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

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Segment condensations were performed to construct peptide fragments related to *Sulfolobus solifataricus* Ribonuclease. At each condensation step, the new protecting groups were stable. The protected peptide fragments were treated with a low-high HF procedure to give the desired peptide fragments. These peptide fragments were also prepared by the solid-phase method, and the obtained peptides were compared with those obtained by the solution method. The peptide fragments obtained by the solution method were identical with those obtained by the solid-phase method on analytical HPLC, indicating that the new protecting groups could be easily removed by HF, and no racemization occurred during the synthesis of the protected peptides.

Key words 2-adamantyl ester; 2-adamantyloxycarbonyl; peptide fragment; *Sulfolobus solifataricus* Ribonuclease; RNase activity; DNA-binding activity

From the thermoacidophilic archaebacterium, *Sulfolobus solifataricus*, three proteins with ribonuclease (RNase) activity, referred to as P1, P2 and P3, were isolated.[3)] The complete amino acid sequence of P2 displayed high sequence similarity to the 7-kDa DNA-binding proteins. Additionally, protein P2 and P3 demonstrated non-specific DNA-binding.[3)] Protein P2 consists of 62 amino acid residues and, interestingly, it has no His residue, which is essential for catalysis in bovine pancreatic RNase A. Some questions have arisen from the identification of these proteins: how they cleave their substrates in contrast to the RNases with His residue, and which parts in their sequences are involved in RNase activity. These questions have motivated us to investigate the structure–activity relationship of P2.

The synthesis of eight protected peptide segments as building blocks for peptide fragments (I—VIII) related to *Sulfolobus solifataricus* Ribonuclease[3)] (Fig. 1) was reported in the preceding paper.[1)]

This paper deals with the segment condensation of peptide fragments containing protecting groups derived from adamantane, the final deprotection of the protected peptide fragments, and examination of RNase activity and DNA binding activity.

As illustrated in Fig. 2, Boc-(52—62)-OBzl, Boc-(47—62)-OBzl, Boc-(44—62)-OBzl, Boc-(37—62)-OBzl, Boc-(33—62)-OBzl, Boc-(27—62)-OBzl and Boc-(17—62)-OBzl were prepared by the segment condensation method, starting from the C-terminal hexapeptide ester, H-(57—62)-OBzl. Amino acid derivatives bearing protecting groups removable by treatment with HF at 0 °C for 60 min,[4)] *i.e.*, Asp(O-2-Ada),[5,6)] Glu(O-cHex),[7)] Lys(2-Adoc),[8,9)] Tyr(O-2-Adoc),[10)] Trp(Mts),[11)] Arg(Mts),[12)] were employed in combination with the TFA labile Boc-group as the *N*-protecting group. The protected peptides were deblocked by the low-high HF method.[13)] The deprotected crude peptide was treated with Amberlite IRA 45 and then purified with Sephadex G-15 or Sephadex G-25, and finally with HPLC. The purified I—VIII exhibited a single peak on analytical HPLC. The results of amino acid analysis and mass spectrometry are summarized in Table 1.

In order to further confirm the usefulness of the protecting groups developed by us, peptides I—VIII obtained above were compared with peptides I—VIII synthesized by the usual solid-phase method using an automatic peptide synthesizer (Vega peptide synthesizer). Starting from Boc-Lys(2-Cl-Z)-Pam resin, N^{α}-Boc-amino acid was coupled successively by the DCC–HOBt method to give Boc-(57—62)-Pam resin, Boc-(52—62)-Pam resin, Boc-(47—62)-Pam resin, Boc-(44—62)-Pam resin, Boc-(37—62)-Pam resin, Boc-(33—62)-Pam resin, Boc-(27—62)-Pam resin, Boc-(17—62)-Pam resin according to the protocol shown in Table 2. It is well known that stepwise elongation from the COOH-terminal, by one amino acid at a time using urethane-protected amino acids such as *tert*-butyloxycarbonyl amino acid, is advantageous for the avoidance of racemization during the peptide bond-forming reaction.[14—16)] In the maximum protection strategy, the maximum number of functional groups is masked in order to avoid side reactions.[17)] Therefore, in this solid-phase synthesis, racemization-free and pure peptides will be obtained.

Each protected peptide resin was treated with anhydrous HF (low-high method) to give compounds (I—VIII). Each crude peptide was purified by preparative HPLC. The puri-

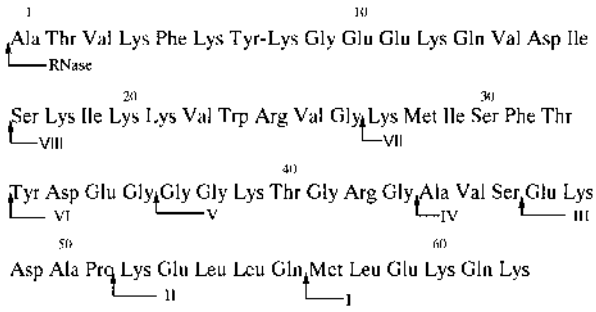

Fig. 1. Amino Acid Sequence of *Sulfolobus solifataricus* Ribonuclease and Related Peptide Fragments (I—VIII)

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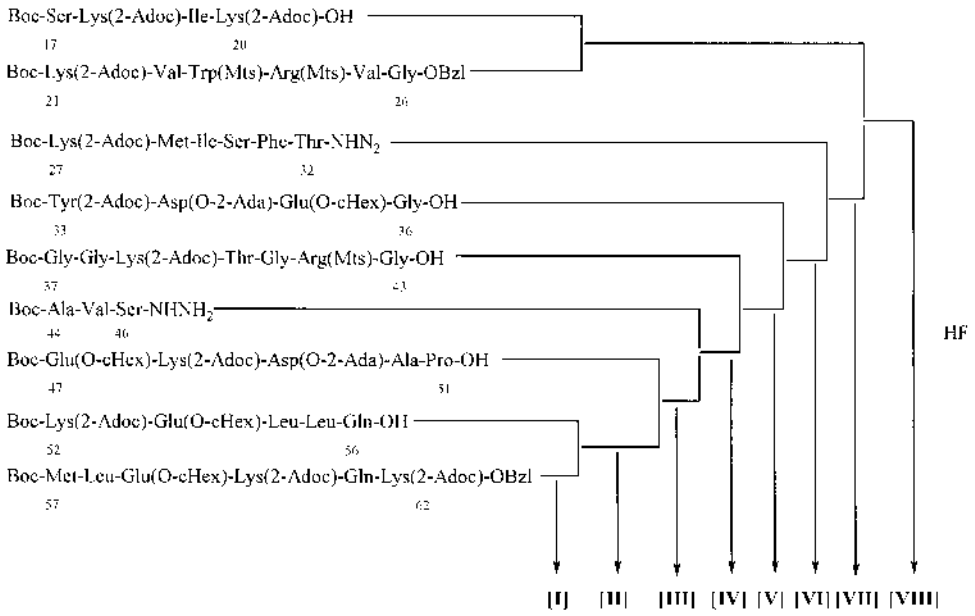

Fig. 2. Synthetic Scheme for Compounds I—VIII

Table 1. Amino Acid Composition and Mass Spectral Data of Peptides I—VIII

Amino acid	H-(57—62)-OH I	H-(52—62)-OH II	H-(47—62)-OH III	H-(44—62)-OH IV	H-(37—62)-OH V	H-(33—62)-OH VI	H-(27—62)-OH VII	H-(17—62)-OH VIII
Asp	—	—	1.01 (1)	1.03 (1)	0.99 (1)	1.92 (2)	1.99 (2)	2.08 (2)
Thr	—	—	—	—	0.96 (1)	0.91 (1)	1.82 (2)	1.95 (2)
Ser	—	—	—	0.62 (1)	0.60 (1)	0.86 (1)	1.28 (2)	2.58 (3)
Glx	2.00 (2)	4.30 (4)	4.66 (5)	4.75 (5)	4.71 (5)	6.13 (6)	5.67 (6)	6.25 (6)
Gly	—	—	—	—	3.75 (4)	5.41 (5)	4.97 (5)	6.80 (6)
Ala	—	—	—	2.45 (2)	1.64 (2)	1.84 (2)	1.58 (2)	1.43 (2)
Val	—	—	1.25 (1)	1.04 (1)	1.02 (1)	0.91 (1)	1.03 (1)	2.23 (3)
Met	0.77 (1)	0.86 (1)	0.75 (1)	0.68 (1)	0.72 (1)	0.96 (1)	1.13 (2)	1.86 (2)
Ile	—	—	—	—	—	—	0.93 (1)	1.76 (2)
Leu	1.00 (1)	3.00 (3)	3.00 (3)	3.00 (3)	3.00 (3)	3.00 (3)	3.00 (3)	3.00 (3)
Tyr	—	—	—	—	—	0.94 (1)	0.68 (1)	1.10 (1)
Phe	—	—	—	—	—	—	0.91 (1)	1.03 (1)
Lys	1.84 (2)	2.93 (3)	3.95 (4)	4.00 (4)	4.98 (5)	5.66 (5)	5.95 (6)	10.1 (9)
Arg	—	—	—	—	0.92 (1)	1.04 (1)	0.97 (1)	2.03 (2)
Pro	—	—	1.02 (1)	1.10 (1)	1.33 (1)	1.44 (1)	1.10 (1)	1.20 (1)
Trp	—	—	—	—	—	—	—	N.D. (1)
MS (*m/z*)	FAB-MS: 776 (M^++1)	FAB-MS: 1387 (M^++1)	FAB-MS: 1928.6 (M^++1)	FAB-MS: 2186.5 (M^++1)	FAB-MS: 2799.6 (M^++1)	FAB-MS: 3263.8 (M^++1)	TOF-MS: 3972.4 (M^++1)	TOF-MS: 5154.6 (M^++1)
[Calcd]	[776]	[1387]	[1928.0]	[2186.1]	[2799.5]	[3263.6]	[3971.0]	[5153.7]

Numbers in parentheses are theoretical values. N.D.=not determined.

fied compounds (I—VIII) exhibited a single peak on analytical HPLC, and each fragment was co-injected with the fragment prepared by the solution method to exhibit a single peak on analytical HPLC. Two examples are shown in Fig. 3A and Fig. 3B. These results showed that the application of Lys(2-Adoc), Tyr(2-Adoc) and Asp(O-2-Ada) to the synthesis of large peptides in the solution method was successful.

Finally, the RNase activity and DNA binding activity of compounds I—VIII were examined. Compounds I—VIII exhibited neither RNase activity nor DNA binding activity. Presumably, the N-terminal part of *Sulfolobus solifataricus* Ribonuclease is very important for the manifestation of RNase activity or DNA binding activity.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co., Ltd.). Amino acid analyses were performed on an amino acid analyzer, K-202 SN (Kyowa Seimitsu Co., Ltd.). The peptides were hydrolyzed in 6 N HCl (Nakarai tesque, Ltd.) at 110 °C for 18 h. Fast-atom bombardment mass spectra (FAB-MS) and Malditof-mass spectra (TOF-MS) were obtained on a JEOL SX-102 mass spectrometer (Japan Spectroscopic Co., Ltd.) and a Kratos MALDI II mass spectrometer (Kratos Analytical), respectively. Analytical HPLC was carried out on a Waters 600 System Controller (Waters, Ltd.) equipped with a Waters 481 LC spectrometer (Waters, Ltd.) on a Vydac 218TP54 column (4.6×250 mm); flow rate, 1 ml/min; detection, 220 nm. 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-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl, Boc-SSR(52—62)-OBzl To a solution of H-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·TFA

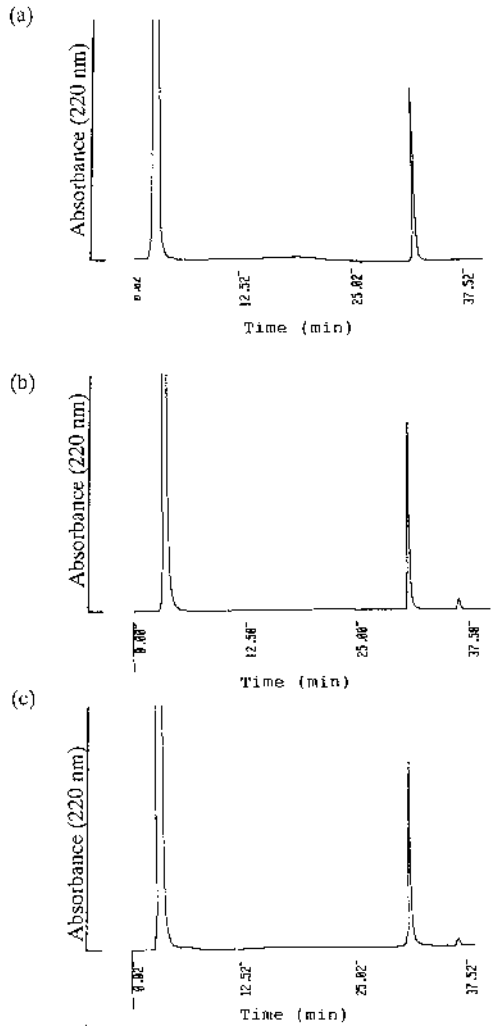

Fig. 3A. Analytical HPLC Profile of H-(27—62)-OH

(a) H-(27—62)-OH synthesized by the solution-phase method; (b) H-(27—62)-OH synthesized by the solid-phase method; (c) co-injection of a and b. Conditions: column, Vydac 218TP54 (4.6×250 mm); solvent, A=H_2O (0.05% TFA), B=MeCN (0.05% TFA); gradient, (A : B) 90 : 10 to 50 : 50 in 40 min; flow rate, 1.0 ml/min; detection, 220 nm.

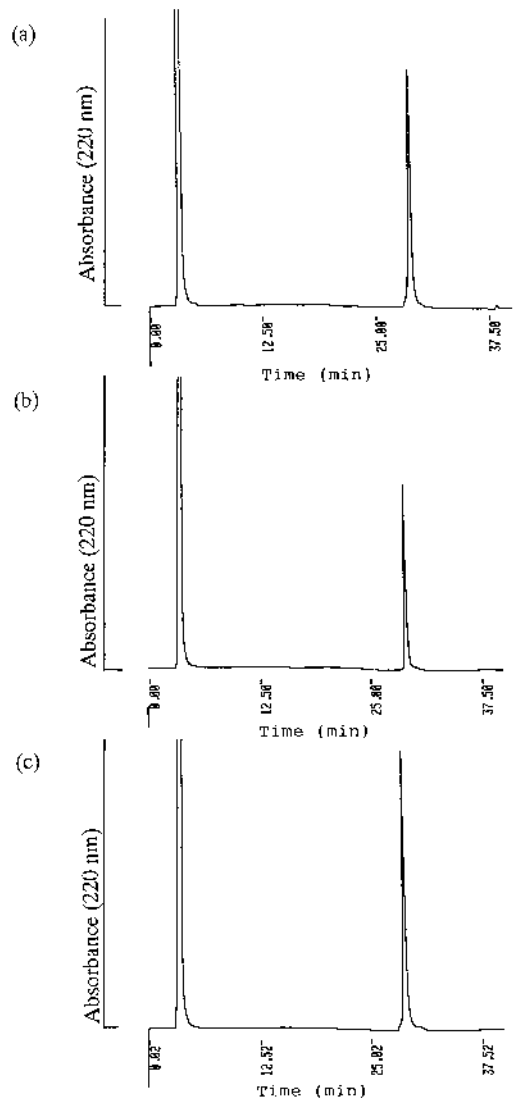

Fig. 3B. Analytical HPLC Profile of H-(37—62)-OH

(a) H-(37—62)-OH synthesized by the solution-phase method; (b) H-(37—62)-OH synthesized by the solid-phase method; (c) co-injection of a and b. Conditions: column, Vydac 218TP54 (4.6×250 mm); solvent, A=H_2O (0.05% TFA), B=MeCN (0.05% TFA); gradient, (A : B) 90 : 10 to 50 : 50 in 40 min; flow rate, 1.0 ml/min; detection, 220 nm.

[prepared from Boc-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (5.00 g, 3.56 mmol) and anisole (1.15 ml, 10.7 mmol) and TFA (2.74 ml, 35.6 mmol) as usual], Boc-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-OH (3.52 g, 3.56 mmol) and HOBt (0.48 g, 3.56 mmol) in DMF (150 ml), containing Et_3N (0.50 ml, 3.56 mmol), DCC (0.88 g, 4.27 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 7.59 g (93.5%), mp 170—171 °C, $[\alpha]_D^{25}$ −21.6° (c=1.0, DMF). *Anal*. Calcd for $C_{118}H_{186}N_{16}O_{26}S$: C, 62.3; H, 8.23; N, 9.84. Found: C, 62.0; H, 8.09; N, 9.75. Amino acid analysis: Lys 2.85, Glu 3.95, Leu 3.00, Met 0.73, (average recovery 93.1%).

Boc-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl, Boc-SSR(47—62)-OBzl A reaction mixture of H-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·TFA [prepared from Boc-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (5.00 g, 2.20 mmol) and anisole (0.71 ml, 6.59 mmol) and TFA (1.69 ml, 22.0 mmol) as usual], Boc-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-OH (2.31 g, 2.20 mmol) HOBt (0.45 g, 3.29 mmol) and benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)-reagent (1.46 g, 3.29 mmol) in DMF (300 ml) containing Et_3N (0.31 ml, 2.20 mmol) was stirred at 4 °C overnight. After removal of the solvent, water was added to the residue to afford crystals which were collected by

Table 2. The Protocol of Synthesis of Peptides I—VIII

Step	Operation	Time
1	Wash with DCM	3 min×1
2	Cleavage with 50% TFA in DCM	3 min×1
3	Cleavage with 50% TFA in DCM	20 min×1
4	Wash with DCM	3 min×2
5	Wash with isopropanol	3 min×1
6	Neutralization with 5% DIEA in DCM	4 min×2
7	Wash with DCM	3 min×1
8	Wash with methanol	3 min×2
9	Wash with DCM	3 min×2
10	Coupling by DCC-HOBt in DCM/DMF	2 to 72 h
11	Wash with DMF	3 min×1
12	Wash with methanol	3 min×2
13	Wash with DCM	3 min×2
14	Kaiser Test	
15	Recoupling	
16	Acetylation with acetic anhydride-DIEA*	30 min×1

* DIEA, *N*,*N*-Diisopropylethylamine.

filtration and recrystallized from MeOH. Yield 5.61 g (79.5%), mp 262—264 °C, $[\alpha]_D^{25}$ −11.2° (c=1.0, DMF). *Anal.* Calcd for $C_{168}H_{260}N_{22}O_{37}S$: C, 62.8; H, 8.16; N, 9.56. Found: C, 62.6; H, 8.14; N, 9.43. Amino acid analysis: Glu 5.10, Lys 3.84, Asp 1.14, Ala 1.22, Pro 1.03, Leu 3.00, Met 0.75 (average recovery 98.9%).

Boc-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl, Boc-SSR(44—62)-OBzl To a cold solution of H-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·TFA [prepared from Boc-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu-(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (5.00 g, 1.56 mmol), anisole (0.50 ml, 4.67 mmol) and TFA (1.20 ml, 15.6 mmol) as usual] in DMF (150 ml) containing Et_3N (0.22 ml, 1.56 mmol), Boc-Ala-Val-Ser-N_3 [prepared from Boc-Ala-Val-Ser-$NHNH_2$ (1.82 g, 4.68 mmol), 5.4 N HCl/dioxane (2.67 ml, 14.0 mmol) and isopentyl nitrite (0.63 ml, 4.68 mmol) as usual] in DMF (20 ml) was added under cooling in an ice bath. The reaction mixture was stirred at 0 °C for 2 h and at 4 °C for 3 d. After removal of the solvent, water was added to the residue to afford crystals which were then collected by filtration and recrystallized from MeOH. Yield 4.61 g (85.3%), mp 240—242 °C, $[\alpha]_D^{25}$ −9.5° (c=1.0, DMF). *Anal.* Calcd for $C_{179}H_{279}N_{25}O_{41}S$: C, 61.3; H, 8.14; N, 9.99. Found: C, 61.4; H, 8.05; N, 9.81. Amino acid analysis: Ala 2.07, Val 1.10, Ser 0.74, Glu 4.88, Lys 3.86, Asp 0.97, Pro 0.85, Leu 3.00, Met 0.77 (average recovery 99.9%).

Boc-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl, Boc-SSR(37—62)-OBzl A reaction mixture of H-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·TFA [prepared from Boc-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp-(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu-(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (4.00 g, 1.55 mmol), anisole (0.37 ml, 3.46 mmol) and TFA (4.44 ml, 57.7 mmol) as usual], Boc-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-OH (1.89 g, 1.73 mmol), HOBt (0.35 g, 2.60 mmol) and BOP-reagent (1.15 g, 2.60 mmol) in DMF (300 ml) containing Et_3N (0.16 ml, 1.15 mmol) was stirred at 4 °C overnight. After removal of the solvent, water was added to the residue to afford crystals which were then collected by filtration and recrystallized from MeOH. Yield 4.49 g (87.8%), mp 230—234 °C, $[\alpha]_D^{25}$ −6.5° (c=1.0, DMF). *Anal.* Calcd for $C_{223}H_{346}N_{36}O_{53}S_2 \cdot 4H_2O$: C, 59.3; H, 7.90; N, 11.2. Found: C, 59.4; H, 7.90; N, 11.0. Amino acid analysis: Gly 3.86, Lys 4.95, Thr 0.94, Arg 1.22, Ala 1.88, Val 0.88, Ser 0.81, Glu 4.81, Asp 0.85, Pro 1.01, Leu 3.00, Met 0.77 (average recovery 98.9%).

Boc-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl, Boc-SSR(33—62)-OBzl A reaction mixture of H-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg-(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·TFA [prepared from Boc-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (4.00 g, 0.90 mmol), anisole (0.29 ml, 2.70 mmol) and TFA (3.47 ml, 45.0 mmol) as usual], Boc-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-OH (1.32 g, 1.35 mmol), HOBt (0.27 g, 2.03 mmol) and BOP-reagent (0.90 g, 2.03 mmol) in DMF (300 ml) containing Et_3N (0.13 ml, 0.90 mmol) was stirred at 4 °C overnight. After removal of the solvent, water was added to the residue to afford crystals which were then collected by filtration and recrystallized from MeOH. Yield 3.74 g (78.9%), mp 229—234 °C, $[\alpha]_D^{25}$ −8.4° (c=1.0, DMF). *Anal.* Calcd for $C_{270}H_{408}N_{40}O_{64}S_2 \cdot 5H_2O$: C, 60.1; H, 7.81; N, 10.4. Found: C, 60.0; H, 7.68; N, 10.3. Amino acid analysis: Tyr 1.09, Asp 2.18, Glu 6.07, Gly 5.18, Lys 4.90, Thr 1.05, Arg 0.96, Ala 1.88, Val 0.99, Ser 0.83, Pro 1.09, Leu 3.00, Met 0.76 (average recovery 87.3%).

Boc-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl, Boc-SSR(27—62)-OBzl To a solution of H-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·TFA [prepared from Boc-Tyr(2-Adoc)-Asp-(O-2-Ada)-Glu(O-cHex)-Gly-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (3.00 g, 0.57 mmol), anisole (0.18 ml, 1.71 mmol) and TFA (2.18 ml, 28.3 mmol) as usual], in DMF (150 ml) containing Et_3N (0.08 ml, 0.57 mmol), Boc-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-N_3 [prepared from Boc-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-$NHNH_2$ (1.74 g, 1.71 mmol), 5.4 N HCl/dioxane (1.01 ml, 5.13 mmol) and isopentyl nitrite (0.23 ml, 1.71 mmol) as usual] in DMF (20 ml) was added under cooling in an ice bath. The reaction mixture was stirred at 0 °C for 2 h and at 4 °C for 3 d. After removal of the solvent, water was added to the residue to afford crystals which were then collected by filtration and recrystallized from MeOH. Yield 2.93 g (82.5%), mp 265—272 °C, $[\alpha]_D^{25}$ −23.6° (c=0.25, hexamethylphosphoramide (HMPA)]. *Anal.* Calcd for $C_{314}H_{475}N_{47}O_{74}S_3 \cdot 8H_2O$: C, 59.6; H, 7.81; N, 10.4. Found: C, 59.5; H, 7.78; N, 10.4. Amino acid analysis: Lys 5.85, Met 1.65, Ile 1.04, Ser 1.86, Phe 0.95, Thr 2.06, Tyr 0.85, Asp 2.24, Glu 6.31, Gly 5.27, Arg 1.02, Ala 1.82, Val 0.87, Pro 1.00, Leu 3.00, (average recovery 90.7%).

Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Adoc)-Lys(2-Adoc)-Val-Trp(Mts)-Arg(Mts)-Val-Gly-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl, Boc-SSR(17—62)-OBzl A reaction mixture of H-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-Tyr(2-Adoc)-Asp-(O-2-Ada)-Glu(O-cHex)-Gly-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl·TFA [prepared from Boc-Lys(2-Adoc)-Met-Ile-Ser-Phe-Thr-Tyr(2-Adoc)-Asp(O-2-Ada)-Glu(O-cHex)-Gly-Gly-Gly-Lys(2-Adoc)-Thr-Gly-Arg(Mts)-Gly-Ala-Val-Ser-Glu(O-cHex)-Lys(2-Adoc)-Asp(O-2-Ada)-Ala-Pro-Lys(2-Adoc)-Glu(O-cHex)-Leu-Leu-Gln-Met-Leu-Glu(O-cHex)-Lys(2-Adoc)-Gln-Lys(2-Adoc)-OBzl (1.00 g, 0.16 mmol), anisole (0.05 ml, 0.48 mmol) and TFA (1.25 ml, 16.2 mmol) as usual], Boc-Ser-Lys(2-Adoc)-Ile-Lys(2-Ado)-Lys(2-Adoc)-Val-Trp(Mts)-Arg(Mts)-Val-Gly-OH (0.71 g, 0.32 mmol), HOBt (0.07 g, 0.48 mmol) and BOP-reagent (0.21 g, 0.48 mmol) in HMPA (150 ml) and DMF (50 ml) containing Et_3N (0.05 ml, 0.32 mmol), was stirred at 4 °C overnight. After the removal of DMF, water was added to the residue to afford crystals which were then collected by filtration and recrystallized from MeOH, yield 1.10 g (81.3%), mp 288—294 °C (dec.), $[\alpha]_D^{25}$ −16.8° (c=0.1, HMPA). *Anal.* Calcd for $C_{421}H_{632}N_{64}O_{95}S_5 \cdot 10H_2O$: C, 59.8; H, 7.78; N, 10.6. Found: C, 59.7; H, 7.62; N, 10.3. Amino acid analysis: Ser 2.45, Lys 9.16, Ile 1.94, Val 2.84, Arg 1.92, Gly 5.81, Met 1.45, Phe 0.96, Thr 1.76, Tyr 0.86, Asp 2.02, Glu 5.88, Ala 1.85, Pro 0.89, Leu 3.00, (average recovery 97.2%).

General Procedure for Deprotection by HF The protected peptide (0.15 mmol) was treated with HF (2 ml) containing *m*-cresol (0.5 ml), thioanisole (0.25 ml), ethanedithiol (0.25 ml) and dimethyl sulfide (7 ml) at −4 °C for 60 min. After the removal of HF and dimethyl sulfide, HF (9 ml) was added to the residue and the reaction mixture was stirred at −4 °C for 60 min. After the removal of HF, dry ether was added to the residue to afford a white powder which was collected by filtration. A solution of the crude peptide in water was treated with Amberlite IRA 45. After removal of the resin, the filtrate was lyophilized to give a white crude peptide. This crude peptide in water (4 ml) was applied to a Sephadex G-15 column (2×70 cm), which is equilibrated and eluted with water. The obtained peptide was further purified by Sephadex G-25 column chromatography. The peptide was further purified by preparative HPLC. Preparative HPLC Conditions: column, YMC Pack R & DR ODS-5-A (20×250 mm); solvents, A : B 80 : 20 for 5 min to 60 : 40 in 20 min, 60 : 40 for 5 min to 80 : 20 in 5 min; flow rate, 10 ml/min; detection, 220 nm. Results of amino acid analysis and mass spectrometry are summarized in Table 1.

Synthesis of Compounds (I—VIII) by the Solid-Phase Method The side-chain protecting groups were Bzl for Ser and Thr, O-cHex for Asp and Glu, 2-Cl-Z for Lys, 2-Br-Z for Tyr, Tos for Arg, formyl for Trp, Xan for Gln, sulfoxide for Met except for the synthesis of I and II, in which cases Met instead of methionine sulfoxide was used. Starting from Boc-Lys(2-ClZ)-Pam resin, Boc-amino acids were coupled successively by the DCC-HOBt method to give Boc-Ser(Bzl)-Lys(2-Cl-Z)-Ile-Lys(2-Cl-Z)-Lys(2-Cl-Z)-Val-Trp(formyl)-Arg(Tos)-Val-Gly-Lys(2-Cl-Z)-Met(O)-Ile-Ser(Bzl)-Phe-Thr(Bzl)-Tyr(2-Br-Z)-Asp-(O-cHex)-Glu(O-cHex)-Gly-Gly-Gly-Lys(2-Cl-Z)-Thr(Bzl)-Gly-Arg(Tos)-Gly-Ala-Val-Ser(Bzl)-Glu(O-cHex)-Lys(2-Cl-

Z)-Asp(O-cHex)-Ala-Pro-Lys(2-Cl-Z)-Glu(O-cHex)-Leu-Leu-Gln(Xan)-Met(O)-Leu-Glu(O-cHex)-Lys(2-Cl-Z)-Gln(Xan)-Lys(2-Cl-Z)-Pam resin, Boc-(17—62)-Pam resin. By the same procedure, Boc-(47—62)-Pam resin, Boc-(44—62)-Pam resin, Boc-(37—62)-Pam resin, Boc-(33—62)-Pam resin, and Boc-(27—62)-Pam resin were prepared. Boc-Lys(2-Cl-Z)-Glu(O-cHex)-Leu-Leu-Gln(Xan)-Met-Leu-Glu(O-cHex)-Lys(2-Cl-Z)-Gln(Xan)-Lys(2-Cl-Z)-Pam resin, Boc-(51—62)-Pam resin, and Boc-Met-Leu-Glu(O-cHex)-Lys(2-Cl-Z)-Gln(Xan)-Lys(2-Cl-Z)-Pam resin, Boc-(57—62)-Pam resin were also prepared.

2.0 g of each peptide resin, 0.8 g of *p*-thiocresol, 3.2 g of *p*-cresol and 26 ml of dimethylsulfide (DMS) were placed in a reactor made of polystyrene. The reactor was then chilled with acetone/dry ice, and 10 ml of anhydrous HF was condensed into the reactor. The acetone/dry ice bath was removed and substituted with an ice bath to keep the reaction temperature between 0—5 °C during stirring for 2 h. After the removal of DMS and HF, 40 ml of HF was again condensed into the reactor. The reaction mixture was stirred at 0—5 °C for 1 h. After the removal of HF, dry ether was added to the residue to give a precipitate which was then collected by filtration and washed with ether. The precipitate was extracted with 5% acetic acid. After removal of resin by filtration, the filtrate was lyophilized.

The crude peptide was purified by RP-HPLC, first by triethylammonium phosphate (TEAP system) to an acetonitrile linear gradient and then by TFA/water to an acetonitrile linear gradient.

Fractions were checked in the following systems: TEAP system: column, Water Delta PAK 5 μ C18 300A (3.9×150 mm); solvents: solvent A, HPLC water (950 ml); TEA (6 ml); H_3PO_4 (85% solution, 5.7 ml); HPLC acetonitrile (50 ml); solvent B, solvent A (200 ml), HPLC acetonitrile (800 ml), A/B from 85/15 to 60/40 in 20 min. TFA system: column, Waters Delta PAK 5 μ C18 300A (3.9×150 mm); solvent, solvent A, HPLC water (1000 ml), TFA (1 ml), solvent B, HPLC water (200 ml); HPLC acetonitrile (800 ml); TFA (1 ml). A/B from 80/20 to 55/45 in 20 min.

The purified I—VIII exhibited single peaks on analytical HPLC at the same retention time as those prepared by the solution method. Amino acid ratios in acid hydrolysates were in good agreement with the theoretically expected values, as shown in Table 1.

Assay for RNase Activity The RNase activity of peptides (I—VIII) was determined according to the procedure described by Fusi *et al*.,[3] with minor modification. The solution, which consisited of 0.2 mg/ml of peptide (I—VIII) and 1.6 mg/ml of RNA in 40 mM sodium phosphate buffer, pH 7.8, in a final volume of 0.25 ml, was incubated for 10 min at 60 °C. The reaction was stopped by the addition of 0.25 ml of 2 mM lantanum nitrate/15% $HClO_4$. After standing on ice for 20 min, the reaction mixture was centrifuged for 5 min at 3000 rpm, then a 300 μl aliquot of the supernatant was withdrawn and diluted to 2 ml with water. The absorbancy of the solution was measured at 260 nm.

Assay for DNA-Binding Activity The DNA-binding activity of peptide (I—VIII) was determined on 1% agarose gels.[18] Peptide (I—VIII, 16 μg) was incubated with pU-RCh2—4 plasmid DNA (48 μg) at 50 °C for 30 min. After incubation of the peptide (I—VIII)-DNA complex with restriction endonuclease EcoR1 (8 U) at 37 °C for 2 h, the products were analyzed on 1% agarose gels.

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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); *ibid*., **6**, 362—364 (1966); and *ibid*., **11**, 1726—1732 (1972). The following additional abbreviations are used: AcOEt, ethyl acetate; DMF dimethylformamide; DCM, dichloromethane; 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; 2-Cl-Z, 2-chlorobenzyloxycarbonyl; Tos, *p*-toluenesulfonyl; Bzl, benzyl; 2-Br-Z, 2-bromobenzyloxycarbonyl; Xan, xanthyl; Pam, 4-hydroxymethylphenylacetamidomethyl; BOP, benzotriazole-1-yl-oxy-tris(dimethylamino)phosphoniumhexafluorophosphate 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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