Synthesis of a Partial Sequence of Proinsulin Using the A-Chain of Natural Insulin. III.[1] Synthesis of a Peptide Corresponding to Positions 41—81 of Bovine Proinsulin

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(Received May 23, 1979)

For the synthesis of bovine proinsulin, the *S*-sulfonate of the Leu–Glu–Gly–Pro–Pro–Gln–Lys[Z(2-Cl)]–Arg–A-chain, which was synthesized using the *S*-sulfonate of the A-chain of natural bovine insulin and which corresponds to positions 53—81 of bovine proinsulin, was coupled with Boc–Ala–Leu–Glu(OBu^t)–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–N_3. After removal of the protecting groups from the resulting material the *S*-sulfonate of the Ala–Leu–Glu–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–Leu–Glu–Gly–Pro–Pro–Gln–Lys[Z(2-Cl)]–Arg–A-chain, corresponding to positions 41—81 of bovine proinsulin, was purified by chromatography on QAE-Sephadex A-25.

In the preceding paper[1] we reported the synthesis of a peptide corresponding to positions 53—81 of bovine proinsulin,[2] the *S*-sulfonate of the Leu–Glu–Gly–Pro–Pro–Gln–Lys[Z(2-Cl)]–Arg–A-chain,[3] using the *S*-sulfonate of the A-chain of natural bovine insulin. The synthesis has the merit that the *S*-sulfonates not only of the A-chain but also of its elongated derivatives can react with acylpeptides in homogeneous solution, since they are very soluble in organic solvents such as DMF and DMSO in the presence of water. The solubility of the reaction products makes it easy to purify them, and pure products can be obtained free from starting materials and side-reaction products by chromatography. The present paper reports the synthesis of a 41-amino acid sequence corresponding to positions 41—81 of bovine proinsulin by this procedure, as a step in the synthesis of the prohormone.

In the synthesis of this partial sequence of the prohormone, shown in Fig. 1, the *S*-sulfonate of the Leu-Glu-Gly-Pro-Pro-Gln-Lys[Z(2-Cl)]-Arg-A-chain[1] was allowed to react with a protected peptide azide with the sequence, Ala–Leu–Glu–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly. Boc–Ala–Leu–Glu(OBu^t)–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–OBzl (X) was synthesized, according to the scheme shown in Fig. 2. Before synthesis of this protected dodecapeptide, its two building fragments, Boc–Ala–Leu–Glu(OBu^t)–Leu–Ala–Gly–OH (V) and Boc–Gly–Pro–Gly–Ala–Gly–Gly–OBzl (IX) were synthesized separately. The synthesis of the former hexapeptide (V) was started by coupling Boc–Ala–OH[4] with H–Gly–OBzl by DCC to give Boc–Ala–Gly–OBzl (I). The protecting group on the amino terminus was removed by TFA and the peptide chain was elongated further by two single-reactions using Boc–Leu–ONSu[5] and Z–Glu(OBu^t)–ONSu[6] for acylation. The tetrapeptide, Z–Glu(OBu^t)–Leu–Ala–Gly–OBzl (III), thus synthesized was catalytically hydrogenated and the resulting free tetrapeptide was condensed *in situ* with Z–Leu–ONSu[5] to give Z–Leu–Glu(OBu^t)–Leu–Ala–Gly–OH (IV). The protected pentapeptide (IV) was once again catalytically hydrogenated and the resulting peptide was allowed to react with Boc–Ala–ONSu[5] to give the hexapeptide (V), Boc–Ala–Leu–Glu(OBu^t)–Leu–Ala–Gly–OH.

The hexapeptide (IX), Boc–Gly–Pro–Gly–Ala–Gly–Gly–OBzl, was synthesized by the condensation of Boc–Gly–Pro–OH[7] with the tetrapeptide benzyl ester, which was prepared by treatment of Boc–Gly–Ala–Gly–Gly–OBzl (VIII) with TFA, by DCC. The tetrapeptide (VIII) was synthesized from Boc–Gly–Gly–OBzl (VI), which was prepared by coupling Boc–Gly–OH[4] with H–Gly–OBzl by DCC, by a stepwise elongation procedure using Boc-amino acid *N*-hydroxysuccinimide ester. The hexapeptide (IX) thus synthesized was treated with TFA. The resulting peptide was not isolated, but coupled directly with the hexapeptide (V) by DCC in the presence of HOBt.[8] The crude material was purified on a column of LH-20 to give pure Boc–Ala–Leu–Glu(OBu^t)–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–OBzl (X).

The *S*-sulfonate of Leu–Glu–Gly–Pro–Pro–Gln–Lys-[Z(2-Cl)]–Arg–A-chain[1] was coupled with a protected peptide with the sequence Ala–Leu–Glu–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly. The dodecapeptide benzyl ester (X) was converted to the corresponding acid (XI) by catalytic hydrogenation. Attempts were made to couple the dodecapeptide acid (XI) thus obtained with HONSu in the presence of DCC in order to obtain the corresponding active ester. However, pure active ester could not be obtained. The dodecapeptide ester (X) was converted to its corresponding

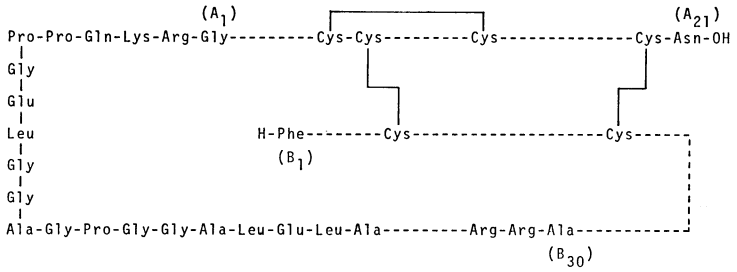

Fig. 1. Strutural model of bovine proinsulin.[2]

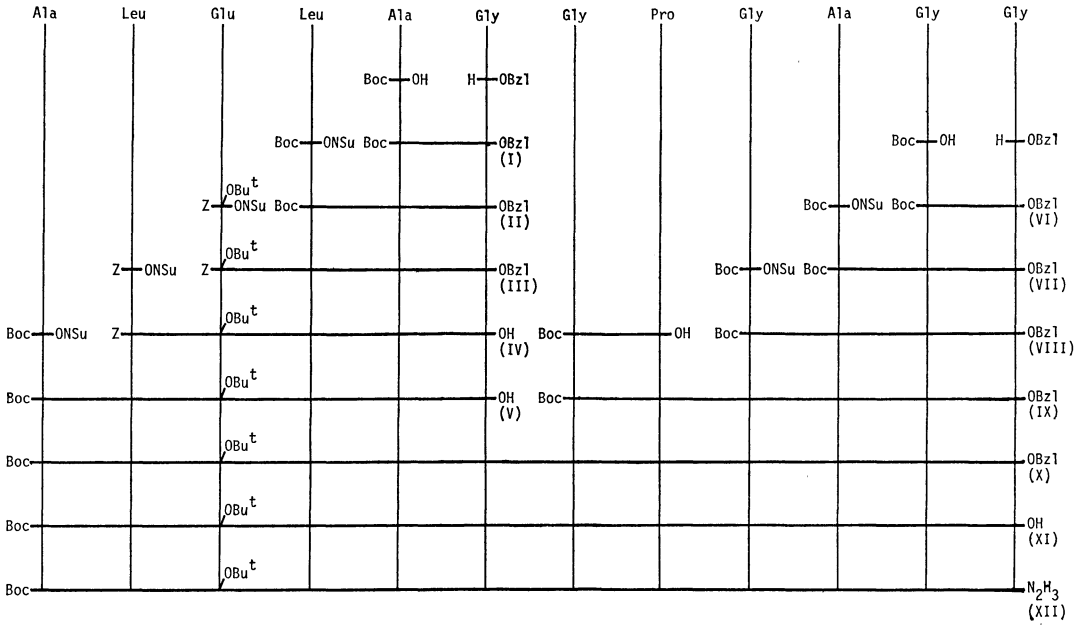

Fig. 2. Scheme for synthesis of protected dodecapeptide (sequence corresponding to positions C_9 to C_{20} in bovine proinsulin).

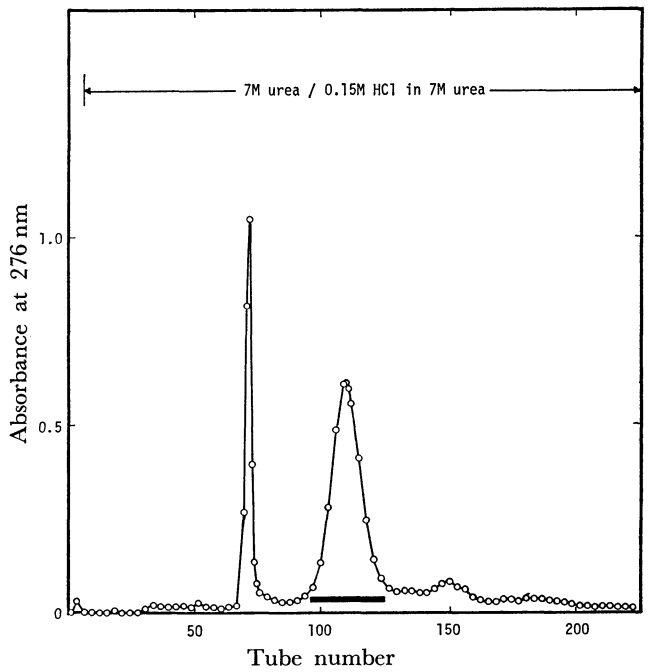

Fig. 3. Chromatogram of the *S*-sulfonate of the Ala–Leu–Glu–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–Leu–Glu–Gly–Pro–Pro–Gln–Lys[Z(2-Cl)]–Arg–A-chain on QAE-Sephadex A-25 obtained under the conditions described in the text.

hydrazide (XII) by the usual method of hydrazinolysis, and the hydrazide (XII) was converted to the corresponding azide by Rudinger's method[9]. The peptide azide was allowed to react *in situ* with the *S*-sulfonate of the Leu–Glu–Gly–Pro–Pro–Gln–Lys-[Z(2-Cl)]–Arg–A-chain. The resulting *S*-sulfonate of the Boc–Ala–Leu–Glu(OBu^t)–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–Leu–Glu–Gly–Pro–Pro–Gln–Lys[Z-(2-Cl)]–Arg–A-chain was treated with TFA and then

TABLE 1. AMINO ACID RATIO OF PEPTIDES SYNTHESIZED[a]

Amino acid	Protected [41—53]	*S*-Sulfonate of [53—81][b]	*S*-Sulfonate of [41—81]
Lys		1.00 (1)	1.00 (1)
Arg		1.00 (1)	1.00 (1)
Asp		1.94 (2)	2.09 (2)
Ser		1.79 (2)	1.98 (2)
Glu	1.03 (1)	6.73 (6)	8.39 (7)
Pro	1.06 (1)	2.09 (2)	3.04 (3)
Gly	4.85 (5)	1.95 (2)	7.40 (7)
Ala	3.00 (3)	1.00 (1)	4.00 (4)
Cys		nd[c]	nd[c]
Val		1.59 (2)	1.60 (2)
Ile		0.63 (1)	0.55 (1)
Leu	1.92 (2)	2.96 (3)	5.09 (5)
Tyr		1.94 (2)	1.86 (2)

a) Molar ratios of individual amino acids are shown relative to that of alanine. Numbers in parentheses are theoretical values. b) Values are cited from Ref. 1. c) Cystine was not determined.

subjected to chromatography on QAE-Sephadex A-25 (Fig. 3). The amino acid ratio of the acid hydrolysate of the *S*-sulfonate synthesized is given in Table 1. The N-terminal amino acid residue of the purified *S*-sulfonate was identified as alanine by dinitrophenylation.[10]

Thus, the *S*-sulfonate of a peptide corresponding to positions 41—81 of bovine proinsulin was synthesized and purified. Studies on the further elongation of this peptide will be described in the subsequent paper.[11]

Experimental

The general experimental and analytical methods used were described in the preceding paper.[1]

Boc–Ala–Gly–OBzl (I). H–Gly–OBzl·TosOH (40.4 g,

120 mmol) and TEA (16.8 ml) were dissolved in a mixture of DMF (100 ml) and THF (200 ml). The solution was mixed with Boc-Ala-OH[4] (18.9 g, 100 mmol) and cooled to −5 °C—−10 °C. DCC (20.6 g, 100 mmol) was added to the cooled mixture. The mixture was stirred at the same temperature for 1 h and at room temperature for 1 d. The precipitate formed was filtered off and the filtrate was concentrated to a syrup under reduced pressure. The syrup was dissolved in AcOEt and washed successively with 0.1 M HCl, 5% aqueous $NaHCO_3$ and water. The washed solution was dried over Na_2SO_4 and concentrated to a syrup *in vacuo*. The syrup was crystallized from AcOEt and hexane; wt 26.0 g (77.4%). The crude material was recrystallized from AcOEt and hexane; wt 21.5 g (64.0%), mp 86—88 °C, $[\alpha]_D^{18}$ −11.7° (*c* 2.2, DMF).

Found: C, 60.75; H, 7.34; N, 8.65%. Calcd for $C_{17}H_{24}O_5N_2$: C, 60.70; H, 7.19; N, 8.33%.

Boc-Leu-Ala-Gly-OBzl (II). Compound I (20.2 g, 60.1 mmol) was dissolved in TFA (40 ml). The solution was stirred at room temperature for 90 min and then concentrated to a syrup. The syrup was washed with ether and redissolved with TEA (8.4 ml) and Boc-Leu-ONSu[5] (21.0 g, 64.0 mmol) in THF (200 ml). The solution was stirred for 2 d and then concentrated to a syrup. The syrup was dissolved in AcOEt and washed successively with 0.1 M HCl, 5% aqueous $NaHCO_3$ and water. The washed solution was dried and concentrated to an oily residue, which was crystallized from a mixture of AcOEt, ether and hexane; wt 26.5 g. The crude material was recrystallized from ether; wt 20.5 g (75.9%), mp 83—86 °C, $[\alpha]_D^{18}$ −19.2° (*c* 2.0, DMF).

Found: C, 61.24; H, 7.88; N, 9.52%. Calcd for $C_{23}H_{35}O_6N_3$: C, 61.45; H, 7.85; N, 9.35%.

Z-Glu(OBu^t)-Leu-Ala-Gly-OBzl (III). Compound II (18.0 g, 40.0 mmol) was dissolved in TFA (50 ml), stirred at room temperature for 90 min and then concentrated to a syrup. The syrup was solidified in ether, dissolved with TEA (5.6 ml) and Z-Glu(OBu^t)-ONSu[6] (18.4 g, 42.4 mmol) in $CHCl_3$ (300 ml), and stirred at room temperature for 2 d. The solution was diluted with $CHCl_3$, washed successively with 0.1 M HCl, 5% aqueous $NaHCO_3$ and water, dried, and then concentrated to a solid. The solid was crystallized from AcOEt and hexane; wt 23.5 g. Recrystallization of the crude product from AcOEt and hexane gave 23.0 g of material (85.8% yield), mp 162—164 °C, $[\alpha]_D^{18}$ −17.5° (*c* 2.0, DMF).

Found: C, 62.68; H, 7.25; N, 8.52%. Calcd for $C_{35}H_{48}O_9N_4$: C, 62.85; H, 7.24; N, 8.38%.

Z-Leu-Glu(OBu^t)-Leu-Ala-Gly-OH (IV). Compound III (6.70 g, 10.0 mmol) was dissolved in hot MeOH (150 ml), cooled to room temperature and mixed with AcOH (50 ml). Catalytic hydrogenation was carried out over 5% palladium-charcoal catalyst at atmospheric pressure. The catalyst was filtered off and the filtrate was concentrated to a solid. The solid was suspended with TEA (2 ml) and Z-Leu-ONSu[5] (4.32 g, 11.9 mmol) in a mixture of DMF (100 ml) and THF (50 ml), and dissolved gradually during the course of stirring. The solution was stirred overnight at room temperature and concentrated to a solid, which was suspended in AcOEt and washed with 0.1 M HCl and water. The organic layer was concentrated to a solid, which was crystallized from EtOH; wt 5.10 g. The crude material was recrystallized from EtOH; wt 4.58 g (66.2%), mp 226—228 °C (dec), $[\alpha]_D^{20}$ −26.0° (*c* 2.0, DMF).

Found: C, 58.90; H, 7.84; N, 10.18%. Calcd for $C_{34}H_{53}O_{10}N_5$: C, 59.03; H, 7.72; N, 10.12%.

Boc-Ala-Leu-Glu(OBu^t)-Leu-Ala-Gly-OH (V). Compound IV (4.15 g, 6.00 mmol) was dissolved in hot MeOH (150 ml), cooled to room temperature, and hydrogenated in the presence of AcOH (50 ml) and 5% palladium-charcoal catalyst. AcOH (100 ml) was added to dissolve the solid formed during the course of hydrogenation. The catalyst was filtered off and the filtrate was concentrated to a solid. The solid was suspended with Boc-Ala-ONSu[5] (2.00 g, 6.99 mmol) in DMF (300 ml) and stirred at room temperature for 3 d. The solution thus obtained was concentrated to a gelatinous solid, which was collected by filtration with $CHCl_3$. The crude material was recrystallized from MeOH; wt 3.52 g (79.5%), mp 230 °C (dec), $[\alpha]_D^{20}$ −34.2° (*c* 2.0, DMF). Amino acid ratio in the acid hydrolysate: Glu, 1.10 (1); Gly, 1.00 (1); Ala, 2.00 (2); Leu, 2.00 (2).

Found: C, 55.07; H, 8.25; N, 11.38%. Calcd for $C_{34}H_{60}O_{11}N_6 \cdot 1/2H_2O$: C, 55.34; H, 8.33; N, 11.39%.

Boc-Gly-Gly-OBzl (VI). H-Gly-OBzl·TosOH (24.3 g, 72.1 mmol) was dissolved with TEA (10.1 ml) and Boc-Gly-OH[4] (10.5 g, 60.0 mmol) in THF (300 ml). The solution was cooled to 0 °C, mixed with a solution of DCC (12.4 g, 60.2 mmol) in THF (50 ml), and stirred overnight at room temperature. The precipitate formed was filtered off and the filtrate was concentrated to an oily residue. The residue was dissolved in AcOEt, washed successively with 0.1 M HCl, 5% aqueous $NaHCO_3$ and water, dried, and concentrated to a syrup. The syrup was crystallized from a mixture of AcOEt and hexane; wt 16.5 g. The crude material was recrystallized from AcOEt and hexane; wt 16.0 g (82.9%), mp 83—84.5 °C.

Found: C, 59.78; H, 7.02; N, 8.84%. Calcd for $C_{16}H_{22}O_5N_2$: C, 59.61; H, 6.88; N, 8.69%.

Boc-Ala-Gly-Gly-OBzl (VII). Compound VI (13.0 g, 40.4 mmol) was dissolved in 3.55 M HCl in dioxane (113 ml) and stirred at room temperature for 1 h. The crystalline material formed was collected by filtration with ether and dissolved with TEA (5.4 ml) and Boc-Ala-ONSu[5] (13.7 g, 47.9 mmol) in a mixture of $CHCl_3$ (200 ml) and DMF (100 ml). The solution was stirred at room temperature for 1 d and then concentrated to a syrup. The syrup was dissolved in AcOEt and washed successively with 0.1 M HCl, 5% aqueous $NaHCO_3$ and water, dried and then concentrated to an oil. The oil was triturated in a mixture of AcOEt and hexane; wt 14.0 g. The crude material was recrystallized from AcOEt and hexane; wt 13.9 g (87.4%), mp 108.5—110.5 °C, $[\alpha]_D^{17}$ +0.2° (*c* 2.0, DMF).

Found: C, 58.21; H, 6.70; N, 10.69%. Calcd for $C_{19}H_{27}O_6N_3$: C, 58.00; H, 6.92; N, 10.68%.

Boc-Gly-Ala-Gly-Gly-OBzl (VIII). Compound VII (9.80 g, 24.9 mmol) was dissolved in 3.90 M HCl in dioxane (47.5 ml), stirred at room temperature for 60 min and concentrated to an oily residue. The oil was triturated in ether and dissolved with TEA (3.5 ml) and Boc-Gly-ONSu[5] (8.20 g, 30.1 mmol) in a mixture of DMF (100 ml) and $CHCl_3$ (100 ml). The solution was stirred overnight at room temperature and concentrated to a syrup. The syrup was dissolved in AcOEt, washed successively with 0.1 M HCl, 5% aqueous $NaHCO_3$ and water, dried, and concentrated to a crystalline solid. The solid was collected by filtration with a mixture of AcOEt and hexane; wt 9.00 g. The crude material was recrystallized from AcOEt and hexane; wt 8.54 g (76.3%), mp 126—128 °C, $[\alpha]_D^{17}$ −1.0° (*c* 2.0, DMF).

Found: C, 55.74; H, 6.84; N, 11.96%. Calcd for $C_{21}H_{30}O_7N_4$: C, 55.99; H, 6.71; N, 12.44%.

Boc-Gly-Pro-Gly-Ala-Gly-Gly-OBzl (IX). Compound VIII (4.05 g, 9.00 mmol) was dissolved in 4.0 M HCl in dioxane (25 ml), stirred at room temperature for

60 min and then concentrated to a crystalline residue. The residue was collected by filtration with ether and dried over NaOH. The crystalline material was dissolved with *N*-methylmorpholine (1.0 ml), Boc–Gly–Pro–OH[7] (2.45 g, 9.01 mmol) and HOBt (2.02 g, 15.0 mmol) in DMF (40 ml). The mixture was cooled to 0 °C, mixed with DCC (2.30 g, 11.2 mmol), and then stirred at the same temperature for 1 h and at room temperature overnight. The precipitate formed was filtered off and the filtrate was concentrated to dryness. The residue was dissolved in $CHCl_3$, washed successively with 0.1 M HCl, 5% aqueous $NaHCO_3$ and water, dried, and concentrated to a syrup. The syrup was crystallized from AcOEt, EtOH, and hexane; wt 5.17 g. The crude product was recrystallized from AcOEt, EtOH, and hexane; wt 4.73 g (86.8%), mp 164 °C, $[\alpha]_D^{18}$ −21.0° (*c* 2.0, DMF). Amino acid ratio in the acid hydrolysate: Gly, 3.84 (4); Ala, 1.00 (1); Pro, 1.02 (1).

Found: C, 55.67; H, 6.78; N, 13.83%. Calcd for $C_{28}H_{40}O_9N_6$: C, 55.62; H, 6.67; N, 13.90%.

Boc–Ala–Leu–Glu(OBu^t)–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–OBzl (X). Compound IX (3.00 g, 4.96 mmol) was dissolved in TFA (10 ml), stirred at room temperature for 30 min and concentrated to a syrup, which was triturated with ether. The solid was dissolved with *N*-methylmorpholine (0.85 ml), compound V (3.70 g, 5.01 mmol) and HOBt (1.00 g, 7.41 mmol) in DMF (300 ml) and cooled below 0 °C. The solution was stirred with DCC (1.00 g, 4.85 mmol) at the same temperature for 1 h and at room temperature for 1 d. The solution was stirred with HOBt (0.30 g, 2.2 mmol) and DCC (0.5 g, 2.4 mmol) at room temperature for 3 d. The precipitate formed was filtered off and the filtrate was concentrated to dryness. The residue was taken up in DMF and water and purified on a column (3×50 cm) of LH-20 using DMF as a solvent. The fractions containing the product were collected and concentrated to a solid, which was filtered with hot AcOEt; wt 4.09 g (67.4%), mp 210 °C (sintered) and 227 °C (dec), $[\alpha]_D^{20}$ −31.0° (*c* 2.0, DMF). Amino acid ratio in the acid hydrolysate: Glu, 1.03 (1); Pro, 1.06 (1); Gly, 4.85 (5); Ala, 3.00 (3); Leu, 1.92 (2).

Found: C, 55.86; H, 7.64; N, 13.68%. Calcd for $C_{57}H_{90}O_{17}N_{12}\cdot 1/2H_2O$: C, 55.91; H, 7.49; N, 13.73%.

Boc–Ala–Leu–Glu(OBu^t)–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–OH (XI). Compound X (1.22 g, 1.00 mmol) was dissolved in DMF (100 ml) by gentle heating and hydrogenated over 5% palladium–charcoal catalyst in a water bath at 35 °C. The catalyst was filtered off and the filtrate was concentrated to a solid, which was collected with AcOEt by filtration; wt 1.14 g. The crude product was reprecipitated from DMF and AcOEt; wt 1.12 g (97.4%), mp 217 °C (dec), $[\alpha]_D^{16.5}$ −25.9° (*c* 1.0, DMF).

Found: C, 52.16; H, 7.43; N, 14.51%. Calcd for $C_{50}H_{84}O_{17}N_{12}\cdot 3/2H_2O$: C, 52.11; H, 7.61; N, 14.59%.

Boc–Ala–Leu–Glu(OBu^t)–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–N_2H_3 (XII). Compound X (2.79 g, 2.28 mmol) was dissolved in *N*-methylpyrrolidone (20 ml) by gentle heating. The solution was cooled to room temperature, mixed with hydrazine hydrate (1.2 ml) and stirred at room temperature overnight. The precipitate formed was filtered and dried; wt 2.26 g (81.0%), mp 222—224 °C (dec).

Found: C, 51.89; H, 7.62; N, 16.79%. Calcd for $C_{50}H_{86}O_{16}N_{14}\cdot H_2O$: C, 51.89; H, 7.66; N, 16.95%.

S-Sulfonate of the Ala–Leu–Glu–Leu–Ala–Gly–Gly–Pro–Gly–Ala–Gly–Gly–Leu–Glu–Gly–Pro–Pro–Gln–Lys[Z(2-Cl)]–Arg–A-Chain. Compound XII (1.68 g, 1.38 mmol) was suspended in *N*-methylpyrrolidone (20 ml), cooled to −20 °C—−30 °C and mixed with 3.0 M HCl in dioxane (2.3 ml). The solution thus obtained was mixed with isopentyl nitrite (0.19 g, 1.66 mmol) and stirred at −15 °C——20 °C for 60 min. The *S*-sulfonate of the Leu–Glu–Gly–Pro–Pro–Gln–Lys[Z(2-Cl)]–Arg–A-chain (510 mg) in water (2 ml) was added and its solution was stirred at 2 °C—3 °C in a refrigerator for 5 d and then poured into 0.1 M NH_4HCO_3 (100 ml). The precipitate formed was centrifuged, dissolved in DMSO, mixed with 0.1 M NH_4HCO_3 and filtered through a column of Sephadex G-25 (3×60 cm). The supernatant was also filtered through a similar Sephadex G-25 column. The eluates with absorption at 280 nm were pooled and lyophilized; wt 639 mg. Amino acid ratio in the acid hydrolysate: Lys, 1.00 (1); Arg, 1.00 (1); Asp, 2.09 (2); Ser, 1.98 (2); Glu, 8.39 (7); Pro, 3.04 (3); Gly, 7.40 (7); Ala, 4.00 (4); Cys, not determined; Val, 1.60 (2); Ile, 0.55 (1); Leu, 5.09 (5); Tyr, 1.86 (2). The powder (325 mg) was dissolved in TFA (10 ml) in an ice–water bath, stirred at room temperature for 60 min and concentrated to a syrup. The syrup was dissolved in 0.1 M NH_4HCO_3 (pH *ca.* 8.0) and lyophilized. The powder was dissolved with Na_2SO_3 (0.5 g), $Na_2S_4O_6$ (0.5 g) and EDTA·2Na (0.01 g) in 8 M urea (12.5 ml). The solution was adjusted to pH 8.0 and stirred at room temperature for 2 d. Then it was charged on Sephadex G-25 (3×60 cm) and the material was eluted with 0.1 M NH_4HCO_3. Fractions with absorption at 280 nm were collected and lyophilized; wt 287 mg. The lyophilized powder (49.1 mg) was charged on a column (0.9×8 cm) of QAE-Sephadex A-25 (acetate cycle) equilibrated with 7 M urea, and the material was eluted with a linear gradient of 7 M urea (250 ml) to 0.15 M HCl in 7 M urea (250 ml), as shown in Fig. 3. The fraction with an absorption at 276 nm, shown by a bar in Fig. 3, was collected and desalted on a column (3×60 cm) of Sephadex G-25 using 0.1 M NH_4HCO_3 and lyophilized; wt 38.3 mg.

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3) The abbreviations used are those recommended by the IUPAC-IUB: *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations: DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; HOBt, 1-hydroxybenzotriazole; TEA, triethylamine; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran.

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