

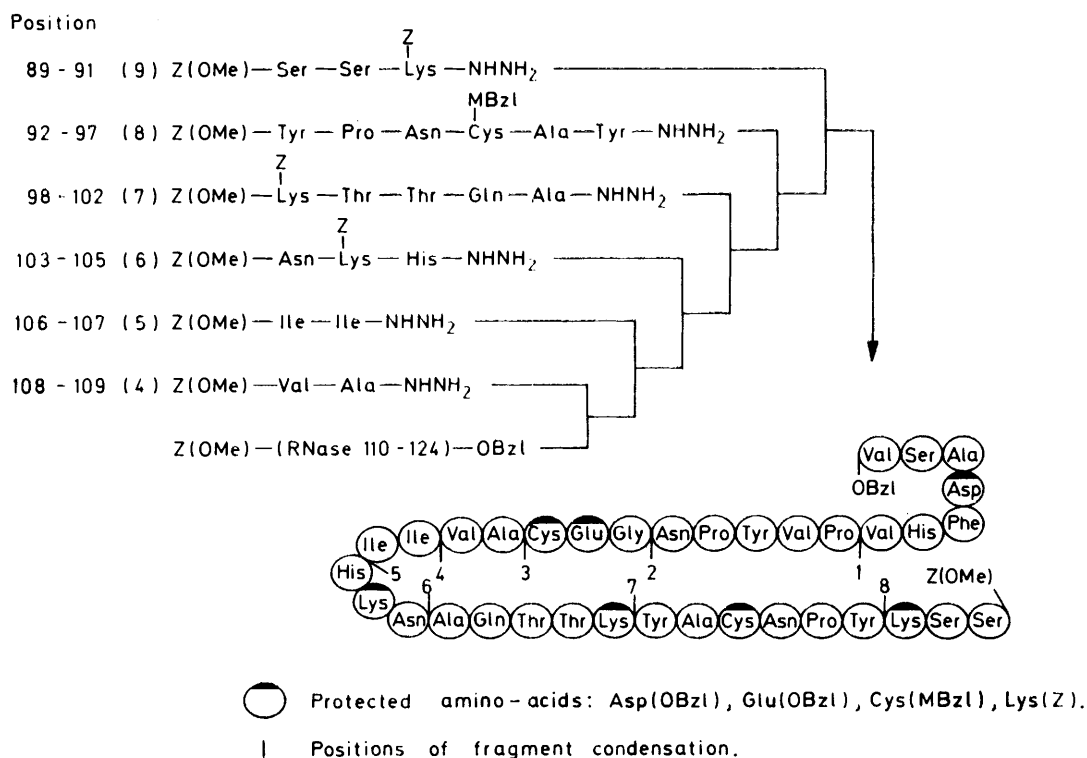
Total Synthesis of Bovine Pancreatic Ribonuclease A. Part 2.¹ Synthesis of the Protected Hexatriacontapeptide Ester (Positions 89—124) †

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Commencing with the protected C-terminal pentadecapeptide of bovine pancreatic RNase, Z(OMe)-(RNase 110—124)-OBzl, chain elongation was accomplished to form the hexatriacontapeptide, Z(OMe)-(RNase 89—124)-OBzl, by six successive azide condensations of the peptide fragments, Z(OMe)-Val-Ala-NHNH₂ (4), Z(OMe)-Ile-Ile-NHNH₂ (5), Z(OMe)-Asn-Lys(Z)-His-NHNH₂ (6), Z(OMe)-Lys(Z)-Thr-Thr-Gln-Ala-NHNH₂ (7), Z(OMe)-Tyr-Pro-Asn-Cys(MBzl)-Ala-Tyr-NHNH₂ (8), and Z(OMe)-Ser-Ser-Lys(Z)-NHNH₂ (9).

THE synthesis of the protected C-terminal pentadecapeptide (positions 110—124) was described in Part I of this series;¹ its chain elongation to the hexatriacontapeptide (positions 89—124) is now reported. Chain

(5). These two hydrazides were obtained by the usual treatment with hydrazine of the corresponding esters, prepared by the mixed anhydride procedure⁴ rather than the DCC procedure. The latter procedure gave con-



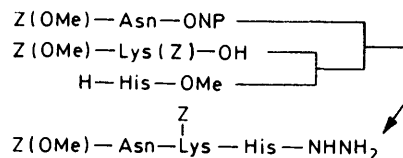
SCHEME 1 Synthetic route to the protected hexatriacontapeptide ester, Z(OMe)-(RNase 89—124)-OBzl

elongation was accomplished by successive azide condensations² of six peptide fragments as shown in Scheme 1.

Positions 106—108 of RNase consist of the sterically hindered amino-acids Ile-Ile-Val. Initially, we prepared Z(OMe)-Ile-Ile-Val-Ala-OMe in a stepwise manner by DCC condensation³ without difficulty. However, the corresponding hydrazide was very insoluble even in hot DMF or DMSO. Because of this difficulty, we decided to introduce this tetrapeptide unit in two steps as Z(OMe)-Val-Ala-NHNH₂ (4) and Z(OMe)-Ile-Ile-NHNH₂

considerable amounts of the acylurea compounds⁵ in both cases.

The fragment Z(OMe)-Asn-Lys(Z)-His-NHNH₂ (6) (positions 103—105) was prepared as shown in Scheme 2. Rink and Riniker⁶ have pointed out that the DCC

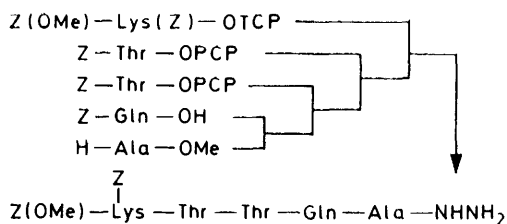


SCHEME 2 Synthetic scheme for the protected tripeptide hydrazide, Z(OMe)-(RNase 103—105)-NHNH₂ (6)

† This paper is regarded as Part 89 in the series 'Studies on Peptides' [Part 88, ref. 1 (preceding paper)].

condensation of histidine-containing peptides gives a DCC adduct at the imidazole ring. After the DCC condensation of Z(OMe)-Lys(Z)-OH and H-His-OMe, the product was, therefore, treated with methanol and 3% acetic acid to remove this adduct. Z(OMe)-Asn-OH was condensed by the NP method⁷ with the TFA-treated sample of Z(OMe)-Lys(Z)-His-OMe thus obtained, and subsequently the product was converted into (6) by the usual treatment with hydrazine.

Fragment (7), Z(OMe)-Lys(Z)-Thr-Thr-Gln-Ala-NH-NH₂ (7) (positions 98–102), was synthesized, according to Scheme 3, starting with Z-Gln-Ala-OMe,⁸ prepared by DCC condensation in the presence of HOBT.⁹ No CN bond¹⁰ vibration was observed in the i.r. spectrum of this starting material. After removing the Z group by catalytic hydrogenolysis, the PCP¹¹ and TCP¹² active-ester procedures were employed to introduce threonine and Lys(Z), respectively, since these esters are crystalline compounds.^{13,14} The resulting pentapeptide ester, Z-(OMe)-Lys(Z)-Thr-Thr-Gln-Ala-OMe, was smoothly converted into (7) by hydrazine in the usual manner.



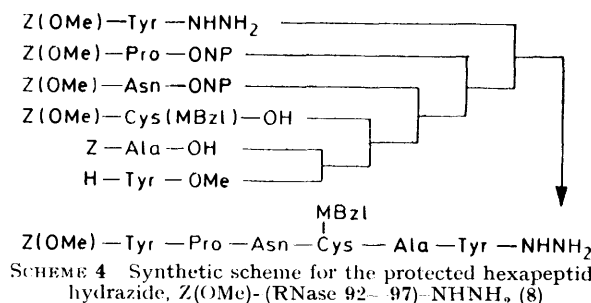
SCHEME 3 Synthetic scheme for the protected pentapeptide hydrazide, Z(OMe)-(RNase 98–102)-NHNH₂ (7)

We next tried to elongate the peptide chain by adding the two sub-units Z(OMe)-Pro-Asn-Cys(MBzl)-Ala-Tyr-NH-NH₂ and Z(OMe)-Ser-Ser-Lys(Z)-Tyr-NH-NH₂. However, preliminary experiments showed that the azide prepared from the latter peptide, after *N*-deprotection, coupled only incompletely with the hindered Pro-terminated peptide, obtained by way of the former intermediate, to give a poor yield of a product with a markedly lower optical rotatory power than that of authentic Z(OMe)-(RNase 89–124)-OBzl. We therefore synthesized the latter compound by using instead Z(OMe)-Tyr-Pro-Asn-Cys(MBzl)-Ala-Tyr-NH-NH₂ (8) and Z(OMe)-Ser-Ser-Lys(Z)-NHNH₂ (9). The former hydrazide was synthesized in a stepwise manner starting with Z-Ala-Tyr-OMe¹⁵ as shown in Scheme 4. Z(OMe)-Cys(MBzl)-OH was introduced by DCC, followed by two amino-acids, Z(OMe)-Asn-OH and Z(OMe)-Pro-OH, by the NP method, and finally Z(OMe)-Tyr-NH-NH₂ by the azide method. The resulting hexapeptide ester was converted into (8) in the usual manner.

Fragment (9) (positions 89–91) was prepared by two successive azide condensations of Z(OMe)-Ser-NH-NH₂ with H-Lys(Z)-OMe followed by treatment of the resulting tripeptide ester, Z(OMe)-Ser-Ser-Lys(Z)-OMe, with hydrazine (Scheme 5). Previously, we used Z(OMe)-Ser-OPCP for the preparation of the aforementioned tetrapeptide hydrazide. However some difficulty was

encountered in preparing the starting material, Z(OMe)-Ser-OH,¹⁶ because of its high solubility in water.

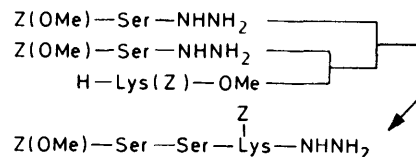
The six peptide hydrazides, the purities of which were assessed by elemental and amino-acid analysis after acid hydrolysis, were then assembled successively by the



SCHEME 4 Synthetic scheme for the protected hexapeptide hydrazide, Z(OMe)-(RNase 92–97)-NHNH₂ (8)

Rudinger azide procedure. The possibility of a certain degree of racemization occurring during the azide coupling has been pointed out by several authors.¹⁷ However, on the basis of the available information, and our own experience, we still considered the azide method to be the preferred method for obtaining peptides with high optical purity, particularly as we were synthesizing the peptides without masking the hydroxy-group of serine and threonine or the phenolic group of tyrosine. Bodanszky *et al.*¹⁸ have reported over-activation of active esters, which took place at these functional groups in the presence of histidine. We felt it safer to carry out our synthesis using the azide procedure, as demonstrated by Denkwalter *et al.*,¹⁹ since much less risk seemed to be involved in this method, which can be performed with cooling.

Among the condensation reactions involved in this section, the one which most concerned us was the bond formation between isoleucine and valine (positions 107 and 108) *via* the azide condensation of fragment (5) with the Val-terminal amino-component (position 108–124), because of the steric hindrance of these amino-acids. Indeed, conversion of Z(OMe)-Ile-Ile-OMe into the corresponding hydrazide needed slight warming to 50 °C; however the azide reaction went smoothly in better than expected yield (79%). Acid hydrolysis of the protected peptide, Z(OMe)-(RNase 106–124)-OBzl, gave a low recovery of isoleucine, as predicted from



SCHEME 5 Synthetic scheme for the protected tripeptide hydrazide, Z(OMe)-(RNase 89–91)-NHNH₂ (9)

earlier observations.²⁰ We also noted this phenomenon²¹ during the synthesis of basic trypsin inhibitor, which contained the identical Ile-Ile sequence.²² Thus, hydrolysis for 72 h was performed at this stage to ensure satisfactory incorporation of this dipeptide unit. Analy-

sis showed the presence of D-allo-Ile (2.3%). Acid hydrolysis of the above starting hydrazide did not give any detectable amount of the allo-compound, which is the diagnostic compound in the racemization test (cf. ref. 23). We had to admit the possibility of the occurrence of racemization in not only the above coupling step; however the extent was likely to be less than 0.4%.^{17c}

Every condensation was performed with a ca. 1.3–2.5-fold excess of the azide component, until the solution became negative to ninhydrin. The progress of the reaction was followed by a Shimadzu dual-wavelength t.l.c. scanner. DMF–DMSO or DMF–HMPA was employed as solvent depending on the solubility of the amino-components. In particular, after incorporation of the Val-Ala unit (4), succeeding condensations of the fragments, from (5) to (9), were performed in such solvent systems. Under these conditions also, improvements in solubility were noted after incorporation of fragments bearing protecting groups, such as Lys(Z) and Cys(MBzl). The excess of peptide azide could be removed by repeated precipitation from DMF or a mixture of DMF and DMSO with methanol or ethyl acetate. Coupling yields were between 72 and 93%. The purity of the protected intermediates was assessed by t.l.c. and amino-acid analysis (Table). The recovery

Amino-acid ratios of Z(OMe)-(RNase 89–124)-OBzl and intermediates

Position Residue	108—124 (17)	106—124 (19) *	103—124 (22)	98—124 (27)	92—124 (33)	89—124 (36)
Asp	1.97(2)	2.02(2)	3.09(3)	3.09(3)	4.24(4)	4.20(4)
Thr				2.04(2)	1.80(2)	1.60(2)
Ser	0.89(1)	0.77(1)	0.78(1)	0.82(1)	0.72(1)	2.47(3)
Glu	1.06(1)	1.08(1)	1.12(1)	2.34(2)	2.26(2)	2.08(2)
Pro	2.00(2)	2.18(2)	2.17(2)	2.20(2)	3.05(3)	2.62(3)
Gly	1.05(1)	1.01(1)	1.09(1)	1.06(1)	1.08(1)	1.08(1)
Ala	2.03(2)	2.01(2)	1.91(2)	2.98(3)	4.18(4)	3.89(4)
Val	4.09(4)	3.89(4)	3.76(4)	3.85(4)	3.86(4)	4.03(4)
Ile		1.84(2) *	1.82(2)	1.76(2)	1.86(2)	1.84(2)
Tyr	0.94(1)	0.97(1)	0.91(1)	0.99(1)	2.97(3)	2.65(3)
Phe	1.00(1)	1.00(1)	1.00(1)	1.00(1)	1.00(1)	1.00(1)
Lys			1.13(1)	2.10(2)	1.81(2)	2.99(3)
His	0.83(1)	0.88(1)	1.84(2)	1.98(2)	1.78(2)	1.82(2)
Cys	(1)	(1)	(1)	(1)	(2)	(2)
Recovery	92%	97%	94%	84%	87%	86%

* Ile 2.02 in 72-h hydrolysate.

of phenylalanine was taken as the basis of calculation, as described in the preceding paper.¹ Elemental analysis offers little information about the purity of relatively large peptides, but we carried out such analyses to obtain as much information as possible about homogeneity.

The docosapeptide (position 103–124) synthesized by Jenkins *et al.*²⁰ was similar to the one reported here with the exception of the protecting groups employed.

EXPERIMENTAL

General experimental procedures are described in Part I.¹ The N^α-protecting group, Z(OMe), was cleaved by TFA in the presence of anisole (2 mol equiv. or more) in an ice-bath for 45 to 60 min. The DCC and the active ester condensations were performed at room temperature (17–

25 °C). The azide condensations were performed according to ref. 2. The mixed anhydrides were prepared according to ref. 4.

Z(OMe)-Val-Ala-OMe.—A mixed anhydride [from Z(OMe)-Val-OH (23.90 g, 85 mmol)] in dry THF (200 ml) was added to an ice-chilled solution of H-Ala-OMe [from the hydrochloride (10.70 g, 85 mmol) with Et₃N (11.7 ml, 85 mmol)] in DMF (80 ml) and the mixture was stirred in an ice-bath for 1 h and at room temperature for 4 h. The solvent was evaporated off and the residue was purified by procedure A followed by recrystallization from AcOEt and ether to give the *protected dipeptide ester* (19.91 g, 64%), m.p. 167–169 °C, [α]_D²⁰ –43.1° (c, 0.8 in MeOH), R_{F1} 0.85 (Found: C, 58.95; H, 7.3; N, 7.8. C₁₈H₂₆N₂O₆ requires C, 59.00; H, 7.15; N, 7.65%).

Z(OMe)-Val-Ala-NHNH₂ (4).—Z(OMe)-Val-Ala-OMe (19.90 g, 54 mmol) in MeOH (150 ml) was treated with 80% hydrazine hydrate (27.0 ml, 8 mol equiv.) for 24 h. Precipitation of the resulting solid from DMF with MeOH gave the *dipeptide hydrazide* (17.42 g, 88%), m.p. 240–243 °C, [α]_D²² –6.6° (c, 0.7 in DMSO), R_{F1} 0.54 (Found: C, 55.6; H, 7.4; N, 15.3. C₁₇H₂₆N₄O₅ requires C, 55.72; H, 7.15; N, 15.29%).

Z(OMe)-Ile-Ile-OMe.—A mixed anhydride [from Z(OMe)-Ile-OH (16.50 g, 56 mmol)] in dry THF (100 ml) was added to an ice-chilled solution of H-Ile-OMe [from the hydrochloride (10.20 g, 56 mmol) with Et₃N (7.7 ml, 56 mmol)] in DMF (100 ml). After stirring in an ice-bath for 1 h and at room temperature for 4 h, the solution was concentrated and the residue was purified by procedure A followed by recrystallization from AcOEt–ether to give the *protected dipeptide ester* (16.31 g, 69%), m.p. 114–117 °C, [α]_D²² –19.6° (c, 1.0 in MeOH), R_{F1} 0.98, R_{F2} 0.87 (Found: C, 62.45; H, 8.25; N, 6.8. C₂₂H₃₄N₂O₆ requires C, 62.54; H, 8.11; N, 6.63%).

Z(OMe)-Ile-Ile-NHNH₂ (5).—Z(OMe)-Ile-Ile-OMe (16.30 g, 39 mmol) in MeOH (150 ml) was treated with 80% hydrazine hydrate (19.5 ml, 8 mol equiv.) at 50 °C for 5 h and then at room temperature overnight. The resulting solid was precipitated from DMF with MeOH to give the *hydrazide* (12.02 g, 73%), m.p. 234–237 °C, [α]_D²² –4.5° (c, 1.1 in DMSO), R_{F1} 0.56 (Found: C, 59.7; H, 8.2; N, 13.15. C₂₁H₃₄N₄O₅ requires C, 59.69; H, 8.11; N, 13.26%). No D-allo-Ile was detected in the 6N-HCl (24 h) hydrolysate.

Z(OMe)-Lys(Z)-His-OMe.—DCC (22.09 g, 0.11 mol) was added to a mixture of Z(OMe)-Lys(Z)-OH (44.45 g, 0.1 mol) and H-His-OMe [from the hydrochloride (20.52 g, 0.1 mol) with Et₃N (27.6 ml, 0.2 mol)] in DMF (350 ml) and the solution, after stirring for 48 h, was filtered; the filtrate was concentrated and the residue was treated with ether and 5% NaHCO₃ to afford a powder, which was washed with 5% NaHCO₃, H₂O, and ether. The product was further treated with MeOH containing 3% AcOH at 50 °C for 7 h and recrystallized from MeOH–AcOEt to give the *dipeptide ester* (44.55 g, 75%), m.p. 140–142 °C, [α]_D²² +7.8° (c, 0.9 in DMF), R_{F1} 0.60 (Found: C, 60.75; H, 6.2; N, 11.45. C₃₀H₃₇N₅O₈ requires C, 60.49; H, 6.26; N, 11.76%).

Z(OMe)-Asn-Lys(Z)-His-OMe.—Z(OMe)-Lys(Z)-His-OMe (29.78 g, 50 mmol) was treated with TFA–anisole (66 ml; 52:14 v/v) as usual and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 h, and dissolved in DMF (200 ml) together with Et₃N (13.8 ml, 0.1 mol) and Z(OMe)-Asn-ONP (25.0 g, 60 mmol). After 48 h, evaporation of the solvent followed by trituration of the residue with

AcOEt afforded a powder, which was washed with 5% Na_2CO_3 and H_2O and then recrystallized from MeOH-AcOEt to give the *protected tripeptide ester* (25.47 g, 72%), m.p. 184–187 °C, $[\alpha]_D^{22} +4.6^\circ$ (*c*, 0.9 in DMF), R_F 0.43 (Found: C, 56.4; H, 6.2; N, 13.55. $\text{C}_{34}\text{H}_{43}\text{N}_7\text{O}_{10}\cdot\text{H}_2\text{O}$ requires C, 56.11; H, 6.23; N, 13.47%).

Z(OMe)-Asn-Lys(Z)-His-NHNH₂, *Z(OMe)-(RNase 103–105)-NHNH₂* (6).—*Z(OMe)-Asn-Lys(Z)-His-OMe* (14.90 g, 21 mmol) in MeOH (150 ml) was treated with 80% hydrazine hydrate (8.4 ml, 6.4 mol equiv.) overnight. A gelatinous mass was formed which was crystallized from MeOH to give the *tripeptide hydrazide* (12.40 g, 83%), m.p. 180–181 °C, $[\alpha]_D^{23} -6.9^\circ$ (*c*, 0.6 in DMSO), R_F 0.48. Amino-acid analysis: Asp 1.10, Lys 1.00, His 0.86 (average recovery 90%) (Found: C, 54.7; H, 6.2; N, 17.2. $\text{C}_{33}\text{H}_{43}\text{N}_9\text{O}_9\cdot\text{H}_2\text{O}$ requires C, 54.46; H, 6.23; N, 17.32%).

Z-Thr-Gln-Ala-OMe.—In the presence of 1*N*-HCl (40 ml, 1 mol equiv.), *Z-Gln-Ala-OMe* (16.02 g, 40 mmol) in THF (200 ml) was hydrogenated over Pd in the usual manner for 8 h. The filtered solution was neutralized with Et_3N (5.5 ml, 40 mmol). After addition of *Z-Thr-OPCP* (21.96 g, 44 mmol) and Et_3N (5.5 ml, 40 mmol), the mixture was stirred for 48 h and the solvent was evaporated off. Treatment of the residue with ether afforded a fine powder, which was purified by procedure B followed by recrystallization from MeOH to give the *protected tripeptide ester* (10.82 g, 58%), m.p. 199–202 °C, $[\alpha]_D^{22} -9.0^\circ$ (*c*, 1.0 in DMF), R_F 0.32, R_F 0.58 (Found: C, 54.15; H, 6.65; N, 12.05. $\text{C}_{21}\text{H}_{30}\text{N}_4\text{O}_8$ requires C, 54.07; H, 6.48; N, 12.01%).

Z-Thr-Thr-Gln-Ala-OMe.—In the presence of 1*N*-HCl (44 ml, 1 mol equiv.), *Z-Thr-Gln-Ala-OMe* (20.53 g, 44 mmol) in THF (150 ml) was hydrogenated over a Pd catalyst as mentioned above. To this filtered solution were added Et_3N (12.1 ml, 88 mmol) and *Z-Thr-OPCP* (23.95 g, 48 mmol) in DMF (120 ml) and the mixture was stirred for 48 h. Evaporation of the solvent followed by trituration of the residue with AcOEt afforded a gelatinous mass, which was purified by procedure B. Recrystallization from MeOH afforded the *protected tetrapeptide ester* (18.92 g, 76%), m.p. 238–239 °C, $[\alpha]_D^{22} +4.9^\circ$ (*c*, 1.0 in DMF), R_F 0.46 (Found: C, 52.75; H, 6.75; N, 12.4. $\text{C}_{25}\text{H}_{37}\text{N}_5\text{O}_{10}$ requires C, 52.25; H, 6.58; N, 11.72%).

Z(OMe)-Lys(Z)-Thr-Thr-Gln-Ala-OMe.—*Z-Thr-Thr-Gln-Ala-OMe* (8.82 g, 16 mmol) in MeOH (80 ml) containing 1*N*-HCl (16 ml, 1 mol equiv.) was hydrogenated as above. The filtered solution was concentrated and the residue was dissolved in DMF (100 ml) together with Et_3N (4.3 ml, 31 mmol) and *Z(OMe)-Lys(Z)-OTCP* (10.70 g, 17 mmol). After stirring for 48 h, the solution was concentrated. Trituration of the residue with AcOEt afforded a gelatinous solid, which was purified by procedure B followed by precipitation from DMF with AcOEt to give the *protected pentapeptide ester* (8.87 g, 67%), m.p. 169–172 °C, $[\alpha]_D^{22} +3.9^\circ$ (*c*, 1.2 in DMF), R_F 0.39 (Found: C, 54.65; H, 7.0; N, 11.3. $\text{C}_{40}\text{H}_{57}\text{N}_7\text{O}_{14}\cdot\text{H}_2\text{O}$ requires C, 54.72; H, 6.77; N, 11.17%).

Z(OMe)-Lys(Z)-Thr-Thr-Gln-Ala-NHNH₂, *Z(OMe)-(RNase 98–102)-NHNH₂* (7).—*Z(OMe)-Lys(Z)-Thr-Gln-Ala-OMe* (8.60 g, 10 mmol) in DMF (60 ml) was treated with 80% hydrazine hydrate (6.2 ml, 10 mol equiv.) overnight. The resulting gelatinous mass was collected by filtration and washed with MeOH to give the *hydrazide* (7.78 g, 90%), m.p. 206–209 °C, $[\alpha]_D^{22} +0.8^\circ$ (*c*, 0.9 in DMSO), R_F 0.16. Amino-acid analysis: Lys 1.14, Thr 2.12, Glu 1.10, Ala 1.00 (average recovery 83%) (Found: C,

53.45; H, 6.9; N, 14.5. $\text{C}_{39}\text{H}_{57}\text{N}_9\text{O}_{13}\cdot\text{H}_2\text{O}$ requires C, 53.35; H, 6.77; N, 14.36%).

Z(OMe)-Cys(MBzl)-Ala-Tyr-OMe.—*Z-Ala-Tyr-OMe* (15 (20.02 g, 50 mmol) in MeOH (150 ml) and 1*N*-HCl (50 ml, 1 mol equiv.) was hydrogenated over Pd for 8 h. The catalyst was removed by filtration, the filtrate was concentrated, and the residue was dissolved in DMF (150 ml). Et_3N (6.9 ml, 50 mmol), *Z(OMe)-Cys(MBzl)-OH* (20.27 g, 50 mmol), and DCC (11.30 g, 55 mmol) were successively added and the mixture was stirred for 48 h. The solution was filtered, the filtrate was concentrated, and the resulting solid residue was purified by procedure B followed by recrystallization from dioxan-MeOH to give the *protected tripeptide ester* (21.05 g, 66%), m.p. 182–185 °C, $[\alpha]_D^{23} -8.5^\circ$ (*c*, 1.1 in DMF), R_F 0.73 (Found: C, 60.4; H, 6.0; N, 6.55. $\text{C}_{33}\text{H}_{39}\text{N}_3\text{O}_9\text{S}$ requires C, 60.63; H, 6.01, 6.43%).

Z(OMe)-Asn-Cys(MBzl)-Ala-Tyr-OMe.—*Z(OMe)-Cys-(MBzl)-Ala-Tyr-OMe* (16.22 g, 25 mmol) was treated with TFA-anisole (25 ml; 4 : 1 v/v) as usual and dry ether was added. The resulting oily precipitate was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (160 ml) together with Et_3N (6.9 ml, 50 mmol) and *Z(OMe)-Asn-ONP* (10.34 g, 25 mmol). After stirring for 48 h, the solution was concentrated and the solid residue was purified by procedure B followed by precipitation from DMF with AcOEt to give the *protected tetrapeptide ester* (16.33 g, 86%), m.p. 210–213 °C, $[\alpha]_D^{22} -16.3^\circ$ (*c*, 1.0 in DMF), R_F 0.54 (Found: C, 57.4; H, 6.0; N, 9.25. $\text{C}_{37}\text{H}_{45}\text{N}_5\text{O}_{11}\text{S}$ requires C, 57.87; H, 5.90; N, 9.12%).

Z(OMe)-Pro-Asn-Cys(MBzl)-Ala-Tyr-OMe.—*Z(OMe)-Asn-Cys(MBzl)-Ala-Tyr-OMe* (11.63 g, 15 mmol) was treated with TFA-anisole (21 ml; 5 : 2 v/v) as usual and dry ether was added. The resulting powder was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (100 ml) together with Et_3N (4.1 ml, 30 mmol) and *Z(OMe)-Pro-ONP* (6.67 g, 17 mmol). After stirring for 48 h, the solution was concentrated and the residue was treated with ether. The resulting powder was purified by procedure B followed by precipitation from DMF with AcOEt to give the *protected pentapeptide ester* (11.48 g, 89%), m.p. 156–159 °C, $[\alpha]_D^{23} -33.4^\circ$ (*c*, 0.8 in DMF), R_F 0.46 (Found: C, 57.15; H, 6.05; N, 9.6. $\text{C}_{42}\text{H}_{52}\text{N}_8\text{O}_{12}\text{S}\cdot\text{H}_2\text{O}$ requires C, 57.13; H, 6.16; N, 9.95%).

Z(OMe)-Tyr-Pro-Asn-Cys(MBzl)-Ala-Tyr-OMe.—The above protected pentapeptide ester (16.66 g, 19 mmol) was treated with TFA-anisole (68 ml; 50 : 18 v/v) as usual and the N^α -deprotected peptide isolated as stated above was dissolved in DMF (120 ml) containing Et_3N (5.2 ml, 38 mmol). To this ice-chilled solution was added the azide [from *Z(OMe)-Tyr-NHNH₂* (8.34 g, 23 mmol)] in DMF (50 ml). After stirring for 24 h, the solution was concentrated and the residue was triturated with 5% citric acid and ether. The resulting powder was purified by procedure B followed by recrystallization from MeOH to give the *hexapeptide ester* (17.88 g, 89%), m.p. 139–144 °C, $[\alpha]_D^{23} -33.9^\circ$ (*c*, 1.0 in DMF), R_F 0.61 (Found: C, 58.4; H, 5.9; N, 9.4. $\text{C}_{51}\text{H}_{61}\text{N}_7\text{O}_{14}\text{S}\cdot\text{H}_2\text{O}$ requires C, 58.54; H, 6.07; N, 9.39%).

Z(OMe)-Tyr-Pro-Asn-Cys(MBzl)-Ala-Tyr-NHNH₂, *Z(OMe)-(RNase 92–97)-NHNH₂* (8).—The above protected hexapeptide ester (15.31 g, 15 mmol) dissolved in DMF-MeOH (150 ml; 1 : 2 v/v) was treated with 80% hydrazine hydrate (7.3 ml, 8 mol equiv.) overnight. Precipitation of the resulting mass from DMF with MeOH afforded the *hydrazide* (13.29 g, 87%), m.p. 174–177 °C, $[\alpha]_D^{21} -38.8^\circ$

(*c*, 0.8 in DMSO), R_F , 0.72. Amino-acid analysis: Tyr 1.96, Pro 1.00, Asp 1.18, Ala 1.20 (average recovery 82%) (Found: C, 57.2; H, 5.85; N, 12.25. $C_{50}H_{81}N_9O_{13} \cdot H_2O$ requires C, 57.40; H, 6.07; N, 12.05%).

Z(OMe)-Ser-Lys(Z)-OMe.—The azide [from *Z(OMe)-Ser-NHNH₂* (16.20 g, 57 mmol)] in DMF (80 ml) and Et_3N (7.9 ml, 57 mmol) were added to a solution of *H-Lys(Z)-OMe* [from the hydrochloride (18.90 g, 57 mmol) with Et_3N (7.9 ml, 57 mmol)] in DMF (150 ml). After stirring for 24 h, the solution was concentrated. The residue was purified by procedure A followed by recrystallization from AcOEt and ether to give the *protected dipeptide ester* (20.61 g, 66%), m.p. 104–106 °C, $[\alpha]_D^{23} - 6.9^\circ$ (*c*, 1.0 in DMF), R_F , 0.88 (Found: C, 59.7; H, 6.45; N, 7.7. $C_{27}H_{35}N_3O_9$ requires C, 59.44; H, 6.47; N, 7.70%).

Z(OMe)-Ser-Ser-Lys(Z)-OMe.—*Z(OMe)-Ser-Lys(Z)-OMe* (20.41 g, 37 mmol) was treated with TFA–anisole (100 ml; 4 : 1 v/v) as usual and dry ether was added. The resulting powder was dissolved in DMF (150 ml) containing Et_3N (5.1 ml, 37 mmol). To this ice-chilled solution were added the azide [from *Z(OMe)-Ser-NHNH₂* (11.64 g, 41 mmol)] in DMF (50 ml) and Et_3N (5.1 ml, 37 mmol). After stirring for 24 h, the solution was concentrated and the residue was purified by procedure A followed by recrystallization from MeOH–ether to give the *protected tripeptide ester* (21.40 g, 91%), m.p. 94–96 °C, $[\alpha]_D^{22} + 7.0^\circ$ (*c*, 0.9 in DMF), R_F , 0.67 (Found: C, 56.65; H, 6.45; N, 9.1. $C_{30}H_{40}N_4O_{11}$ requires C, 56.95; H, 6.37; N, 8.86%).

Z(OMe)-Ser-Ser-Lys(Z)-NHNH₂, *Z(OMe)-(RNase 89–91)-NHNH₂* (9).—*Z(OMe)-Ser-Ser-Lys(Z)-OMe* (21.10 g, 33 mmol) in MeOH (200 ml) was treated with 80% hydrazine hydrate. Precipitation of the resulting mass from DMF with MeOH gave the *hydrazide* (19.03 g, 87%), m.p. 204–206 °C, $[\alpha]_D^{22} + 4.7^\circ$ (*c*, 0.8 in DMSO), R_F , 0.39. Amino-acid analysis: Ser 1.62, Lys 1.00 (average recovery 90%) (Found: C, 54.75; H, 6.2; N, 13.5. $C_{29}H_{40}N_6O_{10}$ requires C, 55.05; H, 6.37; N, 13.28%).

Z(OMe)-Val-Ala-Cys(MBzl)-Glu(OBzl)-Gly-Asn-Pro-Tyr-Val-Pro-Val-His-Phe-Asp(OBzl)-Ala-Ser-Val-OBzl, *Z(OMe)-(RNase 108–124)-OBzl*.—*Z(OMe)-(RNase 110–124)-OBzl* (12.90 g, 5.9 mmol) was treated with TFA–anisole (50 ml; 4 : 1 v/v) as usual and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMF (100 ml) containing Et_3N (1.63 ml, 11.8 mmol). To this ice-chilled solution were added the azide [from *Z(OMe)-Val-Ala-NHNH₂* (2.59 g, 7.1 mmol)] in DMF (10 ml) and Et_3N (0.98 ml, 7.1 mmol). After stirring for 48 h, the solution was concentrated. Trituration of the residue with AcOEt and H_2O afforded a powder, which was purified by procedure B followed by precipitation twice from DMF with MeOH to give the *protected heptadecapeptide ester* (10.10 g, 72%), m.p. 238–243 °C, $[\alpha]_D^{22} - 50.9^\circ$ (*c*, 0.8 in DMSO), R_F , 0.46 (Found: C, 59.75; H, 6.75; N, 11.65; S, 1.5. $C_{110}H_{152}N_{20}O_{29}S \cdot 2H_2O$ requires C, 59.68; H, 6.57; N, 11.70; S, 1.34%).

Z(OMe)-Ile-Ile-Val-Ala-Cys(MBzl)-Glu(OBzl)-Gly-Asn-Pro-Tyr-Val-Pro-Val-His-Phe-Asp(OBzl)-Ala-Ser-Val-OBzl, *Z(OMe)-(RNase 106–124)-OBzl*.—*Z(OMe)-(RNase 108–124)-OBzl* (12.01 g, 5 mmol) was treated with TFA–anisole (52 ml; 10 : 3 v/v) and the N^α -deprotected peptide, isolated as stated above, was then dissolved in DMF–HMPA (100 ml; 3 : 2 v/v) containing Et_3N (1.38 ml, 10 mmol). To this ice-chilled solution were added the azide [from *Z(OMe)-Ile-Ile-NHNH₂* (5.08 g, 12 mmol)] in DMF (20 ml) and Et_3N (1.66 ml, 12 mmol). After stirring for

48 h, the solution was concentrated and the residue was treated with 5% $NaHCO_3$ and ether to afford a fine powder, which was purified by procedure B followed by precipitation twice from DMSO with MeOH to give the *protected nonadecapeptide ester* (10.37 g, 79%), m.p. 249–259 °C, $[\alpha]_D^{22} - 36.8^\circ$ (*c*, 0.8 in DMSO), R_F , 0.50 (Found: C, 60.05; H, 6.8; N, 11.8. $C_{131}H_{174}N_{22}O_{31}S \cdot 2H_2O$ requires C, 60.01; H, 6.85; N, 11.76%).

Z(OMe)-Asn-Lys(Z)-His-Ile-Ile-Val-Ala-Cys(MBzl)-Glu(OBzl)-Gly-Asn-Pro-Tyr-Val-Pro-Val-His-Phe-Asp(OBzl)-Ala-Ser-Val-OBzl, *Z(OMe)-(RNase 103–124)-OBzl*.—*Z(OMe)-(RNase 106–124)-OBzl* (12.03 g, 4.6 mmol) was treated with TFA–anisole (60 ml; 4 : 1 v/v) and the N^α -deprotected peptide, isolated as mentioned above, was dissolved in DMF–HMPA (30 ml; 3 : 1 v/v) containing Et_3N (1.3 ml, 9.2 mmol). To this ice-chilled solution were added the azide [from *Z(OMe)-Asn-Lys(Z)-His-NHNH₂* (8.02 g, 11 mmol)] in DMF (80 ml) and *N*-methylmorpholine (1.21 ml, 11 mmol). After stirring for 48 h, the solution was concentrated and the residue was treated with 5% $NaHCO_3$ to give a fine powder, which was purified by procedure B followed by precipitation twice from DMSO with MeOH to give the *protected docosapeptide ester* (11.72 g, 81%), m.p. 251–256 °C, $[\alpha]_D^{23} - 35.5^\circ$ (*c*, 0.9 in DMSO), R_F , 0.61, R_F , 0.85 (Found: C, 59.0; H, 6.95; N, 13.15; S, 1.3. $C_{155}H_{205}N_{29}O_{37}S \cdot 3H_2O$ requires C, 59.05; H, 6.75; N, 12.89; S, 1.02%).

Z(OMe)-Lys(Z)-Thr-Thr-Gln-Ala-Asn-Lys(Z)-His-Ile-Ile-Val-Ala-Cys(MBzl)-Glu(OBzl)-Gly-Asn-Pro-Tyr-Val-Pro-Val-His-Phe-Asp(OBzl)-Ala-Ser-Val-OBzl, *Z(OMe)-(RNase 98–124)-OBzl*.—*Z(OMe)-(RNase 103–124)-OBzl* (13.00 g, 4.1 mmol) was treated with TFA–anisole (63 ml; 50 : 13 v/v) and the N^α -deprotected peptide isolated as mentioned above was dissolved in DMF–DMSO (100 ml; 1 : 1 v/v) containing Et_3N (1.71 ml, 12.4 mmol). To this ice-chilled solution were added the azide [from *Z(OMe)-Lys(Z)-Thr-Thr-Gln-Ala-NHNH₂* (9.04 g, 10.3 mmol)] in DMF (60 ml) and Et_3N (1.42 ml, 10.3 mmol). After stirring for 48 h, the solution was concentrated and the residue was treated with 5% $NaHCO_3$ and ether to give a fine powder, which was purified by procedure B followed by precipitation twice from DMSO with MeOH to give the *protected heptacosapeptide ester* (14.09 g, 89%), m.p. 250–256 °C, $[\alpha]_D^{23} - 35.9^\circ$ (*c*, 1.0 in DMSO), R_F , 0.54, R_F , 0.78 (Found: C, 57.7; H, 6.65; N, 12.85; S, 1.15. $C_{185}H_{250}N_{36}O_{47}S \cdot 4H_2O$ requires C, 57.95; H, 6.78; N, 13.15; S, 0.84%).

Z(OMe)-Tyr-Pro-Asn-Cys(MBzl)-Ala-Tyr-Lys(Z)-Thr-Thr-Gln-Ala-Asn-Lys(Z)-His-Ile-Ile-Val-Ala-Cys(MBzl)-Glu(OBzl)-Gly-Asn-Pro-Tyr-Val-Pro-Val-His-Phe-Asp(OBzl)-Ala-Ser-Val-OBzl, *Z(OMe)-(RNase 92–124)-OBzl*.—*Z(OMe)-(RNase 98–124)-OBzl* (13.88 g, 3.6 mmol) was treated with TFA–anisole (63 ml; 50 : 13 v/v) and the N^α -deprotected peptide, isolated as above, was dissolved in DMF–DMSO (90 ml; 1 : 1 v/v) containing Et_3N (1.50 ml, 10.9 mmol). To this ice-chilled solution were added the azide [from *Z(OMe)-Tyr-Pro-Asn-Cys(MBzl)-Ala-Tyr-NHNH₂* (9.30 g, 9 mmol)] in DMF (50 ml) and Et_3N (1.25 ml, 9 mmol). After stirring for 48 h, the solution was concentrated and the residue was treated with ether and 5% $NaHCO_3$. The resulting powder was purified by procedure B followed by precipitation three times from DMSO with MeOH to give the *protected tritriacontapeptide ester* (14.89 g, 87%), m.p. 236–241 °C, $[\alpha]_D^{23} - 40.0^\circ$ (*c*, 0.8 in DMSO), R_F , 0.71 (Found: C, 57.25; H, 6.45; N, 12.8. $C_{228}H_{299}N_{43}O_{57}S_2 \cdot 8H_2O$ requires C, 57.28; H, 6.66; N, 12.71%).

Z(OMe)-Ser-Ser-Lys(Z)-Tyr-Pro-Asn-Cys(MBzl)-Ala-Tyr-Lys(Z)-Thr-Thr-Gln-Ala-Asn-Lys(Z)-His-Ile-Ile-Val-Ala-Cys(MBzl)-Glu(OBzl)-Gly-Asn-Pro-Tyr-Val-Pro-Val-His-Phe-Asp(OBzl)-Ala-Ser-Val-OBzl, Z(OMe)-(RNase 89—124)-OBzl.—*Z(OMe)-(RNase 92—124)-OBzl* (12.20 g, 2.6 mmol) was treated with TFA–anisole (62 ml; 25:6 v/v) and the N^{α} -deprotected peptide isolated as stated above was dissolved in DMF–DMSO (100 ml; 1:1 v/v) containing Et_3N (1.06 ml, 7.7 mmol). To this ice-chilled solution were added the azide [from *Z(OMe)-Ser-Ser-Lys(Z)-NHNH_2* (4.08 g, 6.4 mmol)] in DMF (30 ml) and Et_3N (0.89 ml, 6.4 mmol). After stirring for 48 h, the solution was concentrated. The residue was purified as above and finally precipitated three times from DMSO with AcOEt to give the *protected hexatriacontapeptide ester* (12.33 g, 93%), m.p. 241–250°, $[\alpha]_D^{23} -36.1^\circ$ (*c*, 0.8 in DMSO), R_{F_1} 0.76 (Found: C, 57.26; H, 6.52; N, 12.51; S, 1.44. $\text{C}_{246}\text{H}_{327}\text{N}_{47}\text{O}_{64}\text{S}_2 \cdot 7\text{H}_2\text{O}$ requires C, 57.29; H, 6.67; N, 12.77; S, 1.24%).

Trial Experiments.—*Z-Ile-Val-Ala-OMe.* *Z-Val-Ala-OMe* ²⁴ (4.70 g, 14 mmol) was treated with 2.7*N*-HBr–AcOH (12 ml) at room temperature for 40 min. The HBr salt precipitated by ether was dissolved in DMF (25 ml) together with Et_3N (1.9 ml, 14 mmol) and *Z-Ile-OH* (3.71 g, 14 mmol). After addition of DCC (3.07 g, 15 mmol), the mixture was stirred for 24 h, filtered, and the filtrate was concentrated. Treatment of the residue with ether afforded a gelatinous mass, which was purified by procedure B followed by recrystallization from MeOH to give the *protected tripeptide ester* (4.72 g, 75%), m.p. 190–194°C, $[\alpha]_D^{23} -8.7^\circ$ (*c*, 0.6 in DMF), R_{F_1} 0.77 (Found: C, 61.9; H, 7.95; N, 9.65. $\text{C}_{23}\text{H}_{35}\text{N}_3\text{O}_6$ requires C, 61.45; H, 7.85; N, 9.35%).

Z(OMe)-Ile-Ile-Val-Ala-OMe. *Z-Ile-Val-Ala-OMe* (1.53 g, 3.4 mmol) in THF (30 ml) containing 1*N*-HCl–dioxan (3.4 ml) was hydrogenated over Pd for 8 h and the catalyst removed by filtration. To this filtrate were added Et_3N (0.47 ml, 3.4 mmol), *Z(OMe)-Ile-OH* (1.01 g, 3.4 mmol), and DCC (0.77 g, 3.7 mmol), and the mixture was stirred overnight. After filtration, the filtrate was concentrated. Trituration of the residue with AcOEt afforded a powder which was purified by procedure B followed by precipitation from DMF with AcOEt to give the *protected tetrapeptide ester* (1.34 g, 66%), m.p. 241–246°C, $[\alpha]_D^{22} -25.0^\circ$ (*c*, 1.1 in DMSO), R_{F_1} 0.52, R_{F_3} 0.61 (Found: C, 60.5; H, 8.4; N, 9.5. $\text{C}_{30}\text{H}_{48}\text{N}_4\text{O}_8$ requires C, 60.79; H, 8.16; N, 9.45%).

Z(OMe)-Ile-Ile-Val-Ala-NHNH_2 (positions 106–109). *Z(OMe)-Ile-Ile-Val-Ala-OMe* (0.84 g, 1.4 mmol) in DMF (8 ml) was treated with 80% hydrazine hydrate (0.8 ml, 9 mol equiv.) overnight. A gelatinous mass formed which was washed with MeOH to give the *hydrazide*, which had low solubility even in hot DMF (0.77 g, 92%), m.p. 277°C (decomp.), $[\alpha]_D^{22} -29.3^\circ$ (*c*, 0.6 in DMSO), R_{F_1} 0.44 (Found: C, 59.0; H, 8.35; N, 14.3. $\text{C}_{29}\text{H}_{48}\text{N}_6\text{O}_7$ requires C, 58.76; H, 8.16; N, 14.18%).

Z(OMe)-Pro-Asn-Cys(MBzl)-Ala-Tyr-NHNH_2 (positions 93–97). *Z(OMe)-Pro-Asn-Cys(MBzl)-Ala-Tyr-OMe* (8.83 g, 10 mmol) in DMF (50 ml) was treated with 80% hydrazine hydrate (6 ml, 10 mol equiv.) overnight. A gelatinous mass, formed by addition of MeOH, was collected by filtration and washed with MeOH to give the *hydrazide* (7.62 g, 86%), m.p. 208–211°C, $[\alpha]_D^{24} -38.5^\circ$ (*c*, 0.9 in DMSO), R_{F_1} 0.44, R_{F_3} 0.89 (Found: C, 56.1; H, 6.35; N, 12.7. $\text{C}_{41}\text{H}_{52}\text{N}_8\text{O}_{11}\text{S} \cdot \text{H}_2\text{O}$ requires C, 55.76; H, 6.16; N, 12.69%).

Z(OMe)-Lys(Z)-Tyr-OMe. DCC (4.08 g, 20 mmol) was

added to a mixture of *Z(OMe)-Lys(Z)-OH* (8.88 g, 20 mmol) and *H-Tyr-OMe* [from the hydrochloride (4.64 g, 20 mmol) with Et_3N (2.8 ml, 20 mmol)] in DMF (80 ml). After 24 h, the solution was filtered, the filtrate was concentrated, and the residue was purified by procedure A. Recrystallization from AcOEt–ether afforded the *dipeptide ester* (4.39 g, 55%), m.p. 106–109°C, $[\alpha]_D^{22} -7.3^\circ$ (*c*, 1.0 in MeOH), R_{F_1} 0.62 (Found: C, 64.05; H, 6.55; N, 6.9. $\text{C}_{33}\text{H}_{39}\text{N}_3\text{O}_9$ requires C, 63.75; H, 6.32; N, 6.76%).

Z(OMe)-Ser-Lys(Z)-Tyr-OMe. *Z(OMe)-Lys(Z)-Tyr-OMe* (2.46 g, 6.2 mmol) was treated with TFA–anisole (7 ml; 5:2 v/v) as usual and dry ether was added. The oily precipitate was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (40 ml) together with Et_3N (1.7 ml, 12 mmol) and *Z(OMe)-Ser-OPCP* (3.84 g, 7.4 mmol). After stirring for 24 h, the solution was concentrated and the residue was purified by procedure A. Recrystallization from dioxan–MeOH gave the *protected tripeptide ester* (2.99 g, 68%), m.p. 178–181°C, $[\alpha]_D^{22} -2.3^\circ$ (*c*, 0.9 in DMF), R_{F_1} 0.76 (Found: C, 60.7; H, 6.45; N, 7.9. $\text{C}_{36}\text{H}_{44}\text{N}_4\text{O}_{11}$ requires C, 61.00; H, 6.26; N, 7.91%).

Z(OMe)-Ser-Ser-Lys(Z)-Tyr-OMe. *Z(OMe)-Ser-Lys(Z)-Tyr-OMe* (2.13 g, 3 mmol) was treated with TFA–anisole (4.5 ml; 2:1 v/v) as usual and dry ether was added. The resulting powder was dissolved in DMF (20 ml) together with Et_3N (0.8 ml, 6 mmol) and *Z(OMe)-Ser-OPCP* (1.56 g, 3 mmol). After stirring for 24 h, the solution was concentrated and the residue was treated with AcOEt. The resulting powder was purified by procedure B and recrystallized from MeOH–AcOEt to afford the *protected tetrapeptide ester* (1.88 g, 79%), m.p. 134–137°C, $[\alpha]_D^{22} +6.9^\circ$ (*c*, 0.9 in DMF), R_{F_1} 0.55 (Found: C, 59.1; H, 6.3; N, 8.7. $\text{C}_{39}\text{H}_{49}\text{N}_5\text{O}_{13}$ requires C, 58.86; H, 6.20; N, 8.80%).

Z(OMe)-Ser-Ser-Lys(Z)-Tyr-NHNH_2 (positions 89–92). *Z(OMe)-Ser-Ser-Lys(Z)-Tyr-OMe* (1.26 g, 1.6 mmol) in MeOH (12 ml) was treated with 80% hydrazine hydrate (1.0 ml, 10 mol equiv.) overnight. A gelatinous mass formed which was collected by filtration and washed with MeOH to give the *hydrazide* (1.10 g, 87%), m.p. 206–208°C, $[\alpha]_D^{22} +2.7^\circ$ (*c*, 0.7 in DMSO), R_{F_1} 0.62 (Found: C, 56.6; H, 6.4; N, 11.4. $\text{C}_{38}\text{H}_{49}\text{N}_7\text{O}_{12} \cdot \text{H}_2\text{O}$ requires C, 56.07; H, 6.32; N, 12.04%).

Z(OMe)-Pro-Asn-Cys(MBzl)-Ala-Tyr-(98—124)-OBzl,

Z(OMe)-(RNase 93—124)-OBzl. *Z(OMe)-(RNase 98—124)-OBzl* (4.20 g, 1.1 mmol) was treated with TFA–anisole (19 ml; 15:4 v/v) and the N^{α} -deprotected peptide, isolated as above, was dissolved in DMF–DMSO (30 ml; 1:1 v/v) containing Et_3N (0.46 ml, 3.3 mmol). To this ice-chilled solution were added the azide [from *Z(OMe)-Pro-Asn-Cys(MBzl)-Ala-Tyr-NHNH_2* (2.37 g, 2.7 mmol)] in DMF (10 ml) and Et_3N (0.37 ml, 2.7 mmol). After stirring for 48 h, the solution was concentrated and the residue was treated with ether and 5% NaHCO_3 . The resulting powder was purified by procedure B followed by precipitation twice from DMSO with MeOH to give the *protected dotriacontapeptide ester* (3.86 g, 78%), m.p. 241–251°C, $[\alpha]_D^{22} -53.6^\circ$ (*c*, 0.9 in DMSO), R_{F_1} 0.61, R_{F_3} 0.79. Amino-acid analysis: Asp 4.03, Thr 1.66, Ser 0.94, Glu 2.06, Pro 3.04, Gly 1.00, Ala 3.92, Val 4.24, Ile 1.63, Tyr 1.87, Phe 1.00, Lys 1.70, His 1.97 (average recovery 86%) (Found: C, 57.35; H, 6.85; N, 13.05; S, 1.65. $\text{C}_{217}\text{H}_{302}\text{N}_{42}\text{O}_{55}\text{S}_2 \cdot 6\text{H}_2\text{O}$ requires C, 57.42; H, 6.71; N, 12.96; S, 1.41%).

Alternative Synthesis of Z(OMe)-(RNase 89—124)-OBzl.—The above protected dotriacontapeptide ester, *Z(OMe)-(RNase 93—124)-OBzl*, (3.86 g, 0.85 mmol) was

treated with TFA–anisole (20 ml; 4:1 v/v) and the N α -deprotected peptide, isolated as above, was dissolved in DMF–DMSO (20 ml; 1:1 v/v) containing Et₃N (0.35 ml, 2.6 mmol). To this ice-chilled solution, the azide [from Z(OMe)-Ser-Ser-Lys(Z)-Tyr-NHNH₂ (1.69 g, 2.13 mmol)] in DMF (15 ml) and Et₃N (0.29 ml, 2.13 mmol) were added. After 48 h, additional azide (3 mol equiv.) was added and the mixture was stirred for 48 h. The solvent was evaporated off and the product was isolated in essentially the same manner as above; 2.51 g (57%), m.p. 246–251 °C, $[\alpha]_D^{25} -29.6^\circ$ (c, 0.7 in DMSO), R_F 0.46, R_F 0.76. Amino-acid analysis: Asp 4.24, Thr 1.67, Ser 2.95, Glu 2.10, Pro 3.18, Gly 1.00, Ala 4.30, Val 4.08, Ile 1.70, Tyr 2.96, Phe 1.00, Lys 2.74, His 2.04 (average recovery 91%).

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