Total Synthesis of Bovine Pancreatic Ribonuclease A. Part 5.[1] Synthesis of the Protected S-Protein (Positions 21—124) and the Protected S-Peptide (Positions 1—20) †

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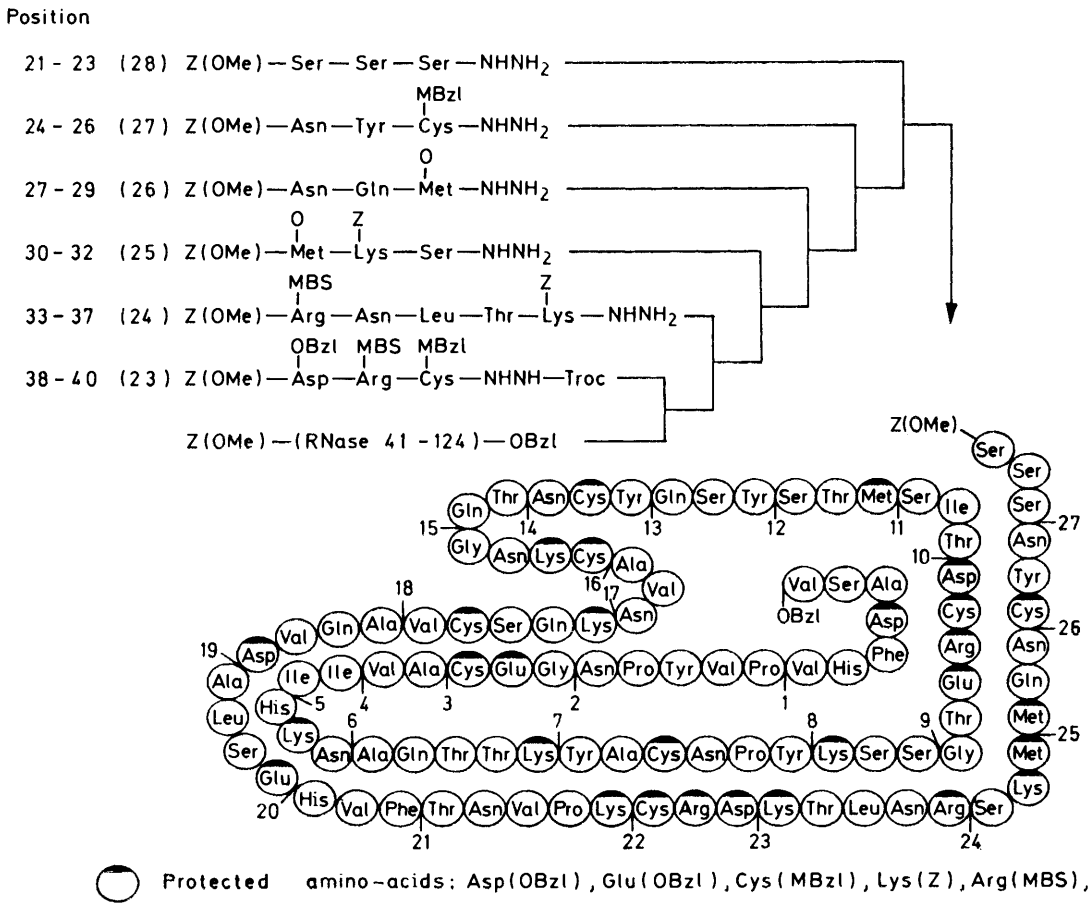
Starting from the protected tetraoctacontapeptide corresponding to the sequence Z(OMe)-(41—124)-OBzl of bovine pancreatic RNase, the protected S-protein Z(OMe)-(RNase 21—124)-OBzl was synthesized by six successive azide condensations of the peptide fragments Z(OMe)-Asp(OBzl)-Arg(MBS)-Cys(MBzl)-NHNH-Troc (23), Z(OMe)-Arg(MBS)-Asn-Leu-Thr-Lys(Z)-$NHNH_2$ (24), Z(OMe)-Met(O)-Lys(Z)-Ser-$NHNH_2$ (25), Z(OMe)-Asn-Gln-Met(O)-$NHNH_2$ (26), Z(OMe)-Asn-Tyr-Cys(MBzl)-$NHNH_2$ (27), and Z(OMe)-Ser-Ser-Ser-$NHNH_2$ (28). In addition, the S-peptide was synthesized in a protected form, Z-Lys(Z)-Glu(OBu^t)-Thr-Ala-Ala-Ala-Lys(Z)-Phe-Glu(OBzl)-Arg(MBS)-Gln-His-Met(O)-Asp-Ser-Ser-Thr-Ser-Ala-Ala-$NHNH_2$, by assembling three sub-units: (A) (14—20), (B) (9—13), and (C) (1—8).

SUBSEQUENT to the synthesis of the protected tetraoctacontapeptide ester Z(OMe)-(RNase 41—124)-OBzl,[1] this peptide chain was elongated to the protected tetrahectapeptide Z(OMe)-(RNase 21—124)-OBzl, termed shown in Scheme 1. In addition, the protected icosapeptide hydrazide Z-(RNase 1—20)-$NHNH_2$, termed the protected S-peptide, was synthesized. We now present a detailed account of these syntheses.



SCHEME 1 Synthetic route to the protected tetrahectapeptide ester, Z(OMe)-(RNase 21—124)-OBzl (protected S-protein)

the protected S-protein, by successive azide condensation [2] of six relatively small peptide fragments, as

We first planned construction of the N-terminal portion of the protected S-protein by addition of fragments (23), (24), and (25), together with two additional sub-units, Z(OMe)-Ser-Ser-Ser-Asn-Tyr-$NHNH_2$ (21—

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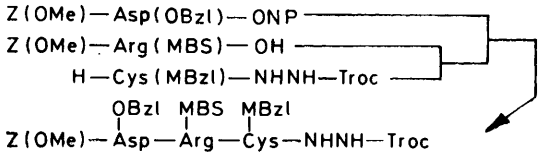
25) and Z(OMe)-Cys(MBzl)-Asn-Gln-Met(O)-$NHNH_2$ (26—29). Preliminary experiments indicated that the Rudinger azide condensation of the latter proceeded satisfactorily, but, for some reason, the final condensation of the former sub-unit onto the amino-component possessing the Cys(MBzl)-Asn unit at the N-terminal portion did not proceed quantitatively, even when a large excess of the acyl component was used. In the acid hydrolysate of the isolated peptide, recoveries of the newly incorporated amino-acids remained at around 76%, indicating that the product was heavily contaminated with unchanged amino-component. If such a product were to be submitted to subsequent coupling reactions, it is obvious that the product would be contaminated with a peptide containing a failure sequence. At earlier stages of the synthesis, each condensation reaction was driven to completion by use of an excess of the acyl component, removal of the unchanged acyl component being the main consideration in obtaining homogeneous products. In the present situation, where the reactivity of the amino-components has apparently decreased significantly and removal of unchanged material has become extremely difficult, it is absolutely necessary to drive the reaction as near to completion as possible, even by the use of larger excesses of acyl components, preferably those with a higher reactivity. To this end, we decided to subdivide the above two peptides into three sub-units, (26), (27), and (28), as shown in Scheme 1.

Fragment (23), Z(OMe)-Asp(OBzl)-Arg(MBS)-Cys-(MBzl)-NHNH-Troc (positions 38—40), was prepared in a stepwise manner starting from H-Cys(MBzl)-NHNH-Troc (Scheme 2). The DCC-plus-HOBT [3] and the NP

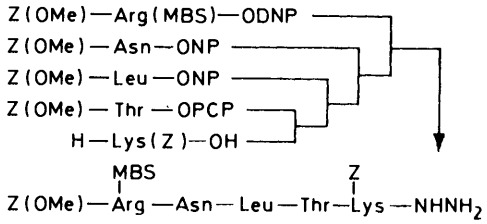


Scheme 2 Synthetic scheme for the protected tripeptide hydrazide derivative Z(OMe)-(RNase 38—40)-NHNH-Troc (23)

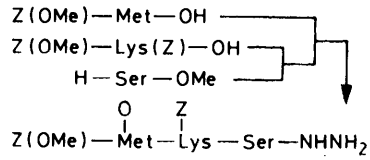
procedures [4] were employed to introduce Z(OMe)-Arg(MBS)-OH [5] and Z(OMe)-Asp(OBzl)-OH respectively. The Troc group [6] was removed from the resulting protected tripeptide (23) as usual, by treatment with Zn in acetic acid [7] to yield Z(OMe)-Asp(OBzl)-Arg(MBS)-Cys(MBzl)-$NHNH_2$.

The next fragment, Z(OMe)-Arg(MBS)-Asn-Leu-Thr-Lys(Z)-$NHNH_2$(24) (positions 33—37), was also prepared in a stepwise manner, but without protection of the C-terminal end. Z(OMe)-Thr-Lys(Z)-OH and Z(OMe)-Leu-Thr-Lys(Z)-OH, prepared by the active-ester procedures, were easily purified by extraction. Z(OMe)-Asn-OH was then introduced by the NP method, as usual, and Z(OMe)-Arg(MBS)-OH by the DNP method.[8] In the latter instance, the corresponding active ester was not isolated and the progress of the reaction was monitored by t.l.c. Thus, any intramolecular lactam formation [9] by Arg(MBS) was suppressed. The resulting pentapeptide was esterified and subsequently exposed to hydrazine to give the desired hydrazide (Scheme 3).

The tripeptide hydrazide Z(OMe)-Met(O)-Lys(Z)-Ser-$NHNH_2$ (25) (positions 30—32) was prepared according to Scheme 4. Z(OMe)-Met-Lys(Z)-Ser-OMe, prepared by



Scheme 3 Synthetic scheme for the protected pentapeptide hydrazide Z(OMe)-(RNase 33—37)-$NHNH_2$ (24)



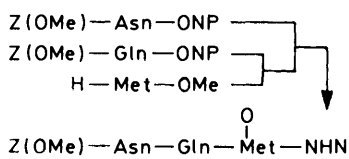
Scheme 4 Synthetic scheme for the protected tripeptide hydrazide Z(OMe)-(RNase 30—32)-$NHNH_2$ (25)

two successive DCC condensations [10] was oxidized by sodium metaperiodate,[11] instead of hydrogen peroxide [12] or tetrachlorauric(III) acid [13] adopted previously for the preparation of Z(OMe)-Ser-Thr-Met(O)$NHNH_2$(12).[1c] A convenient procedure for the preparation of Met(O) derivatives was available, the mild oxidation producing a mixture of diastereoisomeric sulphoxides, but little sulphone.

In order to prepare fragment (26), Z(OMe)-Asn-Gln-Met(O)-$NHNH_2$ (positions 27—29), the methionine residue was oxidized by the aforementioned oxidant, sodium metaperiodate, at the dipeptide stage. The resulting dipeptide, Z(OMe)-Gln-Met(O)-OMe, was converted into Z(OMe)-Asn-Gln-Met(O)-OMe by the NP method and subsequently into the hydrazide (26) by the usual hydrazine treatment (Scheme 5).

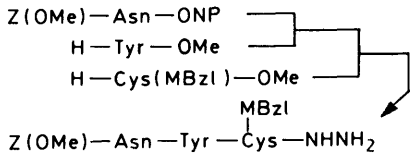
Z(OMe)-Asn-Tyr-Cys(MBzl)-$NHNH_2$(27) (positions 24—26), a fragment selected for the reasons stated earlier, was formed by condensation of Z(OMe)-Asn-Tyr-$NHNH_2$ (*via* the azide) with H-Cys(MBzl)-OMe, the resulting tripeptide ester being converted into the hydrazide (27), in the usual manner (Scheme 6).

The N-terminal tripeptide unit Z(OMe)-Ser-Ser-Ser-$NHNH_2$ (28) (positions 21—23) was prepared by two

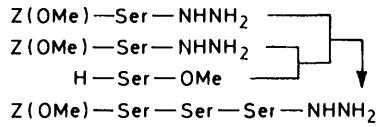


Scheme 5 Synthetic scheme for the protected tripeptide hydrazide Z(OMe)-(RNase 27—29)-$NHNH_2$ (26)

successive azide condensations followed by the usual hydrazine treatment (Scheme 7).



SCHEME 6 Synthetic scheme for the protected tripeptide hydrazide Z(OMe)-(RNase 24—26)-$NHNH_2$ (27)



SCHEME 7 Synthetic scheme for the protected tripeptide hydrazide Z(OMe)-(RNase 21—23)-$NHNH_2$ (28)

The six successive azide condensations of these peptide fragments were performed according to Scheme 1. In the azide condensations of (23) and (24), two additions of acyl components (3 + 2 molar equivalents) at intervals of 72 h were sufficient to bring the reactions to completion, as mentioned in the preceding paper.[1d] The condensation of (25) was performed similarly and the reaction mixture became almost ninhydrin negative. When a sample of the product was isolated at this stage and submitted to amino-acid analysis, incorporation of acyl component was found to be only around 80%. Even after purification by repeated washing and precipitation, the situation was not altered. In order to avoid laborious purification procedures, after 48 h a third portion of acyl component (2 molar equivalents) was added to drive the last 20% of the reaction to completion. Based on these experiences, the subsequent condensations of fragments (26), (27), and (28) were performed using 9—12 molar equivalents of acyl component in each step. Each reaction was started with 5—6 molar equivalents of acyl component and after 72 h, the second azide (2 mol equiv.) was added, at which the reaction mixture became almost ninhydrin negative. In order to secure complete condensation, 2 mol equiv. of the third azide were added for fragments (26) and (27) followed by 2 mol equiv. of the fourth azide for fragment (28). Amino-acid ratios in the acid hydrolysates of the protected S-protein and the intermediates, synthesized under the above conditions, are listed in the Table. These values corresponded well with those predicted by theory. Phenylalanine, in addition to leucine, still played a very important role as a diagnostic amino-acid for assessment of homogeneity. In order to obtain additional proof for the homogeneity of synthetic Z(OMe)-(RNase 21—124)-OBzl, the sample was submitted to gel-filtration on Sephacryl S-200. When eluted with DMSO–H_2O (19 : 1), the desired compound emerged from the column as a single component, thereby excluding the possibility of contamination by acyl components.

After successive condensations of 28 peptide fragments and column chromatographic examination at three points (positions 69, 41, and 21), we were able to obtain 4.9 g of the protected S-protein having a high degree of homogeneity. Hirschmann *et al.*[14] have prepared the protected S-protein by the azide condensation of two fragments, Boc-(21—64)-$NHNH_2$ and H-(65—124)-OH, and this material, without purification and characterization, was submitted to the final deprotection. No information about the coupling yield and the purity of the protected S-protein was given.

Following the synthesis of the protected S-protein, we synthesized the S-peptide in the form of the protected hydrazide, expecting to reach the final goal by a single azide condensation. Based on the earlier observations made by Richards and his co-workers[15] that equimolar combinations of S-peptide and S-protein brought about full regeneration of enzymatic activity (RNase S'), Hofmann *et al.*[16] synthesized an S-peptide in 1966, using protecting groups removable by TFA and claimed the semi-synthesis of RNase S', after combination with natural S-protein. Information about structure–function relationships of S-peptides has been presented by the above authors[17] and by others.[18]

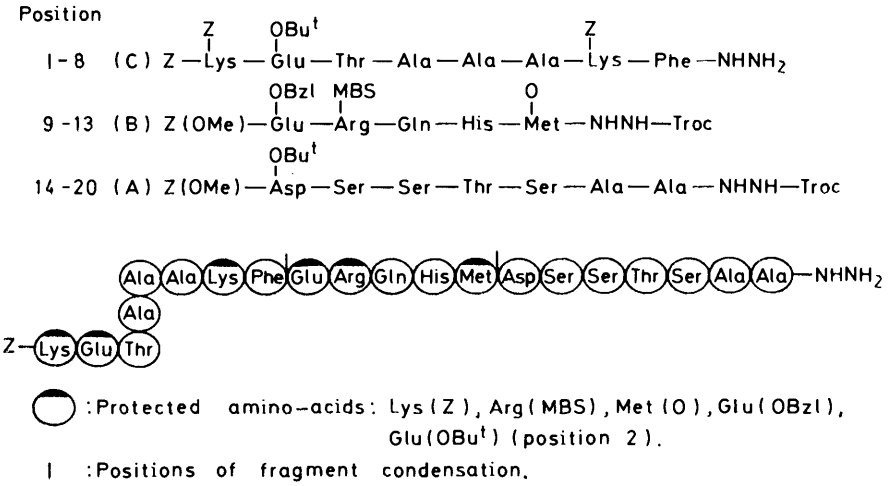
Amino-acid ratios of Z(OMe)-(RNase 21—124)-OBzl and intermediates

Position	38—124	33—124	30—124	27—124	24—124	21—124
Residue	(87)	(92)	(95)	(98)	(101)	(104)
Asp	11.28(11)	12.34(12)	12.34(12)	13.26(13)	14.23(14)	14.20(14)
Thr	6.91(7)	8.01(8)	7.96(8)	7.48(8)	7.52(8)	7.69(8)
Ser	6.52(8)	6.48(8)	7.18(9)	7.29(9)	7.28(9)	9.59(12)
Glu	8.21(8)	8.12(8)	8.12(8)	9.44(9)	9.36(9)	9.39(9)
Pro	3.69(4)	3.79(4)	3.76(4)	3.99(4)	3.77(4)	3.81(4)
Gly	3.23(3)	3.29(3)	3.17(3)	3.22(3)	3.26(3)	3.02(3)
Ala	6.81(7)	6.83(7)	6.86(7)	6.91(7)	6.88(7)	6.85(7)
Val	8.79(9)	8.85(9)	8.68(9)	8.65(9)	8.77(9)	8.53(9)
Met *	0.69(1)	0.70(1)	1.55(2)	2.57(3)	2.37(3)	2.41(3)
Ile	2.47(3)	2.41(3)	2.38(3)	2.16(3)	2.09(3)	2.01(3)
Leu	1.01(1)	2.04(2)	2.12(2)	1.91(2)	1.99(2)	1.97(2)
Tyr	5.40(5)	5.32(5)	5.29(5)	5.11(5)	6.28(6)	6.26(6)
Phe	2.00(2)	2.00(2)	2.00(2)	2.00(2)	2.00(2)	2.00(2)
Lys	6.20(6)	7.34(7)	8.32(8)	8.41(8)	8.36(8)	8.37(8)
His	2.71(3)	2.67(3)	2.71(3)	2.31(3)	2.55(3)	2.23(3)
Arg	1.97(2)	3.05(3)	2.87(3)	2.99(3)	2.81(3)	2.86(3)
Cys	(7)	(7)	(7)	(7)	(8)	(8)
Recovery (%)	87	84	89	86	82	89

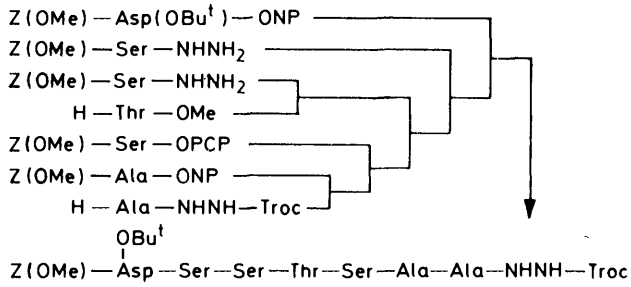
* Met(O) was not calculated.

We synthesized, with the aid of the substituted hydrazine, the protected S-peptide hydrazide Z-(RNase 1—20)-$NHNH_2$, by the condensation of three building blocks as shown in Scheme 8. The C-terminal heptapeptide fragment Z(OMe)-Asp(OBu^t)-Ser-Ser-Thr-Ser-Ala-Ala-NHNH-Troc, Z(OMe)-(RNase 14—20)-NHNH-Troc (A), was prepared according to Scheme 9. Z(OMe)-Ser-Ala-Ala-NHNH-Troc, prepared in a stepwise manner by the active-ester procedure, was condensed successively with Z(OMe)-Ser-Thr-$NHNH_2$ and Z(OMe)-Ser-$NHNH_2$ *via* the azide procedure. Next, Z(OMe)-Asp(OBzl)-OH was added onto the resulting hexapeptide. However, when exposed to Et_3N, the protected heptapeptide was heterogeneous on t.l.c. This phenomenon seems due to formation of succinimide by the Asp(OBzl)-Ser unit, as reported by Bodanszky *et al.*[19] Therefore, we decided to introduce Z(OMe)-Asp(OBu^t)-OH and to remove both protecting groups by TFA, prior to the next condensation.

The middle fragment, Z(OMe)-Glu(OBzl)-Arg(MBS)-Gln-His-Met(O)-NHNH-Troc, Z(OMe)-(RNase 9—13)-NHNH-Troc (B) containing Glu(OBzl), was synthesized starting from H-Met(O)-NHNH-Troc as shown in Scheme 10. Z(OMe)-Gln-His-$NHNH_2$[20] was then introduced *via* the azide, Z(OMe)-Arg(MBS)-OH by the mixed anhydride method,[21] and Z(OMe)-Glu (OBzl)-OH by the NP method. The Troc group was removed from the resulting pentapeptide (B) using Zn, as usual, to yield Z(OMe)-Glu(OBzl)-Arg(MBS)-Gln-His-Met(O)-NH-NH_2.

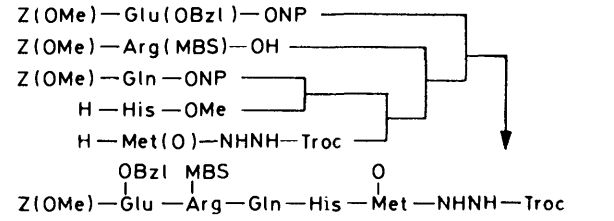


SCHEME 8 Synthetic route to the protected icosapeptide hydrazide, Z-(RNase 1—20)-$NHNH_2$ (protected S-peptide)

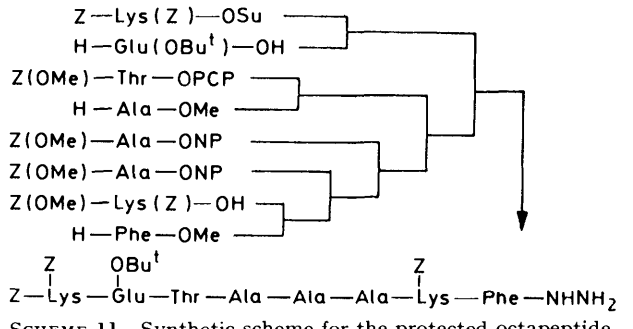


SCHEME 9 Synthetic scheme for the protected heptapeptide hydrazide derivative Z(OMe)-(RNase 14—20)-NHNH-Troc (A)

The N-terminal octapeptide hydrazide Z-Lys(Z)-Glu(OBu^t)-Thr-Ala-Ala-Ala-Lys(Z)-Phe-$NHNH_2$, Z-(RNase 1—8)-$NHNH_2$ (C), contains the glutamic acid residue. Glu(OBu^t), instead of Glu(OBzl), was used in this case since this ester resists the action of hydrazine[22] and further chain elongation is not necessary. As shown in Scheme 11, Z(OMe)-Ala-Ala-Lys(Z)-Phe-OMe was prepared in a stepwise manner using either the DCC or NP method, and two dipeptide units, Z(OMe)-Thr-Ala-$NHNH_2$ and Z-Lys(Z)-Glu(OBu^t)-NH-NH_2, were successively condensed onto it. Finally, the resulting octapeptide ester was converted into the cor-



SCHEME 10 Synthetic scheme for the protected pentapeptide hydrazide derivative Z(OMe)-(RNase 9—13)-NHNH-Troc (B)



SCHEME 11 Synthetic scheme for the protected octapeptide hydrazide Z-(RNase 1—8)-$NHNH_2$ (C)

responding hydrazide using HMPA–MeOH as the solvent system (because of the low solubility of the ester in DMF).

According to Scheme 8, successive azide condensations of the three fragments led to the formation of the protected icosapeptide derivative, from which the Troc group was removed with Zn in HMPA–NMP–acetic acid. The Troc group was for some reason not removed from this relatively large fragment in DMSO–acetic acid. As mentioned above, the side chain of the aspartic acid residue at position 14 was free in our protected S-peptide. The homogeneity of the protected S-peptide was assessed by t.l.c., acid hydrolysis, and elemental analysis. We were thus able to obtain the two peptide units necessary to construct the entire amino-acid sequence of RNase A. The final steps of the total synthesis of RNase are reported in the following paper.

EXPERIMENTAL

General experimental procedures are essentially the same as described in the Part 1.[1a] The N^{α}-protecting group, *Z*(OMe), was removed by TFA in the presence of anisole (≥2 mol equiv.) in an ice-bath for 45—60 min. The DCC and the active ester condensations were performed at room temperature (17—25 °C). The azide condensation was performed according to Honzl and Rudinger.[2] A mixed anhydride was prepared according to Vaughan and Osato.[21b]

Z(OMe)-Cys(MBzl)-NHNH-Troc.—DCC (11.30 g, 55 mmol) was added to a stirred solution of Z(OMe)-Cys(MBzl)-OH (20.27 g, 50 mmol) and Troc-$NHNH_2$ (11.41 g, 55 mmol) in AcOEt (250 ml). After 48 h, the solution was filtered, the filtrate washed with 10% citric acid, 5% Na_2CO_3, and H_2O, dried over Na_2SO_4, and concentrated. Trituration with ether followed by recrystallization from AcOEt–ether afforded the *substituted hydrazide* (20.20 g, 68%), m.p. 126—129 °C, $[\alpha]_D^{27}$ −21.8° (*c*, 0.9 in DMF), R_{F_2} 0.51 (Found: C, 46.6; H, 4.45; N, 7.05. $C_{23}H_{26}Cl_3N_3O_7S$ requires C, 46.43; H, 4.41; N, 7.06%).

Z(OMe)-Arg(MBS)-Cys(MBzl)-NHNH-Troc.—Z(OMe)-Cys(MBzl)-NHNH-Troc (8.15 g, 14 mmol) was treated with TFA–anisole (20 ml; 4 : 1 v/v) as usual, then the excess of TFA was removed by evaporation. The oily residue was washed with n-hexane and dissolved in 4.2N-HCl–dioxan (4.0 ml, 16 mmol). The solvent was evaporated off and the resulting oil, after washing with n-hexane, was dissolved in DMF (80 ml) together with Et_3N (1.9 ml, 14 mmol), HOBT (2.22 g, 16 mmol), and Z(OMe)-Arg(MBS)-OH (7.66 g, 15 mmol). After addition of DCC (3.39 g, 16 mmol), the solution was stirred for 48 h, filtered, and the filtrate was concentrated. The residue was purified by procedure A followed by recrystallization from AcOEt–ether to afford the *protected dipeptide* (11.33 g, 90%), m.p. 95—98 °C, $[\alpha]_D^{22}$ −15.6° (*c*, 0.5 in MeOH), R_{F_1} 0.69 (Found: C, 47.1; H, 4.65; N, 10.65. $C_{36}H_{44}Cl_3N_7O_{11}S_2$ requires C, 46.93; H, 4.81; N, 10.64%).

Z(OMe)-Asp(OBzl)-Arg(MBS)-Cys(MBzl)-NHNH-Troc(23).—The above protected dipeptide (11.33 g, 12 mmol) was treated with TFA (23 ml) and anisole (5.6 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 (100 ml) together with Et_3N (3.4 ml, 25 mmol) and Z(OMe)-Asp(OBzl)-ONP (7.51 g, 15 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 give the *protected tripeptide* (10.50 g, 76%), m.p. 101—105 °C, $[\alpha]_D^{22}$ −11.5° (*c*, 1.3 in DMF), R_{F_1} 0.66 (Found: C, 50.85; H, 4.95; N, 10.45. $C_{47}H_{55}Cl_3N_8O_{14}S_2$ requires C, 50.11; H, 4.92; N, 9.95%).

Z(OMe)-Asp(OBzl)-Arg(MBS)-Cys(MBzl)-NHNH$_2$, *Z(OMe)-(RNase* 38—40)-*NHNH*$_2$.—The above protected tripeptide (10.36 g, 9.2 mmol) in AcOH (100 ml) was treated with Zn powder (6.0 g, 10 mol equiv.) for 48 h and the solvent was evaporated off *in vacuo* at a bath temperature <25 °C. The residue was treated with a saturated solution of EDTA to give a gelatinous mass, which was washed with 5% Na_2CO_3 and H_2O and recrystallized from dioxan–ether to afford the *protected tripeptide hydrazide* (7.01 h, 80%). m.p. 161—167 °C, $[\alpha]_D^{23}$ −23.4° (*c*, 1.4 DMSO), R_{F_1} 0.53. Amino-acid analysis: Asp 1.00, Arg 0.82 (average recovery 87%) (Found: C, 54.4; H, 5.6; N, 11.35. $C_{44}H_{54}N_8O_{12}S_2 \cdot H_2O$ requires C, 54.53; H, 5.82; N, 11.56%).

Z(OMe)-Thr-Lys(Z)-OH.—Z(OMe)-Thr-OPCP (42.40 g, 80 mmol) was added to a stirred solution of Lys(Z) (22.40 g, 80 mmol) in 50% aqueous pyridine (220 ml) containing Et_3N (21.6 ml, 0.16 mol). After 48 h, the solvent was evaporated off and the residue was purified by procedure C followed by recrystallization from MeOH–AcOEt to yield the *protected dipeptide* (27.03 g, 62%), m.p. 141—145 °C, $[\alpha]_D^{22}$ +13.0° (*c*, 0.5 in DMF), R_{F_1} 0.35 (Found: C, 59.7; H, 6.45; N, 7.8. $C_{27}H_{35}N_3O_9$ requires C, 59.43; H, 6.46; N, 7.70%).

Z(OMe)-Leu-Thr-Lys(Z)-OH.—Z(OMe)-Thr-Lys(Z)-OH (12.40 g, 23 mmol) was treated with TFA–anisole (26 ml; 10 : 3 v/v) as usual, then dry ether was added. The resulting powder, isolated as above, was dissolved in DMF (80 ml) together with Et_3N (9.5 ml, 69 mmol) and Z(OMe)-Leu-ONP (9.61 g, 23 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure C followed by recrystallization from AcOEt to give the *protected tripeptide* (12.42 g, 83%), m.p. 99—104 °C, $[\alpha]_D^{23}$ +0.6° (*c*, 0.8 in DMF), R_{F_1} 0.57 (Found: C, 59.9; H, 7.0; N, 8.7. $C_{33}H_{46}N_4O_{10}$ requires C, 60.17; H, 7.04; N, 8.51%).

Z(OMe)-Asn-Leu-Thr-Lys(Z)-OH.—Z(OMe)-Leu-Thr-Lys(Z)-OH (9.01 g, 13.7 mmol) was treated with TFA–anisole (13.5 ml; 2 : 1 v/v) as usual, then dry ether was added. The resulting powder, isolated as above, was dissolved in DMF (90 ml) together with Et_3N (5.7 ml, 41 mmol) and Z(OMe)-Asn-ONP (7.40 g, 18 mmol). After stirring for 48 h, the solution was concentrated and the residue was treated with 5% citric acid and ether to form a powder, which was washed with 5% citric acid and H_2O and precipitated from DMF with AcOEt to afford the *protected tetrapeptide* (8.13 g, 77%), m.p. 178—181 °C, $[\alpha]_D^{22}$ −14.3° (*c*, 1.4 in DMF), R_{F_1} 0.32 (Found: C, 57.45; H, 6.8; N, 10.8. $C_{37}H_{52}N_6O_{12}$ requires C, 57.50; H, 6.78; N, 10.88%).

Z(OMe)-Arg(MBS)-Asn-Leu-Thr-Lys(Z)-OH.—DCC (2.47 g, 12 mmol) was added to a stirred solution of Z(OMe)-Arg(MBS)-OH (6.15 g, 12 mmol) and DNP-OH (2.23 g, 12 mmol) in AcOEt–DMF (35 ml; 6 : 1 v/v). After 5 h, the solution was filtered and the filtrate was submitted to the following condensation reaction. Z(OMe)-Asn-Leu-Thr-Lys(Z)-OH (8.50 g, 11 mmol) was treated with TFA–anisole (21 ml; 4 : 1 v/v) and the N^{α}-deprotected peptide, isolated as mentioned above, was dissolved in DMF (50 ml) containing Et_3N (4.6 ml, 33 mmol). This solution was mixed with the solution containing the DNP ester. 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 pentapeptide* (6.80 g, 56%), m.p. 168—171 °C, $[\alpha]_D^{23}$ −6.8° (*c*, 0.1 in DMF), R_{F_2} 0.14 (Found: C, 54.6; H, 6.6; N, 12.65. $C_{50}H_{70}N_{10}O_{16}S$ requires C, 54.63; H, 6.42; N, 12.74%).

Z(OMe)-Arg(MBS)-Asn-Leu-Thr-Lys(Z)-OMe.—An ethereal solution of diazomethane was added to an ice-chilled solution of Z(OMe)-Arg(MBS)-Asn-Leu-ThrLys(Z)-OH (6.34 g, 5.8 mmol) in DMF–MeOH (1 : 1 v/v; 100 ml) until the yellow colour persisted for 30 min. After addition of a few drops of AcOH, the solution was concentrated and the residue was triturated with ether. The resulting powder was washed with 5% Na_2CO_3 and H_2O and precipitated from DMF with AcOEt to give the *protected pentapeptide ester* (4.48 g, 70%), m.p. 158—160 °C, $[\alpha]_D^{22}$ −12.1° (*c*, 1.0 in DMF), R_{F_1} 0.61 (Found: C, 55.0; H, 6.4; N, 12.55. $C_{51}H_{72}N_{10}O_{16}S$ requires C, 55.02; H, 6.52; N, 12.58%).

Z(OMe)-Arg(MBS)-Asn-Leu-Thr-Lys(Z)-NHNH$_2$, *Z-(OMe)-(RNase 33—37)-NHNH$_2$(24)*.—The above methyl ester (4.01 g, 3.6 mmol) in DMF (40 ml) was treated with 80% hydrazine hydrate (2.3 ml, 10 mol equiv.) overnight. The solvent was evaporated off and the residue was triturated with MeOH. Precipitation of the resulting mass from DMF with MeOH afforded the *hydrazide* (3.30 g, 82%), m.p. 237—240 °C, $[\alpha]_D^{22}$ −11.6° (*c*, 1.0 in DMSO), R_{F_1} 0.55. Amino-acid analysis: Asp 1.01, Thr 0.94, Leu 1.00, Lys 1.13, Arg 0.87 (average recovery 94%) (Found: C, 53.75; H, 6.3; N, 14.9. $C_{50}H_{72}N_{12}O_{15}S$ requires C, 53.94; H, 6.52; N, 15.10%).

Z(OMe)-Met-Lys(Z)-Ser-OMe.—Z(OMe)-Lys(Z)-Ser-OMe [23] (16.37 g, 30 mmol) was treated with TFA-anisole (41.2 ml; 4 : 1 v/v) as usual and dry ether was added. The resulting powder was dissolved in 4.21N-HCl–dioxan (7.13 ml, 30 mmol), the solvent was evaporated off, and the residue was treated with ether. 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 (4.14 ml, 30 mmol) and Z(OMe)-Met-OH (9.40 g, 30 mmol). After addition of DCC (6.80 g, 33 mmol), the solution was stirred for 48 h, filtered and the filtrate was concentrated. The residue was purified by procedure B followed by precipitation from DMF with AcOEt to afford the *protected tripeptide ester* (14.70 g, 72%), m.p. 188—190 °C $[\alpha]_D^{23}$ −12.9° (*c*, 0.2 in DMF), R_{F_1} 0.60 (Found: C, 56.85; H, 6.6; N, 8.4. $C_{32}H_{44}N_4O_{10}S$ requires C, 56.79; H, 6.55; N, 8.28%).

Z(OMe)-Met(O)-Lys(Z)-Ser-OMe.—$NaIO_4$ (2.35 g, 11 mmol) in H_2O (50 ml) was added to an ice-chilled solution of Z(OMe)-Met-Lys(Z)-Ser-OMe (6.77 g, 10 mmol) in DMF–MeOH (80 ml; 3 : 5 v/v). After stirring at 4 °C for 24 h, the solution was concentrated and the residue was treated with ether and H_2O. The resulting powder was precipitated from DMF with MeOH to afford the *sulphoxide* (6.20 g, 90%), m.p. 207—211 °C, $[\alpha]_D^{22}$ +2.1° (*c*, 0.9 in DMF), R_{F_1} 0.79, R_{F_2} 0.62 (Found: C, 55.3; H, 6.4; N, 8.05. $C_{32}H_{44}N_4O_{11}S$ requires C, 55.48; H, 6.40; N, 8.09%).

Z(OMe)-Met(O)-Lys(Z)-Ser-NHNH$_2$, *Z(OMe)-(RNase 30—32)-NHNH$_2$* (25).—Z(OMe)-Met(O)-Lys(Z)-Ser-OMe (6.03 g, 8.7 mmol) in DMF–MeOH (50 ml; 4 : 1 v/v) was treated with 80% hydrazine hydrate (4.4 ml, 8 mol equiv.) overnight. The solvent was evaporated off and the residue was precipitated from DMF with MeOH to give the *hydrazide* (4.97 g, 82%), m.p. 221—224 °C, $[\alpha]_D^{22}$ +2.0° (*c*, 1.0 in DMSO), R_{F_1} 0.61, R_{F_2} 0.20. Amino-acid analysis: Met + Met(O), 0.82, Lys 1.00, Ser 0.71 (average recovery 90%) (Found: C, 53.55; H, 6.65; N, 12.1. $C_{31}H_{44}N_6O_{10}S$ requires C, 53.74; H, 6.40; N, 12.13%).

Z(OMe)-Gln-Met-OMe.—Z(OMe)-Gln-ONP (64.65 g, 0.15 mol) and Et_3N (20.7 ml, 0.15 mol) were added to a stirred solution of H-Met-OMe [from the hydrochloride (29.85 g, 0.15 mol) with Et_3N (20.7 ml, 0.15 mol)] in DMF (300 ml). After 48 h, the solution was concentrated and the residue was purified by procedure B followed by recrystallization from dioxan–MeOH to give the *protected dipeptide ester* (46.41 g, 68%), m.p. 162—170 °C, $[\alpha]_D^{23}$ −7.0° (*c*, 1.4 in DMF), R_{F_1} 0.43 (Found: C, 53.1; H, 6.65; N, 9.35. $C_{20}H_{29}N_3O_7S$ requires C, 52.73; H, 6.42; N, 9.23%).

Z(OMe)-Gln-Met(O)-OMe.—$NaIO_4$ (1.93 g, 9 mmol) in H_2O (20 ml) was added to an ice-chilled solution of Z(OMe)-Gln-Met-OMe (3.65 g, 8 mmol) in MeOH–DMF (65 ml; 8 : 5 v/v). After stirring at 4 °C for 12 h, the solution was concentrated and the residue was washed with H_2O to remove the inorganic salt; some of the product was lost, because of its solubility in water. Precipitation from DMF with ether afforded the *sulphoxide* (1.60 g, 40%), m.p. 185—186 °C, $[\alpha]_D^{23}$ −15.9° (*c*, 1.8 in DMF), R_{F_1} 0.70, R_{F_2} 0.55 (Found: C, 50.95; H, 6.15; N, 8.9. $C_{20}H_{29}N_3O_8S$ requires C, 50.94; H, 6.20; N, 8.91%).

Z(OMe)-Asn-Gln-Met(O)-OMe.—Z(OMe)-Gln-Met(O)-OMe (7.77 g, 16.5 mmol) was treated with TFA–anisole (38 ml; 15 : 4 v/v) as usual, and dry ether was added. The resulting powder isolated as mentioned above, was dissolved in DMF–DMSO (150 ml; 2 : 1 v/v) together with Et_3N (4.55 ml, 33 mmol), HOBT (0.50 g, 3.3 mmol), and Z(OMe)-Asn-ONP (6.89 g, 16.5 mmol). After stirring for 24 h, the solution was concentrated and the residue was purified by washing with 5% citric acid and H_2O followed by precipitation (×2) from DMSO with AcOEt to yield the *protected tripeptide ester* (7.01 g, 73%), m.p. 216—220 °C, $[\alpha]_D^{23}$ −19.6° (*c*, 0.5 in DMSO), R_{F_1} 0.37 (Found: C, 47.2; H, 6.2; N, 11.4. $C_{24}H_{25}N_5O_{10}S\cdot 1.5H_2O$ requires C, 47.05; H, 6.25; N, 11.43%). Attempts to wash with 5% Na_2CO_3 or precipitate from DMSO with MeOH produced a pasty material that was difficult to collect by filtration.

Z(OMe)-Asn-Gln-Met(O)-NHNH$_2$, *Z(OMe)-(RNase 27—29)-NHNH$_2$(26)*.—Z(OMe)-Asn-Gln-Met(O)-OMe (4.82 g, 8.2 mmol) in DMSO–MeOH (25 ml; 4 : 1 v/v) was treated with 80% hydrazine hydrate (2.06 ml, 4 mol equiv.) at 4 °C overnight. The solvent was evaporated off and the residue was precipitated twice from DMSO with MeOH to afford the *hydrazide* (4.68 g, 97%), m.p. 226—229 °C, $[\alpha]_D^{20}$ −23.5° (*c*, 0.9 in DMSO), R_{F_1} 0.14. Amino-acid analysis: Asp 1.00, Glu 1.03, Met + Met(O) 0.71 (average recovery 98%) (Found: C, 46.55; H, 6.1; N, 16.15. $C_{23}H_{35}N_7O_9S\cdot 1/2H_2O$ requires C, 46.45; H, 6.10; N, 16.49%).

Z(OMe)-Asn-Tyr-OMe.—Z(OMe)-Asn-ONP (12.98 g, 31.1 mmol) and Et_3N (4.29 ml, 31.1 mmol) were added to a stirred solution of H-Tyr-OMe [from the hydrochloride (7.29 g, 37.3 mmol)] with Et_3N (5.15 ml, 37.3 mmol)] in DMF (100 ml). After 24 h, the solution was concentrated and the residue was purified by procedure B followed by precipitation (×2) from MeOH with ether to give the *protected dipeptide ester* (10.61 g, 72%), m.p. 171—172 °C, $[\alpha]_D^{20}$ +8.2° (*c*, 1.1 in DMF), R_{F_1} 0.45 (Found: C, 58.35; H, 5.85; N, 8.85. $C_{23}H_{27}N_3O_8$ requires C, 58.34; H, 5.75; N, 8.88%).

Z(OMe)-Asn-Tyr-NHNH$_2$.—Z(OMe)-Asn-Tyr-OMe (6.05

g, 12.8 mmol) in DMF–MeOH (35 ml; 6 : 1 v/v) was treated with 80% hydrazine hydrate (6.4 ml, 8 mol equiv.) overnight and MeOH (200 ml) was added. The resulting gelatinous mass was precipitated twice from DMF with MeOH to afford the *hydrazide* (5.63 g, 93%), m.p. 215—218 °C, $[\alpha]_D^{23}$ −1.7° (*c*, 0.6 in DMSO), R_{F1} 0.22 (Found: C, 56.0; H, 5.8; N, 14.95. $C_{22}H_{27}N_5O_7$ requires C, 55.80; H, 5.75; N, 14.79%).

Z(OMe)-Asn-Tyr-Cys(MBzl)-OMe.—The azide [from Z-(OMe)-Asn-Tyr-$NHNH_2$ (5.02 g, 10.6 mmol)] in DMF (20 ml) and Et_3N (1.61 ml, 11.7 mmol) were added to an ice-chilled solution of H-Cys(MBzl)-OMe [from the Z(OMe)-derivative (6.17 g, 14.7 mmol) by the usual TFA treatment followed by neutralization with Et_3N (2.23 ml, 16.2 mmol)] in DMF (45 ml). After stirring for 24 h, the solution was concentrated and the residue was purified by procedure B followed by precipitation twice from DMF with MeOH to yield the *methyl ester* (6.02 g, 82%), m.p. 198—200 °C, $[\alpha]_D^{23}$ −37.3° (*c*, 0.7 in DMF), R_{F1} 0.68 (Found: C, 58.55; H, 5.65; N, 8.2. $C_{34}H_{40}N_4O_{10}S$ requires C, 58.61; H, 5.79; N, 8.04%).

Z(OMe)-Asn-Tyr-Cys(MBzl)-$NHNH_2$, Z(OMe)-(RNase 24—26)-$NHNH_2$(27).—Z(OMe)-Asn-Tyr-Cys(MBzl)-OMe (6.02 g, 8.7 mmol) in DMF–MeOH (35 ml; 6 : 1 v/v) was treated with 80% hydrazine hydrate (4.3 ml, 10 mol equiv.) overnight. Addition of MeOH (200 ml) gave a gelatinous mass, which was precipitated from DMF with MeOH to give the *hydrazide* (5.33 g, 88%), m.p. 224—226 °C, $[\alpha]_D^{20}$ −20.3° (*c*, 1.2 in DMSO), R_{F1} 0.39. Amino-acid analysis: Asp 1.00, Tyr 1.01 (average recovery 94%) (Found: C, 56.6; H, 5.8; N, 11.95. $C_{38}H_{40}N_6O_9S$ requires C, 56.88; H, 5.79; N, 12.06%).

Z(OMe)-Ser-Ser-OMe.—The azide [from Z(OMe)-Ser-$NHNH_2$ (10.05 g, 36 mmol)] in DMF (70 ml) and Et_3N (4.90 ml, 36 mmol) were added to an ice-chilled solution of H-Ser-OMe [from the hydrochloride (7.18 g, 46 mmol) with Et_3N (6.36 ml, 46 mmol)] in DMF (35 ml). After stirring for 24 h, the solution was concentrated. Trituration with EtOH followed by precipitation from DMF with EtOH afforded the *protected dipeptide ester* (10.22 g, 60%), m.p. 124—127 °C, $[\alpha]_D^{23}$ +12.1° (*c*, 0.4 in DMF), R_{F1} 0.52 (Found: C, 51.45; H, 5.9; N, 7.45. $C_{16}H_{22}N_2O_8$ requires C, 51.89; H, 5.99; N, 7.56%).

Z(OMe)-Ser-Ser-Ser-OMe.—Z(OMe)-Ser-Ser-OMe (8.10 g, 22 mmol) was treated with TFA–anisole (32 ml; 3 : 1 v/v) as usual, and dry ether was added. The resulting powder was dissolved in DMF (80 ml) containing Et_3N (3.02 ml, 22 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Ser-$NHNH_2$(6.20 g, 22 mmol)] in DMF (50 ml) and Et_3N (3.02 ml, 22 mmol). After stirring for 24 h, the solution was concentrated. Trituration with EtOH followed by precipitation from DMF with EtOH afforded the *protected tripeptide ester* (7.62 g, 76%), m.p. 188—189 °C, $[\alpha]_D^{23}$ +7.9° (*c*, 0.5 in DMF), R_{F1} 0.67 (Found: C, 50.2; H, 5.95; N, 8.9. $C_{19}H_{27}N_3O_{10}$ requires C, 49.89; H, 5.95; N, 9.19%).

Z(OMe)-Ser-Ser-Ser-$NHNH_2$, Z(OMe)-(RNase 21—23)-$NHNH_2$ (28).—Z(OMe)-Ser-Ser-Ser-OMe (5.01 g, 11 mmol) in DMF–MeOH (50 ml; 2 : 3 v/v) was treated with 80% hydrazine hydrate (2.74 ml, 4 mol equiv.) overnight. The solvent was evaporated off and the residue was precipitated from DMF with MeOH to give the *hydrazide* (4.42 g, 88%), m.p. 209—214 °C, $[\alpha]_D^{20}$ +5.2° (*c*, 0.8 in DMSO), R_{F1} 0.41 (Found: C, 46.65; H, 5.95; N, 15.05. $C_{18}H_{27}N_5O_9 \cdot 1/2H_2O$ requires C, 46.35; H, 6.05; N, 15.02%).

Z(OMe)-Asp(OBzl)-Arg(MBS)-Cys(MBzl)-Lys(Z)-Pro-Val-Asn-Thr-Phe-Val-His-Glu(OBzl)-Ser-Leu-Ala-Asp-(OBzl)-Val-Gln-Ala-Val-Cys(MBzl)-Ser-Gln-Lys(Z)-Asn-Val-Ala-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 38—124)-OBzl or Z(OMe)-Asp(OBzl)-Arg(MBS)-Cys(MBzl)-(41—124)-OBzl].—Z(OMe)-(RNase 41—124)-OBzl (6.21 g, 0.53 mmol) was treated with TFA–anisole (35 ml; 28 : 7 v/v), then dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 h, and dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v; 50 ml) containing Et_3N (0.29 ml, 2.12 mmol). To this ice-chilled solution, the azide [from Z(OMe)-Asp(OBzl)-Arg-(MBS)-Cys(MBzl)-$NHNH_2$ (1.51 g, 1.59 mmol)] in DMF (12 ml) and Et_3N (0.22 ml, 1.59 mmol) were added and the mixture was stirred at 4 °C for 72 h. The additional azide (prepared from 1.01 g, 1.06 mmol of the hydrazide) in DMF (5 ml) and Et_3N (0.15 ml, 1.06 mmol) were added and the mixture, after stirring for a further 48 h, was concentrated. The residue was purified by procedure B followed by precipitation (×4) from DMSO with MeOH to yield the *protected heptaoctacontapeptide ester* (5.62 g, 85%), m.p. 246 °C (decomp.), $[\alpha]_D^{23}$ −29.4° (*c*, 0.4 in DMSO), R_F 0 (Found: C, 53.6; H, 6.3; N, 12.4. $C_{591}H_{797}N_{117}O_{163}S_{10} \cdot 38H_2O$ requires C, 53.96; H, 6.69; N, 12.46%).

Z(OMe)-(RNase 33—124)-OBzl [Z(OMe)-Arg(MBS)-Asn-Leu-Thr-Lys(Z)-(38—124)-OBzl].—Z(OMe)-(RNase 38—124)-OBzl (5.61 g, 0.45 mmol) was treated with TFA–anisole (30 ml; 4 : 1 v/v) and the N^{α}-deprotected peptide, isolated as mentioned above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v; 40 ml) containing Et_3N (0.25 ml, 1.8 mmol). To this ice-chilled solution, the azide [from Z(OMe)-Arg(MBS)-Asn-Leu-Thr-Lys(Z)-$NHNH_2$ (1.50 g, 1.35 mmol)] in DMF (8 ml) and Et_3N (0.19 ml, 1.35 mmol) were added and the mixture was stirred for 72 h. The additional azide [from the hydrazide (1.00 g, 0.90 mmol)] in DMF (6 ml) and Et_3N (0.12 ml, 0.90 mmol) were added and the mixture, after stirring for an additional 72 h, was concentrated. The product was purified by procedure B followed by precipitation (×2) from DMSO with DMF. Further precipitations from DMSO with Bu^nOH and from DMSO with MeOH afforded the *protected dononacontapeptide ester*, free from contamination of the acyl component (t.l.c.) (4.96 g, 82%), m.p. 251 °C (decomp.). $[\alpha]_D^{23}$ −31.9° (*c*, 0.4 in DMSO), R_F 0 (Found: C, 52.45; H, 6.25, N, 12.1. $C_{632}H_{857}N_{127}O_{175}S_{11} \cdot 50H_2O$ requires C, 53.13; H, 6.75; N, 12.45%).

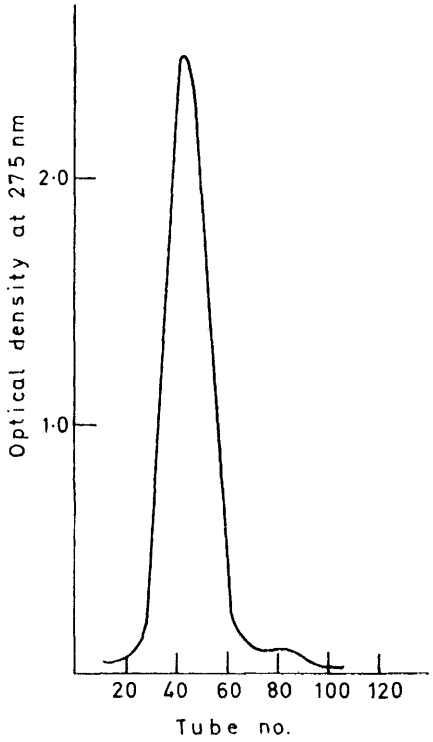
Z(OMe)-(RNase 30—124)-OBzl [Z(OMe)-Met(O)-Lys(Z)-Ser-(33—124)-OBzl].—Z(OMe)-(RNase 33—124)-OBzl (4.95 g, 0.37 mmol) was treated with TFA–anisole (30 ml; 4 : 1 v/v) and the N^{α}-deprotected peptide, isolated as above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v; 40 ml) containing Et_3N (0.21 ml, 1.52 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Met(O)-Lys(Z)-Ser-$NHNH_2$ (0.77 g, 1.11 mmol)] in DMF (6 ml) and Et_3N (0.16 ml, 1.16 mmol), and the mixture was stirred for 72 h. The additional azide [from the hydrazide (0.51 g, 0.74 mmol)] in DMF (5 ml) and Et_3N (0.10 ml, 0.74 mmol) were added and stirring was continued for 48 h. The third batch of azide [from the hydrazide (0.51 g, 0.74 mmol)] in

DMF (5 ml) and Et_3N (0.10 ml, 0.74 mmol) were added and the reaction was continued for an additional 48 h, until the solution became ninhydrin negative. The solvent was evaporated off and the residue was purified by procedure B followed by precipitation (×3) from DMSO with DMF, then once from DMSO with MeOH to afford the *protected pentanonacontapeptide ester* with no contamination of the acyl component (t.l.c.) (4.18 g, 81%), m.p. 248 °C (decomp.), $[\alpha]_D^{23}$ −24.2° (*c*, 0.4 in DMSO), R_F 0 (Found: C, 53.55; H, 6.25; N, 12.5. $C_{632}H_{857}N_{127}O_{175}S_{12}\cdot 40H_2O$ requires C, 53.79; H, 6.69; N, 12.56%).

Z(OMe)-(RNase 27—124)-*OBzl* [*Z(OMe)-Asn-Gln-Met(O)-*(30—124)-*OBzl*].—Z(OMe)-(RNase 30—124)-OBzl (4.17 g, 0.30 mmol) was treated with TFA–anisole (30 ml; 5 : 1 v/v) and the N^{α}-deprotected peptide, isolated as above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v; 40 ml) containing Et_3N (0.17 ml, 1.23 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Asn-Gln-Met(O)-$NHNH_2$ (0.88 g, 1.50 mmol)] in DMF–DMSO (1 : 1 v/v; 7 ml) and Et_3N (0.21 ml, 1.52 mmol). After 72 h, the second batch of the azide [from the hydrazide (0.35 g, 0.60 mmol)] in DMF–DMSO (1 : 1 v/v; 3 ml) and Et_3N (0.09 ml, 0.65 mmol) were added. After an additional 48 h, the 3rd batch of the azide [from the hydrazide (0.35 g, 0.60 mmol)] in DMSO–DMF (1 : 1 v/v; 3 ml) and Et_3N (0.09 ml, 0.65 mmol) were added and stirring was continued for a further 48 h, until the solution became ninhydrin negative. The solvent was evaporated off and the residue was purified by procedure B followed by precipitation from DMSO with DMF (×2). Further precipitations from DMSO with Bu^nOH (×2) and from DMSO with MeOH (×1) afforded the *protected octanonacontapeptide ester*, free from contamination of the acyl component (3.47 g, 81%), m.p. 241 °C (decomp.), $[\alpha]_D^{23}$ −23.4° (*c*, 0.6 in DMSO), R_F 0 (Found: C, 53.7; H, 6.45; N, 12.8. $C_{668}H_{912}N_{136}O_{188}S_{13}\cdot 33H_2O$ requires C, 53.97; H, 6.63; N, 12.81%).

Z(OMe)-(RNase 24—124)-*OBzl* [*Z(OMe)-Asn-Tyr-Cys-(MBzl)-*(27—124)-*OBzl*].—Z(OMe)-(RNase 27—124)-OBzl (3.46 g, 0.24 mmol) was treated with TFA–anisole (24 ml; 5 : 1 v/v) and the N^{α}-deprotected peptide, isolated as above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v; 30 ml) containing Et_3N (0.14 ml, 1.01 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Asn-Tyr-Cys(MBzl)-$NHNH_2$ (0.85 g, 1.22 mmol)] in DMF (7 ml) and Et_3N (0.17 ml, 1.23 mmol), and after 72 h the second batch of the azide [from the hydrazide (0.34 g, 0.49 mmol)] in DMF (3 ml) and Et_3N (67 μl, 0.49 mmol) were added. After an additional 48 h, the same amounts of the azide and Et_3N were added and stirring was continued for 48 h, until the solution became ninhydrin negative. The solution was concentrated and the residue was purified by procedure B followed by precipitation from DMSO with Bu^nOH (×3). Further precipitation from DMSO with MeOH afforded the *protected henhectapeptide ester*, free from contamination by the acyl component (t.l.c.) (3.10 g, 86%), m.p. 240 °C (decomp.), $[\alpha]_D^{23}$ −21.6° (*c*, 0.6 in DMSO), R_F 0 (Found: C, 54.1; H, 6.35; N, 12.6. $C_{692}H_{940}N_{140}O_{194}S_{14}\cdot 33H_2O$ requires C, 54.08; H, 6.60; N, 12.76%).

Z(OMe)-(RNase 21—124)-*OBzl* [*Z(OMe)-Ser-Ser-Ser-*(24—124)-*OBzl, Protected S-Protein*].—Z(OMe)-(RNase 24—124)-OBzl (3.09 g, 0.21 mmol) was treated with TFA–anisole (24 ml; 5 : 1 v/v) and the N^{α}-deprotected peptide, isolated as above, was dissolved in DMSO–DMF–HMPA (1 : 1 : 1 v/v; 25 ml) containing Et_3N (0.12 ml, 0.87 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Ser-Ser-Ser-$NHNH_2$ (0.58 g, 1.26 mmol)] in DMF (5 ml) and Et_3N (0.18 ml, 1.30 mmol) and after stirring for 72 h, three batches of the azide [each from the hydrazide (0.19 g, 0.42 mmol)] in DMF (3 ml) and Et_3N (58 μl, 0.42 mmol each) were further added at 48 h intervals. After addition of the last batch of the azide, stirring was continued for a further 24 h, until the solution became completely ninhydrin negative. H_2O (250 ml) was added and the resulting powder was purified by procedure B followed by precipitation from DMSO with Bu^nOH (×3). Further precipitation from DMSO with MeOH afforded the *protected tetrahectapeptide ester*, free from contamination with the acyl component (t.l.c.) (2.59 g, 82%), m.p. 246 °C (decomp.), $[\alpha]_D^{23}$ −21.1°



Gel-filtration of Z(OMe)-(RNase 21—124)-OBzl on Sephacryl S-200

(*c*, 0.7 in DMSO), R_F 0. For testing of purity, the sample (202 mg) was dissolved in 5% H_2O–DMSO (2 ml) and applied to a column of Sephacryl S-200 (3 × 142 cm), and eluted with 5% H_2O–DMSO. Individual fractions (10 ml each) were collected and absorption at 275 nm was determined. A single peak with a minor tailing was detected (Figure). Fractions corresponding to the main peak (tube nos. 22—55) were collected, the solvent was evaporated off, and the residue was treated with MeOH to give a powder (182 mg, 90%). No significant difference in amino-acid analyses was noted, before (Table 5) and after gel-filtration: Asp 14.21 (14), Thr 7.49 (8), Ser 9.44 (12), Glu 9.09 (9), Pro 3.81 (4), Gly 3.06 (3), Ala 6.71 (7), Val 8.62 (9), Met + Met(O) 2.17 (3), Ile 2.09 (3), Leu 2.05 (2), Tyr 6.42 (6), Phe 2.00 (2), Lys 8.44 (8), His 2.31 (3), Arg 2.84 (3) (average recovery 86%) (Found: C, 53.0; H, 6.25; N, 12.95. $C_{701}H_{955}N_{143}O_{200}S_{14}\cdot 43H_2O$, *M* 150 33.690 + hydration, requires C, 53.26; H, 6.64; N, 12.67%).

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

Troc [24] (37.0 g, 84 mmol) was treated with TFA–anisole (68 ml; 25 : 9 v/v) as usual, 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 (200 ml) together with Et_3N (23.5 ml, 0.17 mol) and Z(OMe)-Ala-ONP (36.02 g, 96 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 give the *dipeptide derivative* (36.04 g, 91%), m.p. 118—120 °C, $[\alpha]_D^{27}$ −11.5° (*c*, 1.4 in DMF, R_{F_1}) 0.63 (Found: C, 42.3; H, 4.5; N, 10.7. $C_{18}H_{23}N_4O_7Cl_3$ requires C, 42.08; H, 4.51; N, 10.91%).

Z(OMe)-Ser-Ala-Ala-NHNH-Troc.—Z(OMe)-Ala-Ala-NHNH-Troc (41.03 g, 80 mmol) was treated with TFA–anisole (55 ml; 8 : 3 v/v), then the excess of TFA was removed by evaporation. The oily residue, isolated, as above, was dissolved in DMF (250 ml) together with Et_3N (22.05 ml, 0.16 mol) and Z(OMe)-Ser-OPCP (41.36 g, 80 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure A followed by recrystallization from MeOH to give the *protected tripeptide derivative* (34.60 g, 72%), m.p. 179—183 °C, $[\alpha]_D^{25}$ −3.8° (*c*, 0.8 in DMF), R_{F_1} 0.53 (Found: C, 42.0; H, 4.65; N, 11.75. $C_{21}H_{28}Cl_3N_5O_9$ requires C, 41.98; H, 4.70; N, 11.66%).

Z(OMe)-Ser-Thr-OMe.—The azide [from Z(OMe)-Ser-$NHNH_2$ (32.76 g, 0.12 mol)] in DMF (200 ml) and Et_3N (15.95 ml, 0.12 mol) were added to an ice-chilled solution of H-Thr-OMe [from the hydrochloride (18.01 g, 0.12 mol)] with Et_3N (15.97 ml, 0.12 mol) in DMF (150 ml). 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 dipeptide* (21.20 g, 48%), m.p. 125—127 °C, $[\alpha]_D^{30}$ +7.2° (*c*, 1.0 in DMF), R_{F_1} 0.66 (Found: C, 53.1; H, 6.5; N, 7.25. $C_{17}H_{24}N_2O_8$ requires C, 53.12; H, 6.29; N, 7.29%).

Z(OMe)-Ser-Thr-$NHNH_2$.—Z(OMe)-Ser-Thr-OMe (13.20 g, 34 mmol) in MeOH (80 ml) was treated with 80% hydrazine hydrate (17 ml, 8 mol equiv.) overnight. The resulting mass was precipitated from DMF with MeOH to yield the *hydrazide* (11.05 g, 85%), m.p. 204—207 °C, $[\alpha]_D^{25}$ +10.5° (*c*, 0.7 in DMSO), R_{F_1} 0.50 (Found: C, 49.85; H, 6.4; N, 14.65. $C_{16}H_{23}N_3O_7$ requires C, 49.99; H, 6.29; N, 14.58%).

Z(OMe)-Ser-Thr-Ser-Ala-Ala-NHNH-Troc.—Z(OMe)-Ser-Ala-Ala-NHNH-Troc (14.77 g, 25 mmol) was treated with TFA–anisole (56 ml; 3 : 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 (120 ml) containing Et_3N (3.39 ml, 25 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Ser-Thr-$NHNH_2$ (10.42 g, 27 mmol)] in DMF (70 ml) and Et_3N (3.74 ml, 27 mmol), and the solution, after stirring for 48 h, was concentrated. The residue was purified by procedure B followed by precipitation from DMF with MeOH (×2) to yield the *protected pentapeptide derivative* (17.15 g, 89%), m.p. 224—228 °C, $[\alpha]_D^{25}$ −7.5° (*c*, 1.6 in DMSO), R_{F_1} 0.32. Amino-acid analysis: Ser 1.39, Thr 0.85, Ala 2.00 (average recovery 91%) (Found: C, 42.65; H, 5.2; N, 12.65. $C_{28}H_{40}Cl_3N_7O_{13}$ requires C, 42.62; H, 5.11; N, 12.43%).

Z(OMe)-Ser-Ser-Thr-Ser-Ala-Ala-NHNH-Troc.—Z(OMe)-Ser-Thr-Ser-Ala-Ala-NHNH-Troc (17.15 g, 22 mmol) was treated with TFA–anisole (67 ml; 50 : 17 v/v) and the N^{α}-deprotected peptide, isolated as above, was dissolved in DMF (150 ml) containing Et_3N (3.0 ml, 22 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Ser-$NHNH_2$ (7.39 g, 26 mmol)] in DMF (70 ml) and Et_3N (3.6 ml, 26 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 (×2) to yield the *protected hexapeptide derivative* (16.99 g, 89%), m.p. 241—244 °C, $[\alpha]_D^{25}$ −7.4° (*c*, 1.1 in DMF), R_{F_1} 0.41. Amino-acid analysis: Ser 2.46, Thr 0.94, Ala 2.00 (average recovery 80%) (Found: C, 42.25; H, 5.4; N, 12.7. $C_{31}H_{45}Cl_3N_8O_{15}$ requires C, 42.50; H, 5.18; N, 12.79%).

Z(OMe)-Asp(OBu^t)-Ser-Ser-Thr-Ser-Ala-Ala-NHNH-Troc, Z(OMe)-(RNase 14—20)-NHNH-*Troc* (A).—Z(OMe)-Ser-Ser-Thr-Ser-Ala-Ala-NHNH-Troc (9.33 g, 10.7 mmol) was treated with TFA–anisole (50 ml; 4 : 1 v/v) and the N^{α}-deprotected peptide, isolated as above, was dissolved in DMSO–DMF (1 : 1 v/v; 100 ml) together with Et_3N (2.94 ml, 21.3 mmol) and Z(OMe)-Asp(OBu^t)-ONP (5.05 g, 10.7 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 (×2) to give the *protected heptapeptide derivative* (8.77 g, 79%), m.p. 221 °C (decomp.), $[\alpha]_D^{20}$ −9.2° (*c*, 0.9 in DMSO), R_{F_1} 0.19, R_{F_3} 0.82. Amino-acid analysis: Asp 1.00, Ser 2.24, Thr 0.86, Ala 2.00 (average recovery 93%) (Found: C, 44.4; H, 5.55; N, 11.65. $C_{39}H_{58}Cl_3N_9O_{18}\cdot 1/2H_2O$ requires C, 44.34; H, 5.63; N, 11.93%).

Z(OMe)-Met(O)-NHNH-Troc.—DCC (10.89 g, 53 mmol) was added to a stirred solution of Z(OMe)-Met(O)-OH (15.84 g, 48 mmol) and Troc-$NHNH_2$ (10.97 g, 53 mmol) in THF (200 ml). After 24 h, the filtered solution was concentrated and the residue was purified by procedure A followed by recrystallization from AcOEt to give the *hydrazide derivative* (16.22 g, 65%), m.p. 133—138 °C, $[\alpha]_D^{25}$ +12.9° (*c*, 1.0 in DMF), R_{F_2} 0.56 (Found: C, 39.25; H, 4.25; N, 8.0. $C_{17}H_{22}Cl_3N_3O_7S$ requires C, 39.35; H, 4.27; N, 8.10%).

Z(OMe)-Gln-His-Met(O)-NHNH-Troc.—Z(OMe)-Met(O)-NHNH-Troc (10.63 g, 20.5 mmol) was treated with TFA–anisole (50 ml; 4 : 1 v/v) as usual, then n-hexane was added. The resulting oily precipitate was dried over KOH pellets *in vacuo* for 3 h and then dissolved in DMF (100 ml) containing Et_3N (2.83 ml, 20.5 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Gln-His-$NHNH_2$ [20] (9.46 g, 20.5 mmol)] in DMF (80 ml) and Et_3N (2.83 ml, 20.5 mmol) were added and the mixture, after stirring for 48 h, was concentrated. The residue was dissolved in Bu^{n}OH saturated previously with H_2O. The organic phase was washed three times with 5% Na_2CO_3 and H_2O, dried over $MgSO_4$, and concentrated. The residue was washed with H_2O and recrystallized from EtOH to give the *protected tripeptide derivative* (12.25 g, 76%), m.p. 142—146 °C, $[\alpha]_D^{25}$ +6.5° (*c*, 1.1 in DMF), R_{F_1} 0.49 (Found: C, 43.0; H, 5.05; N, 14.45. $C_{28}H_{37}Cl_3N_8O_{10}S$ requires C, 42.89; H, 4.76; N, 14.29%).

Z(OMe)-Arg(MBS)-Gln-His-Met(O)-NHNH-Troc.—Z(OMe)-Gln-His-Met(O)-NHNH-Troc (10.58 g, 13.5 mmol) was treated with TFA–anisole (40 ml; 3 : 1 v/v), then dry ether was added. The resulting powder, isolated as mentioned previously, was dissolved in DMF (100 ml) containing Et_3N (3.72 ml, 27 mmol). To this ice-chilled solution was added the mixed anhydride [from Z(OMe)-Arg(MBS)-OH (8.23 g, 16.2 mmol)] in dry THF (60 ml). After stirring in an ice-bath for 3 h, the solution was concentrated and the residue was purified by washing with 5% $NaHCO_3$ and

H_2O followed by precipitation from DMF with MeOH to afford the *protected tetrapeptide derivative* (10.72 g, 72%), m.p. 176—181 °C, $[\alpha]_D^{25}$ −18.0° (*c*, 0.7 in DMF), R_{F1} 0.53 (Found: C, 45.0; H, 5.2; N, 15.4. $C_{41}H_{55}Cl_3N_{12}O_{14}S_2$ requires C, 44.34; H, 4.99; N, 15.14%).

Z(OMe)-Glu(OBzl)-Arg(MBS)-Gln-His-Met(O)-NHNH-Troc (B).—Z(OMe)-Arg(MBS)-Gln-His-Met(O)-NHNH-Troc (11.69 g, 10.5 mmol) was treated with TFA–anisole (52 ml; 10 : 3 v/v) and the N^{α}-deprotected peptide, isolated as above, was dissolved in DMF (100 ml) together with Et_3N (4.36 ml, 31.6 mmol) and Z(OMe)-Glu(OBzl)-ONP (5.50 g, 10.5 mmol). After stirring for 24 h, the solution was concentrated and the residue was purified by procedure B followed by precipitation from DMF with EtOH to yield the *protected pentapeptide derivative* (10.99 g, 79%), m.p. 192—197 °C, $[\alpha]_D^{25}$ −14.4° (*c*, 0.7 in DMF). Amino-acid analysis: Glu 2.08, Arg 0.96, His 1.00, Met + Met(O) 0.75 (average recovery 89%) (Found: C, 46.05; H, 5.1; N, 13.05. $C_{53}H_{68}Cl_3N_{13}O_{17}S_2 \cdot 2.5H_2O$ requires C, 46.30; H, 5.35; N, 13.25%).

Z(OMe)-Glu(OBzl)-Arg(MBS)-Gln-His-Met(O)-NHNH$_2$, *Z(OMe)-(RNase 9—13)-NHNH$_2$*.—The above protected pentapeptide (10.54 g, 7.9 mmol) in DMF–AcOH (120 ml; 5 : 1 v/v) was treated with Zn powder (5.18 g, 10 mol equiv.) in an ice-bath for 10 min and at room temperature for 3 h. The solution was filtered, the filtrate was concentrated *in vacuo* at a bath temperature of <35 °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 twice from DMSO with MeOH to afford the *hydrazide* (6.72 g, 73%), m.p. 229—236 °C, $[\alpha]_D^{20}$ −22.5° (*c*, 0.9 in DMSO), R_{F3} 0.47. Amino-acid analysis: Glu 2.00, Arg 1.06, His 1.01, Met + Met(O) 0.68 (average recovery 93%) (Found: C, 50.3; H, 6.2; N, 14.9. $C_{50}H_{67}N_{13}O_{15}S_2 \cdot 2.5H_2O$ requires C, 50.07; H, 6.05; N, 15.18%).

Z(OMe)-Lys-(Z)-Phe-OMe.—DCC (22.66 g, 0.11 mol) was added to a stirred mixture of Z(OMe)-Lys(Z)-OH (44.45 g, 0.10 mol) and H-Phe-OMe [from the hydrochloride (21.52 g, 0.10 mol) with Et_3N (13.8 ml, 0.10 mol)] in DMF (200 ml). After 48 h, the filtered solution was concentrated and the residue was purified by procedure B followed by recrystallization from MeOH–AcOEt to give the *protected dipeptide ester* (43.33 g, 72%), m.p. 129—131 °C, $[\alpha]_D^{22}$ −3.6° (*c*, 1.1 in DMF), R_{F1} 0.92 (Found: C, 65.35; H, 6.6; N, 7.2. $C_{33}H_{39}N_3O_8$ requires C, 65.44; H, 6.49; N, 6.94%).

Z(OMe)-Ala-Lys(Z)-Phe-OMe.—Z(OMe)-Lys(Z)-Phe-OMe (40.01 g, 66 mmol) was treated with TFA–anisole (60 ml; 2 : 1 v/v) as usual, then n-hexane was added. The resulting oily precipitate, isolated as previously, was dissolved in DMF (250 ml) together with Et_3N (18.2 ml, 0.13 mol) and Z(OMe)-Ala-ONP (26.20 g, 70 mmol), and the mixture, after stirring for 48 h, was concentrated. The residue was purified by procedure B followed by recrystallization from dioxan–MeOH to afford the *protected tripeptide ester* (35.65 g, 80%), m.p. 159—162 °C, $[\alpha]_D^{25}$ −6.9° (*c*, 1.5 in DMF), R_{F1} 0.85 (Found: C, 63.95; H, 6.5; N, 8.0. $C_{36}H_{44}N_4O_9$ requires C, 63.89; H, 6.55; N, 8.28%).

Z(OMe)-Ala-Ala-Lys(Z)-Phe-OMe.—Z(OMe)-Ala-Lys(Z)-Phe-OMe (33.02 g, 49 mmol) was treated with TFA–anisole (49 ml; 33 : 16 v/v) as usual, then dry ether was added. The resulting powder, isolated as above, was dissolved in DMF (250 ml) together with Et_3N (13.5 ml, 98 mmol) and Z(OMe)-Ala-ONP (18.70 g, 50 mmol) and the mixture, after stirring for 48 h, was concentrated. The residue was purified by procedure B followed by precipitation from DMF with MeOH to afford the *protected tetrapeptide ester* (27.32 g, 75%), m.p. 203—204 °C, $[\alpha]_D^{25}$ −9.8° (*c*, 0.6 in DMF), R_{F1} 0.90 (Found: C, 62.5; H, 6.5; N, 9.45. $C_{39}H_{49}N_5O_{10}$ requires C, 62.63; H, 6.60; N, 9.37%).

Z(OMe)-Thr-Ala-OMe.—Z(OMe)-Thr-OPCP (37.11 g, 70 mmol) was added to a stirred solution of H-Ala-OMe [from the hydrochloride (9.77 g, 70 mmol) with Et_3N (9.64 ml, 70 mmol)] in DMF (100 ml). After 48 h, the solvent was evaporated off and the residue was purified by procedure B followed by recrystallization from AcOEt–ether to afford the *protected dipeptide ester* (18.49 g, 72%), m.p. 123—126 °C, $[\alpha]_D^{25}$ −2.6° (*c*, 0.8 in DMF), R_{F1} 0.80 (Found: C, 55.7; H, 6.8; N, 7.7. $C_{17}H_{24}N_2O_7$ requires C, 55.42; H, 6.57; N, 7.61%).

Z(OMe)-Thr-Ala-NHNH$_2$.—Z(OMe)-Thr-Ala-OMe (21.03 g, 57 mmol) in MeOH (150 ml) was treated with 80% hydrazine hydrate (18 ml, 5 mol equiv.) overnight. The resulting mass was recrystallized from MeOH to afford the *hydrazide* (18.48 g, 88%), m.p. 199—200 °C, $[\alpha]_D^{25}$ +7.1° (*c*, 0.7 in DMSO), R_{F1} 0.64 (Found: C, 51.6; H, 6.55; N, 15.0. $C_{16}H_{24}N_4O_6$ requires C, 52.16; H, 6.57; N, 15.21%).

Z(OMe)-Thr-Ala-Ala-Ala-Lys(Z)-Phe-OMe.—Z(OMe)-Ala-Ala-Lys(Z)-Phe-OMe (7.70 g, 10.3 mmol) was treated with TFA–anisole (37 ml; 30 : 7 v/v) and the N^{α}-deprotected peptide, isolated as above, was dissolved in DMF (70 ml) containing Et_3N (1.42 ml, 10.3 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-Thr-Ala-NHNH$_2$ (5.30 g, 14.4 mmol)] in DMF (30 ml) and Et_3N (1.99 ml, 14.4 mmol), and the mixture was stirred for 48 h. The solvent was evaporated off and the residue was purified by procedure B followed by precipitation from DMF with MeOH (×2) to afford the *protected hexapeptide* (8.52 g, 90%), m.p. 218—222 °C, $[\alpha]_D^{25}$ −10.1° (*c*, 0.7 in DMF), R_{F1} 0.73. Amino-acid analysis: Thr 0.93, Ala 2.89, Lys 1.03, Phe 1.00 (average recovery 91%) (Found: C, 59.8; H, 6.7; N, 10.55. $C_{46}H_{61}N_7O_{13}$ requires C, 60.05; H, 6.68; N, 10.66%).

Z-Lys(Z)-Glu(OBu^t)-OH.—Z-Lys(Z)-OSu (12.48 g, 24 mmol) in THF (120 ml) was added to a stirred solution of H-Glu(OBu^t)-OH (4.92 g, 24 mmol) and Et_3N (6.68 ml, 48 mmol) in H_2O (50 ml). After 24 h, the solvent was evaporated off and the residue was extracted with AcOEt. The organic phase was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4, and concentrated. Trituration with ether and n-hexane followed by recrystallization from AcOEt–n-hexane afforded the *protected dipeptide* (12.23 g, 84%), m.p. 86—89 °C, $[\alpha]_D^{25}$ −4.3° (*c*, 0.7 in DMF), R_{F1} 0.68 (Found: C, 62.15; H, 6.95; N, 6.95. $C_{31}H_{41}N_3O_9$ requires C, 62.09; H, 6.89; N, 7.01%).

Z-Lys(Z)-Glu(OBu^t)-OMe.—An ethereal solution of diazomethane was added to an ice-chilled solution of Z-Lys(Z)-Glu(OBu^t)-OH (8.10 g, 13.5 mmol) in MeOH (80 ml), until the yellow colour persisted for 30 min. After addition of a few drops of AcOH, the solvent was evaporated off. Trituration with ether followed by recrystallization from AcOEt–n-hexane afforded the *dipeptide ester* (7.12 g, 86%), m.p. 64—67 °C, $[\alpha]_D^{25}$ −5.9° (*c*, 0.9 in DMF), R_{F1} 0.97 (Found: C, 62.35; H, 7.0; N, 6.8. $C_{32}H_{43}N_3O_9$ requires C, 62.62; H, 7.06; N, 6.85%).

Z-Lys(Z)-Glu(OBu^t)-NHNH$_2$.—Z-Lys(Z)-Glu(OBu^t)-OMe (7.11 g, 11.6 mmol) in EtOH (70 ml) was treated with 80% hydrazine hydrate (3.63 ml, 5 mol equiv.) at 4 °C for 48 h, then ether was added. The resulting gelatinous mass was recrystallized from MeOH–ether to afford the *hydrazide* (5.72 g, 80%), m.p. 101—105°, $[\alpha]_D^{25}$ −7.7° (*c*, 1.0 in DMF),

R_{F1} 0.73 (Found: C, 60.05; H, 7.0; N, 11.85. $C_{31}H_{43}N_5O_8$ requires C, 60.65; H, 7.05; N, 11.4%).

Z-Lys(Z)-Glu(OBu^t)-Thr-Ala-Ala-Ala-Lys(Z)-Phe-OMe.—Z(OMe)-Thr-Ala-Ala-Ala-Lys(Z)-Phe-OMe (7.74 g, 8.4 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 DMSO–DMF (1 : 1 v/v, 80 ml) containing Et_3N (1.17 ml, 8.4 mmol). To this ice-chilled solution were added the azide [from Z-Lys(Z)-Glu(OBu^t)-$NHNH_2$ (6.22 g, 10.1 mmol)] in DMF (30 ml) and Et_3N (1.40 ml, 10.1 mmol), and the mixture, after stirring for 48 h, was concentrated. The residue was purified by procedure B followed by precipitation from DMSO with MeOH (×2) to afford the *protected octapeptide ester* (9.67 g, 86%), m.p. 233—236 °C, $[\alpha]_D^{25}$ −12.0° (*c*, 0.8 in DMF), R_{F1} 0.76, R_{F6} 0.69. Amino-acid analysis: Lys 2.02, Glu 1.00, Thr 0.89, Ala 2.86, Phe 1.00 (average recovery 87%) (Found: C, 60.85; H, 6.9; N, 10.35. $C_{68}H_{92}N_{10}O_{18}$ requires C, 61.06. H, 6.93; N, 10.47%).

Z-Lys(Z)-Glu(OBu^t)-Thr-Ala-Ala-Ala-Lys(Z)-Phe-NHNH$_2$, Z(OMe)-(RNase 1—8)-NHNH$_2$ (C).—The above protected octapeptide ester (7.21 g, 5.4 mmol) in HMPA–MeOH (4 : 1 v/v; 70 ml) was treated with 80% hydrazine hydrate (1.69 ml, 5 mol equiv.) for 24 h, then H_2O (300 ml) was added. The resulting powder was washed with H_2O and precipitated from DMSO with MeOH to yield the *protected octapeptide hydrazide* (6.31 g, 88%), m.p. 234—238 °C, $[\alpha]_D^{20}$ −15.1° (*c*, 1.1 in DMSO), R_{F3} 0.86, R_{F5} 0.28. Amino-acid analysis: Lys 2.00, Glu 1.01, Thr 0.96, Ala 2.90, Phe 1.00 (average recovery 92%) (Found: C, 59.95; H, 7.0; N, 12.3. $C_{67}H_{92}N_{12}O_{17}$ requires C, 60.16; H, 6.93; N, 12.57%).

Z(OMe)-Glu(OBzl)-Arg(MBS)-Gln-His-Met(O)-Asp-Ser-Ser-Thr-Ser-Ala-Ala-NHNH-Troc.—Z(OMe)-(RNase 14—20)-NHNH-Troc (A) (4.28 g, 4.1 mmol) was treated with TFA–anisole (20 ml; 4 : 1 v/v) in an ice-bath for 3 h and at room temperature for 30 min, then dry ether was added. The resulting powder, isolated as above, was dissolved in DMSO–DMF (1 : 1 v/v; 40 ml) containing Et_3N (1.13 ml, 8.2 mmol). To this ice-chilled solution were added the azide [from Z(OMe)-(RNase 9—13)-$NHNH_2$ (4.71 g, 4.1 mmol)] in DMSO–DMF (1 : 1 v/v; 30 ml) and Et_3N (0.56 ml, 4.1 mmol), and the solution, after stirring for 24 h, was concentrated. The residue was purified by procedure B followed by precipitation from DMF with AcOEt (×2) to afford the *protected dodecapeptide derivative* (6.14 g, 77%), m.p. 200—203 °C, $[\alpha]_D^{25}$ −13.6° (*c*, 0.9 in DMSO), R_{F3} 0.66. Amino-acid analysis: Asp 1.04, Thr 0.89, Ser 2.35, Glu 2.11, Ala 2.00, Met + Met(O) 0.93, His 1.05, Arg 1.04 (average recovery 88%) (Found: C, 46.15; H, 5.8; N, 13.65. $C_{76}H_{105}Cl_3N_{20}O_{30}S_2 \cdot 2.5H_2O$ requires C, 45.77; H, 5.56; N, 14.04%).

Z-Lys(Z)-Glu(OBu^t)-Thr-Ala-Ala-Ala-Lys(Z)-Phe-Glu(OBzl)-Arg(MBS)-Gln-His-Met(O)-Asp-Ser-Ser-Thr-Ser-Ala-Ala-NHNH-Troc.—The above protected dodecapeptide (1.24 g, 0.64 mmol) was treated with TFA–anisole (5 ml; 4 : 1 v/v) and the N^{α}-deprotected peptide, isolated as above, was dissolved in DMSO–DMF (1 : 1 v/v; 7 ml) containing Et_3N (0.18 ml, 1.27 mmol) and *N*-methylmorpholine (0.07 ml, 0.64 mmol). To this ice-chilled solution were added the azide [from Z-(RNase 1—8)-$NHNH_2$ (C) (0.85 g, 0.64 mmol)] in DMSO–DMF (1 : 1 v/v 6 ml) and *N*-methylmorpholine (0.07 ml, 0.64 mmol). After stirring for 48 h, the solution was concentrated and the residue was purified by procedure B followed by precipitation from DMSO with MeOH (×2) to afford the *protected icosapeptide derivative* (1.02 g, 52%), m.p. 228 °C (decomp.), $[\alpha]_D^{25}$ −13.7° (*c*, 0.7 in DMSO), R_{F3} 0.76. Amino-acid analysis: Asp 0.94, Thr 1.77, Ser 2.22, Glu 2.98, Ala 5.10, Met + Met(O) 0.62, Phe 1.00, Lys 2.08, His 0.90, Arg 0.95 (average recovery 92%) (Found: C, 51.45; H, 6.1; N, 13.25. $C_{134}H_{185}Cl_3N_{30}O_{44}S_2 \cdot 2H_2O$ requires C, 51.47; H, 6.09; N, 13.44%).

Z-Lys(Z)-Glu(OBu^t)-Thr-Ala-Ala-Ala-Lys(Z)-Phe-Glu(OBzl)-Arg(MBS)-Gln-His-Met(O)-Asp-Ser-Ser-Thr-Ser-Ala-Ala-NHNH$_2$, Z-(RNase 1—20)-NHNH$_2$. Protected S-peptide.—The above protected icosapeptide derivative (0.53 g, 0.17 mmol) in HMPA–NMP (2 : 1 v/v; 5 ml) and AcOH (1 ml) was treated with Zn powder (0.56 g, 50 mol equiv.) at 40 °C for 3 h. The solvent was evaporated off and the residue was treated with 5% EDTA. The resulting gelatinous mass was purified by washing with H_2O followed by precipitation from DMSO with MeOH to yield the *hydrazide* (0.41 g, 83%), m.p. 251 °C (decomp.), $[\alpha]_D^{20}$ −28.9° (*c*, 0.8 in DMSO), R_{F3} 0.61. Amino-acid analysis: Asp 0.93, Thr 1.74, Ser 2.09, Glu 3.02, Ala 4.85, Met + Met(O) 0.77, Phe 1.00, Lys 2.13, His 0.91, Arg 0.91 (average recovery 93%) (Found: C, 53.4; H, 6.5; N, 13.3. $C_{131}H_{184}N_{30}O_{42}S_2 \cdot 3H_2O$ requires C, 52.99; H, 6.45; N, 14.15%).

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