Peptide Synthesis in Aqueous Solution. III. Synthesis and Biological Activity of Cyclohexylamide Derivatives of Peptides Related to a Molluscan Neuropeptide, FMRFamide (Phe-Met-Arg-Phe-NH_2)

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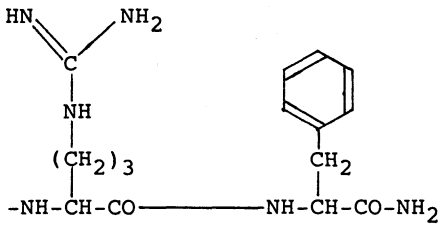
In order to investigate the role of C-terminal Phe^4-NH_2 of FMRFamide (Phe-Met-Arg-Phe-NH_2) to the biological activity, 30 kinds of cyclohexylamide derivatives of peptides related to FMRFamide were synthesized and their FMRFamide-like activity was measured. From the results, it was found that the peptide which was placed at C-terminal Phe^4-NH_2 by D-Ala-CHA showed only a relaxing activity. Furthermore, it was recognized that Met-Arg-Asp-diCHA inhibited FMRFamide contraction selectively.

The molluscan neuropeptide FMRFamide(Phe-Met-Arg-Phe-NH_2)[1] which was isolated from the ganglia of the clam *Macrocallista nimbosa* by Price and Greenberg,[2] causes a relaxation or a contraction, depending on its concentration, in the anterior byssus retractor muscle of *Mytilus*. Low concentrations of this peptide(10^{-8}—10^{-7}M, 1M=1mol dm^{-3}) relax acetylcholine-induced catch-tension,[3] whereas high concentrations(higher than 10^{-7}M) cause contraction.[4] In a previous paper,[5] we reported the synthesis of FMRFamide analogs for N-terminal moiety and discussed their structure-activity relations. Consequently, it was found that the precise structure of the N-terminal moiety is indispensable for the FMRFamide-like activity. However, we did not deal with the structure-activity relations of the C-terminal moiety in that paper. Muneoka and Saitoh[6] already reported that the substitution of D-Arg or Lys for Arg,[3] the substitution of D-Phe for C-terminal Phe, and the removal of the C-terminal amide eliminated contractile activity and greatly reduced relaxing activity in the relations of the C-terminal moiety. From their report, it is assumed that the C-terminal moiety (Arg^3-Phe^4-NH_2) of this peptide serves as a binding unit to the FMRFamide receptor, so that it gives rise to a FMRFamide-like activity.

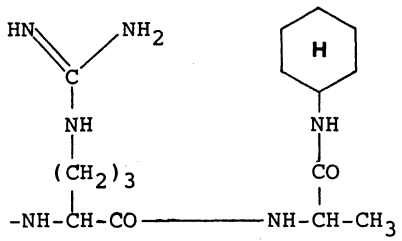
To learn more about the role of C-terminal Phe^4-NH_2 to the activity, we designed FMRFamide analogs and fragments, which possess the fundamental structure shown in Fig. 1. Since the substitution of cyclohexylamide of D-amino acid for C-terminal Phe^4-NH_2 seems to substantially keep the molecular structure of FMRFamide, we expected that these peptides were designed to exhibit the same activity as the FMRFamide one.

In this paper, for the first step of this study, about thirty kinds of cyclohexylamide derivatives of di-, tri-, and tetrapeptide were synthesized and the FMRFamide-like activity of these peptides were measured. Further, antagonistic effects of these peptides to FMRFamide- or acetylcholine-induced contractions were examined in order to determine the interaction of FMRFamide and its receptor.

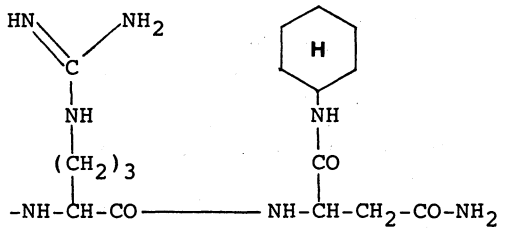
The synthetic route to the cyclohexylamide deriva-



-Arg-Phe-NH_2(FMRFamide)



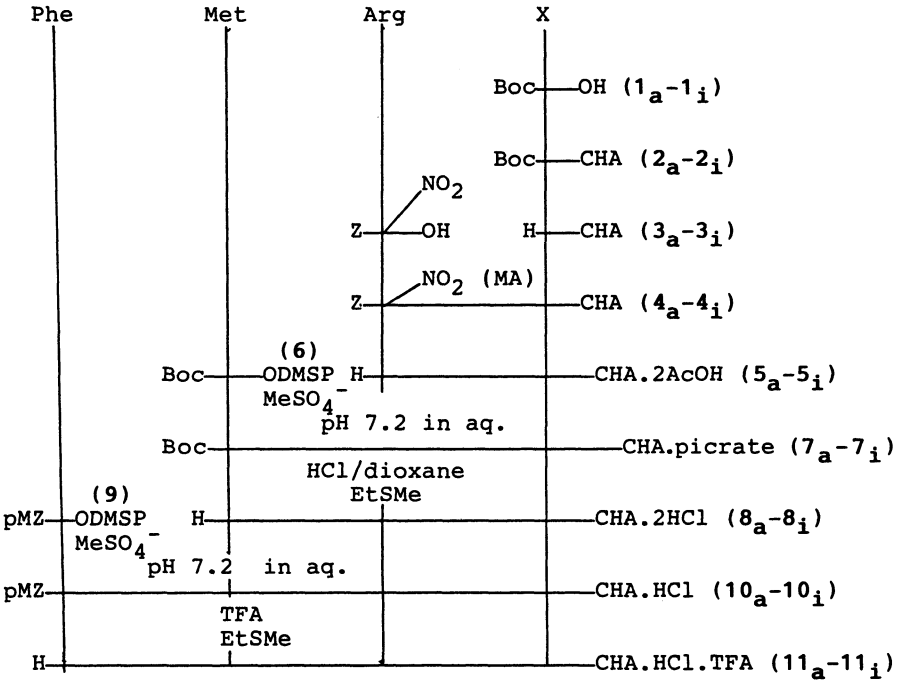
-Arg-D-Ala-CHA



-Arg-D-Asn-CHA

Fig. 1. Cyclohexylamide derivatives of FMRFamide.

tives of di-, tri-, and tetrapeptide is shown in Fig. 2. For the synthesis of FMRFamide, the method of selective acylation to Arg residue in aqueous solution was employed.[7] Boc-X-OH(X; Gly, D/L-Ala, Abu, D/L-Asn, Gln, Asp, or Gln was coupled with cyclohexylamide by the mixed anhydride(MA) method to yield $\mathbf{2_a}$—$\mathbf{2_i}$. The N-terminal protecting group of $\mathbf{2_a}$—$\mathbf{2_i}$ was removed by the action of hydrogen chloride in dioxane to yield $\mathbf{3_a}$—$\mathbf{3_i}$, and then Z-Arg-(NO_2)-OH and $\mathbf{3_a}$—$\mathbf{3_i}$ were condensed to yield $\mathbf{4_a}$—$\mathbf{4_i}$. The removal of the Z-group and NO_2-group from $\mathbf{4_a}$—$\mathbf{4_i}$ by hydrogenation afforded the free dipeptide cyclohexylamide($\mathbf{5_a}$—$\mathbf{5_i}$). Boc-Met-OH was esterified by DCC and *p*-(hydroxyphenyl)dimethylsulfonium methyl sulfate (HODMSP·$MeSO_4^-$) to afford the water-soluble active ester (**6**). In an aqueous solution, compound **6** was allowed to react with $\mathbf{5_a}$—$\mathbf{5_i}$ in the presence of 1M Na_2CO_3 (1M=1 mol dm^{-3}) at pH 7.2. To the reaction



X: Gly(a),Ala(b),D-Ala(c), Abu(d), Asn(e),D-Asn(f),Gln(g),
Asp(h),Glu(i),

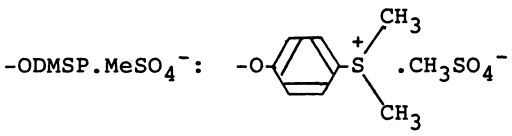


Fig. 2. Synthesis of cyclohexylamide derivatives of peptides.

Table 1. Contractile and Relaxing Effect of Synthesized Peptide

Peptide		Contractile effect/M				Relaxing effect/M				
		10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Phe-Met-Arg-Phe-NH_2 (FMRFamide)		±	+	++		+				−
Phe-Met-Arg-Gly-CHA	(**11a**)				−					−
Phe-Met-Arg-Ala-CHA	(**11b**)				−					−
Phe-Met-Arg-D-Ala-CHA	(**11c**)				−		±	+	++	++
Phe-Met-Arg-Abu-CHA	(**11d**)				−					−
Phe-Met-Arg-Asn-CHA	(**11e**)				−					−
Phe-Met-Arg-D-Asn-CHA	(**11f**)				−					−
Phe-Met-Arg-Gln-CHA	(**11g**)				−					−
Phe-Met-Arg-Asp-diCHA	(**11h**)				−					−
Phe-Met-Arg-Glu-diCHA	(**11i**)				−					−

+: Effect, −: no effect.

Table 2. Antagonistic Action of Dipeptides

Peptide	Antagonistic action			
	Ach (10^{-4} M) contraction/M		FMRFa (10^{-6} M) contraction/M	
	10^{-5}	10^{-4}	10^{-5}	10^{-4}
Arg-Gly-CHA (**5a**)		*		*
Arg-Ala-CHA (**5b**)		−		−
Arg-D-Ala-CHA (**5c**)		*		*
Arg-Abu-CHA (**5d**)		*		*
Arg-Asn-CHA (**5e**)		−		−
Arg-D-Asn-CHA (**5f**)		−		−
Arg-Gln-CHA (**5g**)		−		−
Arg-Asp-diCHA (**5h**)		+	+	
Arg-Glu-diCHA (**5i**)		±		+

+: Effect, −: no effect, *: increasing contraction.

Table 3. Antagonistic Action of Tripeptides

Peptide	Antagonistic action			
	Ach (10^{-4} M) contraction/M		FMRFa (10^{-6} M) contraction/M	
	10^{-5}	10^{-4}	10^{-5}	10^{-4}
Met-Arg-Gly-CHA (**8a**)		−		−
Met-Arg-Ala-CHA (**8b**)		−		−
Met-Arg-D-Ala-CHA (**8c**)		−		−
Met-Arg-Abu-CHA (**8d**)		−		−
Met-Arg-Asn-CHA (**8e**)		−		±
Met-Arg-D-Asn-CHA (**8f**)		−		−
Met-Arg-Gln-CHA (**8g**)		−		±
Met-Arg-Asp-diCHA (**8h**)		±	+	++
Met-Arg-Glu-diCHA (**8i**)		+	+	++

+: Effect, −: no effect.

mixture containing the Boc-tripeptide cyclohexylamide acetate, 1% picric acid was added and then the resulting Boc-tripeptide cyclohexylamide picrate(**7a**—**7i**) was isolated by extraction with ethyl acetate. The Boc-group was removed from **7a**—**7i** with hydrogen chloride in the presence of ethyl methyl sulfide to yield tripeptide cyclohexylamide(**8a**—**8i**). pMZ-Phe-OH was esterified by DCC and HODMSP·$MeSO_4^-$ to afford the water-soluble active ester(**9**), which was allowed to react with **8a**—**8i** in the same manner as described for the preparation of Boc-tripeptide cyclohexylamide to yield pMZ-tetrapeptide cyclohexylamide(**10a**—**10i**). Finally, the pMZ-group was removed from **10a**—**10i** with trifluoroacetic acid to yield free tetrapeptide cyclohexylamide(**11a**—**11i**).

The purity of the synthetic peptides was confirmed by TLC in two solvent systems and by elemental analysis. The homogeneity of the tri- or tetrapeptides were confirmed by amino acid analysis.

The FMRFamide-like activity of these synthesized peptides on the anterior byssus retractor muscle was determined,[8] as shown in Table 1. All of the cyclohexylamide derivatives of di- or tripeptide synthesized showed neither contractile activity nor relaxing activity between 10^{-8} and 10^{-4} M. In the tetrapeptides, the

Table 4. Antagonistic Action of Tetrapeptides

Peptide	Antagonistic action			
	Ach (10^{-4} M) contraction/M		FMRFa (10^{-6} M) contraction/M	
	10^{-5}	10^{-4}	10^{-5}	10^{-4}
Phe-Met-Arg-Gly-CHA (**11a**)		−	−	+
Phe-Met-Arg-Ala-CHA (**11b**)		−	−	+
Phe-Met-Arg-D-Ala-CHA (**11c**)		+		+
Phe-Met-Arg-Abu-CHA (**11d**)	+	+		+
Phe-Met-Arg-Asn-CHA (**11e**)		+		++
Phe-Met-Arg-D-Asn-CHA (**11f**)		+		+
Phe-Met-Arg-Gln-CHA (**11g**)		+		++
Phe-Met-Arg-Asp-diCHA (**11h**)		++		++
Phe-Met-Arg-Glu-diCHA (**11i**)		++		++

+: Effect, −: no effect.

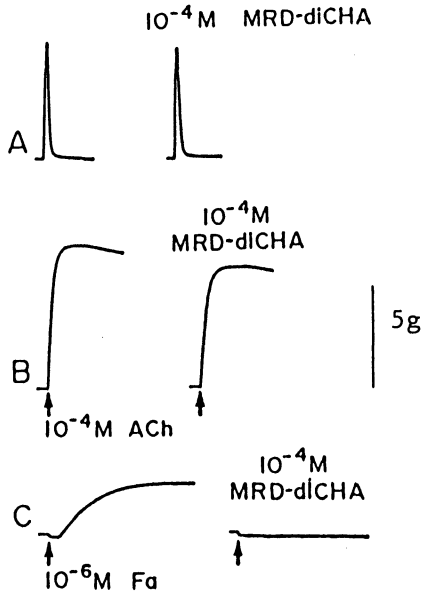


Fig. 3. Antagonistic action of Met-Arg-Asp-diCHA(MRD-diCHA) on contractions produced by electrical pulses (A), Acetylcholine (B), and FMRFamide (C). Fa: FMRFamide, Ach: Acetylcholine.

peptide which was replaced at C-terminal Phe^4-NH_2 by D-Ala-CHA showed only a relaxing activity. Its potency was one tenth as compared with that of FMRFamide, but the fact that this peptide had only a relaxing activity is noteworthy from the viewpoint of pharmacology.

Muneoka and Saitoh[6] reported that Phe-Met-Arg-D-Phe-NH_2 antagonized strongly FMRFamide contraction. It is very important to determine the antagonists

Table 5. Yields and Analytical Data of Protected and Free Dipeptides

Compound	Yield %	Mp θ_m/°C	$[\alpha]_D$/° (Solvent)	Formula	Found(Calcd)/% C	H	N	R_{f_1}	R_{f_2}
4a	74	124(decomp)	+8 (*c* 0.5, DMF)	$C_{22}H_{33}O_6N_7$	53.49 (53.75	6.70 6.71	19.81 19.94)	0.80	0.55
4b	70	149(decomp)	+6 (*c* 1, DMF)	$C_{23}H_{35}O_6N_7$	53.49 (54.64	6.88 6.92	19.30 19.38)	0.82	0.61
4c	68	151(decomp)	−4 (*c* 0.5, DMF)	$C_{23}H_{35}O_6N_7$	54.39 (54.64	6.90 6.92	19.42 19.38)	0.82	0.62
4d	72	181(decomp)	+6 (*c* 0.5, DMF)	$C_{24}H_{37}O_6N_7$	55.18 (55.47	7.11 7.12	18.59 18.86)	0.83	0.61
4e	60	216(decomp)	−3 (*c* 1, DMF)	$C_{24}H_{36}O_7N_8 \cdot 1/2H_2O$	50.01 (50.25	6.40 6.45	19.28 19.53)	0.87	0.48
4f	74	188(decomp)	+13 (*c* 1, DMF)	$C_{24}H_{36}O_7N_8 \cdot H_2O$	49.45 (49.48	6.51 6.52	18.99 19.22)	0.85	0.48
4g	84	195(decomp)	+5 (*c* 1, DMF)	$C_{25}H_{38}O_7N_8 \cdot 2/3H_2O$	52.20 (52.25	6.88 6.85	19.21 19.49)	0.91	0.79
4h	75	205(decomp)	+4 (*c* 0.5, DMF)	$C_{30}H_{45}O_7N_8$	56.98 (57.26	7.05 7.15	17.57 17.80)	0.91	0.79
4i	82	265(decomp)	−2 (*c* 1, DMF)	$C_{31}H_{47}O_7N_8$	57.58 (57.88	7.29 7.31	17.28 17.41)	0.93	0.76
5a	94	Hygroscopic	—	$C_{14}H_{28}O_2N_6 \cdot 2CH_3COOH$	—	—	—	0.54	0.21
5b	92	Hygroscopic	—	$C_{15}H_{30}O_2N_6 \cdot 2CH_3COOH$	—	—	—	0.55	0.20
5c	96	Hygroscopic	—	$C_{15}H_{30}O_2N_6 \cdot 2CH_3COOH$	—	—	—	0.60	0.23
5d	88	Hygroscopic	—	$C_{16}H_{32}O_2N_6 \cdot 2CH_3COOH$	—	—	—	0.58	0.30
5e	91	Hygroscopic	—	$C_{16}H_{31}O_3N_7 \cdot 2CH_3COOH$	—	—	—	0.60	0.39
5f	94	115—118	+22 (*c* 0.5, MeOH)	$C_{16}H_{31}O_3N_7 \cdot 2CH_3COOH \cdot H_2O$	47.11 (47.37	8.03 8.08	19.08 19.32)	0.60	0.40
5g	80	110—113	+22 (*c* 0.5, MeOH)	$C_{17}H_{33}O_3N_7 \cdot 2CH_3COOH \cdot 3/2H_2O$	47.29 (47.57	8.27 8.30	18.43 18.49)	0.63	0.38
5h	97	113—115	+4 (*c* 1, MeOH)	$C_{22}H_{40}O_3N_7 \cdot 2CH_3COOH \cdot 2H_2O$	51.28 (51.51	8.54 8.58	16.01 16.17)	0.72	0.43
5i	91	116—118	+12 (*c* 0.5, MeOH)	$C_{23}H_{42}O_3N_7 \cdot 2CH_3COOH \cdot 2H_2O$	52.02 (52.29	8.68 8.71	15.71 15.80)	0.71	0.42

of FMRFamide for the elucidation of the mechanism of its biological actions. Therefore, these synthesized peptides were tested regarding their abilities to block FMRFamide-induced contractions or the contractions produced by other actions.[8] The results of antagonistic activity tests are shown in Tables 2—4. As shown in Table 2, Arg-Asp-diCHA(**5h**) and Arg-Glu-diCHA(**5i**) antagonized FMRFamide contrations and acetylcholine contractions. From Table 3, it was found that Met-Arg-Asp-diCHA(**8h**) and Met-Arg-Glu-diCHA(**8i**) exhibit higher activities than the corresponding dipeptides to block FMRFamide-induced contractions, respectively. In particular, Met-Arg-Asp-diCHA(**8h**) inhibited FMRFamide contraction selectively and had no effects upon acetylcholine contractions, as shown in Fig. 3. Most of the synthesized tetrapeptides antagonized FMRFamide contraction and acetylcholine contraction. The ability of the inhibition generally enhanced with the increasing of the hydrophobicity of C-terminal, but the selective inhibition observed in Met-Arg-Asp-diCHA(**8h**) was not observed.

From the results described above, we could synthesize a relaxing peptide by the subsutitution of cyclohexylamide derivatives for C-terminal Phe^4-NH_2. This fact will lead us to design more useful relaxing peptide. Furthermore, the result that the synthesized peptide possessing high hydrophobicity at C-terminal antagonized FMRFamide contraction, was compatible with the hypothesis that the hydrophobic C-terminal of FMRFamide serves as a binding unit to the receptor.

Experimental

All the melting points were uncorrected. The optical rotations were measured on a Union PM-101 polarimeter. TLC was carried out on Merck Silicagel G: R_{f_1}, 1-butanol-acetic acid-pyridine-water(4 : 1 : 1 : 2, v/v), R_{f_2}, $CHCl_3$-methanol (5 : 1, v/v). Spots of material possessing free amino group on TLC plate were detected by spraying with ninhydrin, and those of amino group blocked materials by spraying with 25% HBr in acetic acid and then ninhydrin. Amino acid analysis was performed by HPLC using JASCO TRI ROTAR-V and UVIDEC-100 V apparatus.

Synthesis of Cyclohexylamide Derivatives of Peptides. Z-Arg(NO_2)-X-CHA(4a—4i): To a solution of Z-Arg(NO_2)-OH in THF and equimolar NMM, equimolar ECF was added at −5 °C. After 10 min, a solution of equimolar H-X-CHA · HCl(**3a**—**3i**) and NMM in $CHCl_3$ was added. The reaction mixture was stirred in an ice bath for 1 h and then at room temperature overnight. The mixture was evaporated in vacuo, and the oily residue was dissolved in ethyl acetate. The solution was washed with water, 4% sodium hydrogencarbonate, 0.5M hydrochloric acid, and water, successively, and then dried over anhydrous sodium

Table 6. Yields and Analytical Data of Protected and Free Tripeptides

Compound	Yield %	Mp θ_m/°C	$[\alpha]_D$/° (Solvent)	Formula	Found(Calcd)/% C	H	N	R_{f_1}	R_{f_2}
7a	65	130(decomp)	−6 (*c* 0.5, DMF)	$C_{24}H_{45}O_5N_7S\cdot C_6H_3O_7N_3$	46.49 (46.74	6.21 6.23	17.94 18.16)	0.80	0.20
7b	48	150(decomp)	−4 (*c* 0.5, DMF)	$C_{25}H_{47}O_5N_7S\cdot C_6H_3O_7N_3\cdot 1/2H_2O$	47.01 (47.25	6.38 6.47	17.51 17.77)	0.80	0.30
7c	54	152(decomp)	+10 (*c* 0.5, DMF)	$C_{25}H_{47}O_5N_7S\cdot C_6H_3O_7N_3\cdot H_2O$	46.58 (46.72	6.23 6.52	17.41 17.57)	0.81	0.31
7d	56	156(decomp)	−6 (*c* 0.5, DMF)	$C_{26}H_{49}O_5N_7S\cdot C_6H_3O_7N_3$	47.79 (48.02	6.47 6.50	17.31 17.49)	0.82	0.31
7e	70	154(decomp)	−8 (*c* 0.5, DMF)	$C_{26}H_{48}O_6N_8S\cdot C_6H_3O_7N_3\cdot H_2O$	45.69 (45.98	6.30 6.34	18.42 18.43)	0.82	0.31
7f	43	158(decomp)	+14 (*c* 0.5, DMF)	$C_{26}H_{48}O_6N_8S\cdot C_6H_3O_7N_3\cdot 2/3H_2O$	46.30 (46.31	6.11 6.31	18.28 18.56)	0.82	0.31
7g	47	164(decomp)	−12 (*c* 0.5, DMF)	$C_{27}H_{50}O_6N_8S\cdot C_6H_3O_7N_3\cdot 2H_2O$	45.39 (45.66	6.50 6.57	17.11 17.20)	0.81	0.30
7h	62	158(decomp)	−2 (*c* 1, DMF)	$C_{32}H_{57}O_6N_8S\cdot C_6H_3O_7N_3$	49.97 (50.13	6.57 6.59	16.67 16.92)	0.82	0.31
7i	71	182(decomp)	−14 (*c* 0.5, DMF)	$C_{33}H_{59}O_6N_8S\cdot C_6H_3O_7N_3$	51.38 (50.67	6.62 6.71	16.47 16.66)	0.80	0.28
8a	84	115(decomp)	−6 (*c* 0.5, MeOH)	$C_{19}H_{37}O_3N_7S\cdot 2HCl\cdot 2H_2O$	41.28 (41.33	7.75 7.78	17.48 17.74)	0.58	0.21
8b	91	121(decomp)	−12 (*c* 0.5, MeOH)	$C_{20}H_{39}O_3N_7S\cdot 2HCl\cdot 3/4H_2O$	43.82 (44.15	7.73 7.81	17.88 18.01)	0.59	0.25
8c	78	123(decomp)	+24 (*c* 0.5, MeOH)	$C_{20}H_{39}O_3N_7S\cdot 2HCl\cdot 3/2H_2O$	43.05 (43.08	7.54 7.89	17.44 17.58)	0.58	0.25
8d	80	105(decomp)	−8 (*c* 0.5, MeOH)	$C_{21}H_{41}O_3N_7S\cdot 2HCl\cdot H_2O$	44.69 (44.86	7.88 8.00	17.31 17.43)	0.58	0.24
8e	94	125(decomp)	−6 (*c* 0.5, MeOH)	$C_{21}H_{40}O_4N_8S\cdot 2HCl\cdot 3H_2O$	39.94 (40.19	7.62 7.65	17.59 17.85)	0.60	0.30
8f	85	127(decomp)	+10 (*c* 0.5, MeOH)	$C_{21}H_{40}O_4N_8S\cdot 2HCl\cdot 3/2H_2O$	41.78 (41.99	7.38 7.49	18.63 18.65)	0.61	0.31
8g	88	130(decomp)	−3 (*c* 0.5, MeOH)	$C_{22}H_{42}O_4N_8S\cdot 2HCl\cdot 2H_2O$	42.19 (42.37	7.64 7.70	17.78 17.96)	0.67	0.30
8h	84	129(decomp)	−10 (*c* 0.5, MeOH)	$C_{27}H_{49}O_4N_8S\cdot 2HCl\cdot H_2O$	48.01 (48.24	7.83 7.88	16.41 16.66)	0.70	0.32
8i	92	138(decomp)	−8 (*c* 0.5, MeOH)	$C_{28}H_{51}O_4N_8S\cdot 2HCl\cdot 2H_2O$	47.61 (47.75	8.02 8.09	15.69 15.90)	0.70	0.34

sulfate. The filtrate was evaporated in vacuo. The oily residue was crystallized with ether. In the case of peptides containing D/L-Asn, Gln, Asp, and Glu, the reaction mixture was evaporated in vacuo, and the resulting residue was poured into ice-water. The solid was filtered and the filter cake was washed with 4% sodium hydrogencarbonate, 0.5M hydrochloric acid, and water, successively. The product thus obtained was recrystallized from ethyl acetate. These results were summarized in Table 5.

H-Arg-X-CHA·2AcOH(5$_a$—5$_i$): A solution of Z-Arg-(NO_2)-X-CHA(**4$_a$—4$_i$**) in acetic acid was hydrogenated in the presence of palladium black at room temperature for 21 h. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo. The residue was crystallized with ether. These results are summarized in Table 5.

Boc-Met-ODMSP·MeSO$_4^-$(6)(Water-Soluble Active Ester): To a chilled solution of Boc-Met-OH (2.49g, 10 mmol) and HODMSP·$MeSO_4^-$(2.66g, 10 mmol) in CH_3CN (80 ml), DCC (2.06g, 10 mmol) was added. The reaction mixture was held overnight at 0 °C and the DCurea which formed was filtered off. The filtrate was concentrated in vacuo, and the residual oil was washed with ether by decantation; Yield 3.93g (80%); $[\alpha]_D^{20}$ −30° (*c* 1, MeOH); R_{f_1} 0.82; R_{f_2} 0.34.

Boc-Met-Arg-X-CHA(7$_a$—7$_i$): H-Arg-X-CHA·2AcOH (**5$_a$—5$_i$**) was dissolved in H_2O and the pH of the solution was adjusted to 7.3 by addition of 1M Na_2CO_3. Equimolar Boc-Met-ODMSP·$MeSO_4^-$ was added to the stirred solution below 20 °C, the pH being maintained automatically at 7.2 with 1M Na_2CO_3. After 12 h, 1% picric acid was added to the solution and the pH of the solution was adjusted to 5.6. Yellow precipitate was filtered off and it was dissolved in ethyl acetate. The solution was washed successively with 4% sodium hydrogencarbonate, 4% citric acid and water, and then dried over anhydrous sodium sulfate. The solution was concentrated to an oily residue which was crystallized by addition of ether. It was recrystallized from $CHCl_3$. These results are summarized in Table 6.

H-Met-Arg-X-CHA·2HCl(8$_a$—8$_i$): To a solution of Boc-Met-Arg-X-CHA(**7$_a$—7$_i$**), TFA and ethyl methyl sulfide was added. The reaction mixture was concentrated in vacuo. To a residue, 4M HCl in dioxane was added and then the resulting hydrochloride salt was solidified with acetone. These results are summarized in Table 6.

pMZ-Phe-ODMSP·MeSO$_4^-$(9)(Water-Soluble Active Ester): pMZ-Phe-OH (3.29g, 10 mmol) and HODMSP·$MeSO_4^-$ (2.66g, 10 mmol) were coupled by the same method as **6**. It was an oily product; Yield of the oily product 5.20 g

Table 7. Yields and Analytical Data of Protected and Free Tetrapeptides

Compound	Yield %	Mp θ_m/°C	$[\alpha]_D$/° (Solvent)	Formula	Found(Calcd)/% C	H	N	R_{f_1}	R_{f_2}
10a	74	101(decomp)	−4 (*c* 0.5, DMF)	$C_{37}H_{54}O_7N_8S\cdot HCl$	56.01 (56.19	6.85 6.95	14.02 14.16)	0.81	0.25
10b	68	96(decomp)	−8 (*c* 0.5, DMF)	$C_{38}H_{56}O_7N_8S\cdot HCl\cdot 1/2H_2O$	56.18 (56.07	7.10 7.13	13.48 13.76)	0.83	0.29
10c	65	98(decomp)	+4 (*c* 0.5, DMF)	$C_{38}H_{56}O_7N_8S\cdot HCl\cdot 1/2H_2O$	55.84 (56.07	6.98 7.13	13.53 13.76)	0.83	0.30
10d	58	132(decomp)	−5 (*c* 0.5, DMF)	$C_{39}H_{58}O_7N_8S\cdot HCl$	57.01 (57.20	7.16 7.21	13.48 13.68)	0.82	0.31
10e	71	178(decomp)	−8 (*c* 0.5, DMF)	$C_{39}H_{57}O_8N_9S\cdot HCl\cdot H_2O$	54.21 (54.10	6.48 6.93	14.43 14.55)	0.83	0.33
10f	70	182(decomp)	+10 (*c* 0.5, DMF)	$C_{39}H_{57}O_8N_9S\cdot HCl\cdot H_2O$	53.88 (54.10	6.77 6.93	14.31 14.55)	0.83	0.33
10g	63	163(decomp)	−20 (*c* 0.5, DMF)	$C_{40}H_{59}O_8N_9S\cdot HCl\cdot 1/2H_2O$	54.92 (55.17	6.81 7.00	14.29 14.47)	0.84	0.32
10h	43	175(decomp)	−10 (*c* 0.5, DMF)	$C_{45}H_{66}O_8N_9S\cdot HCl$	58.11 (58.18	6.99 7.11	13.24 13.56)	0.83	0.38
10i	67	181(decomp)	−10 (*c* 0.3, DMF)	$C_{46}H_{68}O_8N_9S\cdot HCl$	58.32 (58.59	7.18 7.21	13.18 13.36)	0.81	0.40
11a	84	98(decomp)	−6 (*c* 0.5, MeOH)	$C_{28}H_{46}O_4N_8S\cdot HCl\cdot CF_3COOH\cdot H_2O$	47.15 (47.45	6.58 6.59	14.58 14.75)	0.62	0.19
11b	89	115(decomp)	−8 (*c* 0.5, MeOH)	$C_{29}H_{48}O_4N_8S\cdot HCl\cdot CF_3COOH\cdot 3/2H_2O$	49.38 (49.66	7.03 7.07	14.80 14.94)	0.64	0.30
11c	87	117(decomp)	+6 (*c* 0.5, MeOH)	$C_{29}H_{48}O_4N_8S\cdot HCl\cdot CF_3COOH\cdot 2H_2O$	46.81 (47.05	6.80 6.82	14.00 14.15)	0.65	0.30
11d	90	110(decomp)	−8 (*c* 0.5, MeOH)	$C_{30}H_{50}O_4N_8S\cdot HCl\cdot CF_3COOH\cdot H_2O$	48.57 (48.85	6.80 6.86	14.17 14.24)	0.65	0.31
11e	91	121(decomp)	−8 (*c* 0.5, MeOH)	$C_{30}H_{49}O_5N_9S\cdot HCl\cdot CF_3COOH\cdot 3/2H_2O$	47.31 (47.43	6.45 6.46	15.21 15.55)	0.67	0.33
11f	85	120(decomp)	+7 (*c* 0.5, MeOH)	$C_{30}H_{49}O_5N_9S\cdot HCl\cdot CF_3COOH\cdot 2H_2O$	45.78 (46.06	6.47 6.59	15.01 15.10)	0.67	0.34
11g	94	115(decomp)	−10 (*c* 0.5, MeOH)	$C_{31}H_{51}O_5N_9S\cdot HCl\cdot CF_3COOH\cdot H_2O$	45.41 (47.73	6.60 6.62	15.01 15.17)	0.66	0.35
11h	80	134(decomp)	−10 (*c* 0.5, MeOH)	$C_{36}H_{58}O_5N_9S\cdot HCl\cdot CF_3COOH\cdot H_2O$	50.61 (50.89	6.83 6.91	13.81 14.05)	0.73	0.41
11i	83	142(decomp)	−30 (*c* 0.5, MeOH)	$C_{37}H_{60}O_5N_9S\cdot HCl\cdot CF_3COOH\cdot 1/2H_2O$	51.66 (51.94	6.81 6.99	13.81 13.97)	0.73	0.43

(90%); $[\alpha]_D^{20}$ −7° (*c* 1, MeOH); R_{f_1} 0.85; R_{f_2} 0.51.

pMZ-Phe-Met-Arg-X-CHA · HCl(10$_a$—10$_i$): H-Met-Arg-X-CHA (**8$_a$—8$_i$**) was dissolved in H_2O and the pH of the solution was adjusted to 7.2 by addition of 1M Na_2CO_3. A slight excess of pMZ-Phe-ODMSP · $MeSO_4^-$ was added to the stirred solution below 20 °C, the pH being maintained automatically at 7.2 with 1M Na_2CO_3. After 12 h, the crude product was precipitated from the reaction mixture. It was filtered and then washed with 4% sodium hydrogencarbonate and water. After removal of moisture by drying, it was recrystallized from methanol or ethyl acetate. These results are summarized in Table 7.

H-Phe-Met-Arg-X-CHA · HCl · TFA(11$_a$—11$_i$): pMZ-Phe-Met-Arg-X-CHA · HCl (**10$_a$—10$_i$**) and ethyl methyl sulfide were dissolved in TFA. The reaction mixture was allowed to stand at room temperature. After 2 h, the solution was concentrated in vacuo. The residue was crystallized with ether. These results were summarized in Table 7.

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References

1) Abbreviations used are according to IUPAC-IUB Commissions, *Eur, J. Biochem.*, **138**, 9 (1984). Other abbreviations: DCC, dicyclohexylcarbodiimide; DCurea, *N,N'*-dicyclohexylurea; ECF, ethyl chloroformate; NMM, *N*-methylmorpholine; TFA, trifluoroacetic acid; CHA, cyclohexylamide group.

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8) We asked Professor Yojiro Muneoka of Hiroshima University to measure the biological assays. He measured these assays according to the Ref. 6.