yield, %	Mp, °C	$[\alpha]_D$, deg	Anal., %[a]		
				C	H	N
Phe-OMe | Asp	88 90AC	196–197 W	+4 W	57.13 57.11	6.17 6.26	9.52 9.60
Tyr-OMe | Asp	65 90AC	202–204 P–W	+13 W	54.19 53.66	5.85 6.18	9.03 9.02
Glu-Phe-OMe	93 90AC	Soften from 140 M–EA	+1 W	58.43 58.95	6.54 6.83	9.09 8.91
Phe-OMe | Glu·0.5H$_2$O	91 90AC	182–183 W	−2 M	56.77 56.57	6.67 6.69	8.83 9.11
Glu-Tyr-OMe	71 90AC	144–145 M–W	+21 M	52.62 52.71	6.48 6.60	8.18 8.07
Asp-Phe-OMe L D	98 90AC	157–159, 212–213 W	+19 W	57.13 57.47	6.17 6.34	9.52 9.42
Asp-Phe-OMe D L	78 90AC	159–160, 212–213 W	−18 W	57.13 57.02	6.17 6.00	9.52 9.48
Asp-Phe-OMe D D	80 90AC	190, 244–245 E–W	0 W	57.13 57.17	6.17 6.49	9.52 9.52

[a] Calculated values on first line, found on second.

OMe·HCl, Trp-Phe-OMe·HCl, Tyr-Phe-OMe·HCl, Val-Phe-OMe·HCl.

Intermediates. It was necessary to synthesize many derivatives of amino acids during this work. Methyl, ethyl, and propyl ester hydrochlorides were prepared by the thionyl chloride procedure[14] and benzyl ester *p*-toluenesulfonates by azeotropic distillation of water from a solution of the amino acid and *p*-toluenesulfonic acid in benzyl alcohol–benzene. The melting points of the products agreed well with literature values. Previously known compounds, other than methyl, ethyl, and benzyl esters of unmodified amino acids, are Z-Asp(OBzl)-ONp,[15] Z-Asp(ONp)-OBzl,[16] Z-Asp(OMe)-ONp,[17] Z-Asp(NH$_2$)-ONp,[18] Cys(Me)-OMe·HCl,[19] Z-Glu(OBzl)-

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Table V. Protected Functional Group Analogs. Phenylalanine

Compound	Yield, %	Mp, °C	[α]D, deg	Anal., %[e] C	H	N
OBzl | Z-Asp-Phe-OBzl[a]	99 EA	99–102 EA–CY	−20 M	70.69 70.69	5.76 5.59	4.71 4.86
—Phe-OBzl | Z-Asp-OBzl	83 D	138–142 EA–CY	−8 D	70.69 70.77	5.76 6.05	4.71 4.73
OMe | Z-Asp-Phe-OBzl	85 EA	103–105 EA–CY	−26 M	67.17 67.38	5.83 5.76	5.40 5.49
OBzl | Z-Asp-Phe-OEt	40 EA	85–95 P	−10 M	67.65 67.68	6.06 6.22	5.26 5.33
OBzl | Z-Asp-Phe-OPr^n	86 EA	90–91 PA–CY	−12 M	68.11 68.32	6.27 6.37	5.13 5.08
OBzl | Z-Asp-Phe-OPr^i	65 EA	95–101 EA–CY	−9 M	68.11 67.96	6.27 6.02	5.13 5.39
OBzl | Z-Asp-Phe-OBu^t	78 EA	69–70.5 C–S	−9 M	68.55 68.69	6.47 6.73	5.00 4.93
OBzl | Z-Asp-Phe-NH$_2$[b,c]	94 PA	173–174.5 PA	−27 D	66.78 66.87	5.81 6.17	8.35 8.51
—Phe-NH$_2$· | Z-Asp-OBzl	94 D	209–210 M	−4 D	66.78 66.49	5.81 5.84	8.35 8.40
NH$_2$ | Z-Asp-Phe-OBzl	76 D	183–184 M–ET	−13 D	66.78 66.77	5.81 5.98	8.35 8.42
OBzl | Z-Asp-Phe-NHMe[c,d]	85 PA	190.5–192 PA	−14 AC	67.29 67.40	6.04 6.03	8.12 8.18
OBzl | Z-Asp-Phe-NMe$_2$	100 D	Oil	−6 M	67.87 67.45	6.26 6.31	7.91 7.60
OBzl | Z-Asp-Phe-NH(CH$_2$)$_2$OH	86 D	136–140 PA	−30 M	65.80 65.61	6.70 6.33	7.67 7.98
OBzl | Z-Asp-Phe-NHNMe$_2$	57 D	177–178 ET	−40 M	65.92 65.79	6.27 6.28	10.25 10.27

[a] Reference 24, mp 97–99°. [b] Reference 9, mp 170–171°, $[\alpha]^{22}\text{D}$ −25.9° (*c* 1, DMF). [c] The product crystallized from the reaction solution. [d] Reference 24, mp 180–183°, $[\alpha]^{24}\text{D}$ −22.7° (*c* 1, DMF). [e] Calculated values on first line, found on second.

ONp,[20] Z-Glu(ONp)-OBzl,[20] Phe-OPr^n·HCl,[21] Phe-OPr^i·HCl,[21] Phe-OBu^t,[22] Phe-NH$_2$,[23] Phe-NHMe·HOAc,[24] Tyr(Me)-OMe·HCl,[25] Tyr(Et)-OMe·HCl,[26] Tyr-NH$_2$,[23] and Tyr-NHNH$_2$.[27] New compounds are described individually in detail.

D-Z-Asp(OBzl)-ONp. D-Z-Asp(OBzl)[9] (25.0 g, 0.07 mol) and 12.5 g (0.09 mol) of *p*-nitrophenol were dissolved in 70 ml of EtOAc; the solution was cooled in an ice bath to 10° and 16.1 g(0.078 mol) of dicyclohexylcarbodiimide in 30 ml of EtOAc added. The mixture was stirred 1 hr at room temperature; the dicyclohexylurea was filtered and washed with EtOAc. The combined filtrates were washed with 1 *N* HCl and H_2O, four times with 1 *M* K_2CO_3, and three times with H_2O, and dried over Na_2SO_4; the EtOAc was distilled (bath 50°). The residue was crystallized from 125 ml of *i*-PrOH to give needles, 31.6 g (94%), mp 80–81°, $[\alpha]\text{D}^{26}$ −9° (*c* 1, $CHCl_3$). *Anal.* Calcd for $C_{25}H_{22}N_2O_8$: C, 62.76; H, 4.64; N, 5.86. Found: C, 62.93; H, 4.66; N, 5.99.

Cys[Me(O$_2$)]-OMe·HCl. Cys[Me(O$_2$)][28] (4.24 g, 0.025 mol) was dissolved in 6.0 g (0.05 mol) of $SOCl_2$ in 50 ml of MeOH and the solution heated 2 hr under reflux. Dilution with Et_2O gave 5.25 g (96%), mp 168–170° dec, $[\alpha]^{26}\text{D}$ +6° (*c* 1, H_2O). *Anal.* Calcd for $C_5H_{11}NO_4S \cdot HCl$: N, 6.44; Cl, 16.29. Found: N, 6.43; Cl, 16.18.

Met(O$_2$)-OMe·HCl. The above procedure was used starting with 6.0 g (0.033 mol) of Met(O$_2$). The yield was 7.43 g (96%), mp 164–167° dec, $[\alpha]^{25}\text{D}$ +25° (*c* 1, H_2O). *Anal.* Calcd for $C_6H_{13}NO_4S \cdot HCl$: N, 6.05; Cl, 15.30; S, 13.84. Found: N, 6.26; Cl, 15.30; S, 13.88.

Z-Phe-NHNMe$_2$. Z-Phe (11.96 g, 0.040 mol) was dissolved in 60 ml of anhydrous THF; 4.5 ml (0.04 mol) of N-methylmorpholine was added and the solution cooled to −20°. Ethyl chloro-

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Table VI. Functional Group Analogs. Phenylalanine

Compound	Yield, %	Mp, °C	$[\alpha]_D$, deg	*Anal.*, %[h] C	H	N	Taste
Asp-Phe[a]	78 75AC	232–235 P–W	+16 75AC	55.71 55.67	5.75 5.78	10.00 10.15	–
Phe | Asp	99 90AC	198–203 A–W	+37 AC	55.71 55.46	5.75 6.10	10.00 9.91	–
Asp-Phe[b]	*c*	277–278 M–W	–68 M	59.53 59.25	5.38 5.37	10.68 10.98	0
OMe | Asp-Phe·H_2O	50 75AC	200–207 P–W	+64 W	53.84 53.76	6.45 6.48	8.97 8.92	–
Asp-Phe-OEt	74 75AC	184, 244–246 M–W	–6 M	58.43 58.13	6.54 6.61	9.09 8.99	++
Asp-Phe-OPr^n	89 75AC	155–160, 242–245 M–W	–6 M	59.61 59.58	6.88 7.27	8.69 8.53	+
Asp-Phe-OPr^i	92 75AC	180–181, 232–235 M–W	–2 H	59.61 59.57	6.88 6.99	8.69 8.92	+
Asp-Phe-OBu^t·0.5H_2O	100 75AC	Amorphous	–8 H	59.11 59.32	7.30 7.13	8.11 8.36	+
Me | Me-Asp-Phe-OMe	64[d] 90AC	134–135 P–ET	–25 W	59.61 59.91	6.88 7.11	8.69 8.60	–
Asp-Phe-NH_2[e]	71 75AC	181–191, 225–227 W	+14 W	55.90 55.94	6.14 6.07	15.05 14.99	0
Phe-NH_2[f] | Asp	95 90AC	252–254 P–W	+12 W	55.90 55.97	6.14 6.38	15.05 15.07	0
NH_2 | Asp-Phe	73 90AC	262–265 P–W	+26 W	55.90 56.09	6.14 6.26	15.05 15.07	0
Asp-Phe-NHMe[g]	75 75AC	204–205 W	+29 H	57.32 57.01	6.53 6.87	14.33 14.43	–
Asp-Phe-NMe_2	60 75AC	107–114 M–ET	+39 H	58.62 58.55	6.89 7.22	13.67 13.64	–
Asp-Phe-$NH(CH_2)_2OH$	62 75AC	200 M–W	+24 W	55.72 55.70	6.55 6.72	13.00 13.17	+
Asp-Phe-$NHNMe_2$	95 90AC	186–187 M–P	+26 W	55.88 56.07	6.88 7.03	17.38 17.21	–

[a] Reference 24, mp 220–223°. [b] Reference 9, mp 256–258°. [c] Prepared by heating the filtrate from the crystallization of Asp-Phe-OMe overnight on the steam bath. [d] Prepared by reductive alkylation of Asp-Phe-OMe with formaldehyde over palladium black. [e] Reference 9, mp 188–189°, $[\alpha]^{22}_D$ +20.6° (*c* 1, DMF + 1 *N* HCl). [f] H. Gregory, J. S. Morley, J. M. Smith, and M. J. Smithers, *J. Chem. Soc.*, 715 (1968), reported as the hydrochloride. No rotation or analysis. [g] Reference 24, mp 201–203°, $[\alpha]^{24}_D$ +0.85° (*c* 1, DMF). [h] Calculated values on first line, found on second.

formate (4.0 ml, 0.042 mol) was added with stirring so that the temperature remained below –10° and the mixture stirred 5 min at –15°. N,N-Dimethylhydrazine (3.3 ml, 0.044 mol) was added with stirring so that the temperature remained below –5° and the mixture allowed to stand overnight at 5°. The mixture was diluted with 200 ml of EtOAc; the organic layer was washed with H_2O, 2 *N* HOAc, H_2O, and twice with 1 *N* $KHCO_3$ and dried over Na_2SO_4, and the solvents were distilled (bath 50°). The residue was shaken with cyclohexane and the product filtered and washed with cyclohexane, yield 11.41 g (84%), mp 138–141°. Recrystallization from *i*-PrOH gave needles, mp 144–146°, $[\alpha]^{28}_D$ +4° (*c* 1, MeOH). *Anal.* Calcd for $C_{19}H_{23}N_3O_3$: C, 66.84; H, 6.79; N, 12.30. Found: C, 67.05; H, 6.67; N, 12.33.

Phe-NMe_2·HOAc. The procedure for Z-Phe-$NHNMe_2$ was followed using 29.9 g (0.10 mol) of Z-Phe and 30 ml of 4 *M* Me_2NH in THF. Z-Phe-NMe_2 was obtained as an oil (25.5 g, 78%). The crude product was hydrogenated in 200 ml of 75% HOAc over 8 g of palladium black. The solvents were distilled (bath 50°), and the residue was dissolved in H_2O and taken to dryness. The crude product was crystallized from MeOH-Et_2O to give 13.2 g (67%), mp 98–110°, $[\alpha]^{26}_D$ +62° (*c* 1, H_2O). *Anal.* Calcd for $C_{11}H_{16}N_2O \cdot C_2H_4O_2$: C, 61.88; H, 7.99; N, 11.10. Found: C, 61.79; H, 7.78; N, 11.34.

Z-Phe-$NHCH_2CH_2OH$. The procedure for Z-Phe-$NHNMe_2$ was followed using 12.0 g (0.040 mol) of Z-Phe and 2.5 ml (0.040 mol) of ethanolamine. The crude product (12.7 g) was crystallized twice from *i*-PrOAc to give 7.87 g (54%), mp 132–134°, $[\alpha]^{25}_D$ –4° (*c* 1, MeOH). *Anal.* Calcd for $C_{19}H_{22}N_2O_4$: C, 66.65; H, 6.48; N, 8.18. Found: C, 66.66; H, 6.83; N, 8.34.

Phe-$NHCH_2CH_2OH$·HCl. Hydrogenation of 5.38 g (0.0156 mol) of Z-Phe-$NHCH_2CH_2OH$ was carried out as described for Phe-NMe_2. The crude product was dissolved in 25 ml of 1 *N* HCl and taken to dryness and the residue crystallized from 75 ml of 1:5 MeOH-*i*-PrOH, yield 2.78 g (72%), mp 188–190°, $[\alpha]^{26}_D$ +54° (*c* 1, H_2O). *Anal.* Calcd for $C_{11}H_{16}N_2O_2 \cdot HCl$: C, 53.98; H, 7.00; N, 11.45. Found: C, 53.65; H, 6.96; N, 11.68.

Z-Tyr(Z)-NHMe. The procedure for Z-Phe-$NHNME_2$ was followed using 22.5 g (0.050 mol) of Z-Tyr(Z) and 27.5 ml of 2 *M* $MeNH_2$ in THF. The reaction mixture was filtered and the product washed with H_2O. Crude Z-Tyr(Z)-NHMe (16.2 g) was crystallized twice from MeOH to give 15.9 g (69%), mp 163–167°, $[\alpha]^{24}_D$ +3° (*c* 1, DMF). *Anal.* Calcd for $C_{26}H_{26}N_2O_6$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.84; H, 5.73; N, 6.08.

Tyr-NHMe·HCl. Z-Tyr(Z)-NHMe (13.87 g, 0.030 mol) was hydrogenated as described for Phe-NHMe. The crude product was treated with excess HCl in *i*-PrOH and the hydrochloride precipitated with Et_2O. Crystallization from *i*-PrOH gave 5.76 g (83%), mp 235–240° dec, $[\alpha]^{25}_D$ +70° (*c* 0.5, H_2O). *Anal.* Calcd for $C_{10}H_{14}N_2O_2 \cdot HCl$: C, 52.06; H, 6.55; N, 12.15. Found: C, 51.84; H, 6.81; N, 12.03.

Table VII. Protected Functional Group Analogs. Tyrosine

Compound	Yield, %	Mp, °C	[α]D, deg	Anal., %[c]		
				C	H	N
OBzl | Z-Asp-Tyr-OBzl	67 EA	96–101 P	−21 M	68.84 68.69	5.61 5.79	4.59 4.48
OBzl | Z-Asp-Tyr-OEt	50 EA	122–124 PA-ET	−9 M	65.68 65.90	5.88 5.96	5.11 5.35
OBzlMe | | Z-Asp-Tyr-OMe	76 EA	114–115 EA-ET	−4 M	65.68 65.85	5.88 5.78	5.11 5.00
OBzlEt | | Z-Asp-Tyr-OMe	82 EA	115–116 EA-ET	−6 M	66.18 66.19	6.09 6.03	4.98 4.85
OBzl | Z-Asp-Tyr-NH_2	72[a] EA	177.5–179 M	−19 M	64.73 64.91	5.63 5.79	8.09 8.32
OBzl | Z-Asp-Tyr-NHMe	58 D	194–196 M	−19 D	65.28 65.06	5.86 5.98	7.88 7.78
OBzl | Z-Asp-Tyr-NMe_2	88 D	Amorphous	0 M	65.80 66.09	6.07 6.14	7.67 7.61
OBzl | Z-Asp-Tyr-$NHNH_2$	54 D	164–166 P	−19 D	62.91 62.70	5.66 5.66	10.48 10.19
OBzl | Z-Asp-Tyr-$NHNMe_2$[b]	86 D	184–188 M-EA	−19 M	64.04 63.91	6.09 6.09	9.96 9.86

[a] The product crystallized from the reaction solution. [b] Z-Tyr(Z)-$NHNMe_2$ was deprotected with 2 N HBr in HOAc and the crude hydrobromide used directly. [c] Calculated values on first line, found on second.

Table VIII. Functional Group Analogs. Tyrosine

Compound	Yield, %	Mp, °C	[α]D, deg	Anal., %[b]			Taste
				C	H	N	
Asp-Tyr·H_2O	66 75AC	180, 217–225 E-W	+15 W	51.61 51.80	6.03 5.90	9.24 9.42	−
Asp-Tyr	a	235–237 M-W	−24 AC	56.11 55.92	5.07 5.39	10.07 9.87	0
Asp-Tyr-OEt	88 75AC	189–190 E-W	+20 75AC	55.55 55.06	6.22 6.66	8.64 8.49	++
Me | Asp-Tyr-OMe	92 75AC	138–140, 185–235 E-W	0 M	55.55 55.32	6.22 6.34	8.64 8.58	+
Et | Asp-Tyr-OMe	83 75AC	185–240 E-W	−2 M	56.79 56.32	6.55 6.44	8.28 8.32	+
Asp-Tyr-NH_2·H_2O	71 75AC	197–200 W	+27 H	49.83 50.18	6.11 6.23	13.41 13.50	0
Asp-Tyr-NHMe	93 75AC	208–209 W	+34 H	54.36 54.24	6.19 6.44	13.59 13.44	−
Asp-Tyr-NMe_2	52 75AC	145–155 M-W	+57 W	55.72 55.56	6.55 6.58	13.00 12.92	−
Asp-Tyr-$NHNH_2$· 1.25H_2O	96 90AC	> 175 dec P-W	+28 W	46.91 47.12	6.21 6.31	16.84 16.50	0
Asp-Tyr-$NHNMe_2$	89 90AC	> 175 dec M-P	−2 M	53.24 52.89	6.55 6.78	16.56 16.02	0

[a] Prepared by heating the filtrate from the crystallization of Asp-Tyr-OMe overnight on the steam bath. [b] Calculated values on first line, found on second.

Tyr-NMe_2·HOAc. Z-Tyr(Z)-ONp[29] (13.5 g, 0.024 mol) was dissolved in 125 ml of EtOAc and 6.5 ml of 4 M Me_2NH in THF added. After 4 days at room temperature, the solution was washed with 1 N HCl and H_2O, four times with 1 M K_2CO_3, and H_2O and dried over Na_2SO_4 and the EtOAc distilled (bath 50°) to give Z-Tyr(Z)-NMe_2 as an oil. The crude product was hydrogenated as described for Phe-NMe_2. The acetate was crystallized from MeOH to yield 3.6 g (56%), mp 170–180°, $[\alpha]^{28}D$ + 62° (c 1, H_2O). *Anal.* Calcd for $C_{11}H_{16}N_2O_2 \cdot C_2H_4O_2$: C, 58.19; H, 7.51; N, 10.44. Found: C, 58.34; H, 7.55; N, 10.47.

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Z-Tyr(Z)-NHNMe$_2$. The procedure for Z-Phe-NHNMe$_2$ was followed using 18.0 g (0.040 mol) of Z-Tyr(Z). The crude product was washed with cyclohexane to yield 17.6 g (90%), mp 152–159°. Crystallization from *i*-PrOH gave small needles, mp 160–163°, $[\alpha]^{28}\mathrm{D}$ +4° (*c* 1, MeOH). *Anal.* Calcd for $C_{27}H_{29}N_3O_6$: C, 65.97; H, 5.95; N, 8.55. Found: C, 66.22; H, 6.06; N, 8.57.

Dipeptides. Coupling reactions were carried out by the *p*-nitrophenyl ester method.[12] When dimethylformamide was the solvent, the amino ester salt could be used directly followed by 1 equiv of a tertiary amine. In other cases, it was necessary first to liberate the amino ester and isolate it. The peptide reaction mixture was usually diluted with ethyl acetate and washed with dilute hydrochloric acid and thoroughly with potassium carbonate solution. The desired product was readily separated from unreacted active ester by crystallization.

Hydrogenations were done in 75 or 90% acetic acid over 5–10% by weight of palladium black at room temperature and up to 4 atm pressure. No difficulties were experienced even with S-methylcysteine and methionine at these high catalyst ratios.

Solid-Phase Synthesis of the Cyclododecadepsipeptide Valinomycin[1]

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Abstract: The method of automated solid-phase peptide synthesis was applied to the preparation of the antibiotic valinomycin, a cyclic dodecadepsipeptide containing D-valyl, L-valyl, D-α-hydroxyisovaleryl, and L-lactyl residues. The open-chain depsipeptide was synthesized by coupling alternately the N-Boc-protected didepsipeptides L-valyl-D-α-hydroxyisovaleric acid and D-valyl-L-lactic acid to resin-bound D-valyl-L-lactate using dicyclohexylcarbodiimide as coupling agent. After cleavage from the resin the peptide was cyclized by the acid chloride method to give valinomycin. The crystalline product that was obtained in an over-all yield of 33% had the same physical and chemical properties as the natural antibiotic and showed the same characteristics in making lipid bilayers selectively permeable to potassium ions.

The antibiotic valinomycin was isolated from *Streptomyces fulvissimus* by Brockmann[2] in 1955, and a cyclooctadepsipeptide[3] structure was proposed for it.[4] The correct structure was finally established when Shemyakin, *et al.*,[5] synthesized the cyclododecadepsipeptide of the formula in Figure 1 and showed it to be identical with natural valinomycin. It contains two amino acids (L-valine and D-valine) and two hydroxy acids (D-α-hydroxyisovaleric acid and L-lactic acid),[6] which are arranged in a 36-membered ring regularly alternating between amino and hydroxy acids.

In recent years valinomycin has attracted the attention of several groups of investigators because of its remarkable effect on the permeability of biological and artificial lipid membranes to monovalent cations. The compound produces marked selectivity for K$^+$, compared with Na$^+$, in membranes of mitochondria,[7] in red blood cells,[8] and in several types of lipid bilayers.[9-12] Furthermore, under nonaqueous conditions this depsipeptide forms complexes much more readily with K$^+$ than with Na$^+$,[13,14] a property which is undoubtedly related to its effects on membranes.

The present investigation was undertaken in order to acquire more information about the relationship between the primary structure of the depsipeptide and its effects on the permeability of membranes to monovalent cations. Since a rapid way to prepare analogs of valinomycin was required, a method involving the principles of solid-phase peptide synthesis[15,16] was adapted to the synthesis of this depsipeptide.

Solid-phase peptide synthesis was first used to make a depsipeptide by Semkin, Smirnova, and Shchukina[17] who prepared an angiotensin analog containing one hydroxy

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[1] Supported by Contract 14-01-001-1309, Office of Saline Water, U. S. Department of Interior, and in part by U. S. Public Health Service Grant AM 1260.

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[6] The abbreviations recommended by the IUPAC–IUB commission on Biochemical Nomenclature (*J. Biol. Chem.*, **241**, 2491 (1966); **242**, 555 (1967)) have been used throughout. In addition: Hyv = α-hydroxyisovaleric acid and Lac = lactic acid.

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