Design and Synthesis of Peptides Passing through the Blood-Brain Barrier

Tateaki Wakamiya,* Makoto Kamata,† Shoichi Kusumoto,† Hiroyuki Kobayashi,†† Yoshimichi Sai,†† Ikumi Tamai,†† and Akira Tsuji††

Department of Chemistry, Faculty of Science and Technology, Kinki University, Higashi-osaka, Osaka 577

†Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560

††Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Ishikawa 920

(Received September 11, 1997)

The blood-brain barrier (BBB) is a highly selective membranous barrier regulating the transport of substances in blood into the brain parenchyma. At present, delivery of biologically active peptides or peptide drugs into the brain is quite an important subject from the standpoint of chemotherapy for brain diseases. H–MeTyr–Arg–MeArg–D-Leu–$NH(CH_2)_8NH_2$ termed 001-C8 was first synthesized to elucidate the structural specificity of peptides for passing through the BBB. The N^{α}-methylamino acid and D-amino acid residues were appropriately situated in this peptide to protect against the digestion by peptidase. Furthermore, a number of basic peptides were prepared as 001-C8 analogs for studying the relationship between structure and BBB permeability of peptides.

The blood-brain barrier (BBB) is formed by brain capillary endothelial cells (BCEC) which make up an epithelial-like tight junction. The characteristic structure of brain capillary accounts for the action of BBB as a highly selective membranous barrier; the substances flowing in brain capillary are strictly controlled to be transported into the brain parenchyma for maintaining the regular functions of the brain. Therefore, it was believed for a long time that not only nutritive or medicinal substances in blood but also even biologically important peptides locating in brain can hardly penetrate the BBB. However, the BBB transport of dynorphin-like analgesic peptide (DLAP), E-2078 (**1**),[1,2] and a novel adrenocorticotropic hormone (ACTH) analog, ebiratide (**2**)[3,4] have recently been confirmed (Chart 1).

Although the mechanisms for transport of peptides across the BBB have not been fully clarified yet, they can be classified into the following four categories at present: (1) passive transport, (2) carrier-mediated transport, (3) receptor-mediated transcytosis (RMT), and (4) adsorptive-mediated transcytosis (AMT).[5] The above-mentioned E-2078 and ebiratide were confirmed to penetrate into the brain via the last mechanism. This result suggests that suitable basicity and/or lipophilicity in the molecule may be required for peptides to pass through the BBB based on the AMT mechanism.[1—4]

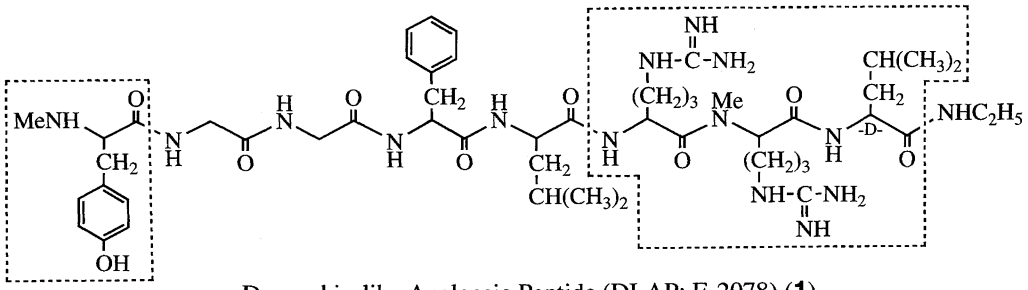
In order to clarify the structure-permeability relationship of peptides passing through the BBB, we first designed and synthesized a tetrapeptide amide, H–MeTyr–Arg–MeArg–D-Leu–$NH(CH_2)_8NH_2$[6] termed 001-C8 (**3**).[7] (Fig. 1) This compound consists of the basic tetrapeptide part in the molecule of E-2078 (**1**) and the lipophilic amide part in the molecule of ebiratide (**2**); these parts are enclosed with a dotted line in each structure. The N^{α}-methylamino acid and D-amino acid residues are appropriately situated in 001-C8 to prevent the hydrolytic cleavage of peptide bonds by peptidase locating in BCEC. We next prepared various 001-C8 analogs **4**—**13** (Fig. 2); these peptides were designed for changing the basicity, lipophilicity, or molecular size of 001-C8.

In the present paper the synthetic procedures of 001-C8 and its analog peptides are described in detail. In addition, we briefly report preliminary results concerning the BBB permeability of these peptides on the basis of an in vitro study using the primary cultured bovine BCEC.

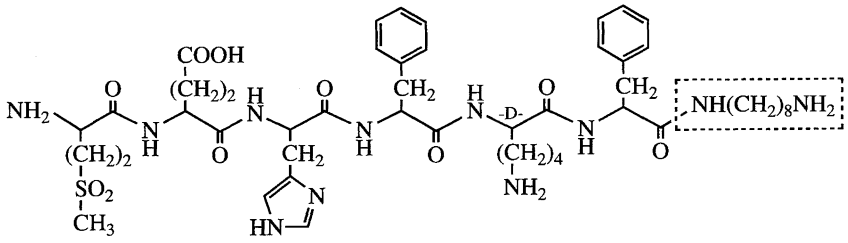
Results and Discussion

Synthesis of 001-C8. Of two kinds of N^{α}-methylamino acids used in the present study, N^{α}-methyltyrosine (MeTyr) derivative was easily prepared by Benoiton's method; Boc–Tyr(Bzl)–OH was treated with CH_3I and NaH in anhydrous THF.[8] On the other hand, this method was not helpful in preparing N^{α}-methylarginine (MeArg) derivative at all, since the methylation of the guanidino group occurs concurrently to give a complex mixture of N^{α}- and N^{g}-methylated products. However, we confirmed that the selective N^{α}-methylation of the Arg residue can be carried out by Grieco's method based on the retro-aza-Diels–Alder reaction as illustrated in Scheme 1.[9] In the present study, we demonstrated the application of this method to the *N*-terminal arginine residue in peptides.

The synthesis of 001-C8 was primarily carried out by conventional Boc-mode solution method using DCC or EDC·HCl as a coupling agent and HOBt as an additive as

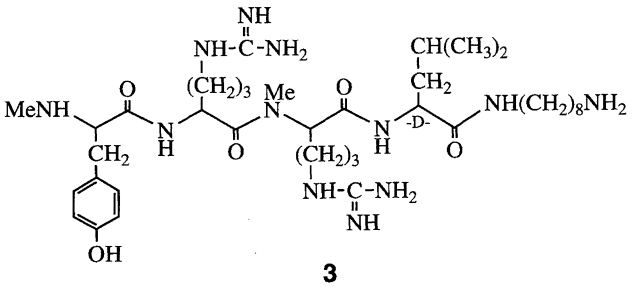


Dynorphin-like Analgesic Peptide (DLAP; E-2078) (**1**)



Ebiratide ($ACTH_{4-9}$ analog) (**2**)

Chart 1.

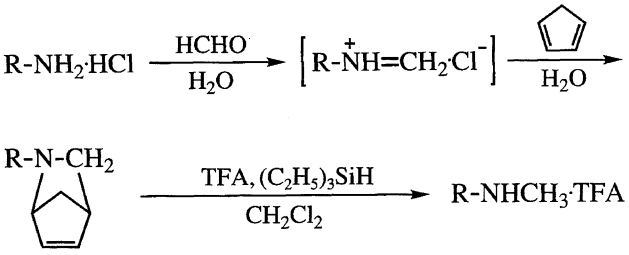


3

Fig. 1. Structure of 001-C8 (**3**).

shown in Scheme 2. After the successive coupling of the Leu and Arg residues to *N*-benzyloxycarbonyl-1,8-octanediamine (H-Oda-Z) (**14**) used as a *C*-terminal amide part, the *N*-terminal Arg residue of the peptide **18** was then converted into the MeArg residue by Grieco's method. The reaction proceeded successfully to afford N^{α}-methylarginine-containing peptide (MeArg-peptide) **19** in a reasonable yield.

The thus-prepared MeArg-peptide **19** was then subjected to the coupling with an acid component Boc–Arg(Ts)–OH by means of carbodiimide–HOBt method. However, we realized that this conventional method is not favorable for the coupling between these components. So far as we examined, the best result was obtained by means of symmetrical



Scheme 1.

001-C8	H-MeTyr-Arg-MeArg-D-Leu-$NH(CH_2)_8NH_2$ (**3**)

Tetrapeptide analogs

001-EA	H-MeTyr-Arg-MeArg-D-Leu-$NHCH_2CH_3$ (**4**)
001-OH	H-MeTyr-Arg-MeArg-D-Leu-OH (**5**)
002-C8	H-MeTyr-Leu-MeArg-D-Leu-$NH(CH_2)_8NH_2$ (**6**)
003-C8	H-MeTyr-Arg-MeArg-D-Arg-$NH(CH_2)_8NH_2$ (**7**)
004-C8	H-MeTyr-Leu-D-Leu-D-Leu-$NH(CH_2)_8NH_2$ (**8**)

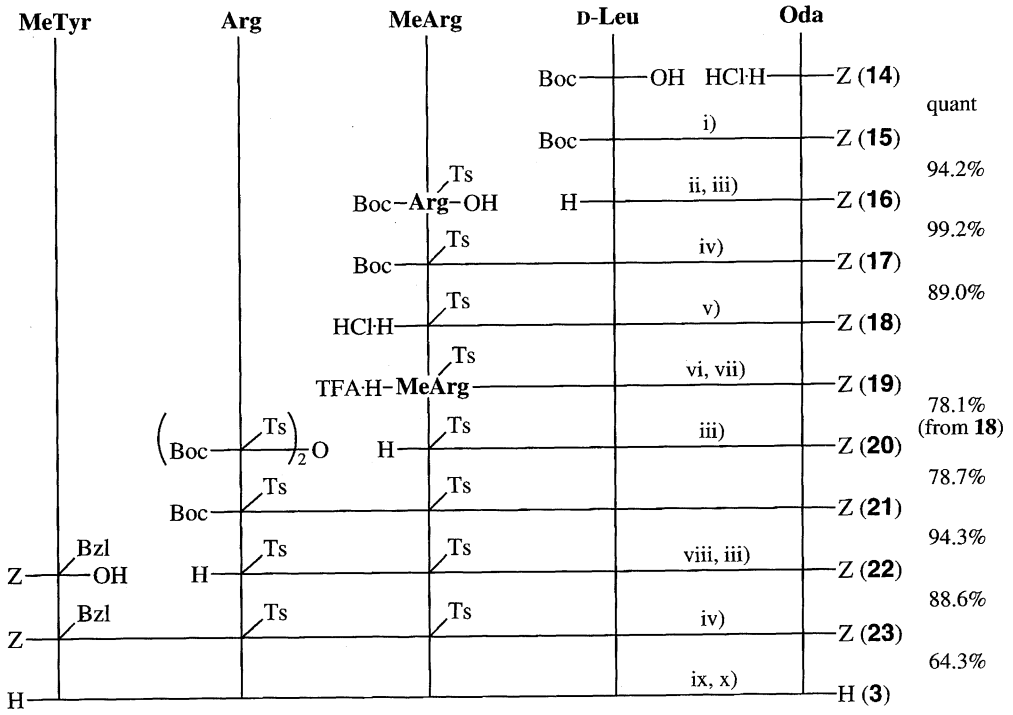
Dipeptide analogs

101-C8	H-MeTyr-Arg-$NH(CH_2)_8NH_2$ (**9**)
101-C5	H-MeTyr-Arg-$NH(CH_2)_5NH_2$ (**10**)
101-C2	H-MeTyr-Arg-$NH(CH_2)_2NH_2$ (**11**)
101-EA	H-MeTyr-Arg-$NHCH_2CH_3$ (**12**)
101-A	H-MeTyr-Arg-NH_2 (**13**)

Fig. 2. Synthetic analogs of 001-C8.

acid anhydride method; TFA salt of MeArg-peptide **19** was first treated with saturated aqueous $NaHCO_3$, and the thus-obtained amino-free segment **20** was then coupled with an excess amount of $[Boc–Arg(Ts)]_2O$ prepared in advance to give tripeptide derivative **21**. *N*-Terminal MeTyr derivative was finally coupled with the amine segment **22** prepared from **21**, and the thus-obtained fully protected peptide **23** was treated with anhydrous HF, followed by preparative RPHPLC purification to afford 001-C8 (**3**).

Syntheses of Tetrapeptide Analogs. As a result of preliminary tests concerning the BBB transport of 001-C8, we confirmed that this compound is internalized to the primary cultured monolayers of BCEC much more effectively than E-2078 or ebiratide by the adsorptive-mediated endocytosis (AME) mechanism. In order to elucidate the relationship between structure and BBB permeability of peptides, we newly synthesized five kinds of tetrapeptide analogs **4**—**8** depicted



Scheme 2. i) DCC/HOBt/TEA; ii) 50% TFA in CH_2Cl_2; iii) sat. $NaHCO_3$ aq; iv) EDC·HCl/HOBt; v) 1.5 M HCl in AcOH; vi) HCHO/cyclopentadiene; vii) TES/TFA; viii) TFA; ix) HF/thioanisole; x) preparative RPHPLC.

in Fig. 2; they were designed to change the basicity and/or lipophilicity of 001-C8 molecule. All these peptides were basically prepared in a similar manner for the synthesis of 001-C8; the yields of *N*-methylation of the Arg residue in intermediate peptides **24**—**27** are summarized in Table 1. The coupling of Boc–Arg(Ts)–OH or Boc–Leu–OH with MeArg-peptides **28**—**31** were carried out by means of the symmetrical acid anhydride method; the thus-obtained tripeptides **32**—**35** were subjected to further reactions for synthesizing the corresponding 001-C8 analogs **4**—**7**, as shown in Scheme 3. The synthesis of 004-C8 (**8**) with no basic amino acid residues was prepared by the conventional carbodiimide–HOBt method.

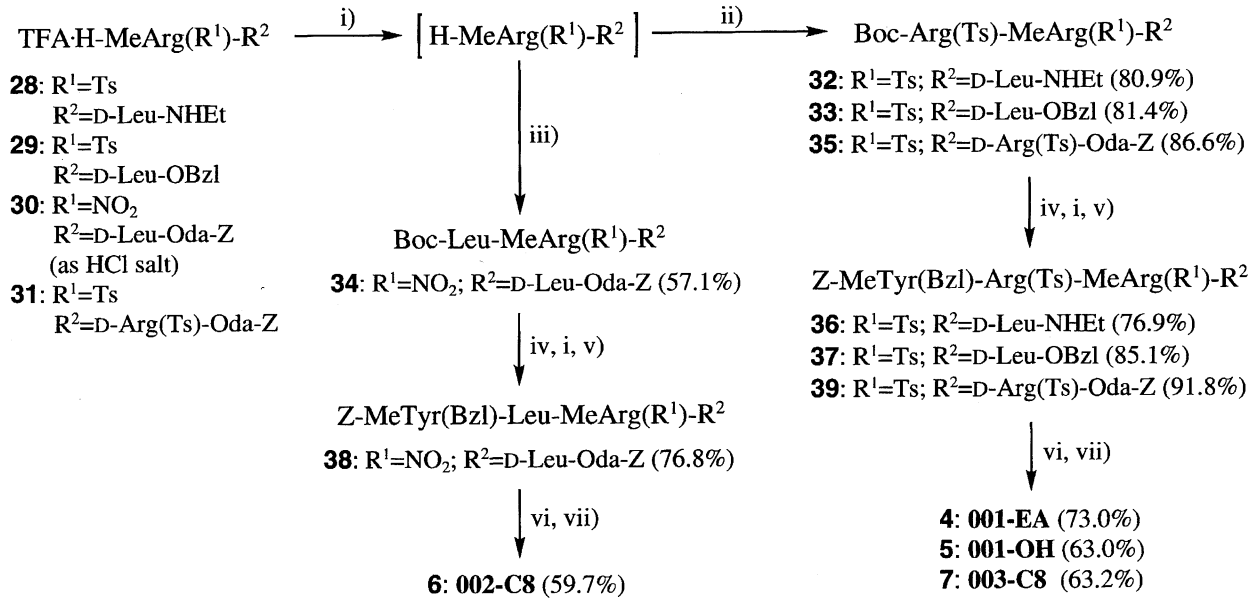
Throughout the syntheses of Arg- and/or MeArg-containing peptides, the guanidino groups of these amino acid residues were protected with the Ts group which is removable with anhydrous HF. However, in order to demonstrate the applicability of N^g-NO_2-protection for the Arg residue, we examined the synthesis of 002-C8 by use of Boc–Arg-(NO_2)–OH as a starting material. As a result, we realized that the N^g-NO_2 group is rather difficult to remove by conventional catalytic hydrogenation using Pd catalyst, and thus it also must be removed by HF.

Syntheses of Dipeptide Analogs. In order to elucidate the relationship between molecular size and BBB permeability of peptides, we next prepared five kinds of dipeptide analogs **9**—**13** as shown in Scheme 4. Of three kinds of alkanediamines used as the amide part, *N*-benzyloxycarbonyl-1,5-pentanediamine (H-Pda-Z) (**40**) was prepared in a similar manner to that for the preparation of 1,8-octanediamine derivative **14**. On the other hand, *N*-benzyloxycarbonyl-1,2-ethanediamine (H-Eda-Z) (**44**) was prepared from N^{β}-benzyloxycarbonyl-β-alaninamide (Z-β-Ala-NH_2) (**42**) by means of the modified Hofmann reaction[10] as shown in Scheme 5, since direct acylation of 1,2-ethanediamine (**45**) with ZOSu and TEA resulted in a formation of 2-imidazolidinone (**46**). Furthermore, the synthesis of 101-A (**13**) was carried out via Boc–Arg(Ts)–$NHBzh(Me)_2$ (**53**), since the use of Boc–Arg(Ts)–NH_2 resulted in a low overall yield of this simple amide analog; the amine component bis(4-meth-

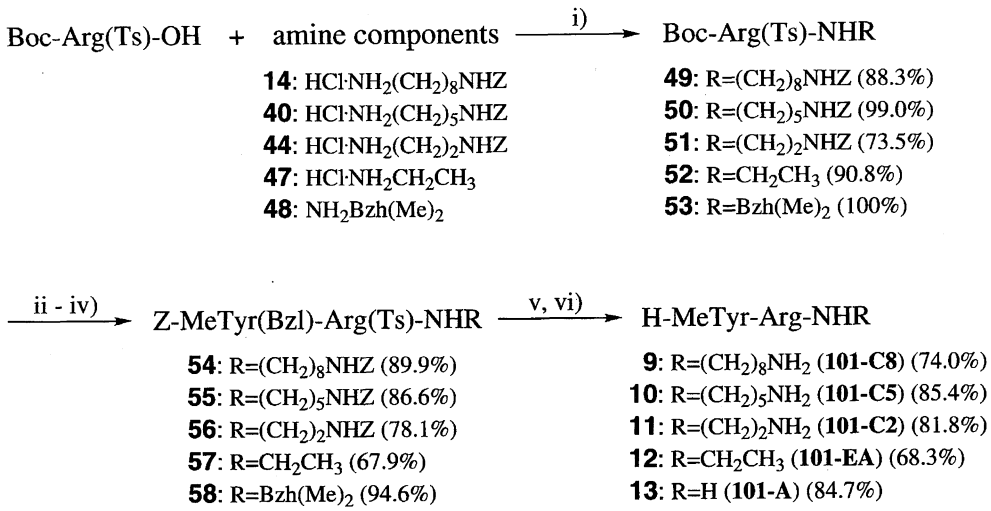
Table 1. *N*-Methylation Yields of Arg-Peptides [HCl·H–Arg(R^1)–R^2 (**24**—**27**) ⟶ TFA·H–MeArg(R^1)–R^2 (**28**—**31**)]

MeArg-peptides	Intermediate of	Arg-peptides		Yield (%)
		R^1	R^2	
28	001-EA	Ts	D-Leu–NHEt (**24**)	95.9
29	001-OH	Ts	D-Leu–OBzl (**25**)	86.9
30[a)]	002-C8	NO_2	D-Leu–$NH(CH_2)_8NHZ$ (**26**)	73.3
31	003-C8	Ts	D-Arg(Ts)–$NH(CH_2)_8NHZ$ (**27**)	91.1

a) Only this compound was prepared as HCl salt.



Scheme 3. i) sat. $NaHCO_3$ aq; ii) $[Boc–Arg(Ts)]_2O$; iii) $(Boc–Leu)_2O$; iv) TFA; v) Z–MeTyr(Bzl)–OH/EDC·HCl/HOBt; vi) HF/thioanisole; vii) preparative RPHPLC.



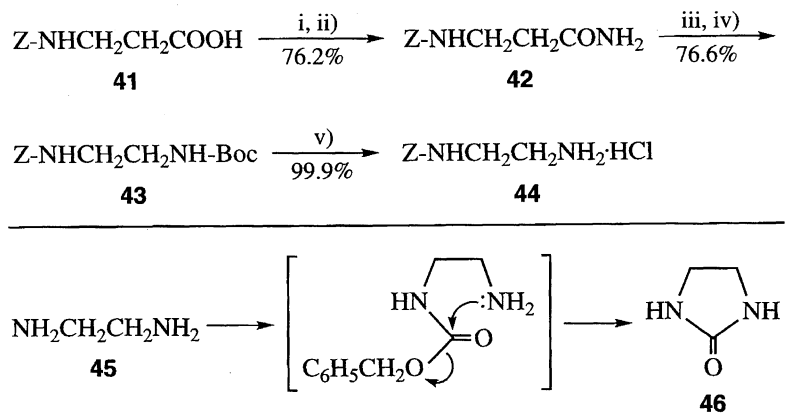
Scheme 4. i) EDC·HCl/HOBt/DIEA; ii) 50% TFA in CH_2Cl_2; iii) sat. $NaHCO_3$ aq; iv) Z–MeTyr(Bzl)–OH/EDC·HCl/HOBt; v) HF/thioanisole; vi) preparative RPHPLC.

ylphenyl)methanamine [$NH_2Bzh(Me)_2$] (**48**) for this purpose was prepared as shown in Scheme 6.

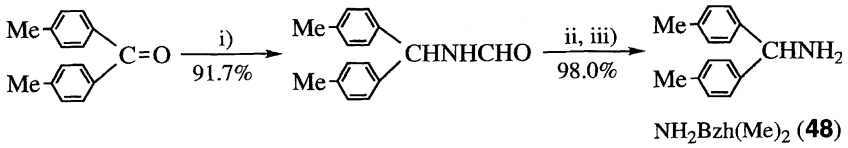
The BBB Permeability of Synthetic Peptides. In vitro study using BCEC is advantageous to assess the BBB permeability of peptides, because primary cultured BCEC has favorable characteristics as a BBB model morphologically.[11,12] Actually good agreement between in vitro uptake study using BCEC and in vivo transport study using a capillary depletion method or a brain microdialysis method was observed in the case of not only E-2078 or ebiratide but 001-C8 and its fluorescence-labeled analog as well.[1–4,13] The uptake of peptides labeled with ^{125}I to BCEC is evaluated by their acid resistant binding[14] to the cells as shown in Fig. 3. Among all of the synthetic peptides, the excellent uptake to BCEC was observed in 001-C8 (**3**). Other tetrapeptide analogs such as 002-C8 (**6**), 003-C8 (**7**), and 004-C8 (**8**) showed a little better uptake than E-2078 (**1**) or ebiratide (**2**), whereas 001-EA (**4**) and 001-OH (**5**) were hardly internalized to BCEC. Of the dipeptide analogs, only 101-C8 (**9**) showed comparable uptake to E-2078 or ebiratide, and the others were not internalized to BCEC at all. Although details about the results of elucidation concerning the BBB permeability of synthetic peptides had already been reported separately,[15] we herein suggest that a suitable balance between basicity and lipophilicity of peptide molecule is an important requisite for the BBB transport of peptides. In particular, the 1,8-octanediamine (Oda) residue as an amide component plays an important role for enhancing both basicity and lipophilicity of the peptide molecule.

Experimental

All of the melting points are uncorrected; they were measured by



Scheme 5. i) *i*-BuOCOCl/Bu_3N; ii) 25% aqueous ammonia; iii) $C_6H_5[(CF_3COO)_2I]$/pyridine; iv) $Boc_2O/NaHCO_3$; v) 1.5 M HCl in AcOH.



Scheme 6. i) $HCOOH/HCONH_2$; ii) 6 M HCl; iii) 2 M NaOH.

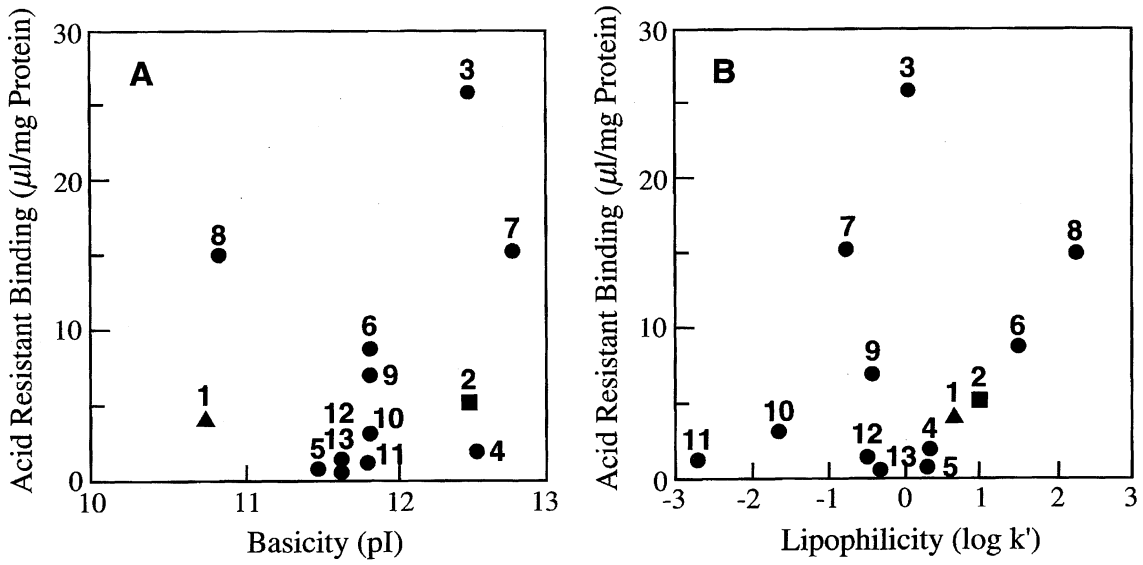


Fig. 3. Plots of basicity (A) or lipophilicity (B) vs. acid resistant binding of E-2078 (**1**), ebiratide (**2**), 001-C8 (**3**), and 001-C8 analogs **4**—**13** to primary cultured monolayers of BCEC. Basicity of peptides was represented by the theoretical isoelectric point (pI), and lipophilicity of those was evaluated in terms of the capacity factor (k') obtained by reversed-phase HPLC; details of the measurements are described in Ref. 15.

a Yanaco MP-J3 (Yanaco Co Ltd., Kyoto, Japan). Melting points of powdery samples prepared from oily substances by lyophilization and/or trituration with diethyl ether or hexane were not measured. Amino acid derivatives were purchased from Peptide Institute Inc., (Osaka, Japan). E-2078 and ebiratide were kindly supplied by Eisai Co., Ltd., (Tokyo, Japan) and Hoechst Japan Ltd., (Kawagoe, Japan), respectively. The specific rotations were measured on a Perkin–Elmer 241 polarimeter. Fast-atom bombardment mass spectra (FAB-MS) were obtained on a JEOL JMS SX-270 mass spectrometer. Silica-gel column chromatography was carried out with Merck silica gel 60 (Art. 9385, 230—400 mesh) at medium pressure (1—5 $kg\,cm^{-2}$). Final deprotection with anhydrous HF was carried out in the HF-reaction apparatus developed by Peptide Institute Inc., (Osaka, Japan). Preparative RPHPLC was performed on Cosmosil $5C_{18}$-AR 20×250 mm (Nacalai Tesque, Kyoto, Japan).

After each reaction, we generally worked up as follows; 1) the reaction mixture was concentrated in vacuo, and the residue was dissolved in AcOEt; 2) the solution was washed successively with 10% aqueous citric acid, brine, saturated aqueous $NaHCO_3$, and brine (**work-up procedure A**) or washed successively with saturated aqueous $NaHCO_3$, brine, 10% aqueous citric acid, and brine (**work-up procedure B**); 3) the organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo.

H–MeTyr–Arg–MeArg–D-Leu–NH(CH$_2$)$_8$NH$_2$ (001-C8) (3).

***N*-Benzyloxycarbonyl-1,8-octanediamine Hydrochloride (HCl·H–Oda–Z) (14).** To a solution of 1,8-octanediamine (5.76

g, 40.0 mmol) in diethyl ether (600 ml) was added dropwise a solution of ZOSu (4.98 g, 20.0 mmol) and TEA (2.77 ml, 20.0 mmol) in diethyl ether (800 ml) at 0 °C over a period of 40 h. The reaction mixture was additionally stirred overnight at 0 °C, and then concentrated in vacuo. The residue was dissolved in methanol (180 ml) and 6 M HCl (10 ml, 1 M = 1 mol dm^{-3}), and it was then diluted with water (1 dm^3). The precipitate was filtered off, and the filtrate was allowed to stand overnight in a refrigerator. The precipitated crystalline substance was filtered off again, and the filtrate was applied to Diaion® HP 20 column (Mitsubishi Chemical Co., Tokyo, 6.0×20 cm). The column was thoroughly washed with water, and then HCl·H–Oda–Z adsorbed on the column was eluted with 80% MeOH. The eluate was concentrated in vacuo, and the thus-obtained crystalline residue was recrystallized from methanol and diethyl ether. Yield 3.70 g (58.9% from ZOSu); mp 183—185.5 °C (decomp); FAB-MS *m/z* 279.1 $(M+H)^+$. Found: C, 59.33; H, 8.73; N, 8.62; Cl, 11.18%. Calcd for $C_{16}H_{27}O_2N_2Cl\cdot 1/2H_2O$: C, 59.33; H, 8.71; N, 8.65; Cl, 10.94%.

N^1-Benzyloxycarbonyl-N^8-(*t*-butoxycarbonyl-D-leucyl)-1,8-octanediamine (Boc–D-Leu–Oda–Z) (15). (General Procedure 1). To a solution of **14** (3.66 g, 11.6 mmol), Boc–D-Leu–OH·H_2O (3.42 g, 12.8 mmol), and HOBt (1.88 g, 11.6 mmol) in DMF (50 ml) were added DDC (2.87 g, 13.9 mmol) and TEA (1.78 ml, 12.8 mmol) at 0 °C; the mixture was then stirred overnight at r.t. AcOH (224 μl, 3.48 mmol) was added to the reaction mixture, and stirred for a further 1 h. The precipitate was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in AcOEt, and treated by work-up procedure A. The thus-obtained crystalline residue was recrystallized from diethyl ether and hexane to give **15** (5.70 g, 100%). Mp 52—56 °C; $[\alpha]_D^{20}$ +20.4° (*c* 1.04, $CHCl_3$); FAB-MS *m/z* 492.3 $(M+H)^+$. Found: C, 66.00; H, 9.29; N, 8.57%. Calcd for $C_{27}H_{45}O_5N_3$: C, 65.95; H, 9.22; N, 8.55%.

N^1-Benzyloxycarbonyl-N^8-D-leucyl-1,8-octanediamine (H–D-Leu–Oda–Z) (16). (General Procedure 2). A solution of **15** (670 mg, 1.36 mmol) in CH_2Cl_2 (2 ml) and TFA (2 ml) was stirred for 15 min at r.t., and concentrated in vacuo. The residue was dissolved in AcOEt, and the solution was washed with saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo to obtain **16** as a crystalline substance which was used for a subsequent coupling reaction after confirming the structure by measurement of FAB-MS. Yield 534 mg (94.2%); FAB-MS *m/z* 392.3 $(M+H)^+$.

N^1-Benzyloxycarbonyl-N^8-(N^α-*t*-butoxycarbonyl-N^g-tosylarginyl-D-leucyl)-1,8-octanediamine [Boc–Arg(Ts)–D-Leu–Oda–Z] (17). (General Procedure 3). EDC·HCl (53.9 mg, 0.281 mmol) was added to a solution of **16** (100 mg, 0.255 mmol), Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (140 mg, 0.281 mmol), and HOBt (38.0 mg, 0.281 mmol) in DMF (1.5 ml) at 0 °C. The mixture was stirred for 5.5 h at r.t., and concentrated in vacuo. The residue was dissolved in AcOEt, and treated by work-up procedure A. The thus-obtained oily residue was triturated with hexane to give **17** (203 mg, 99.2%) as a powdery substance that was pure enough to use for subsequent reactions. $[\alpha]_D^{20}$ +25.3° (*c* 0.910, MeOH); FAB-MS *m/z* 802.7 $(M+H)^+$. Found: C, 59.38; H, 7.85; N, 11.88%. Calcd for $C_{40}H_{63}O_8N_7S\cdot 1/2H_2O$: C, 59.23; H, 7.95; N, 12.08%.

N^1-Benzyloxycarbonyl-N^8-(N^α-methyl-N^g-tosylarginyl-D-leucyl)-1,8-octanediamine [H–MeArg(Ts)–D-Leu–Oda–Z] (20). (General Procedure 4: The *N*-Methylation by the Diels–Alder reaction). The compound **17** (163 mg, 0.204 mmol) was dissolved in 1.5 M HCl in AcOH (2.04 ml). The solution was stirred for 20 min at r.t., and concentrated in vacuo. The residue was dissolved in dioxane, and the solution was then lyophilized to give **18** (134 mg, 89.0%) as a powder hydrochloride.

To a solution of **18** (134 mg, 0.181 mmol) in H_2O (1 ml) was added 39.0 μl (0.483 mmol) each of 37% formaldehyde for three times over a period of 1 h. To the solution was added 56 μl (0.66 mmol) each of freshly distilled cyclopentadiene twice over a period of 30 min with vigorous stirring, and the mixture was stirred for a further 1 h at r.t. The heterogeneous reaction mixture was washed several times with hexane by decantation, and neutralized with aqueous $NaHCO_3$. The neutralized mixture was extracted several times with $CHCl_3$. The combined extracts were dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in dioxane, and lyophilized to give N^1-Z-N^8-[2-*N*-(2-azanorbornenyl)-5-(tosylguanidino)valeryl-D-Leu]-1,8-octanediamine (139 mg, 98.4%) as a powdery substance.

To the thus-obtained 2-azanorbornene derivative (139 mg, 0.178 mmol) in CH_2Cl_2 (0.9 ml) were added TFA (0.9 ml) and TES (85.2 μl, 0.533 mmol) in an atmosphere of argon. The solution was stirred for 3 h at r.t., and concentrated in vacuo. The residue was column chromatographed on silica gel (10 g,[16] $CHCl_3$–MeOH–AcOH = 6 : 1 : 0.1), and the fractions containing TFA·H–MeArg(Ts)–D-Leu–Oda–Z (**19**) were concentrated in vacuo. The residue was dissolved in $CHCl_3$, and the solution was washed with aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **20** (102 mg, 79.4%) as an oily substance; the thus-obtained **20** was subjected to subsequent reaction without any characterization.

N^1-Benzyloxycarbonyl-N^8-(N^α-*t*-butoxycarbonyl-N^g-tosylarginyl-N^α-methyl-N^g-tosylarginyl-D-leucyl)-1,8-octanediamine [Boc–Arg(Ts)–MeArg(Ts)–D-Leu–Oda–Z] (21). (General Procedure 5: The Coupling of Boc–Amino Acids with MeArg–Peptides by Symmetrical Acid Anhydride Method). EDC·HCl (942 mg, 4.92 mmol) was added to a solution of Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (2.45 g, 4.92 mmol) in DMF (10 ml), and the mixture was stirred for 1 h under cooling in an ice-salt bath. To the chilled mixture was added **20** (1.17 g, 1.64 mmol) in DMF (10 ml), and the mixture was stirred for 3 h in an ice-salt bath and overnight at r.t. The reaction mixture was concentrated in vacuo, and the residue was treated by work-up procedure A. The thus-obtained oily residue was column-chromatographed on silica gel (30 g, $CHCl_3$–MeOH = 15 : 1). The fractions containing **21** were combined, and concentrated in vacuo. The residue was triturated with diethyl ether to give **21** (1.46 g, 78.7%) as a powdery substance which was used for a subsequent reaction without further purification. $[\alpha]_D^{24}$ − 17.5° (*c* 1.10, MeOH); FAB-MS *m/z* 1127.0 $(M+H)^+$. Found: C, 56.48; H, 7.47; N, 13.48%. Calcd for $C_{54}H_{83}O_{11}N_{11}S_2\cdot H_2O$: C, 56.67; H, 7.49; N, 13.46%.

N^α-Benzyloxycarbonyl-N^α-methyl-*O*-benzyltyrosine [Z–MeTyr(Bzl)–OH]. To a solution of Z–Tyr(Bzl)–OH (3.59 g, 8.85 mmol) in anhydrous THF (23 ml) were added NaH (60% oil suspension, 1.06 g, 26.6 mmol) and 98% CH_3I (4.41 ml, 70.8 mmol) at 0 °C. The solution was stirred for 15 min at 0 °C and overnight at r.t. The reaction mixture was acidified with 1 M HCl (26 ml), and concentrated in vacuo. The residue was dissolved in AcOEt, and the solution was washed three times with brine. The AcOEt layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo. To the residue was added saturated aqueous $NaHCO_3$, and the thus-precipitated Z–MeTyr(Bzl)–ONa was filtered with suction. The sodium salt was treated with 1 M HCl and AcOEt, and the organic layer was washed three times with brine. The AcOEt layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was recrystallized from AcOEt and hexane. Yield 2.90 g (78.3%); mp 93—94 °C (sintered at 66 °C); $[\alpha]_D^{22}$ −45.5°

(*c* 1.01, MeOH); FAB-MS *m/z* 420.0 $(M+H)^+$. Found: C, 71.36; H, 5.87; N, 3.30%. Calcd for $C_{25}H_{25}O_5N$: C, 71.58; H, 6.01; N, 3.34%.

N^1-Benzyloxycarbonyl-N^8-(N^α-benzyloxycarbonyl-N^α-methyl-*O*-benzyltyrosyl-N^g-tosylarginyl-N^α-methyl-N^g-tosylarginyl-D-leucyl)-1,8-octanediamine [Z–MeTyr(Bzl)–Arg(Ts)–MeArg(Ts)–D-Leu–Oda–Z] (23). (General Procedure 6: The Coupling of Z–MeTyr(Bzl)–OH with Amine Segments). Compound **21** (500 mg, 0.444 mmol) was dissolved in TFA (513 μl), and the solution was stirred for 20 min at r.t. TFA was removed in vacuo, and the residue was worked up in a similar manner to that mentioned for preparing **16** to afford H–Arg(Ts)–MeArg(Ts)–D-Leu–Oda–Z (**22**) as an oily substance (430 mg, 94.3%). A part of the thus-obtained amine segment was subjected to the following reaction without further purification.

EDC·HCl (10.3 mg, 56.3 μmol) was added to a solution of **22** (50.0 mg, 48.7 μmol), Z–MeTyr(Bzl)–OH (22.5 mg, 53.6 μmol), and HOBt (7.4 mg, 54 μmol) in DMF (200 μl) at 0 °C. The mixture was worked up according to General Procedure 3. The thus-obtained crude product was column-chromatographed on silica-gel (30 g, $CHCl_3$–MeOH = 20 : 1). The fractions containing **23** were combined, and concentrated in vacuo. The residue was dissolved in dioxane, and lyophilized to give **23** (61.7 mg, 88.6%) as a powdery substance: $[\alpha]_D^{28}$ −19.3° (*c* 1.04, MeOH); FAB-MS *m/z* 1428.2 $(M+H)^+$. Found: C, 60.94; H, 6.93; N, 11.42%. Calcd for $C_{74}H_{98}O_{13}N_{12}S_2\cdot 2H_2O$: C, 60.71; H, 7.02; N, 11.48%.

***N*-(N^α-Methyltyrosylarginyl-N^α-methylarginyl-D-leucyl)-1,8-octanediamine (H–MeTyr–Arg–MeArg–D-Leu–Oda–H) (3). (General Procedure 7: The Final Deprotection with Anhydrous HF).** HF (2 ml) was added to **23** (25.0 mg, 17.5 μmol) and thioanisole ((methylthio)benzene) (190 μl) at −78 °C. The reaction mixture was stirred for 1 h at 0 °C, and concentrated in vacuo. The residue was dissolved in 4% acetic acid, and the solution was washed with diethyl ether. The aqueous layer was passed through Dowex 1×8 column (AcO^- form), and the eluate was lyophilized. The thus-obtained crude product was purified by RPHPLC (10—40% CH_3CN–0.1% TFA aq, 8.0 ml min^{-1}). The fractions containing **3** were combined, and lyophilized. The residue was dissolved 1.5 M HCl in AcOH, and the solution was lyophilized again to give **3** (13.7 mg, 64.3%) as a powdery hydrochloride. $[\alpha]_D^{25}$ −18.3° (*c* 1.02, MeOH); FAB-MS *m/z* 761.6 $(M+H)^+$. Found: C, 46.15; H, 7.97; N, 17.19%. Calcd for $C_{37}H_{68}O_5N_{12}\cdot 4HCl\cdot 3H_2O$: C, 46.24; H, 8.18; N, 17.49%.

H–MeTyr–Arg–MeArg–D-Leu–NHCH$_2$CH$_3$ (001-EA) (4).

N-(N^α-*t*-Butoxycarbonyl-D-leucyl)ethanamine (Boc–D-Leu–NHEt). Boc–D-Leu–OH·H_2O (5.00 g, 20.1 mmol) was coupled with HCl·NH_2Et (1.80 g, 22.1 mmol) according to General Procedure 1, except for the use of EDC·HCl as a coupling agent. Recrystallization from hexane gave Boc–D-Leu–NHEt (5.04 g, 97.3%) as colorless crystals: Mp 103—103.5 °C; $[\alpha]_D^{23}$ +20.1° (*c* 1.00, MeOH); FAB-MS *m/z* 258.9 $(M+H)^+$. Found: C, 60.75; H, 10.24; N, 10.73%. Calcd for $C_{13}H_{26}O_3N_2$: C, 60.43; H, 10.14; N, 10.84%.

N-(N^α-*t*-Butoxycarbonyl-N^g-tosylarginyl-D-leucyl)ethanamine [Boc–Arg(Ts)–D-Leu–NHEt]. Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (425 mg, 0.852 mmol) was coupled with TFA·H–D-Leu–NHEt (211 mg, 0.774 mmol) prepared from Boc–D-Leu–NHEt (200 mg, 0.774 mmol) by conventional TFA–CH_2Cl_2 treatment in a quantitative yield; the coupling was carried out in a similar manner to that in General Procedure 1, except for the use of EDC·HCl as a coupling agent and DIEA as a base. Recrystallization from AcOEt and diethyl ether gave colorless crystals: Yield 417 mg (94.7%); mp 118—121 °C; $[\alpha]_D^{23}$ +30.1° (*c* 1.00, MeOH); FAB-MS *m/z* 569.4 $(M+H)^+$. Found: C, 54.74; H, 7.77; N, 14.77%. Calcd for $C_{26}H_{44}O_6N_6S$: C, 54.90; H, 7.80; N, 14.77%.

N-(N^α-Methyl-N^g-tosylarginyl-D-leucyl)ethyanamine Trifluoroacetate [TFA·H–MeArg(Ts)–D-Leu–NHEt] (28). *N*-Methylation of HCl·H–Arg(Ts)–D-Leu–NHEt (**24**) prepared from Boc–Arg(Ts)–D-Leu–NHEt (120 mg, 0.211 mmol) was carried out according to General Procedure 4, and TFA·H–MeArg(Ts)–D-Leu–NHEt (**28**) was once isolated as a powdery substance by trituration with diethyl ether: Yield 122 mg (95.9%).

N-(N^α-*t*-Butoxycarbonyl-N^g-tosylarginyl-N^α-methyl-N^g-tosylarginyl-D-leucyl)ethanamine [Boc–Arg(Ts)–MeArg(Ts)–D-Leu–NHEt] (32). H–MeArg(Ts)–D-Leu–NHEt (171 mg, 0.354 mmol) prepared from **28** (220 mg, 0.367 mmol) by base treatment as described in General Procedure 4 was coupled with Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (530 mg, 1.06 mmol) according to General Procedure 5. Purification by silica-gel column chromatography (30 g, $CHCl_3$–MeOH = 15 : 1) gave **32** as an oily substance; yield 265 mg (80.9% from **28**). $[\alpha]_D^{25}$ −22.7° (*c* 1.01, MeOH); FAB-MS *m/z* 739.8 $[(M-Ts+H)+H]^+$.[17] Found: C, 52.17; H, 7.22; N, 15.22%. Calcd for $C_{40}H_{64}O_9N_{10}S_2\cdot 1.5H_2O$: C, 52.21; H, 7.34; N, 15.22%.

N-(N^α-Benzyloxycarbonyl-N^α-methyl-*O*-benzyltyrosyl-N^g-tosylarginyl-N^α-methyl-N^g-tosylarginyl-D-leucyl)ethanamine [Z–MeTyr(Bzl)–Arg(Ts)–MeArg(Ts)–D-Leu–NHEt] (36). Z–MeTyr(Bzl)–OH (104 mg, 0.247 mmol) was coupled with H–Arg(Ts)–MeArg(Ts)–D-Leu–NHEt prepared from **32** (200 mg, 0.224 mmol) and worked up as described in General Procedure 6. Compound **36** was thus obtained as a powdery substance; yield 206 mg (76.9%). $[\alpha]_D^{25}$ −26.6° (*c* 1.02, MeOH); FAB-MS *m/z* 1194.7 $(M+H)^+$. Found: C, 59.23; H, 6.70; N, 12.48%. Calcd for $C_{60}H_{79}O_{11}N_{11}S_2\cdot 1.5H_2O$: C, 58.99; H, 6.77; N, 12.61%.

N-(N^α-Methyltyrosylarginyl-N^α-methylarginyl-D-leucyl)-ethanamine (H–MeTyr–Arg–MeArg–D-Leu–NHEt) (4). Deprotection of **36** (100 mg, 83.7 μmol) was carried out according to General Procedure 7. RPHPLC (10—40% CH_3CN–0.1% TFA, 8.0 ml min^{-1}) purification gave **4** as a powdery TFA salt; yield 61.3 mg (73.0%). $[\alpha]_D^{26}$ −18.9° (*c* 1.29, MeOH); FAB-MS *m/z* 662.4 $(M+H)^+$. Found: C, 41.77; H, 5.64; N, 14.39%. Calcd for $C_{31}H_{55}O_5N_{11}\cdot 3.5TFA\cdot 1.5H_2O$: C, 41.95; H, 5.70; N, 14.16%.

H–MeTyr–Arg–MeArg–D-Leu–OH (001-OH).

D-Leucine Benzyl Ester Hydrochloride (HCl·H–D-Leu–OBzl). Dicyclohexylamine (3.98 ml, 22.0 mmol) and benzyl bromide (2.38 ml, 22.0 mmol) were added to a solution of Boc–D-Leu–OH·H_2O (4.99 g, 20.0 mmol) in DMF (50 ml) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and overnight at r.t. The precipitate was filtered off, and the filtrate was concentrated in vacuo. The residue was worked up by procedure A except for the use of anhydrous $MgSO_4$ as a desiccant. The oily residue was then column-chromatographed on silica gel (100 g, toluene–AcOEt = 19 : 1) to give Boc–D-Leu–OBzl as an oily substance.

The thus-obtained Boc–D-Leu–OBzl was dissolved in 1.5 M HCl in AcOH (215 ml), and the solution was stirred for 80 min. After concentration in vacuo, the residue was lyophilized from dioxane to give HCl·H–D-Leu–OBzl as a colorless oil; yield 4.97 g (96.1%).

***t*-Butoxycarbonyl-N^g-tosylarginyl-D-leucine Benzyl Ester [Boc–Arg(Ts)–D-Leu–OBzl].** Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (109 mg, 0.218 mmol) was coupled with HCl·H–D-Leu–OBzl (51.0 mg, 0.198 mmol) according to General Procedure 1, except for the use of EDC·HCl as a coupling agent, to give Boc–Arg(Ts)–D-Leu–OBzl as a colorless oil; yield 112 mg (89.4%). $[\alpha]_D^{23}$ +11.3° (*c* 0.400, MeOH); FAB-MS *m/z* 632.3 $(M+H)^+$. Found: C, 57.76; H, 7.01; N, 10.95%. Calcd for $C_{31}H_{45}O_7N_5S\cdot 3/4H_2O$: C,

57.70; H, 7.26; N, 10.85%.

N^{α}-Methyl-N^{g}-tosylarginyl-D-leucine Benzyl Ester Trifluoroacetate [TFA·H–MeArg(Ts)–D-Leu–OBzl] (29). *N*-Methylation of HCl·H–Arg(Ts)–D-Leu–OBzl (**25**) prepared from Boc–Arg(Ts)–D-Leu–OBzl (112 mg, 0.177 mmol) was carried out according to General Procedure 4, and the thus-obtained crude product was purified by silica-gel column chromatography (20 g,[16] $CHCl_3$–MeOH–AcOH = 6 : 1 : 0.1) gave **29** (101 mg, 86.9%).

N^{α}-*t*-Butoxycarbonyl-N^{g}-tosylarginyl-N^{α}-methyl-N^{g}-tosylarginyl-D-leucine Benzyl Ester [Boc–Arg(Ts)–MeArg(Ts)–D-Leu–OBzl] (33). H–MeArg(Ts)–D-Leu–OBzl prepared from **29** (87.9 mg, 0.133 mmol) was coupled with Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (199 mg, 0.399 mmol) according to General Procedure 5 as described in preparing **32**. Compound **33** was obtained only as an oily substance even after lyophilization from dioxane; yield 103 mg (81.4%). $[\alpha]_D^{23}$ −17.1° (*c* 1.17, MeOH); FAB-MS *m/z* 956.4 $[(M - Ts + H) + H]^+$.[17] Found: C, 52.40; H, 6.99; N, 11.17%. Calcd for $C_{45}H_{65}O_{10}N_9S_2$·4/5$C_4H_8O_2$ (dioxane)·4H_2O: C, 52.69; H, 7.28; N, 11.47%.

N^{α}-Benzyloxycarbonyl-N^{α}-methyl-*O*-benzyltyrosyl-N^{g}-tosylarginyl-N^{α}-methyl-N^{g}-tosylarginyl-D-leucine Benzyl Ester [Z–MeTyr(Bzl)–Arg(Ts)–MeArg(Ts)–D-Leu–OBzl] (37). Z–MeTyr(Bzl)–OH (29.7 mg, 70.8 μmol) was coupled with H–Arg(Ts)–MeArg(Ts)–D-Leu–OBzl prepared from **33** (61.6 mg, 64.4 μmol) according to General Procedure 6. Compound **37** was obtained only as an oily substance even after lyophilization from dioxane; yield 69.0 mg (85.1%). $[\alpha]_D^{23}$ −22.8° (*c* 1.00, DMF); FAB-MS *m/z* 1257.9 $(M+H)^+$. Found: C, 59.12; H, 6.52; N, 10.25%. Calcd for $C_{65}H_{80}O_{12}N_{10}S_2$·1/2$C_4H_8O_2$·3$H_2O$: C, 59.36; H, 6.69; N, 10.33%.

N^{α}-Methyltyrosylarginyl-N^{α}-methylarginyl-D-leucine (H–MeTyr–Arg–MeArg–D-Leu–OH) (5). Compound **37** (28.0 mg, 22.2 μmol) was worked up according to General Procedure 7, and tetrapeptide **5** was obtained as a powdery hydrochloride: Yield 9.98 mg (63.0%); $[\alpha]_D^{20}$ −36.7° (*c* 1.37, MeOH); FAB-MS *m/z* 635.4 $(M+H)^+$. Found: C, 43.31; H, 7.18; N, 17.40%. Calcd for $C_{29}H_{53}O_6N_{10}$·3HCl·3H_2O: C, 43.63; H, 7.45; N, 17.54%.

H–MeTyr–Leu–MeArg–D-Leu–NH$(CH_2)_8NH_2$ (002-C8) (6).

N^{1}-Benzyloxycarbonyl-N^{8}-(N^{α}-*t*-butoxycarbonyl-N^{g}-nitroarginyl-D-leucyl)-1,8-octanediamine [Boc–Arg(NO_2)–D-Leu–Oda–Z]. EDC·HCl (2.25 g, 11.7 mmol) was added to a solution of Boc–Arg(NO_2)–OH·1/2AcOEt·1/4H_2O (3.95 g, 10.7 mmol), **16** (3.82 g, 9.76 mmol), and HOBt (1.58 g, 11.7 mmol) in DMF (25 ml) at 0 °C. The mixture was worked up as described in General Procedure 3. The thus-obtained oily residue was column chromatographed on silica gel (200 g, $CHCl_3$–MeOH = 25 : 1) to give Boc–Arg(NO_2)–D-Leu–Oda–Z as an oily substance. Yield 6.83 g (quant); $[\alpha]_D^{20}$ +9.5° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 693.4 $(M+H)^+$. Found: C, 56.88; H, 8.15; N, 15.88%. Calcd for $C_{33}H_{56}O_8N_8$·1/3H_2O: C, 56.72; H, 8.17; N, 16.03%.

N^{1}-Benzyloxycarbonyl-N^{8}-(N^{α}-methyl-N^{g}-nitroarginyl-D-leucyl)-1,8-octanediamine Hydrochloride [HCl·H–MeArg(NO_2)–D-Leu–Oda-Z] (30). *N*-Methylation of N^{g}-NO_2–Arg peptide was carried out in a similar manner to that in General Procedure 4. Boc–Arg(NO_2)–D-Leu–Oda–Z (1.84 g, 2.66 mmol) was dissolved in 1.5 M HCl in AcOH (9 ml), and the solution was stirred for 70 min at r.t., followed by concentration in vacuo. The residue was triturated with diethyl ether to give HCl·H–Arg(NO_2)–D-Leu–Oda–Z (**26**) (1.62 g, 97.0%) as a powdery substance.

A part of the thus-obtained hydrochloride **26** (225 mg, 0.181 mmol) was dissolved in H_2O (2 ml). To the solution was added 62.0 μl (0.622 mmol) each of 37% formaldehyde four times over a period of 1 h, and then 73.0 μl (0.895 mmol) each of freshly distilled cyclopentadiene twice over a period of 40 min with vigorous stirring; the mixture was stirred for a further 100 min at r.t. The heterogeneous reaction mixture was worked up according to the General Procedure 4 to give N^{1}-Z-N^{8}-[2-*N*-(2-azanorbornenyl)-5-(nitroguanidino)valeryl-D-Leu]-1,8-octanediamine (240 mg, quant) as an oily substance.

To the thus-obtained 2-azanorbornene derivative (240 mg, 0.358 mmol) in CH_2Cl_2 (2.0 ml) were added TFA (2.0 ml) and TES (170 μl, 1.43 mmol) in an atmosphere of argon. The mixture was stirred for 7 h at r.t., and concentrated in vacuo. The residue was column-chromatographed on silica gel (10 g,[16] $CHCl_3$–MeOH–AcOH = 6 : 1 : 0.1), and the fractions containing TFA·H–MeArg(Ts)–D-Leu–Oda–Z were concentrated in vacuo. The residue was dissolved in a small amount of MeOH, and 12.5 M HCl in MeOH (100 μl) was added to the solution, followed by concentration in vacuo. The residue was triturated with diethyl ether to give **30** (169 mg, 73.3%) as a powdery substance; the thus-obtained MeArg-peptide was used for subsequent reaction without any characterization.

N^{1}-Benzyloxycarbonyl-N^{8}-(N^{α}-*t*-butoxycarbonylleucyl-N^{α}-methyl-N^{g}-nitroarginyl-D-leucyl)-1,8-octanediamine [Boc–Leu–MeArg(NO_2)–D-Leu–Oda–Z] (34). The hydrochloride **30** (636 mg, 0.988 mmol) prepared as shown above was treated with AcOEt and saturated $NaHCO_3$. The organic layer was separated, and dried over anhydrous Na_2SO_4. The thus-obtained H–MeArg(Ts)–D-Leu–Oda–Z [386 mg (64.3%),[18] 0.636 mmol] was coupled with Boc–Leu–OH·H_2O (793 mg, 3.18 mmol) according to General Procedure 5. After column-chromatographic purification on silica gel (30 g, AcOEt–toluene = 9 : 1), **34** was obtained as a powdery substance by lyophilization from dioxane; yield 464 mg (57.1% from **30**). $[\alpha]_D^{23}$ −41.4° (*c* 1.05, MeOH); FAB-MS 775.4 $[(M - NO_2 + H) + H]^+$. Found: C, 58.23; H, 8.49; N, 14.37%. Calcd for $C_{37}H_{68}O_5N_{12}$·1/2$C_4H_8O_2$: C, 58.38; H, 8.51; N, 14.58%.

N^{1}-Benzyloxycarbonyl-N^{8}-(N^{α}-benzyloxycarbonyl-N^{α}-methyl-*O*-benzyltyrosylleucyl-N^{α}-methyl-N^{g}-nitroarginyl-D-leucyl)-1,8-octanediamine [Z–MeTyr(Bzl)–Leu–MeArg(NO_2)–D-Leu–Oda–Z] (38). Preparation of **38** was carried out in a similar manner to that in General Procedure 6, i.e., compound **34** (464 mg, 0.566 mmol) was worked up according to General Procedure 2, and the thus-obtained H–Leu–MeArg(NO_2)–D-Leu–Oda–Z was coupled with Z–MeTyr(Bzl)–OH (238 mg, 0.566 mmol) according to General Procedure 3. The thus-obtained crude product was column-chromatographed on silica gel (30 g, $CHCl_3$–MeOH = 29 : 1). The fractions containing **38** were combined, and concentrated in vacuo. The residue was dissolved in dioxane, and lyophilized to give **38** (486 mg, 76.8%) as a powdery substance: $[\alpha]_D^{30}$ −20.0° (*c* 1.07, DMF); FAB-MS *m/z* 1077.0 $[(M - NO_2 + H) + H]^+$. Found: C, 63.50; H, 7.59; N, 12.25%. Calcd for $C_{60}H_{84}O_{11}N_{10}$·H_2O: C, 63.24; H, 7.61; N, 12.29%.

***N*-(N^{α}-Methyltyrosylleucyl-N^{α}-methylarginyl-D-leucyl)-1,8-octanediamine [H–MeTyr–Leu–MeArg–D-Leu–Oda–H] (6).** Compound **38** (310 mg, 0.276 mmol) was worked up according to General Procedure 7. The thus-obtained crude product was purified by RPHPLC (20—55% CH_3CN–0.1% TFA aq, 8.0 ml min^{-1}), and the fractions containing **6** were combined, followed by concentration in vacuo. The residue was dissolved in 1.5 M HCl in AcOH, and the solution was lyophilized to give **6** (137 mg, 59.7%) as a powdery hydrochloride. $[\alpha]_D^{20}$ −23.3° (*c* 0.750, MeOH); FAB-MS *m/z* 718.7. Found: C, 49.64; H, 8.30; N, 13.71%. Calcd for $C_{37}H_{67}O_5N_9$·4HCl·2H_2O: C, 49.38; H, 8.40; N, 14.00%.

H–MeTyr–Arg–MeArg–D-Arg–NH(CH$_2$)$_8$NH$_2$ (003-C8) (7). **N^1-Benzyloxycarbonyl-N^8-(N^α-*t*-butoxycarbonyl-N^g-tosyl-D-arginyl)-1,8-octanediamine [Boc–D-Arg(Ts)–Oda–Z].** Boc–D-Arg(Ts)–OH·3/5AcOEt·1/5H$_2$O (678 mg, 1.40 mmol) was coupled with **14** (400 mg, 1.27 mmol) according to General Procedure 1, except for the use of EDC·HCl as a coupling agent and DIEA as a base. After purification by silica-gel column chromatography (30 g, CHCl$_3$–MeOH = 19 : 1), Boc–D-Arg(Ts)–Oda–Z was obtained as a powdery substance by lyophilization from dioxane: Yield 751 mg (85.9%); $[\alpha]_D^{35}$ +0.80° (*c* 1.0, MeOH). Found: C, 58.65; H, 7.70; N, 11.99%. Calcd for C$_{34}$H$_{52}$O$_7$N$_6$S·1/2H$_2$O: C, 58.51; H, 7.65; N, 12.04%.

N^1-Benzyloxycarbonyl-N^8-(N^g-tosyl-D-arginyl)-1,8-octanediamine [H–D-Arg(Ts)–Oda–Z]. Boc–D-Arg(Ts)–Oda–Z (650 mg, 0.944 mmol) was worked up according to General Procedure 2 to give H–D-Arg(Ts)–Oda–Z as a powdery substance after lyophilization from dioxane; yield 490 mg (88.0%).

N^1-Benzyloxycarbonyl-N^8-(N^α-*t*-butoxycarbonyl-N^g-tosylarginyl-N^g-tosyl-D-arginyl)-1,8-octanediamine [Boc–Arg(Ts)–D-Arg(Ts)–Oda–Z]. Boc–Arg(Ts)–OH·3/4AcOEt·1/4H$_2$O (56.4 mg, 0.113 mmol) was coupled with H–D-Arg(Ts)–Oda–Z (60.5 mg, 0.103 mmol) according to General Procedure 3. The crude product was purified by silica-gel column chromatography (20 g, CHCl$_3$: MeOH = 20 : 1), and Boc–Arg(Ts)–D-Arg(Ts)–Oda–Z was obtained as a colorless oil; yield 79.5 mg (77.2%). $[\alpha]_D^{23}$ +9.7° (*c* 0.54, MeOH); FAB-MS *m/z* 999.5 (M+H)$^+$. Found: C, 53.64; H, 7.12; N, 12.62%. Calcd for C$_{47}$H$_{70}$O$_{10}$N$_{10}$S$_2$·3/4C$_4$H$_8$O$_2$·3H$_2$O: C, 53.64; H, 7.38; N, 12.51%.

N^1-Benzyloxycarbonyl-N^8-(N^α-methyl-N^g-tosylarginyl-N^g-tosyl-D-arginyl)-1,8-octanediamine Trifluoroacetate [TFA·H–MeArg(Ts)–D-Arg(Ts)–Oda–Z] (31). *N*-Methylation of HCl·H–Arg(Ts)–D-Arg(Ts)–Oda–Z (**27**) prepared from Boc–Arg(Ts)–D-Arg(Ts)–Oda–Z (69.5 mg, 69.6 μmol) was carried out according to General Procedure 4, and an oily product was triturated with diethyl ether to give **31** as a powdery substance; yield 68.7 mg (91.1%).

N^1-Benzyloxycarbonyl-N^8-(N^α-*t*-butoxycarbonyl-N^g-tosylarginyl-N^α-methyl-N^g-tosylarginyl-N^g-tosyl-D-arginyl)-1,8-octanediamine [Boc–Arg(Ts)–MeArg(Ts)–D-Arg(Ts)–Oda–Z] (35). Boc–Arg(Ts)–OH·3/4AcOEt·1/4H$_2$O (274 mg, 0.549 mmol) was coupled with **31** (167 mg, 0.183 mmol) according to General Procedure 5, and compound **35** was obtained as a powdery substance; yield 210 mg (86.6%). $[\alpha]_D^{25}$ −17.3° (*c* 1.03, MeOH); FAB-MS *m/z* 1324.0 (M+H)$^+$. Found: C, 53.57; H, 6.81; N, 14.43%. Calcd for C$_{61}$H$_{90}$O$_{13}$N$_{14}$S$_3$·2H$_2$O: C, 53.88; H, 6.97; N, 14.42%.

N^1-Benzyloxycarbonyl-N^8-(N^α-benzyloxycarbonyl-N^α-methyl-*O*-benzyltyrosyl-N^g-tosylarginyl-N^α-methyl-N^g-tosylarginyl-N^g-tosyl-D-arginyl)-1,8-octanediamine [Z–MeTyr(Bzl)–Arg(Ts)–MeArg(Ts)–D-Arg(Ts)–Oda–Z] (39). Z–MeTyr(Bzl)–OH (26.0 mg, 62.0 mmol) was coupled with H–Arg(Ts)–MeArg(Ts)–D-Arg(Ts)–Oda–Z prepared from **35** (75.0 mg, 56.7 μmol) according to General Procedure 6, and compound **39** was obtained as a powdery substance; yield 84.1 mg (91.8%). $[\alpha]_D^{25}$ −23.0° (*c* 0.240, MeOH); FAB-MS *m/z* 1625.0 (M+H)$^+$. Found: C, 58.84; H, 6.54; N, 12.71%. Calcd for C$_{81}$H$_{105}$O$_{15}$N$_{15}$S$_3$·1.5H$_2$O: C, 58.89; H, 6.59; N, 12.71%.

***N*-(N^α-Methyltyrosylarginyl-N^α-methylarginyl-D-arginyl)-1,8-octanediamine [H–MeTyr–Arg–MeArg–D-Arg–Oda–H] (7).** Compound **39** (35.0 mg, 21.6 μmol) was worked up according to General Procedure 7. Preparative RPHPLC (10—20% CH$_3$CN–0.1% TFA, 8.0 ml min^{-1}) gave **7** as a powdery TFA salt; yield 18.7 mg (63.2%). $[\alpha]_D^{26}$ −15.3° (*c* 0.810, MeOH); FAB-MS *m/z* 804.9 (M+H)$^+$. Found: C, 38.39; H, 5.48; N, 14.06%. Calcd for C$_{37}$H$_{69}$O$_5$N$_{15}$·5.5TFA·3.5H$_2$O: C, 38.58; H, 5.50; N, 14.06%.

H–MeTyr–Leu–D-Leu–D-Leu–NH(CH$_2$)$_8$NH$_2$ (004-C8) (8). **N^1-Benzyloxycarbonyl-N^8-(N^α-*t*-butoxycarbonyl-D-leucyl-D-leucyl)-1,8-octanediamine (Boc–D-Leu–D-Leu–Oda–Z).** Boc–D-Leu–OH·H$_2$O (352 mg, 1.41 mmol) was coupled with **16** (503 mg, 1.28 mmol) according to General Procedure 6. An oily product was triturated with hexane to give Boc–D-Leu–D-Leu–Oda–Z as a powdery substance; yield 774 mg (99.7%). $[\alpha]_D^{24}$ +34.3° (*c* 0.640, MeOH); FAB-MS *m/z* 605.1 (M+H)$^+$. Found: C, 65.16; H, 9.32; N, 9.22%. Calcd for C$_{33}$H$_{56}$O$_6$N$_4$: C, 65.53; H, 9.33; N, 9.26%.

N^1-Benzyloxycarbonyl-N^8-(N^α-*t*-butoxycarbonylleucyl-D-leucyl-D-luecyl)-1,8-octanediamine (Boc–Leu–D-Leu–D-Leu–Oda–Z). Boc–D-Leu–D-Leu–Oda–Z (760 mg, 1.26 mmol) was worked up in a similar manner as General Procedure 2 to prepare H–D-Leu–D-Leu–Oda–Z, and the thus-obtained amine segment was coupled with Boc–Leu–OH·H$_2$O (342 mg, 1.37 mmol) as described in General Procedure 6. An oily product was triturated with AcOEt and hexane to give Boc–Leu–D-Leu–D-Leu–Oda–Z as a powdery substance; yield 679 mg (75.8%). $[\alpha]_D^{25}$ +27.8° (*c* 1.05, MeOH); FAB-MS *m/z* 718.6 (M+H)$^+$. Found: C, 64.81; H, 9.38; N, 9.66%. Calcd for C$_{39}$H$_{67}$O$_7$N$_5$·1/4H$_2$O: C, 64.83; H, 9.42; N, 9.69%.

N^1-Benzyloxycarbonyl-N^8-(N^α-benzyloxycarbonyl-N^α-methyl-*O*-benzyltyrosylleucyl-D-leucyl-D-leucyl)-1,8-octanediamine [Z–MeTyr(Bzl)–Leu–D-Leu–D-Leu–Oda–Z]. Z–MeTyr(Bzl)–OH (193 mg, 0.459 mmol) was coupled with H–Leu–D-Leu–D-Leu–Oda–Z prepared from Boc–Leu–D-Leu–D-Leu–Oda–Z (300 mg, 0.418 mmol) as described in General Procedure 6. Z–MeTyr(Bzl)–Leu–D-Leu–D-Leu–Oda–Z was obtained as an oily substance; yield 348 mg (81.7%). $[\alpha]_D^{25}$ +3.5° (*c* 1.0, MeOH); FAB-MS *m/z* 1019.9 (M+H)$^+$. Found: C, 68.80; H, 8.08; N, 8.23%. Calcd for C$_{59}$H$_{82}$O$_9$N$_6$·1/2H$_2$O: C, 68.91; H, 8.14; N, 8.17%.

***N*-(N^α-Methyltyrosylleucyl-D-leucyl-D-leucyl)-1,8-octanediamine (H–MeTyr–Leu–D-Leu–D-leu–Oda–H) (8).** Z–MeTyr(Bzl)–Leu–D-Leu–D-Leu–Oda–Z (10.6 mg, 10.4 μmol) was dissolved in MeOH (1 ml) and 1 M HCl (31.2 μl, 31.2 μmol), and hydrogenated for 80 min in the presence of Pd black (5 mg). The catalyst was filtered off, and the filtrate was concentrated in vacuo. The thus-obtained crude product was purified by preparative RPHPLC (25—40% CH$_3$CN–0.1% TFA, 8.0 ml min^{-1}) to give **8** as a powdery TFA salt; yield 5.7 mg (62%). $[\alpha]_D^{28}$ +41.3° (*c* 0.920, MeOH): FAB-MS *m/z* 661.4 (M+H)$^+$. Found: C, 50.18; H, 7.19; N, 8.84%. Calcd for C$_{36}$H$_{64}$O$_5$N$_6$·2.5TFA·2H$_2$O: C, 50.14; H, 7.24; N, 8.56%.

H–MeTyr–Arg–NH(CH$_2$)$_8$NH$_2$ (101-C8) (9). **N^1-Benzyloxycarbonyl-N^8-(*t*-butoxycarbonyl-N^g-tosylarginyl)-1,8-octanediamine [Boc–Arg(Ts)–Oda–Z] (49).** Compound **49** was prepared in a similar manner to that for the preparation of Boc–D-Arg(Ts)–Oda–Z mentioned above, and obtained as a powdery substance in a 88.3% yield. $[\alpha]_D^{28}$ −0.73° (*c* 0.96, MeOH); FAB-MS *m/z* 489.5 (M+H)$^+$. Found: C, 58.50; H, 7.57; N, 12.07%. Calcd for C$_{34}$H$_{52}$O$_7$N$_6$S·1/2H$_2$O: C, 58.51; H, 7.65; N, 12.04%.

N^1-Benzyloxycarbonyl-N^8-(N^α-benzyloxycarbonyl-N^α-methyl-*O*-benzyltyrosyl-N^g-tosylarginyl)-1,8-octanediamine [Z–MeTyr(Bzl)–Arg(Ts)–Oda–Z] (54). Compound **49** (472 mg, 0.685 mmol) was worked up according to General Procedure 2. The thus-obtained H–Arg(Ts)–Oda–Z was coupled with Z–MeTyr(Bzl)–OH (312 mg, 0.745 mmol) as described in General Procedure 6. Compound **54** was obtained as an oily substance; yield 609 mg (89.9%).

$[\alpha]_D^{27}$ −3.4° (*c* 0.99, DMF); FAB-MS *m/z* 990.8 $(M+H)^+$. Found: C, 64.70; H, 6.77; N, 10.00%. Calcd for $C_{54}H_{67}O_9N_7S\cdot 1/2H_2O$: C, 64.90; H, 6.86; N, 9.81%.

***N*-(N^{α}-Methyltyrosylarginyl)-1,8-octanediamine (H–MeTyr–Arg–Oda–H) (9).** Compound **54** (316 mg, 0.319 mmol) was worked up according to General Procedure 7, and compound **9** was obtained as a powdery HCl salt; yield 138 mg (74.0%). $[\alpha]_D^{20}$ +4.0° (*c* 0.93, MeOH); FAB-MS *m/z* 478.5 $(M+H)^+$. Found: C, 47.12; H, 8.17; N, 15.69%. Calcd for $C_{24}H_{46}O_3N_7\cdot 3HCl\cdot 1/4CH_3COOH\cdot 5/4H_2O$: C, 47.12; H, 7.99; N, 15.69%.

H–MeTyr–Arg–NH$(CH_2)_5NH_2$ (101-C5) (10).

***N*-Benzyloxycarbonyl-1,5-pentanediamine Hydrochloride (HCl·H–Pda–Z) (40).** Compound **40** was prepared from 1,5-pentanediamine and ZOSu in a similar manner to that described for preparing **14**: yield 54.8% from ZOSu. Mp 166—167 °C; FAB-MS *m/z* 237.0 $(M+H)^+$. Found: C, 56.88; H, 7.55; N, 10.11%. Calcd for $C_{13}H_{21}O_2N_2Cl\cdot 1/5H_2O$: C, 56.49; H, 7.80; N, 10.13%.

***N*^{1}-Benzyloxycarbonyl-*N*^{5}-(*N*$^{\alpha}$-*t*-butoxycarbonyl-*N*^{g}-tosylarginyl)-1,5-pentanediamine [Boc–Arg(Ts)–Pda–Z] (50).** Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (604 mg, 1.21 mmol) was coupled with **40** (300 mg, 1.10 mmol) according to General Procedure 1, except for the use of EDC·HCl as a coupling agent and DIEA as a base. Compound **50** was obtained as a powdery substance by trituration with hexane; yield 704 mg (99.0%). $[\alpha]_D^{28}$ −0.2° (*c* 1.0, MeOH); FAB-MS *m/z* 647.1 $(M+H)^+$. Found: C, 57.56; H, 7.44; N, 12.46%. Calcd for $C_{31}H_{46}O_7N_6S\cdot 1/4C_6H_{14}$ (hexane): C, 57.46; H, 7.23; N, 12.56%.

***N*^{1}-Benzyloxycarbonyl-*N*^{5}-(*N*$^{\alpha}$-benzyloxycarbonyl-*N*$^{\alpha}$-methyl-*O*-benzyltyrosyl-*N*^{g}-tosylarginyl)-1,5-pentanediamine [Z–MeTyr(Bzl)–Arg(Ts)–Pda–Z] (55).** Compound **50** (328 mg, 0.507 mmol) was worked up according to General Procedure 2. An oily product (238 mg, 85.8%, 0.435 mmol) was coupled with Z–MeTyr(Bzl)–OH (182 mg, 0.435 mmol) as described in General Procedure 6. Compound **55** was obtained as an oily substance; yield 361 mg (87.5%). $[\alpha]_D^{27}$ −18.9° (*c* 1.02, MeOH); FAB-MS *m/z* 948.7 $(M+H)^+$. Found: C, 64.09; H, 6.49; N, 10.27%. Calcd for $C_{51}H_{61}O_9N_7S\cdot 1/2H_2O$: C, 63.99; H, 6.53; N, 10.24%.

***N*-(N^{α}-Methyltyrosylarginyl)-1,5-pentanediamine (H–MeTyr–Arg–Pda–H) (10).** Compound **96** (274 mg, 0.289 mmol) was worked up according to General Procedure 7, and compound **10** was obtained as a very hygroscopic TFA salt after preparative RPHPLC; yield 192 mg (85.4%).[19] FAB-MS *m/z* 436.3 $(M+H)^+$.

H–MeTyr–Arg–Eda (101-C2) (11).

***N*$^{\beta}$-Benzyloxycarbonyl-β-alaninamide (Z–β-Ala–NH_2) (42).** To a stirred solution of Z–β-Ala–OH (200 mg, 0.896 mmol) in THF (2 ml) were added *i*-butoxycarbonyl chloride (128 μl, 0.986 mmol) and tributylamine (235 μl, 0.986 mmol) under cooling in an ice-salt bath (−20 °C); the reaction mixture was stirred for a further 10 min at the same temperature. To the solution was added 25% aqueous ammonia (193 μl), and the reaction mixture was stirred for 1 h at 0 °C, followed by concentration in vacuo and work-up Procedure B. The thus-obtained crystalline residue was recrystallized from AcOEt and hexane; yield 152 mg (76.2%). Mp 158—159 °C; FAB-MS *m/z* 223.1 $(M+H)^+$. Found: C, 59.14; H, 6.30; N, 12.73%. Calcd for $C_{11}H_{14}O_3N_2$: C, 59.44; H, 6.35; N, 12.60%.

***N*^{1}-Benzyloxycarbonyl-*N*^{2}-*t*-butoxycarbonyl-1,2-ethanediamine (Boc–Eda–Z) (43).** To a solution of **42** (300 mg, 1.35 mmol) in DMF (2.5 ml) and H_2O (2.5 ml) were added pyridine (218 μl, 2.70 mmol) and [bis(trifluoroacetoxy)iodo]benzene[20] (871 mg, 2.02 mmol) at 0 °C; the mixture was stirred for 3 h at r.t. The reaction mixture was washed with diethyl ether several times, and then concentrated in vacuo. To the residue in dioxane (2 ml) and H_2O (2 ml) were added $NaHCO_3$ (227 mg, 2.70 mmol) and Boc_2O (310 μl, 1.35 mmol) at 0 °C. The reaction mixture was stirred overnight at r.t., and concentrated in vacuo. The residue was dissolved in AcOEt, and subjected to work-up Procedure B. The thus-obtained crystalline residue was recrystallized from AcOEt and hexane; yield 304 mg (76.6%). Mp 120 °C; FAB-MS *m/z* 295.1 $(M+H)^+$. Found: C, 60.97; H, 7.36; N, 9.86%. Calcd for $C_{15}H_{22}O_4N_2$: C, 61.20; H, 7.53; N, 9.52%.

***N*-Benzyloxycarbonyl-1,2-ethanediamine Hydrochloride (HCl·H–Eda–Z) (44).** Compound **43** (220 mg, 0.747 mmol) was dissolved in 1.5 M HCl in AcOH (7.5 ml). The solution was stirred for 15 min at r.t., and concentrated in vacuo, followed by lyophilization from dioxane; yield 172 mg (99.9%).

***N*^{1}-Benzyloxycarbonyl-*N*^{2}-(N^{α}-*t*-butoxycarbonyl-*N*^{g}-tosylarginyl)-1,2-ethanediamine [Boc–Arg(Ts)–Eda–Z] (51).** Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (686 mg, 1.51 mmol) was coupled with **44** (267 mg, 1.38 mmol) in a similar manner to that described in preparing **50**; yield 611 mg (73.5%). $[\alpha]_D^{26}$ +3.5° (*c* 1.0, MeOH); FAB-MS *m/z* 605.3 $(M+H)^+$. Found: C, 55.52; H, 6.86; N, 14.08%. Calcd for $C_{28}H_{40}O_7N_6S$: C, 55.61; H, 6.67; N, 13.89%.

***N*^{1}-Benzyloxycarbonyl-*N*^{2}-(N^{α}-benzyloxycarbonyl-N^{α}-methyl-*O*-benzyltyrosyl-*N*^{g}-tosylarginyl)-1,2-ethanediamine [Z–MeTyr(Bzl)–Arg(Ts)–Eda–Z] (56).** Compound **51** (561 mg, 0.927 mmol) was worked up according to General Procedure 2 to prepare H–Arg(Ts)–Eda–Z. The thus-obtained amino-free segment was coupled with Z–MeTyr(Bzl)–OH (400 mg, 0.953 mmol) as described in General Procedure 6, and **56** was obtained as a powdery substance by trituration with hexane; yield 656 mg (78.1%). $[\alpha]_D^{27}$ −20.4° (*c* 1.11, MeOH); FAB-MS *m/z* 906.3 $(M+H)^+$. Found: C, 62.43; H, 6.13; N, 10.72%. Calcd for $C_{48}H_{55}O_9N_7S\cdot H_2O$: C, 62.38; H, 6.22; N, 10.61%.

***N*-(N^{α}-Methyltyrosylarginyl)-1,2-ethanediamine (H–MeTyr–Arg–Eda–H) (11).** Compound **56** (250 mg, 0.276 mmol) was worked up in a similar manner as described for preparing **10**, yield 166 mg (81.8%).[19] FAB-MS *m/z* 394.3 $(M+H)^+$.

H–MeTyr–Arg–NHCH_2CH_3 (101-EA) (12).

***N*-(N^{α}-*t*-Butoxycarbonyl-*N*^{g}-tosylarginyl)ethanamine [Boc–Arg(Ts)–NHEt] (52).** Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (5.84 g, 11.7 mmol) was coupled with HCl·NH_2Et (1.05 g, 12.8 mmol) in a similar manner as described for preparing **50**, and compound **52** was obtained as an oily substance even after lyophilization from dioxane; yield 4.83 g (90.8%). $[\alpha]_D^{28}$ +2.8° (*c* 0.94, MeOH); FAB-MS *m/z* 456.3 $(M+H)^+$. Found: C, 52.01; H, 7.36; N, 14.32%. Calcd for $C_{20}H_{33}O_5N_5S\cdot 1/5C_4H_8O_2\cdot 1/2H_2O$: C, 51.80; H, 7.44; N, 14.52%.

***N*-(N^{α}-Benzyloxycarbonyl-N^{α}-methyl-*O*-benzyltyrosyl-*N*^{g}-tosylarginyl)ethanamine [Z–MeTyr(Bzl)–Arg(Ts)–NHEt] (57).** Z–MeTyr(Bzl)–OH (533 mg, 1.27 mmol) was coupled with H–Arg(Ts)–NHEt (452 mg, 1.27 mmol) that was prepared from **52** according to General Procedure 2. The same work up as General Procedure 6 gave compound **57** as an oily substance; yield 752 mg (67.9%). $[\alpha]_D^{27}$ −7.3° (*c* 1.0, DMF); FAB-MS *m/z* 757.7 $(M+H)^+$. Found: C, 62.89; H, 6.36; N, 10.94%. Calcd for $C_{40}H_{48}O_7N_6S\cdot 1/2H_2O$: C, 62.72; H, 6.45; N, 10.97%.

***N*-(N^{α}-Methyltyrosylarginyl)ethanamine [H–MeTyr–Arg–NHEt] (12).** Compound **57** (300 mg, 0.396 mmol) was worked up according to General Procedure 7, and **12** was obtained as a powdery TFA salt after preparative RPHPLC; yield 122 mg (68.3%).[19] FAB-MS *m/z* 379.3 $(M+H)^+$.

H–MeTyr–Arg–NH_2 (101-A) (13).

Bis(4-methylphenyl)methanamine [NH_2Bzh(Me)$_2$] (48). A mixture of bis(4-methylphenyl) ketone (14.2 g, 67.7 mmol), formic acid (3.26 ml, 84.6 mmol), and formamide (13.4 ml, 339 mmol) was stirred overnight at 168 °C. The reaction mixture was dissolved in AcOEt, and the solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The thus-obtained crystalline residue was recrystallized from $CHCl_3$ and hexane; yield 14.9 g (91.7%).

The above-obtained *N*-[bis(4-methylphenyl)methyl]formamide (100 mg, 0.418 mmol) was suspended in 6 M HCl (292 μl) and formic acid (146 μl), and the suspension was stirred for 30 min at 75 °C. The reaction mixture was neutralized with 2 M NaOH at 0 °C, and the product was extracted several times with AcOEt. The combined extracts were washed several times with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The thus-obtained crystalline residue was recrystallized from hexane; yield 86.3 mg (98.0%). Mp 88—89 °C. Found: C, 85.58; H, 8.04; N, 6.56%. Calcd for $C_{15}H_{17}N$: C, 85.26; H, 8.11; N, 6.63%.

***N*-(N^{α}-*t*-Butoxycarbonyl-N^{g}-tosylarginyl)bis(4-methylphenyl)methanamine [Boc–Arg(Ts)–NHBzh(Me)$_2$] (53).** Boc–Arg(Ts)–OH·3/4AcOEt·1/4H_2O (665 mg, 1.33 mmol) was coupled with **48** (256 mg, 1.21 mmol) according to General Procedure 3. The thus-obtained crude product was purified by silica-gel column chromatography (30 g, $CHCl_3$–MeOH = 20 : 1), yield 753 mg (100%). $[\alpha]_D^{25}$ −3.4° (*c* 1.0, MeOH); FAB-MS *m/z* 622.1 $(M+H)^+$. Found: C, 63.48; H, 7.17; N, 11.07%. Calcd for $C_{33}H_{43}O_5N_5S$: C, 63.74; H, 6.97; N, 11.26%.

***N*-(N^{α}-Benzyloxycarbonyl-N^{α}-methyl-*O*-benzyltyrosyl-N^{g}-tosylarginyl)bis(4-methylphenyl)methanamine [Z–MeTyr(Bzl)–Arg(Ts)–NHBzh(Me)$_2$] (58).** Z–MeTyr(Bzl)–OH (139 mg, 0.332 mmol) was coupled with H–Arg(Ts)–NHBzh(Me)$_2$ (173 mg, 0.332 mmol) that was prepared from **53** according to General Procedure 2. The work-up was carried out as described in General Procedure 6, and compound **58** was obtained as an oily substance; yield 290 mg (94.6%). $[\alpha]_D^{25}$ −16.0° (*c* 0.990, MeOH); FAB-MS *m/z* 923.0 $(M+H)^+$. Found: C, 68.59; H, 6.13; N, 8.69%. Calcd for $C_{53}H_{58}O_7N_6S$·1/2H_2O: C, 68.29; H, 6.38; N, 9.02%.

N^{α}-Methyltyrosylargininamide [H–MeTyr–Arg–NH_2] (13). Compound **58** (85.0 mg, 92.1 μmol) was worked up according to General Procedure 7, and **13** was obtained as a powdery HCl salt; yield 44.9 mg (84.7%). $[\alpha]_D^{20}$ +10.4° (*c* 0.890, MeOH); FAB-MS *m/z* 351.1 $(M+H)^+$. Found: C, 41.68; H, 6.86; N, 17.66%. Calcd for $C_{16}H_{26}O_3N_6$·2HCl·1/5CH_3CO_2H·2H_2O: C, 41.78; H, 7.01; N, 17.82%.

Uptake Studies Using Cultured BCEC. Isolation and culture of BCEC, radioiodination of peptides, and measurements of uptake of [^{125}I]peptides into cultured monolayers of BCEC were carried out by the methods described in a previous paper.[15]

This work was supported in part by a Grant-in-Aid for Scientific Research No. 07229245 from the Japanese Ministry of Education, Science, Sports and Culture, and by a Grant from the Japan Health Sciences Foundation, Drug Innovation Project.

References

1) T. Terasaki, K. Hirai, H. Sato, Y. S. Kang, and A. Tsuji, *J. Pharmacol. Exp. Ther.*, **251**, 351 (1989).

2) T. Terasaki, Y. Deguchi, H. Sato, K. Hirai, and A. Tsuji, *Pharm. Res.*, **8**, 815 (1991).

3) T. Shimura, S. Tabata, T. Ohnishi, T. Terasaki, and A. Tsuji, *J. Pharmacol Exp. Ther.*, **258**, 459 (1991).

4) T. Shimura, S. Tabata, T. Ohnishi, T. Terasaki, Y. Deguchi, and A. Tsuji, *J. Pharm. Pharmacol.*, **44**, 583 (1992).

5) A. Tsuji, "Trends and Future Perspectives in Peptides and Protein Drug Delivery," ed by V. H. L. Lee, M. Hashida, and Y. Mizushima, Harwood Academic Publishers, Switzerland (1995), pp. 153—174.

6) Abbreviations according to IUPAC-IUB commission, *Eur. J. Biochem.*, **138**, 9 (1984), are used. AcOEt: ethyl acetate; Arg: arginine; Boc: *t*-butoxycarbonyl; Bzh: benzhydryl (diphenylmethyl); Bzl: benzyl; DCC; dicyclohexylcarbodiimide; DIEA: *N,N*-diisoprpylethylamine; DMF: *N,N*-dimethylformamide; Eda: 1,2-ethanediamine; EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; FAB-MS: fast atom bombardment-mass spectrometry; HOBt: 1-hydroxybenzotriazole; Leu: leucine; MeArg: N^{α}-methylarginine; MeTyr: N^{α}-methyltyrosine; Oda: 1,8-octanediamine; Pda: 1,5-pentanediamine; TEA: triethylamine; TES: triethylsilane; TFA: trifluoroacetic acid; THF: tetrahydrofuran; Ts: *p*-toluenesulfonyl or tosyl; Z: benzyloxycarbonyl; ZOSu: *N*-(benzyloxycarbonyloxy)succinimide.

7) A part of this work was presented in "the 31st Symposium on Peptide Chemistry," Akashi, October, 1993, and reported in "Peptide Chemistry 1993," ed by Y. Okada, Protein Research Foundation, Osaka (1994), pp. 213—216.

8) S. T. Cheung and N. L. Benoiton, *Can. J. Chem.*, **55**, 906 (1977).

9) P. A. Grieco and Ali Bahsas, *J. Org. Chem.*, **52**, 5746 (1987).

10) M. Waki, Y. Kitajima, and N. Izumiya, *Synthesis*, **1981**, 266.

11) A. Tsuji, T. Terasaki, Y. Takabatake, Y. Tenda, I. Tamai, T. Yamashima, S. Moritani, T. Tsuruo, and J. Yamashita, *Life Sci.*, **51**, 1427 (1992).

12) A. Tsuji, I. Tamai, A. Sakata, Y. Tenda, and T. Terasaki, *Biochem. Pharmacol.*, **46**, 1096 (1993).

13) Y. Sai, M. Kajita, I. Tamai, M. Kamata, T. Wakamiya, and A. Tsuji, *J. Neurochem.*, in submission (1997).

14) C. C. W. Hughes and P. L. Rantos, *Brain Res.*, **480**, 126 (1989).

15) I. Tamai, Y. Sai, H. Kobayashi, M. Kamata, T. Wakamiya, and A. Tsuji, *J. Pharmacol. Exp. Ther.*, **280**, 410 (1997).

16) Silica gel was pre-washed with the eluent before the use.

17) The presence of the Ts group was confirmed by ^{1}H NMR measurement.

18) The amine component with the N^{g}-NO_2 protection seems to be a little hydrophilic , and thus amino-free segment was obtained in a less yield than **20** with N^{g}-Ts protection.

19) Because of very hygroscopic character, measurements of $[\alpha]_D$ and elemental analysis were not carried out.

20) M. E. Parham and G. M. Koudon, *Biochem. Biophys. Res. Commun.*, **80**, 1 (1978).