Amino Acids and Peptides. LIV. Application of 2-Adamantyl Derivatives as Protecting Groups to the Synthesis of Peptide Fragments Related to *Sulfolobus solifataricus* Ribonuclease. I[1,2)]

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The 2-adamantyloxycarbonyl group was employed for the protection of the ε-amino group of Lys and the hydroxyl group of Tyr, and the 2-adamantyl ester was employed for the protection of the β-carboxyl group of Asp in order to construct eight peptide segments as building blocks for the preparation of peptide fragments related to the sequence of *Sulfolobus solifataricus* Ribonuclease. The usefulness of the above protecting groups developed in our laboratory was confirmed.

Key words protecting group; 2-adamantyloxycarbonyl; 2-adamantyl ester; protected peptide segment; building block; *Sulfolobus solifataricus* Ribonuclease

For the preparation of large peptides or proteins by the solution method, the condensation of peptide segments is an attractive strategy. The convergent solid-phase method[3)] is also a useful procedure for the above purpose; the separation of product from truncated or deleted sequences is considerably facilitated. However, one frustrating problem incurred in the solution method and the convergent solid-phase method is the solubility of the protected intermediates, even in solvents as powerful as DMF, hexamethylphosphoramude (HMPA) and *N*-methylpyrolidone (NMP). The difficulty in obtaining homogeneous products increases rapidly as the size of the target peptide increases. Low solubility in commonly used solvents and low molecular concentrations because of the high molecular weight of large segments cause the low reaction rates. If intermediate peptides are soluble in solvents, purification of the intermediates will be easy even at the intermediate stage. In order to maintain the solubility of peptide intermediates suitable for a coupling reaction, two main approaches have been attempted. One is the development of solvent systems which have high solubilizing potential[4,5)] and the other is the development of protecting groups which can render peptide intermediates soluble in organic solvents. In 1973, a protected benzyloxycarbonylhydrazide, corresponding to the sequence of residues 1—13 of porcine gastric inhibitory peptide, which had a benzyl side-chain protection, was described,[6)] but the poor solubility of the intermediates caused difficulty. With the objective of increasing solubility, a picolyl group instead of the benzyl group was employed to prepare protected tridecapeptide 4-picolyloxycarbonylhydrazide.[7)] However, there was no significant improvement in the solubility of the higher protected peptide in DMF. The same authors also attempted to increase the solubility of peptide fragments having benzyl-based protection by introducing dimethylcarbamoyl into the phenyl ring, with the hope that the presence of almost the whole DMF structure would increase their miscibility with the solvent.[8)] However, in such cases examined so far, the molar solubility in DMF has not been increased significantly. The 1-adamantyloxycarbonyl (1-Adoc) group improves the solubility of amino acids and peptides protected by 1-Adoc in organic solvents due to its strong hydrophobicity.[9,10)] Lys(1-Adoc) was successfully employed for synthesis of PHI[11)] and the peptide fragments of a lysozyme.[12)]

With the objective of developing these protecting groups, as described above, we have been searching for new protecting groups for the side-chain functional groups of amino acids. At present, we have developed H-Asp(O-2-Ada)-OH,[13,14)] H-Glu(O-2-Ada)-OH,[15)] H-Lys(2-Adoc)-OH,[16,17)] H-His(τ-2-Adoc)-OH,[18,19)] H-His(π-2-Adom)-OH[20,21)] and H-Tyr(2-Adoc)-OH[22)] and their N$^{\alpha}$-Boc and Fmoc derivatives in order to construct peptide fragments. These protecting groups, derived from the 2-adamantyl group, are stable under TFA treatment and hydrogenation over a Pd catalyst, and can be easily removed by 1 M TFMSA–thioanisole/TFA and anhydrous HF, although the protecting groups derived from the 1-adamantyl group, such as H-Asp(O-1-Ada)-OH[13,14)] and H-His(π-1-Adom)-OH[23,24)] can be easily removed by TFA. Therefore, in combination with the above protecting groups with benzyl ester instead of phenacyl ester[25)] as a *C*-terminal protecting group, the peptide fragments as building blocks for large peptides can easily be prepared using the hydrogenolysis method. This paper deals with the application of H-Asp(O-2-Ada)-OH, H-Lys(2-Adoc)-OH and H-Tyr(2-Adoc)-OH to the synthesis of peptide fragments related to *Sulfolobus solifataricus* Ribonuclease[26)] in order to evaluate the usefulness of our protecting groups. Boc-Glu(O-cHex)-OH[27)] was employed instead of Boc-Glu(O-2-Ada)-OH, the synthetic procedure of which in large quantity is not yet established. Beside Boc-Glu(O-cHex)-OH can prevent glutarimide formation[28)] and pyrolidone formation[29)] during peptide synthesis.

Sulfolobus solifataricus Ribonuclease consists of 62 amino acids (Fig. 1) and exhibits RNase activity, although it does not contain a histidine residue.[26)] The sequence of this RNase showed close similarity to that of DNA-binding proteins previously isolated from *Sulfolobus* strains.[30,31)] In order to study the structure–activity relationship of *Sulfolobus solifataricus* Ribonuclease, we planned the preparation of peptide fragments (I-VIII) related to *Sulfolobus solifataricus* Ribonuclease (Fig. 1). In order to construct the foregoing peptide frag-

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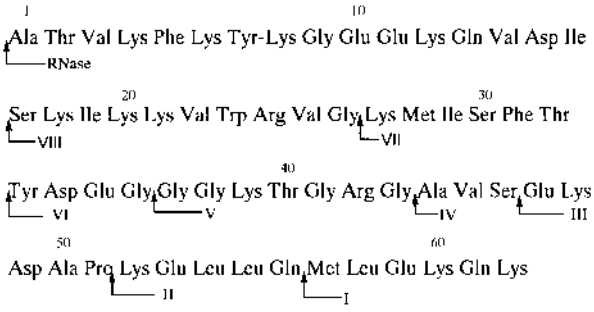

Fig. 1. Amino Acid Sequence of *Sulfolobus solifataricus* Ribonuclease and Peptide Fragments (I—VIII)

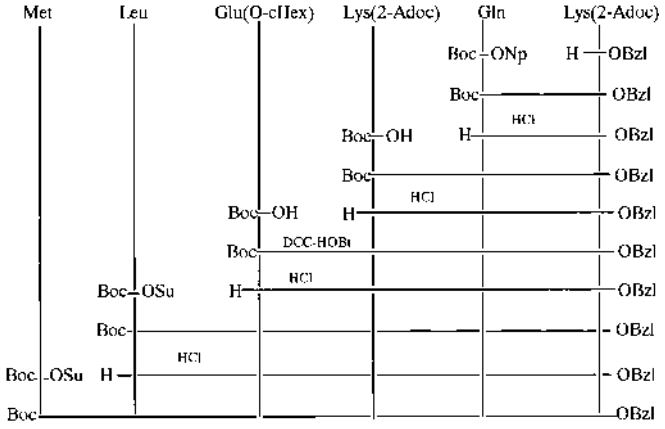

Chart 1. Synthetic Route to Boc-(57—62)-OBzl

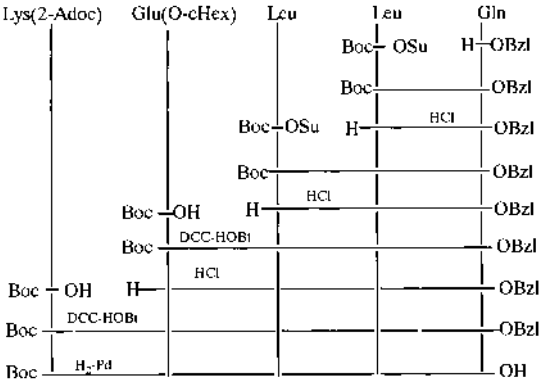

Chart 2. Synthetic Route to Boc-(52—56)-OH

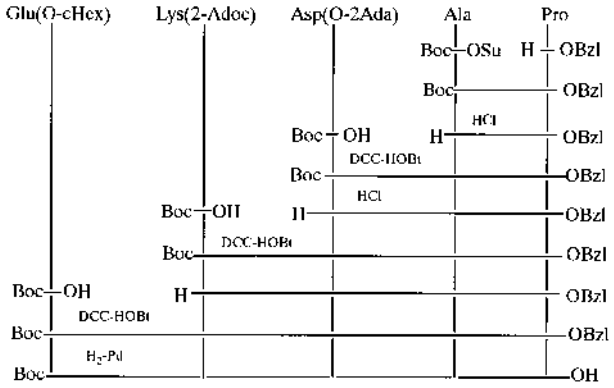

Chart 3. Synthetic Route to Boc-(47—51)-OH

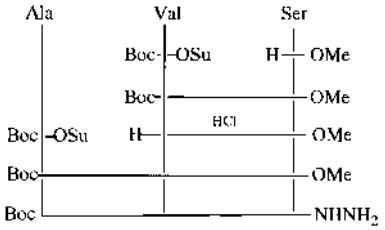

Chart 4. Synthetic Route to Boc-(44—46)-$NHNH_2$

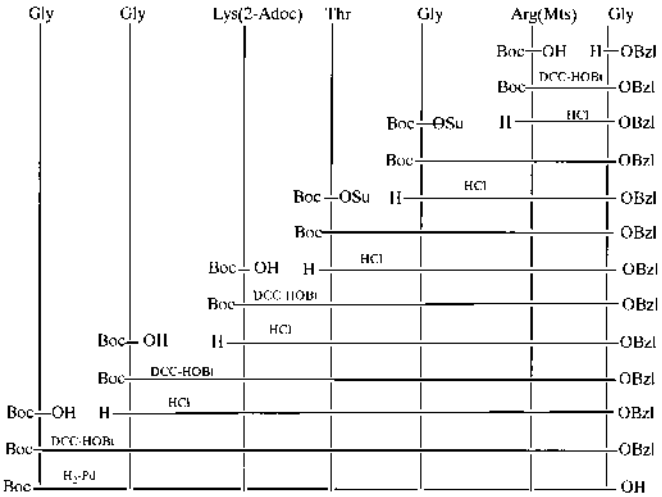

Chart 5. Synthetic Route to Boc-(37—43)-OH

ments, eight peptide segments were prepared. Those segments have Gly, Pro or hydrazide at their *C*-terminal in order to prevent racemization during segment coupling, except for Gln at position 56 of the ribonuclease (RNase).

Boc-(57—62)-OBzl was prepared as shown in Chart 1. Starting from H-Lys(2-Adoc)-OBzl,[17] Boc-Gln-ONp,[32] Boc-Lys(2-Adoc)-OH,[17] Boc-Glu(O-cHex)-OH,[29] Boc-Leu-OSu,[33] and Boc-Met-OSu,[33] were successively coupled to give Boc-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl in an analytically pure form.

The synthetic route to Boc-(52—56)-OH is shown in Chart 2. Starting from H-Gln-OBzl, Boc-Leu-OSu, Boc-Leu-OSu, Boc-Glu(O-cHex)-OH and Boc-Lys(2-Adoc)-OH were coupled successively to give Boc-(52—56)-OBzl. Boc-(52—56)-OBzl was hydrogenated over a Pd catalyst to yield Boc-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-OH, Boc-(52—56)-OH, in a pure form.

Boc-(47—51)-OH was synthesized according to the scheme shown in Chart 3. Starting from H-Pro-OBzl,[34] Boc-Ala-OSu,[33] Boc-Asp(O-2-Ada)-OH,[14] Boc-Lys(2-Adoc)-OH and Boc-Glu(O-cHex)-OH were coupled successively to yield Boc-(47—51)-OBzl, which was hydrogenated over a Pd catalyst to give Boc-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-OH, Boc-(47—51)-OH, in a pure form.

Boc-(44—46)-$NHNH_2$ was synthesized by the route shown in Chart 4. Starting from H-Ser-OMe, Boc-Val-OSu[33] and Boc-Ala-OSu were successively coupled to give Boc-(44—46)-OMe. Boc-(44—46)-OMe was treated with hydrazine hydrate to yield Boc-Ala-Val-Ser-$NHNH_2$, Boc-(44—46)-$NHNH_2$ in pure form.

Boc-(37—43)-OH was synthesized by the route shown in Chart 5. Starting from H-Gly-OBzl,[35] Boc-Arg(Mts)-OH,[36]

Boc-Gly-OSu,[33] Boc-Thr-OSu,[37] Boc-Lys(2-Adoc)-OH, Boc-Gly-OH, and Boc-Gly-OH were coupled successively to give Boc-(37—43)-OBzl, which was hydrogenated over a Pd catalyst to yield Boc-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OH, Boc-(37—43)-OH, in a pure form.

The synthetic procedure of Boc-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-OH, Boc-(33—36)-OH, was reported previously,[22] and the synthetic procedure of Boc-(27—32)-$NHNH_2$ is shown in Chart 6. Starting from H-Thr-OBzl,[38] Boc-Phe-OH,[39] Boc-Ser-OH,[40] Boc-Ile-OSu,[33] Boc-Met-OSu and Boc-Lys(2-Adoc)-OH were coupled successively to give Boc-(27—32)-OBzl. Boc-(27—32)-OBzl was treated with hydrazine hydrate in DMF to yield Boc-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-$NHNH_2$, Boc-(27—32)-$NHNH_2$ in a pure form.

Boc-(21—26)-OBzl was prepared by the route shown in Chart 7. Starting from H-Gly-OBzl, Boc-Val-OSu, Boc-Arg(Mts)-OH, Boc-Trp(Mts)-OH,[41] Boc-Val-OSu, and Boc-Lys(-2-Adoc)-OH were coupled successively to give Boc-(21—26)-OBzl in a pure form. Using this fragment, Boc-(17—26)-OH was prepared according to the route shown in Chart 8. The Boc group of Boc-(21—26)-OBzl was removed by TFA treatment to give H-(21—26)-OBzl, which was coupled with Boc-(17—18)-OH to give Boc-(17—26)-OBzl. Boc-(17—26)-OBzl was hydrogenated over a Pd catalyst to give Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Adoc)-Lys(2-Adoc)-Val-Trp(Mts)-Arg(Mts)-Val-Gly-OH, Boc-(17—26)-OH in a pure form.

Thus, we prepared all requisite segments to construct RNase (17—62), VIII, in a pure form, indicating that our newly developed protecting groups containing a 2-adamantyl group are stable under the conditions required to construct peptide segments as building blocks, and each segment presents no problem concerning solubility in organic solvents, such as DMF, as described in the Experimental Section. The segment condensation reactions of the above building blocks and the removal of the protecting groups in large peptides will be described in a continued issue (Amino Acids and

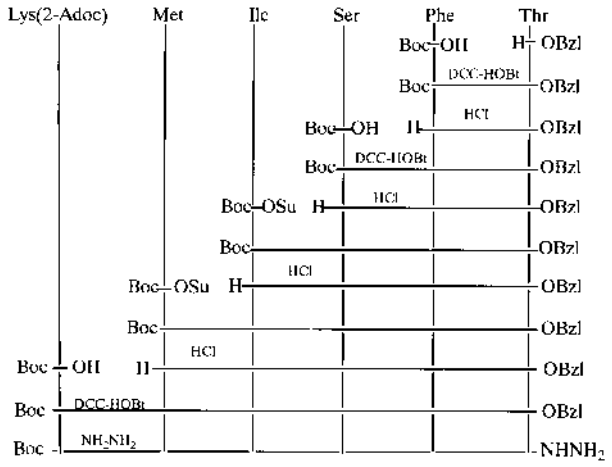

Chart 6. Synthetic Route to Boc-(27—32)-$NHNH_2$

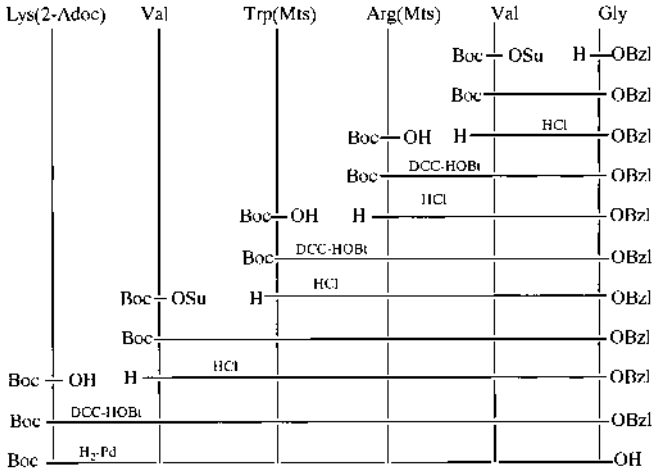

Chart 7. Synthetic Route to Boc-(21—26)-OBzl

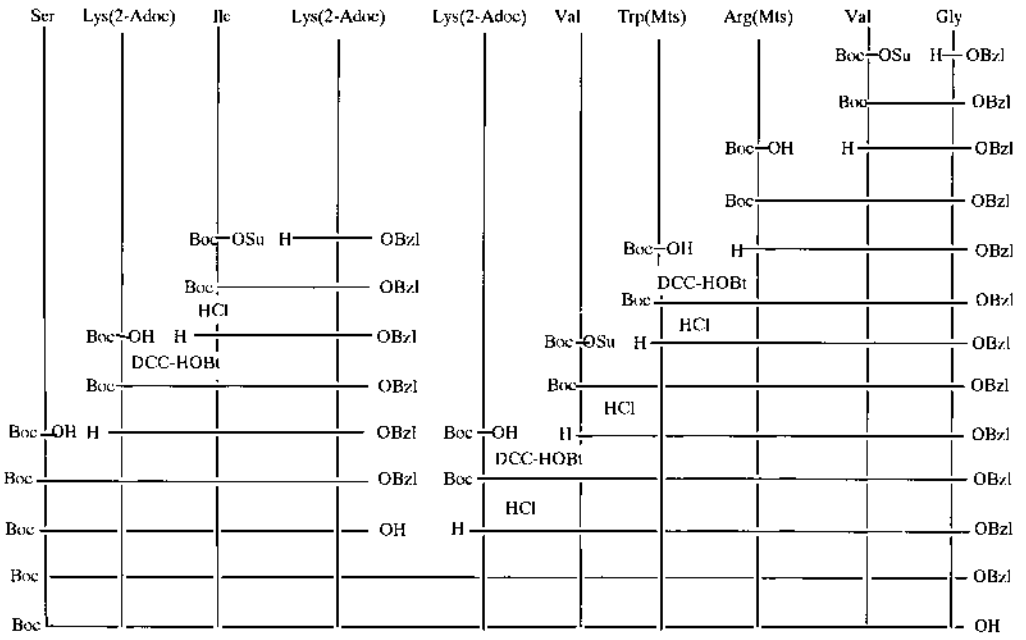

Chart 8. Synthetic Route to Boc-(17—26)-OH

Peptides. LV).

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co., Ltd.). On TLC (Kieselgel G. Merck), Rf^1 and Rf^2 values refer to the systems of $CHCl_3$, MeOH and AcOH (90 : 8 : 2) and $CHCl_3$, MeOH and H_2O (8 : 3 : 1, lower phase), respectively.

Boc-Gln-Lys(2-Adoc)-OBzl To a solution of H-Lys(2-Adoc)-OBzl·HCl (15.8 g, 35 mmol) in DMF (200 ml) containing Et_3N (4.9 ml, 35 mmol), Boc-Gln-ON*p* (12.9 g, 35 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and water, dried over Na_2SO_4 and evaporated down to give an oily product, yield 18.5 g (82.5%), Rf^2 0.83.

Boc-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl To a solution of H-Gln-Lys(2-Aoc)-OBzl·HCl [prepared from Boc-Gln-Lys(2-Adoc)-OBzl (15.5 g, 27.3 mmol) and 5.8 N HCl/dioxane (47.1 ml, 273 mmol) as usual], Boc-Lys(2-Adoc)-OH (11.6 g, 27.3 mmol), HOBt (3.7 g, 27.3 mmol) in DMF (200 ml) containing Et_3N (3.8 ml, 27.3 mmol), DCC (6.8 g, 32.7 mmol) was added under cooling with ice-salt. The reaction mixture was stirred at 4 °C overnight. After removal of the DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 23.0 g (88.8%), mp 123—125 °C, $[\alpha]_D^{25}$ −13.5° (c=1.0, DMF), Rf^1 0.49, Rf^2 0.72. *Anal.* Calcd for $C_{51}H_{76}N_6O_{11}$: C, 64.5; H, 8.07; N, 8.85. Found: C, 64.6; H, 8.26; N, 8.85. Amino acid analysis: Glu 0.97, Lys 2.00 (average recovery 83.1%).

Boc-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl To a solution of H-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·HCl [prepared from Boc-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (15.0 g, 15.8 mmol) and 7.2 N HCl/dioxane (21.9 ml, 157.7 mmol) as usual], Boc-Glu(O-cHex)-OH (5.2 g, 15.8 mmol), and HOBt (2.1 g, 15.8 mmol) in DMF (150 ml) containing Et_3N (2.2 ml, 15.8 mmol), DCC (3.9 g, 18.9 mmol) was added under cooling with ice-salt. The reaction mixture was stirred at 4 °C overnight. After removal of the DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 15.5 g (84.5%), mp 125—127 °C, $[\alpha]_D^{25}$ −11.5° (c=1.0, DMF), Rf^1 0.45, Rf^2 0.71. *Anal.* Calcd for $C_{62}H_{93}N_7O_{14}$: C, 64.2; H, 8.08; N, 8.45. Found: C, 64.2; H, 8.27; N, 8.46. Amino acid analysis: Glu 1.86, Lys 2.00 (average recovery 88.7%).

Boc-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl A reaction mixture of H-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·HCl [prepared from Boc-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (13.0 g, 11.2 mmol) and 7.2 N HCl/dioxane (15.5 ml, 111.6 mmol) as usual] and Boc-Leu-OSu (3.7 g, 11.2 mmol) in DMF (100 ml) containing Et_3N (1.9 ml, 13.4 mmol) was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 11.8 g (82.7%), mp 163—165 °C, $[\alpha]_D^{25}$ −10.5° (c=1.0, DMF), Rf^1 0.43, Rf^2 0.71. *Anal.* Calcd for $C_{68}H_{104}N_8O_{15}$: C, 64.1; H, 8.23; N, 8.80. Found: C, 63.8; H, 8.29; N, 8.68. Amino acid analysis: Leu 1.02, Glu 2.14, Lys 2.00 (average recovery 95.1 %).

Boc-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl, Boc-SSR(57—62)-OBzl A reaction mixture of H-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·HCl[prepared from Boc-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (9.4 g, 7.4 mmol) and 5.0 N HCl/dioxane (14.8 ml, 74.0 mmol) as usual] and Boc-Met-OSu (2.6 g, 7.4 mmol) in DMF (100 ml) containing Et_3N (1.3 ml, 7.4 mmol) was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 9.5 g (90.8%), mp 180—182 °C, $[\alpha]_D^{25}$ −19.9° (c=1.0, DMF), Rf^1 0.46, Rf^2 0.72. *Anal.* Calcd for $C_{73}H_{113}N_9O_{16}\cdot 0.5H_2O$: C, 62.0; H, 8.13; N, 8.92. Found: C, 61.9; H, 8.18; N, 9.00. Amino acid analysis: Met 0.76, Leu 1.06, Glu 2.16, Lys 2.00 (average recovery 89.7%).

Boc-Leu-Gln-OBzl A reaction mixture of H-Gln-OBzl·HCl [prepared from Boc-Gln-OBzl (26.8 g, 80 mmol) and 6.8 N HCl/dioxane (117 ml, 796 mmol) as usal] and Boc-Leu-OSu (26.3 g, 80 mmol) in DMF (200 ml) containing Et_3N (13.4 ml, 96 mmol) was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 31.1 g (86.5%), mp 180—182 °C, $[\alpha]_D^{25}$ −19.5° (c=1.0, DMF), Rf^1 0.64, Rf^2 0.74. *Anal.* Calcd for $C_{23}H_{35}N_3O_6$: C, 61.5; H, 7.85; N, 9.35. Found: C, 61.2; H, 7.92; N, 9.4. Amino acid analysis: Leu 0.98, Glu 1.00 (average recovery 90.6%).

Boc-Leu-Leu-Gln-OBzl A reaction mixture of H-Leu-Gln-OBzl·HCl [prepared from Boc-Leu-Gln-OBzl (15.3 g, 34 mmol) and 6.8 N HCl/dioxane (50 ml, 340 mmol) as usual] and Boc-Leu-OSu (11.2 g, 34 mmol) in DMF (200 ml) containing Et_3N (5.7 ml, 40.8 mmol) was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, and then dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 18.0 g (93.6%), mp 182—183 °C, $[\alpha]_D^{25}$ −35.7° (c=1.0, DMF), Rf^1 0.51, Rf^2 0.71. *Anal.* Calcd for $C_{29}H_{46}N_4O_7$: C, 61.1; H, 8.28; N, 9.83. Found: C, 61.3; H, 8.37; N, 9.97. Amino acid analysis: Leu 2.00, Glu 0.98 (average recovery 94.7%).

Boc-Glu(O-cHex)-Leu-Leu-Gln-OBzl To a solution of H-Leu-Leu-Gln-OBzl·HCl [prepared from Boc-Leu-Leu-Gln-OBzl (11.7 g, 20.6 mmol) and 5.8 N HCl/dioxane (35.5 ml, 206 mmol) as usual], Boc-Glu(O-cHex)-OH (6.8 g, 20.6 mmol) and HOBt (2.8 g, 20.6 mmol) in DMF (200 ml) containing Et_3N (2.9 ml, 20.6 mmol), DCC (5.1 g, 24.8 mmol) was added under cooling with ice-salt and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 10.1 g (63.2%), mp 170—173 °C, $[\alpha]_D^{25}$ −34.1° (c=1.0, DMF), Rf^1 0.49, Rf^2 0.72. *Anal.* Calcd for $C_{40}H_{63}N_5O_{10}$: C, 62.1; H, 8.20; N, 9.05. Found: C, 62.3; H, 8.19; N, 9.35. Amino acid analysis: Glu 1.95, Leu 2.00 (average recovery 99.8%).

Boc-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-OBzl To a solution of H-Glu(O-cHex)-Leu-Leu-Gln-OBzl·HCl [prepared from Boc-Glu(O-cHex)-Leu-Leu-Gln-OBzl (9.3 g, 12.0 mmol) and 7.2 N HCl/dioxane (16.7 ml, 120 mmol) as usual], Boc-Lys(2-Adoc)-OH (5.1 g, 12.0 mmol) and HOBt (1.6 g, 12.0 mmol) in DMF (150 ml) containing Et_3N (1.7 ml, 12.0 mmol), DCC (3.0 g, 14.4 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 10.3 g (79.4%), mp 192—194 °C, $[\alpha]_D^{25}$ −21.0° (c=1.0, DMF), Rf^1 0.48, Rf^2 0.74. *Anal.* Calcd for $C_{57}H_{89}N_7O_{13}$: C, 63.4; H, 8.30; N, 9.08. Found: C, 63.6; H, 8.58; N, 8.82. Amino acid analysis: Lys 1.00, Glu 2.15, Leu 2.21 (average recovery 94.8%).

Boc-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-OH, Boc-SSR(52—56)-OH Boc-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-OBzl (2.2 g, 2 mmol) in MeOH (150 ml) was hydrogenated over a Pd catalyst for 2 h. After the removal of Pd and the solvent, petroleum ether was added to the residue to afford a powder, yield 1.8 g (98.4%), mp 181—183 °C, $[\alpha]_D^{25}$ −22.0° (c=1.0, DMF), Rf^1 0.10, Rf^2 0.29. *Anal.* Calcd for $C_{50}H_{83}N_7O_{13}\cdot H_2O$: C, 59.6; H, 8.50; N, 9.72. Found: C, 59.8; H, 8.38; N, 9.52.

H-Ala-Pro-OBzl·HCl A reaction mixture of H-Pro-OBzl·HCl (15.0 g, 62.1 mmol) and Boc-Ala-OSu (17.8 g, 62.1 mmol) in DMF (200 ml) containing Et_3N (10.4 ml, 73.4 mmol) was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down to give an oily product (Rf^2 0.82). This product was converted to the title compound by treatment with 5.8 N HCl/dioxane (90.7 ml, 526 mmol) in the usual manner, yield 17.5 g (90.1%), mp 172—174 °C, $[\alpha]_D^{25}$ −84.5° (c=1.0, DMF), Rf^1 0.12, Rf^2 0.45. *Anal.* Calcd for $C_{15}H_{21}ClN_2O_3$: C, 57.6; H, 6.77; N, 8.96. Found: C, 57.4; H, 6.72; N, 8.91. Amino acid analysis: Ala 1.00, Pro 0.94 (average recovery 96.5%).

Boc-Asp(O-2-Ada)-Ala-Pro-OBzl To a solution of H-Ala-Pro-OBzl·HCl (12.0 g, 38.4 mmol), Boc-Asp(O-2-Ada)-OH (14.1 g, 38.4 mmol) and HOBt (5.2 g, 38.4 mmol) in DMF (200 ml) containing Et_3N (5.37 ml, 38.4 mmol), DCC (9.5 g, 24.8 mmol) was added under cooling with ice-salt and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and

evaporated down. Petroleum ether was added to the residue to afford an amorphous powder, which was collected by filtration, yield 17.5 g (72.9%), mp 90—93 °C, $[\alpha]_D^{25}$ −35.6° (c=1.0, DMF), Rf^1 0.76. *Anal.* Calcd for $C_{34}H_{47}N_3O_8$: C, 65.3; H, 7.57; N, 6.72. Found: C, 65.5; H, 7.31; N, 6.60. Amino acid analysis: Asp 0.91, Ala 1.00, Pro 0.94 (average recovery 91.6%).

Boc-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-OBzl To a solution of H-Asp(O-2-Ada)-Ala-Pro-OBzl·HCl [prepared from Boc-Asp(O-2-Ada)-Ala-Pro-OBzl (17.5 g, 28.0 mmol) and 5.8 N HCl/dioxane (42.8 ml, 248.2 mmol) as usual], Boc-Lys(2-Adoc)-OH (11.9 g, 28.0 mmol) and HOBt (3.8 g, 28.0 mmol) in DMF (200 ml) containing Et_3N (3.92 ml, 28.0 mmol), DCC (6.9 g, 33.6 mmol) was added under cooling with ice-salt and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 16.2 g (62.0%), mp 83—85 °C, $[\alpha]_D^{25}$ −40.9° (c=1.0, DMF), Rf^1 0.81, Rf^2 0.92. *Anal.* Calcd for $C_{51}H_{73}N_5O_{11}\cdot 0.5H_2O$: C, 65.1; H, 7.92; N, 7.44. Found: C, 65.2; H, 7.98; N, 7.61. Amino acid analysis: Lys 0.96, Asp 0.90, Ala 1.00, Pro 1.11 (average recovery 81.7%).

Boc-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-OBzl To a solution of H-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-OBzl·HCl [prepared from Boc-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-OBzl (9.3 g, 10.0 mmol) and 5.4 N HCl/dioxane (18.5 ml, 99.9 mmol) as usual], Boc-Glu(O-cHex)-OH (3.29 g, 10.0 mmol) and HOBt (1.4 g, 10.0 mmol) in DMF (150 ml) containing Et_3N (1.4 ml, 10.0 mmol), DCC (2.5 g, 12.0 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 7.4 g (64.7%), mp 110—112 °C, $[\alpha]_D^{25}$ −37.4° (c=1.0, DMF), Rf^1 0.85, Rf^2 0.95. *Anal.* Calcd for $C_{62}H_{90}N_6O_{14}$: C, 65.1; H, 7.93; N, 7.35. Found: C, 65.4; H, 8.18; N, 7.50. Amino acid analysis: Glu 0.88, Lys 0.88, Asp 1.00, Ala 1.00, Pro 1.05 (average recovery 84.5%).

Boc-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-OH, Boc-SSR(47—51)-OH Boc-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-OBzl (3.0 g, 2.6 mmol) in MeOH (150 ml) was hydrogenated over a Pd catalyst for 2 h. After the removal of Pd and the solvent, petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 2.7 g (97.7%), mp 118—121 °C, $[\alpha]_D^{25}$ −24.4° (c=1.0, DMF), Rf^1 0.15, Rf^2 0.23. *Anal.* Calcd for $C_{55}H_{84}N_6O_{14}$: C, 62.7; H, 8.04; N, 7.98. Found: C, 62.6; H, 8.26; N, 8.21.

Boc-Val-Ser-OMe A reaction mixture of H-Ser-OMe.HCl (12.4 g, 80 mmol) and Boc-Val-OSu (25.1 g, 80 mmol) in DMF (300 ml) containing Et_3N (13.4 ml, 96 mmol) was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 23.4 g (91.9%), mp 76—78 °C, $[\alpha]_D^{25}$ −3.4° (c=1.0, DMF), Rf^1 0.51. *Anal.* Calcd for $C_{14}H_{26}N_2O_6$: C, 52.8; H, 8.23; N, 8.80. Found: C, 52.9; H, 8.02; N, 8.84. Amino acid analysis: Val 1.00, Ser 0.88 (average recovery 86.3%).

Boc-Ala-Val-Ser-OMe A reaction mixture of H-Val-Ser-OMe·HCl [prepared from Boc-Val-Ser-OMe (12.7 g, 40 mmol) and 5.8 N HCl/dioxane (68.8 ml, 399 mmol) as usual] and Boc-Ala-OSu (11.5 g, 40 mmol) in DMF (200 ml) containing Et_3N (6.7 ml, 48 mmol) was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 14.5 g (93.1%), mp 155—157 °C, $[\alpha]_D^{25}$ −12.7° (c=1.0, DMF), Rf^1 0.46, Rf^2 0.68. *Anal.* Calcd for $C_{17}H_{31}N_3O_7$: C, 52.4; H, 8.02; N, 10.8. Found: C, 52.6; H, 8.15; N, 10.8. Amino acid analysis: Ala 1.10, Val 1.00, Ser 0.93 (average recovery 94.6%).

Boc-Ala-Val-Ser-NHNH$_2$, Boc-SSR(44—46)-NHNH$_2$ To a solution of Boc-Ala-Val-Ser-OMe (3.0 g, 7.7 mmol) in DMF (200 ml), hydrazine hydrate (3.8 g, 77 mmol) was added, and the reaction mixture was stirred at room temperature for 2 d. After removal of the solvent, water was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 2.5 g (83.4%), mp 95—98 °C, $[\alpha]_D^{25}$ −16.2° (c=1.0, DMF), Rf^1 0.42. *Anal.* Calcd for $C_{16}H_{31}N_5O_6$: C, 49.4; H, 8.02; N, 18.0. Found: C, 49.2; H, 8.05; N, 18.2.

Boc-Arg(Mts)-Gly-OBzl To a solution of H-Gly-OBzl·TosOH (10.1 g, 30 mmol), Boc-Arg(Mts)-OH (13.7 g, 30 mmol) and HOBt (4.05 g, 30 mmol) in DMF (200 ml) containing Et_3N (1.4 ml, 10.0 mmol), DCC (7.4 g, 36 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down to afford Boc-Arg(Mts)-Gly-OBzl as an oily product, yield, 17.2 g (95.0%), Rf^2 0.48, amino acid analysis: Arg 1.00, Gly 0.93 (average recovery 72.5%).

Boc-Gly-Arg(Mts)-Gly-OBzl A reaction mixture of H-Arg(Mts)-Gly-OBzl·HCl (5.4 g, 10 mmol) [prepared from Boc-Arg(Mts)-Gly-OBzl (7.4 g, 12.2 mmol) and 5.8 N HCl/dioxane (25.8 ml, 150 mmol) as usual] and Boc-Gly-OSu (2.7 g, 10 mmol) in DMF (100 ml) containing Et_3N (1.7 ml, 12 mmol) was stirred at room temperature for 3 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 5.4 g (80.0%), mp 82—83 °C, $[\alpha]_D^{25}$ −3.6° (c=1.0, DMF), Rf^1 0.66. *Anal.* Calcd for $C_{31}H_{44}N_6O_8S$: C, 56.4; H, 6.71; N, 12.7. Found: C, 56.4; H, 6.87; N, 12.5. Amino acid analysis: Gly 2.10, Arg 1.00 (average recovery 92.3%).

Boc-Thr-Gly-Arg(Mts)-Gly-OBzl A reaction mixture of H-Gly-Arg(Mts)-Gly-OBzl·HCl [prepared from Boc-Gly-Arg(Mts)-Gly-OBzl (1.98 g, 3 mmol) and 5.4 N HCl/dioxane (5.6 ml, 30 mmol) as usual] and Boc-Thr-OSu (0.95 g, 3 mmol) in DMF (50 ml) containing Et_3N (0.5 ml, 3.6 mmol) was stirred at room temperature for 3 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration, yield 1.6 g (70.0%), mp 112—114 °C, $[\alpha]_D^{25}$ −10.3° (c=1.0, DMF), Rf^2 0.31. *Anal.* Calcd for $C_{35}H_{51}N_7O_{10}S\cdot H_2O$: C, 54.1; H, 6.74; N, 12.5. Found: C, 53.9; H, 6.85; N, 12.6. Amino acid analysis: Thr 1.00, Gly 2.07, Arg 0.89 (average recovery 75.3%).

Boc-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OBzl To a solution of H-Thr-Gly-Arg(Mts)-Gly-OBzl·HCl [prepared from Boc-Thr-Gly-Arg(Mts)-Gly-OBzl (1.6 g, 2.1 mmol) and 5.4 N HCl/dioxane (3.9 ml, 21 mmol) as usual], Boc-Lys(2-Adoc)-OH (1.16 g, 2.73 mmol), and HOBt (0.29 g, 2.1 mmol), in DMF (50 ml) containing Et_3N (0.44 ml, 3.15 mmol), DCC (0.52 g, 2.52 mmol) was added under cooling with ice-salt and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration, yield 1.66 g (74%), mp 123—125 °C, $[\alpha]_D^{25}$ −11.6° (c=1.0, DMF), Rf^1 0.70. *Anal.* Calcd for $C_{52}H_{77}N_9O_{13}S\cdot H_2O$: C, 57.5; H, 7.33; N, 11.6. Found: C, 57.6; H, 7.24; N, 11.6. Amino acid analysis: Lys 1.02, Thr 0.97, Arg 1.00, Gly 2.18 (average recovery 81.2%).

Boc-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OBzl To a solution of H-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OBzl·HCl [prepared from Boc-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OBzl (1.6 g, 1.5 mmol) and 5.4 N HCl/dioxane (3.0 ml, 16.2 mmol) as usual], Boc-Gly-OH (0.32 g, 1.8 mmol) and HOBt (0.20 g, 1.5 mmol) in DMF (50 ml) containing Et_3N (0.32 ml, 2.25 mmol), DCC (0.37 g, 1.8 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were collected by filtration, yield 1.32 g (78.1%), mp 137—139 °C, $[\alpha]_D^{25}$ −7.3° (c=1.0, DMF), Rf^1 0.80. *Anal.* Calcd for $C_{54}H_{80}N_{10}O_{14}S$: C, 57.6; H, 7.17; N, 12.5. Found: C, 57.9; H, 7.36; N, 12.3. Amino acid analysis: Lys 1.02, Thr 0.91, Arg 1.00, Gly 2.85 (average recovery 80.2%).

Boc-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OBzl, Boc-SSR(37—43)-OBzl To a solution of H-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OBzl·HCl [prepared from Boc-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OBzl (1.24 g, 1.1 mmol) and 5.4 N HCl/dioxane (2.0 ml, 11 mmol) as usual], Boc-Gly-OH (0.29 g, 1.65 mmol) and HOBt (0.15 g, 1.1 mmol) in DMF (50 ml) containing Et_3N (0.23 ml, 1.65 mmol), DCC (0.27 g, 1.32 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down. Ether was added to the residue to afford crystals, which were

collected by filtration, yield 1.0 g (76.9%), mp 137—138 °C, $[\alpha]_D^{25}$ −9.4° (c=1.0, DMF), Rf^1 0.68. *Anal.* Calcd for $C_{56}H_{83}N_{11}O_{15}S$: C, 56.9; H, 7.08; N, 13.0. Found: C, 56.9; H, 7.20; N, 12.8. Amino acid analysis: Lys 1.03, Thr 0.97, Arg 1.00, Gly 3.88 (average recovery 76.5%).

Boc-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OH, Boc-SSR(37—43)-OH Boc-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OBzl (1.0 g, 0.85 g) in DMF (50 ml) was hydrogenated over a Pd catalyst for 24 h. After the removal of Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 0.88 g (95.0%), mp 129—132 °C. *Anal.* Calcd for $C_{49}H_{77}N_{11}O_{15}S\cdot 1.5H_2O$: C, 52.6; H, 7.20; N, 13.8. Found: C, 52.5; H, 7.36; N, 13.5.

Boc-Phe-Thr-OBzl To a solution of H-Thr-OBzl·HCl (18.0 g, 73.3 mmol), Boc-Phe-OH (19.4 g, 73.3 mmol) and HOBt (9.9 g, 73.3 mmol) in DMF (200 ml) containing Et_3N (10.3 ml, 73.3 mmol), DCC (18.1 g, 87.9 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 26.2 g (78.3%), mp 102—106 °C, $[\alpha]_D^{25}$ −10.1° (c=1.0, DMF), Rf^1 0.68, Rf^2 0.62. *Anal.* Calcd for $C_{25}H_{32}N_2O_6$: C, 65.8; H, 7.06; N, 6.14. Found: C, 65.9; H, 7.32; N, 5.99. Amino acid analysis: Thr 0.92, Phe 1.00 (average recovery 78.9%).

Boc-Ser-Phe-Thr-OBzl To a solution of H-Phe-Thr-OBzl·HCl [prepared from Boc-Phe-Thr-OBzl (13.7 g, 30.0 mmol) and 7.2 N HCl/dioxane (41.7 ml, 300 mmol) as usual], Boc-Ser-OH (6.2 g, 30.0 mmol) and HOBt (4.1 g, 30.0 mmol) in DMF (200 ml) containing Et_3N (4.2 ml, 30.0 mmol), DCC (7.4 g, 36.0 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 14.0 g (85.8%), mp 108—110 °C, $[\alpha]_D^{25}$ −7.9° (c=1.0, DMF), Rf^1 0.45, Rf^2 0.63. *Anal.* Calcd for $C_{28}H_{37}N_3O_8$: C, 61.9; H, 6.86; N, 7.73. Found: C, 61.6; H, 6.77; N, 7.69. Amino acid analysis: Ser 0.87, Phe 1.00, Thr 0.95 (average recovery 87.1%).

Boc-Ile-Ser-Phe-Thr-OBzl A reaction mixture of H-Ser-Phe-Thr-OBzl·HCl [prepared from Boc-Ser-Phe-Thr-OBzl (12.0 g, 22 mmol) and 7.2 N HCl/dioxane (30.7 ml, 221 mmol) as usual] and Boc-Ile-OSu (7.3 g, 22 mmol) in DMF (200 ml) containing Et_3N (3.7 ml, 26 mmol) was stirred at room temperature for 2 h. After removal of the solvent, AcOEt and water were added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 10.5 g (72.7%), mp 155—157 °C, $[\alpha]_D^{25}$ −10.1° (c=1.0, DMF), Rf^1 0.46, Rf^2 0.65. *Anal.* Calcd for $C_{34}H_{48}N_4O_9$: C, 60.5; H, 7.47; N, 8.30. Found: C, 60.5; H, 7.43; N, 8.28. Amino acid analysis: Ile 0.92, Ser 0.84, Phe 1.00, Thr 0.92 (average recovery 87.1%).

Boc-Met-Ile-Ser-Phe-Thr-OBzl A reaction mixture of H-Ile-Ser-Phe-Thr-OBzl·HCl [prepared from Boc-Ile-Ser-Phe-Thr-OBzl (13.1 g, 20 mmol) and 6.8 N HCl/dioxane (41.0 ml, 279 mmol) as usual] and Boc-Met-OSu (6.9 g, 20 mmol) in DMF (200 ml) containing Et_3N (3.5 ml, 24 mmol) was stirred at room temperature for 2 h. After removal of the solvent, AcOEt and water were added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 13.6 g (86.3%), mp 213—215 °C, $[\alpha]_D^{25}$ −16.3° (c=1.0, DMF), Rf^1 0.47, Rf^2 0.64. *Anal.* Calcd for $C_{39}H_{57}N_5O_{10}S\cdot 0.5H_2O$: C, 58.8; H, 7.34; N, 8.79. Found: C, 58.5; H, 7.40; N, 8.76. Amino acid analysis: Met 0.76, Ile 0.97, Ser 0.85, Phe 1.00, Thr 1.03 (average recovery 92.4%).

Boc-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-OBzl To a solution of H-Met-Ile-Ser-Phe-Thr-OBzl·HCl [prepared from Boc-Met-Ile-Ser-Phe-Thr-OBzl (10.0 g, 12.7 mmol) and 6.5 N HCl/dioxane (19.5 ml, 127 mmol) as usual], Boc-Lys(2-Adoc)-OH (5.4 g, 12.7 mmol), HOBt (1.7 g, 12.7 mmol) in DMF (100 ml) containing Et_3N (1.8 ml, 12.7 mmol), DCC (3.2 g, 15.2 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 9.8 g (70.5%), mp 212—214 °C, $[\alpha]_D^{25}$ −15.4° (c=1.0, DMF), Rf^1 0.48, Rf^2 0.67. *Anal.* Calcd for $C_{56}H_{83}N_7O_{13}S$: C, 60.5; H, 7.70; N, 8.81. Found: C, 60.7; H, 7.78; N, 8.90. Amino acid analysis: Lys 1.00, Met 0.86, Ile 1.03, Ser 0.95, Phe 1.00, Thr 1.18 (average recovery 87.1%).

Boc-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-NHNH$_2$, Boc-SSR(27—32)-NHNH$_2$ To a solution of Boc-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-OBzl (4.0 g, 3.6 mmol) in DMF (100 ml), hydrazine hydrate (1.8 g, 36.0 mmol) was added. The reaction mixture was stored at room temperature for 2 d. After removal of the solvent, water was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 3.2 g (87.3%), mp 185—189 °C, $[\alpha]_D^{25}$ −22.5° (c=1.0, DMF), Rf^1 0.40. *Anal.* Calcd for $C_{49}H_{79}N_9O_{12}S$: C, 57.8; H, 7.82; N, 12.4. Found: C, 57.9; H, 7.95; N, 12.6.

Boc-Val-Gly-OBzl A reaction mixture of H-Gly-OBzl·Tos-OH (27 g, 80 mmol) and Boc-Val-OSu (25 g, 80 mmol) in DMF (200 ml) containing Et_3N (12.3 ml, 88 mmol) was stirred at room temperature for 3 h. After removal of the solvent, petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 25.2 g (86.3%), mp 72—73 °C, $[\alpha]_D^{25}$ −29.4° (c=1.0, MeOH), Rf^1 0.83. *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.6; H, 7.74; N, 7.69. Found: C, 62.5; H, 7.72; N, 7.75. Amino acid analysis: Val 1.16, Gly 1.00 (average recovery 93.2%).

Boc-Arg(Mts)-Val-Gly-OBzl To a solution of H-Val-Gly-OBzl·HCl [prepared from Boc-Val-Gly-OBzl (18.0 g, 50 mmol) and 7.2 N HCl/dioxane (70 ml, 504 mmol) as usual], Boc-Arg(Mts)-OH (22.8 g, 50 mmol) and HOBt (6.75 g, 50 mmol) in DMF (200 ml) containing Et_3N (8.4 ml, 60 mmol), DCC (12.4 g, 60 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 30.5 g (86.0%), mp 126—128 °C, $[\alpha]_D^{25}$ −26.5° (c=1.0, DMF), Rf^2 0.53. *Anal.* Calcd for $C_{34}H_{50}N_6O_8S$: C, 55.9; H, 7.32; N, 11.5. Found: C, 55.5; H, 6.94; N, 11.6. Amino acid analysis: Arg 1.00, Val 1.10, Gly 0.97 (average recovery 88.5%).

Boc-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl To a solution of H-Arg(Mts)-Val-Gly-OBzl·HCl [prepared from Boc-Arg(Mts)-Val-Gly-OBzl (15.0g, 21 mmol) and 6.5 N HCl/dioxane (32.8 ml, 210 mmol) as usual], Boc-Trp(Mts)-OH [prepared from Boc-Trp(Mts)-OH·dicyclohexylamine (DCHA) (13.4 g, 20 mmol) and 10% citric acid (50 ml) as usual] and HOBt (2.7 g, 20 mmol) in DMF (200 ml) containing Et_3N (4.2 ml, 30 mmol), DCC (4.9 g, 24 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford an amorphous powder, yield 20 g (93.5%), Rf^1 0.79. Amino acid analysis: Arg 1.00, Val 1.00, Gly 1.03 (average recovery 93.5%). Trp was not determined.

Boc-Val-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl A reaction mixture of H-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl·HCl [prepared from Boc-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl (20 g, 18.7 mmol) and 5.0 N HCl/dioxane (37 ml, 185 mmol) as usual] and Boc-Val-OSu (5.9 g, 19 mmol) in DMF (200 ml) containing Et_3N (4.0 ml, 28 mmol) was stirred at room temperature for 3 h. After removal of the solvent, petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 19 g (84.2%), mp 122—124 °C, $[\alpha]_D^{25}$ −19.9° (c=1.0, MeOH), Rf^1 0.69. *Anal.* Calcd for $C_{59}H_{79}N_9O_{12}S_2\cdot 1.8H_2O$: C, 59.4; H, 7.09; N, 11.6. Found: C, 59.1; H, 6.91; N, 11.3. Amino acid analysis: Val 2.36, Arg 0.95, Gly 1.00 (average recovery 93.2%). Trp was not determined.

Boc-Lys(2-Adoc)-Val-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl Boc-SSR(21—26)-OBzl To a solution of H-Val-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl·HCl [prepared from Boc-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl (14.0 g, 12 mmol) and 5.0 N HCl/dioxane (25 ml, 125 mmol) as usual], Boc-Lys(2-Adoc)-OH (5.1 g, 12 mmol) and HOBt (1.6 g, 12 mmol) in DMF (200 ml) containing Et_3N (2.0 ml, 14 mmol), DCC (3.0 g, 14.6 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down. The residue in $CHCl_3$ was applied to a silica gel column (3×45 cm), equilibrated and eluted with $CHCl_3$. Eluate containing the desired product was collected and the solvent removed by evaporation. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 4.1 g (23.3%), mp 164—166 °C, $[\alpha]_D^{25}$ −10.8° (c=1.0, DMF), Rf^1 0.83. *Anal.* Calcd for $C_{76}H_{105}N_{11}O_{15}S_2\cdot 0.2H_2O$: C, 61.7; H, 7.18; N, 10.4. Found: C, 61.4; H, 7.18; N, 10.2. Amino acid analysis: Lys 1.00, Val 1.87, Arg 1.13, Gly 0.94 (average recovery 89.9%).

Boc-Ile-Lys(2-Adoc)-OBzl A reaction mixture of H-Lys(2-Adoc)-

OBzl·HCl (15.8 g, 35 mmol) and Boc-Ile-OSu (11.5 g, 35 mmol) in DMF (200 ml) containing Et_3N (5.9 ml, 42 mmol) was stirred at room temperature for 2 h. After removal of the solvent, the residue in $CHCl_3$ (5 ml) was applied to a silica gel column (3.5×43 cm), equilibrated and eluted with $CHCl_3$. The solvent of the eluate (1500—2300 ml) was removed by evaporation to give an oily product, yield 18.1 g (82.1%), Rf^1 0.58.

H-Ile-Lys(2-Adoc)-OBzl·HCl The title compound was prepared from the Boc-Ile-Lys(2-Adoc)-OBzl obtained above and 5.0 N HCl/dioxane (70 ml, 350 mmol), as usual, to give crystals which were collected by filtration, yield 11.7 g, (81.5%), mp 110—112 °C, $[\alpha]_D^{25}$ +7.9° (c=1.0, DMF), Rf^1 0.72, Rf^2 0.90. *Anal.* Calcd for $C_{30}H_{46}ClN_3O_5$: C, 62.3; H, 8.29; N, 7.26. Found: C, 62.0; H, 8.24; N, 7.57. Amino acid analysis: Ile 1.16, Lys 1.00 (average recovery 89.9%).

Boc-Lys(2-Adoc)-Ile-Lys(2-Adoc)-OBzl To a solution of H-Ile-Lys(2-Adoc)-OBzl·HCl (9.1 g, 16 mmol), Boc-Lys(2-Adoc)-OH (6.9 g, 16 mmol) and HOBt (2.2 g, 16 mmol) in DMF (150 ml) containing Et_3N (2.3 ml, 16 mmol), DCC (4.0 g, 19.4 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 12.6 g (84.3%), mp 110—115 °C, $[\alpha]_D^{25}$ −12.9° (c=1.0, DMF), Rf^1 0.72, Rf^2 0.90. *Anal.* Calcd for $C_{52}H_{79}N_5O_{10}$: C, 66.9; H, 8.52; N, 7.50. Found: C, 67.1; H, 8.67; N, 7.41. Amino acid analysis: Lys 2.00, Ile 1.00 (average recovery 96.9%).

Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Adoc)-OBzl To a solution of H-Lys(2-Adoc)-Ile-Lys(2-Adoc)-OBzl·HCl [prepared from Boc-Lys(2-Adoc)-Ile-Lys(2-Adoc)-OBzl (12.2 g, 13 mmol) and 5.0 N HCl/dioxane (26.1 ml, 131 mmol) as usual], Boc-Ser-OH (2.7 g, 13 mmol) and HOBt (1.8 g, 13 mmol) in DMF (100 ml) containing Et_3N (1.8 ml, 13 mmol), DCC (3.2 g, 15.7 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, then dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 19.5 g (71.6%), mp 98—100 °C, $[\alpha]_D^{25}$ −11.9° (c=1.0, DMF), Rf^1 0.94, Rf^2 0.85. *Anal.* Calcd for $C_{55}H_{84}N_6O_{12}$: C, 64.7; H, 8.29; N, 8.23. Found: C, 64.7; H, 8.59; N, 8.13. Amino acid analysis: Ser 1.07, Lys 2.00, Ile 1.03 (average recovery 83.6%).

Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Adoc)-OH, Boc-SSR(17—20)-OH Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Adoc)-OBzl (4.0 g, 4.0 mmol) in MeOH (200 ml) was hydrogenated over a Pd catalyst for 2 h. After the removal of Pd and the solvent, petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 3.5 g (94.0%), mp 130—133 °C, $[\alpha]_D^{25}$ −6.6° (c=1.0, DMF), Rf^1 0.42, Rf^2 0.63. *Anal.* Calcd for $C_{48}H_{78}N_6O_{12}$: C, 61.3; H, 8.47; N, 8.94. Found: C, 61.2; H, 8.22; N, 9.07.

Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Adoc)-Lys(2-Adoc)-Val-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl To a solution of H-Lys(2-Adoc)-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl·HCl [prepared from Boc-Lys(2-Adoc)-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl (2.0 g, 1.35 mmol) and 7.2 N HCl/dioxane (1.88 ml, 13.5 mmol) as usual], Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Adoc)-OH (1.26 g, 1.35 mmol) and HOBt (0.18 g, 1.35 mmol) in DMF (100 ml) containing Et_3N (0.19 ml, 1.35 mmol), DCC (0.34 g, 1.63 mmol) was added under cooling with ice-salt, and the reaction mixture was stirred at 4 °C overnight. After the removal of DCC urea and the solvent, water was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 2.51 g (81.5%), mp 255—257 °C, $[\alpha]_D^{25}$ −9.6° (c=1.0, DMF), Rf^1 0.48, Rf^2 0.89. *Anal.* Calcd for $C_{119}H_{173}N_{17}O_{24}S_2$: C, 62.4; H, 7.61; N, 10.4. Found: C, 62.4; H, 7.81; N, 10.3. Amino acid analysis: Ser 1.07, Lys 3.00, Ile 0.88, Val 2.25, Arg 1.09, Gly 0.99 (average recovery 91.2%). Trp was not determined.

Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Adoc)-Lys(2-Adoc)-Val-Trp(Mts)-Arg(Mts)-Val-Gly-OH, Boc-SSR(17—26)-OH Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Adoc)-Lys(2-Adoc)-Val-Trp(Mts)-Arg(Mts)-Val-Gly-OBzl (2.00 g, 0.87 mmol) in DMF (150 ml) was hydrogenated over a Pd catalyst for 3 h. After the removal of Pd and the solvent, ether was added to the residue to afford an amorphous powder, which was collected by filtration, yield 1.76 g (88.5 %), $[\alpha]_D^{25}$ −8.4° (c=1.0, DMF), Rf^1 0.26, Rf^2 0.58. *Anal.* Calcd for $C_{112}H_{167}N_{17}O_{24}S_2$: C, 60.4; H, 7.69; N, 10.7. Found: C, 60.1; H, 7.92; N, 11.0.

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References and Notes

1) Part LIII: Fukumizu A., Tsuda Y., Wanaka K., Tada M., Okamoto S., Hijikata-Okunomiya A., Okada Y., *Chem. Pharm. Bull.*, **47**, in press (1676).
2) The customary L-configuration for amino acid residues is omitted. Abbreviations used in this report for amino acids, peptides and the derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485—2489 (1966); *ibid*. **6**, 362—364 (1966); *ibid*. **11**, 1726—1732 (1972). The following additional abbreviations are used: AcOEt, ethyl acetate; DMF, dimethylformamide; TFA, trifluoroacetic acid; AcOH, acetic acid; Boc, *tert*-butyloxycarbonyl; Mts, mesitylenesulfonyl (1999). OSu, *N*-succinimidyl ester; O-2-Ada, 2-adamantyl ester; 2-Adoc, 2-adamantyloxycarbonyl; 2-Adom, 2-adamantyloxymethyl; O-cHex, cyclohexyl ester; DCC, *N*,*N*′-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; TFMSA, trifluoromethanesulfonic acid; MSA, methanesulfonic acid, TEA, triethylamine; $(Boc)_2O$, di-*tert*-butyldicarbonate; SSR, *Sulfolobus solifataricus* Ribonuclease.
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