Inhibition of Urease Activity by Dipeptidyl Hydroxamic Acids

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A series of dipeptidyl hydroxamic acids (H–X–Gly–NHOH: X = amino acid residues) was synthesized, and the inhibitory activity against Jack bean and *Proteus mirabilis* ureases [EC 3.5.1.5] was examined. A number of H–X–Gly–NHOH inhibited Jack bean urease with an I_{50} of the order of 10^{-6} M and inhibited *Proteus mirabilis* urease with an I_{50} of the order of 10^{-5} M. The inhibition against Jack bean urease was more potent than that with the corresponding aminoacyl hydroxamic acids (H–X–NHOH).

Keywords urease; hydroxamic acid; inhibitor; dipeptide

Introduction

Urease is widely distributed in bacteria, invertebrates, and plants. This enzyme hydrolyzes urea which is the best substrate to produce ammonia and carbon dioxide. In mammalian body, no urease activity is detected but infected urea-splitting bacteria have urease activity.[1] Ammonia produced by this urease causes some severe diseases, for example, hyperammonemia and the following hepatic coma,[2] or hyperammonuria and the subsequent magnesium ammonium phosphate (struvite stones) when bacterial infection occurs in urinary tracts.[3]

Hydroxamic acids (HXAs, R–NHOH: R = acyl groups) were found to be potent and specific inhibitors of urease activities of plant and bacterial origin.[4] Therefore, much effort has been focussed upon developing hydroxamic acids as a therapeutic agent against diseases caused by urease.

Kobashi *et al.* have synthesized a series of HXAs (R–NHOH) and have investigated the correlation between the chemical structures or the physicochemical properties of the acyl residues (R) and their inhibitory effects on the enzyme activity.[5] Among them, acylglycino-HXAs (R′–Gly–NHOH, R′ = aliphatic or aromatic acyl groups) showed potent inhibition against ureases from bacteria and plants. Structures of some acyl groups of these acylglycino-HXAs are similar to those of amino acids, for example, R′ = 3-phenylpropionyl = deamino-phenylalanine. This similarity made us wonder whether the acyl groups changed to amino acids. It has also reported that peptidyl hydroxamic acids are potent inhibitors for Zn-metalloproteases,[6] however, little research has been undertaken on the inhibitory activities of these HXAs against urease.[7] Urease does not show any peptidase or protease activity. Therefore, in this study, we prepared a series of dipeptidyl hydroxamic acids (H–X–Gly–NHOH: X = amino acid residues) and investigated their inhibitory effects against Jack bean and *Proteus mirabilis* ureases.

Results and Discussion

Dipeptidyl hydroxamic acids (IV) were synthesized according to the methods shown in Chart 1.

Boc-dipeptidyl HXAs (III) were prepared by the following two routes: Dipeptide ethyl ester (I) was synthesized from Boc-amino acids and Gly–OEt by the EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide)–HOBt (1-hydroxybenzotriazole) condensation method[8] and was converted to dipeptidyl HXA (III) with hydroxylamine in alkaline solution (route A). Dipeptide *O*-benzyl hydroxamate (II) was prepared from Gly–NHOBzl and Boc-amino acid by EDC–HOBt method and was converted to III by catalytic hydrogenation (route B). The Boc group of dipeptidyl HXA (III) was eliminated with HCl/AcOEt to obtain the final compound (IV). Bz–Phe–Gly–NHOH and Ac–Phe–Gly–NHOH were also synthesized from Bz–Phe–Gly–OEt and Ac–Phe–Gly–OEt by the same manner as route A.

To investigate the inhibitory effect of these peptidyl HXAs on urease activity, Jack bean and *Proteus mirabilis* ureases were used in the previous studies.[5] Jack bean urease has been isolated as crystals and is usually used as a urease of plant origin.[9] *Proteus mirabilis* is one of the urea-splitting bacteria obtained from patients with urolithiasis and its infection to the rat urinary tracts causes development of struvite stones.[10] The inhibitory activities of dipeptidyl HXAs (IV) against these two ureases are listed in Table I.

All dipeptidyl HXAs potently inhibited Jack bean urease and some of the notable findings between amino acid residues (X) and their inhibitory activities were as follows.

Firstly, hydrophobic amino acid derivatives (X = Phe (IV-1), Tyr (IV-5), Ser(Bzl) (IV-6)) were more inhibitory than hydrophilic amino acid derivatives (X = Gly (IV-9), Ser (IV-12), Lys (IV-15)). Secondly, blocking of N-terminal amino group reduced the inhibitory activities (IV-17, IV-18). This result was supported by weak inhibitory activities against Jack bean urease of Boc–X–Gly–NHOH (III-1—15)

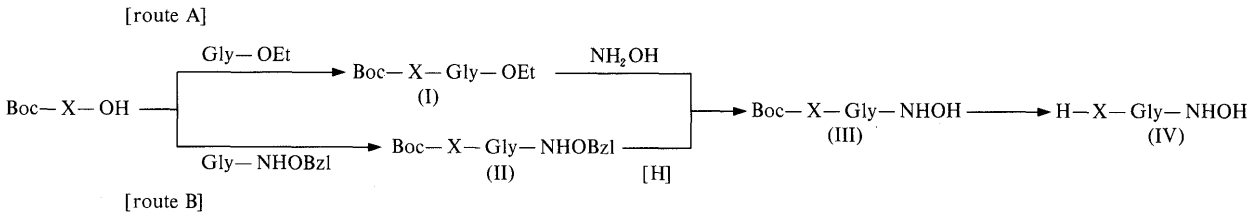

Chart 1. The Synthetic Route to Dipeptidyl Hydroxamic Acids

TABLE I. Inhibition of Jack Bean and *Proteus Mirabilis* Ureases by Dipeptidyl Hydroxamic Acids (H–X–Gly–NHOH)

Compd. No.	X	Jack bean urease[a]	*P. mirabilis* urease	
			Cell free[b]	Intact cell[c]
IV -1	Phe	2.4	80	70
IV -2	Leu	1.8	79	77
IV -3	Ile	15	81	80
IV -4	Tyr(Bzl)	3.8	72	53
IV -5	Tyr	3.3	59	54
IV -6	Ser(Bzl)	2.0	74	70
IV -7	Met	2.6	73	69
IV -8	D-Phe	3.8	41	35
IV -9	Gly	21	73	75
IV-10	Ala	21	69	73
IV-11	Pro	6.0	84	81
IV-12	Ser	9.3	66	65
IV-13	Glu	6.0	27	33
IV-14	Gln	16	43	53
IV-15	Lys	20	54	60
IV-16	Arg	8.4	52	56
IV-17	Bz–Phe	16	54	72
IV-18	Ac–Phe	35	66	47

a) I_{50} (μM). *b*) Inhibition (%) at 0.1 mM. *c*) Inhibition (%) at 1 mM.

(I_{50} values were near the order of mM, data not shown), which were intermediates of aiming compounds (IV). It has been reported that hydrophobicity of acyl residues (R) of acyl HXAs (R–NHOH) had considerable effect on the inhibitory activity against urease, and that more hydrophobic compounds were more potent inhibitors.[4g] The second finding is in disagreement with the previous results, however, it suggests the participation of the amino group in the binding between urease and dipeptidyl HXAs. On the other hand, it also suggests that some steric effect by N-blocking groups decreases the inhibitory activities on Jack bean urease since weaker inhibition of the Ile analog (IV-3) than the Leu analog (IV-2) indicates the serious effect of steric hindrance of binding between urease and these inhibitors.

The third finding concerns the enantiomeric effect on inhibitory activity. The enantiomer effect of acyl residues has not been investigated in the inhibition study of acyl HXAs. Here, we investigated the effect of D- and L-isomers of Phe (IV-1 and -8) on plant urease activity, and found their inhibitions against Jack bean urease to be almost equal, indicating no enantiomeric effect on inhibitory activity.

Against *Proteus mirabilis* urease, a number of dipeptidyl HXAs showed inhibition of over 50% at 0.1 mM. These activities were about 10 fold lower than those against Jack bean urease and the three findings described above on the inhibition of Jack bean urease were not observed. Thus, the inhibition by dipeptidyl HXAs was affected by the different nature of the ureases from plant and bacteria. The inhibition activities against *Proteus mirabilis* urease in intact cells were about one tenth those against cell-free urease, indicating that the membrane permeability of dipeptidyl HXAs is low.

The inhibitory activities of dipeptidyl HXAs were compared with those of the aminoacyl-[11] and acylglycino-[5] HXAs which were lead compounds of this research.

As shown in Table II, all the dipeptidyl HXAs tested inhibited Jack bean urease more potently than the corresponding aminoacyl HXAs. One remarkable difference

TABLE II. Inhibition of Jack Bean Urease by Aminoacyl (H–X–NHOH), Acylglycino (R′–Gly–NHOH), and Dipeptidyl (H–X–Gly–NHOH) Hydroxamic Acids

X	I_{50} (μM)		
	H–X–Gly–NHOH	H–X–NHOH	R′–Gly–NHOH
Phe	2.4	5.4[a]	0.9[c]
Leu	1.8	13	1.9[d]
Ile	15	20	
Tyr	3.3	24	
Met	2.6	3.9	
Glu	6.0	620[b]	
Lys	20	90	
Arg	8.4	16	

a) I_{50} value of D,L-compound. *b*) I_{50} value of γ-hydroxamate. *c*) R′: 3-phenylpropionyl. *d*) R′: 4-methyl-*n*-pentanoyl.

was the case of glutamic acid derivatives (about a 100 fold increase). This can be explained by the fact that the α-carboxyl group of glutamyl γ-HXA is considered to interfere with the formation of enzyme–inhibitor complex,[4f] but γ-carboxyl group of glutamylglycino-HXA is remote from the active site of urease and therefore does not affect the inhibition.

A comparison of dipeptidyl HXAs with the corresponding acylglycino-HXAs showed their inhibitory activities to be almost equal. Thus, the amino group of dipeptidyl HXAs does not significantly affect inhibitory activity. However, it has been reported that hydrophobicity of acyl group of acylglycino-HXAs was important for the inhibitory activity against urease.[5a] This result supports the interaction between the amino group of dipeptidyl HXAs and Jack bean urease described above.

Kobashi *et al.* reported that hydroxamic acid is a specific inhibitor of urease activity.[4a,12] However, it has also been reported that peptidic hydroxamic acids inhibited Zn-metalloproteases.[6] Therefore, the inhibitory activities of dipeptidyl HXAs against Zn-metalloproteases has been examined. One potent urease inhibitor, H–Leu–Gly–NHOH (IV-2), little inhibited tadpole and *Clostridium histolyticum* collagenases and thermolysin, which are well known as Zn-metalloproteases.[7]

In this study, we prepared a series of dipeptidyl HXAs and investigated their efficiencies for inhibitory activities of urease and clarified the structure–activity relationships. In order to develop dipeptidyl HXAs to therapeutic drugs, knowledge of their bioavailability and toxicity is required. With regard to toxicity several HXAs have been reported to be teratogenic.[13] However, it has been reported that acylglycino-HXAs in the case of R′=aliphatic acyl group were non-teratogenic and non-mutagenic.[5] Dipeptidyl HXAs is expected to be safer than acylglycino-HXAs in the body since the acyl moiety is naturally occurring amino acids.

Experimental

Melting points were determined on a Yanagimoto melting apparatus (Kyoto) without correction. Specific rotation was measured with a Jasco DIP-140 apparatus (Tokyo). The purity of all compounds was monitored by analytical thin layer chromatography (TLC) on Merck silica gel plate in the following solvent systems: Rf^1, $CHCl_3$–MeOH–AcOH (95:5:3, v/v); Rf^2, $CHCl_3$–MeOH–AcOH (80:10:5, v/v); Rf^3, *n*-BuOH–AcOH–H_2O (4:1:1, v/v).

TABLE III. Physicochemical and Analytical Data for Boc–X–Gly–OEt

Compd. No.	X	mp (°C) Recryst. solv.	Yield (%)	$[\alpha]_D^{25}$ (°) (*c*, solv.)	Rf^1	Formula	Analysis (%) Calcd (Found)		
							C	H	N
I-2	Leu	84—86 AcOEt–*n*-hexane	95	−34.1 (1.0, EtOH)	0.70	$C_{15}H_{28}N_2O_5$	56.95 (57.23	8.92 8.85	8.85 8.96)
I-3	Ile	103—104 AcOEt–*n*-hexane	84	−33.6 (1.0, EtOH)	0.72	$C_{15}H_{28}N_2O_5$	56.95 (57.03	8.92 9.05	8.85 8.83)
I-4	Tyr(Bzl)	134—136 AcOEt–*n*-hexane	93	−3.9 (1.0, EtOH)	0.81	$C_{25}H_{32}N_2O_6$	65.76 (65.79	7.08 7.14	6.13 6.19)
I-5	Ser(Bzl)	—[a]	90	+9.1 (1.0, EtOH)	0.79	$C_{19}H_{28}N_2O_6$			
I-6	Met	—[a]	88		0.62	$C_{14}H_{26}N_2O_5S$			
I-7	D-Phe	90—91 AcOEt–*n*-hexane	93	+5.1 (0.5, EtOH)	0.75	$C_{21}H_{24}N_2O_5$	61.7 (61.67	7.48 7.5	7.99 8.02)

a) Oily compound: chromatographed on silica gel with $CHCl_3$.

TABLE IV. Physicochemical and Analytical Data for Boc–X–Gly–NHOBzl

Compd. No.	X	mp (°C) Recryst. solv.	Yield (%)	$[\alpha]_D^{25}$ (°) (*c*, solv.)	Rf^1	Formula	Analysis (%) Calcd (Found)		
							C	H	N
II-2	Ala	—[a]	99	−5.0 (1.0, EtOH)	0.49	$C_{17}H_{25}N_3O_5$			
II-3	Pro	—[a]	99	−25.8 (1.0, EtOH)	0.61	$C_{19}H_{27}N_3O_5$			
II-4	Ser		94	−7.4 (1.0, EtOH)	0.25	$C_{17}H_{25}N_3O_6$	55.58 (55.48	6.86 6.7	11.44 11.37)
II-5	Glu(OBzl)	86—87 Et_2O	90	−2.1 (1.0, EtOH)	0.52	$C_{26}H_{33}N_3O_7$	62.51 (62.47	6.65 6.52	8.41 8.42)
II-6	Gln	162.5—164.5 EtOH	64	0 (1.0, DMF)	0.20	$C_{19}H_{28}N_4O_6$	55.87 (55.6	6.9 7.23	13.71 13.53)
II-7	Lys(*Z*)	90—96.5 AcOEt–*n*-hexane	97	−4.2 (1.0, DMF)	0.33	$C_{28}H_{38}N_4O_7$	61.97 (62.05	7.05 6.88	10.32 10.33)

a) Oily compound: purified as described for II-1.

Boc–Phe–Gly–OEt (I-1) As a typical example of Boc–X–Gly–OEt synthesis. To a tetrahydrofuran (THF) solution of Boc–Phe–OH (5.3 g, 20 mmol), H–Gly–OEt·HCl (3.0 g, 22 mmol), and HOBt (1-hydroxybenzotriazole, 2.7 g, 20 mmol) was added EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, 3.8 ml, 21 mmol) at −20 °C. The reaction mixture was stirred overnight at 5 °C and concentrated *in vacuo*. The residue was dissolved in AcOEt and the solution was washed with 1 N-HCl, 10% Na_2CO_3, and H_2O and dried over $MgSO_4$. After removal of AcOEt, the residue was recrystallized from AcOEt–*n*-hexane to give I-1 as colorless crystals (6.4 g, 91%). mp 90—91 °C, $[\alpha]_D^{25}$ −5.1° (*c*=0.5, EtOH). Rf^1, 0.75. *Anal.* Calcd for $C_{18}H_{26}N_2O_5$: C, 61.7; H, 7.48; N, 7.99. Found: C, 61.71; H, 7.36; N, 8.05. Other Boc–X–Gly–OEt, I-2—7 were prepared as described for I-1 and the results are shown in Table III.

Boc–Gly–Gly–NHOBzl (II-1) As a typical example of Boc–X–Gly–NHOBzl synthesis. To a THF solution of Boc–Gly–OH (0.88 g, 5.0 mmol), H–Gly–NHOBzl·HCl (1.2 g, 5.5 mmol), and HOBt (0.72 g, 5.3 mmol) was added EDC (1.0 ml, 5.5 mmol) at −20 °C. The reaction mixture was stirred overnight at 5 °C and concentrated *in vacuo*. The residue was dissolved in AcOEt and the solution was washed with 1 N-HCl, 10% Na_2CO_3, and H_2O and dried over $MgSO_4$. AcOEt was evaporated off to give II-1 as colorless oil (1.5 g, 91%). This product was used for the next reaction without further purification since it was one spot on TLC (Rf^1, 0.42). Other Boc–X–Gly–NHOBzl, II-2—8 were prepared as described for II-1 and the results are shown in Table IV.

Boc–Phe–Gly–NHOH (III-1) As a typical example of Boc–X–Gly–NHOH synthesis from Boc–X–Gly–OEt. 1 M NH_2OH/MeOH (30 ml) [a solution of NH_2OH·HCl (2.8 g, 40 mmol) in MeOH (25 ml) was added to a solution of 85% KOH (3.7 g, 56 mmol) in MeOH (15 ml), and KCl formed was filtered off] was added to Boc–Phe–Gly–OEt (5.0 g, 14 mmol) at 4 °C. The reaction mixture was stirred for 20 h and concentrated *in vacuo*, and dissolved in AcOEt. The AcOEt solution was washed with 1 N-HCl, 10% Na_2CO_3, and H_2O and dried over Na_2SO_4, and then AcOEt was evaporated off. The crude product was purified by chromatography on silica gel with $CHCl_3$–AcOEt (1 : 1, v/v) and solidified by Et_2O to give III-1 as a white powder (6.4 g, 71%). mp 148—150 °C, $[\alpha]_D^{25}$ +10.5° (*c*=1.0, MeOH), Rf^2, 0.65. *Anal.* Calcd for $C_{16}H_{23}N_3O_5$: C, 56.97; H, 6.87; N, 12.46. Found: C, 57.14; H, 6.92; N, 12.45. Other Boc–X–Gly–NHOH from Boc–X–Gly–OEt, III-2—8 were prepared as described for III-1 and the results are shown in Table V.

Boc–Tyr–Gly–NHOH (III-5) A mixture of Boc–Tyr(Bzl)–Gly–NHON (III-4) (0.58 g 1.3 mmol) and 5% Pd–C (0.2 g) in MeOH was vigorously stirred for 1 h at room temperature under a hydrogen flow. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was reprecipitated from EtOH–Et_2O to give III-5 as a white hygroscopic powder (0.27 g, 59%). $[\alpha]_D^{25}$ +13.8° (*c*=1.0, MeOH), Rf^2, 0.59. *Anal.* Calcd for $C_{16}H_{23}N_3O_6$: C, 54.38; H, 6.56; N, 11.89. Found: C, 54.14; H, 6.72; N, 12.1.

Boc–Gly–Gly–NHOH (III-9) As a typical example of Boc–X–Gly–NHOH synthesis from Boc–X–Gly–NHOBzl. A mixture of Boc–Gly–Gly–NHOBzl (II-1) (0.82g, 2.4 mmol) and 5% Pd–C (0.2 g) in EtOH was vigorously stirred for 1 h at room temperature under a hydrogen flow. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was reprecipitated from EtOH–Et_2O to give III-9 as a white powder (0.49 g, 82%). mp 141 °C, Rf^2, 0.28. *Anal.* Calcd for $C_9H_{17}N_3O_5$: C, 43.72; H, 6.93; N, 17.0. Found: C, 43.93; H, 7.21; N, 17.19. Other Boc–X–Gly–NHOH from Boc–X–Gly–NHOBzl, III-10—15 were prepared as described for III-9 and the results are shown in Table V.

H–Phe–Gly–NHOH·HCl (IV-1) As a typical example of H–X–Gly–NHOH·HCl synthesis. Boc–Phe–Gly–NHOH (I-1) (0.2 g, 0.59 mmol) was dissolved in 4.2 M HCl/AcOEt (2 ml) at room temperature for 1 h and

TABLE V. Physicochemical and Analytical Data for Boc–X–Gly–NHOH

Compd. No.	X	mp (°C) Recryst. solv.	Yield (%)	$[\alpha]_D^{25}$ (°) (*c*, solv.)	*Rf*[2]	Formula	Analysis (%) Calcd (Found)		
							C	H	N
III- 2	Leu	121—123 EtOH–Et_2O	81	−10.5 (1.0, MeOH)	0.60	$C_{13}H_{25}N_3O_5$	51.47 (51.39	8.31 8.09	13.85 13.87)
III- 3	Ile	140—140.5 AcOH–H_2O	84	−5.4 (1.0, MeOH)	0.63	$C_{13}H_{25}N_3O_5$	51.47 (51.48	8.31 8.33	13.85 13.82)
III- 4	Tyr(Bzl)	138—139 EtOH–iso-Pr_2O	83	+14.6 (1.0, MeOH)	0.66	$C_{23}H_{29}N_3O_6 \cdot 1/3H_2O$	61.45 (61.42	6.65 6.61	9.35 9.08)
III- 6	Ser(Bzl)	147—150 EtOH–Et_2O	59	+7.6 (1.0, DMF)	0.73	$C_{17}H_{25}N_3O_6$	55.58 (55.64	6.86 6.93	11.44 11.26)
III- 7	Met	—[a]	89		0.52	$C_{12}H_{23}N_3O_5S$			
III- 8	D-Phe	83—88 AcOEt–*n*-hexane	93	−11.2 (1.0, MeOH)	0.64	$C_{16}H_{23}N_3O_5$	56.97 (56.81	6.87 6.95	12.46 12.22)
III-10	Ala	152—156 EtOH–Et_2O	75	+7.3 (1.0, EtOH)	0.36	$C_{10}H_{19}N_3O_5$	45.97 (46.13	7.33 7.3	16.08 15.82)
III-11	Pro	147.5—148.5 EtOH–Et_2O	95	−43.0 (1.0, MeOH)	0.56	$C_{12}H_{21}N_3O_5$	50.17 (50.1	7.37 7.42	14.63 14.45)
III-12	Ser	116—119 EtOH–iso-Pr_2O	94	−7.8 (1.0, MeOH)	0.22	$C_{10}H_{19}N_3O_6$	43.32 (43.46	6.91 6.9	15.15 15.33)
III-13	Glu	—[a]			0.11	$C_{12}H_{21}N_3O_7$			
III-14	Gln	—[a]			0.08	$C_{12}H_{22}N_4O_6$			
III-15	Lys	127—130 EtOH–Et_2O	97	−6.4 (1.0, MeOH)	0.09	$C_{13}H_{26}N_4O_5$	49.04 (49.04	8.23 8.22	17.59 17.34)

a) Oily compound: used for next reaction without purification.

TABLE VI. Physicochemical and Analytical Data for H–X–Gly–NHOH

Compd. No.	X	mp (°C) Recryst. solv.	Yield (%)	$[\alpha]_D^{25}$ (°) (*c*, solv.)	*Rf*[3]	Formula	Analysis (%) Calcd (Found)		
							C	H	N
IV- 2	Leu	190—201 EtOH–MeOH–Et_2O	79	+52.9 (1.0, H_2O)	0.44	$C_8H_{18}ClN_3O_3$	40.08 (39.87	7.56 7.55	17.53 17.75)
IV- 3	Ile	215—223 EtOH–MeOH–Et_2O	87	+56.5 (1.0, H_2O)	0.42	$C_8H_{18}ClN_3O_3$	40.08 (39.9	7.56 7.59	17.53 17.32)
IV- 4	Tyr(Bzl)	—[a] EtOH–Et_2O	90	+62.5 (1.0, H_2O)	0.54	$C_{18}H_{22}ClN_3O_4$			
IV- 5	Tyr	—[a] EtOH–Et_2O	87	+59.3 (1.0, H_2O)	0.42	$C_{11}H_{16}ClN_3O_4$			
IV- 6	Ser(Bzl)	92—99 EtOH–Et_2O	85	+32.6 (1.0, H_2O)	0.49	$C_{12}H_{18}ClN_3O_4 \cdot 1/6H_2O$	46.98 (47.23	6.02 6.24	13.69 13.48)
IV- 7	Met	132—141 MeOH–H_2O–Et_2O	52	+57.5 (1.0, H_2O)	0.37	$C_7H_{16}ClN_3O_3S$	32.62 (37.72	6.26 6.33	16.3 16.49)
IV- 8	D-Phe	100—106 EtOH–AcOEt	90	−67.9 (1.0, H_2O)	0.45	$C_{11}H_{16}ClN_3O_3 \cdot 1/2H_2O$	46.73 (46.46	6.06 5.94	14.86 14.57)
IV- 9	Gly	137—140 EtOH–Et_2O	67		0.14	$C_4H_{10}ClN_3O_3$	26.16 (26.39	5.48 5.45	22.88 23.05)
IV-10	Ala	155—157 EtOH–Et_2O	79	+31.5 (1.0, H_2O)	0.19	$C_5H_{12}ClN_3O_3$	30.38 (30.63	6.12 6.14	21.26 21.35)
IV-11	Pro	—[a] EtOH–Et_2O	62	−17.7 (1.0, H_2O)	0.13	$C_7H_{14}ClN_3O_3$			
IV-12	Ser	—[a] EtOH–Et_2O	81	+33.5 (1.0, H_2O)	0.16	$C_5H_{12}ClN_3O_4$			
IV-13	Glu	—[a] MeOH–Et_2O	95	+58.7 (1.0, H_2O)	0.19	$C_7H_{14}ClN_3O_5$			
IV-14	Gln	—[a] H_2O–THF	32	+39.8 (1.0, H_2O)	0.16	$C_7H_{15}ClN_4O_4$			
IV-15	Lys	—[a] EtOH–Et_2O	96	+28.4 (1.0, H_2O)	0.05	$C_8H_{20}Cl_2N_4O_3$			

a) A hygroscopic powder.

concentrated *in vacuo*. The residue was reprecipitated from AcOEt to give compound IV-1 as a white powder (0.16 g, 99%). mp 110—118 °C, $[\alpha]_D^{25}$ +64.8° (*c* = 1.0, H_2O). *Anal.* Calcd for $C_{11}H_{16}ClN_3O_3$: C, 48.26; H, 5.89; N, 15.35. Found: C, 47.9; H, 6.07; N, 15.11. Other H–X–Gly–NHOH, IV-2—15 were prepared as described for IV-1 and the results are shown in Table VI.

H–Arg–Gly–NHOH·HCl (IV-16) To a dimethylformamide (DMF) solution of Z–Arg–OH (1.85 g, 6.0 mmol), H–Gly–NHOBzl·HCl (1.51 g,

7.0 mmol), and HOBt (1.78 g, 13 mmol) was added EDC (1.3 ml, 7.0 mmol) at −20 °C. The reaction mixture was stirred overnight at 5 °C and concentrated *in vacuo*. The crude residue was purified by HP-20 (washed with 5% Na_2CO_3 and H_2O, and then eluted with MeOH) to give Z–Arg–Gly–NHOBzl (1.6 g, 57%) as colorless oil. A mixture of Z–Arg–Gly–NHOBzl (0.7 g, 1.6 mmol), 4.5 M HCl/AcOEt (1.5 ml), and 5% Pd–C (0.2 g) in EtOH was vigorously stirred for 1 h at room temperature under a hydrogen flow. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was reprecipitated from MeOH–iso-Pr_2O to give IV-16 as a white hygroscopic powder (0.1 g, 15%). $[\alpha]_D^{25}$ +32.3° (c=1.0, H_2O), Rf^3, 0.08. *Anal.* Calcd for $C_8H_{20}Cl_2N_6O_3 \cdot 2/3H_2O$: C, 29.01; H, 6.49; N, 25.37. Found: C, 28.29; H, 6.48; N, 25.01.

Bz–Phe–Gly–NHOH (IV-17) Boc–Phe–Gly–OEt (I-1) (1.26 g, 3.6 mmol) was treated with 4.2 M HCl/AcOEt (20 ml) at room temperature for 1 h and concentrated *in vacuo*. To a THF solution of the residue was added Bz–Cl (0.49 ml, 4.23 mmol) and triethylamine (TEA, 1.2 ml, 8.6 mmol) at −10 °C. The reaction mixture was stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in AcOEt and washed with H_2O and dried over $MgSO_4$. After evaporation of AcOEt, the residue was recrystallized from iso-PrOH to give Bz–Phe–Gly–OEt (0.82 g, 66%) as colorless needles. mp 134—136 °C, $[\alpha]_D^{25}$ −48.5° (c=1.0, MeOH), Rf^1, 0.43. *Anal.* Calcd for $C_{20}H_{22}N_2O_4$: C, 67.78; H, 6.26; N, 7.9. Found: C, 67.69; H, 6.47; N, 7.74. Bz–Phe–Gly–OEt (0.33 g, 0.93 mmol) was converted to Bz–Phe–Gly–NHOH by the same manner as described for Boc–Phe–Gly–NHOH (III-1). The crude product was purified by recrystallization from AcOEt–*n*-hexane to give IV-17 as colorless crystals (0.22 g, 69%). mp 139—140 °C, $[\alpha]_D^{25}$ +16.9° (c=1.0, EtOH), Rf^2, 0.51. *Anal.* Calcd for $C_{18}H_{19}N_3O_4 \cdot 1/4H_2O$: C, 62.51; H, 5.68; N, 12.1. Found: C, 62.62; H, 5.72; N, 11.87.

Ac–Phe–Gly–NHOH (IV-18) This compound was prepared by the same manner as described for Bz–Phe–Gly–NHOH (IV-17). Colorless needles. mp 167—170 °C, $[\alpha]_D^{25}$ +29.2° (c=1.0, MeOH), Rf^2, 0.38. *Anal.* Calcd for $C_{13}H_{17}N_3O_4$: C, 55.91; H, 6.14; N, 15.05. Found: C, 55.95; H, 5.92; N, 14.89.

Preparation of Urease Jack bean urease was partially purified according to the method of Uehara and Kobashi[9] and its specific activity was 9.25 units/mg protein. Jack bean urease activity was assayed according to the method of van Slyke and Archibald.[14] *Proteus mirabilis* was isolated from the urine of a patient with urinary tract infection and cultured in bouillon broth. An intact cell suspension and a cell free extract by the sonication were used as the urease preparation. Bacterial urease activity was assayed by determining the amount of ammonia produced by the indophenol method.[15]

Urease Inhibitory Assay I_{50} value (μl) which gives 50% inhibition in a final concentration was measured according to the method of Kobashi *et al.*[11] Inhibition percentages of *Proteus mirabilis* urease using an intact cell suspension and a cell free extract were measured in the presence of 1 and 0.1 mM test compounds, respectively, in a final concentration according to the method of Kobashi *et al.*[5a]

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