Development of Active Center-Directed Plasmin and Plasma Kallikrein Inhibitors and Studies on the Structure–Inhibitory Activity Relationship[1)]

Naoki TENO,[a] Keiko WANAKA,[b] Yoshio OKADA,*[,a] Hiroaki TAGUCHI,[a] Utako OKAMOTO,[b] Akiko HIJIKATA-OKUNOMIYA[c] and Shosuke OKAMOTO[b]

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University,[a] Nishi-ku, Kobe 651–21, Japan, Kobe Research Projects on Thrombosis and Haemostasis,[b] Asahigaoka 3–15–18, Tarumi-ku, Kobe 655, Japan and School of Allied Medical Sciences, Kobe University,[c] Suma-ku, Kobe 654–01, Japan. Received September 28, 1992

The molecule of *trans*-4-aminomethylcyclohexanecarbonylphenylalanine 4-carboxymethylanilide (8), which is a potent and selective inhibitor of plasma kallikrein, can be devided into three parts (P_1, $P_{1'}$ and $P_{2'}$), each of which contains one of the rings. In order to study the role of each part in the manifestation of potent and selective inhibitory activity and the relationship between the structure and inhibitory activities toward plasmin, plasma kallikrein, urokinase and thrombin, each part was substituted with various other moieties to give many kinds of analogs and their inhibitory activities against the above enzymes were examined. Among them, *trans*-4-aminomethylcyclohexanecarbonyl-*O*-2-bromobenzyloxycarbonyltyrosine 4-acetylanilide (12) inhibited plasmin and plasma kallikrein with IC_{50} values of 2.3×10^{-7} M and 3.7×10^{-7} M, and K_i values of 1.2×10^{-7} M and 1.3×10^{-7} M, respectively.

Keywords inhibitor; plasmin; plasma kallikrein; chemical synthesis; structure–activity relationship

It is well known that proteinases and their natural inhibitors regulate biological functions cooperatively to maintain homeostasis, and imbalances between proteinases and their natural inhibitors can cause serious disorders.[2,3)] With regard to plasmin (PL), α_2-macroglobulin (α_2-M)[4)] and α_2-plasmin inhibitor (α_2-PI)[5)] are known as endogenous inhibitors, and an imbalance between plasmin and its natural inhibitors causes serious syndromes, such as hyperfibrinolysis.[6–8)] α_2-M[9)] and C_1-inactivator[10)] are endogenous inhibitors of plasma kallikrein (PK). Besides liberating bradykinin from high molecular weight kininogen,[11)] PK can activate factor XII,[12)] prourokinase[13)] and plasminogen[14)] and may enhance blood polymorphonuclear leukocyte chemotaxis.[15)] However, the roles of PK still remain to be established in detail.

With the objectives of obtaining a powerful tool for studies of the roles of PL and PK, and developing new types of clinical therapy, our attention was directed to the synthesis of a potent inhibitors of PL and PK. Previously, we reported the development of active center-directed inhibitors of PL[16,17)] and of PK,[18,19)] and studies on the structure–inhibitory activity relationship.

This paper deals with further studies on the structure–inhibitory activity relationship, and the development of inhibitors of PL and PK.

Our previous report demonstrated that *trans*-4-aminomethylcyclohexanecarbonyl(Tra)–Phe–4-carboxymethylanilide (**8**) is a selective inhibitor of PK.[19)] This is a Phe derivative having a very simple structure. Tra is located at the N-terminal of Phe and 4-carboxymethylaniline at the C-terminal position. Regarding the interaction of this compound (**8**) with PK, we hypothesized that the amino group in the Tra moiety at the P_1 position[20)] might interact with a negatively charged group of the enzyme and that the phenyl groups of the Phe and anilide moieties were also able to interact with the enzyme to manifest a potent inhibitory activity.

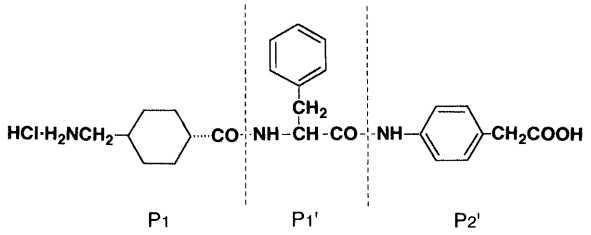


Fig. 1. Structure of *trans*-4-Aminomethylcyclohexanecarbonyl–Phe–4-Carboxymethylanilide (**8**)

First of all, the role of each moiety of the compound (**8**) in the manifestation of inhibitory activity was studied. The Tra moiety is at the P_1 position as shown in Fig. 1. Therefore, the carbonyl group of the Tra moiety might interact with the hydroxy group of the Ser residue in the active center of the enzyme. However, *trans*-4-aminomethylcyclohexanechloromethyl ketone (**1**) did not show any detectable inhibitory activity against PL, PK, urokinase (UK) or thrombin (TH), as summarized in Table I, indicating that besides the Tra moiety, the phenyl groups at the $P_{1'}$ and $P_{2'}$ positions would be required for manifestation of the inhibitory activity. H–Tra–4-acetylphenoxymethyl ketone (**2**) and H–Tra–4-benzoylphenoxymethyl ketone (**3**) exhibited weak inhibitory activity against UK, indicating that a phenyl moiety at the $P_{1'}$ position might weakly interact with the enzyme.

H–Tra–Phe–CH_2Cl (**4**) inhibited PL activity toward S-2251 and fibrin with IC_{50} values of 1200 and 730 μM, respectively as summarized in Table I. H–Tra–Tyr(Bzl)–CH_2Cl (**5**) inhibited PL and PK with IC_{50} values of 340 and 600 μM, respectively. The Bzl group in the side chain of the Tyr residue increased the inhibitory activity against both PL and PK, although the inhibitory activities were still very weak.

H–Tra–Phe–4-acetylanilide (**6**)[21)] exhibited inhibitory activity against PL, PK and UK with IC_{50} values of 36, 0.85 and 58 μM, respectively and H–Tra–Tyr(Bzl)–4-acetylanilide (**7**)[21)] inhibited PL, PK and UK with IC_{50} values of 1.8, 0.63 and 31 μM, respectively. These results suggested the need for another phenyl group at the $P_{2'}$ position for manifestation of potent inhibitory activity. It can be deduced that the Phe residue at the $P_{1'}$ position

TABLE I. IC_{50} Values (μM) of Compounds **1**—**24** for Various Enzymes

No.	P_1	$P_{1'}$	$P_{2'}$	PL		PK	UK	TH	
				S-2251	Fn	S-2302	S-2444	S-2238	Fg
1	H_2NCH_2-(H)-CO	CH_2Cl		>1000 (0%)[a]	>1000 (26%)	>1000 (0%)	1300	>1000 (0%)	>1000 (0%)
2	H_2NCH_2-(H)-CO	$CH_2-O-C_6H_4-COCH_3$		>1000 (22%)	>1000 (41%)	>1000 (18%)	830	>1000 (0%)	>1000 (0%)
3	H_2NCH_2-(H)-CO	$CH_2-O-C_6H_4-CO-C_6H_5$		>1000 (20%)	>500 (38%)	>1000 (29%)	1000	>1000 (0%)	>500 (0%)
4	H_2NCH_2-(H)-CO	Phe	CH_2Cl	1200	730	>500 (0%)	>500 (17%)	>500 (0%)	>500 (0%)
5	H_2NCH_2-(H)-CO	Tyr(-CH_2-)	CH_2Cl	340	120	600	>1000 (34%)	>1000 (10%)	>500 (0%)
6	H_2NCH_2-(H)-CO	Phe	NH-C_6H_4-$COCH_3$	36	21	0.85	58	>1000 (19%)	>1000 (0%)
7	H_2NCH_2-(H)-CO	Tyr(-CH_2-)	NH-C_6H_4-$COCH_3$	1.8	0.40	0.63	31	>200 (11%)	>100 (0%)
8	H_2NCH_2-(H)-CO	Phe	NH-C_6H_4-CH_2COOH	620	350	1.3	350	>1000 (0%)	>1000 (0%)
9	H_2NCH_2-(H)-CO	Phe	NH-C_6H_4-CH_2COOCH_2-C_6H_5	26	11	1.1	140	>100 (0%)	>100 (0%)
10	H_2NCH_2-(H)-CO	Tyr(-CO_2CH_2-)	NH-C_6H_4-$COCH_3$	0.64	0.29	0.58	45	>100 (23%)	>100 (0%)
11	H_2NCH_2-(H)-CO	Tyr(-CO_2CH_2-(Cl))	NH-C_6H_4-$COCH_3$	0.56	0.075	0.75	31	86	>50 (0%)
12	H_2NCH_2-(H)-CO	Tyr(-CO_2CH_2-(Br))	NH-C_6H_4-$COCH_3$	0.23	0.051	0.37	43	63	>50 (0%)
13	H_2NCH_2-(H)-CO	Phe	$CH_2O-C_6H_4-COCH_3$	>1000 (40%)	390	78	>500 (36%)	>500 (0%)	>500 (0%)
14	H_2NCH_2-(H)-CO	Tyr(-CH_2-)	$CH_2O-C_6H_4-COCH_3$	26	13	26	>400 (41%)	>200 (0%)	>50 (0%)
15	H_2NCH_2-(H)-CO	Tyr(-CO_2CH_2-(Br))	$CH_2O-C_6H_4-COCH_3$	8.0	1.8	22	150	>50 (27%)	>20 (0%)
16	H_2NCH_2-(H)-CO	Phe	$CH_2-NH-CH_2$-(N)	>500 (8%)	>500 (22%)	>1000 (25%)	>500 (13%)	ND[b]	>500 (0%)
17	H_2NCH_2-(H)-CO	Phe	$CH_2-NH-CH_2$-(N)	>500 (18%)	>500 (42%)	>1000 (18%)	>500 (33%)	ND	>500 (0%)
18	H_2NCH_2-(H)-CO	Phe	$CH_2-NH-CH_2-C_6H_4-COOC_2H_5$	>1000 (11%)	>1000 (47%)	>1000 (4%)	ND	ND	ND
18'	$(H_2NCH_2$-(H)-CO-Phe-$CH_2)_2$-N-$CH_2-C_6H_4-COOC_2H_5$			>200 (10%)	>200 (45%)	>200 (10%)	ND	ND	ND
19	H_2NCH_2-(H)-CO	Phe	$CH_2-NH-C_6H_4-COOC_2H_5$	>1000 (47%)	450	300	>1000 (39%)	ND	>500 (0%)
20	H_2NCH_2-(H)-CO	Tyr(-CO_2CH_2-)	$CH_2NH-CH_2-C_6H_4-COOC_2H_5$	12	4.5	30	160	ND	>100 (0%)
20'	$[H_2NCH_2$-(H)-CO-Tyr(-CO_2CH_2-)-$CH_2]_2$-N-$CH_2-C_6H_4-COOC_2H_5$			6.5	6.6	ND	ND	ND	ND
21	H_2NCH_2-(H)-CO	Tyr(-CO_2CH_2-(Br))	CH_2O-(N)	2.1	2.9	ND	110	ND	ND
22	H_2NCH_2-(H)-CO	Tyr(-CO_2CH_2-(Br))	CH_2O-(N)	4.2	5.3	100 (30%)	ND	ND	ND
23	H_2NCH_2-(H)-CO	CH_2-Tyr(-CO_2CH_2-)	NH-C_6H_4-$COCH_3$	43	21	ND	120	ND	>40 (0%)
24	H_2NCH_2-(H)-CO	CH_2-Tyr(-CO_2CH_2-(Br))	NH-C_6H_4-$COCH_3$	26	7.9	55	ND	24	>50 (0%)

a) Values in parenthesis are inhibition % at the concentration described (μM). *b*) ND; not determined.

interacts with PK more strongly than with PL, while the Tyr(Bzl) residue at the $P_{1'}$ position can interact with both PL and PK. H–Tra–Phe–4-benzyloxycarbonylmethylanilide (**9**) inhibited PL and PK with IC_{50} values of 26 and 1.1 μM, respectively, while H–Tra–Phe–4-carboxymethylanilide (**8**) inhibited PL and PK with IC_{50} values of higher than 620 and 1.3 μM, respectively.[19] The benzyl ester group in compound **9** increases the inhibitory activity against PL.

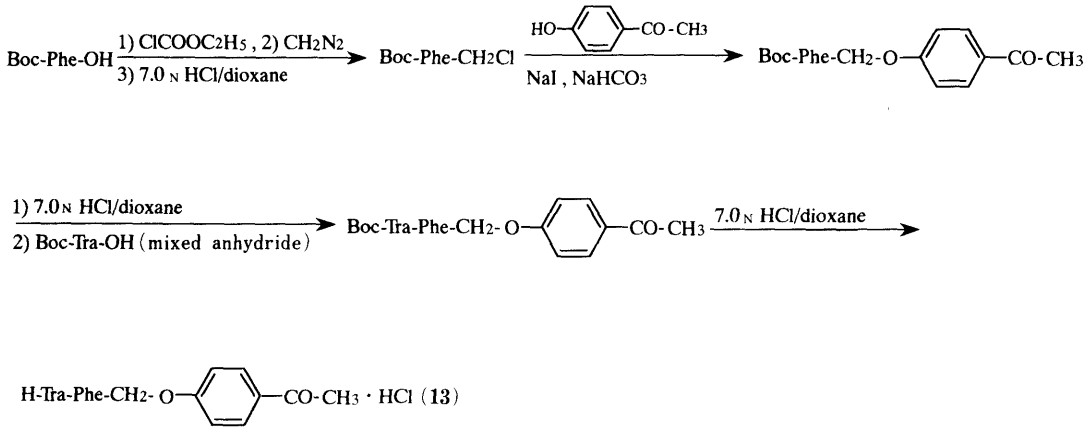


Fig. 2. Synthetic Route to H–Tra–Phe–4-Acetylphenoxymethyl Ketone

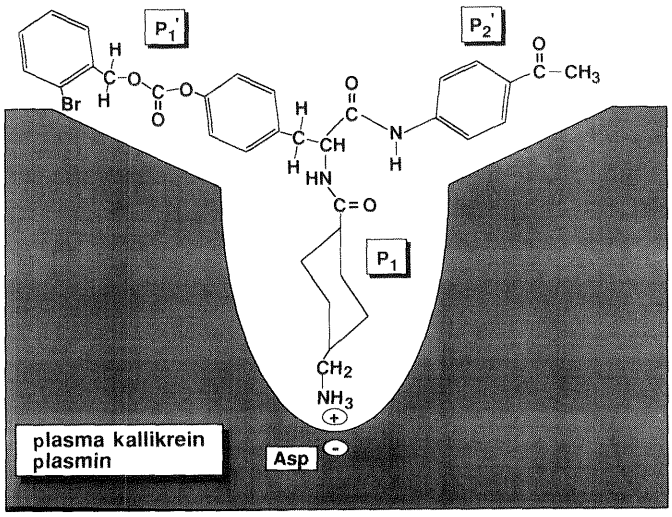


Fig. 3. Schematic Representation of Interaction of Compound **12** with Enzymes

These results suggested the existence of differences in stereogeometry at the $S_{1'}$ and $S_{2'}$ positions[20] between PL and PK, indicating that there is scope to design selective inhibitors.

In order to increase the interaction of the inhibitor with enzyme, H–Tra–Tyr(2-X–Z)–4-acetylanilide (**10**, X: H; **11**, X: Cl; **12**, X: Br) were synthesized and their inhibitory activities examined. Compounds **10**, **11** and **12** inhibited PL with IC_{50} values of 0.64, 0.56 and 0.23 μM, respectively, and PK with IC_{50} values of 0.58, 0.75 and 0.37 μM, respectively.

H–Tra–X–4-acetylphenoxymethyl ketones [**13**, X: Phe; **14**, X: Tyr(Bzl); **15**, X: Tyr(2-Br–Z)] were prepared by the route shown in Fig. 2 according to the method described previously,[22,23] and their inhibitory activities were examined. The inhibitory activities of this series (**13**, **14**, **15**) increased in a similar manner to the series **6**, **7**, **12**, although the former compounds exhibited weaker inhibitory activities than the anilide derivatives (**6**, **7**, **12**). Anilide type structure is more suitable for the manifestation of potent inhibitory activity than the corresponding ketomethylene type structure, although the former is more vulnerable to enzymatic hydrolysis than the latter.

These results further confirmed our hypothesis that the phenyl group of a Phe or Tyr residue can interact with PK more strongly than with PL, while the phenyl group of a Bzl, Z, Cl–Z or Br–Z in the side chain of a Tyr residue can interact with both PK and PL. The interaction between compound **12** and the enzymes is schematically represented in Fig. 3.

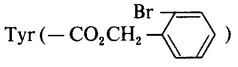
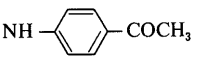
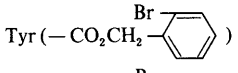
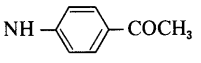
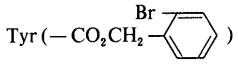
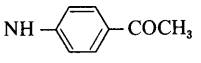
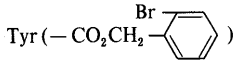
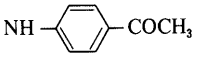
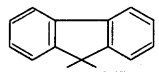
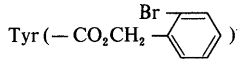
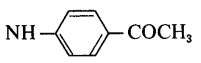
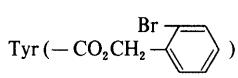
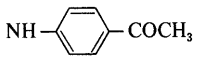
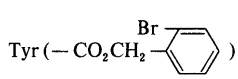
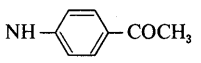
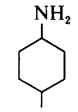
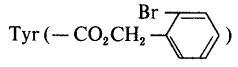
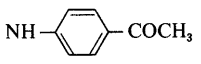
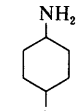
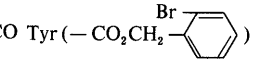
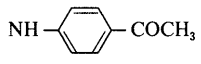
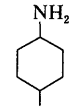
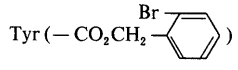
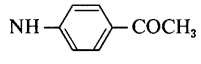
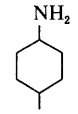
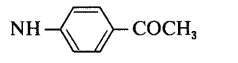
Among the Phe derivatives (**16**—**19**), only compound **19** inhibited PK with an IC_{50} value of 300 μM. These results support our hypothesis that anilide type structure at the $P_{2'}$ position of the compound **6** is more suitable for the manifestation of potent inhibitory activity against PL and PK than the corresponding methyl ketone structure (**16**—**19**). Compound **20** inhibited PL and PK with IC_{50} values of 12 and 30 μM, respectively, while compound **18** exhibited only weak inhibitory activity against PL and PK. These results showed that the Tyr(Z) moiety at the $P_{1'}$ position has a very important role in the manifestation of strong inhibitory activity against PL and PK. Compounds **18′** and **20′** exhibited stronger inhibitory activity against PL than compounds **18** and **20**, although the reason is not clear. Compounds **21** and **22** inhibited PL with IC_{50} values of 2.1 and 4.2 μM, respectively. The latter compound (**22**) inhibited PK by 30% at the concentration of 100 μM. In **23** and **24**, a methylene group ($-CH_2-$) is inserted between the Tra and Tyr moieties. These compounds exhibited much weaker inhibitory activities than **10** and **12**. The insertion of a methylene group made the Tra moiety at the P_1 position unfavorable for binding with negatively charged regions of the enzymes.

So far as examined, H–Tra–Tyr(2-Br–Z)–4-acetylanilide (**12**) inhibited PL and PK most strongly. The Tyr(Br–Z)–4-acetylanilide moiety presumably interacts with PL and PK, resulting in potent inhibitory activities. Therefore, the Tra moiety of **12** was substituted with a basic amino acid. Lys derivatives (**25**—**29**) were prepared and their inhibitory activities were examined. The results are summarized in Table II. These compounds exhibited inhibitory activities against PL and PK, but their IC_{50} values were higher than those of **12**.

4-Amino–Phe derivatives (**30** and **31**) exhibited very weak inhibitory activity against PL and PK.

The amino group of the Tra moiety can interact with a negatively charged moiety of the enzymes. Therefore, 4-aminocyclohexylalanine derivatives (**32**—**36**) were prepared by the route shown in Fig. 4. Compounds **32**—**34** inhibited PL and PK but their IC_{50} values are higher than

TABLE II. IC_{50} Values (μM) of Compounds **25**—**36** for Various Enzymes

No.	P_2	P_1	$P_{1'}$	$P_{2'}$	PL		PK	UK	TH	
					S-2251	Fn	S-2302	S-2444	S-2238	Fg
25		Lys			40	25	19	>200 (0%)[a]	>50 (0%)	>25 (0%)
26		Lys			11	19	29	>100 (0%)	>200 (48%)	>25 (0%)
27		Lys			28	>10 (22%)	30	>40 (0%)	>40 (29%)	>10 (0%)
28		Lys			8.8	>10 (36%)	27	>50 (0%)	>25 (32%)	>10 (0%)
29		Lys			7.2	>10 (18%)	28	>25 (0%)	>12.5 (26%)	>10 (0%)
30		 Phe			>25 (9%)	>25 (0%)	>25 (19%)	ND[b]	ND	ND
31		 Phe			>100 (12%)	>100 (9%)	>100 (46%)	ND	ND	ND
32	 (*trans*)				52	>10 (15%)	16	>200 (32%)	ND	>10 (0%)
33	 (*trans*)				3.0—47	>10 (15%)	24	>200 (38%)	ND	>10 (0%)
34	 (*cis*)				14	>20 (0%)	33	>100 (36%)	ND	>20 (0%)
35					>1000 (47%)	>500 (36%)	>1000 (0%)	>1000 (14%)	ND	>500 (0%)
36					180	84	>1000 (36%)	>1000 (20%)	ND	>200 (0%)

a) Values in parenthesis are inhibition % at the concentration described (μM). *b*) ND; not determined.

those of **12**. In **35** and **36**, the Tyr(2-Br–Z) moiety was eliminated in order to decrease steric hindrance. Only compound **36** inhibited PL with an IC_{50} value of 180 μM.

In conclusion, based on studies of the structure–activity relationship, potent inhibitors of PL and PK were designed and synthesized. H–Tra–Phe–4-carboxymethylanilide (**8**) inhibited PK selectively[19] and H–Tra–Tyr(2-Br–Z)–2-pyridyloxymethyl ketone (**22**) inhibited PL selectively, as summarized in Table III.

H–Tra–Tyr(Br–Z)–4-acetylanilide (**12**) could inhibit PL

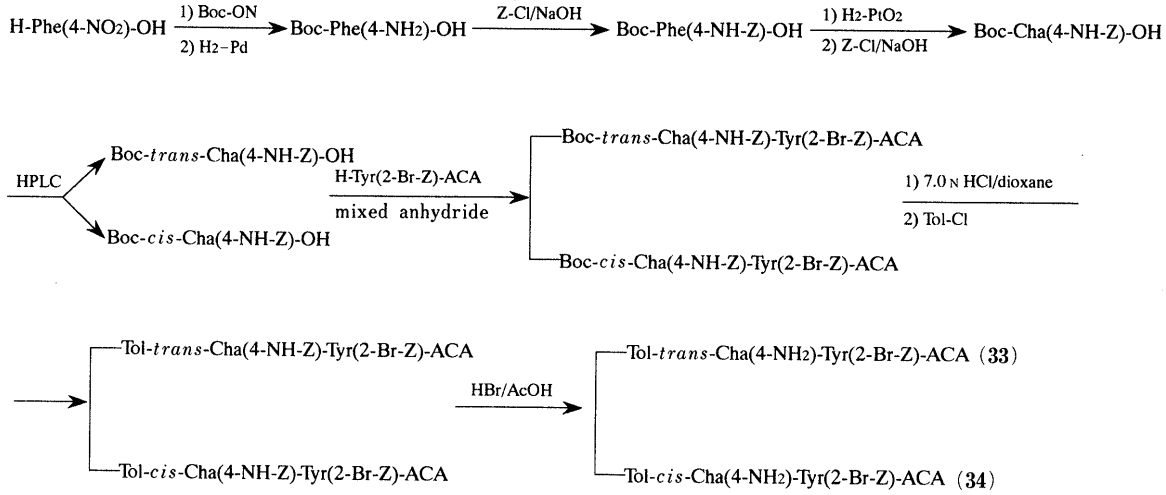


Fig. 4. Synthetic Route to Compounds **33** and **34**

ACA, 4-acetylanilide; Boc–ON, 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile; *trans*-Cha(4-NH_2), *trans*-4-aminocyclohexylalanine; *cis*-Cha(4-NH_2), *cis*-4-aminocyclohexylalanine.

TABLE III. Comparison of IC_{50} Values (μM) of Compounds **8**, **12** and **22** for PL and PK

No.	P_1	$P_{1'}$	$P_{2'}$	PL	PK
				S-2251	S-2302
8	H_2NCH_2–(H)–CO	Phe	NH–CH_2COOH	620	1.3
12	H_2NCH_2–(H)–CO	Tyr(–CO_2CH_2–(Br))	NH–$COCH_3$	0.23	0.37
22	H_2NCH_2–(H)–CO	Tyr(–CO_2CH_2–(Br))	CH_2O–(N)	4.2	>100 (30%)[a]

a) Values in parenthesis are inhibition % at the concentration described (μM).

not only toward S-2251 but also toward fibrin with IC_{50} values of 2.3×10^{-7} M (K_i value: 1.2×10^{-7} M) and 5.1×10^{-8} M, respectively, and PK with an IC_{50} value of 3.7×10^{-7} M (K_i value: 1.3×10^{-7} M). These results suggest that some similarity of active site structures may exist between these two kinds of proteases.

Moreover, this compound (**12**) exhibited a relatively high LD_{50} value (>100 mg/kg, mice, i.v.). Therefore, it should be a powerful experimental tool for investigating the roles of PL and PK and it may also play an important role in the development of new types of clinical therapy.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid hydrolysates (6 N HCl, 110 °C, 18 h) were determined with an amino acid analyzer (K-101 AS, Kyowa Seimitsu). ^{1}H-NMR spectra were measured with a Bruker (400 MHz) spectrometer. Chemical shift values are expressed as ppm downfield from tetramethylsilane used as an internal standard (δ-value). For thin-layer chromatography (TLC) (Kieselgel G, Merck), Rf^1, Rf^2, Rf^3, Rf^4, Rf^5, Rf^6, Rf^7, Rf^8, Rf^9, Rf^{10} and Rf^{11} values refer to the systems of $CHCl_3$–MeOH–AcOH (90:8:2), $CHCl_3$–MeOH–H_2O (89:10:1), $CHCl_3$–MeOH–H_2O (8:3:1, lower phase), *n*-BuOH–AcOH–H_2O (4:1:5, upper phase), *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2), $CHCl_3$–AcOEt–*n*-hexane (4:1:4), $CHCl_3$–AcOEt–*n*-hexane (2:1:2), $CHCl_3$–ether (4:1), $CHCl_3$–MeOH (9:1), $CHCl_3$–MeOH (8:2) and iso-PrOH–H_2O–AcOEt–NH_4OH (5:1:2:1), respectively.

Boc–Tra–CH_2Cl Diazomethane [prepared from nitrosomethylurea (7.1 g, 70 mmol)] was added to a mixed anhydride [prepared from Boc–Tra–OH (4.5 g, 17 mmol), ethyl chloroformate (1.7 ml, 17 mmol) and Et_3N (2.4 ml, 17 mmol) as usual] in tetrahydrofuran (THF) (200 ml) at −15 °C. The reaction mixture was stirred at 4 °C for 5 h. After addition of 7.3 N HCl/dioxane (9.6 ml, 70 mmol) at −15 °C, the reaction mixture was stirred at the same temperature for 3 h and neutralized with Et_3N. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated to a small volume. The crude product in EtOH (3 ml) was applied to a column of Sephadex LH-20 (3.4 × 155 cm), equilibrated and eluted with EtOH. Individual fractions (5 g each) were collected and the solvent of the effluent (tube Nos. 53—60) was removed by evaporation. Ether was added to the residue to afford crystals, which were collected by filtration, yield 0.5 g (10.6%), mp 86—89 °C, Rf^8 0.70, Rf^9 0.88. *Anal.* Calcd for $C_{14}H_{24}ClNO_3$: C, 58.1; H, 8.29; N, 4.83. Found: C, 58.3; H, 8.29; N, 4.83.

H–Tra–CH_2Cl (1) A solution of Boc–Tra–CH_2Cl (0.1 g, 0.35 mmol) in 7.0 N HCl/dioxane (0.5 ml, 3.5 mmol) was kept at room temperature for 70 min. Ether was added to the solution to form a precipitate, which was collected by filtration. The crude product in EtOH (2 ml) was applied to a column of Sephadex LH-20 (3.4 × 155 cm), equilibrated and eluted with EtOH. Individual fractions (5 g each) were collected and the solvent of the effluent (tube Nos. 115—121) was removed by evaporation. The residue was dissolved in water and lyophilized, yield 50 mg (63.1%), Rf^4 0.16, Rf^5 0.53. *Anal.* Calcd for $C_9H_{16}ClNO \cdot HCl \cdot 2H_2O$: C, 47.7; H, 7.27; N, 6.18. Found: C, 47.3; H, 7.25; N, 6.14.

Boc–Tra–4-Acetylphenoxymethyl Ketone Boc–Tra–CH_2Cl (0.4 g, 1.6 mmol), 4-hydroxyacetophenone (0.2 g, 1.6 mmol), NaI (0.2 g, 1.6 mmol) and $NaHCO_3$ (0.1 g, 1.6 mmol) were dissolved in DMF (10 ml). The reaction mixture was stirred at 45 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. Ether was added

to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 0.28 g (45.0%), mp 113—115 °C, Rf^8 0.38, Rf^9 0.67. ^{1}H-NMR ($CDCl_3$) δ: 0.96—1.06 (2H, m), 1.44 (9H, s), 1.38—1.48 (3H, m), 1.85—1.86 (2H, m), 1.94—1.97 (2H, m), 2.58 (3H, s), 2.58—2.65 (1H, m), 2.98—3.01 (2H, m), 4.58 (1H, br), 4.71 (2H, s), 7.74 (2H, d, J=8.9 Hz), 6.90 (2H, d, J=8.9 Hz). *Anal.* Calcd for $C_{22}H_{31}NO_5 \cdot 1/4H_2O$: C, 67.1; H, 8.00; N, 3.56. Found: C, 67.3; H, 7.96; N, 3.64.

H-Tra-4-Acetylphenoxymethyl Ketone (2) A solution of the corresponding Boc derivative (47 mg, 0.12 mmol) in 7.0 N HCl/dioxane (0.2 ml, 1.4 mmol) containing anisole (0.03 ml, 0.24 mmol) was kept at room temperature for 70 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 35 mg (89.6%), mp 181—184 °C, Rf^4 0.10, Rf^5 0.47. ^{1}H-NMR ($CDCl_3$) δ: 1.09—1.19 (2H, m), 1.39—1.50 (2H, m), 1.61—1.67 (1H, m), 1.91—1.95 (2H, m), 2.01—2.06 (2H, m), 2.59 (3H, s), 2.64—2.72 (1H, m), 2.81 (2H, d, J=7.0 Hz), 4.95 (2H, s), 7.97 (2H, d, J=9.0 Hz), 6.93 (2H, d, J=9.0 Hz). *Anal.* Calcd for $C_{17}H_{23}NO_3 \cdot HCl \cdot 1/4H_2O$: C, 61.9; H, 7.27; N, 4.24. Found: C, 61.9; H, 7.27; N, 4.25.

Boc-Tra-4-Benzoylphenoxymethyl Ketone Boc–Tra–CH_2Cl (0.25 g, 0.9 mmol), 4-hydroxybenzophenone (0.17 g, 0.9 mmol), NaI (0.13 g, 0.9 mmol) and $NaHCO_3$ (0.07 g, 0.9 mmol) were dissolved in DMF (10 ml). The reaction mixture was stirred at 45 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. EtOH was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 0.08 g (20.1%), mp 141—142 °C, Rf^1 0.49, Rf^{10} 0.60. ^{1}H-NMR ($CDCl_3$) δ: 0.96—1.06 (2H, m), 1.44 (9H, s), 1.38—1.48 (3H, m), 1.85—1.86 (2H, m), 1.94—1.97 (2H, m), 2.58—2.65 (1H, m), 2.98—3.01 (2H, m), 4.58 (1H, br), 4.71 (2H, s), 6.20—6.39 (5H, m), 7.94 (2H, d, J=8.9 Hz), 6.90 (2H, d, J=8.9 Hz). *Anal.* Calcd for $C_{27}H_{33}NO_5$: C, 71.9; H, 7.31; N, 3.10. Found: C, 71.7; H, 7.36; N, 3.05.

H-Tra-4-Benzoylphenoxymethyl Ketone (3) A solution of the corresponding Boc derivative (63 mg, 0.14 mmol) in 7.0 N HCl/dioxane (0.2 ml, 1.4 mmol) was kept at room temperature for 70 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 46 mg (86.4%), mp 190—194 °C, Rf^4 0.23, Rf^5 0.51. ^{1}H-NMR (CD_3OD) δ: 1.09—1.19 (2H, m), 1.39—1.50 (2H, m), 1.61—1.67 (1H, m), 1.91—1.95 (2H, m), 2.01—2.06 (2H, m), 2.64—2.72 (1H, m), 2.81 (2H, d, J=7.0 Hz), 4.95 (2H, s), 6.20—6.39 (5H, m), 7.97 (2H, d, J=9.0 Hz), 6.98 (2H, d, J=9.0 Hz). *Anal.* Calcd for $C_{22}H_{25}NO_3 \cdot HCl$: C, 68.2; H, 6.71; N, 3.61. Found: C, 67.7; H, 6.65; N, 3.63.

Boc-Phe-CH_2Cl Diazomethane [prepared from nitrosomethylurea (15.7 g, 0.15 mol)] was added to a mixed anhydride [prepared from Boc–Phe–OH (10.0 g, 0.038 mol), ethyl chloroformate (3.6 ml, 0.038 mol) and Et_3N (5.3 ml, 0.038 mol) as usual] in THF (180 ml) at −15 °C and the reaction mixture was stirred at 4 °C for 5 h. After addition of 7.6 N HCl/dioxane (20 ml, 0.15 mol) at −15 °C, the reaction mixture was stirred at the same temperature for 3 h and the pH of the solution was adjusted to 7 with Et_3N. The solvent was removed by evaporation and the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. The crude product in $CHCl_3$ (5 ml) was applied to a column of silica gel (2.0×28 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (100 ml each) were collected and the solvent of the effluent (tube Nos. 1—5) was removed by evaporation. Ether and petroleum ether were added to the residue to afford crystals, which were collected by filtration, yield 9.0 g (79.6%), mp 93—94 °C, $[\alpha]_D^{25}$ +17.0° (c=1.4, $CHCl_3$), Rf^7 0.77, Rf^8 0.82. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 2.94—3.11 (2H, m), 4.08, 4.19 (2H, each d, J=44, 21 Hz), 4.61—4.67 (1H, m), 5.05—5.08 (1H, m), 7.12—7.17 (2H, m), 7.25—7.34 (3H, m). *Anal.* Calcd for $C_{15}H_{20}ClNO_3$: C, 60.4; H, 6.74; N, 4.78. Found: C, 60.4; H, 6.72; N, 4.70.

Boc-Tra-Phe-CH_2Cl A solution of mixed anhydride [prepared from Boc–Tra–OH (5.1 g, 20 mmol) and ethyl chloroformate (1.9 ml, 20 mmol) as usual] in THF (150 ml) was added to an ice-cold solution of H–Phe–$CH_2Cl \cdot HCl$ [prepared from Boc–Phe–CH_2Cl (4.0 g, 13.4 mmol) and 7.0 N HCl/dioxane as usual] in DMF (20 ml) containing Et_3N (1.9 ml, 13.4 mmol). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, AcOEt and water were added to the residue to give crystals, which were collected by filtration and recrystallized from AcOEt, yield 3.2 g (54.7%), mp 170—173 °C, $[\alpha]_D^{25}$ +13.6° (c=1.3, $CHCl_3$), Rf^1 0.88, Rf^{10} 0.69. ^{1}H-NMR ($CDCl_3$) δ: 0.87—0.98 (2H, m), 1.44 (9H, s), 1.32—1.41 (3H, m), 1.79—2.06 (5H, m), 2.95—3.14 (4H, m), 3.98, 4.18 (2H, each d, J=80, 16.1 Hz), 4.60 (1H, br), 4.90—4.95 (1H, m), 6.05—6.07 (1H, m), 7.12—7.15 (2H, m), 7.25—7.34 (3H, m). *Anal.* Calcd for $C_{23}H_{33}ClN_2O_4$: C, 63.3; H, 7.56; N, 6.41. Found: C, 63.2; H, 7.55; N, 6.49.

H-Tra-Phe-CH_2Cl (4) A solution of the corresponding Boc-derivative (70.7 mg, 0.17 mmol) in 7.0 N HCl/dioxane (0.24 ml, 1.7 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 47.1 mg (76.1%), mp 149—152 °C, $[\alpha]_D^{25}$ −38.1° (c=1.2, MeOH), Rf^4 0.24, Rf^5 0.64. *Anal.* Calcd for $C_{18}H_{25}ClN_2O_2 \cdot HCl \cdot 3/4H_2O$: C, 55.9; H, 7.11; N, 7.24. Found: C, 56.1; H, 6.87; N, 7.32.

Boc-Tyr(Bzl)-CH_2Cl Diazomethane [prepared from nitrosomethylurea (5.4 g, 52 mmol)] was added to mixed anhydride [prepared from Boc–Tyr(Bzl)–OH (4.9 g, 13 mmol), ethyl chloroformate (1.2 ml, 13 mmol) and Et_3N (1.8 ml, 13 mmol)] in THF (150 ml) at −15 °C and the reaction mixture was stirred at 4 °C for 5 h. After addition of 7.3 N HCl/dioxane (7.1 ml, 52 mmol) at −15 °C, the reaction mixture was stirred at −15 °C for 3 h and neutralized with Et_3N. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were collected by filtration and reprecipitated from AcOEt and ether, 2.0 g (40.6%), mp 113 °C, $[\alpha]_D^{25}$ −39.8° (c=0.5, MeOH), Rf^6 0.65, Rf^7 0.69. *Anal.* Calcd for $C_{22}H_{26}ClNO_4 \cdot 1/4H_2O$: C, 64.7; H, 6.49; N, 3.43. Found: C, 65.0; H, 6.32; N, 3.63.

Boc-Tra-Tyr(Bzl)-CH_2Cl A solution of mixed anhydride [prepared from Boc–Tra–OH (0.8 g, 3.1 mmol) and ethyl chloroformate (0.3 ml, 3.1 mmol) as usual] in THF (50 ml) was added to an ice-cold solution of H–Tyr(Bzl)–CH_2Cl [prepared from Boc–Tyr(Bzl)–CH_2Cl (1.0 g, 2.6 mmol) and 7.3 N HCl/dioxane (3.6 ml, 26 mmol) as usual] in DMF (25 ml) containing Et_3N (0.36 ml, 2.6 mmol). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration and reprecipitated from EtOH and ether, yield 0.3 g (21.3%), mp 127—128 °C, $[\alpha]_D^{25}$ −47.9° (c=0.3, DMF), Rf^1 0.76, Rf^2 0.71. *Anal.* Calcd for $C_{30}H_{39}ClN_2O_5$: C, 66.4; H, 7.19; N, 5.16. Found: C, 66.1; H, 7.15; N, 5.06.

H-Tra-Tyr(Bzl)-CH_2Cl (5) A solution of Boc–Tra–Tyr(Bzl)–CH_2Cl (0.16 g, 0.3 mmol) in 7.3 N HCl/dioxane (0.42 ml, 3 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 0.13 g (90.4%), mp 151—154 °C, $[\alpha]_D^{25}$ −16.6° (c=1.1, MeOH), Rf^4 0.38, Rf^5 0.66. *Anal.* Calcd for $C_{25}H_{31}ClN_2O_3 \cdot HCl \cdot 1/2H_2O$: C, 61.5; H, 6.76; N, 5.74. Found: C, 61.3; H, 6.53; N, 5.70.

H-Tra-Phe-4-Benzyloxycarbonylmethylanilide (9) The title compound was prepared from Boc–Tra–Phe–4-benzyloxycarbonylmethylanilide[19] (140 mg, 0.22 mmol) in the same manner as described above. The TFA salt was lyophilized from dioxane containing HCl, yield 100 mg (80%), $[\alpha]_D^{25}$ +21.7° (c=0.5, MeOH), Rf^3 0.38.

Boc-Tyr(Z)-4-Acetylanilide A solution of mixed anhydride [prepared from Boc–Tyr(Z)–OH (8.6 g, 27.6 mmol) and ethyl chloroformate (2.6 ml, 27.6 mmol) as usual] in THF (200 ml) was added to an ice-cold solution of 4-aminoacetophenone (3.7 g, 27.6 mmol) in DMF (100 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 6.2 g (42.3%), mp 197—199 °C, $[\alpha]_D^{25}$ +51.4° (c=0.4, DMF), Rf^1 0.61, Rf^2 0.53. *Anal.* Calcd for $C_{30}H_{32}N_2O_7 \cdot 1/4H_2O$: C, 67.1; H, 6.05; N, 5.22. Found: C, 67.2; H, 5.96; N, 5.25.

Boc-Tra-Tyr(Z)-4-Acetylanilide A solution of mixed anhydride [prepared from Boc–Tra–OH (0.26 g, 1.0 mmol) and ethyl chloroformate (0.1 ml, 1.0 mmol) as usual] in THF (20 ml) was added to an ice-cold solution of H–Tyr(Z)–4-acetylanilide·HCl [prepared from Boc–Tyr(Z)–4-acetylanilide (0.36 g, 0.68 mmol) and 7.0 N HCl/dioxane (1.0 ml, 7.0 mmol) as usual] in DMF (5 ml) containing Et_3N (0.1 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 76 mg (16.3%), mp 208—210 °C, $[\alpha]_D^{25}$ +15.3° (c=0.2, DMF), Rf^{10} 0.49. *Anal.* Calcd for

$C_{38}H_{45}N_3O_8$: C, 68.0; H, 6.70; N, 6.26. Found: C, 67.7; H, 6.60; N, 6.17.

H–Tra–Tyr(Z)–4-Acetylanilide (10) A solution of Boc–Tra–Tyr(Z)–4-acetylanilide (42 mg, 0.06 mmol) in 7.0 N HCl/dioxane (0.1 ml, 0.70 mmol) containing anisole (0.07 ml, 0.62 mmol) was kept at room temperature for 70 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 34 mg (91.0%), mp 123—127 °C, $[\alpha]_D^{25}$ +27.9° (c=0.4, MeOH), Rf^4 0.33, Rf^5 0.57. *Anal.* Calcd for $C_{33}H_{37}N_3O_6 \cdot HCl \cdot 3/2H_2O$: C, 62.4; H, 6.46; N, 6.62. Found: C, 62.2; H, 6.12; N, 6.66.

Boc–Tyr(2-Cl–Z)–4-Acetylanilide A solution of mixed anhydride [prepared from Boc–Tyr(2-Cl–Z)–OH (1.0 g, 2.8 mmol) and ethyl chloroformate (0.3 ml, 2.8 mmol) as usual] in THF (50 ml) was added to an ice-cold solution of 4-aminoacetophenone (0.4 g, 2.8 mmol) in DMF (20 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, AcOEt and water were added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 0.6 g (37.8%), mp 183—185 °C, $[\alpha]_D^{25}$ +21.8° (c=1.1, DMF), Rf^1 0.61, Rf^{10} 0.64. *Anal.* Calcd for $C_{30}H_{31}ClN_2O_7 \cdot 1/4H_2O$: C, 63.1; H, 5.51; N, 4.90. Found: C, 63.1; H, 5.53; N, 4.97.

Boc–Tra–Tyr(2-Cl–Z)–4-Acetylanilide A solution of mixed anhydride [prepared from Boc–Tra–OH (60 mg, 0.25 mmol) and ethyl chloroformate (0.02 ml, 0.25 mmol) as usual] in THF (10 ml) was added to an ice-cold solution of H–Tyr(2-Cl–Z)–4-acetylanilide·HCl [prepared from Boc–Tyr(2-Cl–Z)–4-acetylanilide (0.1 g, 0.17 mmol) and 7.0 N HCl/dioxane (0.24 ml, 1.7 mmol) as usual] in DMF containing Et_3N (0.02 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 50 mg (42.0%), mp 135—139 °C, $[\alpha]_D^{25}$ +21.1° (c=1.0, DMF), Rf^1 0.51, Rf^2 0.62. *Anal.* Calcd for $C_{38}H_{44}ClN_3O_8 \cdot 3/4H_2O$: C, 63.5; H, 6.33; N, 5.84. Found: C, 63.7; H, 6.03; N, 5.59.

H–Tra–Tyr(2-Cl–Z)–4-Acetylanilide (11) A solution of Boc–Tra–Tyr(2-Cl–Z)–4-acetylanilide (42 mg, 0.06 mmol) in 7.0 N HCl/dioxane (0.1 ml, 0.70 mmol) containing anisole (0.03 ml, 0.3 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 36 mg (92.8%), mp 120—124 °C, $[\alpha]_D^{25}$ +34.3° (c=0.1, MeOH), Rf^4 0.24, Rf^5 0.63. *Anal.* Calcd for $C_{33}H_{36}ClN_3O_6 \cdot HCl \cdot 5/4H_2O$: C, 59.6; H, 5.94; N, 6.32. Found: C, 59.6; H, 5.76; N, 6.23.

Boc–Tyr(2-Br–Z)–4-Acetylanilide A solution of mixed anhydride [prepared from Boc–Tyr(2-Br–Z)–OH (1.8 g, 4.6 mmol) and ethyl chloroformate (0.44 ml, 4.6 mmol) as usual] in THF (50 ml) was added to an ice-cold solution of 4-aminoacetophenone (0.62 g, 4.6 mmol) in DMF (20 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, AcOEt and water were added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 0.4 g (15.5%), mp 182—184 °C, $[\alpha]_D^{25}$ +47.8° (c=1.0, DMF). Rf^2 0.71. *Anal.* Calcd for $C_{30}H_{31}BrN_2O_7 \cdot 1/4H_2O$: C, 58.5; H, 5.11; N, 4.54. Found: C, 58.5; H, 5.27; N, 4.47.

Boc–Tra–Tyr(2-Br–Z)–4-Acetylanilide A solution of mixed anhydride [prepared from Boc–Tra–OH (0.19 g, 0.75 mmol) and ethyl chloroformate (0.07 ml, 0.75 mmol) as usual] in THF (15 ml) was added to an ice-cold solution of H–Tyr(2-Br–Z)–4-acetylanilide·HCl [prepared from Boc–Tyr(2-Br–Z)–4-acetylanilide (0.4 g, 0.50 mmol) and 7.0 N HCl/dioxane (0.70 ml, 5.0 mmol) as usual] in DMF (5 ml) containing Et_3N (0.07 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 77 mg (39.2%), mp 213—216 °C, $[\alpha]_D^{25}$ +32.9° (c=1.5, DMF), Rf^1 0.76, Rf^2 0.57. *Anal.* Calcd for $C_{38}H_{44}BrN_3O_8 \cdot 1/2H_2O$: C, 60.1; H, 5.93; N, 5.53. Found: C, 60.2; H, 5.79; N, 5.50.

H–Tra–Tyr(2-Br–Z)–4-Acetylanilide (12) A solution of Boc–Tra–Tyr(2-Br–Z)–4-acetylanilide (20 mg, 0.29 mmol) in 7.3 N HCl/dioxane (0.4 ml, 2.9 mmol) containing anisole (0.12 ml, 1.1 mmol) was kept at room temperature for 70 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 37 mg (74.4%), mp 117—120 °C, $[\alpha]_D^{25}$ +32.2° (c=1.3, MeOH), Rf^4 0.32, Rf^5 0.65. *Anal.* Calcd for $C_{33}H_{36}BrN_3O_6 \cdot HCl \cdot 5/2H_2O$: C, 55.6; H, 5.56; N, 5.56. Found: C, 55.5; H, 5.30; N, 5.71.

Boc–Phe–4-Acetylphenoxymethyl Ketone A solution of Boc–Phe–CH_2Cl (1.0 g, 3.4 mmol), 4-hydroxyacetophenone (0.46 g, 3.4 mmol), NaI (0.51 g, 3.4 mmol) and $NaHCO_3$ (0.28 g, 3.4 mmol) in DMF (30 ml) was stirred at 45 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. EtOH was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 0.40 g (29.4%), mp 125—132 °C, $[\alpha]_D^{25}$ −12.5° (c=0.8, DMF), Rf^8 0.72, Rf^9 0.64. *Anal.* Calcd for $C_{23}H_{27}NO_5$: C, 69.6; H, 6.80; N, 3.52. Found: C, 69.7; H, 6.92; N, 3.65.

Boc–Tra–Phe–4-Acetylphenoxymethyl Ketone A solution of mixed anhydride [prepared from Boc–Tra–OH (0.28 g, 1.14 mmol) and ethyl chloroformate (0.11 ml, 1.14 mmol) as usual] in THF (20 ml) was added to an ice-cold solution of H–Phe–acetylphenoxymethyl ketone·HCl [prepared from Boc–Phe–4-acetylphenoxymethyl ketone (0.3 g, 0.76 mmol) and 7.0 N HCl/dioxane (1.1 ml, 7.7 mmol) as usual] in DMF (20 ml) containing Et_3N (0.11 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from AcOEt, yield 87.5 mg (21.5%), mp 139—142 °C, $[\alpha]_D^{25}$ −11.5° (c=0.5, MeOH), Rf^1 0.78, Rf^9 0.61. *Anal.* Calcd for $C_{31}H_{40}N_2O_6 \cdot 1/4H_2O$: C, 68.9; H, 7.49; N, 5.18. Found: C, 68.9; H, 7.53; N, 5.16.

H–Tra–Phe–4-Acetylphenoxymethyl Ketone (13) A solution of the corresponding Boc-derivative (69.4 mg, 0.13 mmol) in 7.0 N HCl/dioxane (0.19 ml, 1.32 mmol) was stirred at room temperature for 60 min. Ether was added to the solution to afford a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 53.0 mg (84.9%), mp 149—152 °C, $[\alpha]_D^{25}$ −8.2° (c=1.0, MeOH), Rf^4 0.21, Rf^5 0.68. *Anal.* Calcd for $C_{26}H_{32}N_2O_4 \cdot HCl \cdot H_2O$: C, 63.6; H, 7.13; N, 5.71. Found: C, 63.5; H, 6.92; N, 5.93.

Boc–Tyr(Bzl)–4-Acetylphenoxymethyl Ketone Boc–Tyr(Bzl)–CH_2Cl (1.2 g, 3 mmol), 4-hydroxyacetophenone (0.4 g, 3 mmol), NaI (0.4 g, 3 mmol) and $NaHCO_3$ (0.3 g, 3 mmol) were dissolved in DMF (20 ml). The reaction mixture was stirred at 45 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. EtOH was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 0.38 g (25.3%), mp 118—119 °C, $[\alpha]_D^{25}$ −11.2° (c=0.8, DMF), Rf^6 0.36. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 2.56 (3H, s), 3.08 (2H, m), 4.53, 4.75 (2H, each d, J=92, 17.3 Hz), 4.69—4.74 (1H, m), 5.02 (1H, s), 5.04—5.08 (1H, m), 6.84 (2H, d, J=8.9 Hz), 7.10 (2H, d, J=8.9 Hz), 6.92 (2H, d, J=8.6 Hz), 7.38 (2H, d, J=8.6 Hz), 7.32—7.42 (5H, m). *Anal.* Calcd for $C_{30}H_{33}NO_6 \cdot 1/2H_2O$: C, 70.3; H, 6.64; N, 2.73. Found: C, 70.7; H, 6.60; N, 2.77.

Boc–Tra–Tyr(Bzl)–4-Acetylphenoxymethyl Ketone A solution of mixed anhydride [prepared from Boc–Tra–OH (0.19 g, 0.74 mmol) and isobutyl chloroformate (0.1 ml, 0.74 mmol) as usual] in THF (8 ml) was added to an ice-cold solution of H–Tyr(Bzl)–4-acetylphenoxymethyl ketone·HCl [prepared from Boc–Tyr(Bzl)–4-acetylphenoxymethyl ketone (0.23 g, 0.46 mmol) and 7.3 N HCl/dioxane (0.63 ml, 4.6 mmol) as usual] in DMF (4 ml) containing Et_3N (0.06 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH and ether, yield 0.13 g (44.5%), mp 138—139 °C, $[\alpha]_D^{25}$ −28.8° (c=1.1, DMF), Rf^2 0.79. *Anal.* Calcd for $C_{38}H_{46}N_2O_7 \cdot 1/2H_2O$: C, 70.1; H, 7.22; N, 4.29. Found: C, 70.1; H, 7.15; N, 4.31.

H–Tra–Tyr(Bzl)–4-Acetylphenoxymethyl Ketone (14) A solution of Boc–Tra–Tyr(Bzl)–4-acetylphenoxymethyl ketone (87 mg, 0.14 mmol) in 7.3 N HCl/dioxane (0.2 ml, 1.4 mmol) was kept at room temperature for 70 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 72 mg (91.1%), mp 158—159 °C, $[\alpha]_D^{25}$ +3.1° (c=0.1, MeOH), Rf^4 0.24, Rf^5 0.69. ^{1}H-NMR (DMSO-d_6) δ: 0.96—1.06 (2H, m), 1.38—1.48 (3H, m), 1.85—1.86 (2H, m), 1.94—1.97 (2H, m), 2.58—2.65 (1H, m), 2.98—3.01 (2H, m), 4.55—4.61 (1H, m), 4.91, 5.09 (2H, each d, J=72, 8.9 Hz), 7.10 (2H, d, J=8.9 Hz), 6.84 (2H, d, J=8.9 Hz), 7.38 (2H, d, J=8.6 Hz), 6.92 (2H, d, J=8.6 Hz), 7.32—7.42 (5H, m). *Anal.* Calcd for $C_{33}H_{38}N_2O_5 \cdot HCl \cdot 3/2H_2O$: C, 65.4; H, 6.93; N, 4.62. Found: C, 65.2; H, 7.24; N, 4.61.

Boc–Tyr(2-Br–Z)–CH_2Cl Diazomethane [prepared from nitrosomethylurea (3.2 g, 31 mmol)] was added to a mixed anhydride [prepared from Boc–Tyr(2-Br–Z)–OH (3.0 g, 7.7 mmol) and ethyl chloroformate

(0.8 ml, 7.7 mmol) as usual] in THF (80 ml) at −15°C and the reaction mixture was stirred at 4 °C for 4 h. After addition of 7.3 N HCl/dioxane (4.2 ml, 31 mmol) at −15 °C, the reaction mixture was stirred at the same temperature for 3 h and neutralized with Et_3N. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated to a small volume. Ether was added to the residue to give crystals, which were collected by filtration and reprecipitated from AcOEt and ether, yield 0.5 g (12.1%), mp 102—103 °C, $[\alpha]_D^{25}$ +1.7° (c=0.8, $CHCl_3$), Rf^6 0.47. *Anal.* Calcd for $C_{23}H_{25}BrClNO_6$: C, 52.5; H, 4.75; N, 2.69. Found: C, 52.3; H, 4.74; N, 2.65.

Boc-Tyr(2-Br-Z)-4-Acetylphenoxymethyl Ketone Boc-Tyr(2-Br-Z)-CH_2Cl (0.45 g, 0.85 mmol), 4-hydroxyacetophenone (0.12 g, 0.85 mmol), NaI (0.13 g, 0.85 mmol) and $NaHCO_3$ (0.07 g, 0.85 mmol) were dissolved in DMF (10 ml). The reaction mixture was stirred at 45 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. EtOH was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 70 mg (12.4%), mp 116—118 °C, $[\alpha]_D^{25}$ −9.3° (c=0.2, $CHCl_3$), Rf^6 0.18, Rf^7 0.33. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 2.55 (3H, s), 2.96—3.17 (2H, m), 4.53, 4.76 (2H, each d, J=92, 17.3 Hz), 5.06—5.08 (1H, m), 5.37 (2H, s), 7.15 (2H, d, J=8.6 Hz), 7.26 (2H, d, J=8.6 Hz), 7.21—7.27 (1H, m), 7.33—7.38 (1H, m), 7.40—7.52 (1H, m), 7.60—7.63 (1H, m), 7.68 (2H, d, J=8.8 Hz), 7.95 (2H, d, J=8.8 Hz). *Anal.* Calcd for $C_{31}H_{32}BrNO_8$: C, 59.5; H, 5.11; N, 2.24. Found: C, 59.4; H, 5.15; N, 2.16.

Boc-Tra-Tyr(2-Br-Z)-4-Acetylphenoxymethyl Ketone A solution of mixed anhydride [prepared from Boc-Tra-OH (0.13 g, 0.51 mmol) and ethyl chloroformate (0.05 ml, 0.51 mmol) as usual] in THF (20 ml) was added to an ice-cold solution of H-Tyr(2-Br-Z)-4-acetylphenoxymethyl ketone·HCl [prepared from Boc-Tyr(2-Br-Z)-4-acetylphenoxymethyl ketone (0.21 g, 0.34 mmol) and 7.3 N HCl/dioxane (0.47 ml, 3.4 mmol) as usual] in DMF (7 ml) containing Et_3N (0.05 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from AcOEt, yield 0.08 g (30.7%), mp 154—156 °C, $[\alpha]_D^{25}$ −42.5° (c=0.5, DMF), Rf^1 0.77, Rf^2 0.69. *Anal.* Calcd for $C_{39}H_{45}BrN_2O_9$: C, 61.2; H, 5.88; N, 3.66. Found: C, 61.2; H, 6.04; N, 3.65.

H-Tra-Tyr(2-Br-Z)-4-Acetylphenoxymethyl Ketone (15) A solution of Boc-Tyr(2-Br-Z)-4-acetylphenoxymethyl ketone (55 mg, 71.9 μmol) in TFA (0.11 ml, 1.4 mmol) containing anisole (0.04 ml, 0.36 mmol) was kept at room temperature for 90 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 50 mg (89.2%), mp 101—104 °C, $[\alpha]_D^{25}$ −4.0° (c=0.1, MeOH), Rf^4 0.26, Rf^5 0.63. *Anal.* Calcd for $C_{34}H_{37}BrN_2O_7 \cdot CF_3COOH \cdot 1/2H_2O$: C, 54.9; H, 4.82; N, 3.55. Found: C, 55.0; H, 5.09; N, 3.41.

Boc-Tra-Phe-Isonicotinylaminomethyl Ketone Boc-Tra-Phe-CH_2Cl (1.2 g, 2.8 mmol), 4-aminomethylpyridine (0.30 g, 2.8 mmol), NaI (0.42 g, 2.8 mmol) and $NaHCO_3$ (0.24 g, 2.8 mmol) were dissolved in DMF (15 ml). The reaction mixture was stirred at 45 °C for 24 h. After removal of the solvent, AcOEt and 10% citric acid were added to the residue. The pH of the water layer was adjusted to 9 with Na_2CO_3 and extracted with AcOEt. The extract was dried over Na_2SO_4 and evaporated down. The oily product in $CHCl_3$ (2 ml) was applied to a column of silica gel (2.0 × 16 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (20 ml each) were collected. The solvent of the effluent (tube Nos. 9—13) was removed by evaporation. Ether was added to the residue to afford crystals, which were collected by filtration, yield 80 mg (5.6%), mp 91—95 °C, $[\alpha]_D^{25}$ −2.7° (c=0.3, $CHCl_3$), Rf^1 0.38, Rf^2 0.63, Rf^3 0.86. *Anal.* Calcd for $C_{29}H_{40}N_4O_4 \cdot 1/2H_2O$: C, 67.3; H, 7.92; N, 10.8. Found: C, 67.7; H, 7.82; N, 10.8.

H-Tra-Phe-Isonicotinylaminomethyl Ketone (16) A solution of Boc-Tra-Phe-isonicotinylaminomethyl ketone (94 mg, 0.18 mmol) in 7.6 N HCl/dioxane (0.24 ml, 1.8 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 91 mg (97.2%), $[\alpha]_D^{25}$ −33.0° (c=1.2, MeOH), Rf^4 0.10, Rf^5 0.22, Rf^{11} 0.23. *Anal.* Calcd for $C_{24}H_{32}N_4O_2 \cdot 3HCl \cdot H_2O$: C, 53.8; H, 6.90; N, 10.5. Found: C, 54.0; H, 7.23; N, 10.5.

Boc-Tra-Phe-Nicotinylaminomethyl Ketone Boc-Tra-Phe-CH_2Cl (1.0 g, 2.3 mmol), 3-aminomethylpyridine (0.25 g, 2.3 mmol), NaI (0.344 g, 2.3 mmol) and $NaHCO_3$ (0.19 g, 2.3 mmol) were dissolved in DMF (15 ml). The reaction mixture was stirred at 45 °C for 24 h. After removal of the solvent, AcOEt and 10% citric acid were added. The pH of the water layer was adjusted to 9 with Na_2CO_3 and extracted with AcOEt, then the extract was dried over Na_2SO_4 and evaporated down. The oily product in $CHCl_3$ (2 ml) was applied to a column of silica gel (2.0 × 16 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (20 ml each) were collected. The solvent of the effluent (tube Nos. 9—13) was removed by evaporation. Ether was added to the residue to afford crystals, which were collected by filtration, yield 50 mg (4.3%), mp 86—89 °C, $[\alpha]_D^{25}$ −11.1° (c=0.3, $CHCl_3$), Rf^1 0.40, Rf^2 0.58. *Anal.* Calcd for $C_{29}H_{40}N_4O_4 \cdot 1/4H_2O$: C, 67.3; H, 7.92; N, 10.8. Found: C, 67.4; H, 7.74; N, 10.8.

H-Tra-Phe-Nicotinylaminomethyl Ketone (17) A solution of Boc-Tra-Phe-nicotinylaminomethyl ketone (80 mg, 0.16 mmol) in 7.6 N HCl/dioxane (0.23 ml, 1.7 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 51 mg (61.6%), $[\alpha]_D^{25}$ −6.8° (c=1.0, MeOH), Rf^5 0.44, Rf^{11} 0.27. *Anal.* Calcd for $C_{24}H_{32}N_4O_2 \cdot 3HCl \cdot 2H_2O$: C, 52.1; H, 7.04; N, 9.11. Found: C, 51.8; H, 6.82; N, 9.43.

Boc-Tra-Phe-4-Ethoxycarbonylbenzylaminomethyl Ketone Boc-Tra-Phe-CH_2Cl (0.2 g, 0.46 mmol), 4-ethoxycarbonylbenzylamine (0.25 g, 1.4 mmol), NaI (0.07 g, 0.46 mmol) and $NaHCO_3$ (0.04 g, 0.46 mmol) were dissolved in EtOH-H_2O (200—15 ml). The reaction mixture was stirred at 25 °C for 1 week. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. The oily product in $CHCl_3$ (2 ml) was applied to a column of silica gel (1.4 × 12 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (5 ml each) were collected and the solvent of the effluent (tube Nos. 5—9) was removed by evaporation. Petroleum ether was added to the residue to afford an amorphous powder, yield 0.06 g (22.5%), $[\alpha]_D^{25}$ −5.5° (c=1.1, $CHCl_3$), Rf^1 0.82, Rf^2 0.86. ^{1}H-NMR ($CDCl_3$) δ: 0.92—1.02 (2H, m), 1.40 (3H, t), 1.44 (9H, s), 1.47—1.54 (2H, m), 1.63—2.12 (5H, m), 2.46 (3H, m), 2.98 (2H, m), 3.16, 3.61 (2H, each d, J=180, 20 Hz), 3.25, 3.75 (each d, J=200, 16 Hz), 4.39 (2H, q), 4.58 (1H, m), 6.74 (1H, br), 6.96—6.98 (2H, m), 7.21—7.24 (3H, m), 8.00 (2H, d, J=8.3 Hz), 7.38 (2H, d, J=8.3 Hz). *Anal.* Calcd for $C_{33}H_{45}N_3O_6 \cdot 1/4H_2O$: C, 67.7; H, 7.75; N, 7.28. Found: C, 67.9; H, 7.79; N, 7.19.

H-Tra-Phe-4-Ethoxycarbonylbenzylaminomethyl Ketone (18) A solution of Boc-Tra-Phe-4-ethoxycarbonylbenzylaminomethyl ketone (35 mg, 0.06 mmol) in 7.0 N HCl/dioxane (0.1 ml, 0.7 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 30 mg (90.6%), $[\alpha]_D^{25}$ −5.2° (c=0.5, MeOH), Rf^4 0.45, Rf^5 0.64, Rf^{11} 0.49. *Anal.* Calcd for $C_{28}H_{37}N_3O_4 \cdot 2HCl \cdot 7/2H_2O$: C, 54.7; H, 7.48; N, 6.82. Found: C, 54.4; H, 7.22; N, 7.02.

***N,N*-Bis(Boc-Tra-Phe-CH_2)-4-Ethoxycarbonylbenzylamine** Boc-Tra-Phe-CH_2Cl (1.4 g, 3.3 mmol), 4-ethoxycarbonylbenzylamine (0.58 g, 3.3 mmol), NaI (0.49 g, 3.3 mmol) and $NaHCO_3$ (0.27 g, 3.3 mmol) were dissolved in DMF (50 ml). The reaction mixture was stirred at 45 °C for 48 h. After removal of the solvent, AcOEt and water were added to the residue to afford crystals, which were collected by filtration and reprecipitated from DMF-MeOH, yield 0.45 g (23.9%), mp 192—195 °C, $[\alpha]_D^{25}$ −0.3° (c=1.2, $CHCl_3$), Rf^1 0.66, Rf^2 0.70. ^{1}H-NMR ($CDCl_3$) δ: 0.85—0.95 (4H, m), 1.39 (3H, t), 1.43 (18H, s), 1.25—1.37 (6H, m), 1.71—1.98 (10H, m), 2.76—3.03 (8H, m), 3.27, 3.65 (4H, each d, J=152, 18 Hz), 3.58, 3.88 (2H, each d, J=120, 13.8 Hz), 4.36 (2H, q), 4.68 (2H, br), 4.80—4.85 (2H, m), 6.08—6.10 (2H, m), 7.06—7.08 (4H, m), 7.18—7.26 (6H, m), 7.43 (2H, d, J=8.4 Hz), 7.97 (2H, d, J=8.4 Hz). *Anal.* Calcd for $C_{56}H_{77}N_5O_{10} \cdot 1/4H_2O$: C, 68.4; H, 7.88; N, 7.11. Found: C, 68.5; H, 7.91; N, 6.98.

***N,N*-Bis(H-Tra-Phe-CH_2)-4-Ethoxycarbonylbenzylamine (18′)** A solution of the corresponding N^{α}-Boc derivative (0.34 g, 0.6 mmol) in 7.6 N HCl/dioxane (0.82 ml, 6.2 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration. The crude product in EtOH (4 ml) was applied to a column of Sephadex LH-20 (3.4 × 150 cm), equilibrated and eluted with EtOH. Individual fractions (5 g each) were collected and the solvent of the effluent (tube Nos. 23—32) was removed by evaporation, yield 38 mg (7.0%), $[\alpha]_D^{25}$ −24.2° (c=0.7, MeOH), Rf^{11} 0.45. *Anal.* Calcd for $C_{48}H_{59}N_5O_6 \cdot 2HCl$: C, 65.9; H, 6.98; N, 8.01. Found: C, 65.8; H, 7.22; N, 8.30.

Boc-Tra-Phe-4-Ethoxycarbonylanilinomethyl Ketone Boc-Tra-Phe-CH_2Cl (1.5 g, 3.4 mmol), 4-ethoxycarbonylaniline (0.56 g, 3.4 mmol), NaI

(0.52 g, 3.4 mmol) and $NaHCO_3$ (0.29 g, 3.4 mmol) were dissolved in DMF (15 ml). The reaction mixture was stirred at 45 °C for 48 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated down. The oily product in $CHCl_3$ (5 ml) was applied to a column of silica gel (1.4 × 12 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (20 ml each) were collected and the solvent of the effluent (tube Nos. 13—26) was removed by evaporation. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 0.12 g (6.2%), mp 174—180 °C, $[\alpha]_D^{25}$ −5.4° (c=0.1, $CHCl_3$), Rf^1 0.58, Rf^2 0.88. ^{1}H-NMR ($CDCl_3$) δ: 0.92—0.98 (2H, m), 1.36 (3H, t, J=7.0 Hz), 1.44 (9H, s), 1.37—1.41 (3H, m), 1.60—2.07 (5H, m), 2.95—3.12 (4H, m), 3.76, 4.08 (2H, each d, J=128, 19.8 Hz), 4.31 (2H, q), 4.60 (1H, br), 4.85—4.90 (1H, m), 6.00—6.02 (1H, br), 7.13—7.15 (2H, m), 7.25—7.33 (3H, m), 6.44 (2H, d, J=8.8 Hz), 7.75 (2H, d, J=8.8 Hz). *Anal.* Calcd for $C_{32}H_{43}N_3O_6$: C, 68.0; H, 7.16; N, 7.43. Found: C, 67.8; H, 7.17; N, 7.43.

H–Tra–Phe–4-Ethoxycarbonylanilinomethyl Ketone (19) A solution of Boc–Tra–Phe–4-ethoxycarbonylanilinomethyl ketone (42 mg, 0.07 mmol) in 7.0 N HCl/dioxane (0.1 ml, 0.7 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 26 mg (69.0%), $[\alpha]_D^{25}$ −16.1° (c=0.4, MeOH), Rf^4 0.39; Rf^5 0.53. *Anal.* Calcd for $C_{27}H_{35}N_3O_4 \cdot 2HCl$: C, 60.6; H, 7.14; N, 7.85. Found: C, 60.3; H, 6.88; N, 7.80.

Boc–Tyr(Z)–CH_2Cl Diazomethane [prepared from nitrosomethylurea (10.3 g, 0.1 mol)] was added to a mixed anhydride [prepared from Boc–Tyr(Z)–OH (10.0 g, 24 mmol) and ethyl chloroformate (2.3 ml, 24 mmol) as usual] in THF (150 ml) at −15 °C and the reaction mixture was stirred at 4 °C for 5 h. After addition of 3.2 N HCl/dioxane (31 ml, 0.1 mol) at −15 °C, the reaction mixture was stirred at the same temperature for 3 h and neutralized with Et_3N. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated to a small volume. The crude product in $CHCl_3$ (6 ml) was applied to a column of silica gel (3.4 × 35 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (200 ml each) were collected and the solvent of the effluent (tube Nos. 3—7) was removed by evaporation. Ether was added to the residue to afford crystals, which were collected by filtration, yield 4.8 g (44.7%), mp 78—81 °C, $[\alpha]_D^{25}$ +1.5° (c=0.3, $CHCl_3$), Rf^7 0.63, Rf^8 0.80. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 2.94—3.15 (2H, m), 4.08—4.21 (2H, dd, J=68, 21 Hz), 4.61—4.67 (1H, m), 5.26 (2H, s), 7.12—7.20, 7.32—7.45 (9H, m). *Anal.* Calcd for $C_{23}H_{26}ClNO_6$: C, 61.7; H, 5.81; N, 3.13. Found: C, 61.7; H, 5.84; N, 3.24.

Boc–Tra–Tyr(Z)–CH_2Cl A solution of mixed anhydride [prepared from Boc–Tra–OH (6.3 g, 24.6 mmol) and ethyl chloroformate (2.4 ml, 24.6 mmol) as usual] in THF (200 ml) was added to an ice-cold solution of H–Tyr(Z)–CH_2Cl·HCl [prepared from Boc–Tyr(Z)–CH_2Cl (7.3 g, 16.4 mmol) and 7.6 N HCl/dioxane (23 ml, 174 mmol) as usual] in DMF (200 ml) containing Et_3N (2.3 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, AcOEt and water were added to the residue to give crystals, which were collected by filtration and recrystallized from AcOEt, yield 5.2 g (54.0%), mp 177—180 °C, $[\alpha]_D^{25}$ +10.8° (c=1.1, DMF), Rf^1 0.68, Rf^2 0.66, Rf^3 0.78. *Anal.* Calcd for $C_{31}H_{39}ClN_2O_7$: C, 63.5; H, 6.64; N, 4.77. Found: C, 63.3; H, 6.94; N, 5.04.

Boc–Tra–Tyr(Z)–4-Ethoxycarbonylbenzylaminomethyl Ketone Boc–Tra–Tyr(Z)–CH_2Cl (2.7 g, 4.7 mmol), 4-ethoxycarbonylbenzylamine (0.84 g, 4.7 mmol), NaI (0.7 g, 4.7 mmol) and $NaHCO_3$ (0.39 g, 4.7 mmol) were dissolved in DMF (150 ml). The reaction mixture was stirred at 45 °C for 48 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. The oily product in $CHCl_3$ (5 ml) was applied to a column of silica gel (1.4 × 16 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (30 ml each) were collected and the solvent of the effluent (tube Nos. 2—8) was removed by evaporation. Ether was added to the residue to afford *N,N*-bis(Boc–Tra–Tyr(Z)–CH_2)–4-ethoxycarbonylbenzylamine, yield 0.53 g (8.8%), mp 191—194 °C, $[\alpha]_D^{25}$ −4.3° (c=1.1, $CHCl_3$), Rf^1 0.63, Rf^2 0.89. ^{1}H-NMR ($CDCl_3$) δ: 0.84—0.93 (4H, m), 1.37 (3H, t, J=7.2 Hz), 1.43 (18H, s), 1.21—1.34 (6H, m), 1.70—2.04 (10H, m), 2.73—3.03 (8H, m), 3.28, 3.63 (4H, each d, J=140, 18 Hz), 3.59, 3.87 (2H, each d, J=112, 13.5 Hz), 4.34 (2H, q), 4.69 (2H, br), 4.79—4.85 (2H, m), 5.24 (4H, s), 6.12—6.14 (2H, m), 7.03—7.11, 7.36—7.45 (20H, m), 7.96 (2H, d, J=8.3 Hz). *Anal.* Calcd for $C_{72}H_{89}N_5O_{16} \cdot 5/2H_2O$: C, 65.3; H, 7.09; N, 5.28. Found: C, 64.9; H, 6.71; N, 5.29. After removal of the solvent of the effluent (tube Nos. 9—20), the residue in EtOH (3 ml) was applied to a column of Sephadex LH-20 (3.4 × 150 cm), equilibrated and eluted with EtOH. Individual fractions (10 g each) were collected and the solvent of the effluent (tube Nos. 43—59) was removed by evaporation. Ether was added to the residue to give the title compound, yield 0.21 g (6.1%), mp 124—127 °C, $[\alpha]_D^{25}$ +6.5° (c=1.1, $CHCl_3$), Rf^1 0.34, Rf^2 0.82. ^{1}H-NMR ($CDCl_3$) δ: 0.87—0.97 (2H, m), 1.39 (3H, t), 1.44 (9H, s), 1.32—1.41 (3H, m), 1.78—2.02 (5H, m), 2.92—3.09 (4H, m), 3.42, 3.53 (2H, each d, J=44, 19 Hz), 3.74, 3.45 (2H, dd, J=13.8, 2.4 Hz), 4.36 (2H, q), 4.57 (1H, br), 4.78—4.83 (1H, m), 5.26 (2H, s), 5.97—5.99 (1H, m), 7.10 (3H, s), 7.33—7.45 (8H, m), 7.99 (2H, d, J=8.3 Hz). *Anal.* Calcd for $C_{41}H_{51}N_3O_9$: C, 67.5; H, 6.99; N, 5.76. Found: C, 67.2; H, 6.99; N, 5.49.

H–Tra–Tyr(Z)–4-Ethoxycarbonylbenzylaminomethyl Ketone (20) A solution of the corresponding N^{α}-Boc derivative (65 mg, 0.09 mmol) in 7.6 N HCl/dioxane (0.12 ml, 0.9 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 55 mg (87.7%), $[\alpha]_D^{25}$ −28.5° (c=1.1, MeOH), Rf^4 0.30, Rf^5 0.56, Rf^{11} 0.32. *Anal.* Calcd for $C_{36}H_{43}N_3O_7 \cdot 2HCl \cdot H_2O$: C, 60.0; H, 6.53; N, 5.83. Found: C, 60.3; H, 6.64; N, 5.89.

***N,N*-Bis[H–Tra–Tyr(Z)–CH_2]–4-Ethoxycarbonylbenzylamine (20′)** A solution of the corresponding N^{α}-Boc derivative (0.14 g, 0.1 mmol) in TFA (0.07 ml, 1.0 mmol) containing anisole (0.05 ml, 0.5 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and converted to the corresponding acetate form by Amberlite IRA 45 (acetate form). This crude product in 3% AcOH (3 ml) was applied to a column of Sephadex G-25 (2.8 × 132 cm), equilibrated and eluted with 3% AcOH. Individual fractions (3 g each) were collected and the solvent of the effluent (tube Nos. 52—60) was removed by lyophilization, yield 90 mg (75.2%), $[\alpha]_D^{25}$ −9.58° (c=0.2, MeOH), Rf^4 0.25, Rf^5 0.52. *Anal.* Calcd for $C_{62}H_{71}N_5O_{12} \cdot 2CH_3COOH \cdot H_2O$: C, 65.2; H, 6.67; N, 5.76. Found: C, 65.0; H, 6.60; N, 5.54.

Boc–Tra–Tyr(2-Br–Z)–4-Pyridyloxymethyl Ketone A solution of mixed anhydride [prepared from Boc–Tra–OH (0.11 g, 0.42 mmol) and ethyl chloroformate (0.04 ml, 0.42 mmol) as usual] in THF (10 ml) was added to an ice-cold solution of H–Tyr(2-Br–Z)–4-pyridyloxymethyl ketone·HCl [prepared from the corresponding Boc derivative (0.19 g, 0.34 mmol) and 7.6 N HCl/dioxane (0.4 ml, 3.04 mmol) as usual] in DMF (5 ml) containing Et_3N (0.05 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from AcOEt, yield 50 mg (24.7%), $[\alpha]_D^{25}$ −24.8° (c=0.1, MeOH), Rf^1 0.76, Rf^2 0.89.

H–Tra–Tyr(2-Br–Z)–4-Pyridyloxymethyl Ketone (21) A solution of the corresponding Boc derivative (20 mg, 27 μmol) in 7.0 N HCl/dioxane (0.1 ml, 0.7 mmol) was stored at room temperature for 60 min. Ether was added to the solution to afford a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 10 mg (52.4%), amorphous powder, $[\alpha]_D^{25}$ −10.5° (c=0.1, MeOH), Rf^4 0.51, Rf^5 0.75.

Boc–Tra–Tyr(2-Br–Z)–2-Pyridyloxymethyl Ketone A mixed anhydride [prepared from Boc–Tra–OH (0.11 g, 0.42 mmol) and ethyl chloroformate (0.04 ml, 0.42 mmol) as usual] in THF (10 ml) was added to an ice-cold solution of H–Tyr(2-Br–Z)–2-pyridyloxymethyl ketone·HCl [prepared from Boc–Tyr(2-Br–Z)–2-pyridyloxymethyl ketone (0.19 g, 0.34 mmol) and 7.6 N HCl/dioxane (0.4 ml, 3.04 mmol) as usual] in DMF (5 ml) containing Et_3N (0.05 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford an amorphous powder, yield 45 mg (22.2%), $[\alpha]_D^{25}$ −21.3° (c=0.1, MeOH), Rf^1 0.68, Rf^2 0.85.

H–Tra–Tyr(2-Br–Z)–2-Pyridyloxymethyl Ketone (22) A solution of the corresponding Boc derivative (20 mg, 27 μmol) in 7.0 N HCl/dioxane (0.1 ml, 0.7 mmol) was stored at room temperature for 60 min. Ether was added to the solution to afford a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 12 mg (62.9%), amorphous powder, $[\alpha]_D^{25}$ −12.7° (c=0.1, MeOH), Rf^4 0.46, Rf^5 0.72.

Boc–Tra–CH_2–Tyr(Z)–4-Acetylanilide Boc–Tra–CH_2Cl (1.0 g, 3.5 mmol), H–Tyr(Z)–4-acetylanilide [prepared from Boc–Tyr(Z)–4-acetylanilide (2.0 g, 3.5 mmol), 7.0 N HCl/dioxane (5.0 ml, 35 mmol) and

$NaHCO_3$ (0.29 g, 3.5 mmol)], NaI (0.5 g, 3.5 mmol) and $NaHCO_3$ (0.29 g, 3.5 mmol) were dissolved in DMF (20 ml). The reaction mixture was stirred at 45 °C for 48 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. The oily product in $CHCl_3$ (1.5 ml) was applied to a column of silica gel (1.8 × 17 cm), equilibrated and eluted with $CHCl_3$. Individual fractions (30 ml each) were collected and the solvent of the effluent (tube Nos. 2—4) was removed by evaporation. The residue in EtOH (2 ml) was further applied to a column of Sephadex LH-20 (3.4 × 148 cm). Individual fractions (5 g each) were collected and the solvent of the effluent (tube Nos. 55—59) was removed by evaporation. Ether was added to the residue to afford crystals, which were collected by filtration. Ether was added to the residue to afford crystals, which were collected by filtration, yield 0.15 g (9.5%), mp 85—87 °C, $[\alpha]_D^{25}$ −103.8° (c=0.5, $CHCl_3$), Rf^1 0.64, Rf^7 0.85, Rf^{10} 0.32. ^{1}H-NMR ($CDCl_3$) δ: 0.86—0.96 (2H, m), 1.43 (9H, s), 1.26—1.36 (3H, m), 1.80—1.83 (4H, m), 2.15—2.23 (1H, m), 2.58 (3H, s), 2.88—2.97 (3H, m), 3.26—3.31 (1H, m), 3.35—3.38 (1H, br), 3.39, 3.59 (2H, each d, J=84, 20 Hz), 4.56 (1H, br), 5.26 (2H, s), 7.25 (2H, d, J=8.6 Hz), 7.13 (2H, d, J=8.6 Hz), 7.38—7.42 (5H, m), 7.95 (2H, d, J=8.6 Hz), 7.67 (2H, d, J=8.6 Hz). *Anal.* Calcd for $C_{39}H_{47}N_3O$: C, 68.4; H, 6.86; N, 6.13. Found: C, 68.1; H, 6.86; N, 6.10.

H-Tra-CH_2-Tyr(Z)-4-Acetylanilide (23) A solution of Boc-Tra-CH_2-Tyr(Z)-4-acetylanilide (0.13 g, 0.19 mmol) in 7.0 N HCl/dioxane (0.3 ml, 2.1 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 0.11 g (88.0%), $[\alpha]_D^{25}$ +62.9° (c=1.0, MeOH), Rf^4 0.10, Rf^{11} 0.45. *Anal.* Calcd for $C_{34}H_{39}N_3O_6 \cdot 2HCl \cdot 5/4H_2O$: C, 60.0; H, 6.39; N, 6.17. Found: C, 59.8; H, 6.15; N, 6.21.

Boc-Tra-CH_2-Tyr(2-Br-Z)-4-Acetylanilide The title compound was prepared from Boc-Tra-CH_2Cl (0.59 g, 2.0 mmol), H-Tyr(2-Br-Z)-4-acetylanilide [prepared from Boc-Tyr(2-Br-Z)-4-acetylanilide (1.0 g, 1.5 mmol), 7.0 N HCl/dioxane (2.3 ml, 16.1 mmol) and Na_2CO_3 (0.1 g, 1.5 mmol)], NaI (0.3 g, 2.0 mmol) and $NaHCO_3$ (0.17 g, 2.0 mmol). The crude product was purified by silica gel column chromatography and Sephadex LH-20 column chromatography, yield 0.18 g (11.8%), mp 126—128 °C, $[\alpha]_D^{25}$ −80.2° (c=1.2, $CHCl_3$), Rf^1 0.64, Rf^{10} 0.90. ^{1}H-NMR ($CDCl_3$) δ: 0.87—0.92 (2H, m), 1.43 (9H, s), 1.26—1.39 (3H, m), 1.80—1.83 (4H, m), 2.17—2.23 (1H, m), 2.60 (3H, s), 2.89—2.95 (3H, m), 3.26—3.31 (1H, m), 3.35—3.38 (1H, br), 3.39, 3.60 (2H, each d, J=84, 20 Hz), 4.55 (1H, br), 5.48 (2H, s), 7.26 (2H, d, J=8.6 Hz), 7.15 (2H, d, J=8.6 Hz), 7.21—7.27 (1H, m), 7.33—7.38 (1H, m), 7.40—7.52 (1H, m), 7.60—7.63 (1H, m), 7.95 (2H, d, J=8.8 Hz), 7.69 (2H, d, J=8.8 Hz). *Anal.* Calcd for $C_{39}H_{46}BrN_3O_8$: C, 61.3; H, 6.02; N, 5.50. Found: C, 61.4; H, 6.07; N, 5.21.

H-Tra-CH_2-Tyr(2-Br-Z)-4-Acetylanilide (24) A solution of Boc-Tra-CH_2-Tyr(2-Br-Z)-4-acetylanilide (81 mg, 0.11 mmol) in 7.6 N HCl/dioxane (0.2 ml, 1.5 mmol) was kept at room temperature for 60 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 66 mg (81.5%), mp 131—133 °C, $[\alpha]_D^{25}$ +51.1° (c=1.0, MeOH), Rf^4 0.10, Rf^{11} 0.65. *Anal.* Calcd for $C_{34}H_{38}BrN_3O_6 \cdot 2HCl \cdot 3/2H_2O$: C, 53.4; H, 5.63; N, 5.50. Found: C, 53.6; H, 5.46; N, 5.60.

Boc-Lys(Z)-Tyr(2-Br-Z)-4-Acetylanilide A solution of mixed anhydride [prepared from Boc-Lys(Z)-OH (0.32 g, 0.84 mmol) and ethyl chloroformate (0.08 ml, 0.84 mmol) as usual] in THF (15 ml) was added to an ice-cold solution of H-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-Tyr(2-Br-Z)-4-acetylanilide (0.35 g, 0.58 mmol), 7.0 N HCl/dioxane (1.0 ml, 7.0 mmol) and anisole (0.62 ml) as usual] in DMF (5 ml) containing Et_3N (0.1 ml). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from AcOEt, yield 0.29 g (56.9%), mp 162—163 °C, $[\alpha]_D^{25}$ +14.7° (c=1.1, DMF), Rf^2 0.69, Rf^{10} 0.67. *Anal.* Calcd for $C_{44}H_{49}BrN_4O_{10}$: C, 60.5; H, 5.61; N, 6.41. Found: C, 60.5; H, 5.58; N, 6.38.

Tos-Lys(Z)-Tyr(2-Br-Z)-4-Acetylanilide Tos-Cl (0.04 g, 0.21 mmol) and H-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from the corresponding N^{α}-Boc derivative (0.12 g, 0.14 mmol) and 7.0 N HCl/dioxane (0.23 ml, 1.6 mmol)] were dissolved in DMF (5 ml) containing Et_3N (0.03 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 0.05 g (38.5%), mp 142—145 °C, $[\alpha]_D^{25}$ +5.4° (c=0.1, DMF), Rf^1 0.73, Rf^{10} 0.69. *Anal.* Calcd for $C_{46}H_{47}BrN_4O_{10}S$: C, 59.6; H, 5.07; N, 6.04. Found: C, 59.7; H, 4.90; N, 6.22.

Tos-Lys-Tyr(2-Br-Z)-4-Acetylanilide (25) A solution of Tos-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide (33 mg, 0.04 mmol) in 25% HBr/AcOH (0.04 ml, 0.11 mmol) was kept at room temperature for 40 min. Ether was added to the solution to form a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*, yield 25 mg (78.5%), mp 122—123 °C, $[\alpha]_D^{25}$ −10.7° (c=0.1, MeOH), Rf^4 0.44, Rf^5 0.66. *Anal.* Calcd for $C_{38}H_{41}BrN_4O_8S \cdot HBr \cdot H_2O$: C, 51.2; H, 4.93; N, 6.27. Found: C, 51.2; H, 4.87; N, 6.39.

Tol-Lys(Z)-Tyr(2-Br-Z)-4-Acetylanilide The title compound was prepared from Tol-Cl (0.03 g, 0.21 mmol) and H-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from the N^{α}-Boc derivative (0.12 g, 0.14 mmol) and 7.0 N HCl/dioxane (0.2 ml, 1.4 mmol)], yield 0.08 g (68.9%), mp 173—177 °C, $[\alpha]_D^{25}$ +6.56° (c=0.1, DMF), Rf^1 0.63, Rf^{10} 0.63. *Anal.* Calcd for $C_{47}H_{47}BrN_4O_9 \cdot 3/2H_2O$: C, 61.5; H, 6.44; N, 6.65. Found: C, 61.2; H, 6.44; N, 6.81.

Tol-Lys-Tyr(2-Br-Z)-4-Acetylanilide (26) The title compound was prepared from Tol-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide (76 mg, 0.09 mmol) and 25% HBr/AcOH (0.08 ml, 0.26 mmol), yield 56 mg (78.0%), mp 115—118 °C, $[\alpha]_D^{25}$ +11.2° (c=1.1, MeOH), Rf^4 0.44, Rf^5 0.66. *Anal.* Calcd for $C_{39}H_{41}BrN_4O_7 \cdot HBr \cdot 3H_2O$: C, 52.5; H, 5.48; N, 6.27. Found: C, 52.7; H, 5.75; N, 6.11.

NaPh-SO_2-Lys(Z)-Tyr(2-Br-Z)-4-Acetylanilide The title compound was prepared from NaPh-SO_2Cl (0.06 g, 0.26 mmol) and H-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide (0.11 g, 0.13 mmol) and 7.0 N HCl/dioxane (0.2 ml, 1.4 mmol)], yield 0.04 g (33.5%), mp 126—127 °C, $[\alpha]_D^{25}$ +2.0° (c=0.1, DMF), Rf^1 0.65, Rf^{10} 0.78. *Anal.* Calcd for $C_{49}H_{47}BrN_4O_{10}S \cdot 3/4H_2O$: C, 60.4; H, 4.87; N, 5.46. Found: C, 60.2; H, 4.96; N, 5.73.

NaPh-SO_2-Lys-Tyr(2-Br-Z)-4-Acetylanilide (27) The title compound was prepared from NaPh-SO_2-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide (39 mg, 0.04 mmol) and 25% HBr/AcOH (0.05 ml, 0.12 mmol), yield 31 mg (86.2%), mp 122—124 °C, $[\alpha]_D^{25}$ −30.0° (c=0.1, MeOH), Rf^5 0.62. *Anal.* Calcd for $C_{41}H_{41}BrN_4O_8S \cdot HBr \cdot 3H_2O$: C, 51.1; H, 4.54; N, 6.00. Found: C, 51.1; H, 4.98; N, 5.81.

NaPh-CO-Lys(Z)-Tyr(2-Br-Z)-4-Acetylanilide The title compound was prepared from NaPhCOCl (58 mg, 0.26 mmol) and H-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from the corresponding Boc derivative (0.11 g, 0.13 mmol) and 7.0 N HCl/dioxane (0.2 ml, 1.4 mmol)], yield 58.7 mg (48.7%), mp 135—136 °C, $[\alpha]_D^{25}$ +16.0° (c=0.1, DMF), Rf^1 0.74, Rf^9 0.70. *Anal.* Calcd for $C_{50}H_{47}BrN_4O_9$: C, 64.8; H, 5.07; N, 6.04. Found: C, 64.6; H, 5.14; N, 5.90.

NaPh-CO-Lys-Tyr(2-Br-Z)-4-Acetylanilide (28) The title compound was prepared from NaPh-CO-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide (35 mg, 0.04 mmol) and 25% HBr/AcOH (0.05 ml, 0.12 mmol), yield 25 mg (86.0%), amorphous powder, $[\alpha]_D^{25}$ +10.2° (c=0.90, MeOH), Rf^5 0.62. *Anal.* Calcd for $C_{42}H_{41}BrN_4O_7 \cdot HBr \cdot 3/2H_2O$: C, 56.0; H, 4.99; N, 6.21. Found: C, 55.8; H, 4.89; N, 6.47.

Fmoc-Lys(Z)-Tyr(2-Br-Z)-4-Acetylanilide The title compound was prepared from Fmoc-Cl (0.05 g, 0.18 mmol) and H-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide (0.15 g, 0.18 mmol) and 7.0 N HCl/dioxane (0.3 ml, 2.1 mmol)], yield 0.08 g (44.7%), mp 128—131 °C, $[\alpha]_D^{25}$ +8.2° (c=0.6, DMF), Rf^1 0.70, Rf^{10} 0.88. *Anal.* Calcd for $C_{54}H_{51}BrN_4O_{10}$: C, 64.9; H, 5.13; N, 5.49. Found: C, 65.2; H, 5.12; N, 5.63.

Fmoc-Lys-Tyr(2-Br-Z)-4-Acetylanilide (29) The title compound was prepared from Fmoc-Lys(Z)-Tyr(2-Br-Z)-4-acetylanilide (60 mg, 0.06 mmol) and 25% HBr/AcOH (0.06 ml, 0.18 mmol), yield 30 mg (53.8%), $[\alpha]_D^{25}$ +2.3° (c=0.8, MeOH), Rf^5 0.60. *Anal.* Calcd for $C_{46}H_{45}BrN_4O_8 \cdot HBr \cdot 3/2H_2O$: C, 57.2; H, 4.91; N, 6.00. Found: C, 57.0; H, 5.06; N, 5.78.

Boc-Phe(4-NO_2)-OH H-Phe(4-NO_2)-OH (5.0 g, 24 mmol) and Boc-ON (7.1 g, 29 mmol) were dissolved in H_2O (100 ml) and dioxane (200 ml) containing Et_3N (4.1 ml, 29 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, ether and 5% $NaHCO_3$ were added to the residue. The water layer was acidified with citric acid to pH 3. The precipitate was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give crystals, which were collected by filtration, yield 5.0 g (67.2%), mp 92—93 °C, $[\alpha]_D^{25}$ +8.1° (c=1.6, MeOH), Rf^1 0.46. *Anal.* Calcd for $C_{14}H_{18}N_2O_6$: C, 54.1; H, 5.80; N, 8.85. Found: C, 54.2; H, 5.80; N, 9.03.

Boc-Phe(4-NH_2)-OH Boc-Phe(4-NO_2)-OH (0.5 g, 1.6 mmol) in MeOH (8 ml) was hydrogenated over a Pd catalyst for 4 h. After removal of Pd and the solvent, petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 0.38 g (84.9%), mp 118 °C, $[\alpha]_D^{25}$ +25.8° (c=1.1, MeOH), Rf^1 0.21. *Anal.* Calcd for $C_{14}H_{20}N_2O_4$: C, 59.9; H, 7.21; N, 10.0. Found: C, 60.0; H, 7.14; N, 10.0.

Boc-Phe(4-NH-Z)-OH The title compound was prepared from Boc-Phe(4-NH_2)-OH (1.2 g, 4.3 mmol) and Z-Cl (1.1 g, 6.5 mmol) in a usual manner using NaOH (0.34 g, 8.6 mmol), yield 1.5 g (84.2%), mp 152—157 °C, $[\alpha]_D^{25}$ +19.4° (c=1.4, MeOH), Rf^1 0.63. *Anal.* Calcd for $C_{22}H_{26}N_2O_6$: C, 63.8; H, 6.32; N, 6.76. Found: C, 63.9; H, 6.12; N, 6.65.

Boc-Phe-(4-NH-Z)-Tyr(2-Br-Z)-4-Acetylanilide A solution of mixed anhydride [prepared from Boc-Phe(4-NH-Z)-OH (0.19 g, 0.46 mmol) and ethyl chloroformate (0.044 ml, 0.46 mmol) as usual] in THF (10 ml) was added to an ice-cold solution of H-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-Tyr(2-Br-Z)-4-acetylanilide (0.19 g, 0.31 mmol) and 7.0 N HCl/dioxane (0.44 ml, 3.1 mmol)] in DMF (10 ml) containing Et_3N (0.04 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, AcOEt and water were added to the residue to give crystals, which were collected by filtration and recrystallized from MeOH, yield 0.20 g (71.1%), mp 182—187 °C, $[\alpha]_D^{25}$ +14.3° (c=0.3, DMF), Rf^1 0.66, Rf^2 0.86.

Tos-Phe(4-NH-Z)-Tyr(2-Br-Z)-4-Acetylanilide The title compound was prepared from Tos-Cl (0.04 g, 0.21 mmol) and H-Phe(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide [prepared from Boc-Phe(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (0.1 g, 0.1 mmol) and 7.0 N HCl/dioxane (0.16 ml, 1.1 mmol)], yield 53 mg (50.1%), mp 227—229 °C, $[\alpha]_D^{25}$ +9.3° (c=0.3, DMF), Rf^3 0.66. *Anal.* Calcd for $C_{49}H_{45}BrN_4O_{10}S$: C, 61.2; H, 4.68; N, 5.83. Found: C, 61.5; H, 4.51; N, 6.01.

Tos-Phe(4-NH_2)-Tyr(2-Br-Z)-4-Acetylanilide (30) The title compound was prepared from Tos-Phe(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (43 mg, 0.05 mmol) and 25% HBr/AcOH (0.05 ml, 0.15 mmol), yield 34 mg (78%), $[\alpha]_D^{25}$ +9.3° (c=0.3, MeOH), Rf^5 0.87. *Anal.* Calcd for $C_{41}H_{39}BrN_4O_8S \cdot HBr \cdot 3/2H_2O$: C, 52.6; H, 4.62; N, 6.80. Found: C, 52.6; H, 4.60; N, 6.88.

Tol-Phe(4-NH-Z)-Tyr(2-Br-Z)-4-Acetylanilide The title compound was prepared from Tol-Cl (0.03 g, 0.21 mmol) and H-Phe(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-Phe(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (0.1 g, 0.1 mmol) and 7.0 N HCl/dioxane (0.16 ml, 1.1 mmol)], yield 0.051 g (50.1%), mp 280—283 °C, $[\alpha]_D^{25}$ −20.0° (c=0.3, DMF), Rf^3 0.62. *Anal.* Calcd for $C_{50}H_{45}BrN_4O_9$: C, 64.9; H, 4.89; N, 6.05. Found: C, 65.1; H, 4.59; N, 6.22.

Tol-Phe(4-NH_2)-Tyr(2-Br-Z)-4-Acetylanilide (31) The title compound was prepared from Tol-Phe(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (44.7 mg, 0.05 mmol) and 25% HBr/AcOH (0.05 ml, 0.15 mmol), yield 36 mg (85.5%), $[\alpha]_D^{25}$ −5.3° (c=0.5, MeOH), Rf^5 0.87. *Anal.* Calcd for $C_{42}H_{39}BrN_4O_7 \cdot HBr \cdot 4H_2O$: C, 53.3; H, 5.78; N, 7.38. Found: C, 53.4; H, 5.58; N, 7.62.

Boc-Cha(4-NH-Z)-OH PtO_2 (30 mg) in AcOH (10 ml) was reduced with H_2 for 15 min. Boc-Phe(4-NH-Z)-OH (0.28 g, 0.9 mmol) was added to the above solution and the reaction mixture was stirred at room temperature for 36 h under an H_2 atmosphere. After removal of Pt and the solvent, ether was added to the residue to give crystals, which were collected by filtration. The crude products contained two components (Rf^{11} 0.40 and 0.80). The products (1.5 g) were treated with Z-Cl (0.88 g) and 1 N NaOH (8.6 ml) to give a crude amorphous powder. The purification of the products was performed by reversed-phase HPLC on a YMC D-ODS-5 column (20 × 250 mm). A part of the above amorphous powder was dissolved in MeOH (0.2 ml) and the solution was applied to the column, which was eluted with MeOH : 0.1% aqueous TFA (6 : 4) at a flow rate of 10 ml/min. Two peaks (retention times 59 and 67 min) were obtained. The effluent corresponding to each peak was collected and the solvent of each fraction was removed by lyophilization to give a white fluffy powder. A: a powder corresponding to the product with the retention time of 59 min, yield 0.35 g (19.4%), $[\alpha]_D^{25}$ −5.6° (c=0.5, MeOH), Rf^1 0.63, Rf^4 0.76. *Anal.* Calcd for $C_{22}H_{32}N_2O_6 \cdot H_2O$: C, 60.3; H, 7.76; N, 6.39. Found: C, 60.5; H, 7.55; N, 6.42. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 0.90—1.94 (10H, m), 3.30 (2H, s), 4.11—4.14 (1H, m), 5.04 (2H, s), 7.27—7.34 (5H, m). NMR data confirmed that this compound is a *trans*-cyclohexane derivative. B: a powder corresponding to the product with the retention time of 67 min, yield 0.18 g (9.9%), $[\alpha]_D^{25}$ −3.3° (c=1.6, MeOH), Rf^1 0.63, Rf^4 0.76. *Anal.* Calcd for $C_{22}H_{32}N_2O_6 \cdot H_2O$: C, 60.3; H, 7.76; N, 6.39. Found: C, 60.2; H, 7.35; N, 6.24. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.27—1.78 (10H, m), 3.30 (2H, s), 4.10—4.14 (1H, m), 5.06 (2H, s), 7.27—7.34 (5H, m). NMR data confirmed that this compound is a *cis*-cyclohexane derivative.

Boc-*trans*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-Acetylanilide A solution of mixed anhydride [prepared from Boc-*trans*-Cha(4-NH-Z)-OH (0.35 g, 0.87 mmol) and ethyl chloroformate (0.08 ml, 0.87 mmol) as usual] in THF (6 ml) was added to an ice-cold solution of H-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-Tyr(2-Br-Z)-4-acetylanilide (0.35 g, 0.58 mmol) and 7.0 N HCl/dioxane (0.82 ml, 5.8 mmol) in DMF (4 ml) containing Et_3N (0.08 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 0.40 g (84.2%), mp 205—208 °C, $[\alpha]_D^{25}$ +12.1° (c=1.0, DMF), Rf^1 0.56, Rf^2 0.71. *Anal.* Calcd for $C_{47}H_{53}BrN_4O_{10}$: C, 61.8; H, 5.80; N, 6.13. Found: C, 61.6; H, 5.85; N, 6.08. ^{1}H-NMR (DMSO-d_6) δ: 1.35 (9H, s), 0.82—1.76 (12H, m), 2.48 (3H, s), 2.90—3.09 (2H, m), 3.94—3.95 (1H, m), 4.69—4.70 (1H, m), 4.97 (2H, s), 5.29 (2H, s), 6.86—6.88 (1H, m), 7.11—7.14, 7.29—7.36 (13H, m), 7.69 (2H, d, J=8.8 Hz), 7.92 (2H, d, J=8.8 Hz), 8.07—8.09 (1H, m), 10.39 (1H, s).

Tos-*trans*-Cha(4-NH-Z)-Tyr(2-Br-Z)-Acetylanilide The title compound was prepared from Tos-Cl (0.03 g, 0.17 mmol) and H-*trans*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-*trans*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (0.1 g, 0.11 mmol) and 7.0 N HCl/dioxane (0.16 ml, 1.1 mmol) as usual], yield 52 mg (48.9%), mp 245—248 °C, $[\alpha]_D^{25}$ −0.8° (c=1.7, DMF), Rf^1 0.48, Rf^{10} 0.72. *Anal.* Calcd for $C_{49}H_{51}BrN_4O_{10}S$: C, 60.8; H, 5.27; N, 5.79, Found: C, 60.8; H, 5.20; N, 5.53.

Tos-*trans*-Cha(4-NH_2)-Tyr(2-Br-Z)-4-Acetylanilide (32) The title compound was prepared from Tos-*trans*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (42.5 mg, 0.044 mmol) and 25% HBr/AcOH (0.1 ml, 0.19 mmol), yield 37 mg (92.0%), $[\alpha]_D^{25}$ −25.7° (c=1.1, MeOH), Rf^4 0.55, Rf^{11} 0.38. *Anal.* Calcd for $C_{41}H_{45}BrN_4O_8S \cdot HBr \cdot 3/2H_2O$: C, 52.3; H, 5.21; N, 5.95. Found: C, 52.4; H, 5.22; N, 6.24.

Boc-*cis*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-Acetylanilide A mixed anhydride [prepared from Boc-*cis*-Cha(4-NH-Z)-OH (0.17 g, 0.4 mmol) and ethyl chloroformate (0.04 ml, 0.4 mmol)] in THF (6 ml) was added to an ice-cold solution of H-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-Tyr(2-Br-Z)-4-acetylanilide (0.2 g, 0.33 mmol) and 7.0 N HCl/dioxane (0.5 ml, 3.5 mmol)] in DMF (4 ml) containing Et_3N (0.05 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give crystals, which were collected by filtration and reprecipitated from EtOH and petroleum ether, yield 0.08 g (26.5%), mp 92—94 °C, $[\alpha]_D^{25}$ +21.9° (c=1.1, DMF), Rf^1 0.57. ^{1}H-NMR (DMSO-d_6) δ: 1.36 (9H, s), 1.25—1.49 (12H, m), 2.48 (3H, s), 2.91—3.11 (2H, m), 3.94—3.95 (1H, m), 4.69—4.70 (1H, m), 5.01 (2H, s), 5.31 (2H, s), 6.86—6.88 (1H, m), 7.11—7.14, 7.29—7.36 (13H, m), 7.69 (2H, d, J=8.7 Hz), 7.92 (2H, d, J=7.7 Hz), 8.07—8.09 (1H, m), 10.39 (1H, s). *Anal.* Calcd for $C_{47}H_{53}BrN_4O_{10}$: C, 61.8; H, 5.80; N, 6.13. Found: C, 61.6; H, 5.70; N, 6.11.

Tol-*trans*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-Acetylanilide The title compound was prepared from Tol-Cl (0.03 g, 0.17 mmol) and H-*trans*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-*trans*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (0.1 g, 0.11 mmol) and 7.0 N HCl/dioxane (0.16 ml, 1.1 mmol) as usual], yield 75 mg (73.2%), mp 254—257 °C, $[\alpha]_D^{25}$ +10.0° (c=0.2, DMF), Rf^1 0.63, Rf^{10} 0.86. *Anal.* Calcd for $C_{50}H_{51}BrN_4O_9$: C, 64.5; H, 5.48; N, 6.01. Found: C, 64.3; H, 5.51; N, 6.01.

Tol-*trans*-Cha(4-NH_2)-Tyr(2-Br-Z)-4-Acetylanilide (33) The title compound was prepared from Tol-*trans*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (58.3 mg, 0.063 mmol) and 25% HBr/AcOH (0.1 ml, 0.19 mmol), yield 52 mg (94.4%), $[\alpha]_D^{25}$ +5.3° (c=0.9, MeOH), Rf^4 0.55, Rf^{11} 0.38. *Anal.* Calcd for $C_{42}H_{45}BrN_4O_7 \cdot HBr \cdot 2H_2O$: C, 55.2; H, 5.47; N, 6.13. Found: C, 54.9; H, 5.32; N, 6.45.

Tol-*cis*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-Acetylanilide The title compound was prepared from Tol-Cl (0.03 g, 0.17 mmol) and H-*cis*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide·HCl [prepared from Boc-*cis*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (0.1 g, 0.11 mmol) and 7.0 N HCl/dioxane (0.16 ml, 1.1 mmol)], yield 0.1 g (97.6%), mp 94—98 °C, $[\alpha]_D^{25}$ +53.0° (c=0.1, DMF), Rf^1 0.50, Rf^{10} 0.52. *Anal.* Calcd for $C_{50}H_{51}BrN_4O_9 \cdot 1/2H_2O$: C, 63.9; H, 5.53; N, 5.96. Found: C, 63.9; H, 5.39; N, 6.05.

Tol-*cis*-Cha(4-NH_2)-Tyr(2-Br-Z)-4-Acetylanilide (34) The title compound was prepared from Tol-*cis*-Cha(4-NH-Z)-Tyr(2-Br-Z)-4-acetylanilide (93.8 mg, 0.1 mmol) and 25% HBr/AcOH (0.15 ml, 0.3 mmol), yield 86 mg (98.0%), $[\alpha]_D^{25}$ +3.8° (c=1.2, MeOH), Rf^4 0.58, Rf^5 0.69. *Anal.* Calcd for $C_{42}H_{45}BrN_4O_7 \cdot HBr \cdot 7/2H_2O$: C, 53.6; H, 5.63; N, 5.95. Found: C, 53.8; H, 5.51; N, 5.92.

Boc-Cha(4-NH-Z)-4-Acetylanilide A mixed anhydride [prepared from Boc-Cha(4-NH-Z)-OH (1.7 g, 4 mmol) and ethyl chloroformate (0.38 g, 4 mmol)] in THF (60 ml) was added to an ice-cold solution of 4-acetylaniline (0.54 g, 4 mmol) in DMF (20 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. EtOH was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 1.0 g (46.5%), mp 208—211 °C, $[\alpha]_D^{25}$ −15.5° (c=1.0, $CHCl_3$), Rf^1 0.59, Rf^2 0.61. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (9H, s), 0.91—1.80 (12H, m), 2.50 (3H, s), 4.14 (1H, m), 4.99 (2H, s), 7.10—7.16 (2H, m), 7.28—7.38 (3H, m), 7.74 (2H, d, J=8.8 Hz), 7.94 (2H, d, J=8.8 Hz). *Anal.* Calcd for $C_{30}H_{39}N_3O_6 \cdot 1/4H_2O$: C, 66.5; H, 7.34; N, 7.75. Found: C, 66.5; H, 7.31; N, 7.75.

Tol-Cha(4-NH-Z)-4-Acetylanilide The title compound was prepared from Tol-Cl (0.05 g, 0.35 mmol) and H-Cha(4-NH-Z)-4-acetylanilide·HCl [prepared from Boc-Cha(4-NH-Z)-4-acetylanilide (0.24 g, 0.46 mmol) and 7.0 N HCl/dioxane (0.7 ml, 4.9 mmol)], yield 90 mg (70.5%), mp 176—179 °C, $[\alpha]_D^{25}$ −68.0° (c=0.4, $CHCl_3$), Rf^1 0.61, Rf^2 0.69. *Anal.* Calcd for $C_{33}H_{37}N_3O_5$: C, 71.4; H, 6.66; N, 7.56. Found: C, 71.6; H, 6.89; N, 7.42.

Tol-Cha(4-NH_2)-4-Acetylanilide (35) The title compound was prepared from Tol-Cha(4-NH-Z)-4-acetylanilide (58.0 mg, 0.1 mmol) and 25% HBr/AcOH (0.1 ml, 0.3 mmol), yield 40 mg (81.9%), $[\alpha]_D^{25}$ −4.2° (c=0.8, MeOH), Rf^4 0.29, Rf^{11} 0.33. *Anal.* Calcd for $C_{25}H_{31}N_3O_3 \cdot HBr \cdot 2H_2O$: C, 55.8; H, 6.69; N, 7.81. Found: C, 55.5; H, 6.78; N, 8.01.

Tos-Cha(4-NH-Z)-4-Acetylanilide The title compound was prepared from Tos-Cl (0.06 g, 0.35 mmol) and H-Cha(4-NH-Z)-4-acetylanilide·HCl [prepared from Boc-Cha(4-NH-Z)-4-acetylanilide (0.1 g, 0.23 mmol) and 7.0 N HCl/dioxane (0.35 ml, 2.5 mmol)], yield 80 mg (58.8%), mp 165—166 °C, $[\alpha]_D^{25}$ −96.0° (c=0.1, $CHCl_3$), Rf^1 0.06, Rf^2 0.67. *Anal.* Calcd for $C_{32}H_{37}N_3O_6S$: C, 65.0; H, 6.26; N, 7.10. Found: C, 65.3; H, 6.52; N, 7.11.

Tos-Cha(4-NH_2)-4-Acetylanilide (36) The title compound was prepared from Tos-Cha(4-NH-Z)-4-acetylanilide (61.6 mg, 0.1 mmol) and 25% HBr/AcOH (0.1 ml, 0.3 mmol), yield 23 mg (43.9%), $[\alpha]_D^{25}$ −2.5° (c=1.5, MeOH), Rf^4 0.29, Rf^{11} 0.40. *Anal.* Calcd for $C_{24}H_{31}N_3O_4S \cdot HBr \cdot 2H_2O$: C, 53.2; H, 5.35; N, 7.75. Found: C, 53.1; H, 5.09; N, 7.65.

Assay Procedure The enzymes used were as follows: human plasmin and plasma kallikrein (KABI Co.), bovine thrombin (Mochida Seiyaku Co.), and human urokinase (Green Cross). The enzymatic activities of plasmin, plasma kallikrein, thrombin and urokinase were determined by the method described previously,[24] using D-Val-Leu-Lys-*p*NA (S-2251), D-Pro-Phe-Arg-*p*NA (S-2302), D-Phe-Pip-Arg-*p*NA (S-2266) and ⟨Glu-Gly-Arg-*p*NA (S-2444), respectively. Fibrin and fibrinogen were also used as substrates for plasmin and thrombin, respectively. The IC_{50} values were determined as follows. 1) Antiamidolytic assay[16]: The IC_{50} value was taken as the concentration of inhibitor which decreased the absorbancy at 405 nm by 50% compared with the absorbancy measured under the same conditions without the inhibitor. 2) Antifibrinolytic assay[16]: The IC_{50} value was taken as the concentration of inhibitor which prolongs the complete lysis time twofold in comparison with that in the absence of the inhibitor. 3) Antifibrinogenolytic assay: Bovine thrombin 4 U/ml (0.1 ml) was added to a solution of various concentrations of a peptide to be tested in a borate saline buffer (pH 7.4) (0.5 ml) and 0.2% bovine fibrinogen in the above buffer (0.4 ml). The assay was carried out at 37 °C and the clotting time was measured. The IC_{50} value was taken as the concentration of inhibitor which prolonged the clotting time twofold in comparison with that in the absence of the inhibitor.

References and Notes

1) Standard abbreviations for amino acids and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2548 (1966); *ibid.*, **6**, 362 (1967), *ibid.*, **11**, 1728 (1976). Other abbreviations used are Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Tos, tosyl; Tol, toluoyl; NaPh, β-naphthyl; Bzl, benzyl; Fmoc, 9-fluorenylmethyloxycarbonyl; Pip, pipecolyl; *p*NA, *p*-nitroanilide; NMM, *N*-methylmorpholine; Boc-ON, 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile; Cha, cyclohexylalanine; DCC, *N,N'*-dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; AcOH, acetic acid; *n*-BuOH, *n*-butanol; AcOEt, ethyl acetate; DMF, dimethylformamide; iso-PrOH, isopropanol; DMSO, dimethyl sulfoxide.
2) N. Katsunuma and E. Kominami, *Rev. Physiol. Biochem. Pharmacol.*, **108**, 1 (1987).
3) R. M. Senior, H. Tegner, C. Kuhn, K. Ohlsson, B. C. Starcher and J. A. Pierce, *Am. Rev. Respir. Dis.*, **116**, 469 (1977).
4) M. Iwamoto and Y. Abiko, *Biochim. Biophys. Acta*, **214**, 402 (1970).
5) M. Mori and N. Aoki, *J. Biol. Chem.*, **251**, 5956 (1976).
6) N. Aoki, *Semin. Thromb. Hemostasis*, **10**, 42 (1984).
7) J. Romisch, G. Dickneite, E-P. Paques and N. Heimburg, *Jpn. J. Thromb. Hemostasis*, **3**, 73 (1992).
8) G. Markus, *Semin. Thromb. Hemostasis*, **10**, 61 (1984).
9) D. J. McCornnel, *J. Clin. Invest.*, **51**, 1611 (1972).
10) L. J. Kagen, *Brit. J. Exp. Path.*, **45**, 604 (1964).
11) M. Yano, S. Nagasawa and T. Suzuki, *J. Biochem.* (Tokyo), **69**, 471 (1971).
12) C. C. Cochrana, S. D. Revak and K. D. Wuepper, *J. Exp. Med.*, **138**, 1564 (1973).
13) A. Ichinose, K. Fujikawa and T. Suyama, *J. Biol. Chem.*, **261**, 3486 (1986).
14) R. W. Colman, *Biochem. Biophys. Res. Commun.*, **35**, 273 (1969).
15) A. P. Kaplan, A. B. Kay and K. F. Austen, *J. Exp. Med.*, **135**, 81 (1972).
16) Y. Okada, Y. Tsuda, N. Teno, K. Wanaka, M. Bohgaki, A. Hijikata-Okunomiya, T. Naito and S. Okamoto, *Chem. Pharm. Bull.*, **36**, 1289 (1988).
17) N. Teno, K. Wanaka, Y. Okada, Y. Tsuda, U. Okamoto, A. Hijikata-Okunomiya, T. Naito and S. Okamoto, *Chem. Pharm. Bull.*, **39**, 2340 (1991).
18) N. Teno, K. Wanaka, Y. Okada, Y. Tsuda, U. Okamoto, A. Hijikata-Okunomiya, T. Naito and S. Okamoto, *Chem. Pharm. Bull.*, **39**, 2930 (1991).
19) K. Wanaka, Y. Okada, Y. Tsuda, U. Okamoto, A. Hijikata-Okunomiya and S. Okamoto, *Chem. Pharm. Bull.*, **40**, 1814 (1992).
20) I. Schechter and A. Berger, *Biochem. Biophys. Res. Commun.*, **27**, 157 (1967).
21) This compound was prepared in our laboratory and the synthetic procedure will be described in detail elsewhere.
22) S. Natarajan, E. M. Gordon, E. F. Sabo, J. D. Godfrey, H. N. Weller, J. Pluscec, M. B. Rom and D. W. Cushman, *Biochem. Biophys. Res. Commun.*, **124**, 141 (1984).
23) R. G. Almquist, J. Crase, D. Jennings-White, R. F. Meyer, M. L. Hoefle, R. D. Smith, A. D. Essenburg and H. R. Kaplan, *J. Med. Chem.*, **25**, 1292 (1982).
24) Y. Tsuda, N. Teno, Y. Okada, K. Wanaka, M. Bohgaki, A. Hijikata-Okunomiya, U. Okamoto, T. Naito and S. Okamoto, *Chem. Pharm. Bull.*, **37**, 3108 (1989).