Total Synthesis of Bovine Pancreatic Ribonuclease A. Part 4.[1] Synthesis of the Protected Tetraoctacontapeptide Ester (Positions 41—124) †

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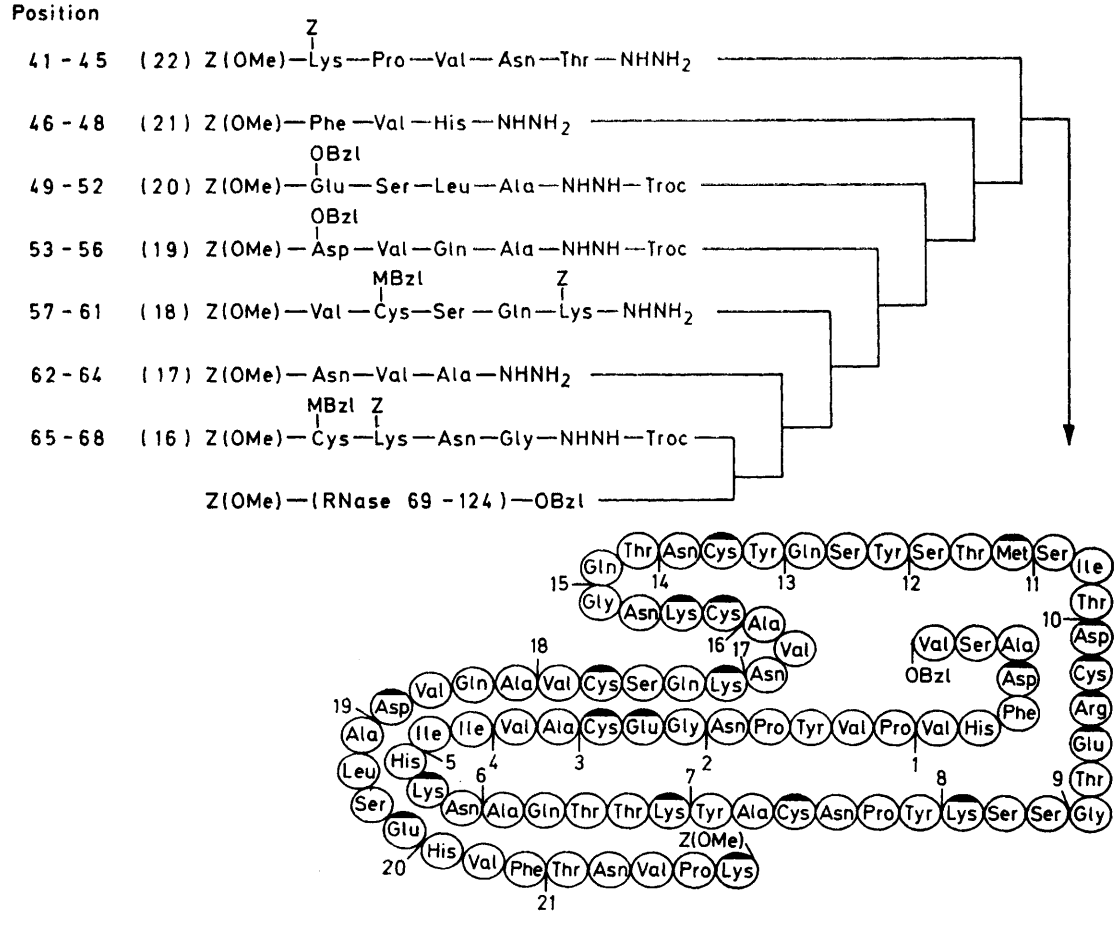
Commencing with the protected hexapentacontapeptide corresponding to the sequence Z(OMe)-(69—124)-OBzl of bovine pancreatic RNase A, chain elongation was carried out to the tetraoctacontapeptide, Z(OMe)-(RNase 41—124)-OBzl, by seven successive azide condensations of the peptide fragments Z(OMe)-Cys(MBzl)-Lys(Z)-Asn-Gly-NHNH-Troc (16), Z(OMe)-Asn-Val-Ala-$NHNH_2$ (17), Z(OMe)-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-$NHNH_2$ (18), Z(OMe)-Asp(OBzl)-Val-Gln-Ala-NHNH-Troc (19), Z(OMe)-Glu(OBzl)-Ser-Leu-Ala-NHNH-Troc (20), Z(OMe)-Phe-Val-His-$NHNH_2$ (21), and Z(OMe)-Lys(Z)-Pro-Val-Asn-Thr-$NHNH_2$ (22). The Troc group was removed from fragments (16), (19), and (20) by treatment with Zn prior to each condensation. T.l.c. assessment of the homogeneity of the products was not possible, due to lack of a suitable solvent system. The ratios of newly incorporated amino-acids in the acid hydrolysate to phenylalanine, however, gave an important clue to purity. The purity of Z(OMe)-(RNase 41—124)-OBzl was confirmed by gel-filtration on Sephacryl S-200.

In the preceding paper,[1c] we described the synthesis of the protected hexapentacontapeptide ester Z(OMe)-(RNase 69—124)-OBzl, together with a purification procedure for the protected peptides by chromatography on Sephacryl S-200. We now report the synthesis of the protected tetraoctacontapeptide ester Z(OMe)-(RNase 41—124)-OBzl, which was obtained by further chain elongation with seven peptide fragments as shown in Scheme 1.



SCHEME 1 Synthetic route to the protected tetraoctacontapeptide ester, Z(OMe)-(RNase 41—124)-OBzl

The tetrapeptide fragment selected, Z(OMe)-Cys-(MBzl)-Lys(Z)-Asn-Gly-NHNH-Troc (16) (positions 65—68), contains the Asn–Gly bond. Gráf *et al.*[2] and Riniker

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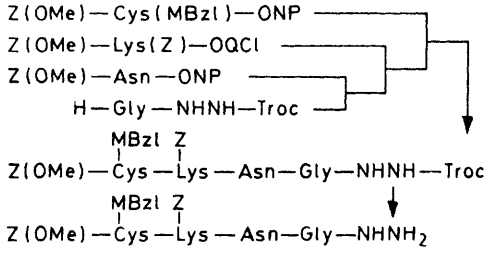
et al.[3] observed that this type of Asn residue has a great tendency to undergo preferential deamination under alkaline conditions. This fact led to the structural revision of porcine and human adrenocorticotropins.[2,3] This indicates that the hydrazide cannot be obtained unambiguously by the usual treatment of the corresponding ester with hydrazine. Thus the substituted hydrazine, Troc-$NHNH_2$,[4] mentioned previously, was used in the synthesis of this fragment. As shown in Scheme 2, Z(OMe)-Asn-ONP was allowed to condense with H-Gly-NHNH-Troc.[5] The resulting dipeptide, Z(OMe)-Asn-Gly-NHNH-Troc, was treated with TFA [6] and, after neutralization with Et_3N, was coupled to Z(OMe)-Lys(Z)-OH *via* the 5-chloro-8-quinolyl ester procedure [7] to give Z(OMe)-Lys(Z)-Asn-Gly-NHNH-Troc. This compound was alternatively prepared by the DCC–HOBT condensation [8] of Troc-$NHNH_2$ with Z(OMe)-Lys(Z)-Asn-Gly-OH, prepared similarly, but starting from glycine. In the latter procedure, the yield of Z(OMe)-Asn-Gly-OH was only 38%. Condensation of

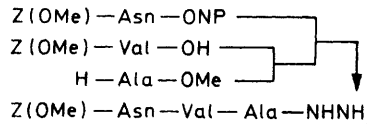


Scheme 2 Synthetic scheme for the protected tetrapeptide hydrazide derivative Z(OMe)-(RNase 65—68)-NHNH-Troc (16)

Z(OMe)-Cys(MBzl)-OH with the TFA-treated sample of the above tripeptide derivative by the NP method [9] afforded (16), from which the Troc group was removed by Zn to yield the protected tetrapeptide hydrazide Z(OMe)-Cys(MBzl)-Lys(Z)-Asn-Gly-$NHNH_2$.

The same tetrapeptide hydrazide unit was selected by Jenkins *et al.*[10] for the preparation of Boc-(RNase 65—85)-OMe. Although they mentioned the base-lability of the Asn–Gly bond, treatment of the tetrapeptide ester with hydrazine was performed and again the above henicosapeptide ester was exposed to hydrazine to prepare the corresponding hydrazide. Independently, alternative syntheses of fragments 65—68 and 65—72 were reported.[11] After our synthesis, we noted a report by Meienhofer *et al.*,[12] in which protected peptides with free carboxy-groups were converted into the hydrazides by DCC and hydrazine in the presence of HOBT.[8] We are convinced that the procedure we employed is the most suitable method for the preparation of base-sensitive hydrazides, because the risk of deamination accompanying exposure of such peptides to strongly basic hydrazine could be eliminated.

The next fragment, Z(OMe)-Asn-Val-Ala-$NHNH_2$(17) (positions 62—64), was prepared without particular difficulty by condensation of Z(OMe)-Asn-ONP with H-Val-Ala-OMe, derived from the known Z(OMe)-derivative,[1b] followed by the usual treatment with hydrazine (Scheme 3).

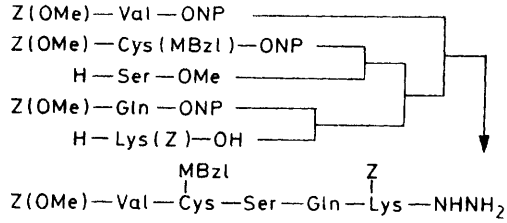


Scheme 3 Synthetic scheme for the protected tripeptide hydrazide Z(OMe)-(RNase 62—64)-$NHNH_2$ (17)

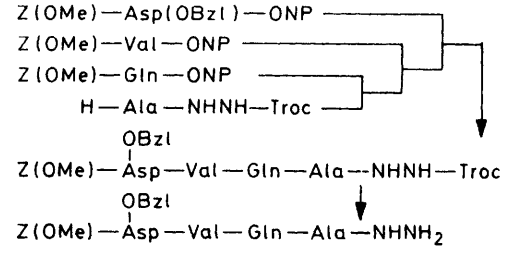
For the synthesis of the protected pentapeptide hydrazide Z(OMe)-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-NH-NH_2(18) (positions 57—61), two dipeptide units, Z(OMe)-Cys(MBzl)-Ser-$NHNH_2$ and H-Gln-Lys(Z)-OH (prepared by the NP method), were joined together by the Rudinger azide procedure [13] (Scheme 4). The latter dipeptide with a free carboxy-group was obtained more easily in pure form than the methyl ester. Introduction of Z(OMe)-Val-OH by the NP method followed by methylation and subsequent hydrazinolysis gave fragment (18). This fragment has a sterically hindered valine residue at the N-terminus, but this did not notably interfere with the condensation with the subsequent fragment (19).

Fragment (19), Z(OMe)-Asp(OBzl)-Val-Gln-Ala-NH-NH-Troc (positions 53—56), was prepared with the aid of the substituted hydrazine H-Ala-NHNH-Troc [14] (Scheme 5). The usual NP method served to introduce Z(OMe)-Gln-OH, Z(OMe)-Val-OH, and Z(OMe)-Asp(OBzl)-OH in a stepwise manner. The Troc group was removed from the resulting protected tetrapeptide derivative (19) with Zn, as above, to yield Z(OMe)-Asp(OBzl)-Val-Gln-Ala-$NHNH_2$.

The next fragment, Z(OMe)-Glu(OBzl)-Ser-Leu-Ala-NHNH-Troc (20) (positions 49—52), contains Glu(OBzl). This unit was prepared from the same substituted hydrazine derivative, H-Ala-NHNH-Troc, as employed for fragment (19). As shown in Scheme 6,

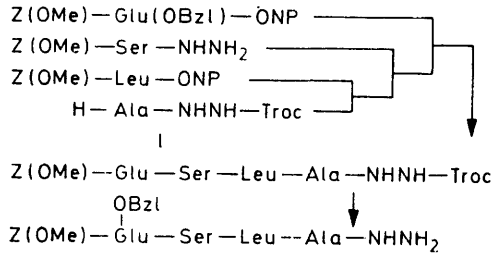


Scheme 4 Synthetic scheme for the protected pentapeptide hydrazide Z(OMe)-(RNase 57—61)-$NHNH_2$ (18)

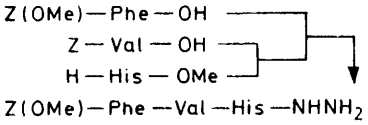


Scheme 5 Synthetic scheme for the protected tetrapeptide hydrazide derivative Z(OMe)-(RNase 53—56)-NHNH-Troc (19)

Z(OMe)-Leu-OH and Z(OMe)-Glu(OBzl)-OH were introduced by the NP method and Z(OMe)-Ser-$NHNH_2$ by the azide procedure. Treatment with Zn removed the Troc group from the resulting tetrapeptide derivative (20) without difficulty to yield Z(OMe)-Glu(OBzl)-Ser-Leu-Ala-$NHNH_2$.



SCHEME 6 Synthetic scheme for the protected tetrapeptide hydrazide derivative Z(OMe)-(RNase 49—52)-NHNH-Troc (20)



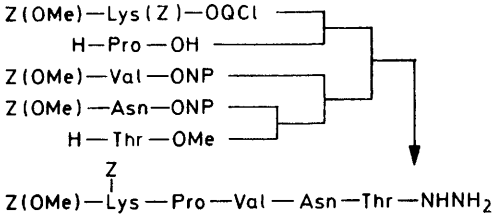
SCHEME 7 Synthetic scheme for the protected tripeptide hydrazide, Z(OMe)-(RNase 46—48)-$NHNH_2$ (21)

Z(OMe)-Phe-Val-His-$NHNH_2$ (21) (positions 46—48) was prepared starting from the known dipeptide Z-Val-His-OMe [15] which, after hydrogenolysis, was condensed with Z(OMe)-Phe-OH using DCC.[16] The product, after treatment with methanol in the presence of acetic acid for the reasons stated earlier,[17] was converted into the hydrazide (21), as shown in Scheme 7.

The next fragment, Z(OMe)-Lys(Z)-Pro-Val-Asn-Thr-$NHNH_2$ (22) (positions 41—45), was prepared by the PCP active-ester condensation [18] of Z(OMe)-Lys(Z)-Pro-OH and H-Val-Asn-Thr-OMe. The former active-ester is a known compound; [19] the latter was synthesized by two successive NP condensations starting with H-Thr-OMe (Scheme 8). The protected pentapeptide ester was subsequently converted into (22) by the usual hydrazine treatment.

These fragments were then assembled according to Scheme 1. Although these reactions were performed principally by the azide procedure mentioned in the preceding paper,[1c] we wish to mention some technical points regarding the lengthening of the peptide chain, with special reference to the reactivity, solubility, and homogeneity of the products.

In the course of the stepwise azide condensations from



SCHEME 8 Synthetic scheme for the protected pentapeptide hydrazide, Z(OMe)-(RNase 41—45)-$NHNH_2$ (22)

fragment (16) through to (20), single additions of 3.0—3.5 equivalents of an acyl component were sufficient to bring each reaction to completion. However, this was not the case for the condensations of fragments (21) and (22). In these instances, after reaction at 4 °C for 48 h, addition of further azide (2 equiv.) was necessary to complete the reactions. As the chain lengthened, the rate of the condensation reaction decreased, presumably due to a decrease in the nucleophilicity of the amino-components. This tendency seems to reflect the poor solubility of both the protected and N^{α}-deprotected peptides. Every condensation had to be performed in a mixture of three solvents, *viz.* DMF–DMSO–NMP or DMF–DMSO–HMPA. No single solvent succeeded in solubilizing the amino-components at the low temperatures required for the azide reactions.

The poor solubility of the peptide intermediates in this synthesis had both advantages and disadvantages. Because of the great difference in solubility between the larger protected products and the smaller activated carboxy-components used in excess during the coupling, the latter could be removed readily by repeated precipitations of the peptide from DMSO with methanol, except in the case of Z(OMe)-(RNase 41—124)-OBzl. The contaminant derived from fragment (22) was found to be less soluble in methanol. In this instance, we were able to purify the desired compound by precipitation from DMSO with DMF, although some compound was lost in the mother-liquid.

Despite these advantages of low solubility, a serious problem was encountered because we were not able to find suitable solvent systems for t.l.c. At the step at which fragment (16) was introduced, the protected peptides and even the N^{α}-deprotected peptides remained at the origin of the plate in all the solvent systems examined. Thus, at this stage, we lacked a useful tool for the assessment of homogeneity of the peptides, as well as for monitoring the progress of the reactions. The usefulness of t.l.c. was thus greatly limited. However, we could at least use the ninhydrin test as a guide to coupling reactions and could examine the presence or absence of acyl components in the isolated products.

The increased insolubility of the larger peptides resulted in a corresponding difficulty in the assessment of their homogeneity. Here, the ratios of newly incorporated amino-acids in the acid hydrolysate to that of phenylalanine gave a very important clue. It is, of course, very important to confirm that observed amino-acid ratios are not due to contamination with unchanged amino and acyl components. In this respect, ratios of phenylalanine and leucine furnish additional information about the homogeneity of the peptides, since one mole of leucine was first introduced with fragment (20). The combination of t.l.c. and amino-acid analysis played a very important role in assessing the purity of the peptides obtained from successive condensations. In addition, the purity of Z(OMe)-(RNase 41—124)-OBzl was checked by gel-filtration on Sephacryl S-200. This was felt to be

desirable at this stage since we had some difficulty in removing the unchanged acyl component. This was our second check-point of purity by chromatographic means. The synthetic tetraoctacontapeptide ester emerged from the column as a single component. Its amino-acid ratios and those of the intermediates synthesized under the conditions mentioned above are listed in the Table. Within the limit of experimental error, these values matched those predicted by theory. We were thus able to synthesize *ca.* 13 g of Z(OMe)-(RNase 41—124)-OBzl, having a high degree of homogeneity.

Amino-acid ratios of Z(OMe)-(RNase 41—124)-OBzl and intermediates

Position	65—124	62—124	57—124	53—124	49—124	46—124	41—124
Residue	(60)	(63)	(68)	(72)	(76)	(79)	(84)
Asp	7.12(7)	8.14(8)	8.16(8)	9.22(9)	9.17(9)	9.18(9)	10.20(10)
Thr	5.78(6)	5.74(6)	5.79(6)	5.83(6)	5.89(6)	5.96(6)	6.88(7)
Ser	4.59(6)	4.79(6)	5.44(7)	5.49(7)	6.43(8)	6.44(8)	6.46(8)
Glu	5.07(5)	5.16(5)	6.08(6)	7.08(7)	8.10(8)	8.16(8)	8.23(8)
Pro	2.77(3)	2.79(3)	2.86(3)	2.88(3)	2.93(3)	2.88(3)	3.79(4)
Gly	3.11(3)	3.12(3)	3.13(3)	3.11(3)	3.21(3)	3.15(3)	3.18(3)
Ala	3.99(4)	4.89(5)	4.91(5)	5.86(6)	6.88(7)	6.91(7)	6.89(7)
Val	3.89(4)	4.91(5)	5.89(6)	7.04(7)	6.99(7)	7.92(8)	8.87(9)
Met	0.66(1)	0.64(1)	0.69(1)	0.71(1)	0.67(1)	0.64(1)	0.73(1)
Ile	2.41(3)	2.42(3)	2.41(3)	2.49(3)	2.49(3)	2.44(3)	2.47(3)
Leu					1.01(1)	1.01(1)	1.03(1)
Tyr	5.30(5)	5.35(5)	5.33(5)	5.31(5)	5.39(5)	5.34(5)	5.37(5
Phe	1.00(1)	1.00(1)	1.00(1)	1.00(1)	1.00(1)	2.00(2)	2.00(2)
Lys	4.27(4)	4.28(4)	5.31(5)	5.26(5)	5.21(5)	5.18(5)	6.21(6)
His	1.72(2)	1.69(2)	1.72(2)	1.74(2)	1.71(2)	2.76(3)	2.70(3)
Arg	0.91(1)	0.88(1)	0.86(1)	0.87(1)	0.86(1)	0.89(1)	0.93(1)
Cys	(5)	(5)	(6)	(6)	(6)	(6)	(6)
Recovery (%)	84	87	88	88	84	90	88

Of the intermediates mentioned above, the corresponding sequence 65—124 has been used as an amino-component for condensation with the sequence 21—64 by Jenkins *et al.*[10] In this report amino-acid ratios of Boc-(65—124)-OH were given, but with no diagnostic amino-acid such as phenylalanine.

EXPERIMENTAL

General experimental procedures were described in Part 1.[1a] The N^{α}-protecting group, Z(OMe), was removed by TFA in the presence of anisole (⩾10 mol equiv.) in an ice-bath for 45—60 min. The DCC and active-ester condensations were performed at room temperature (17—25 °C). The azide condensation was performed according to the method of Honzl and Rudinger.[13]

Z(OMe)-Asn-Gly-OH.—Z(OMe)-Asn-ONP (20.85 g, 50 mmol) in DMF (150 ml) was added to a stirred solution of Gly (11.26 g, 0.15 mol) and Et_3N (20.7 ml, 0.15 mol) in H_2O (80 ml). After 48 h, the solution was concentrated and the residue was treated with ether and 5% citric acid. The resulting powder was washed three times with 5% citric acid and H_2O and recrystallized from MeOH–AcOEt to afford the *protected dipeptide* (6.70 g, 38%), m.p. 165—167 °C, $[\alpha]_D^{23}$ −1.4° (*c*, 0.7 in DMF), R_{F_2} 0.07 (Found: C, 50.95; H, 5.45; N, 11.9. $C_{15}H_{19}N_3O_7$ requires C, 50.99; H, 5.42; N, 11.89%).

Z(OMe)-Lys(Z)-Asn-Gly-OH.—Z(OMe)-Asn-Gly-OH (10.59 g, 30 mmol) was treated with a mixture of TFA (21 ml) and anisole (5.3 ml) as usual, then dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 h, and dissolved in DMF (150 ml) together with Et_3N (8.3 ml, 60 mmol) and Z(OMe)-Lys(Z)-OQCl (20.59 g, 33 mmol). After stirring for 48 h, the solution was concentrated and the residue was treated with ether and 5% citric acid. The resulting powder was washed as above and recrystallized from MeOH–ether to afford the *protected tripeptide* (4.22 g, 23%), m.p. 198—200 °C, $[\alpha]_D^{22}$ −6.1° (*c*, 0.5 in DMF), R_{F_2} 0.15 (Found: C, 56.35; H, 6.1; N, 11.4. $C_{29}H_{37}N_5O_{10}$ requires C, 56.57; H, 6.06; N, 11.38%).

Z(OMe)-Asn-Gly-NHNH-Troc.—Z(OMe)-Gly-NHNH-Troc [5] (20.60 g, 48 mmol) was treated with a mixture of TFA (41 ml) and anisole (10 ml) as usual, then the excess of TFA was evaporated off. The oily residue was washed with n-hexane, dried over KOH pellets *in vacuo* for 3 h, and dissolved in DMF (100 ml) together with Et_3N (12.1 ml, 88 mmol) and Z(OMe)-Asn-ONP (16.68 g, 40 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by recrystallization from MeOH to afford the *protected dipeptide* (13.61 g, 52%), m.p. 175—178 °C, $[\alpha]_D^{23}$ −6.9° (*c*, 0.6 in DMF), R_{F_2} 0.15 (Found: C, 39.75; H, 4.2; N, 12.7. $C_{18}H_{22}Cl_3N_5O_8$ requires C, 39.83; H, 4.09; N, 12.90%).

Z(OMe)-Lys(Z)-Asn-Gly-NHNH-Troc —(*a*) Z(OMe)-Asn-Gly-NHNH-Troc (10 85 g, 20 mmol) was treated with a mixture of TFA (22 ml) and anisole (5.4 ml) as usual, then dry ether was added The resulting powder was filtered off, dried over KOH pellets *in vacuo* for 3 h, and dissolved in DMF (80 ml) containing Et_3N (5.5 ml, 40 mmol) and Z(OMe)-Lys(Z)-OQCl (12.58 g, 20 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by recrystallization from MeOH to afford the *protected tripeptide* (13.73 g, 85%), m.p. 190—193 °C, $[\alpha]_D^{23}$ −6.8° (*c*, 1.0 in DMF), R_{F_1} 0.51, R_{F_2} 0.20 (Found: C, 47.9; H, 4.85; N, 12.3. $C_{32}H_{40}Cl_3N_7O_{11}$ requires C, 47.74; H, 5.01; N, 12.18%).

(*b*) DCC (1.68 g, 8.2 mmol) was added to a mixture of Z(OMe)-Lys(Z)-Asn-Gly-OH (4.18 g, 6.8 mmol), HOBT (1.11 g, 8.2 mmol), and Troc-$NHNH_2$ (1.70 g, 8.2 mmol) in DMF (20 ml). After stirring for 24 h, the solution was filtered, the filtrate was concentrated, and the product was isolated as above; yield 4.74 g (87%), m.p. 190—193 °C, $[\alpha]_D^{25}$ −6.7° (*c*, 0.7 in DMF).

Z(OMe)-Cys(MBzl)-Lys(Z)-Asn-Gly-NHNH-Troc (16).—Z(OMe)-Lys(Z)-Asn-Gly-NHNH-Troc (12.08 g, 15 mmol) was treated with TFA–anisole (30 ml; 4 : 1 v/v) as usual,

then dry ether was added. The resulting powder, isolated as above, was dissolved in DMF (100 ml) together with Et_3N (4.1 ml, 30 mmol) and Z(OMe)-Cys(MBzl)-ONP (9.48 g, 18 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B. Precipitation from DMF with MeOH afforded the *protected tetrapeptide* (11.91 g, 77%), m.p. 213—216 °C, $[\alpha]_D^{23}$ −17.7° (*c*, 0.5 in DMF), R_{F_1} 0.44 (Found: C, 50.25; H, 5.2; N, 10.8. $C_{43}H_{53}Cl_3N_8O_{13}S$ requires C, 50.22; H, 5.20; N, 10.90%).

Z(OMe)-Cys(MBzl)-Lys(Z)-Asn-Gly-NHNH$_2$, *Z(OMe)-(RNase 65—68)-NHNH*$_2$.—Zn powder (6.53 g, 10 mol. equiv.) was added to a stirred solution of Z(OMe)-Cys-(MBzl)-Lys(Z)-Asn-Gly-NHNH-Troc (10.28 g, 10 mmol) in DMF–AcOH (1 : 1 v/v, 200 ml). After 72 h the solution was filtered and the filtrate was concentrated *in vacuo* at 30 °C. Treatment of the residue with a saturated solution of EDTA afforded a gelatinous mass, which was washed with H_2O and precipitated from DMF with MeOH to yield the *protected tetrapeptide hydrazide* (6.27 g, 74%), m.p. 212—215 °C, $[\alpha]_D^{23}$ −12.8° (*c*, 0.9 in DMSO), R_{F_1} 0.53. Amino-acid analysis: Lys 1.00, Asp 1.18, Gly 1.12 (average recovery 99%) (Found: C, 56.15; H, 6.05; N, 12.9. $C_{40}H_{52}N_8O_{11}S$ requires C, 56.32; H, 6.15; N, 13.14%).

Z(OMe)-Asn-Val-Ala-OMe.—Z(OMe)-Val-Ala-OMe [1b] (15.50 g, 42 mmol) was treated with TFA (31 ml) and anisole (7.7 ml) as usual, then the excess of TFA was evaporated off. The oily residue was washed with n-hexane, dried over KOH pellets *in vacuo* for 2 h, and dissolved in DMF (80 ml) together with Et_3N (11.9 ml, 86 mmol) and Z(OMe)-Asn-ONP (17.93 g, 43 mmol). After stirring for 48 h, the solution was concentrated and the product was purified by procedure B followed by precipitation from DMF with MeOH to yield the *protected tripeptide ester* (16.50 g, 82%), m.p. 243—245 °C, $[\alpha]_D^{23}$ −14.6° (*c*, 0.5 in DMF), R_{F_2} 0.35 (Found: C, 54.75; H, 6.6; N, 11.5. $C_{22}H_{32}N_4O_8$ requires C, 54.99; H, 6.71; N, 11.66%).

Z(OMe)-Asn-Val-Ala-NHNH$_2$, *Z(OMe)-(RNase 62—64)-NHNH*$_2$*(17)*.—Z(OMe)-Asn-Val-Ala-OMe (16.50 g, 34 mmol) in DMF (160 ml) was treated with 80% hydrazine hydrate (21 ml, 10 mol equiv.) overnight and then MeOH (160 ml) was added. The resulting mass was reprecipitated from DMF with MeOH to give the *protected tripeptide hydrazide* (15.20 g, 93%), m.p. 246—248 °C, $[\alpha]_D^{23}$ −14.5° (*c*, 0.3 in DMSO), R_{F_1} 0.46. Amino-acid analysis: Asp 1.04, Val 1.00, Ala 0.94 (average recovery 92%) (Found: C, 52.7; H, 6.75; N, 17.45. $C_{21}H_{32}N_6O_7$ requires C, 52.49; H, 6.71; N, 17.49%).

Z(OMe)-Gln-Lys(Z)-OH.—Z(OMe)-Gln-ONP (21.57 g, 50 mmol) in pyridine (70 ml) was mixed with a solution of Lys(Z) (14.02 g, 50 mmol) and Et_3N (13.8 ml, 0.1 mol) in H_2O (70 ml). After stirring for 48 h, the solution was concentrated and the residue was treated with 5% citric acid and ether. The resulting powder was purified by washing with 5% citric acid and H_2O followed by recrystallization from MeOH and AcOEt to yield the *protected dipeptide* (16.0 g, 56%), m.p. 172—174 °C, $[\alpha]_D^{23}$ +4.9° (*c*, 0.7 in DMF), R_{F_2} 0.15 (Found: C, 58.7; H, 6.3; N, 9.7. $C_{28}H_{36}N_4O_9$ requires C, 58.73; H, 6.34; N, 9.79%).

Z(OMe)-Cys(MBzl)-Ser-OMe.—Z(OMe)-Cys(MBzl)-ONP (21.10 g, 40 mmol) in DMF (50 ml) and Et_3N (5.5 ml, 40 mmol) were added to a solution of H-Ser-OMe [from the hydrochloride (7.50 g, 48 mmol) with Et_3N (6.5 ml, 48 mmol)] in DMF (50 ml). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by recrystallization from THF–MeOH to afford the *protected dipeptide ester* (15.50 g, 76%), m.p. 134—135 °C, $[\alpha]_D^{23}$ −28.7° (*c*, 1.2 in DMF), R_{F_1} 0.58 (Found: C, 57.1; H, 6.05; N, 5.65. $C_{24}H_{30}N_2O_8S$ requires C, 56.90; H, 5.97; N, 5.53%).

Z(OMe)-Cys(MBzl)-Ser-NHNH$_2$.—Z(OMe)-Cys(MBzl)-Ser-OMe (15.20 g, 30 mmol) in DMF (60 ml) was treated with 80% hydrazine hydrate (19 ml, 10 mol equiv.) and the gelatinous mass formed on standing overnight was precipitated from DMF with MeOH to give the *hydrazide* (13.20 g, 87%), m.p. 193—194 °C, $[\alpha]_D^{23}$ −4.8° (*c*, 1.4 in DMSO), R_{F_1} 0.53 (Found: C, 54.65; H, 5.95; N, 10.75. $C_{23}H_{30}N_4O_7S$ requires C, 54.53; H, 5.97; N, 11.06%).

Z(OMe)-Cys(MBzl)-Ser-Gln-Lys(Z)-OH.—Z(OMe)-Gln-Lys(Z)-OH (7.44 g, 13 mmol) was treated with TFA (14.8 ml) and anisole (3.7 ml) as usual and the TFA salt, precipitated with dry ether as a fine powder, was filtered off, dried over KOH pellets *in vacuo* for 3 h, and dissolved in DMF (40 ml) containing Et_3N (3.6 ml, 26 mmol). To this ice-chilled solution, the azide [from Z(OMe)-Cys(MBzl)-Ser-$NHNH_2$ (7.89 g, 16 mmol)] in DMF (50 ml) and Et_3N (2.2 ml, 16 mmol) were added. After stirring for 48 h, the solution was concentrated and the residue was purified by washing with 5% citric acid and H_2O followed by recrystallization from MeOH–AcOEt to afford the *protected tetrapeptide* (7.41 g, 65%), m.p. 168—169 °C, $[\alpha]_D^{23}$ −8.8° (*c*, 0.8 in DMF), R_{F_2} 0.18 (Found: C, 57.35; H, 6.3; N, 9.5. $C_{42}H_{54}N_6O_{13}S$ requires C, 57.13; H, 6.16; N, 9.52%).

Z(OMe)-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-OH.—The above protected tetrapeptide (13.65 g, 15 mmol) was treated with TFA (27 ml) and anisole (6.8 ml) as usual, and then dry ether was added. The resulting powder, isolated as above, was dissolved in DMF (70 ml) together with Et_3N (4.1 ml, 30 mmol) and Z(OMe)-Val-ONP (6.84 g, 17 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by washing with 5% citric acid and H_2O followed by precipitation from DMF with MeOH to yield the *protected pentapeptide* (13.70 g, 90%), m.p. 203—204 °C, $[\alpha]_D^{22}$ −8.0° (*c*, 1.1 in DMF), R_{F_1} 0.12 (Found: C, 57.4; H, 6.2; N, 10.05. $C_{47}H_{63}N_7O_{14}S$ requires C, 57.48; H, 6.47; N, 9.98%).

Z(OMe)-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-OMe.—An ethereal solution of diazomethane was added to an ice-chilled solution of Z(OMe)-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-OH (13.70 g, 14 mmol) in DMF–MeOH (200 ml; 3 : 1 v/v) till the yellow colour persisted for 30 min. After addition of a few drops of AcOH, the solvent was evaporated off and the resulting mass was reprecipitated from DMF with MeOH to give the *pentapeptide ester* (11.65 g, 84%), m.p. 227—229 °C, $[\alpha]_D^{23}$ −8.2° (*c*, 0.4 in DMF), R_{F_1} 0.58 (Found: C, 57.6; H, 6.55; N, 9.85. $C_{48}H_{65}N_7O_{14}S$ requires C, 57.87; H, 6.58; N, 9.84%).

Z(OMe)-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-NHNH$_2$,*Z(OMe)-(RNase 57—61)-NHNH*$_2$ (18).—Z(OMe)-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-OMe (11.60 g, 12 mmol) in DMF (100 ml) was treated with 80% hydrazine hydrate (7.3 ml, 10 mol equiv.). The gelatinous mass which formed on standing overnight was filtered off and precipitated from DMF with MeOH to give the *hydrazide* (10.50 g, 91%), m.p. 241—243 °C, $[\alpha]_D^{23}$ 3.8° (*c*, 1.1 in DMSO), R_{F_1} 0.46. Amino-acid analysis: Val 1.11, Ser 0.94, Glu 1.14, Lys 1.00 (average recovery 91%) (Found: C, 56.2; H, 6.4; N, 12.45. $C_{47}H_{65}N_9O_{13}S \cdot 1/2H_2O$ requires C, 56.16; H, 6.62; N, 12.54%).

Z(OMe)-Gln-Ala-NHNH-Troc.—Z(OMe)-Ala-NHNH-

Troc [14] (22.08 g, 50 mmol) was treated with TFA–anisole (55 ml; 4 : 1 v/v) as usual, and then the excess of TFA was removed by evaporation. The oily residue was washed with n-hexane, dried over KOH pellets *in vacuo* for 3 h, and dissolved in DMF (100 ml) together with Et_3N (13.8 ml, 0.1 mol) and Z(OMe)-Gln-ONP (21.57 g, 50 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by recrystallization from MeOH–ether to afford the *protected dipeptide* (16.13 g, 57%), m.p. 193—195 °C, $[\alpha]_D^{23}$ −9.8° (*c*, 0.7 in DMF), R_{F_1} 0.55, R_{F_4} 0.15 (Found: C, 41.85; H, 4.6; N, 12.25. $C_{20}H_{26}Cl_3N_5O_8$ requires C, 42.08; H, 4.59; N, 12.27%).

Z(OMe)-Val-Gln-Ala-NHNH-Troc.—Z(OMe)-Gln-Ala-NHNH-Troc (15.96 g, 28 mmol) was treated with TFA–anisole (40 ml; 4 : 1 v/v) as usual, then 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 (7.7 ml, 56 mmol) and Z(OMe)-Val-ONP (13.67 g, 34 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by recrystallization from MeOH to yield the *protected tripeptide* (13.22 g, 70%), m.p. 201—203 °C, $[\alpha]_D^{22}$ −33.3° (*c*, 0.5 in DMF), R_{F_1} 0.52 (Found: C, 44.6; H, 5.1; N, 12.4. $C_{25}H_{35}Cl_3N_6O_9$ requires C, 44.82; H, 5.27; N, 12.55%).

Z(OMe)-Asp(OBzl)-Val-Gln-Ala-NHNH-Troc (19).—Z(OMe)-Val-Gln-Ala-NHNH-Troc (13.39 g, 20 mmol) was treated with TFA (26 ml) and anisole (6.6 ml) as usual and the N^{α}-deprotected peptide isolated as mentioned above was dissolved in DMF (120 ml) together with Et_3N (5.5 ml, 40 mmol) and Z(OMe)-Asp(OBzl)-ONP (12.19 g, 24 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by precipitation from DMF with MeOH to afford the *protected tetrapeptide* (11.70 g, 67%), m.p. 200—202 °C, $[\alpha]_D^{23}$ −17.1°: (*c*, 1.1 in DMF), R_{F_1} 0.56 (Found: C, 49.6; H, 5.15; N, 11.1. $C_{36}H_{46}Cl_3N_7O_{12}$ requires C, 49.40; H, 5.30; N, 11.20%).

Z(OMe)-Asp(OBzl)-Val-Gln-Ala-NHNH$_2$, Z(OMe)-(RNase 53—56)-NHNH$_2$.—The above protected tetrapeptide (8.0 g, 9 mmol) in DMF–AcOH (100 ml; 1 : 1 v/v) was treated with Zn powder (6.0 g, 10 mol equiv.) for 72 h. Some gelatinous mass which formed was brought into solution with slight warming and the solution was filtered. The filtrate was concentrated *in vacuo* at 30 °C and the residue was treated with a saturated solution of EDTA and 5% $NaHCO_3$. The resulting mass was collected by filtration, washed with H_2O, and precipitated from DMF with MeOH to yield the *hydrazide* (4.04 g, 63%), m.p. 226—228 °C, $[\alpha]_D^{22}$ −13.7° (*c*, 0.4 in DMSO), R_{F_1} 0.53. Amino-acid analysis: Asp 1.10, Val 1.00, Glu 1.08, Ala 0.99 (average recovery 96%) (Found: C, 56.8; H, 6.65; N, 14.0. $C_{33}H_{45}N_7O_{10}$ requires C, 56.64;H, 6.48; N, 14.01%).

Z(OMe)-Leu-Ala-NHNH-Troc.—Z(OMe)-Leu-ONP (22.70 g, 55 mmol) and Et_3N (13.8 ml, 0.1 mol) were added to a solution of H-Ala-NHNH-Troc TFA salt [from the Z(OMe)-derivative (22.10 g, 50 mmol) as stated above] in DMF (100 ml). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure A followed by recrystallization from MeOH–ether to yield the *protected dipeptide* (16.03 g, 58%), m.p. 85—88 °C, $[\alpha]_D^{22}$ −16.2° (*c*, 0.6 in DMF), R_{F_1} 0.85 (Found: C, 45.2; H, 5.2; N, 10.1. $C_{21}H_{29}Cl_3N_4O_7$ requires C, 45.37; H, 5.26; N, 10.08%).

Z(OMe)-Ser-Leu-Ala-NHNH-Troc.—Z(OMe)-Leu-Ala-NHNH-Troc (15.90 g, 29 mmol) was treated with TFA–anisole (40 ml; 4 : 1 v/v) as usual, then dry ether was added. The resulting powder, isolated as earlier, was dissolved in DMF (80 ml). To this ice-chilled solution were added the azide [from Z(OMe)-Ser-$NHNH_2$ (9.70 g, 34 mmol)] in DMF (50 ml) and Et_3N (7.9 ml, 58 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure A followed by recrystallization from AcOEt–ether to yield the *protected tripeptide* (16.15 g, 88%), m.p. 96—99°, $[\alpha]_D^{22}$ −14.1° (*c*, 0.5 in DMF), R_{F_1} 0.67 (Found: C, 44.7: H, 5.25; N, 10.75. $C_{24}H_{34}Cl_3N_5O_9$ requires C, 44.83; H, 5.33; N, 10.89%).

Z(OMe)-Glu(OBzl)-Ser-Leu-Ala-NHNH-Troc(20).—The above protected tripeptide (16.10 g, 25 mmol) was treated with TFA–anisole (40 ml; 4 : 1 v/v) as usual, then dry ether was added. The resulting powder, isolated as above, was dissolved in DMF (130 ml) together with Et_3N (6.9 ml, 50 mmol) and Z(OMe)-Glu(OBzl)-ONP (13.10 g, 25 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by recrystallization from MeOH to afford the *protected tetrapeptide* (17.31 g, 80%), m.p. 182—184 °C, $[\alpha]_D^{22}$ −10.6° (*c*, 0.7 in DMF), R_{F_1} 0.64 (Found: C, 49.88; H, 5.25; N, 9.8. $C_{36}H_{47}N_6O_{12}Cl_3$ requires C, 50.15; H, 5.50; N, 9.75%).

Z(OMe)-Glu(OBzl)-Ser-Leu-Ala-NHNH$_2$, Z(OMe)-(RNase 49—52)-NHNH$_2$.—The above protected tetrapeptide (12.60 g, 15 mmol) in DMF–AcOH (120 ml; 1 : 1 v/v) was treated with Zn powder (9.61 g, 10 mol equiv.) for 72 h. Some gelatinous mass which formed during the reaction was dissolved with slight warming. The solution was filtered, the filtrate was concentrated *in vacuo* at 30 °C and the residue was treated with a saturated solution of EDTA and 5% $NaHCO_3$. The resulting mass was washed with H_2O and precipitated from DMF with MeOH to yield the *hydrazide* (6.50 g, 65%), m.p. 222—224 °C, $[\alpha]_D^{23}$ −8.3° (*c*, 0.4 in DMSO), R_{F_1} 0.65. Amino-acid analysis: Glu 1.07, Ser 0.81, Leu 0.91, Ala 1.00 (average recovery 95%) (Found: C, 57.85; H, 6.8; N, 12.35. $C_{35}H_{46}N_6O_{10}$ requires C, 57.71; H, 6.75; N, 12.24%).

Z(OMe)-Phe-Val-His-OMe.—Z-Val-His-OMe [15] (20.12 g, 50 mmol) in a mixture of THF (150 ml) and 1N-HCl (100 ml, 2 mol equiv.) was hydrogenated over Pd for 8 h, and then the catalyst was removed by filtration. The filtrate was concentrated and the residue, after drying over KOH pellets *in vacuo* for 3 h, was dissolved in DMF (150 ml) together with Et_3N (13.8 ml, 0.1 mol) and Z(OMe)-Phe-OH (16.47 g, 50 mmol). DCC (12.0 g, 58 mmol) was added and the mixture, after stirring for 48 h was filtered. The filtrate was concentrated and the residue was treated with ether. The resulting powder was washed with ether and 5% Na_2CO_3 and incubated in MeOH (150 ml) containing AcOH (6 ml) at 50 °C for 4 h. The solvent was evaporated off and the residue was recrystallized from MeOH–AcOEt to afford the *protected tripeptide ester* (21.42 g, 74%), m.p. 190—192 °C, $[\alpha]_D^{22}$ −7.0° (*c*, 0.3 in DMF), R_{F_1} 0.51 (Found: C, 62.1; H, 6.5; N, 12.1. $C_{30}H_{37}N_5O_7$ requires C, 62.16; H, 6.43; N, 12.08%).

Z(OMe)-Phe-Val-His-NHNH$_2$, Z(OMe)-(RNase 46—48)-NHNH$_2$ (21).—The above protected tripeptide ester (5.80 g, 10 mmol) in DMF (50 ml) was treated with 80% hydrazine hydrate (6.3 ml, 10 mol equiv.) overnight, then the solvent was evaporated off. Trituration of the residue with ether followed by recrystallization from MeOH gave the *protected tripeptide hydrazide* (3.22 g, 56%), m.p. 197—200 °C

$[\alpha]_D^{22}$ −2.9° (*c*, 1.0 in DMSO), R_{F_1} 0.43. Amino-acid analysis: Phe 1.05, Val 1.00, His 0.89 (average recovery 92%) (Found: C, 59.0; H, H, 6.2; N, 16.6. $C_{29}H_{37}N_7O_6 \cdot 1/2H_2O$ requires C, 59.17; H, 6.51; N, 16.66%).

Z(OMe)-Asn-Thr-OMe.—Z(OMe)-Asn-ONP (20.50 g, 49 mmol) and Et_3N (6.8 ml, 49 mmol) were added to a stirred solution of H-Thr-OMe [from the hydrochloride (9.16 g, 54 mmol) with Et_3N (7.5 ml, 54 mmol)] in DMF (100 ml). After 48 h, the solution was concentrated and the residue was purified by procedure B followed by recrystallization from MeOH–AcOEt to give the *protected dipeptide ester* (12.01 g, 60%), m.p. 168—170 °C, $[\alpha]_D^{22}$ −1.5° (*c*, 0.7 in DMF), R_{F_1} 0.82 (Found: C, 52.25; H, 6.2; N, 9.95. $C_{18}H_{25}N_3O_8$ requires C, 52.55; H, 6.13; N, 10.21%).

Z(OMe)-Val-Asn-Thr-OMe.—Z(OMe)-Asn-Thr-OMe (8.23 g, 20 mmol) was treated with TFA–anisole (20 ml; 4 : 1 v/v) as usual, then dry ether was added. The resulting powder, isolated as earlier, was dissolved in DMF (50 ml) together with Et_3N (5.5 ml, 40 mmol) and Z(OMe)-Val-ONP (8.85 g, 22 mmol). After stirring for 24 h, the solution was concentrated and the residue was purified by procedure B followed by precipitation from DMF with MeOH to afford the *protected tripeptide ester* (6.19 g, 61%), m.p. 229—231 °C, $[\alpha]_D^{22}$ −1.0° (*c*, 1.0 in DMF), R_{F_1} 0.15 (Found: C, 53.8; H, 6.65; N, 10.95. $C_{23}H_{34}N_4O_9$ requires C, 54.11; H, 6.71; N, 10.98%).

Z(OMe)-Lys(Z)-Pro-Val-Asn-Thr-OMe.—Z(OMe)-Val-Asn-Thr-OMe (4.09 g, 8.0 mmol) was treated with TFA (15 ml) and anisole (2.6 ml) as usual, then dry ether was added. The resulting powder was dissolved in DMF (25 ml) together with Et_3N (2.3 ml, 17 mmol) and Z(OMe)-Lys(Z)-Pro-OPCP [19] (7.10 g, 9.0 mmol). After stirring overnight, the solution was concentrated and the residue was purified by procedure A followed by precipitation from DMF with MeOH to afford the *protected pentapeptide ester* (4.35 g, 63%), m.p. 193—198 °C, $[\alpha]_D^{22}$ −20.0°: (*c*, 1.3 in DMF), R_{F_1} 0.69 (Found: C, 56.8; H, 6.7; N, 11.05. $C_{42}H_{59}N_7O_{13} \cdot H_2O$ requires C, 56.81; H, 6.92; N, 11.04%).

Z(OMe)-Lys(Z)-Pro-Val-Asn-Thr-NHNH$_2$, Z(OMe)-(RNase 41—45)-NHNH$_2$ (22).—Z(OMe)-Lys(Z)-Pro-Val-Asn-Thr-OMe (10.87 g, 12.5 mmol) in DMF (80 ml) was treated with 80% hydrazine hydrate (7.8 ml, 10 mol equiv.) overnight, then the solvent was evaporated off. The residue was treated with MeOH and the resulting mass was precipitated from DMF with MeOH to give the *protected pentapeptide hydrazide* (6.03 g, 55%), m.p. 192—195 °C, $[\alpha]_D^{23}$ −38.9° (*c*, 1.1 in DMSO), R_{F_1} 0.45. Amino-acid analysis: Lys 1.00, Pro 1.01, Val 0.93, Asp 1.04, Thr 0.88 (average recovery 93%) (Found: C, 55.55; H, 6.9; N, 14.2. $C_{41}H_{59}N_9O_{12} \cdot H_2O$ requires C, 55.45; H, 6.92; N, 14.20%).

Z(OMe)-Cys(MBzl)-Lys(Z)-Asn-Gly-Gln-Thr-Asn-Cys(MBzl)-Tyr-Gln-Ser-Tyr-Ser-Thr-Met(O)-Ser-Ile-Thr-Asp(OBzl)-Cys(MBzl)-Arg(MBS)-Glu(OBzl)-Thr-Gly-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 [*Abbreviated as Z(OMe)-(RNase 65—124)-OBzl or Z(OMe)-Cys(MBzl)-Lys(Z)-Asn-Gly-(RNase 69—124)-OBzl*].—Z(OMe)-(RNase 69—124)-OBzl (9.49 g, 1.2 mmol) was treated with TFA–anisole (60 ml; 5 : 1 v/v) as usual then dry ether was added. The resulting powder was filtered off, dried over KOH pellets *in vacuo* for 3 h, and dissolved in DMSO–DMF–NMP (1 : 1 : 1 v/v; 70 ml) containing Et_3N (0.50 ml, 3.6 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Cys(MBzl)-Lys(Z)-Asn-Gly-NHNH$_2$ (3.07 g, 3.6 mmol)] in DMF (20 ml) and Et_3N (0.50 ml, 3.6 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by precipitation (×6) from DMSO with MeOH to yield the *protected hexacontapeptide ester* (9.04 g, 88%), m.p. 246 °C (decomp.), $[\alpha]_D^{23}$ −22.5° (*c*, 1.0 in DMSO), R_{F_3} 0 (Found: C, 50.8; H, 6.2; N, 12.15. $C_{406}H_{543}N_{79}O_{114}S_7 \cdot 54H_2O$ requires C, 51.05; H, 6.85; N, 11.6%).

Z(OMe)-(RNase 62—124)-OBzl [*Z(OMe)-Asn-Val-Ala-(65—124)-OBzl*].—Z(OMe)-(RNase 65—124)-OBzl (9.03 g, 1.05 mmol) was treated with TFA–anisole (60 ml; 5 : 1 v/v) as usual and the N$^\alpha$-deprotected peptide, isolated as above, was dissolved in DMSO–DMF–NMP (1 : 1 : 1 v/v, 70 ml) containing Et_3N (0.44 ml, 3.16 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Asn-Val-Ala-NHNH$_2$ (0.59 g, 3.16 mmol)] in DMF (5 ml) and Et_3N (0.44 ml, 3.16 mmol) and the solution, after stirring for 48 h, was concentrated. The residue was purified by procedure B followed by precipitation (×8) from DMSO with MeOH to yield the *protected trihexacontapeptide ester* (8.18 g, 88%), m.p. 239 °C (decomp.), $[\alpha]_D^{23}$ −28.2° (*c*, 0.9 in DMSO), R_{F_3} 0 (Found: C, 54.25; H, 6.4; N, 13.0. $C_{418}H_{563}N_{83}O_{118}S_7 \cdot 18H_2O$ requires C, 54.64; H, 6.57; N, 12.66%).

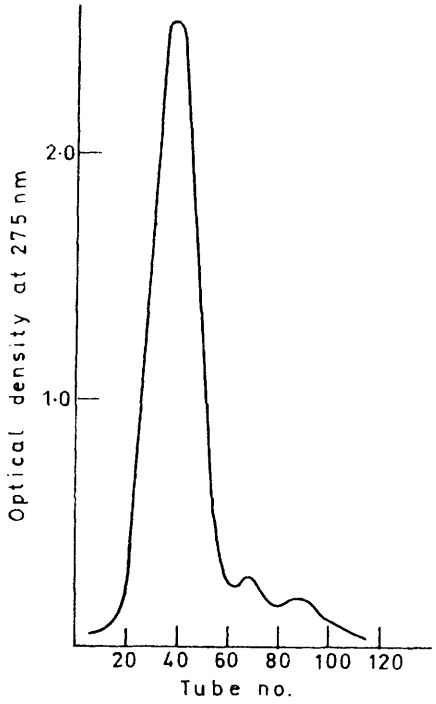
Z(OMe)-(RNase 57—124)-OBzl, [*Z(OMe)-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-(62—124)-OBzl*].—Z(OMe)-(RNase 62—124)-OBzl (8.17 g, 0.92 mmol) was treated with TFA–anisole (60 ml; 5 : 1 v/v) as usual and the N$^\alpha$-deprotected peptide, isolated as above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v; 60 ml) containing Et_3N (0.38 ml, 2.77 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-NHNH$_2$ (3.22 g, 3.23 mmol)] in DMF (20 ml) and Et_3N (0.45 ml, 3.23 mmol) and the solution, after stirring for 48 h, was concentrated. The residue was purified by procedure B followed by precipitation (×6) from DMSO with MeOH to yield the *protected octahexacontapeptide ester* (8.00 g, 90%), m.p. 240 °C (decomp.), $[\alpha]_D^{23}$ −26.5° (*c*, 1.1 in DMSO) (Found: C, 52.4; H, 6.4; N, 12.6. $C_{456}H_{616}N_{90}O_{128}S_8 \cdot 40H_2O$ requires C, 52.74; H, 6.76; N, 12.14%).

Z(OMe)-(RNase 53—124)-OBzl, [*Z(OMe)-Asp(OBzl)-Val-Gln-Ala-(57—124)-OBzl*].—Z(OMe)-(RNase 57—124)-OBzl (7.99 g, 0.83 mmol) was treated with TFA–anisole (48 ml; 5 : 1 v/v) for 80 min and the N$^\alpha$-deprotected peptide, isolated as above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v; 70 ml) containing Et_3N (0.34 ml, 2.48 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Asp(OBzl)-Val-Gln-Ala-NHNH$_2$ (2.03 g, 2.90 mmol)] in DMF (10 ml) and Et_3N (0.40 ml, 2.90 mmol), and after stirring for 96 h, the solution was concentrated. The residue was purified by procedure B followed by precipitation (×4) from DMSO with MeOH to yield the *protected doheptacontapeptide ester* (7.59 g, 90%), m.p. 244 °C (decomp.), $[\alpha]_D^{23}$ −27.4° (*c*, 0.7 in DMSO) (Found: C, 53.15; H, 6.3; N, 12.9. $C_{480}H_{649}N_{95}O_{135}S_8 \cdot 34H_2O$ requires C, 53.48; H, 6.71; N, 12.35%).

Z(OMe)-(RNase 49—124)-OBzl, [*Z(OMe)-Glu(OBzl)-Ser-Leu-Ala-(53—124)-OBzl*].—Z(OMe)-(RNase 53—124)-OBzl (7.58 g, 0.75 mmol) was treated with TFA–anisole (48 ml; 5 : 1 v/v) as usual and the N$^\alpha$-deprotected peptide, isolated as above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v, 80 ml) containing Et_3N (0.31 ml, 2.24 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Glu(OBzl)-Ser-Leu-Ala-NHNH$_2$ (1.79 g, 2.61 mmol)] in

DMF (15 ml) and Et_3N (0.36 ml, 2.61 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by precipitation (×4) from DMSO with MeOH to yield the *protected hexaheptacontapeptide ester* (7.40 g, 93%), m.p. 224 °C (decomp.), $[\alpha]_D^{23}$ −28.3° (*c*, 0.7 in DMSO) (Found: C, 53.8; H, 6.45; N, 12.85. $C_{504}H_{683}N_{99}O_{142}S_8 \cdot 30H_2O$ requires C, 54.06; H, 6.69; N, 12.39%).

Z(OMe)-(RNase 46—124)-OBzl, [*Z(OMe)-Phe-Val-His-(49—124)-OBzl*].—Z(OMe)-(RNase 49—124)-OBzl (7.39 g, 0.69 mmol) was treated with TFA–anisole (48 ml; 5 : 1 v.v) as usual and the Nα-deprotected peptide, isolated as mentioned above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v, 70 ml) containing Et_3N (0.29 ml, 2.08 mmol).



Gel-filtration of Z(OMe)-(RNase 41—124)-OBzl on Sephacryl S-200

To this ice-chilled solution were added the azide [from Z(OMe)-Phe-Val-His-$NHNH_2$ (1.41 g, 2.43 mmol)] in DMF (9 ml) and *N*-methylmorpholine (0.27 ml, 2.43 mmol) and the solution was stirred for 48 h. Additional azide [from the hydrazide (0.81 g, 1.39 mmol)] in DMF (5 ml) and *N*-methylmorpholine (1.5 ml, 1.39 mmol) were added. Stirring was continued for 48 h, until the solution became ninhydrin negative. The solvent was removed by evaporation and the residue was purified by procedure B followed by precipitation (×6) from DMSO with MeOH to yield the *protected nonaheptacontapeptide ester* (7.26 g, 95%), m.p. 240 °C (decomp.), $[\alpha]_D^{23}$ −25.5° (*c*, 0.5 in DMSO) (Found: C, 54.55; H, 6.35; N, 13.2. $C_{524}H_{708}N_{104}O_{145}S_8 \cdot 25H_2O$ requires C, 54.77; H, 6.65; N, 12.68%).

Z(OMe)-(RNase 41—124)-OBzl, [*Z(OMe)-Lys(Z)-Pro-Val-Asn-Thr-(46—124)-OBzl*].—Z(OMe)-(RNase 46—124)-OBzl (6.24 g, 0.57 mmol) was treated with TFA–anisole (42 ml; 5 : 1 v/v) and the Nα-deprotected peptide, isolated as above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v, 50 ml) containing Et_3N (0.32 ml, 2.26 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Lys(Z)-Pro-Val-Asn-Thr-$NHNH_2$ (1.72 g, 1.98 mmol)] in DMF–DMSO (1 : 1 v/v; 12 ml) and Et_3N (0.28 ml, 1.98 mmol) and the solution was stirred for 48 h. Additional azide [from the hydrazide (0.98 g, 1.13 mmol)] in DMF–DMSO (1 : 1 v/v; 8 ml) and Et_3N (0.16 ml, 1.13 mmol) were added and the solution, after further stirring for 48 h, was concentrated. The residue was purified by procedure B followed by precipitation (×2) from DMSO with DMF, then from DMSO with MeOH to yield the *protected tetraoctacontapeptide ester* (5.69 g, 86%), m.p. 249 °C (decomp), $[\alpha]_D^{23}$ −28.3° (*c*, 0.4 in DMSO) (Found: C, 53.4; H, 6.4; N, 13.2. $C_{556}H_{755}N_{111}O_{154}S_8 \cdot 39H_2O$ requires C, 53.77; H, 6.76; N, 12.53%).

As a test of purity, the product (250 mg), in H_2O–DMSO (4 ml; 1 : 19 v/v), was applied to a column of Sephacryl S-200 (2.5 × 131 cm), which was eluted with H_2O–DMSO (1 : 19 v/v) at a flow rate of 39 ml h^{-1}. Individual fractions (7 ml each) were collected and their absorptions at 275 nm measured. A single peak with small tailing was detected (Figure). The desired fractions (tube nos. 32—60) were collected and the solvent was evaporated off and the residue was treated with MeOH to give a powder (233 mg, 93%). No significant difference was observed in the amino-acid analyses before (Table) and after the gel-filtration: Asp 10.17, Thr 6.52, Ser 6.39, Glu 8.07, Pro 3.96, Gly 3.09, Ala 7.03, Val 8.79, Met + Met(O) 0.82, Ile 2.37, Leu 0.97, Tyr 5.25, Phe 2.00, Lys 6.18, His 2.69, Arg 1.02 (recovery 91%).

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