Development of Potent and Selective Plasmin and Plasma Kallikrein Inhibitors and Studies on the Structure–Activity Relationship[1)]

Yoshio OKADA,*[a,b] Yuko TSUDA,[a,b] Mayako TADA,[a,c] Keiko WANAKA,[c] Utako OKAMOTO,[c] Akiko HIJIKATA-OKUNOMIYA,[d] and Shosuke OKAMOTO[c]

Faculty of Pharmaceutical Sciences,[a] and High Technology Research Center,[b] Kobe Gakuin University, Nishi-ku, Kobe 651–2180, Japan, Kobe Research Projects on Thrombosis and Haemostasis,[c] 3–15–18, Asahigaoka, Tarumi-ku, Kobe 655–0033, Japan, and Faculty of Health Sciences, Kobe University School of Medicine,[d] Suma-ku, Kobe 654–0142, Japan.
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Based on structure–activity relationship studies, we designed and synthesized plasmin (PL) and plasma kallikrein (PK) inhibitors. *Trans*-(4-aminomethylcyclohexanecarbonyl)-Tyr(*O*-Pic)-octylamide inhibited PL, PK, urokinase (UK) and thrombin (TH) with IC_{50} values of 0.53, 30, 5.3 and $>400\ \mu$M, respectively. *Trans*-(4-aminomethylcyclohexanecarbonyl)-Tyr(*O*-2-Pyrim)-4-carboxyanilide inhibited PL, PK, UK and TH with IC_{50} values of 36, 0.56, 440 and $>1000\ \mu$M, respectively.

Key words plasmin inhibitor; plasma kallikrein inhibitor; selectivity; structure–activity relationship

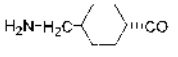
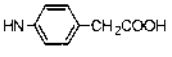
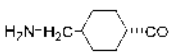
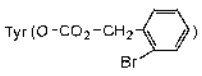
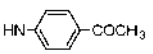
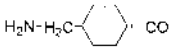
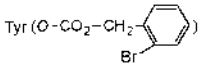
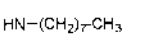
Proteinases and their natural inhibitors regulate biological functions cooperatively to maintain homeostasis, while an imbalance between them may cause serious disorders.[2,3)] With regard to plasmin (PL), α_2-macroglobulin (α_2-M)[4)] and α_2-plasmin inhibitor (α_2-PI)[5)] are known as endogenous inhibitors. Data has shown that α_2-PI consists of two active regions: one binds to the active site (catalytic site) and another to the lysine binding site (LBS) of PL. An imbalance between PL and its natural inhibitors causes a serious syndrome, for instance, over activation of PL may induce hyperfibrinolysis.[6—8)] It was reported that PL selective inhibitor inhibited the increase of tumor cells in sarcoma 180 bearing mice.[9)] Plasma prekallikrein circulates in the blood as the zymogen of plasma kallikrein (PK) and is activated by factor XIIa to form plasma PK.[10)] PK releases bradykinin from high molecular weight kininogen.[11)] It is well known that the PK–kinin system is associated with various diseases, such as disseminated intravascular coagulation (DIC),[12)] allergy[13)] and ARDS (adult respiratory distress syndrome).[14)] Furthermore, it has been reported that PK activates factor XII,[15)] prourokinase,[16)] and plasminogen.[17)] PK is also known to cause neutrophil aggregation[18)] and elastase release.[19)] These observations suggest that although PK has many functions, its precise role remains to be determined.

Keeping these circumstances in mind, our studies were directed toward the development of selective PL and PK inhibitors. Previously, we reported active center-directed inhibitors of PL[20—22)] and studies on their structure–activity relationship.[23)] Our inhibitors consist of three parts, P_1, $P_{1'}$, and $P_{2'}$,[24)] and their structure–activity relationships are summarized in Table 1.

As shown in Table 1, compound I inhibits PK specifically. Compound II inhibits both PL and PK, and compound III specifically inhibits PL. The phenyl ring of Phe in compound I may interact with some functional domain of PK, while it does not appear to react with PL, thereby producing the difference in the inhibitory activity between against PK and PL. The benzene moiety of 2-Br-Z of compound II and III , however, may interact with PK and PL. The octyl amide moiety at the position $P_{2'}$ of compound III interacts with PL more suitably than PK. These results showed that we could design enzyme-selective inhibitors by combining various substituents at positions P_1, $P_{1'}$, and $P_{2'}$. This report deals with design and synthesis of selective PL and PK inhibitors and their structure–activity relationship.

First of all, 2-Br-Z moiety at position $P_{1'}$ of the compound III was modified in order to increase aqueous solubility. As shown in Table 2, the compound **1** exhibited similar inhibitory profile to that of compound III. Compound **2** exhibited an interesting inhibitory profile because it inhibited UK with an IC_{50} value of 5.3 μM. On the other hand, compounds **1** and **3** did not inhibit UK. Interestingly, the compound **5** in-

Table 1. IC_{50} Values (μM) of Compounds I—III for PL and PK

No.	P_1	$P_{1'}$	$P_{2'}$	PL	PK
				S-2251	S-2302
I		Phe		630	1.3
II				0.23	0.37
III				0.80	16

S-2251: D-Val-Leu-Lys-pNA, S-2302: D-Pro-Phe-Arg-*p*NA

* To whom correspondence should be addressed. e-mail: okada@pharm.kobegakuin.ac.jp
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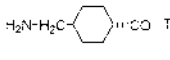
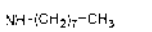
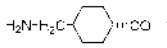
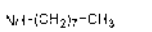
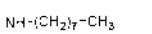
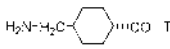
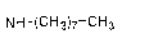
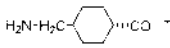
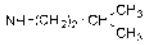
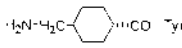
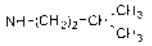
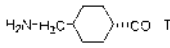
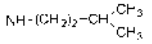
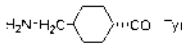
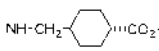
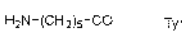
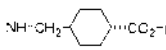
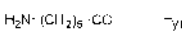
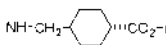
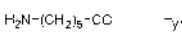
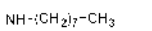
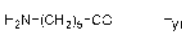
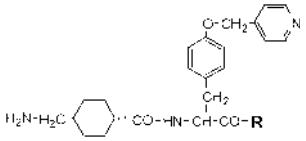
Table 2. IC_{50} Values (μM) of Compounds **1**—**11** for Various Enzymes

No.	P_1	$P_{1'}$	$P_{2'}$	PL		PK	UK	TH	
				S-2251	Fn	S-2302	S-2444	S-2238	Fg
III				0.80	0.23	16	>50	>50	>25
1				0.65	0.29	38	>50	>40	>20
2				0.53	0.36	30	5.3	>400	>100
3				1.0	0.85	12	81	>200	>200
4				0.46	0.056	2.1	260	70	>100
5				1.9	1.7	28	5.9	>1000	>1000
6				2.2	2.0	7.0	75	>1000	>1000
7				1.2	0.6	30	25	>100	>10
8				>100	5.0	>400	>100	>100	>10
9				23	9.5	>1000	>100	>100	>20
10				7.0	1.8	75	>250	>50	>25
11				14	6.6	>400	120	>400	>200

S-2251: D-Val-Leu-Lys-*p*NA, S-2302: D-Pro-Phe-Arg-*p*NA, S-2444: Glp-Gly-Arg-*p*NA, S-2238: D-Phe-Pip-Arg-*p*NA

Table 3. IC_{50} Values (μM) of Compounds **2**, **12** and **13** for Various Enzymes



No.	R	PL		PK	UK	TH	
		S-2251	Fn	S-2302	S-2444	S-2238	Fg
2	-NH-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3	0.53	0.36	30	5.3	>400	>100
12	-NH-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-COOH	5.5	4.6	32	29	>500	>500
13	-NH-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-NH_2	3.8	2.4	330	31	>1000	>1000

S-2251: D-Val-Leu-Lys-*p*NA, S-2302: D-Pro-Phe-Arg-*p*NA, S-2444: Glp-Gly-Arg-*p*NA, S-2238: D-Phe-Pip-Arg-*p*NA

hibited UK with an IC_{50} value of 5.9 μM, while the compounds **4**[23] and **6**, which have bulkier moiety at the position $P_{1'}$ than that in compounds **2** and **5**, only inhibited PL. The combination of the picolyl moiety at position $P_{1'}$ along with alkylamide at position $P_{2'}$ produced UK inhibitors. Compounds **8**,[23] **9**, **10**[23] and **11** exhibited similar inhibitory profiles to each other, although their inhibitory activity was weak. *Trans*-4-aminomethylcyclohexanecarbonyl (Tra) moiety at the position P_1 is more suitable for interaction with active center of PL and PK than ε-aminocaproyl moiety at the position P_1.

As summarized in Table 3, the compound **12**, which has carboxy group at position $P_{2'}$, reduced inhibitory activity against PL and UK compared with the compound **2**, while **12** exhibited similar inhibitory activity against PK relative to **2**. Similar phenomena were reported previously.[25,26] Compound **13**, which contains an amino group at position $P_{2'}$, had a similar inhibitory profile against PL and UK in respect to compound **12**, with decreased inhibitory activity toward PK compared with compound **12**. The effect of carboxyl or amino function at position $P_{2'}$ on enzymatic activity implicated new strategies for the development of enzyme inhibitors.

As shown in Table 1, compound I is a selective PK inhibitor, compound II inhibited both PL and PK and compound III specifically inhibited PL. Figure 1 schematically shows the mode of interaction between the compound II and PK and PL. Although compound II inhibited PL and PK with IC_{50} values of 0.23 and 0.37 μM, respectively, Tra-Phe-ACA (4-acetylanilide) inhibited PL and PK with IC_{50} values of 36 and 0.85 μM, respectively,[22] indicating that Tra-Phe-ACA is

a more PK specific inhibitor. While the benzene moiety of Phe residue interacts with some part in PK, such part appears to be excluded from PL. On the other hand, the benzene moiety of 2-Br-Z at position $P_{1'}$ of compound II interacts with both PL and PK. Based on the above results, we further designed and synthesized PK specific inhibitors and summarized in Table 4. At position $P_{1'}$, Tyr(*O*-2-Pyrim) or D,L-*m*-Tyr(*O*-2-Pyrim) was employed, in which methylene or methyloxycarbonyl group between *O* of Tyr residue and second aromatic moiety was deleted and at position $P_{2'}$, carboxyl function was introduced. Compound **14** inhibited PK, PL, UK and TH with IC_{50} values of 0.56, 36, 440 and >1000 μM, respectively. The compound **15** inhibited the same enzymes with IC_{50} values of 0.71, 32, 650 and >1000 μM, respectively. Both compounds inhibited PK preferentially rather than PL as expected. As shown in Table 4, compounds **16** and **17** exhibited weaker inhibitory activity against PL and PK compared to compound **14** and **15**, although the difference in inhibitory activity against PL and PK is more than those of compounds **14** and **15**.

Compounds **2** and **15** will be candidates for development of novel drugs, such as anti-tumor medicine and anti-allergy medicine, by inhibiting PL and PK, respectively.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus without correction. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co.). Matrix assisted laser desorption ionization time-of-flight mass spectra (MALDI-TOF-MAS) were obtained on a Kratos MALDI II mass spectrometer (Kratos Analysis). Waters model 600E was used for analytical or preparative HPLC. If necessary, peptides were purified by reverse phase HPLC on a C18 semi-preparative column (20×250 mm, Nakalai). The column was eluted in 40 min using a linear gradient from 10 to 50% acetonitrile in water, containing 0.05% TFA at flow rate of 10 ml/min; Detection was at 220 nm. 2-Chloropyrimidine, 4-picolyl chloride and D,L-*m*-tyrosine (D,L-*m*-Tyr) were purchased from Tokyo Kasei (Japan). On TLC (Kieselgel G. Merck), Rf^1, Rf^2, Rf^3, Rf^4, Rf^5, Rf^6, and Rf^7 values refer to the systems of $CHCl_3$, MeOH and AcOH (90 : 8 : 2); $CHCl_3$, MeOH and H_2O (89 : 10 : 1); $CHCl_3$, MeOH and H_2O (8 : 3 : 1, lower phase); *n*-BuOH, AcOH and H_2O (4 : 1 : 5, upper phase); *n*-BuOH, AcOH, pyridine and H_2O (4 : 1 : 1 : 2); *n*-BuOH, AcOH, pyridine and H_2O (1 : 1 : 1 : 1) and $CHCl_3$ and ether (1 : 1), respectively.

***p*-Hydroxymethylbenzoic Acid** To a solution of *p*-formylbenzoic acid (0.50 g, 3.3 mmol) in dry MeOH (30 ml) was added sodium borohydride (0.37 g, 10 mmol) at 0 °C. After 5 min in an ice-bath, the reaction mixture was stirred at room temperature for 2 h. After neutralization with AcOH, the solvent was removed under reduced pressure. The residue was extracted with AcOEt and the extract was washed with 10% AcOH, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give a white precipitate, which was collected by filtration, yield 0.42 g (84%), mp 177—180 °C, Rf^1 0.38. *Anal*. Calcd for $C_8H_8O_3$: C, 63.2; H, 5.30. Found: C, 62.9; H, 5.30.

Methyl *p*-Hydroxymethylbenzoate To dry methanol (30 ml), thionyl chloride (0.70 ml, 8.0 mmol) was added dropwise at −5 to −10 °C. *p*-Hydroxymethylbenzoic acid (1.0 g, 8.0 mmol) was added to the above solution. The reaction mixture was stirred at 50 °C for 3 h. After removal of the solvent, petroleum ether was added to the residue to yield a white precipitate, which was collected by filtration, yield 0.90 g (83%), mp 43.5—44 °C, Rf^1 0.61. *Anal*. Calcd for $C_9H_{10}O_3 \cdot 1/10H_2O$: C, 64.4; H, 6.06. Found; C, 64.5; H, 5.93.

***p*-Methyloxycarbonylbenzyl Chloride** Methyl *p*-hydroxymethylbenzoate (0.32 g, 2.0 mmol) was added to a cooled thionyl chloride (0.34 ml, 4.0 mmol). After all the methyl *p*-hydroxymethylbenzoate had been added, the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was poured into the cracked ice with stirring. The aqueous solution was extracted with ether, and the extract was dried over Na_2SO_4 and evaporated down to give oily material, which was dried over P_2O_5 *in vacuo*, yield 0.29 g (78%), Rf^1 0.77.

Boc-Tyr(*O*-CH_2-C$_6$H$_4$-$COOCH_3$)-octylamide To a solution of Boc-Tyr-octylamide (1.3 g, 4.0 mmol) [prepared from Boc-Tyr(2-Br-Z)-octylamide[23] by catalytic hydrgenation as usual] in DMF (30 ml) was added 50% sodium hydride (0.21 g, 5.2 mmol) at 0 °C. After the evolution of hydrogen gas ceased, *p*-methoxycarbonylbenzyl chloride (1.9 g, 11 mmol) in DMF (10 ml) and sodium iodide (0.69 g, 4.0 mmol) were added to the solution. The reaction mixture was stirred at 40 °C for 6 h. After neutralization with AcOH, the solvent was removed and the residue 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 a precipitate, which was collected by filtration. The crude product dissolved in $CHCl_3$ (2 ml) was applied to a column of silica gel (3.0×30 cm), equilibrated and eluted with $CHCl_3$. The solvent of the effluent (300—600 ml) was removed by evaporation. Pe-

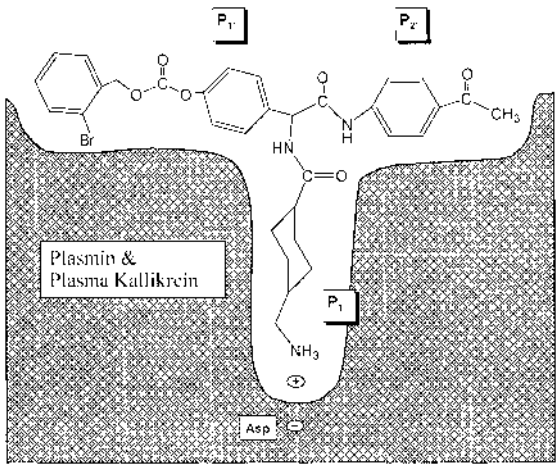


Fig. 1. Schematic Representation of Interaction of Tra-Tyr(*O*-BrZ)-ACA (II) with PL and PK

Table 4. IC_{50} Values (μM) of Compounds **14**—**17** for Various Enzymes

No.	P_1	$P_{1'}$	$P_{2'}$	PL		PK	UK	TH	
				S-2251	Fn	S-2302	S-2444	S-2238	Fg
14	H_2N-H_2C-C$_6$H$_4$-CO	Tyr(*O*-2-Pyrim)	-NH-C$_6$H$_4$-COOH	36	22	0.56	440	>1000	>1000
15	H_2N-H_2C-C$_6$H$_4$-CO	Tyr(*O*-2-Pyrim)	NH-C$_6$H$_4$-COOH	32	29	0.71	650	>1000	>1000
16	H_2N-H_2C-C$_6$H$_4$-CO	D,L-*m*-Tyr(*O*-2-Pyrim)	NH-C$_6$H$_4$-COOH	>400	>400	3.6	>400	>400	>400
17	H_2N-H_2C-C$_6$H$_4$-CO	D,L-*m*-Tyr(*O*-2-Pyrim)	-NH-C$_6$H$_4$-COOH	>500	410	6.8	>500	>500	>500

S-2251: D-Val-Leu-Lys-*p*NA, S-2302: D-Pro-Phe-Arg-*p*NA, S-2444: Glp-Gly-Arg-*p*NA, S-2238: D-Phe-Pip-Arg-*p*NA

troleum ether was added to the residue to yield a precipitate, which was collected by filtration, yield 1.16 g (57%), mp 134—137 °C, $[\alpha]_D^{25}$ +5.1° (c=1.0, MeOH), Rf^1 0.85. *Anal*. Calcd for $C_{31}H_{44}N_2O_6$: C, 67.7; H, 8.25; N, 5.09. Found: C, 68.1; H, 8.02; N, 4.98.

Boc-Tyr(*O*-iNoc)-OH To a solution of Boc-Tyr-OH (2.8 g, 10 mmol) in DMF (50 ml) was added 50% sodium hydride (1.1 g, 22 mmol) at 0 °C. After the evolution of hydrogen gas ceased, isonicotinyl *p*-nitrophenyl carbonate (3.0 g, 11 mmol) was added to the solution. The reaction mixture was stirred at 25—30 °C for 5 h to give a clear solution. After neutralization with AcOH, the solvent was removed *in vacuo*. The solution of the residue in water (30 ml) was washed with ether. The pH of the aqueous phase was adjusted to 6 with AcOH under cooling to give a precipitate, which was collected by filtration and recrystallized from EtOH, yield 1.8 g (43%), mp 161—162 °C, $[\alpha]_D^{25}$ +9.0° (c=0.9, MeOH), Rf^1 0.60, Rf^2 0.12. *Anal*. Calcd for $C_{21}H_{24}N_2O_7 \cdot 1/4\ H_2O$: C, 59.9; H, 5.86; N, 6.65. Found: C, 59.9; H, 5.86; N, 6.74.

Boc-Tyr(*O*-iNoc)-R [R: $NH(CH_2)_7CH_3$, $NH(CH_2)_2CH(CH_3)_2$] A mixed anhydride [prepared from Boc-Tyr(*O*-iNoc)-OH (1.2 g, 2.8 mmol) and isobutyl chloroformate (0.36 ml, 2.6 mmol) as usual] in THF (60 ml) was added to an ice-cooled solution of the corresponding amine (3.0 mmol) in THF (10 ml). The reaction mixture was stirred at 0 °C for 1 h and at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% $NaHCO_3$, 10% citric acid and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give a white precipitate, which was collected by filtration. R: $NH(CH_2)_7CH_3$, yield 81%, mp 118—122 °C, $[\alpha]_D^{25}$ +5.5° (c=0.9, MeOH), Rf^1 0.60, Rf^3 0.12. *Anal*. Calcd for $C_{29}H_{41}N_3O_6 \cdot 1/4H_2O$: C, 65.5; H, 7.83; N, 7.89. Found: C, 65.7; H, 8.04; N, 7.74. R: $NH(CH_2)_2CH(CH_3)_2$, yield 79%, mp 149—151 °C, $[\alpha]_D^{25}$ +4.1° (c=1.0, MeOH), Rf^1 0.67, Rf^3 0.67. *Anal*. Calcd for $C_{26}H_{35}N_3O_6$: C, 64.3; H, 7.27; N, 8.65. Found: C, 64.1; H, 7.39; N, 8.58.

Boc-Tyr-$NH(CH_2)_2CH(CH_3)_2$ Boc-Tyr(2-Br-Z)-$NH(CH_2)_2CH(CH_3)_2$[23] (3.1 g, 5.5 mmol) in MeOH (30 ml) was hydrogenated over a Pd catalyst. After removal of Pd and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give a precipitate, which was collected by filtration, yield 1.7 g (88%), mp 147—150 °C, $[\alpha]_D^{25}$ +6.1° (c=0.3, MeOH), Rf^1 0.70, Rf^2 0.60. *Anal*. Calcd for $C_{19}H_{30}N_2O_4 \cdot 1/4\ H_2O$: C, 64.3; H, 8.66; N, 7.89. Found: C, 64.6; H, 8.60; N, 7.87.

Boc-Tyr(*O*-Pic)-$NH(CH_2)_2CH(CH_3)_2$ To a solution of Boc-Tyr-$NH(CH_2)_2CH(CH_3)_2$ (1.7 g, 4.8 mmol) in DMF (50 ml) was added 60% sodium hydride (0.20 g, 5.0 mmol) at 0 °C. After the evolution of hydrogen gas ceased, 4-picolyl chloride [prepared from 4-picolyl chloride hydrochloride (0.82 g, 5.0 mmol)] in DMF (10 ml) was added to the solution. The reaction mixture was stirred at room temperature overnight. After neutalization with AcOH, the solvent was removed *in vacuo*. The residue was extracted with AcOEt. The extract was washed with 5% $NaHCO_3$, 10% citric acid and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give a precipitate, which was collected by filtration. The crude product in $CHCl_3$ (2 ml) was applied to a column of silica gel (3.0×25 cm), equilibrated and eluted with $CHCl_3$. The solvent of the effluent (1800—3000 ml) was removed by evaporation. Petroleum ether was added to the residue to form a precipitate, which was collected by filtration, yield 1.0 g (47%), mp 131—132 °C, $[\alpha]_D^{25}$ +5.8° (c=0.9, MeOH), Rf^2 0.53. *Anal*. Calcd For $C_{25}H_{35}N_3O_4$: C, 68.2; H, 8.33; N, 9.65. Found: C, 68.2; H, 8.10; N, 9.62.

General Procedure for Preparation of Boc-Tra-Tyr(X)-R [R: $NH(CH_2)_7CH_3$, $NH(CH_2)_2CH(CH_3)_2$, X: iNoc, Pic, CH_2-C$_6$H$_4$-COOMe] Boc-Tra-OH (0.31 g, 1.2 mmol) was added to the corresponding amine component [prepared from the corresponding N^{α}-protected Tyr derivative (1.2 mmol) and TFA (4.6 ml, 60 mmol) in the presence of anisole (0.13 ml, 1.2 mmol) as usual] in acetonitrile (30 ml) containing DIPEA (0.41 ml, 2.4 mmol) and BOP reagent (0.53 g, 1.2 mmol). The reaction mixture was stirred at room temperature for 15 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. Ether was added to the residue to give a white precipitate, which was collected by filtration and recrystallized from AcOEt. Yield, mp, $[\alpha]_D^{25}$ value, elemental analysis and *Rf* values are summarized in Table 5.

General Procedure for Preparation of H-Tra-Tyr(X)-R·HCl [R: $NH(CH_2)_7CH_3$, $NH(CH_2)_2CH(CH_3)_2$, X: iNoc, Pic, CH_2-C$_6$H$_4$-COOMe] Boc-Tra-Tyr(X)-R (0.30 mmol) was dissolved in TFA (1.28 ml, 17 mmol) containing anisole (0.04 ml, 0.34 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 60 min. Ether was added to the solution to yield a white precipitate, which was collected by filtration and dried over KOH pellets *in vacuo*. Each product was lyophilized from 0.1 M HCl (10 ml) to afford an amorphous powder. Yield, mp, $[\alpha]_D^{25}$ value, elemental analysis and *Rf* values are summarized in Table 6.

Boc-Tyr(*O*-Pic)-Tra-Octyl Ester To a solution of Boc-Tyr-Tra-octyl ester (1.75 g, 3.3 mmol) [prepared from Boc-Tyr(2-Br-Z)-Tra-octyl ester by catalytic hydrogenation as usual] in DMF (100 ml) was added 60% sodium hydride (0.33 g, 8.2 mmol) at 0 °C. After the evolution of hydrogen gas ceased, 4-picolyl chloride [prepared from 4-picolyl chloride hydrochloride (0.60 g, 0.82 mmol)] in DMF (30 ml) was added to the solution. The reaction mixture was stirred at room temperature overnight. After neutalization with AcOH, the solvent was removed *in vacuo*. The residue was extracted with AcOEt. The extract was washed with 5% $NaHCO_3$, 10% citric acid and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give an oily product. The crude product in $CHCl_3$ (2 ml) was applied to a column of silica gel (2.0×40 cm), equilibrated and eluted with $CHCl_3$. The solvent of the effluent (500—1300 ml) was removed by evaporation. Petroleum ether was added to the residue to give purified oily material, yield 1.25 g (61%), Rf^1 0.76.

Boc-Tra-Tyr(*O*-Pic)-Tra-Octyl Ester A mixed anhydride [prepared from Boc-Tra-OH (205 mg, 0.80 mmol), isobutyl chloroformate (0.11 ml, 0.80 mmol) and Et_3N (0.11 ml, 0.78 mmol) as usual] in THF (30 ml) was added to a solution of H-Tyr(*O*-Pic)-Tra-octyl ester [prepared from Boc-Tyr(*O*-Pic)-Tra-octyl ester (500 mg, 0.80 mmol), TFA (0.6 ml, 8.0 mmol) and anisole (0.26 ml, 2.4 mmol) as usual] in DMF (40 ml) containing Et_3N (0.11 ml, 0.78 mmol) under cooling with ice-water. The reaction mixture was stirred at room temperature overnight. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3, 10% citric acid and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were col-

Table 5. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Values of Boc-Tra-Tyr(X)-R

X:	R:	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (DMF)	Formula	Elemental analysis Calcd (Found)			TLC
						C	H	N	Rf^1
iNoc	$NH\text{-}(CH_2)_7$	52.0	169—179	−10.2 (c=1.0)	$C_{29}H_{54}N_4O_7 \cdot 1/4H_2O$	65.8 (66.0	8.20 8.10	8.28 8.30)	0.55
iNoc	$NH\text{-}(CH_2)_2\text{-}CH(CH_3)_2$	75.0	196—197	−10.2 (c=1.0)	$C_{34}H_{48}N_4O_7 \cdot 1/2H_2O$	64.4 (64.5	7.79 7.82	7.79 7.82)	0.58
Pic	$NH\text{-}(CH_2)_7$	72.0	188—191	−11.0 (c=1.0)	$C_{36}H_{54}N_4O_5$	69.4 (69.1	8.74 8.86	9.00 8.90)	0.62
Pic	$NH\text{-}(CH_2)_2\text{-}CH(CH_3)_2$	75.0	143—145	−11.0 (c=1.0)	$C_{33}H_{48}N_4O_5 \cdot 1/2H_2O$	65.2 (65.3	8.45 8.08	9.21 9.42)	0.58
CH_2-C$_6$H$_4$-$COOCH_3$	$NH\text{-}(CH_2)_7$	74.0	198.5—201	−9.1 (c=0.9)	$C_{39}H_{57}N_3O_7$	68.9 (68.7	8.45 8.57	6.18 6.15)	0.63

Table 6. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Values of H-Tra-Tyr(X)-R·HCl

X:	R:	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC
						C	H	N	*Rf*[3]
iNoc	NH-$(CH_2)_7$	78.0	Amorphous	+1.9 (c=0.4)	$C_{37}H_{46}N_4O_5$·HCl·TFA	54.5 (54.9	6.45 6.79	7.47 7.76)	0.34
iNoc	NH-$(CH_2)_2$-$CH(CH_3)_2$	83.0	Amorphous	−2.8 (c=0.9)	$C_{29}H_{40}N_4O_5$·2HCl·$2H_2O$	55.0 (54.9	7.31 7.18	8.84 8.78)	0.29
Pic	NH-$(CH_2)_7$	72.0	Amorphous	+2.5 (c=1.0)	$C_{31}H_{46}N_4O_3$·2HCl·$5/2H_2O$	68.7 (68.4	8.33 8.80	8.74 8.66)	0.42
Pic	NH-$(CH_2)_2$-$CH(CH_3)_2$	75.0	Amorphous	+1.0 (c=0.5)	$C_{28}H_{40}N_4O_3$·HCl·TFA·$5/2H_2O$	53.3 (53.1	7.00 6.77	8.28 8.53)	0.29
CH_2-  -$COOCH_3$	NH-$(CH_2)_7$	74.0	Amorphous	+4.4 (c=0.3)	$C_{34}H_{49}N_3O_5$·HCl·$3/2H_2O$	63.5 (63.6	8.30 8.00	6.53 6.38)	0.44

lected by filtration, yield 427 mg (69.8%), mp 153—155 °C, $[\alpha]_D^{25}$ −2.65° (c=1.0, $CHCl_3$), *Rf*[1] 0.79. *Anal*. Calcd for $C_{44}H_{66}N_4O_7 \cdot 1/2H_2O$: C, 68.4; H, 8.76; N, 7.25. Found: C, 68.6; H, 8.83; N, 7.20.

H-Tra-Tyr(*O*-Pic)-Tra-Octyl Ester·2HCl Boc-Tra-Tyr(*O*-Pic)-Tra-octyl ester (150 mg, 0.2 mmol) was dissolved in 7.2 N HCl/dioxane (0.14 ml, 1 mmol) containing anisole (0.7 ml, 0.6 mmol). After dilution of the above reaction mixture with dioxane (0.14 ml), the solution was stored at room temperature for 2 h. Dry ether was added to the solution to afford a precipitate, which was collected by filtration, yield 135 mg (93.8%), mp 214—216 °C, $[\alpha]_D^{25}$ −3.4° (c=1.0, MeOH), *Rf*[3] 0.58. *Anal*. Clacd for $C_{39}H_{58}N_4O_5 \cdot 2HCl \cdot 3/2H_2O$: C, 61.4; H, 8.32; N, 7.31. Found: C, 61.8; H, 8.09; N, 7.00.

Boc-EACA-Tyr(*O*-Pic)-Tra-Octyl Ester A mixed anhydride [prepared from Boc-EACA-OH (185 mg, 0.80 mmol), isobutyl chloroformate (0.11 ml, 0.80 mmol) and Et_3N (0.11 ml, 0.78 mmol) as usual] in THF (30 ml) was added to a solution of H-Tyr(*O*-Pic)-Tra-octyl ester [prepared from Boc-Tyr(*O*-Pic)-Tra-octyl ester (500 mg, 0.80 mmol), TFA (0.6 ml, 8.0 mmol) and anisole (0.26 ml, 2.4 mmol) as usual] in DMF (40 ml) containing Et_3N (0.11 ml, 0.78 mmol) under cooling with ice-water. The reaction mixture was stirred at room temperature overnight. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3, 10% citric acid and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 430 mg (73.1%), mp, 85—88 °C, $[\alpha]_D^{25}$ −3.3° (c=1.0, $CHCl_3$), *Rf*[1] 0.70. *Anal*. Calcd for $C_{42}H_{64}N_4O_7$: C, 68.4; H, 8.77; N, 7.60. Found: C, 68.3; H, 8.94; N, 7.36.

H-EACA-Tyr(*O*-Pic)-Tra-Octyl Ester·2HCl Boc-EACA-Tyr(*O*-Pic)-Tra-octyl ester (150 mg, 0.20 mmol) was dissolved in 7.2 N HCl/dioxane (0.14 ml, 1 mmol) containing anisole (0.7 ml, 0.6 mmol). After dilution of the above reaction mixture with dioxane (0.14 ml), the solution was stored at room temperature for 2 h. Dry ether was added to the solution to afford a precipitate, which was collected by filtration, yield 140 mg (99%), amorphous, $[\alpha]_D^{25}$ +1.5° (c=1.0, MeOH), *Rf*[3] 0.61. *Anal*. Calcd for $C_{37}H_{56}N_4O_5 \cdot 2HCl \cdot 2H_2O$: C, 59.6; H, 8.37; N, 7.51. Found: C, 60.0; H, 8.01; N, 7.36.

Boc-EACA-Tyr(*O*-Pic)-NH-$(CH_2)_7$-CH_3 A mixed anhydride [prepared from Boc-EACA-OH (185 mg, 0.80 mmol), isobutyl chloroformate (0.11 ml, 0.80 mmol) and Et_3N (0.11 ml, 0.78 mmol) as usual] in THF (30 ml) was added to a solution of H-Tyr(*O*-Pic)-octylamide [prepared from Boc-Tyr(*O*-Pic)-octylamide (500 mg, 0.80 mmol), TFA (0.6 ml, 8.0 mmol) and anisole (0.26 ml, 2.4 mmol) as usual] in DMF (40 ml) containing Et_3N (0.11 ml, 0.78 mmol) under cooling with ice-water. The reaction mixture was stirred at room temperature overnight. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3, 10% citric acid 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 washed with ether, yield 420 mg (87.9%), mp, 92—94 °C, $[\alpha]_D^{25}$ −8.7° (c=1.0, DMF), *Rf*[1] 0.56. *Anal*. Calcd for $C_{34}H_{52}N_4O_5$: C, 68.4; H, 8.78; N, 9.39. Found: C, 68.2; H, 8.91; N, 9.29.

H-EACA-Tyr(*O*-Pic)-NH-$(CH_2)_7$-CH_3·2HCl Boc-EACA-Tyr(*O*-Pic)-octylamide (150 mg, 0.25 mmol) was dissolved in 7.2 N HCl/dioxane (0.14 ml, 1 mmol) containing anisole (0.7 ml, 0.6 mmol). After dilution of the above reaction mixture with dioxane (0.14 ml), the solution was stored at room temperature for 2 h. Dry ether was added to the solution to afford a precipitate, which was collected by filtration, yield 140 mg (90%), amorphous, $[\alpha]_D^{25}$ +10.6° (c=1.0, MeOH), *Rf*[3] 0.27. *Anal*. Clacd for $C_{29}H_{44}N_4O_3 \cdot 2HCl \cdot 2H_2O$: C, 57.5; H, 8.32; N, 9.25. Found: C, 57.9; H, 8.09; N, 9.27.

Boc-NH$(CH_2)_7$-COOH $(Boc)_2O$ (8.1 g, 37.2 mmol) in dioxane (30 ml) was added to a solution of 8-aminooctanoic acid (5.0 g, 31 mmol) in water (30 ml) containing Et_3N (6.5 ml, 46.5 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was dissolved in water. The pH of the solution was adjusted to 4 with citric acid and a 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 afford crystals, which were collected by filtration, yield 6.87 g (85.5%), mp 56—56.5 °C, *Rf*[1] 0.37, *Rf*[2] 0.59. *Anal*. Calcd for $C_{13}H_{25}NO_4$: C, 60.2; H, 9.72; N, 5.40. Found: 60.2; H, 9.82; 5.37.

Boc-NH$(CH_2)_7$-COOPac Boc-NH$(CH_2)_7$COOH (2.59 g, 10 mmol) was dissolved in MeOH (20 ml) containing Cs_2CO_3 (1.62 g, 5.0 mmol) in H_2O (8 ml). Phenacyl bromide (1.99 g, 10 mmol) was added to the above solution and the reaction mixture was stirred at room temperature for 4 d. After removal of the white precipitate (CsBr), the solvents were removed by evaporation. The residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 3.8 g (100%), mp 68—69 °C, *Rf*[1] 0.76. *Anal*. Calcd for $C_{21}H_{31}NO_5$: C, 66.8; H, 8.28; N, 3.71. Found: C, 66.7; H, 8.18; N, 3.64.

Boc-Tyr(*O*-Pic)-NH$(CH_2)_7$-COOPac To a solution of Boc-Tyr(*O*-Pic)-OH (0.93 g, 2.5 mmol), H_2N-$(CH_2)_7$-COOPac·TFA [prepared from corresponding Boc derivative (1.0 g, 2.4 mmol), TFA (4.6 ml, 60 mmol) and anisole (0.5 ml, 4.6 mmol) as usual] in DMF (10 ml) containing Et_3N (0.84 ml, 6 mmol), BOP reagent (1.54 g, 3.5 mmol) was added. 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, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 1.03 g (65.3%), mp 131—134 °C, $[\alpha]_D^{25}$ −5.4° (c=1.0, DMF), *Rf*[1] 0.70. *Anal*. Calcd for $C_{36}H_{45}N_3O_7 \cdot H_2O$: C, 66.5; H, 7.29; N, 6.46. Found: C, 66.7; H, 7.10; N, 6.44.

Boc-Tra-Tyr(*O*-Pic)-NH$(CH_2)_7$-COOPac To a solution of H-Tyr(*O*-Pic)-NH$(CH_2)_7$-COOPac·TFA [prepared from corresponding Boc-derivative (720 mg, 1.2 mmol), TFA (1.83 ml, 24 mmol) and anisole (0.18 ml, 1.6 mmol) as usual] and Boc-Tra-OH (308 mg, 1.2 mmol) in DMF (10 ml) containing Et_3N (0.37 ml, 2.6 mmol), BOP reagent was added. 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 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, yield 410 mg (44%), mp 180—181 °C, $[\alpha]_D^{25}$ −10.7° (c=1.0, DMF), *Rf*[1] 0.48. *Anal*. Calcd for $C_{44}H_{58}N_4O_8$: C, 68.5; H, 7.58; N, 7.27. Found: C, 68.3; H, 7.56; N, 7.14.

H-Tra-Tyr(*O*-Pic)-NH-$(CH_2)_7$-COOH·2TFA Boc-Tra-Tyr(*O*-Pic)-NH$(CH_2)_7$-COOPac (200 mg, 0.26 mmol) was dissolved in 90% AcOH (10 ml) and DMF (8 ml). To the solution, Zn powder 1.70 g (26 mmol) was added. The reaction mixture was stirred at 40 °C overnight. After removal of

Zn powder and the solvents, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to produce crystals, which were collected by filtration, yield 160 mg (92.6%), mp 209—211 °C, Rf^1 0.46. Boc-Tra-Tyr(*O*-Pic)-NH$(CH_2)_7$-COOH (70 mg, 0.11 mmol) was dissolved in TFA (0.5 ml, 6.5 mmol) containing anisole (50 μl). The solution was stored at room temperature for 1 h. Ether was added to the solution to give a precipitate, which was collected by centrifugation and lyophilized from water, yield 76 mg. The crude material was purified with preparative HPLC to give 45 mg of purified material (31.9%), Rf^1 0.05. *Anal*. Clacd for $C_{31}H_{44}N_4O_5 \cdot 2TFA \cdot 2H_2O$: C, 52.6; H, 6.06; N, 7.01. Found: 52.3; H, 5.78; N, 7.08.

Fmoc-Tyr(*O*-Pic)-OH To a suspension of H-Tyr(*O*-Pic)-OH·TFA [prepared from Boc-Tyr(*O*-Pic)-OH (7.87 g, 0.02 mol), TFA (30.6 ml, 0.4 mol) and anisole (3.0 ml, 0.028 mol) as usual] in 10% Na_2CO_3 (60 ml), Fmoc-OSu (13.13 g, 0.039 mol) in dimethoxyethane (100 ml) was added dropwise under cooling with ice. The reaction mixture was stirred at room temperature overnight. After removal of insoluble material by filtration, the pH of the filtrate was adjusted to 7 with AcOH and the solvent was removed by evaporation. The residue was extracted with AcOEt. The extract was washed with 0.1 M HCl and water. The AcOEt layer was concentrated to afford crystals, which were collected by filtration and washed with water, yield 9.5 g (92.4%), mp 208—211 °C, $[\alpha]_D^{25}$ −17.7° (*c*=1.0, DMF). *Anal*. Calcd for $C_{30}H_{26}N_2O_5$: C, 72.9; H, 5.30; N, 5.67. Found: C, 73.0; H, 5.23; N, 5.49.

Boc-NH-$(CH_2)_7$-NH_2[27] $(Boc)_2O$ (5 g, 23 mmol) in 50 ml dioxane was added over 3 h to 1,7-diaminoheptane (11.9 g, 92 mmol) in dioxane (100 ml). The mixture was stirred at room temperature for 2 h. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3 (7×200 ml) and water, dried over Na_2SO_4 and evaporated down to give an oily product of 4.2 g (86.8%). The crude product (1.49 g) in $CHCl_3$ (2 ml) was applied to a column of silica gel (1.7×30 cm), equilibrated and eluted with $CHCl_3$. The solvent of the effluent (1200 ml—3900 ml) was removed by evaporation, which was collected as an oily material, yield 1.5 g (79.2%), Rf^4=0.36, MALDI-TOF-MAS: *m/z*=231.50 $(M+1)^+$.

Fmoc-Tyr(*O*-Pic)-NH$(CH_2)_7$NH-Boc A solution of Boc-NH$(CH_2)_7NH_2$ (2 g, 8.7 mmol) in DMF (100 ml) was added to Fmoc-Tyr(*O*-Pic)-OH in DMF (100 ml) in presence of PyBOP (5.41 g, 10.4 mmol), HOBt·H_2O (1.78 g, 10.4 mmol) and DIPEA (1.8 ml, 10.4 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 5 min and was further 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 a white precipitate which was collected by filtration and recrystallized from EtOH, yield 3.68 g (63.2%), mp 134—135.5 °C, $[\alpha]_D^{25}$ −12.6° (*c*=1.0, $CHCl_3$), Rf^1 0.66. *Anal*. Calcd For $C_{42}H_{50}N_4O_6$: C, 71.4; H, 7.13; N, 7.93. Found: C, 71.3; H, 7.24; N, 8.00.

Boc-Tra-Tyr(*O*-Pic)-NH$(CH_2)_7$NH-Boc Piperidine (8 ml) in DMF (40 ml) was added to the solution of Fmoc-Tyr(*O*-Pic)-NH$(CH_2)_7$NH-Boc (1 g, 1.39 mmol) in DMF (50 ml) and stirred at room temperature for 1.5 h. After removal of the solvent, ether was added to the residue to afford crystals [H-Tyr(*O*-Pic)-NH$(CH_2)_7$NH-Boc], which were collected by filtration, yield 0.42 g, (78.7%). A solution of H-Tyr(*O*-Pic)-NH$(CH_2)_7$NH-Boc in DMF (20 ml) was added to Boc-Tra-OH (0.36 g, 1.4 mmol) in DMF (20 ml) in the presence of PyBOP (0.73 g, 1.4 mmol), HOBt·H_2O (0.24 g, 1.6 mmol) and DIPEA (0.24 ml, 1.4 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight. After removal of the solvents, the residue was extracted with AcOEt. The extract was washed with 10% citrtic acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated to dryness. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH, yield 0.45 g (56.5%), mp 175—176 °C, $[\alpha]_D^{25}$ −3.8° (*c*=1.0, $CHCl_3$), Rf^1=0.63. *Anal*. Calcd For $C_{42}H_{61}N_5O_7$: C, 65.6; H, 8.53; N, 9.56. Found: C, 65.6; H, 8.45; N, 9.60.

H-Tra-Tyr(*O*-Pic)-NH$(CH_2)_7NH_2 \cdot 3TFA$ Boc-Tra-Tyr(*O*-Pic)-NH-$(CH_2)_7$NH-Boc (0.25 g, 0.35 mmol) was dissolved in TFA (0.79 ml, 6.9 mmol) containing anisole (0.79 ml, 6.9 mmol) at 0° and the reaction mixture was stirred at 0 °C for 10 min and was further stirred at room temperature for 1 h. After removal of the solvent, dry ether was added to the residue to give a precipitate, which was collected by filtration, yield 0.17 g (94.9%), mp 134—136 °C $[\alpha]_D^{25}$ −9.5° (*c*=1.0, $CHCl_3$), Rf^6 0.7. *Anal*. Calcd For $C_{30}H_{45}N_5O_3 \cdot 3TFA \cdot 2H_2O$: C, 48.0; H, 5.59; N, 7.77. Found: C, 47.7; H, 5.42; N, 7.95. MALDI-TOF-MAS: *m/z*=524.75 $(M+1)^+$.

***N*-Boc-*p*- or -*m*-Aminobenzoic Acid** $(Boc)_2O$ (4.8 g, 22 mmol) in dioxane (100 ml) was added to a solution of *p*- or *m*-aminobenzoic acid (4.3 g, 20 mmol) in water (50 ml) containing Et_3N (4.2 ml, 30 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was dissolved in water. The pH of the solution was adjusted to 4 with citric acid to give an oily precipitate. 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 afford crystals, which were collected by filtration and recrystallized from EtOH. Boc-*p*-amino benzoic acid: yield 2.0 g (41.2%), mp 193—195 °C, Rf^1 0.59, Rf^2 0.77. *Anal*. Calcd for $C_{12}H_{15}NO_4$: C, 60.8; H, 6.37; N, 5.90. Found: C, 60.9; H, 6.39; N, 5.75. Boc-*m*-aminobenzoic acid: yield 1.5 g (32.6%), mp 198—199 °C, Rf^1 0.50. *Anal*. Calcd for $C_{12}H_{15}NO_4$: C, 60.8; H, 6.37; N, 5.90. Found:C, 60.8; H, 6.48; N, 5.94.

Benzyl *N*-Boc-*p*-Aminobenzoate *N*-Boc-*p*-aminobenzoic acid (2.4 g, 10 mmol) was dissolved in MeOH (40 ml) and water (5 ml) and the pH of the solution was adjusted to 8 by adding 5% Cs_2CO_3 in water (5 ml). After removal of the solvents, the residue was dissolved in DMF (30 ml) and the DMF was removed. To a solution of the residue in DMF (25 ml), benzyl bromide (1.34 ml, 11 mmol) was added under cooling with ice. After 4 h, the solvent was removed and water was added to the residue to yield a white powder, which was collected by filtration. The crude material was dissolved in AcOEt. The AcOEt layer was washed with water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford white powder, which was collected by filtration, yield 2.7 g (82.5%), mp 148—150 °C, Rf^2 0.83. *Anal*. Calcd for $C_{19}H_{21}NO_4$: C, 69.7; H, 6.47; N, 4.28. Found: C, 69.6; H, 6.37; N, 4.10.

Benzyl *N*-Boc-*m*-Aminobenzoate *N*-Boc-*m*-aminobenzoic acid (4.7 g, 19.4 mmol) was dissolved in MeOH (80 ml) and water (10 ml) and the pH of the solution was adjusted to 8 by adding 20% Cs_2CO_3 in water (10 ml). After removal of the solvents, the residue was dissolved in DMF (50 ml) and the DMF was removed. To a solution of the residue in DMF (40 ml), benzyl bromide (2.4 ml, 19.6 mmol) was added under cooling with ice. After stirring at 30 °C for 10 h, the solid material (CsBr) and the solvent were removed. The residue was extracted with AcOEt. The AcOEt layer was washed with 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford white powder, which was collected by filtration, yield 3.0 g (47.2%), mp 143—145 °C, Rf^1 0.78. *Anal*. Calcd for $C_{19}H_{21}NO_4$: C, 69.7; H, 6.47; N, 4.28. Found: C, 69.5; H, 6.30; N, 4.20.

Boc-Tyr(*O*-2-Pyrim)-OH To a solution of Boc-Tyr-OH (2.81 g, 10 mmol) in DMF (50 ml) was added 60% sodium hydride (0.88 g, 22 mmol) at 0 °C. After the evolution of hydrogen gas ceased, 2-pyrimidyl chloride (1.37 g, 12 mmol) was added. The reaction mixture was stirred at 33 °C for 4 h. After removal of the solvent, the residue was extracted with AcOEt and water. The pH of the water layer was adjusted to 4 with AcOH to afford a white precipitate, which 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 afford crude product. The crude product in $CHCl_3$ (2 ml) was applied to a column of silica gel (3.0×25 cm), equilibrated and eluted with $CHCl_3$ (1300 ml) followed by 1% MeOH in $CHCl_3$ (500 ml). The solvent of the effluent (800—1700 ml) was removed by evaporation. Petroleum ether and ether were added to the residue to afford a precipitate, which was collected by filtration, yield 1.36 g (37%), mp 145—147 °C, $[\alpha]_D^{25}$ −18.0° (*c*=1.0, DMF), Rf^1 0.56, Rf^2 0.39. *Anal*. Calcd For $C_{18}H_{21}N_3O_5 \cdot 1/4H_2O$: C, 59.4; H, 5.95; N, 11.6. Found: C, 59.6; H, 5.95; N, 11.2.

***N*-Boc-D,L-*m*-Tyr-(*O*-2-Pyrim)-OH** To a solution of H-D,L-*m*-Tyr-OH (4.0 g, 22 mmol) in H_2O (30 ml) containing Et_3N (4.6 ml, 33 mmol), $(Boc)_2O$ (5.8 g, 26 mmol) in dioxnae (30 ml) was added. The reaction mixture was stirred at room temperature for 4 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid 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, yield 5.78 g (93.4%), mp 133—136 °C, Rf^1 0.26, Rf^2 0.13. To a solution of Boc-D,L-*m*-Tyr-OH (5.0 g, 18 mmol) in DMF (100 ml) was added 60% sodium hydride (1.4 g, 40 mmol) at 0 °C. After the evolution of hydrogen gas ceased, 2-pyrimidyl chloride (2.5 g, 22 mmol) was added. The reaction mixture was stirred at 40 °C for 4 h. After removal of the solvent, the residue was extracted with 5% Na_2CO_3 and AcOEt. The pH of the water layer was adjusted to 4 with AcOH to afford an oily material, which 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 afford crude product, which was collected by filtration and recrystallized from petroleum ether and ether, yield 2.21 g (34.1%), mp 160–162 °C, $[\alpha]_D^{25}$ −2.2° (*c*=1.0, DMF), Rf^1 0.55, Rf^2 0.10. *Anal*. Calcd For $C_{18}H_{21}N_3O_5$: C, 60.2; H, 5.89; N, 11.7. Found: C, 59.9; H, 5.89; N, 11.4.

Benzyl *N*-Boc-Tyr(*O*-2-Pyrim)-*p*-Aminobenzoate A mixed anhydride

[prepared from Boc-Tyr(*O*-2-Pyrim)-OH (0.72 g, 2.0 mmol), isobutyl chloroformate (0.26 ml, 2.0 mmol) and Et_3N (0.28 ml, 2.0 mmol) as usual] in THF (20 ml) was added to a solution of benzyl *p*-aminobenzoate [prepared from corresponding Boc derivative (0.65 g, 2.0 mmol), TFA (4.6 ml, 60 mmol) and anisole (0.50 ml, 4.6 mmol) as usual] in THF (20 ml) containing Et_3N (0.42 ml, 3 mmol) under cooling with ice. 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 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down to afford an oily material. The crude material in $CHCl_3$ (2 ml) was applied to a silca gel column (3.0×21.0 cm), equilibrated and eluted with $CHCl_3$. The solvent of the effluent (700—1200 ml) was removed by evaporation. Petroleum ether was added to the residue to afford amorphous powder, which was collected by filtration, yield 0.57 g (25.2%), $[\alpha]_D^{25}$ +47.6° (*c*=0.2, DMF), *Rf*[7] 0.15 *Anal*. Calcd for $C_{32}H_{32}N_4O_6$: C, 67.6; H, 5.67; N, 9.85. Found: C, 67.8; H, 5.96; N, 9.47.

Benzyl *N*-Boc-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoate A mixed anhydride [prepared from Boc-Tyr(*O*-2-Pyrim)-OH (1.57 g, 4.37 mmol), pivaloyl chloride (0.54 ml, 4.5 mmol) and Et_3N (0.63 ml, 4.5 mmol) as usual] in THF (20 ml) was added to a solution of benzyl *m*-aminobenzoate [prepared from the corresponding Boc derivative (0.98 g, 3 mmol), TFA (4.6 ml, 86 mmol) and anisole (0.46 ml, 3 mmol) as usual and converted to HCl salt by 7.1 N HCl/dioxane (0.81 ml, 6 mmol)] in DMF (20 ml) containing Et_3N (0.42 ml, 3 mmol) under cooling with ice. 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 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down to afford an oily material. The crude material in $CHCl_3$ (2 ml) was applied to a silca gel column (3.0×21.0 cm), equilibrated and eluted with $CHCl_3$. The solvent of the effluent (400—800 ml) was removed by evaporation. Petroleum ether was added to the residue to afford amorphous powder, which was collected by filtration, yield 0.51 g (24.0%), mp 74—78 °C, $[\alpha]_D^{25}$ +16.8° (*c*=1.0, DMF), *Rf*[1] 0.59, *Rf*[7] 0.14. *Anal*. Calcd for $C_{32}H_{32}N_4O_6$: C, 67.6; H, 5.67, N, 9.85. Found: C, 67.4; H, 5.81; N, 9.47.

Benzyl *N*-Boc-D,L-*m*-Tyr(*O*-2-Pyrim)-*p*-*A*minobenzoate A mixed anhydride [prepared from Boc-D,L-*m*-Tyr(*O*-2-Pyrim)-OH (0.72 g, 2.0 mmol), pivaloyl chloride (0.24 ml, 2.0 mmol) and Et_3N (0.84 ml, 6.0 mmol) as usual] in THF (20 ml) was added to a solution of benzyl *p*-aminobenzoate [prepared from the corresponding Boc derivative (0.65 g, 2.0 mmol), TFA (3.1 ml, 40 mmol) and anisole (0.30 ml, 3.0 mmol) as usual] in THF (20 ml) containing Et_3N (0.42 ml, 3 mmol) under cooling with ice. 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 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down to afford an oily material. The crude material in $CHCl_3$ (2 ml) was applied to a silca gel column (3.0×19.0 cm), equilibrated and eluted with $CHCl_3$ (1300 ml), followed by 1% MeOH in $CHCl_3$ (600 ml). The solvent of the effluent (500—1700 ml) was removed by evaporation. Petroleum ether was added to the residue to give a powder, which was collected by filtration, yield 0.25 g (22.0%), mp 112—113 °C, $[\alpha]_D^{25}$ −2.3° (*c*=1.0, DMF), *Rf*[1] 0.55, *Rf*[7] 0.11. *Anal*. Calcd for $C_{32}H_{32}N_4O_6$: C, 67.6; H, 5.67, N, 9.85. Found: C, 67.3; H, 5.72; N, 9.81.

Benzyl *N*-Boc-D,L-*m*-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoate A mixed anhydride [prepared from Boc-D,L-*m*-Tyr(O-2-Pyrim)-OH (0.72 g, 2.0 mmol), pivaloyl chloride (0.24 ml, 2.0 mmol) and Et_3N (0.28 ml, 2.0 mmol) as usual] in THF (20 ml) was added to a solution of benzyl *m*-aminobenzoate [prepared from the corresponding Boc derivative (0.65 g, 2.0 mmol), TFA (3.1 ml, 40 mmol) and anisole (0.30 ml, 3.0 mmol) as usual] in THF (20 ml) containing Et_3N (0.42 ml, 3 mmol) under cooling with ice. 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 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the resiude to give a powder. The crude material in $CHCl_3$ (2 ml) was applied to a silca gel column (3.0×19.0 cm), equilibrated and eluted with $CHCl_3$ (1300 ml). The solvent of the effluent (400—900 ml) was removed by evaporation. Petroleum ether was added to the residue to afford a powder, which was collected by filtration, yield 0.70 g (61.6%), mp 70—79 °C $[\alpha]_D^{25}$ −3.3° (*c*=1.0, DMF), *Rf*[1] 0.64, *Rf*[7] 0.20. *Anal*. Calcd for $C_{32}H_{32}N_4O_6$: C, 67.6; H, 5.67, N, 9.85. Found: C, 67.4; H, 5.65; N, 9.72.

Benzyl *N*-Boc-Tra-Tyr(*O*-2-Pyrim)-*p*-Aminobenzoate A mixed anhydride [prepared from Boc-Tra-OH (60 mg, 0.21 mmol), isobutyl chloroformate (27 μl, 0.21 mmol) and Et_3N (29 μl, 0.21 mmol) as usual] in THF (20 ml) was added to a solution of H-Tyr(*O*-2-Pyrim)-*p*-aminobenzoic acid benzyl ester trifluoroacetate [prepared from the corresponding Boc derivative (120 mg, 0.21 mmol), TFA (0.48 ml, 6.3 mmol) and anisole (0.05 ml, 0.46 mmol) as usual] in DMF (20 ml) containing Et_3N (29 μl, 0.21 mmol). 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 afford crystals, which were collected by filtration and recrystallized from EtOH, yield 120.0 mg (80.8%), mp 182.5—184 °C, $[\alpha]_D^{25}$ +37.1° (*c*=0.4, DMF), *Rf*[1] 0.66, *Rf*[2] 0.84. *Anal*. Calcd for $C_{40}H_{45}N_5O_7$: C, 67.8; H, 6.41; N, 9.90. Found: C, 67.8; H, 6.45; N, 9.74.

Benzyl *N*-Boc-Tra-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoate A mixed anhydride [prepared from Boc-Tra-OH (210 mg, 0.81 mmol), isobutyl chloroformate (0.10 ml, 0.81 mmol) and Et_3N (0.11 ml, 0.81 mmol) as usual] in THF (20 ml) was added to a solution of H-Tyr(*O*-2-Pyrim)-*m*-aminobenzoic acid benzyl ester trifluoroacetate [prepared from the corresponding Boc derivative (350 mg, 0.6 mmol), TFA (0.94 ml, 12 mmol) and anisole (0.1 ml, 0.9 mmol) as usual] in THF (20 ml) containing Et_3N (0.10 ml, 0.71 mmol). 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 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from AcOEt, yield 170 mg (40.0%), mp 180—182 °C, $[\alpha]_D^{25}$ +20.5° (*c*=1.0, DMF), *Rf*[1] 0.56, *Rf*[2] 0.79. *Anal*. Calcd for $C_{40}H_{45}N_5O_7 \cdot 1/4H_2O$: C, 67.4; H, 6.43; N, 9.83. Found: C, 67.6; H, 6.46; N, 9.79.

Benzyl *N*-Boc-Tra-D,L-*m*-Tyr(*O*-2-Pyrim)-*p*-Aminobenzoate A mixed anhydride [prepared from Boc-Tra-OH (110 mg, 0.42 mmol), isobutyl chloroformate (50 μl, 0.42 mmol) and Et_3N (60 μl, 0.42 mmol) as usual] in THF (20 ml) was added to a solution of H-D,L-m-Tyr(*O*-2-Pyrim)-*p*-aminobenzoic acid benzyl ester trifluoroacetate [prepared from the corresponding Boc derivative (240 mg, 0.42 mmol), TFA (0.7 ml, 8.4 mmol) and anisole (70 μl, 0.65 mmol) as usual] in DMF (20 ml) containing Et_3N (60 μl, 0.42 mmol). 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 afford crystals, which were collected by filtration. A crude material in $CHCl_3$ (2 ml) was applied to a WAKO gel 300 column (2.5×21 cm), equilibrated and eluted with $CHCl_3$ (255 ml), followed by 1% MeOH in $CHCl_3$ (600 ml). The solvent of an effluent (435—800 ml) was removed by evaporation. Petroleum ether was added to the residue to afford a powder, which was collected by filtration, yield 80 mg (26.9%), mp 152—153 °C, $[\alpha]_D^{25}$ +0.49° (*c*=1.0, MeOH), *Rf*[1] 0.58, *Rf*[7] 0.16. *Anal*. Calcd for $C_{40}H_{45}N_5O_7 \cdot 1/4H_2O$: C, 67.4; H, 6.43; N, 9.83. Found: C, 67.7; H, 6.38; N, 9.87.

Benzyl *N*-Boc-Tra-D,L-*m*-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoate A mixed anhydride [prepared from Boc-Tra-OH (140 mg, 0.53 mmol), isobutyl chloroformate (70 μl, 0.53 mmol) and Et_3N (70 μl, 0.53 mmol) as usual] in THF (20 ml) was added to a solution of H-D,L-*m*-Tyr(*O*-2-Pyrim)-*m*-aminobenzoic acid benzyl ester trifluoroacetate [prepared from the corresponding Boc derivative (300 mg, 0.53 mmol), TFA (1.0 ml, 13 mmol) and anisole (0.1 ml, 0.9 mmol) as usual] in THF (20 ml) containing Et_3N (0.10 ml, 0.71 mmol). 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 afford crystals, which were collected by filtration. A crude material in AcOEt (2 ml) was applied to a silica gel column (3×21 cm), equilibrated and eluted with AcOEt and *n*-hexane (1 : 1, 390 ml), followed by AcOEt and *n*-hexane (2 : 1, 800 ml). The solvent of the effluent (120—1100 ml) was removed by evaporation. Petroleum ether was added to the residue to afford a powder, which was collected by filtration, yield 110 mg (29.3%), mp 178—181 °C, $[\alpha]_D^{25}$ −3.2° (*c*=1.0, DMF), *Rf*[1] 0.60, *Rf*[7] 0.15. *Anal*. Calcd for $C_{40}H_{45}N_5O_7 \cdot 1/4H_2O$: C, 67.4; H, 6.43; N, 9.83. Found: C, 67.5; H, 6.46; N, 9.90.

H-Tra-Tyr(*O*-2-Pyrim)-*p*-aminobenzoic Acid·TFA Benzyl *N*-Boc-Tra-Tyr(*O*-2-Pyrim)-*p*-aminiobenzoate (150 mg, 0.21 mmol) in DMF (2 ml) and MeOH (20 ml) was hydrogenated for 2 h over a Pd catalyst. After removal of Pd and the solvent, ether was added to the residue to yield crystals, which were collected by filtration. The crude material in $CHCl_3$ and MeOH (1 ml+1 ml) was applied to a silica gel column (3×13 cm), equilibrated with $CHCl_3$ and eluted stepwise with $CHCl_3$ (300 ml), 1% MeOH in $CHCl_3$ (200 ml), 3% MeOH in $CHCl_3$ (400 ml), 5% MeOH in $CHCl_3$ (200 ml) and 8% MeOH in $CHCl_3$ (800 ml). The solvent of the effluent (5% and 8% MeOH in $CHCl_3$) was removed by evaporation to yield solid material, which was collected by filtration and recrystallized from AcOEt and ether, yield 117 mg (88%), mp 241—244 °C, *Rf*[1] 0.56, *Rf*[2] 0.42. Boc-Tra-Tyr(*O*-2-Pyrim)-*p*-aminobenzoic acid (80 mg, 0.13 mmol) was dissolved in TFA (0.5

ml, 6.5 mmol) containing anisole (0.05 ml, 0.46 mmol) and the solution was stored at room temperature for 1 h. Ether was added to the solution to give a precipitate, which was collected by centrifugation and washed with ether. The crude material in 3% AcOH (2 ml) was applied to a Sephadex G-15 column (2.3×64 cm), equilibrated and eluted with 5% AcOH. The solvent of the effluent (180—300 ml) was completely removed by evaporation. EtOH was added to the residue to afford a solid material, yield 75.0 mg (67.1%), mp 165—175 °C, $[\alpha]_D^{25}$ +37.4° (c=0.7, MeOH), Rf^4 0.24, Rf^5 0.67. *Anal*. Calcd for $C_{28}H_{31}N_5O_5 \cdot 0.5$TFA: C, 60.6; H, 5.52; N, 12.2. Found: C, 60.7; H, 6/01; N, 12.0.

H-Tra-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoic Acid·TFA Benzyl *N*-Boc-Tra-Tyr(*O*-2-Pyrim)-*m*-aminiobenzoate (180 mg, 0.25 mmol) in DMF (2 ml) and MeOH (20 ml) was hydrogenated for 2 h over a Pd catalyst. After removal of a Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 140 mg (88%), mp 224—229 °C. A solution of *N*-Boc-Tra-Tyr(*O*-2-Pyrim)-*m*-aminiobenzoic acid (80 mg, 0.13 mmol) in TFA (0.70 ml, 5.0 mmol) containing anisole (50 μl) was stored at room temperature for 90 min. Ether was added to the solution to afford a precipitate, which was collected by centrifugation, yield 68 mg (85.0%), mp 241—244 °C, $[\alpha]_D^{25}$ +16.6° (c=0.56, 10% AcOH), Rf^2 0.22. *Anal*. Calcd for $C_{28}H_{31}N_5O_5 \cdot 1.5TFA\cdot H_2O$: C, 52.7; H, 4.92; N, 9.91. Found: C, 52.8; H, 4.83; N, 9.92.

H-Tra-D,L-*m*-Tyr(*O*-2-Pyrim)-*p*-Aminobenzoic Acid·TFA Benzyl *N*-Boc-Tra-D,L-*m*-Tyr(*O*-2-Pyrim)-*p*-aminiobenzoic acid (100 mg, 0.14 mmol) in DMF (2 ml) and MeOH (20 ml) was hydrogenated for 2 h over a Pd catalyst. After removal of Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 80 mg (81%), Rf^1 0.58, Rf^2 0.33. A solution of *N*-Boc-Tra-D,L-*m*-Tyr(*O*-2-Pyrim)-*p*-aminiobenzoic acid (80 mg, 0.13 mmol) in TFA (0.70 ml, 5.0 mmol) containing anisole (50 μl) was stored at room temperature for 90 min. Ether was added to the solution to afford a precipitate, which was collected by centrifugation, purified with HPLC, yield 68 mg (85.0%), amorphous, $[\alpha]_D^{25}$ −1.5° (c= 0.70, 10% AcOH), Rf^3 0.40, Rf^4 0.40, Rf^5 0.47. *Anal*. Calcd for $C_{28}H_{31}N_5O_5 \cdot 1.5$TFA: C, 54.1; H, 4.75; N, 10.2. Found: C, 54.2; H, 5.02; N, 10.6.

H-Tra-D,L-*m*-Tyr(*O*-2-Pyrim)-*m*-Aminobenzoic Acid·TFA Benzyl *N*-Boc-Tra-D,L-*m*-Tyr(*O*-2-Pyrim)-*m*-aminnobebzoic acid (100 mg, 0.14 mmol) in DMF (2 ml) and MeOH (20 ml) was hydrogenated for 2 h over a Pd catalyst. After removal of Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 80 mg (81%), Rf^1 0.58, Rf^2 0.33. A solution of *N*-Boc-Tra-D,L-*m*-Tyr(*O*-2-Pyrim)-*m*-aminiobenzoic acid (80 mg, 0.13 mmol) in TFA (0.70 ml, 5.0 mmol) containing anisole (50 μl) was stored at room temperature for 90 min. Ether was added to the solution to afford a precipitate, which was collected by centrifugation, purified with HPLC, yield 60 mg (75.0%), amorphous, $[\alpha]_D^{25}$ −1.6° (c= 0.45, 10% AcOH), Rf^3 0.39, Rf^4 0.40, Rf^5 0.47. *Anal*. Calcd for $C_{28}H_{31}N_5O_5 \cdot 1.5$TFA: C, 54.1; H, 4.75; N, 10.2. Found: C, 54.2; H, 5.02; N, 10.6.

Assay Procedure The enzymes used were as follows: human PL and PK (KABI Co.), bovine TH (Mochida Seiyaku Co.), porcine glandular kallikrein (GK) (Sigma Chemical Co.) and human UK (Green Cross). Enzymatic activities of PL, PK, TH, GK, and UK were determined by the method described previously,[28] using D-Val-Leu-Lys-*p*NA (S-2251), D-Pro-Phe-Arg-*p*NA (S-2302), D-Phe-Pip-Arg-*p*NA (S-2238), D-Val-Leu-Arg-*p*NA (S-2266), and <Glu-Gly-Arg-*p*NA (S-2444), respectively. Fn and Fg were used as substrates for PL and TH, respectively. IC_{50} values were determined as follows: 1) Antiamidolytic assay[22]; the IC_{50} value was taken as the concentration of inhibitor which reduced the absorbance at 405 nm by 50% compared with the absorbance measured under the same conditions without inhibitor. 2) Antifibrinolytic assay[20]; the IC_{50} value was taken as the concentration of inhibitor which prolonged the complete lysis time two-fold compared with that without inhibitor. 3) Antifibrinogenolytic assay: to a borate saline buffer (pH 7.4) was added solutions containing various concentrations of the inhibitor to be tested (0.5 ml), 0.2% bovine Fg in the above buffer (0.4 ml), and bovine TH 4 U/ml (0.1 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 two-fold compared with that without inhibitor.

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References and Notes

1) The customary L-configuration for amino acid residues is omitted. Abbreviations used in this report for amino acids, peptides and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485—2489 (1966); **6**, 362—364 (1967); **11**, 1726—1732 (1972). The following additional abbreviations are used: AcOEt, ethyl acetate; DMF, *N*,*N*-dimethylformamide; TFA, trifluoroacetic acid; DIPEA, *N*,*N*-diisopropylethylamine; Boc, *tert*-butyloxycarbonyl; TEA, triethylamine; $(Boc)_2O$, di-*tert*-butyldicarbonate; 2-Br-Z, 2-bromobenzyloxycarbonyl; OPac, phenacyl ester; Pic, 4-picolyl; 2-Pyrim, 2-pyrimidyl; EACA, 6-aminohexanoyl; TH, thrombin; UK, urokinase; Fg, fibrinogen; Fn, fibrin; THF, tetrahydrofuran; (DIEA=DIPEA) BOP, benzotriazole-1-yl-oxy-tris(dimethylamino)-phosphoniumhexafluorophosphate; Pac, phenacyl; iNoc, isonicotinyloxycarbonyl; pNA, *p*-nitroanilide; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, N-hydroxybenzotrazole; Py BOP, benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate.
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