# Amino Acids and Peptides. LI. Application of the 2-Adamantyloxycarbonyl (2-Adoc) Group to the Protection of the Hydoxyl Function of Tyrosine in Peptide Synthesis[1,2]

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**A 2-adamantyloxycarbonyl (2-Adoc) group was introduced as a protecting group for the hydroxyl function of tyrosine (Tyr) through the Shotten-Bauman reaction of 2-adamantyloxycarbonyl chloride with the copper complex of Tyr. The 2-Adoc group is stable to trifluoroacetic acid (TFA), 5.0 N HCl/dioxane, hydrogenation over a Pd catalyst and tertiary amine, and is easily removed by treatment with 1 M trifluoromethanesulfonic acid (TFMSA)–thioanisole/TFA and HF. Boc–Tyr(2-Adoc)–OH was prepared by the reaction of $(Boc)_2O$ and H–Tyr(2-Adoc)–OH in the presence of triethylamine. Boc–Tyr(2-Adoc)–OH was successfully applied to the synthesis of Boc–Ala–Thr–Val–Lys(2-Adoc)–Phe–Lys(2-Adoc)–Tyr(2-Adoc)–Lys(2-Adoc)–Gly–OH, corresponding to the sequence 1—9 of *Sulfolobus solfataricus* RNase, and Boc–Tyr(2-Adoc)–Asp(O-2-Ada)–Glu(O-cHex)–Gly–OH, corresponding to the sequence 33—36 of *S. solfataricus* RNase.**

**Boc–Phe–Lys(2-Adoc)–Tyr(2-Adoc)–Lys(2-Adoc)–Gly–OBzl was treated with anhydrous HF to afford H–Phe–Lys–Tyr–Lys–Gly–OH without any side reactions, in a good yield.**

**Key words** protecting group; tyrosine hydroxyl function; 2-adamantyloxycarbonyl; peptide synthesis; *S. solfataricus* RNase

For the preparation of fairly large peptides or proteins by the solution method or convergent solid-phase method,[3] it is very important to employ suitable protecting groups in order to increase the solubility of peptide intermediates in organic solvents as well as to suppress side reactions during the peptide synthesis. For protection of the hydroxyl function of the Tyr residue, the *tert*-butyl ($Bu^t$) group has been employed,[4] especially in the case of the Fmoc strategy in the solid-phase method, since the $Bu^t$ group is stable to alkali and is easily cleavable by TFA. The benzyl (Bzl) group[5] or 2-bromobenzyloxycarbonyl (2-Br-Z) group[6] has also been employed for protection of the hydroxyl function of Tyr. These protecting groups are stable to TFA and cleavable by HF[7] or by hydrogenation over a Pd catalyst. Therefore, for the synthesis of peptide fragments as building blocks of fairly large peptides or proteins, the benzyl ester group can not be employed as a C-terminal carboxyl protecting group in combination with Bzl or 2-Br-Z group as a protecting group for the hydroxyl function of Tyr. In these cases, phenacyl ester[8] instead of Bzl ester is commonly employed for protection of the C-terminal carboxyl function of peptide fragments. However, the phenacyl ester group sometimes can not be removed completely by AcOH–Zn and side reaction may occur during the deprotection reaction.[9] Thus, a new protecting group for the hydroxyl function of Tyr which is stable to TFA and to hydrogenation over a Pd catalyst is required.

This paper deals with the development of a new protecting group for the hydroxyl function of Tyr and its application to peptide synthesis. Previously, we developed the 2-adamantyloxycarbonyl (2-Adoc) group as an ε-amino protecting group for Lys and successfully applied it to peptide synthesis.[10,11] The 2-Adoc group was stable to TFA and alkaline conditions and easily removed by 1 M TFMSA–thioanisole/TFA and HF and, moreover, this protecting group increased solubility of peptide fragments in organic solvents. These results led us to examine the 2-Adoc group for protection of the hydroxyl function of Tyr.

According to Chart 1, H–Tyr(2-Adoc)–OH (Fig. 1) was synthesized from $Tyr_2$–Cu complex and 2-adamantyl chloroformate (2-Adoc-Cl). Reaction of $(Boc)_2O$ with H–

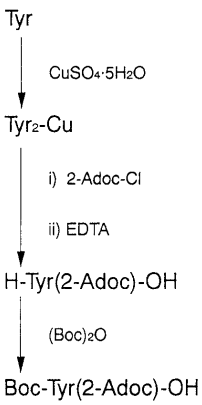


Chart 1. Synthetic Scheme for H–Tyr(2-Adoc)–OH and Boc–Tyr(2-Adoc)–OH

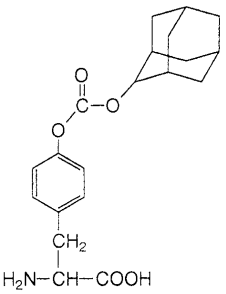


Fig. 1. Structure of H–Tyr(2-Adoc)–OH

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Table 1. Stability of the O-Adoc Group in Various Acids and Bases

| Conditions | % Tyr (in acids)[a] or Boc–Tyr–OH (in bases)[b] regenerated | | | | |
|---|---|---|---|---|---|
| | 10 min | 30 min | 60 min | 2 h | 24 h |
| 5.0 N HCl/dioxane | 0 | 0 | 0 | 0 | 0 |
| TFA | 0 | 0 | 0 | 0 | 2 |
| 25% HBr/AcOH | 0 | 0 | 0 | 0 | 3 |
| MSA | | 33 | 30 | 38 | |
| 1 M TFMSA–thioanisole/TFA | 100 | 100 | 100 | | |
| HF | | 100 | 100 | | |
| 10% TEA/DMF | 0 | 0 | 0 | 0 | 0 |
| 5% $NaHCO_3/H_2O$ | 0 | 0 | 0 | 3 | 7 |
| 5% $Na_2CO_3/H_2O$ | 42 | 67 | 88 | 97 | 97 |
| 20% Piperidine/DMF | 2 | 10 | 18 | 45 | 33 |
| 2 N NaOH | 100 | 100 | 100 | 100 | |
| $H_2$/Pd | | | 0 | 0 | 0 |

a) Determined by an amino acid analyzer. b) Determined by HPLC (column and mobile-phase system are given in the Experimental section).

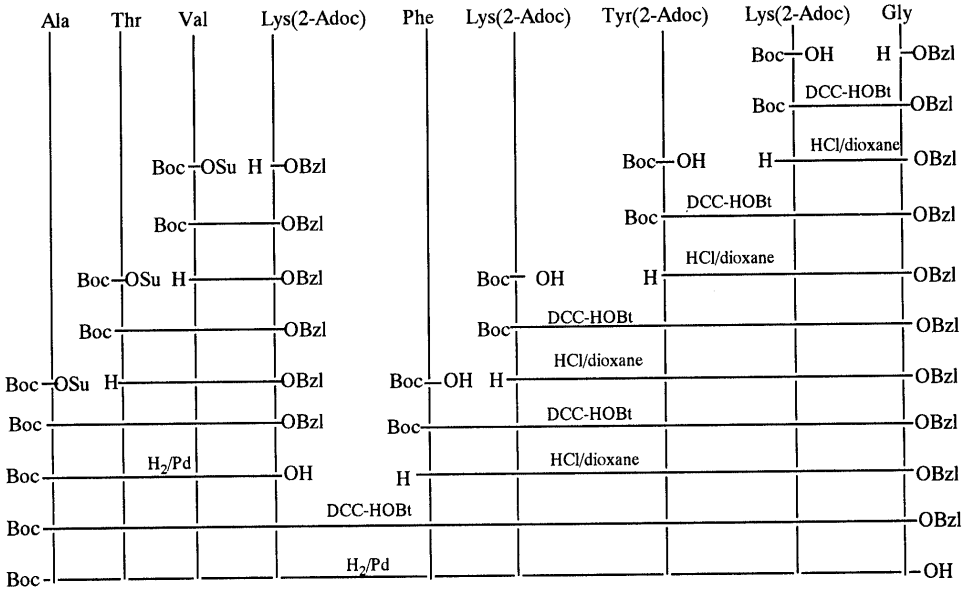


Chart 2. Synthetic Scheme for Boc–(1—9)–OH

Tyr(2-Adoc)–OH afforded Boc–Tyr(2-Adoc)–OH. Using Boc–Tyr(2-Adoc)–OH, the stability and susceptibility of the 2-Adoc group to various acids and bases were examined by measurement of regenerated Tyr with an amino acid analyzer or by measurement of generated Boc–Tyr–OH and intact Boc–Tyr(2-Adoc)–OH by HPLC after treatment with acids or bases, respectively. As summarized in Table 1, the 2-Adoc group was stable to 5.0 N HCl in dioxane, TFA , hydrogenation over a Pd catalyst and 25% HBr in AcOH for up to 24 h. The 2-Adoc group could be removed by treatment with 1 M TFMSA–thioanisole/TFA or anhydrous HF in a few minutes at 0 °C, but it was cleaved very slowly by MSA, and MSA treatment was not practical for its removal. The 2-Adoc group was stable to 10% triethylamine in DMF for up to 24 h. However, the 2-Adoc group was not so stable to 5% $Na_2CO_3$ in water and 20% piperidine in DMF, and was easily cleaved by 2 N NaOH. We therefore concluded that the 2-Adoc group was suitable for protection of the Tyr hydroxyl function in combination with Boc as an $N^{\alpha}$ protecting group.

In order to evaluate the 2-Adoc group for the peptide synthesis employing Boc-chemistry, a protected nonapeptide, Boc–SSR(1—9)–OH, corresponding to sequence 1—9 of *Sulfolobus solfataricus* RNase, and a protected tetrapeptide, Boc–SSR(33—36)–OH, corresponding to sequence 33—36 of *S. solfataricus* RNase,[12] were synthesized using the 2-Adoc group as a protecting group of the Tyr hydroxyl function.

Boc–SSR(1—9)–OH was prepared according to Chart 2. Starting from H–Gly–OBzl, Boc–Lys(2-Adoc)–OH, Boc–Tyr(2-Adoc)–OH, Boc–Lys(2-Adoc)–OH and Boc–Phe–OH were coupled successively by the DCC–HOBt method to afford Boc–Phe–Lys(2-Adoc)–Tyr(2-Adoc)–Lys(2-Adoc)–Gly–OBzl. On the other hand, starting from H–Lys(2-Adoc)–OBzl, Boc–Val–OSu, Boc–Thr–OSu and Boc–Ala–OSu were coupled successively to afford Boc–Ala–Thr–Val–Lys(2-Adoc)–OBzl, which was hydrogenated over a Pd catalyst to give Boc–Ala–Thr–Val–Lys(2-Adoc)–OH. After removal of the Boc group of the former pentapeptide ester, the resultant pentapeptide amine was coupled with the latter Boc-tetrapeptide by the DCC–HOBt method to afford Boc–SSR(1—9)–OBzl, which was hydrogenated over a Pd catalyst to give the

desired Boc–SSR(1—9)–OH.

Boc–SSR(33—36)–OH was prepared according to Chart 3. Starting from H–Gly–OBzl, Boc–Glu(O-cHex)–OH, Boc–Asp(O-2-Ada)–OH and Boc–Tyr(2-Adoc)–OH were coupled successively by the DCC–HOBt method to afford Boc–Tyr(2-Adoc)–Asp(O-2-Ada)–Glu(O-cHex)–Gly–OBzl, which was hydrogenated over a Pd catalyst to afford the desired protected tetrapeptide, Boc–SSR(33—36)–OH in a pure form. In each case described above, the benzyl group was removed completely within several hours and Pd was easily removed by filtration. In contrast, $Zn^{2+}$, which is produced during removal of the phenacyl group, is sometimes difficult to remove completely from peptides by filtration. Thus, the benzyl ester is highly advantageous.

Finally, in order to examine the side reaction of Tyr(2-Adoc) during the deblocking procedure, Boc–Phe–Lys(2-Adoc)–Tyr(2-Adoc)–Lys(2-Adoc)–Gly–OBzl was treated with HF. The HPLC profile of the crude product obtained above is shown in Fig. 2. The desired product (peak A) was purified by preparative HPLC and the purified product exhibited a single peak on HPLC analysis. Peak B and peak C were also isolated by preparative HPLC. After lyophilization, no detectable compound remained at either of these peak positions. These results show that alkylation of the phenyl ring of Tyr did not occur during the deblocking with HF, and the 2-Adoc group is suitable for protection of the Tyr hydroxyl function.

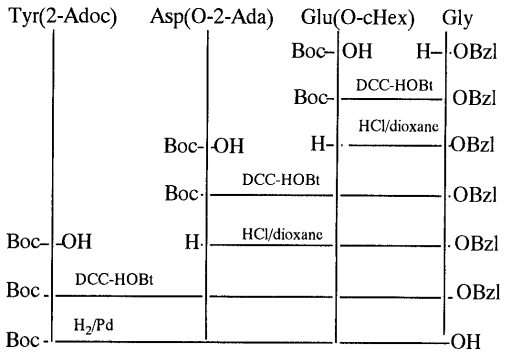


Chart 3. Synthetic Scheme for Boc–(33—36)–OH

During the synthesis of the above two protected peptide fragments, Tyr(2-Adoc) was stable under the various conditions, including hydrogenation over a Pd catalyst. Therefore, it can be concluded that Bzl ester instead of phenacyl ester can be used as a C-terminal protecting group in combination with the Tyr(2-Adoc) residue.

## Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus without correction. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co.). $^1$H-(500 MHz) and $^{13}$C-NMR spectra were recorded on a Bruker ARX500 spectrometer. Chemical shift values are expressed as ppm downfield from tetramethylsilane used as an internal standard ($\delta$-value). The $J$ values are given in Hz. On HPLC, solvent A and solvent B are 0.05% aqueous TFA and 0.05% TFA in MeCN, respectively. 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.

**H–Tyr(2-Adoc)–OH** Tyrosine–copper complex [prepared from Tyr (15.6 g, 86 mmol) and $CuSO_4 \cdot 5H_2O$ as usual] was suspended in dioxane and $H_2O$ (50 ml + 50 ml) and 1 N NaOH (86.0 ml) was added. 2-Adoc–Cl (21.9 g, 103.1 mmol) in dioxane (50 ml) was further added under cooling with ice. The reaction mixture was stirred at the same temperature for 3 h. A precipitate was collected by filtration and the solid material was washed with a mixture of dioxane and $H_2O$ (1 : 1). The copper was removed with saturated EDTA solution as usual and the product was washed with MeOH, yield 19.3 g (62.5%), mp 213—215 °C, $[\alpha]_D^{26}$ −6.2° ($c$ = 1.0, AcOH), $Rf^2$ 0.38. *Anal.* Calcd for $C_{20}H_{25}NO_5 \cdot 0.8H_2O$: C, 64.3; H, 7.17; N, 3.75. Found: C, 64.3; H, 7.01; N, 3.77.

**Boc–Tyr(2-Adoc)–OH** A solution of H–Tyr(2-Adoc)–OH (5.0 g, 13.9 mmol) in DMF (30 ml) containing $Et_3N$ (3.1 ml, 28.0 mmol) was cooled with ice. $(Boc)_2O$ (3.6 g, 16.7 mmol) in DMF (10 ml) was added

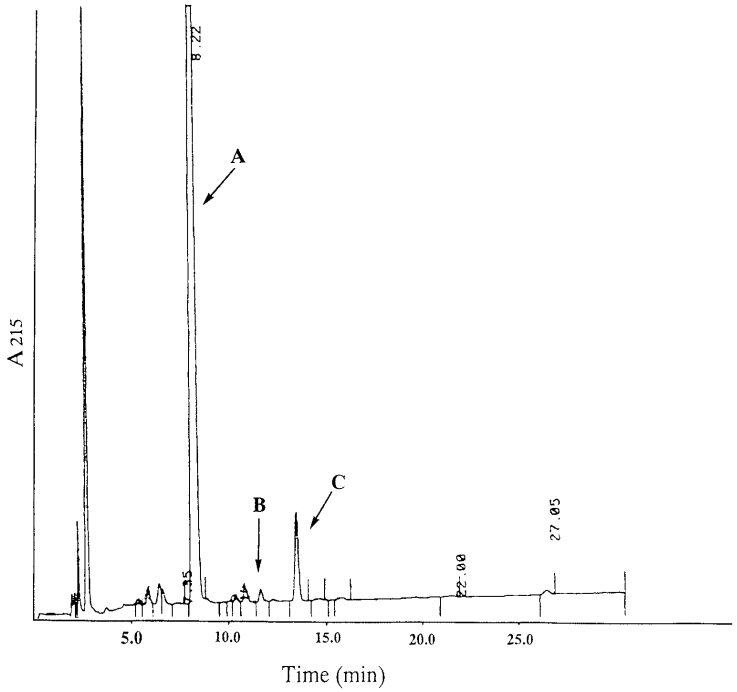


Fig. 2. HPLC Profile of Crude H–Phe–Lys–Tyr–Lys–Gly–OH

Column, Vydac C18 (4.6 × 250 mm); eluent, 0.1% TFA in $H_2O$ containing 5% MeCN (A) and 0.1% TFA in MeCN containing 20% $H_2O$ (B); A : B from 95 : 5 to 50 : 50 in 45 min; flow rates, 1 ml/min, detection, 215 nm.

and the reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and water, dried over $Na_2SO_4$ and evaporated down. Petroleum ether was added to the residue to afford crystals, which were recrystallized from $CHCl_3$ and acetone, yield 5.5 g (85.7%), mp 95—98 °C, $[\alpha]_D^{26}$ −11.5° ($c$=1.0, DMF), $Rf^2$ 0.62. $^1$H-NMR ($CDCl_3$) $\delta$: 1.42 (9H, s, *tert*-butyl), 1.59—2.16 (14H, m, adamantyl), 3.04—3.22 (2H, m, $\beta$-$CH_2$), 4.57—4.59 (1H, m, $\alpha$-CH), 4.88 (1H, s, O-CH), 5.09—5.10 (1H, m, NH), 7.11—7.2.21 (4H, m, Ph). *Anal.* Calcd for $C_{25}H_{33}NO_7 \cdot 0.5H_2O$: C, 64.1; H, 7.31; N, 2.99. Found: C, 64.0; H, 7.40; N, 2.93.

**Examination of Stability and Susceptibility of Boc-Tyr(2-Adoc)-OH to Acids and Bases** 1) Acidic Solution: Boc-Tyr(2-Adoc)-OH (4.5 mg, 10 $\mu$mol) was dissolved in acid (Table 1) at room temperature. Then 0.01 ml of each solution was diluted with water or 0.02—0.5 M $Na_2CO_3$ (0.09 ml) to adjust the pH to about 2. This solution (0.01—0.02 ml) was injected into the amino acid analyzer and the amount of regenerated Tyr was measured as a function of time.

2) Basic Solution: Boc-Tyr(2-Adoc)-OH (4.5 mg, 10 $\mu$mol) was dissolved in a base (Table 1) at room temperature. Then 0.01 ml of each solution was diluted with 0.1—1N HCl (0.09 ml) to adjust the pH to about 2. This solution was used for HPLC analysis. Conditions: column, $\mu$ Bondasphere C18 (3.9 × 150 mm); flow rates, 1 ml/min; eluent, A : B 80 : 20 for 5 min, 80 : 20 to 20 : 80 in 20 min, 80 : 20 for 10 min, and 20 : 80 to 80 : 20 in 5 min. $t_R$ Boc-Tyr-OH: 12.25 min; Boc-Tyr(2-Adoc)-OH: 30.24 min.

**Boc-Lys(2-Adoc)-Gly-OBzl** DCC (3.7 g, 18 mmol) was added to a solution of H-Gly-OBzl·TosOH (5.1 g, 15 mmol), Boc-Lys(2-Adoc)-OH (6.4 g, 15 mmol), and HOBt (2.0 g, 15 mmol) in DMF (150 ml) containing $Et_3N$ (3.2 ml, 22.5 mmol) 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 with10% citric acid, 5% $NaHCO_3$ and water, dried over $Na_2SO_4$ and evaporated down to afford an oily material, yield 6.9 g (80.0%), $Rf^2$ 0.74. Amino acid analysis: Lys 1.07, Gly 1.00 (average recovery 82.7%).

**Boc-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl** H-Lys(2-Adoc)-Gly-OBzl·HCl (5.6 g, 11 mmol) [prepared from Boc-Lys(2-Adoc)-Gly-OBzl (6.5 g, 11.3 mmol) and 5.8 N HCl/dioxane (19.0 ml, 110 mmol) as usual], Boc-Tyr(2-Adoc)-OH (5.1 g, 11 mmol) and HOBt (1.5 g, 11 mmol) were dissolved in DMF (100 ml) containing $Et_3N$ (2.3 ml, 16.5 mmol). DCC (2.7 g, 13.2 mmol) was added to the above solution under cooling with ice-salt and 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. The residue in $CHCl_3$ (5 ml) was applied to a silica gel column (5.0 × 31.0 cm), equilibrated and eluted with $CHCl_3$. The solvent of the eluate (1000—2000 ml) was removed by evaporation. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 5.8 g (57.7%), mp 110—112 °C, $[\alpha]_D^{25}$ −8.1° ($c$=1.0, MeOH), $Rf^1$ 0.78. *Anal.* Calcd for $C_{51}H_{68}N_4O_{11}$: C, 67.1; H, 7.51; N, 6.14. Found: C, 67.0; H, 7.75; N, 5.97. Amino acid analysis: Tyr 0.79, Lys 1.02, Gly 1.00 (average recovery 84.1%).

**Boc-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl** DCC (1.23 g, 5 mmol) was added to a solution of H-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl·HCl (3.88 g, 4.6 mmol) [prepared from Boc-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl (4.56 g, 5 mmol) and 5.8 N HCl/dioxane (8.6 ml, 50 mmol) as usual], Boc-Lys(2-Adoc)-OH (2.1 g, 5 mmol) and HOBt (0.68 g, 5 mmol) in DMF (100 ml) containing $Et_3N$ (0.84 ml, 6 mmol) under cooling with ice-salt, and 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. Ether was added to the residue to afford crystals, which were collected by filtration, yield 4.9 g (87.0%), mp 128 °C, $[\alpha]_D^{25}$ −12.2° ($c$=1.0, MeOH), $Rf^1$ 0.79. *Anal.* Calcd for $C_{68}H_{94}N_6O_{14}$: C, 67.0; H, 7.77; N, 6.89. Found: C, 67.0; H, 7.78; N, 7.11. Amino acid analysis: Tyr 0.86, Lys 2.03, Gly 1.00 (average recovery 72.8%).

**Boc-Phe-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl** DCC (0.74 g, 3.6 mmol) was added to a solution of H-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl·HCl (3.5 g, 3 mmol) [prepared from Boc-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl (4.0 g, 3.3 mmol) and 5.8 N HCl/dioxane (5.7 ml, 3.3 mmol) as usual], Boc-Phe-OH (0.8 g, 3 mmol) and HOBt (0.8 g, 3 mmol) in DMF (100 ml) containing $Et_3N$ (0.5 ml, 3.6 mmol) under cooling with ice-salt, and 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 to give crystals, which were collected by filtration and recrystallized from AcOEt, yield 2.8 g (66.7%), mp 172—174 °C, $[\alpha]_D^{25}$ −10.5° ($c$=1.0, DMF), $Rf^1$ 0.96. *Anal.* Calcd for $C_{77}H_{103}N_7O_{15} \cdot 0.5H_2O$: C, 67.2; H, 7.62; N, 7.13. Found: C, 67.3; H, 7.61; N, 7.20. Amino acid analysis: Phe 0.90, Tyr 0.92, Lys 1.96, Gly 1.00 (average recovery 84.4%).

**Boc-Val-Lys(2-Adoc)-OBzl** Boc-Val-OSu (9.4 g, 30 mmol) was added to a solution of H-Lys(2-Adoc)-OBzl·HCl (13.5 g, 30 mmol) [prepared from Boc-Lys(2-Adoc)-OBzl (17.0 g, 33 mmol) and 5.8 N HCl/dioxane (51.6 ml, 300 mmol) as usual] in DMF (200 ml) containing $Et_3N$ (5.0 ml, 36 mmol), and the reaction mixture 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 to give an oily product, yield 13.5 g (73.3%), $Rf^2$ 0.72.

**H-Val-Lys(2-Adoc)-OBzl·HCl** Boc-Val-Lys(2-Adoc)-OBzl (13.5 g, 22 mmol) was dissolved in 5.0 N HCl/dioxane (44 ml, 220 mmol) and the solution was stirred at 0 °C for 30 min. Dry dioxane (44 ml) was added and the reaction mixture was stirred at room temperature for an additional 1 h. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 11.0 g (90.9%), mp 110—112 °C, $[\alpha]_D^{25}$ +11.0° ($c$=1.0, DMF), $Rf^2$ 0.41. *Anal.* Calcd for $C_{29}H_{44}ClN_3O_5 \cdot 0.5H_2O$: C, 62.3; H, 8.11; N, 7.5. Found: C, 62.4; H, 8.20; N, 7.6.

**Boc-Thr-Val-Lys(2-Adoc)-OBzl** Boc-Thr-OSu (6.3 g, 20 mmol) was added to a solution of H-Val-Lys(2-Adoc)-OBzl·HCl (11.0 g, 20 mmol) in DMF (200 ml) containing $Et_3N$ (4.2 ml, 30 mmol). The reaction mixture 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 to give an oily product, yield 12.8 g (89.5%), $Rf^1$ 0.84.

**H-Thr-Val-Lys(2-Adoc)-OBzl·HCl** Boc-Thr-Val-Lys(2-Adoc)-OBzl (12.8 g, 18 mmol) was dissolved in 5.0 N HCl/dioxane (36 ml, 180 mmol). The reaction mixture was stirred at 0 °C for 30 min. Dry dioxane (36 ml) was added to the above solution and the reaction mixture was stirred at room temperature for an additional 1 h. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration, yield 11.0 g (93.3%), mp 107—110 °C, $[\alpha]_D^{25}$ −0.28° ($c$=1.0, DMF), $Rf^1$ 0.54. *Anal.* Calcd for $C_{33}H_{51}ClN_4O_7 \cdot 1.5H_2O$: C, 58.4; H, 8.02; N, 8.3. Found: C, 58.5; H, 7.98; N, 8.4.

**Boc-Ala-Thr-Val-Lys(2-Adoc)-OBzl** Boc-Ala-OSu (4.6 g, 16 mmol) was added to a solution of H-Thr-Val-Lys(2-Adoc)-OBzl·HCl (10.5 g, 16 mmol) in DMF (200 ml) containing $Et_3N$ (3.4 ml, 24 mmol). The reaction mixture 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 to give crystals, which were collected by filtration and recrystallized from petroleum ether, yield 11.0 g (87.5%), mp 87—89 °C, $[\alpha]_D^{25}$ −13.8° ($c$=1.0, DMF), $Rf^1$ 0.75. *Anal.* Calcd for $C_{41}H_{63}N_5O_{10}$: C, 62.7; H, 8.08; N, 8.9. Found: C, 62.4; H, 8.08; N, 8.7. Amino acid analysis: Ala 1.05, Thr 0.94, Val 1.02, Lys 1.00 (average recovery 84.8%).

**Boc-Ala-Thr-Val-Lys(2-Adoc)-OH** Boc-Ala-Thr-Val-Lys(2-Adoc)-OBzl (2.0 g, 2.64 mmol) in MeOH (100 ml) was hydrogenated over a Pd catalyst for 4 h. After removal of Pd and the solvent, ether was added to the residue to afford amorphous powder, yield 1.65 g (90.0%), mp 122 °C, $[\alpha]_D^{25}$ −5.5° ($c$=1.0, DMF), $Rf^1$ 0.21. *Anal.* Calcd for $C_{34}H_{57}N_5O_{10} \cdot 0.5H_2O$: C, 57.9; H, 8.29; N, 9.94. Found: C, 57.9; H, 8.50; N, 10.1.

**Boc-Ala-Thr-Val-Lys(2-Adoc)-Phe-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl** DCC (0.45 g, 2.20 mmol) was added to a solution of H-Phe-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl·HCl [prepared from Boc-Phe-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl (2.5 g, 1.83 mmol) and 7.2 N HCl/dioxane 2.54 ml, 18.3 mmol) as usual], Boc-Ala-Thr-Val-Lys(2-Adoc)-OH (1.27 g, 1.83 mmol), and HOBt (0.25 g, 1.83 mmol) in DMF (200 ml) containing $Et_3N$ (0.26 ml, 1.83 mmol) under cooling with ice-salt. The reaction mixture was stirred at 4 °C overnight. After removal of the DCC urea and the solvent, water was added to the residue to afford crystals, which were collected by

filtration and recrystallized from MeOH, yield 3.31 g (92.2%), mp 260—263 °C, $[\alpha]_D^{25}$ −12.9° ($c$=1.0, DMF), $Rf^1$ 0.82, $Rf^2$ 0.91. *Anal.* Calcd for $C_{106}H_{150}N_{11}O_{22}$: C, 65.5; H, 7.78; N, 8.64. Found: C, 65.4; H, 7.63; N, 8.62. Amino acid analysis: Ala 0.93, Thr 0.88, Val 1.04, Lys 3.00, Phe 0.88, Tyr 0.91, Gly 1.09 (average recovery 84.5%).

**Boc-Ala-Thr-Val-Lys(2-Adoc)-Phe-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OH** Boc-Ala-Thr-Val-Lys(2-Adoc)-Phe-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl (3.0 g, 1.54 mmol) in DMF (200 ml) was hydrogenated over a Pd catalyst for 3 h. After removal of Pd and the solvent, petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from MeOH, yield 2.45 g (85.7%), mp 210—213 °C, $[\alpha]_D^{25}$ −12.6° ($c$=1.0, DMF), $Rf^1$ 0.35, $Rf^2$ 0.67. *Anal.* Calcd for $C_{99}H_{144}N_{12}O_{22}\cdot 2H_2O$: C, 62.9; H, 7.89; N, 8.89. Found: C, 62.7; H, 7.96; N, 8.91.

**Boc-Glu(O-cHex)-Gly-OBzl** DCC (14.8 g, 71.9 mmol) was added to a solution of H-Gly-OBzl·TosOH (20.2 g, 59.9 mmol), Boc-Glu(O-cHex)-OH (19.7 g, 59.9 mmol), and HOBt (8.1 g, 59.9 mmol) in DMF (200 ml) containing $Et_3N$ (8.4 ml, 59.9 mmol) 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. The residue in $CHCl_3$ (10 ml) was applied to a silica gel column (3.5×43 cm), equilibrated and eluted with $CHCl_3$. The eluate containing the desired compound (1000—1800 ml) was collected and the solvent was removed by evaporation. Petroleum ether was added to the residue to afford crystals, which were collected by filtration, yield 19.1 g (66.9%), mp 60—62 °C, $[\alpha]_D^{25}$ −6.4° ($c$=1.0, DMF), $Rf^1$ 0.74. *Anal.* Calcd for $C_{26}H_{36}N_2O_7$: C, 63.0; H, 7.61; N, 5.88. Found: C, 63.0; H, 7.72; N, 5.97. Amino acid analysis: Glu 1.02, Gly 1.00 (average recovery 82.5%).

**Boc-Asp(O-2-Ada)-Glu(O-cHex)-Gly-OBzl** DCC (7.43 g, 36 mmol) was added to a solution of H-Glu(O-cHex)-Gly-OBzl·HCl [prepared from Boc-Glu(O-cHex)-Gly-OBzl (14.3 g, 30 mmol) and 7.2 N HCl/dioxane (41.7 ml, 300 mmol) as usual], Boc-Asp(O-2-Ada)-OH (11.0 g, 30 mmol) and HOBt (4.1 g, 30 mmol) in DMF (200 ml) containing $Et_3N$ (4.2 ml, 30 mmol) 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. The residue in $CHCl_3$ (10 ml) was applied to a silica gel column (3.5×43 cm), equilibrated and eluted with $CHCl_3$. The eluate containing the desired compound (1200—2000 ml) was collected and the solvent was removed by evaporation to give an oily product. $Rf^2$ 0.83.

**H-Asp(O-2-Ada)-Glu(O-cHex)-Gly-OBzl·HCl** This oily residue was dissolved in 7.2 N HCl/dioxane (41.7 ml, 300 mmol) and the reaction mixture was stirred at 0 °C for 30 min. Dry dioxane (42 ml) was added to the above solution and the reaction mixture was stirred at room temperature for an additional 1 h. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration, total yield 14.7 g (74.0%), mp 90—92 °C, $[\alpha]_D^{25}$ +2.3° ($c$=1.0, DMF), $Rf^1$ 0.44. *Anal.* Calcd for $C_{34}H_{48}ClN_3O_8\cdot 0.5H_2O$: C, 60.8; H, 7.36; N, 6.26. Found: C, 61.0; H, 7.36; N, 6.43. Amino acid analysis: Asp 1.03, Glu 1.04, Gly 1.00 (average recovery 89.1%).

**Boc-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-OBzl** DCC (3.7 g, 18.1 mmol) was added to a solution of H-Asp(O-2-Ada)-Glu(O-cHex)-Gly-OBzl·HCl (10.0 g, 15.1 mmol), Boc-Tyr(2-Adoc)-OH (6.9 g, 15.1 mmol) and HOBt (2.0 g, 15.1 mmol) in DMF (100 ml) containing $Et_3N$ (2.1 ml, 15.1 ml) under cooling with ice-salt, and 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 and recrystallized from MeOH, yield 12.5 g (77.5%), mp 107—108 °C, $[\alpha]_D^{25}$ −9.5° ($c$=1.0, DMF), $Rf^1$ 0.64. *Anal.* Calcd for $C_{59}H_{78}N_4O_{14}$: C, 66.4; H, 7.37; N, 5.25. Found: C, 66.2; H, 7.54; N, 5.51. Amino acid analysis: Tyr 0.89, Asp 1.10, Glu 1.11, Gly 1.00 (average recovery 96.7%).

**Boc-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-OH** Boc-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-OBzl (2.5 g, 2.3 mmol) in MeOH (100 ml) was hydrogenated over a Pd catalyst for 2 h. After removal of Pd and the solvent, ether was added to the residue to afford an amorphous powder, yield 2.0 g (89.0%), $[\alpha]_D^{25}$ −12.5° ($c$=1.0, DMF), $Rf^1$ 0.35. *Anal.* Calcd for $C_{52}H_{72}N_4O_{14}$: C, 63.9; H, 7.43; N, 5.73. Found: C, 64.1; H, 7.28; N, 5.78.

**H-Phe-Lys-Tyr-Lys-Gly-OH** Boc-Phe-Lys(2-Adoc)-Tyr(2-Adoc)-Lys(2-Adoc)-Gly-OBzl (200 mg, 0.146 mmol) was dissolved in HF (2 ml) containing *m*-cresol (0.5 ml), thioanisole (0.25 ml), ethanedithiol (0.25 ml) and dimethyl sulfide (7 ml) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h. After removal of HF and dimethyl sulfide, the residue was dissolved in HF (9 ml) at 0 °C. The solution was stirred at the same temperature for 1 h. After removal of HF, dry ether was added to the residue to afford a precipitate, which was collected by filtration and washed with ether. The HPLC profile of this crude product is shown in Fig. 2. The crude product was purified by preparative HPLC. The purified peptide exhibited a single peak on analytical HPLC, $t_R$ 9.57 min, >99.9% [column, Vydac C18 (4.6×250 mm); eluent, 0.1% TFA in $H_2O$ containing 5% MeCN (A) and 0.1 %TFA in MeCN containing 20% $H_2O$ (B); A:B from 95:5 to 75:25 in 20 min; flow rates, 1 ml/min; detection, 215 nm]. Lyophilization afforded a fluffy powder, 80.6 mg (86%), $C_{32}H_{47}N_7O_7$ (M.W.: 641.77), MS $m/z$: 642.3 $[M+H]^+$, 640.3 $[M-H]^-$. Amino acid analysis: Phe 1.10, Lys 2.03, Tyr 0.98, Gly 1.00 (average recovery 87%).

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**References and Notes**

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2) The customary L-configuration for amino acid residues is omitted. Abbreviations used in this report for amino acids, peptides and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485—2489 (1966); **6**, 362—364 (1966); **11**, 1726—1732 (1972). The following additional abbreviations are used: AcOEt, ethyl acetate; DMF, dimethylformamide; TFA, trifluoroacetic acid; AcOH, acetic acid; Boc, *tert*-butyloxycarbonyl; OSu, *N*-succinimidyl ester; O-2-Ada, 2-adamantyl ester; 2-Adoc, 2-adamantyloxycarbonyl; O-cHex, cyclohexyl ester; DCC, *N,N'*-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; TFMSA, trifluoromethanesulfonic acid; MSA, methanesulfonic acid, TEA, triethylamine; $(Boc)_2O$, di-*tert*-butyldicarbonate; EDTA, ethylenediaminetetraacetic acid disodium salt dihydrate.
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