

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

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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(CH₂)₈NH₂ 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 adrenocorticotrophic hormone (ACTH) analog, ebitratide (**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 ebitratide 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(CH₂)₈NH₂⁶ 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 ebitratide (**2**); these parts are enclosed with

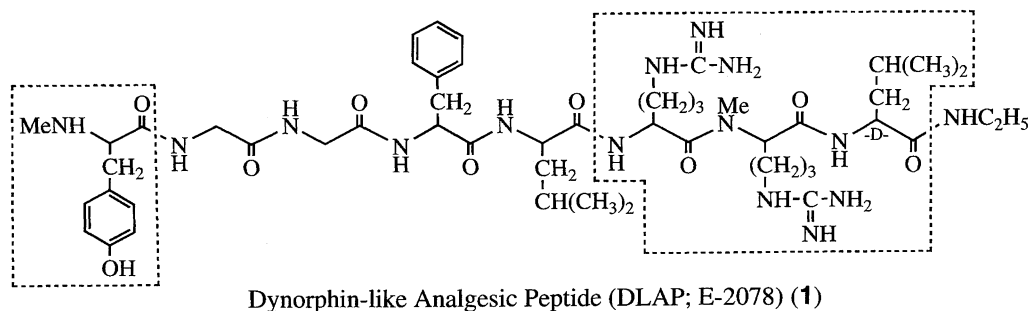
a dotted line in each structure. The *N*^α-methylamino acid and D-amino acid residues are appropriately situated in 001-C8 to prevent the hydrolytic cleavage of peptide bonds by peptidase locating in BCEC. We next prepared various 001-C8 analogs **4–13** (Fig. 2); these peptides were designed for changing the basicity, lipophilicity, or molecular size of 001-C8.

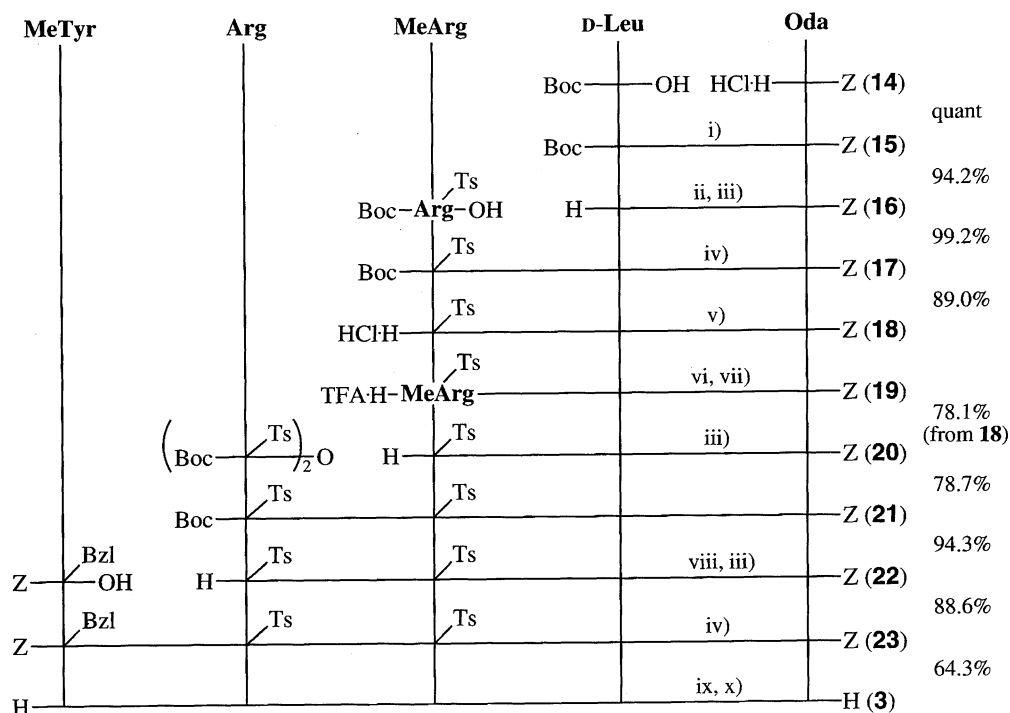
In the present paper the synthetic procedures of 001-C8 and its analog peptides are described in detail. In addition, we briefly report preliminary results concerning the BBB permeability of these peptides on the basis of an in vitro study using the primary cultured bovine BCEC.

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 CH₃I 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*⁸-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





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*⁸-NO₂-protection for the Arg residue, we examined the synthesis of 002-C8 by use of Boc-Arg(NO₂)-OH as a starting material. As a result, we realized

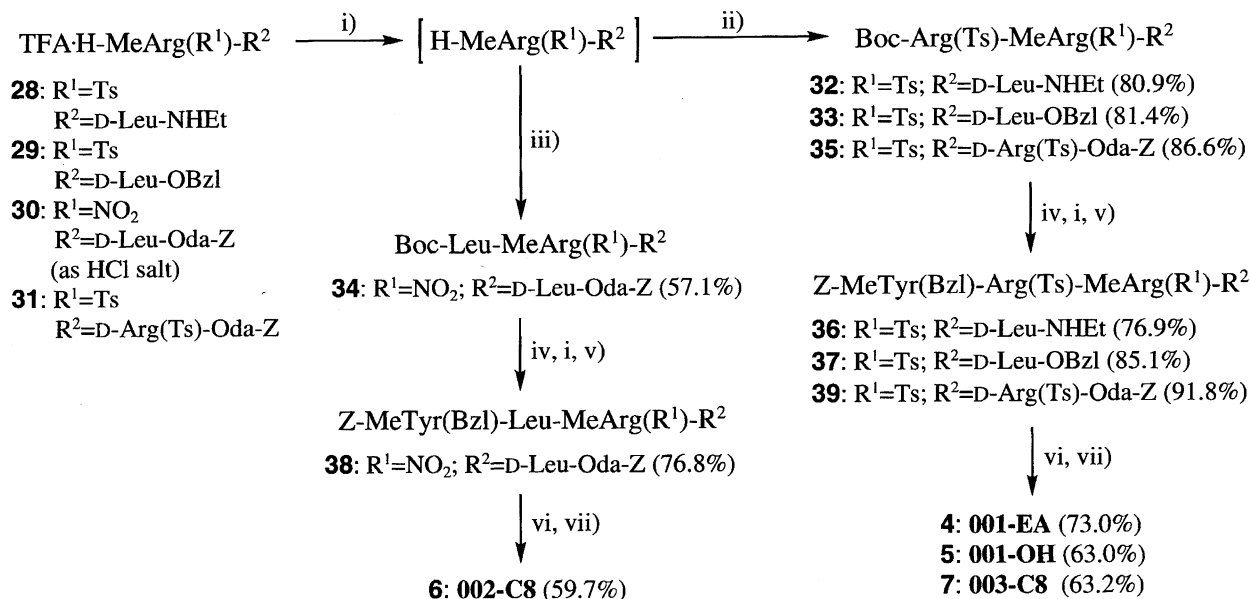
that the *N*⁸-NO₂ group is rather difficult to remove by conventional catalytic hydrogenation using Pd catalyst, and thus it also must be removed by HF.

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-NH₂) (**42**) by means of the modified Hofmann reaction¹⁰) 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)-NH₂ resulted in a low overall yield of this simple amide analog; the amine component bis(4-meth-

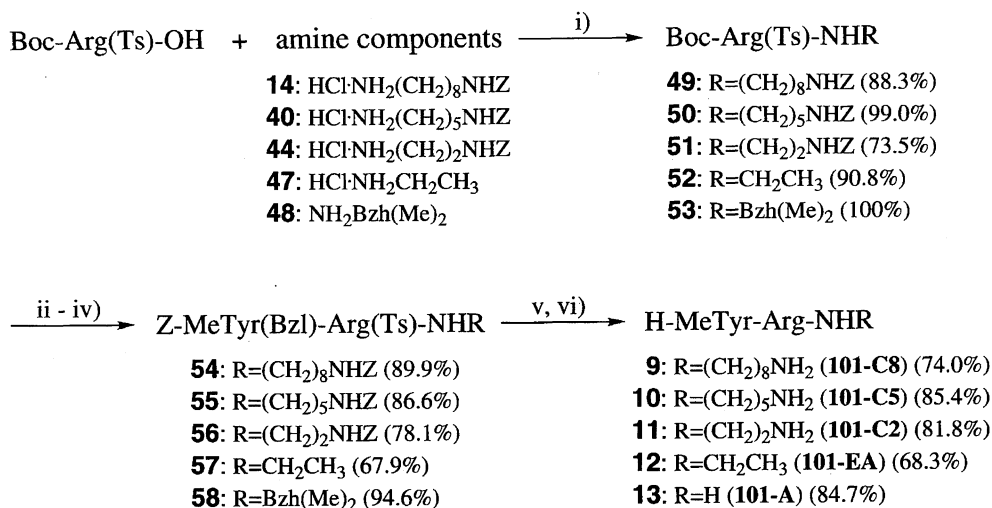
Table 1. *N*-Methylation Yields of Arg-Peptides $\left[\text{HCl} \cdot \text{H} - \text{Arg}(\text{R}^1) - \text{R}^2 \longrightarrow \text{TFA} \cdot \text{H} - \text{MeArg}(\text{R}^1) - \text{R}^2 \right]$

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) ₈ NHZ (26)	73.3
31	003-C8	Ts	D-Arg(Ts)-NH(CH ₂) ₈ NHZ (27)	91.1

a) Only this compound was prepared as HCl salt.



Scheme 3. i) sat. NaHCO_3 aq; ii) $[\text{Boc-Arg}(\text{Ts})]_2\text{O}$; iii) $(\text{Boc-Leu})_2\text{O}$; iv) TFA; v) $\text{Z-MeTyr}(\text{Bzl})\text{-OH/EDC} \cdot \text{HCl/HOBt}$; vi) HF/thioanisole ; vii) preparative RPHPLC.



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

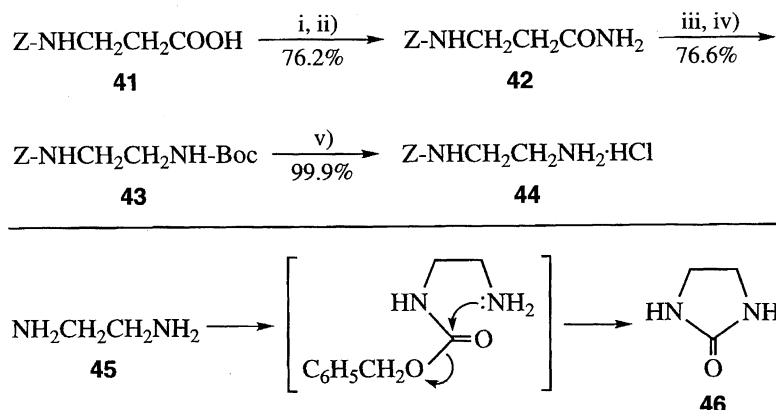
ylphenyl)methanamine [$\text{NH}_2\text{Bzh}(\text{Me})_2$] (**48**) for this purpose was prepared as shown in Scheme 6.

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 ebitatide 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 binding¹⁴ 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

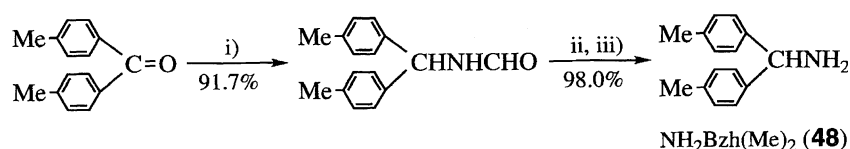
better uptake than E-2078 (**1**) or ebitatide (**2**), whereas 001-EA (**4**) and 001-OH (**5**) were hardly internalized to BCEC. Of the dipeptide analogs, only 101-C8 (**9**) showed comparable uptake to E-2078 or ebitatide, and the others were not internalized to BCEC at all. Although details about 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-octanedi-amine (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*-BuOCOC1/Bu₃N; ii) 25% aqueous ammonia; iii) C₆H₅[(CF₃COO)₂I]/pyridine; iv) Boc₂O/NaHCO₃; v) 1.5 M HCl in AcOH.



Scheme 6. i) HCOOH/HCONH₂; ii) 6 M HCl; iii) 2 M NaOH.

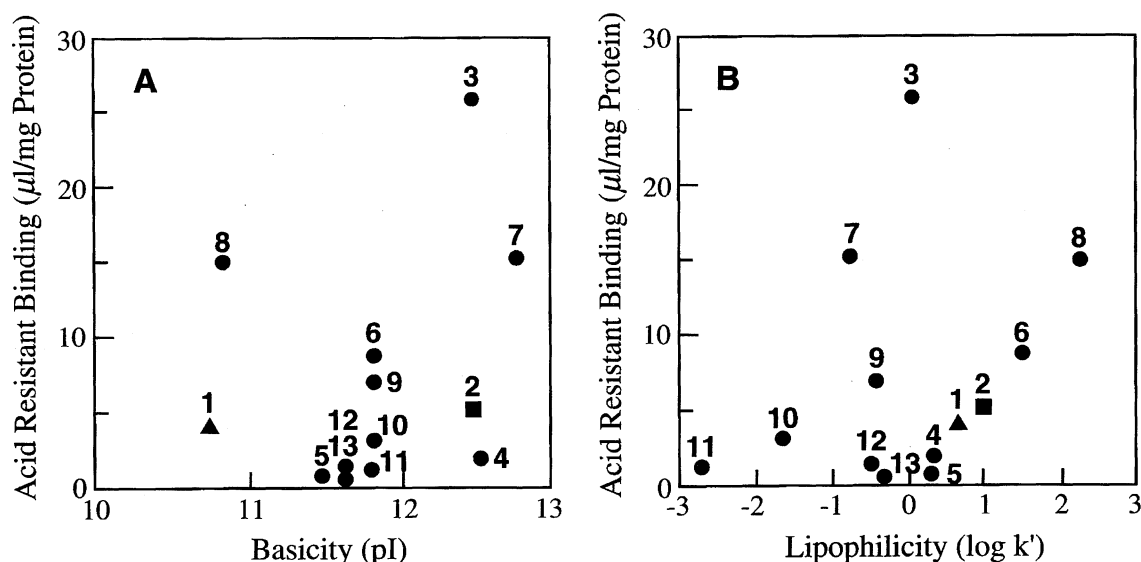


Fig. 3. Plots of basicity (A) or lipophilicity (B) vs. acid resistant binding of E-2078 (1), ebratide (2), 001-C8 (3), and 001-C8 analogs 4–13 to primary cultured monolayers of BCEC. Basicity of peptides was represented by the theoretical isoelectric point (pI), and lipophilicity of those was evaluated 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 ebratide 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 Insti-

tute Inc., (Osaka, Japan). Preparative RPHPLC was performed on Cosmosil 5C₁₈-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 NaHCO₃, and brine (**work-up procedure A**) or washed successively with saturated aqueous NaHCO₃, brine, 10% aqueous citric acid, and brine (**work-up procedure B**); 3) the organic layer was dried over anhydrous Na₂SO₄, and concentrated in vacuo.

H-MeTyr-Arg-MeArg-D-Leu-NH(CH₂)₈NH₂ (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 dm³). 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 C₁₆H₂₇O₂N₂Cl·1/2H₂O: C, 59.33; H, 8.71; N, 8.65; Cl, 10.94%.

N¹-Benzyloxycarbonyl-N⁸-(*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·H₂O (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; [α]_D²⁰ +20.4° (*c* 1.04, CHCl₃); FAB-MS *m/z* 492.3 (M+H)⁺. Found: C, 66.00; H, 9.29; N, 8.57%. Calcd for C₂₇H₄₅O₅N₃: C, 65.95; H, 9.22; N, 8.55%.

N¹-Benzyloxycarbonyl-N⁸-D-leucyl-1,8-octanediamine (H-D-Leu-Oda-Z) (16). (General Procedure 2). A solution of **15** (670 mg, 1.36 mmol) in CH₂Cl₂ (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 NaHCO₃, and brine. The organic layer was dried over anhydrous MgSO₄, 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)⁺.

N¹-Benzyloxycarbonyl-N⁸-(N^α-*t*-butoxycarbonyl-N^ε-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·1/4H₂O (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. [α]_D²⁰ +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 C₄₀H₆₃O₈N₇S·1/2H₂O: C, 59.23; H, 7.95; N, 12.08%.

N¹-Benzyloxycarbonyl-N⁸-(N^α-methyl-N^ε-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 H₂O (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 NaHCO₃. The neutralized mixture was extracted several times with CHCl₃. The combined extracts were dried over anhydrous Na₂SO₄, and concentrated in vacuo. The residue was dissolved in dioxane, and lyophilized to give *N*¹-Z-N⁸-[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 CH₂Cl₂ (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 CHCl₃-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 CHCl₃, and the solution was washed with aqueous NaHCO₃, and brine. The organic layer was dried over anhydrous Na₂SO₄, 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.

N¹-Benzyloxycarbonyl-N⁸-(N^α-*t*-butoxycarbonyl-N^ε-tosylarginyl-N^α-methyl-N^ε-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·1/4H₂O (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, CHCl₃-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. [α]_D²⁴ -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 C₅₄H₈₃O₁₁N₁₁S₂·H₂O: C, 56.67; H, 7.49; N, 13.46%.

N^α-Benzyloxycarbonyl-N^α-methyl-O-benzyltyrosine [Z-Me-Tyr(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% CH₃I (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 Na₂SO₄, and concentrated in vacuo. To the residue was added saturated aqueous NaHCO₃, 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 Na₂SO₄, 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); [α]_D²² -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 C₂₅H₂₅O₅N: C, 71.58; H, 6.01; N, 3.34%.

***N*¹-Benzyloxycarbonyl-*N*⁸-(*N*^α-benzyloxycarbonyl-*N*^α-methyl-*O*-benzyltyrosyl-*N*⁸-tosylarginyl-*N*^α-methyl-*N*⁸-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, CHCl₃-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: [*α*]_D²⁵ -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 C₇₄H₉₈O₁₃N₁₂S₂·2H₂O: 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% CH₃CN–0.1% TFA aq, 8.0 ml min⁻¹). The fractions containing 3 were combined, and lyophilized. The residue was dissolved in 1.5 M HCl in AcOH, and the solution was lyophilized again to give 3 (13.7 mg, 64.3%) as a powdery hydrochloride. [*α*]_D²⁵ -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 C₃₇H₆₈O₅N₁₂·4HCl·3H₂O: C, 46.24; H, 8.18; N, 17.49%.

H-MeTyr-Arg-MeArg-D-Leu-NHCH₂CH₃ (001-EA) (4).

***N*-(*N*^α-*t*-Butoxycarbonyl-D-leucyl)ethanamine (Boc-D-Leu-NHEt).** Boc-D-Leu-OH·H₂O (5.00 g, 20.1 mmol) was coupled with HCl·NH₂Et (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; [*α*]_D²³ +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 C₁₃H₂₆O₃N₂: C, 60.43; H, 10.14; N, 10.84%.

***N*-(*N*^α-*t*-Butoxycarbonyl-*N*⁸-tosylarginyl-D-leucyl)ethanamine [Boc-Arg(Ts)-D-Leu-NHEt].** Boc-Arg(Ts)-OH·3/4AcOEt·1/4H₂O (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-CH₂Cl₂ 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; [*α*]_D²³ +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 C₂₆H₄₄O₆N₆S: C, 54.90; H, 7.80; N, 14.77%.

***N*-(*N*^α-Methyl-*N*⁸-tosylarginyl-D-leucyl)ethanamine Tri-fluoroacetate [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*⁸-tosylarginyl-*N*^α-methyl-*N*⁸-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·1/4H₂O (530 mg, 1.06 mmol) according to General Procedure 5. Purification by silica-gel column chromatography (30 g, CHCl₃-MeOH = 15:1) gave 32 as an oily substance; yield 265 mg (80.9% from 28). [*α*]_D²⁵ -22.7° (*c* 1.01, MeOH); FAB-MS *m/z* 739.8 [(M-Ts+H)+H]⁺. Found: C, 52.17; H, 7.22; N, 15.22%. Calcd for C₄₀H₆₄O₉N₁₀S₂·1.5H₂O: C, 52.21; H, 7.34; N, 15.22%.

***N*-(*N*^α-Benzyloxycarbonyl-*N*^α-methyl-*O*-benzyltyrosyl-*N*⁸-tosylarginyl-*N*^α-methyl-*N*⁸-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%). [*α*]_D²⁵ -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 C₆₀H₇₉O₁₁N₁₁S₂·1.5H₂O: 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% CH₃CN–0.1% TFA, 8.0 ml min⁻¹) purification gave 4 as a powdery TFA salt; yield 61.3 mg (73.0%). [*α*]_D²⁶ -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 C₃₁H₅₅O₅N₁₁·3.5TFA·1.5H₂O: 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·H₂O (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 MgSO₄ 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*⁸-tosylarginyl-D-leucine Benzyl Ester [Boc-Arg(Ts)-D-Leu-OBzl].** Boc-Arg(Ts)-OH·3/4AcOEt·1/4H₂O (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%). [*α*]_D²³ +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 C₃₁H₄₅O₇N₅S·3/4H₂O: C,

57.70; H, 7.26; N, 10.85%.

***N*^α-Methyl-*N*^ε-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 CHCl₃-MeOH-AcOH = 6:1:0.1) gave **29** (101 mg, 86.9%).

***N*^α-*t*-Butoxycarbonyl-*N*^ε-tosylarginyl-*N*^α-methyl-*N*^ε-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·1/4H₂O (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%).

[α]_D²³ -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 C₄₅H₆₅O₁₀N₉S₂·4/5C₄H₈O₂ (dioxane)·4H₂O: C, 52.69; H, 7.28; N, 11.47%.

***N*^α-Benzyloxycarbonyl-*N*^α-methyl-*O*-benzyltyrosyl-*N*^ε-tosylarginyl-*N*^α-methyl-*N*^ε-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%). [α]_D²³ -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 C₆₅H₈₀O₁₂N₁₀S₂·1/2C₄H₈O₂·3H₂O: 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%); [α]_D²⁰ -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 C₂₉H₅₃O₆N₁₀·3HCl·3H₂O: C, 43.63; H, 7.45; N, 17.54%.

H-MeTyr-Leu-MeArg-D-Leu-NH(CH₂)₈NH₂ (002-C8) (6).

***N*¹-Benzyloxycarbonyl-*N*⁸-(*N*^α-*t*-butoxycarbonyl-*N*^ε-nitroarginyl-D-leucyl)-1,8-octanediamine [Boc-Arg(NO₂)-D-Leu-Oda-Z].** EDC·HCl (2.25 g, 11.7 mmol) was added to a solution of Boc-Arg(NO₂)-OH·1/2AcOEt·1/4H₂O (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, CHCl₃-MeOH = 25:1) to give Boc-Arg(NO₂)-D-Leu-Oda-Z as an oily substance. Yield 6.83 g (quant); [α]_D²⁰ +9.5° (c 1.0, CHCl₃); FAB-MS *m/z* 693.4 (M + H)⁺. Found: C, 56.88; H, 8.15; N, 15.88%. Calcd for C₃₃H₅₆O₈N₈·1/3H₂O: C, 56.72; H, 8.17; N, 16.03%.

***N*¹-Benzyloxycarbonyl-*N*⁸-(*N*^α-methyl-*N*^ε-nitroarginyl-D-leucyl)-1,8-octanediamine Hydrochloride [HCl·H-MeArg(NO₂)-D-Leu-Oda-Z] (30).** *N*-Methylation of *N*⁸-NO₂-Arg peptide was carried out in a similar manner to that in General Procedure 4. Boc-Arg(NO₂)-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(NO₂)-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 H₂O (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-azanobornenyl)-5-(nitroguanidino)valeryl-D-Leu]-1,8-octanediamine (240 mg, quant) as an oily substance.

To the thus-obtained 2-azanobornene derivative (240 mg, 0.358 mmol) in CH₂Cl₂ (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 CHCl₃-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*^ε-nitroarginyl-D-leucyl)-1,8-octanediamine [Boc-Leu-MeArg(NO₂)-D-Leu-Oda-Z] (34).** The hydrochloride **30** (636 mg, 0.988 mmol) prepared as shown above was treated with AcOEt and saturated NaHCO₃. The organic layer was separated, and dried over anhydrous Na₂SO₄. The thus-obtained H-MeArg(Ts)-D-Leu-Oda-Z [386 mg (64.3%),¹⁸ 0.636 mmol] was coupled with Boc-Leu-OH·H₂O (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**). [α]_D²³ -41.4° (c 1.05, MeOH); FAB-MS 775.4 [(M - NO₂ + H) + H]⁺. Found: C, 58.23; H, 8.49; N, 14.37%. Calcd for C₃₇H₆₈O₅N₁₂·1/2C₄H₈O₂: 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*^ε-nitroarginyl-D-leucyl)-1,8-octanediamine [Z-MeTyr(Bzl)-Leu-MeArg(NO₂)-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(NO₂)-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, CHCl₃-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: [α]_D³⁰ -20.0° (c 1.07, DMF); FAB-MS *m/z* 1077.0 [(M - NO₂ + H) + H]⁺. Found: C, 63.50; H, 7.59; N, 12.25%. Calcd for C₆₀H₈₄O₁₁N₁₀·H₂O: 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% CH₃CN-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. [α]_D²⁰ -23.3° (c 0.750, MeOH); FAB-MS *m/z* 718.7. Found: C, 49.64; H, 8.30; N, 13.71%. Calcd for C₃₇H₆₇O₅N₉·4HCl·2H₂O: C, 49.38; H, 8.40; N, 14.00%.

H-MeTyr-Arg-MeArg-D-Arg-NH(CH₂)₈NH₂ (003-C8) (7).

N¹-Benzyloxycarbonyl-N⁸-(N^α-*t*-butoxycarbonyl-N^ε-tosyl-D-arginyl)-1,8-octanediamine [Boc-D-Arg(Ts)-Oda-Z]. Boc-D-Arg(Ts)-OH·3/5AcOEt·1/5H₂O (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, CHCl₃-MeOH = 19 : 1), Boc-D-Arg(Ts)-Oda-Z was obtained as a powdery substance by lyophilization from dioxane: Yield 751 mg (85.9%); [α]_D²⁵ +0.80° (c 1.0, MeOH). Found: C, 58.65; H, 7.70; N, 11.99%. Calcd for C₃₄H₅₂O₇N₆S·1/2H₂O: C, 58.51; H, 7.65; N, 12.04%.

N¹-Benzyloxycarbonyl-N⁸-(N^ε-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%).

N¹-Benzyloxycarbonyl-N⁸-(N^α-*t*-butoxycarbonyl-N^ε-tosyl-arginyl-N^ε-tosyl-D-arginyl)-1,8-octanediamine [Boc-Arg(Ts)-D-Arg(Ts)-Oda-Z]. Boc-Arg(Ts)-OH·3/4AcOEt·1/4H₂O (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, CHCl₃ : MeOH = 20 : 1), and Boc-Arg(Ts)-D-Arg(Ts)-Oda-Z was obtained as a colorless oil; yield 79.5 mg (77.2%). [α]_D²³ +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 C₄₇H₇₀O₁₀N₁₀S₂·3/4C₄H₈O₂·3H₂O: C, 53.64; H, 7.38; N, 12.51%.

N¹-Benzyloxycarbonyl-N⁸-(N^α-methyl-N^ε-tosylarginyl-N^ε-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%).

N¹-Benzyloxycarbonyl-N⁸-(N^α-*t*-butoxycarbonyl-N^ε-tosylarginyl-N^α-methyl-N^ε-tosylarginyl-N^ε-tosyl-D-arginyl)-1,8-octanediamine [Boc-Arg(Ts)-MeArg(Ts)-D-Arg(Ts)-Oda-Z] (35). Boc-Arg(Ts)-OH·3/4AcOEt·1/4H₂O (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%). [α]_D²⁵ -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 C₆₁H₉₀O₁₃N₁₄S₃·2H₂O: C, 53.88; H, 6.97; N, 14.42%.

N¹-Benzyloxycarbonyl-N⁸-(N^α-benzyloxycarbonyl-N^α-methyl-*O*-benzyltyrosyl-N^ε-tosylarginyl-N^α-methyl-N^ε-tosylarginyl-N^ε-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 μmol) 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%). [α]_D²⁵ -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 C₈₁H₁₀₅O₁₅N₁₅S₃·1.5H₂O: 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%

CH₃CN-0.1% TFA, 8.0 ml min⁻¹) gave **7** as a powdery TFA salt; yield 18.7 mg (63.2%). [α]_D²⁶ -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 C₃₇H₆₉O₅N₁₅·5.5TFA·3.5H₂O: C, 38.58; H, 5.50; N, 14.06%.

H-MeTyr-Leu-D-Leu-D-Leu-NH(CH₂)₈NH₂ (004-C8) (8).

N¹-Benzyloxycarbonyl-N⁸-(N^α-*t*-butoxycarbonyl-D-leucyl-D-leucyl)-1,8-octanediamine (Boc-D-Leu-D-Leu-Oda-Z). Boc-D-Leu-OH·H₂O (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%). [α]_D²⁴ +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 C₃₃H₅₆O₆N₄: C, 65.53; H, 9.33; N, 9.26%.

N¹-Benzyloxycarbonyl-N⁸-(N^α-*t*-butoxycarbonylleucyl-D-leucyl-D-leucyl)-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·H₂O (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%). [α]_D²⁵ +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 C₃₉H₆₇O₇N₅·1/4H₂O: C, 64.83; H, 9.42; N, 9.69%.

N¹-Benzyloxycarbonyl-N⁸-(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%). [α]_D²⁵ +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 C₅₉H₈₂O₉N₆·1/2H₂O: 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 RPH-PLC (25—40% CH₃CN-0.1% TFA, 8.0 ml min⁻¹) to give **8** as a powdery TFA salt; yield 5.7 mg (62%). [α]_D²⁸ +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 C₃₆H₆₄O₅N₆·2.5TFA·2H₂O: C, 50.14; H, 7.24; N, 8.56%.

H-MeTyr-Arg-NH(CH₂)₈NH₂ (101-C8) (9).

N¹-Benzyloxycarbonyl-N⁸-(*t*-butoxycarbonyl-N^ε-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. [α]_D²⁸ -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 C₃₄H₅₂O₇N₆S·1/2H₂O: C, 58.51; H, 7.65; N, 12.04%.

N¹-Benzyloxycarbonyl-N⁸-(N^α-benzyloxycarbonyl-N^α-methyl-*O*-benzyltyrosyl-N^ε-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%).

$[\alpha]_D^{27} -3.4^\circ$ (*c* 0.99, DMF); FAB-MS *m/z* 990.8 (*M*+*H*)⁺. Found: C, 64.70; H, 6.77; N, 10.00%. Calcd for C₅₄H₆₇O₉N₇S·1/2H₂O: 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%). $[\alpha]_D^{20} +4.0^\circ$ (*c* 0.93, MeOH); FAB-MS *m/z* 478.5 (*M*+*H*)⁺. Found: C, 47.12; H, 8.17; N, 15.69%. Calcd for C₂₄H₄₆O₃N₇·3HCl·1/4CH₃COOH·5/4H₂O: C, 47.12; H, 7.99; N, 15.69%.

H-MeTyr-Arg-NH(CH₂)₅NH₂ (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 C₁₃H₂₁O₂N₂Cl·1/5H₂O: C, 56.49; H, 7.80; N, 10.13%.

***N*¹-Benzyloxycarbonyl-*N*⁵-(*N*^α-*t*-butoxycarbonyl-*N*⁸-tosylarginyl)-1,5-pentanediamine [Boc-Arg(Ts)-Pda-Z] (50).** Boc-Arg(Ts)-OH·3/4AcOEt·1/4H₂O (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%). $[\alpha]_D^{28} -0.2^\circ$ (*c* 1.0, MeOH); FAB-MS *m/z* 647.1 (*M*+*H*)⁺. Found: C, 57.56; H, 7.44; N, 12.46%. Calcd for C₃₁H₄₆O₇N₆S·1/4C₆H₁₄ (hexane): C, 57.46; H, 7.23; N, 12.56%.

***N*¹-Benzyloxycarbonyl-*N*⁵-(*N*^α-benzyloxycarbonyl-*N*^α-methyl-*O*-benzyltyrosyl-*N*⁸-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%). $[\alpha]_D^{27} -18.9^\circ$ (*c* 1.02, MeOH); FAB-MS *m/z* 948.7 (*M*+*H*)⁺. Found: C, 64.09; H, 6.49; N, 10.27%. Calcd for C₅₁H₆₁O₉N₇S·1/2H₂O: 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*^β-Benzyloxycarbonyl-β-alaninamide (Z-β-Ala-NH₂) (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 C₁₁H₁₄O₃N₂: 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 H₂O (2.5 ml) were added pyridine (218 μl, 2.70 mmol) and [bis(trifluoroacetoxy)iodo]benzene²⁰⁾ (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 H₂O (2 ml) were added NaHCO₃ (227 mg, 2.70 mmol) and Boc₂O (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 C₁₅H₂₂O₄N₂: 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*⁸-tosylarginyl)-1,2-ethanediamine [Boc-Arg(Ts)-Eda-Z] (51).** Boc-Arg(Ts)-OH·3/4AcOEt·1/4H₂O (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%). $[\alpha]_D^{26} +3.5^\circ$ (*c* 1.0, MeOH); FAB-MS *m/z* 605.3 (*M*+*H*)⁺. Found: C, 55.52; H, 6.86; N, 14.08%. Calcd for C₂₈H₄₀O₇N₆S: C, 55.61; H, 6.67; N, 13.89%.

***N*¹-Benzyloxycarbonyl-*N*²-(*N*^α-benzyloxycarbonyl-*N*^α-methyl-*O*-benzyltyrosyl-*N*⁸-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%). $[\alpha]_D^{27} -20.4^\circ$ (*c* 1.11, MeOH); FAB-MS *m/z* 906.3 (*M*+*H*)⁺. Found: C, 62.43; H, 6.13; N, 10.72%. Calcd for C₄₈H₅₅O₉N₇S·H₂O: 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-NHCH₂CH₃ (101-EA) (12).

***N*-(*N*^α-*t*-Butoxycarbonyl-*N*⁸-tosylarginyl)ethanamine [Boc-Arg(Ts)-NH₂] (52).** Boc-Arg(Ts)-OH·3/4AcOEt·1/4H₂O (5.84 g, 11.7 mmol) was coupled with HCl·NH₂Et (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%). $[\alpha]_D^{28} +2.8^\circ$ (*c* 0.94, MeOH); FAB-MS *m/z* 456.3 (*M*+*H*)⁺. Found: C, 52.01; H, 7.36; N, 14.32%. Calcd for C₂₀H₃₃O₅N₅S·1/5C₄H₈O₂·1/2H₂O: C, 51.80; H, 7.44; N, 14.52%.

***N*-(*N*^α-Benzyloxycarbonyl-*N*^α-methyl-*O*-benzyltyrosyl-*N*⁸-tosylarginyl)ethanamine [Z-MeTyr(Bzl)-Arg(Ts)-NH₂] (57).** Z-MeTyr(Bzl)-OH (533 mg, 1.27 mmol) was coupled with H-Arg(Ts)-NH₂ (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%). $[\alpha]_D^{27} -7.3^\circ$ (*c* 1.0, DMF); FAB-MS *m/z* 757.7 (*M*+*H*)⁺. Found: C, 62.89; H, 6.36; N, 10.94%. Calcd for C₄₀H₄₈O₇N₆S·1/2H₂O: C, 62.72; H, 6.45; N, 10.97%.

***N*-(*N*^α-Methyltyrosylarginyl)ethanamine [H-MeTyr-Arg-NH₂] (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-NH₂ (101-A) (13).

Bis(4-methylphenyl)methanamine [NH₂Bzh(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 Na₂SO₄, and concentrated in vacuo. The thus-obtained crystalline residue was recrystallized from CHCl₃ 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 Na₂SO₄, 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 C₁₅H₁₇N: C, 85.26; H, 8.11; N, 6.63%.

***N*-(*N*^α-*t*-Butoxycarbonyl-*N*^ε-tosylarginyl)bis(4-methylphenyl)methanamine [Boc-Arg(Ts)-NHBzh(Me)₂] (53).** Boc-Arg(Ts)-OH·3/4AcOEt·1/4H₂O (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, CHCl₃–MeOH = 20 : 1), yield 753 mg (100%). [α]_D²⁵ –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 C₃₃H₄₃O₅N₅S: C, 63.74; H, 6.97; N, 11.26%.

***N*-(*N*^α-Benzyloxycarbonyl-*N*^α-methyl-*O*-benzyltyrosyl-*N*^ε-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%). [α]_D²⁵ –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 C₅₃H₅₈O₇N₆S·1/2H₂O: C, 68.29; H, 6.38; N, 9.02%.

***N*^α-Methyltyrosylargininamide [H-MeTyr-Arg-NH₂] (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%). [α]_D²⁰ +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 C₁₆H₂₆O₃N₆·2HCl·1/5CH₃CO₂H·2H₂O: 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 [¹²⁵I]peptides into cultured monolayers of BCEC were carried out by the methods described in a previous paper.¹⁵⁾

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