Development of Plasmin-Selective Inhibitors and Studies of Their Structure–Activity Relationship[1)]

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Received July 5, 1999; accepted October 9, 1999

Various compounds were synthesized by combining three components at positions P_1, $P_{1'}$ and $P_{2'}$. Of these, *N*-(*trans*-4-aminomethylcyclohexanecarbonyl)-Tyr(*O*-2-bromobenzyloxycarbonyl)-octylamide inhibited plasmin selectively with IC_{50} values of 0.80 and 0.23 μM towards S-2251 and fibrin, respectively. This compound also inhibited plasma kallikrein, urokinase, thrombin and trypsin with IC_{50} values of 10, >50, >50 and 1.6 μM, respectively.

Key words plasmin inhibitor; selectivity; structure–activity relationship; *N*-(*trans*-4-aminomethylcyclohexanecarbonyl)-Tyr(*O*-2-BrZ)-octylamide

It is well known that proteinases and their natural inhibitors regulate biological functions cooperatively to maintain homeostasis, while any imbalance 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. It is also well known that α_2-PI consists of two parts: one part binds to the active site (catalytic site) of PL and another to the lysine binding site (LBS) of PL. An imbalance between PL and its natural inhibitors causes a serious syndrome, such as hyperfibrinolysis.[6—8)] At present, ε-aminocaproic acid[9)] and *trans*-4-aminomethylcyclohexanecarboxylic acid (*trans*-AMCHA)[10)] are used clinically as PL inhibitors. These inhibitors show fairly potent inhibition of fibrinolysis by PL with an IC_{50} value of 60 μM, but poor inhibition of the amidolysis of small peptide substrates and fibrinogenolysis by PL. This is because these inhibitors act on PL by blocking the LBS of an enzyme, which is not the catalytic site.[11)]

With the objectives of obtaining a powerful new tool to study the role of PL and developing novel types of clinical therapy, we focused our attention on the synthesis of potent active center-directed PL inhibitors. Previously, we reported the development of active center-directed inhibitors of PL[12,13)] and studies of their structure–inhibitory activity relationship.[14)] Our inhibitors consist of three parts, P_1, $P_{1'}$ and $P_{2'}$[15)] and their structure–activity relationship is summarized in Table 1.

As shown in Table 1, compound **I** inhibits plasma kallikrein (PK) specifically, compound **II** inhibits both PL and PK, and compound **III** inhibits PL specifically. These results, showed that we could design enzyme-selective inhibitors by combining various kinds of substituents at positions P_1, $P_{1'}$ and $P_{2'}$.

Bearing in mind the above results, we designed and synthesized a series of plasmin-selective inhibitors and this report deals with these PL inhibitors and their structure–activity relationship.

Tyr(*O*-2-BrZ) was selected as the $P_{1'}$ substituent, since it is known that this residue can increase the affinity for some part of the active center of trypsin-like proteinases.[14)] As the P_1 substituent, we chose 6-aminohexanoic acid or *trans*-4-aminomethylcyclohexanecarboxylic acid.

First of all, alkyl amines with various chain lengths were used as the $P_{2'}$ substituent. As illustrated in Chart 1, Boc-Tyr(*O*-2-BrZ)-OH was coupled with alkylamine to give Boc-Tyr(*O*-2-BrZ)-NH-R (R: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl). After removal of Boc with HCl–dioxane, the resulting amine was coupled with Boc-EACA-OH or Boc-Tra-OH to give Boc-EACA- or Boc-Tra-Tyr(*O*-2-BrZ)-NH-R, which were treated with 6 N HCl–dioxane to give compounds **1**—**14**. Their inhibitory activities against a series of enzymes are summarized in Table 2. As the P_1 substituent, the Tra group is more suitable than

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

Compound	P_1	$P_{1'}$	$P_{2'}$	PL	PK
				S-2251	S-2302
I	H_2NH_2C-(cyclohexane)-CO	Phe	HN-(benzene)-CH_2COOH	630	1.3
II	H_2NH_2C-(cyclohexane)-CO	Tyr(O-CO_2CH_2-(2-Br-benzene))	HN-(benzene)-$COCH_3$	0.23	0.37
III	H_2NH_2C-(cyclohexane)-CO	Tyr(O-CO_2CH_2-(2-Br-benzene))	OH_2C-(pyridine, N)	4.2	>100 (30%)[a)]

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

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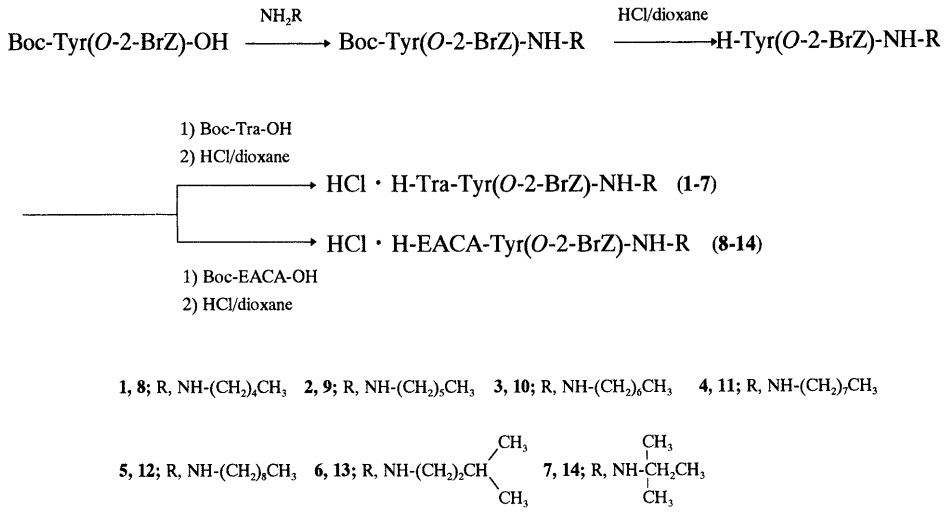

Chart 1. Synthetic Scheme for Compounds **1**—**14**

Table 2. IC_{50} Values (μM) of Compounds **1**—**14** for Various Enzymes

Peptide ID	P_1	$P_{1'}$	$P_{2'}$	PL		PK	UK	TH		TRY
				S-2251	Fn	S-2302	S-2444	S-2238	Fg	S-2238
1	NH_2-$(CH_2)_5$-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_4$-CH_3	10	1.3	110	>250	140	>100	85
2	NH_2-$(CH_2)_5$-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_5$-CH_3	13	2.7	58	>250	>50	>50	80
3	NH_2-$(CH_2)_5$-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_6$-CH_3	11	3.3	60	>500	>50	>50	>75
4	NH_2-$(CH_2)_5$-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_7$-CH_3	7.0	1.8	75	>250	>50	>25	>150
5	NH_2-$(CH_2)_5$-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_8$-CH_3	8.3	1.3	200	>10	100	>10	>150
6	NH_2-$(CH_2)_5$-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_2$-CH(CH_3)$_2$	6.2	1.2	91	>250	200	>100	68
7	NH_2-$(CH_2)_5$-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-C(CH_3)$_2$-CH_2-CH_3	85	19	170	>250	230	>250	>150
8	H_2NH_2C-Ph-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_4$-CH_3	1.1	0.1	8.8	>300	150	>100	3.3
9	H_2NH_2C-Ph-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_5$-CH_3	1.2	0.38	9.0	>50	>50	>25	1.4
10	H_2NH_2C-Ph-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_6$-CH_3	1.1	0.43	10	>50	>50	>25	5.9
11	H_2NH_2C-Ph-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_7$-CH_3	0.8	0.23	16	>50	>50	>25	1.6
12	H_2NH_2C-Ph-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_8$-CH_3	0.5	0.10	22	>10	100	>10	1.9
13	H_2NH_2C-Ph-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-$(CH_2)_2$-CH(CH_3)$_2$	0.46	0.056	2.1	260	70	>100	1.4
14	H_2NH_2C-Ph-CO	Tyr(*O*-CO_2CH_2-Br-Ph)	NH-C(CH_3)$_2$-CH_2-CH_3	13	1.7	69	>200	160	>100	23

the EACA group as far as potent inhibitory activity is concerned, while the EACA group increased the difference in inhibitory activity between PL and the other enzymes examined so far. As far as the $P_{2'}$ substituent is concerned, increasing the chain length resulted in increased inhibitory activity against PL and reduced inhibitory activity against PK. Compounds **11**, **12** inhibited PL potently and selectively. Previously, it was reported that 6-amidino-2-naphthyl *p*-guanidinobenzoate dimethanesulfonate (FUT-175) inhibited PL, PK and thrombin (TH) with IC_{50} values of 0.12, 1.9 and 3.9 μM (substrate: N^{α}-tosylarginine methyl ester, TAME), respectively.[16] Our compounds contain an amide bond, while FUT-175 contains an ester structure and our compounds exhibited much weaker inhibition of TH compared with FUT-175.

Since compound **II** in Table 1 inhibited both PL and PK potently, the 4-acetyl group at the $P_{2'}$ position was exchanged for alkyl groups of various chain lengths. As summarized in Table 3, the inhibitory activity of compounds **15**—**18** was less than that of compounds **19**—**22**. Compound **19**—**22** exhibited similar inhibitory activity against PL and trypsin

Table 3. IC_{50} Values (μM) of Compounds **15**—**22** for Various Enzymes

Peptide ID	P_1	$P_{1'}$	$P_{2'}$	PL		PK	UK	TH		TRY
				S-2251	Fn	S-2302	S-2444	S-2238	Fg	S-2238
15	NH_2-$(CH_2)_5$-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-$(CH_2)_2$-CH_3	9.2	2.0	19	>50	96	>20	1.9
16	NH_2-$(CH_2)_5$-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-Ph-$(CH_2)_3$-CH_3	9.0	2.3	39	>20	>25	>10	>10
17	NH_2-$(CH_2)_5$-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-Ph-$(CH_2)_4$-CH_3	9.0	4.7	58	>25	60	>10	>75
18	NH_2-$(CH_2)_5$-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-Ph-$(CH_2)_5$-CH_3	6.0	3.5	52	>40	>20	>10	>150
19	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-Ph-CH_2-CH_3	0.63	0.098	0.71	>50	89	>10	0.52
20	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-Ph-$(CH_2)_3$-CH_3	0.79	0.090	1.0	>25	>25	>10	0.27
21	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-Ph-$(CH_2)_4$-CH_3	0.57	0.070	1.7	>50	>50	>20	0.33
22	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-Ph-$(CH_2)_5$-CH_3	0.49	0.24	7.9	>40	>20	>10	1.4

Table 4. IC_{50} Values (μM) of Compounds **23**—**26** for Various Enzymes

Peptide ID	P_1	$P_{1'}$	$P_{2'}$	PL		PK	UK	TH		TRY
				S-2251	Fn	S-2302	S-2444	S-2238	Fg	S-2238
23	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-3-pyridyl	0.98	0.17	0.29	19	91	>100	0.74
24	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-CH_2-3-pyridyl	5.3	1.4	14	72	240	>200	4.9
25	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-$(CH_2)_2$-2-pyridyl	1.5	0.52	12	78	310	>200	4.3
26	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	NH-$(CH_2)_2$-Ph	1.1	0.30	7.0	>50	>50	>50	3.0

Table 5. IC_{50} Values (μM) of Compounds **27**—**34** for Various Enzymes

Peptide ID	P_1	$P_{1'}$	$P_{2'}$	PL		PK	UK	TH		TRY
				S-2251	Fn	S-2302	S-2444	S-2238	Fg	S-2238
27	NH_2-$(CH_2)_5$-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	H_2NH_2C-Ph-$COOCH_3$	9.0	6.1	56	>50	>100	>100	90
28	NH_2-$(CH_2)_5$-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	H_2NH_2C-Ph-$COO(CH_2)_5CH_3$	18	4.3	>200	>100	>200	>10	>150
29	NH_2-$(CH_2)_5$-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	H_2NH_2C-Ph-$COO(CH_2)_6CH_3$	24	3.9	>200	>100	>50	>10	>150
30	NH_2-$(CH_2)_5$-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	H_2NH_2C-Ph-$COO(CH_2)_7CH_3$	>100	5.0	>400	>100	>100	>10	>150
31	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	H_2NH_2C-Ph-$COOCH_3$	1.0	0.78	15	>10	>10	>20	0.80
32	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	H_2NH_2C-Ph-$COO(CH_2)_5CH_3$	1.5	0.40	40	>50	>50	>10	4.5
33	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	H_2NH_2C-Ph-$COO(CH_2)_6CH_3$	1.4	0.42	37	>50	>50	>10	10
34	H_2NH_2C-Ph-CO	Tyr(O-CO_2CH_2-3,5-Br$_2$-Ph)	H_2NH_2C-Ph-$COO(CH_2)_7CH_3$	2.5	0.56	45	>50	>50	>10	27

(TRY). The longer the chain length, the weaker the inhibition of PK, although the differences were not very marked.

Next, methylene groups were inserted between NH and the aromatic ring of the $P_{2'}$ moiety. As summarized in Table 4, compound **23** inhibited both PL and PK potently, while insertion of methylene groups reduced the inhibition of PK more than that of PL.

Finally, as the $P_{2'}$ moiety, *trans*-4-aminomethylcyclohexanecarboxylic acid alkyl esters of various chain lengths were used because we believed that the bulkiness of the cleft and stereogeometry of the active site of PL and PK were quite different. Compounds **27**—**34** were prepared and their in-

hibitory activities are summarized in Table 5. The inhibitory activity against PL is more potent than against PK, presumably due to the bulkiness of the $P_{2'}$ moiety.

In conclusion, it was found that the cleft of the active center of PL is larger than that of PK and other enzymes and that the compound **11** interacts with the active center of PL, as shown in Fig. 1. For the further study of PL selective inhibitors, the compound **11** was selected as the lead compound.

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.). On TLC (Kieselgel G. Merck), Rf^1, Rf^2, Rf^3, Rf^4 and Rf^5 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) and *n*-BuOH, AcOH, pyridine and H_2O (4 : 1 : 1 : 2), respectively.

General Procedure for Synthesis of Boc-Tyr(*O*-2-BrZ)-NH-X [X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl] A mixed anhydride of Boc-Tyr(*O*-2-BrZ)-OH [prepared routinely from Boc-Tyr(*O*-2-BrZ)-OH (1.5 g, 3.0 mmol), isobutyl chloroformate (0.45 ml, 3.0 mmol) and Et_3N (0.45 ml, 3.0 mmol)] in tetrahydrofuran (THF, 15 ml) was added to a solution of NH_2X (X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl) (3.3 mmol) in THF (10 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 to dryness. Ether was added to the residue to afford crystals, which were collected by filtration. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 6.

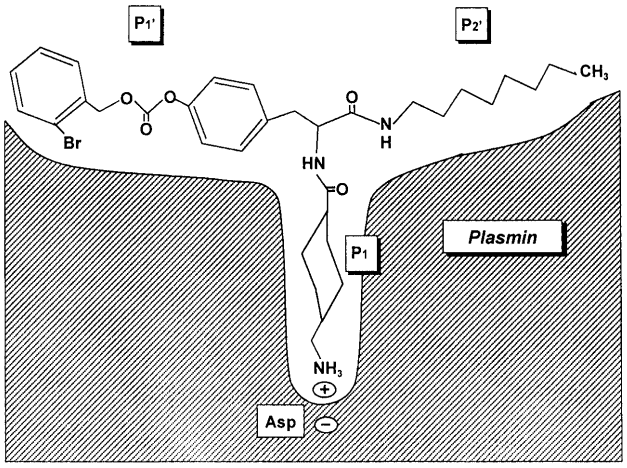

Fig. 1. Schematic Representation of Interaction of Tra-Tyr(*O*-2-BrZ)-octylamide (**11**) with Plasmin

General Procedure for Synthesis of Boc-EACA-Tyr(*O*-2-BrZ)-NH-X [X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl] A mixed anhydride of Boc-EACA-OH [prepared routinely from Boc-EACA-OH (221 mg, 0.89 mmol), isobutyl chloroformate (0.13 ml, 0.89 mmol) and Et_3N (0.12 ml, 0.89 mmol)] in THF (15 ml) was added to a solution of H-Tyr(*O*-2-BrZ)-NH-X·HCl [X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl; prepared routinely from Boc-Tyr(*O*-2-BrZ)-NH-X (0.89 mmol) and 7.2 N HCl–dioxane (2.0 ml, 14.4 mmol)] in DMF (30 ml) containing Et_3N (0.15 ml, 1.1 mmol) at 0 °C. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration and washed with water. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 7.

General Procedure for Synthesis of H-EACA-Tyr(*O*-2-BrZ)-NH-X·HCl (1—7) [X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl] Boc-EACA-Tyr(*O*-2-BrZ)-NH-X [X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl; (0.2 mmol)] was dissolved in 5.4 N HCl–dioxane (0.5 ml, 2.7 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 5 min. After addition of dioxane (0.2 ml), the reaction mixture was stirred at room temperature for 90 min. After removal of the solvent, dry ether was added to the residue to afford a precipitate. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 8.

General Procedure for Synthesis of Boc-Tra-Tyr(*O*-2-BrZ)-NH-X [X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl] A mixed anhydride of Boc-Tra-OH [prepared routinely from Boc-Tra-OH (245 mg, 0.89 mmol), isobutyl chloroformate (0.13 ml, 0.89 mmol) and Et_3N (0.12 ml, 0.89 mmol)] in THF (15 ml) was added to a solution of H-Tyr(*O*-2-BrZ)-NH-X·HCl [X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl; prepared routinely from Boc-Tyr(*O*-2-BrZ)-NH-X (0.89 mmol) and 7.2 N HCl–dioxane (2.0 ml, 14.4 mmol)] in DMF (20 ml) containing Et_3N (0.15 ml, 1.1 mmol) at 0 °C and the reaction mixture was stirred at 4 °C overnight. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration and washed with water. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 9.

General Procedure for Synthesis of H-Tra-Tyr(*O*-2-BrZ)-NH-X·HCl (8—14) [X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl] Boc-Tra-Tyr(*O*-2-BrZ)-NH-X [X: *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, 3-methylbutyl, 1,1-dimethylpropyl; (0.14 mmol)] was dissolved in 5.4 N HCl–dioxane (0.5 ml, 2.7 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 5 min. After addition of dioxane (0.2 ml), the reaction mixture was stirred at room temperature for 90 min. After removal of the solvent, dry ether was added to the residue to afford a precipitate. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf*

Table 6. Yield, Melting Point, Optical Rotation , Elemental Analysis and *Rf* Value of Boc-Tyr(*O*-2-BrZ)-NH-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ ($CHCl_3$)	Formula	Elemental analysis Calcd (Found)			TLC
					C	H	N	Rf^1
n-Pentyl	83	117—118	+2.4 (c=1.0)	$C_{27}H_{35}BrN_2O_6$	57.54 (57.45)	6.27 6.32	4.97 4.98)	0.80
n-Hexyl	82	117—119	+2.9 (c=1.0)	$C_{28}H_{37}BrN_2O_6$	58.23 (58.17)	6.47 6.30	4.85 4.83)	0.78
n-Heptyl	77	118—121	+3.2 (c=1.0)	$C_{29}H_{39}BrN_2O_5$	58.87 (58.83)	6.66 6.64	4.73 4.76)	0.78
n-Octyl	78	113—118	+3.05 (c=1.0)	$C_{30}H_{41}BrN_2O_6$	59.30 (59.39)	6.82 6.99	4.61 4.70)	0.88
n-Nonyl	72	103—105	+4.0 (c=1.0)	$C_{31}H_{43}BrN_2O_6$	60.09 (59.89)	6.99 7.10	4.52 4.50)	0.69
3-Methylbutyl	88	134—136	+2.3 (c=1.0)	$C_{27}H_{35}BrN_2O_6$	57.54 (57.81)	6.27 6.21	4.97 5.02)	0.81
1,1-Dimethylpropyl	83	74—76	+2.1 (c=1.0)	$C_{27}H_{35}BrN_2O_6$	57.54 (57.26)	6.27 6.12	4.97 4.96)	0.80

Table 7. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of Boc-EACA-Tyr(*O*-2-BrZ)-NH-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ ($CHCl_3$)	Formula	Elemental analysis Calcd (Found)			TLC
					C	H	N	Rf^1
n-Pentyl	87	113—115	+1.5 (c=1.0)[a]	$C_{33}H_{46}BrN_3O_7$	58.57 (58.33	6.87 6.85	6.21 6.14)	0.65
n-Hexyl	84	105—107	−0.5 (c=1.0)	$C_{34}H_{48}BrN_3O_7$	59.12 (59.04	7.02 7.01	6.08 5.92)	0.70
n-Heptyl	80	120—122	−1.0 (c=1.0)	$C_{35}H_{50}BrN_3O_7$	59.65 (59.53	7.17 7.21	5.96 5.84)	0.71
n-Octyl	84	128—131	−0.6 (c=1.0)	$C_{36}H_{52}BrN_3O_7$	60.15 (59.91	7.31 7.34	5.84 5.73)	0.80
n-Nonyl	67	124—126	−5.1 (c=1.0)[b]	$C_{37}H_{54}BrN_3O_7$	60.64 (60.38	7.42 7.50	5.73 5.74)	0.85
3-Methylbutyl	92	97—99	+1.5 (c=1.0)[a]	$C_{33}H_{46}BrN_3O_7$	58.57 (58.36	6.87 6.85	6.21 6.08)	0.72
1,1-Dimethylpropyl	65	Amorphous	−1.7 (c=1.0)[a]	$C_{33}H_{46}BrN_3O_7$	58.57 (58.68	6.87 7.15	6.21 6.13)	0.75

a) MeOH, *b*) DMF.

Table 8. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Values of H-EACA-Tyr(*O*-2-BrZ)-NH-X·HCl (**1**—**7**)

X	Peptide ID	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC	
						C	H	N	Rf^1	Rf^3
n-Pentyl	**1**	96	139—143	+8.3 (c=1.0)	$C_{28}H_{38}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	54.07 (54.25	6.48 6.92	6.75 7.48)	0.20	0.67
n-Hexyl	**2**	87	175—177	+5.2 (c=1.0)	$C_{29}H_{40}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	54.76 (54.86	6.66 6.68	6.61 6.79)		0.35
n-Heptyl	**3**	93	178—181	+6.2 (c=1.0)	$C_{30}H_{42}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	55.43 (55.53	6.67 6.87	6.46 6.49)		0.32
n-Octyl	**4**	88	162—165	+7.9 (c=1.0)	$C_{31}H_{44}BrN_3O_5 \cdot HCl$	56.83 (57.07	6.92 7.19	6.41 6.49)		0.35
n-Nonyl	**5**	87	174—176	+6.9 (c=1.0)	$C_{32}H_{46}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	56.67 (56.60	7.13 7.21	6.19 6.38)		0.40
3-Methylbutyl	**6**	74	123—127	+8.3 (c=1.0)	$C_{28}H_{38}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	54.07 (53.53	6.48 6.83	6.75 7.28)		0.32
1,1-Dimethylpropyl	**7**	56	Amorphous	+7.5 (c=1.0)	$C_{28}H_{38}BrN_3O_5 \cdot HCl \cdot 2H_2O$	51.81 (52.13	6.67 7.13	6.47 7.08)		0.32

Table 9. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Values of Boc-Tra-Tyr(*O*-2-BrZ)-NH-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (DMF)	Formula	Elemental analysis Calcd (Found)			TLC	
					C	H	N	Rf^1	Rf^2
n-Pentyl	58	202.5—205	−9.4 (c=1.0)	$C_{35}H_{48}BrN_3O_7$	59.82 (59.52	6.88 7.03	5.98 5.89)	0.60	0.70
n-Hexyl	81	199—200	−8.3 (c=1.0)	$C_{36}H_{50}BrN_3O_7$	60.32 (60.14	7.05 7.01	5.86 5.75)	0.71	
n-Heptyl	62	180—182	−2.3 (c=1.0)[a]	$C_{37}H_{52}BrN_3O_7$	60.81 (60.65	7.19 7.22	5.75 5.70)	0.78	
n-Octyl	65	177—181	−8.4 (c=1.0)	$C_{38}H_{54}BrN_3O_7$	61.27 (61.28	7.32 7.47	5.64 5.61)	0.70	
n-Nonyl	77	189—191	−8.9 (c=1.0)	$C_{39}H_{56}BrN_3O_7$	61.73 (61.66	7.43 7.77	5.53 5.51)	0.87	
3-Methylbutyl	84	186—188	−8.8 (c=1.0)	$C_{35}H_{39}BrN_3O_7$	59.81 (59.83	6.90 7.06	5.98 5.97)	0.76	
1,1-Dimethylpropyl	84	89—92	−8.2 (c=1.0)[b]	$C_{35}H_{39}BrN_3O_7$	59.81 (59.74	6.90 6.74	5.98 5.87)	0.72	

a) $CHCl_3$, *b*) MeOH.

Table 10. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Values of H-Tra-Tyr(*O*-2-BrZ)-NH-X·HCl (**8**—**14**)

X	Peptide ID	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC		
						C	H	N	Rf^3	Rf^4	Rf^5
n-Pentyl	**8**	98	200—204	+0.4 (c=0.9)	$C_{30}H_{40}BrN_3O_5 \cdot HCl \cdot H_2O$	54.84 (54.67	6.59 6.28	6.39 6.41)		0.30	0.27
n-Hexyl	**9**	95	207—209	+1.8 (c=1.0)	$C_{31}H_{42}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	56.23 (56.19	6.70 6.58	6.35 6.29)	0.42		
n-Heptyl	**10**	87	218—220	+6.4 (c=1.0)	$C_{32}H_{44}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	56.85 (56.90	6.71 6.87	6.22 6.25)	0.38		
n-Octyl	**11**	90	210—212	+0.7 (c=1.0)	$C_{33}H_{46}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	57.43 (57.25	7.01 6.91	6.09 6.21)	0.33		
n-Nonyl	**12**	93	208—210	+3.0 (c=1.0)	$C_{34}H_{48}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	57.99 (57.85	7.15 7.05	5.96 5.95)	0.43		
3-Methylbutyl	**13**	92	203—208	+1.3 (c=1.0)	$C_{30}H_{40}BrN_3O_5 \cdot HCl \cdot 0.5H_2O$	55.60 (55.30	6.53 6.54	6.51 6.56)	0.32		
1,1-Dimethylpropyl	**14**	92	126—130	−2.7 (c=1.0)	$C_{30}H_{40}BrN_3O_5 \cdot HCl \cdot 1.5H_2O$	54.10 (53.77	6.66 6.49	6.31 6.38)	0.26		

Table 11. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of Boc-Tyr(*O*-2-BrZ)-NH-⬡-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ ($CHCl_3$)	Formula	Elemental analysis Calcd (Found)			TLC
					C	H	N	Rf^1
Ethyl	92	162—164	−2.8 (c=1.0)	$C_{30}H_{33}BrN_2O_5$	60.30 (60.13	5.58 5.62	4.69 4.74)	0.95
n-Butyl	74	160—163	−1.2 (c=1.0)	$C_{32}H_{37}BrN_2O_6$	61.43 (61.57	5.97 5.96	4.48 4.47)	0.78
n-Pentyl	82	164—168	−3.2 (c=1.0)	$C_{33}H_{39}BrN_2O_6$	61.97 (61.87	6.16 6.09	4.38 4.32)	0.92
n-Hexyl	79	137—141	−3.3 (c=1.0)	$C_{34}H_{41}BrN_2O_6$	62.47 (62.33	6.32 6.44	4.28 4.34)	

Table 12. Yield, Melting Point, Optical Rotation , Elemental Analysis and *Rf* Value of Boc-EACA-Tyr (*O*-2-BrZ)-NH-⬡-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ ($CHCl_3$)	Formula	Elemental analysis Calcd (Found)			TLC
					C	H	N	Rf^1
Ethyl	95	137—139	+23.1 (c=1.0)[a]	$C_{36}H_{44}BrN_3O_7$	60.84 (60.72	6.25 6.34	5.91 5.74)	0.90
n-Butyl	90	120—123	+22.5 (c=1.0)[a]	$C_{38}H_{48}BrN_3O_7$	61.78 (61.91	6.56 6.54	5.69 5.60)	0.70
n-Pentyl	76	134—137	+2.9 (c=1.0)	$C_{39}H_{50}BrN_3O_7$	62.22 (62.08	6.71 6.64	5.58 5.51)	0.70
n-Hexyl	65	134—137	+2.5 (c=1.0)	$C_{40}H_{52}BrN_3O_7$	62.65 (62.54	6.85 6.86	5.48 5.45)	0.68

a) DMF.

values are summarized in Table 10.

General Procedure for Synthesis of Boc-Tyr(*O*-2-BrZ)-NH-⬡-X [X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl] A mixed anhydride of Boc-Tyr(*O*-2-BrZ)-OH [prepared routinely from Boc-Tyr(*O*-2-BrZ)-OH (1.5 g, 3.0 mmol), isobutyl chloroformate (0.45 ml, 3.0 mmol) and Et_3N (0.45 ml, 3.0 mmol)] in THF (15 ml) was added to a solution of NH_2-⬡-X (X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl) (3.3 mmol) in THF (10 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 to dryness. Ether was added to the residue to afford crystals, which were collected by filtration. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 11.

General Procedure for Synthesis of Boc-EACA-Tyr(*O*-2-BrZ)-NH-⬡-X [X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl] A mixed anhydride of Boc-EACA-OH [prepared routinely from Boc-EACA-OH (221 mg, 0.89 mmol), isobutyl chloroformate (0.13 ml, 0.89 mmol) and Et_3N (0.12 ml, 0.89 mmol)] in THF (15 ml) was added to a solution of H-Tyr(*O*-2-BrZ)-NH-⬡-X·HCl [X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl; prepared routinely from Boc-Tyr(*O*-2-BrZ)-NH-⬡-X (0.89 mmol) and 7.2 N HCl–dioxane (2.0 ml, 14.4 mmol)] in DMF (30 ml) containing Et_3N (0.15 ml, 1.1 mmol) at 0 °C. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration and washed with water. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 12.

General Procedure for Synthesis of H-EACA-Tyr(*O*-2-BrZ)-NH-⬡-X·HCl (15—18) [X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl] Boc-EACA-Tyr(*O*-2-BrZ)-NH-⬡-X [X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl (0.2 mmol)]

Table 13. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of H-EACA-Tyr(*O*-2-BrZ)-NH-⬡-X·HCl (**15**—**18**)

X	Peptide ID	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC
						C	H	N	*Rf*[3]
Ethyl	**15**	75	Amorphous	+31.2 (c=1.0)	$C_{31}H_{36}BrN_3O_5$ HCl·H_2O	55.98 (55.90	5.91 5.70	6.31 6.32)	0.56
n-Butyl	**16**	92	148—152	+27.3 (c=1.0)	$C_{33}H_{40}BrN_3O_5$ HCl·0.5H_2O	57.94 (57.56	6.19 6.01	6.14 6.28)	0.57
n-Pentyl	**17**	90	Amorphous	+23.7 (c=1.0)	$C_{34}H_{42}BrN_3O_5$ HCl·0.5H_2O	58.50 (58.28	6.35 6.17	6.02 5.81)	0.45
n-Hexyl	**18**	77	Amorphous	+25.7 (c=1.0)	$C_{35}H_{44}BrN_3O_5$ HCl·H_2O	58.29 (58.14	6.56 6.46	5.82 5.59)	0.76

Table 14. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of Boc-Tra-Tyr(*O*-2-BrZ)-NH-⬡-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (DMF)	Formula	Elemental analysis Calcd (Found)			TLC
					C	H	N	*Rf*[1]
Ethyl	95	219—222	+18.0 (c=1.0)	$C_{38}H_{46}BrN_3O_7$	61.95 (61.72	6.31 6.39	5.70 5.63)	0.90
n-Butyl	90	196—199	+17.2 (c=1.0)	$C_{40}H_{50}BrN_3O_7$	62.81 (62.82	6.60 6.55	5.49 5.51)	0.74
n-Pentyl	77	199—202	+18.7 (c=1.0)	$C_{41}H_{52}BrN_3O_7$	63.22 (64.14	6.74 6.78	5.39 5.28)	0.63
n-Hexyl	65	181—183	+18.3 (c=1.0)	$C_{42}H_{54}BrN_3O_7$	63.62 (63.74	6.88 7.03	5.30 5.32)	0.78

Table 15. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of H-Tra-Tyr(*O*-2-BrZ)-NH-⬡-X·HCl (**19**—**22**)

X	Peptide ID	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC
						C	H	N	*Rf*[3]
Ethyl	**19**	95	200—203	+18.3 (c=1.0)	$C_{33}H_{38}BrN_3O_5$· HCl·1.5H_2O	56.61 (56.45	6.04 5.68	6.00 6.23)	0.58
n-Butyl	**20**	81	Amorphous	+19.4 (c=1.0)	$C_{35}H_{42}BrN_3O_5$· HCl·1.5H_2O	57.74 (58.00	6.37 6.07	5.77 5.80)	0.62
n-Pentyl	**21**	101	175—178	+17.6 (c=1.0)	$C_{36}H_{44}BrN_3O_5$· HCl·H_2O	58.99 (58.73	6.46 6.32	5.73 5.70)	0.43
n-Hexyl	**22**	84	188—190	+17.1 (c=1.0)	$C_{37}H_{46}BrN_3O_5$· HCl·0.75H_2O	59.84 (59.79	6.58 6.42	5.65 5.63)	0.55

was dissolved in 5.4 N HCl–dioxane (0.5 ml, 2.7 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 5 min. After addition of dioxane (0.2 ml), the reaction mixture was stirred at room temperature for 90 min. After removal of the solvent, dry ether was added to the residue to afford a precipitate. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 13.

General Procedure for Synthesis of Boc-Tra-Tyr(*O*-2-BrZ)-NH-⬡-X [X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl] A mixed anhydride of Boc-Tra-OH [prepared routinely from Boc-Tra-OH (245 mg, 0.89 mmol), isobutyl chloroformate (0.13 ml, 0.89 mmol) and Et_3N (0.12 ml, 0.89 mmol)] in THF (15 ml) was added to a solution of H-Tyr(*O*-2-BrZ)-NH-⬡-X·HCl [X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl; prepared routinely from Boc-Tyr(*O*-2-BrZ)-NH-⬡-X (0.89 mmol) and 7.2 N HCl–dioxane (2.0 ml, 14.4 mmol)] in DMF (20 ml) containing Et_3N (0.15 ml, 1.1 mmol) at 0 °C and the reaction mixture was stirred at 4 °C overnight. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration and washed with water. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 14.

General Procedure for Synthesis of H-Tra-Tyr(*O*-2-BrZ)-NH-⬡-X·HCl (19—22) [X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl] Boc-Tra-Tyr(*O*-2-BrZ)-NH-⬡-X [X: ethyl, *n*-butyl, *n*-pentyl, *n*-hexyl; (0.14 mmol)] was dissolved in 5.4 N HCl–dioxane (0.5 ml, 2.7 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 5 min. After addition of dioxane (0.2 ml), the reaction mixture was stirred at room temperature for 90 min. After removal of the solvent, dry ether was added to the residue to afford a precipitate. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 15.

General Procedure for Synthesis of Boc-Tyr(*O*-2-BrZ)-NH-X [X: 4-pyridyl, 4-picolyl, 2-(2-pyridyl)ethyl, β-phenethyl] A mixed anhydride of Boc-Tyr(*O*-2-BrZ)-OH [prepared routinely from Boc-Tyr(*O*-2-BrZ)-OH (1.5 g, 3.0 mmol), isobutyl chloroformate (0.45 ml, 3.0 mmol) and Et_3N (0.45 ml, 3.0 mmol)] in THF (15 ml) was added to a solution of NH_2-X (X: 4-pyridyl, 4-picolyl, 2-(2-pyridyl)ethyl, β-phenethyl) (3.3 mmol) in THF (10 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 to dryness. Ether was added to the residue to afford crystals, which were collected by filtration. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 16.

General Procedure for Synthesis of Boc-Tra-Tyr(*O*-2-BrZ)-NH-X [X: 4-pyridyl, 4-picolyl, 2-(2-pyridyl)ethyl, β-phenethyl] A mixed anhydride of Boc-Tra-OH [prepared routinely from Boc-Tra-OH (245 mg, 0.89 mmol),

Table 16. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Values of Boc-Tyr(*O*-2-BrZ)-NH-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC	
					C	H	N	*Rf*[1]	*Rf*[2]
4-Pyridyl	27	126—128	+36.6 (c=0.9)	$C_{27}H_{28}BrN_3O_6$	56.84 (56.57	4.94 4.94	7.36 7.44)	0.25	0.54
4-Picolyl	59	156.5—158	+1.5 (c=1.0)	$C_{28}H_{30}BrN_3O_6$	57.54 (57.57	5.17 5.17	7.18 7.20)	0.49	0.53
2-(2-Pyridyl)ethyl	75	114—122	−0.11 (c=0.9)	$C_{29}H_{32}BrN_3O_6$	58.19 (57.76	5.38 5.25	7.02 6.87)	0.65	0.62
β-Phenethyl	75	152—155	−1.6 (c=1.0)[a]	$C_{30}H_{33}BrN_2O_6$	60.30 (60.46	5.58 5.57	4.69 4.63)	0.83	

a) $CHCl_3$.

Table 17. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Values of Boc-Tra-Tyr(*O*-2-BrZ)-NH-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC	
					C	H	N	*Rf*[1]	*Rf*[2]
4-Pyridyl	66	178—180	+19.4 (c=1.0)	$C_{35}H_{41}BrN_4O_7$	58.50 (58.72	5.89 5.86	7.80 7.71)	0.40	0.57
4-Picolyl	59	189—192	−13.2 (c=1.0)	$C_{36}H_{43}BrN_4O_7$	59.75 (59.48	5.98 5.86	7.74 7.71)	0.46	0.48
2-(2-Pyridyl)ethyl	65	195—195.5	−14.6 (c=0.9)	$C_{37}H_{45}BrN_4O_7$	59.51 (59.55	6.14 6.13	7.50 7.48)	0.57	0.42
β-Phenethyl	81	198—200	−7.8 (c=1.0)	$C_{38}H_{46}BrN_3O_7$	61.95 (61.77	6.31 6.31	5.70 5.72)	0.76	

Table 18. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of H-Tra-Tyr(*O*-2-BrZ)-NH-X·HCl (**23**—**26**)

X	Peptide ID	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC
						C	H	N	*Rf*[3]
4-Pyridyl	**23**	83	Amorphous	+40.3 (c=0.9)	$C_{30}H_{33}BrN_4O_5\cdot 2HCl\cdot 2.5H_2O$	49.48 (49.53	5.16 5.54	7.42 7.70)	0.39
4-Picolyl	**24**	72	Amorphous	−0.35 (c=0.9)[a]	$C_{31}H_{35}BrN_4O_5\cdot 2HCl\cdot 2.5H_2O$	50.21 (49.93	5.71 5.40	7.56 7.28)	0.39
2-(2-Pyridyl)ethyl	**25**	80	Amorphous	−4.4 (c=0.6)[a]	$C_{32}H_{37}BrN_4O_5\cdot 2HCl\cdot 3H_2O$	50.27 (50.10	5.93 5.30	7.32 7.20)	0.52
β-Phenethyl	**26**	74	204—206	−3.5 (c=1.0)	$C_{33}H_{38}BrN_3O_5\cdot HCl\cdot 0.5H_2O$	58.11 (57.81	5.91 5.84	6.16 6.17)	0.30

a) 0.1 N HCl.

isobutyl chloroformate (0.13 ml, 0.89 mmol) and Et_3N (0.12 ml, 0.89 mmol)] in THF (15 ml) was added to a solution of H-Tyr(*O*-2-BrZ)-NH-X·HCl [X: 4-pyridyl, 4-picolyl, 2-(2-pyridyl)ethyl, β-phenethyl; prepared routinely from Boc-Tyr(*O*-2-BrZ)-NH-X (0.89 mmol) and 7.2 N HCl–dioxane (2.0 ml, 14.4 mmol) as usual] in DMF (20 ml) containing Et_3N (0.15 ml, 1.1 mmol) at 0 °C and the reaction mixture was stirred at 4 °C overnight. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration and washed with water. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 17.

General Procedure for Synthesis of H-Tra-Tyr(*O*-2-BrZ)-NH-X·HCl (23—26) [X: 4-pyridyl, 4-picolyl, 2-(2-pyridyl)ethyl, β-phenethyl] Boc-Tra-Tyr(*O*-2-BrZ)-NH-X [X: 4-pyridyl, 4-picolyl, 2-(2-pyridyl)ethyl, β-phenethyl; (0.14 mmol)] was dissolved in 5.4 N HCl–dioxane (0.5 ml, 2.7 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 5 min. After addition of dioxane (0.2 ml), the reaction mixture was stirred at room temperature for 90 min. After removal of the solvent, dry ether was added to the residue to afford a precipitate. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 18.

General Procedure for Synthesis of Boc-Tyr(*O*-2-BrZ)-Tra-O-X [X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl] A mixed anhydride of Boc-Tyr(*O*-2-BrZ)-OH [prepared routinely from Boc-Tyr(*O*-2-BrZ)-OH (1.5 g, 3.0 mmol), isobutyl chloroformate (0.45 ml, 3.0 mmol) and Et_3N (0.45 ml, 3.0 mmol)] in THF (15 ml) was added to a solution of NH_2-X (X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl) (3.3 mmol) in THF (10 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 to dryness. Ether was added to the residue to afford crystals, which were collected by filtration. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 19.

General Procedure for Synthesis of Boc-EACA-Tyr(*O*-2-BrZ)-Tra-O-X [X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl] A mixed anhydride of Boc-EACA-OH [prepared routinely from Boc-EACA-OH (221 mg, 0.89 mmol), isobutyl chloroformate (0.13 ml, 0.89 mmol) and Et_3N (0.12 ml, 0.89 mmol)] in THF (15 ml) was added to a solution of H-Tyr(*O*-2-BrZ)-Tra-*O*-X·HCl [X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl; prepared routinely from Boc-Tyr(*O*-2-BrZ)-Tra-*O*-X (0.89 mmol) and 7.2 N HCl–dioxane (2.0 ml, 14.4 mmol)] in DMF (30 ml) containing Et_3N (0.15 ml, 1.1 mmol) at 0 °C. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, ether was

Table 19. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of Boc-Tyr(*O*-2-BrZ)-Tra-O-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ ($CHCl_3$)	Formula	Elemental analysis Calcd (Found)			TLC
					C	H	N	*Rf*[1]
Methyl	70	144—147	−0.1 (c=1.0)	$C_{31}H_{39}BrN_2O_8$	57.50 (57.37	6.08 5.92	4.32 4.29)	0.70
n-Hexyl	87	98—101	+1.2 (c=1.0)	$C_{36}H_{49}BrN_2O_8$	60.24 (60.05	6.90 6.95	3.90 3.87)	0.81
n-Heptyl	89	109—112	+1.8 (c=1.0)	$C_{37}H_{51}BrN_2O_8$	60.72 (60.67	7.04 7.08	3.83 3.82)	0.75
n-Octyl	89	95—99	−0.5 (c=1.0)	$C_{38}H_{53}BrN_2O_8$	61.20 (61.17	7.18 6.91	3.75 3.72)	0.95

Table 20. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of Boc-EACA-Tyr(*O*-2-BrZ)-Tra-O-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (DMF)	Formula	Elemental analysis Calcd (Found)			TLC
					C	H	N	*Rf*[1]
Methyl	84	103—106	−2.5 (c=1.0)[a]	$C_{37}H_{48}BrN_3O_9$	58.41 (58.28	6.37 6.56	5.52 5.44)	0.70
n-Hexyl	86	94—97	−4.3 (c=1.0)	$C_{42}H_{60}BrN_3O_9$	60.71 (60.49	7.29 7.31	5.05 5.02)	0.69
n-Heptyl	80	101—103	−5.35 (c=1.0)	$C_{43}H_{62}BrN_3O_9$	61.12 (60.97	7.41 7.33	4.97 4.86)	0.72
n-Octyl	73	118—120	−4.15 (c=1.0)	$C_{44}H_{64}BrN_3O_9$	61.52 (61.30	7.53 7.56	4.89 4.80)	0.71

a) $CHCl_3$.

Table 21. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of H-EACA-Tyr(*O*-2-BrZ)-Tra-O-X·HCl (**27**—**30**)

X	Peptide ID	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC
						C	H	N	*Rf*[3]
Methyl	**27**	82	168—178	+7.2 (c=1.0)	$C_{32}H_{42}BrN_3O_7 \cdot HCl \cdot H_2O$	53.74 (53.70	6.34 6.19	5.88 6.12)	0.21
n-Hexyl	**28**	90	155—157	+6.7 (c=1.0)	$C_{37}H_{52}BrN_3O_7 \cdot HCl \cdot H_2O$	56.60 (56.45	7.06 6.83	5.35 5.29)	0.38
n-Heptyl	**29**	92	156—159	+6.6 (c=1.0)	$C_{38}H_{54}BrN_3O_7 \cdot HCl \cdot H_2O$	57.11 (56.82	7.19 6.96	5.26 5.17)	0.69
n-Octyl	**30**	91	159—161	+7.0 (c=1.0)	$C_{39}H_{56}BrN_3O_7 \cdot HCl \cdot 1.5H_2O$	56.97 (56.95	7.35 7.15	5.11 5.09)	0.61

added to the residue to afford crystals, which were collected by filtration and washed with water. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 20.

General Procedure for Synthesis of H-EACA-Tyr(*O*-2-BrZ)-Tra-O-X·HCl (27—30) [X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl] Boc-EACA-Tyr(*O*-2-BrZ)-Tra-O-X [X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl (0.2 mmol)] was dissolved in 5.4 N HCl–dioxane (0.5 ml, 2.7 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 5 min. After addition of dioxane (0.2 ml), the reaction mixture was stirred at room temperature for 90 min. After removal of the solvent, dry ether was added to the residue to afford a precipitate. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 21.

General Procedure for Synthesis of Boc-Tra-Tyr(*O*-2-BrZ)-Tra-O-X [X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl] A mixed anhydride of Boc-Tra-OH [prepared routinely from Boc-Tra-OH (245 mg, 0.89 mmol), isobutyl chloroformate (0.13 ml, 0.89 mmol) and Et_3N (0.12 ml, 0.89 mmol)] in THF (15 ml) was added to a solution of H-Tyr(*O*-2-BrZ)-Tra-O-X·HCl [X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl; prepared routinely from Boc-Tyr(*O*-2-BrZ)-Tra-O-X (0.89 mmol) and 7.2 N HCl–dioxane (2.0 ml, 14.4 mmol)] in DMF (20 ml) containing Et_3N (0.15 ml, 1.1 mmol) at 0 °C and the reaction mixture was stirred at 4 °C overnight. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration and washed with water. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 22.

General Procedure for Synthesis of H-Tra-Tyr(*O*-2-BrZ)-Tra-O-X·HCl (31—34) [X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl] Boc-Tra-Tyr(*O*-2-BrZ)-O-Tra-X [X: methyl, *n*-hexyl, *n*-heptyl, *n*-octyl (0.14 mmol)] was dissolved in 5.4 N HCl–dioxane (0.5 ml, 2.7 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 5 min. After addition of dioxane (0.2 ml), the reaction mixture was stirred at room temperature for 90 min. After removal of the solvent, dry ether was added to the residue to afford a precipitate. The yield, mp, $[\alpha]_D^{25}$ values, elemental analysis and *Rf* values are summarized in Table 23.

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.), human urokinase (UK) (Green Cross) and TRY (Sigma Chemical Co.). Enzymatic activities of PL, PK, TH, GK, UK and TRY were determined by the method described previously,[17]

Table 22. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of Boc-Tra-Tyr(*O*-2-BrZ)-Tra-O-X

X	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (DMF)	Formula	Elemental analysis Calcd (Found)			TLC
					C	H	N	*Rf*[1]
Methyl	82	219—222	−4.5 (*c*=1.0)[a]	$C_{39}H_{50}BrN_3O_9$	59.53 (59.46	6.66 6.65	5.34 5.31)	0.64
n-Hexyl	67	189—191	−8.5 (*c*=1.0)	$C_{44}H_{62}BrN_3O_9$	61.66 (61.64	7.31 7.44	4.90 4.89)	0.81
n-Heptyl	68	185—187	−7.9 (*c*=1.0)	$C_{45}H_{64}BrN_3O_9$	62.05 (62.06	7.42 7.41	4.82 4.73)	0.72
n-Octyl	88	184—187	−7.8 (*c*=1.0)	$C_{46}H_{66}BrN_3O_9$	62.42 (62.52	7.53 7.63	4.75 4.69)	0.73

a) $CHCl_3$.

Table 23. Yield, Melting Point, Optical Rotation, Elemental Analysis and *Rf* Value of H-Tra-Tyr(*O*-2-BrZ)-Tra-O-X·HCl (**31**—**34**)

X	Peptide ID	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found)			TLC
						C	H	N	*Rf*[3]
Methyl	**31**	95	213—216	−1.1 (*c*=1.0)	$C_{34}H_{44}BrN_3O_7 \cdot HCl \cdot 1.5H_2O$	54.45 (54.79	6.45 6.53	5.60 5.66)	0.17
n-Hexyl	**32**	86	200—203	+1.0 (*c*=1.0)	$C_{39}H_{54}BrN_3O_7 \cdot HCl \cdot 1.5H_2O$	58.38 (58.34	7.03 7.11	5.23 5.22)	0.66
n-Heptyl	**33**	75	212—214	−0.4 (*c*=1.0)	$C_{40}H_{56}BrN_3O_7 \cdot HCl \cdot 0.5H_2O$	58.85 (58.93	7.16 7.22	5.14 5.18)	0.71
n-Octyl	**34**	82	198—202	+1.0 (*c*=1.0)	$C_{41}H_{58}BrN_3O_7 \cdot HCl \cdot 1.5H_2O$	58.05 (58.16	7.38 7.07	4.95 4.94)	0.83

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), <Glu-Gly-Arg-*p*NA (S-2444) and D-Phe-Pip-Arg-*p*NA (S-2238), respectively. Fibrin and fibrinogen were used as substrates for PL and TH, respectively. IC_{50} values were determined as follows: 1) Antiamidolytic assay[18]; 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[18]; 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 fibrinogen 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.

Acknowledgement The authors are grateful for the assistance of Drs. L. H. Lazarus and S. D. Bryant of the National Institute of Environmental Health Sciences (NIEHS) for their kind help during the preparation of this manuscript.

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; Boc, *tert*-butyloxycarbonyl; TEA, triethylamine; $(Boc)_2O$, di-*tert*-butyldicarbonate; 2-BrZ, 2-bromobenzyloxcarbonyl; Tra, 4-aminomethylcyclohexanecarbonyl; EACA, 6-aminohexanoyl; PK, plasma kallikrein; PL, plasmin; TH, thrombin; GK, glandular kallikrein; UK, urokinase; TRY, trypsin; Fg, fibrinogen; Fn, fibrin.

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