Amino Acids and Peptides. Part 32.[1] Total Synthesis of Eglin c. Part 2. Synthesis of a Heptacontapeptide corresponding to the Entire Amino Acid Sequence of Eglin c and of Related Peptides, and Studies on the Relationship between the Structure and Inhibitory Activity against Human Leukocyte Elastase, Cathepsin G and α-Chymotrypsin

Yoshio Okada* and Satoshi Tsuboi
Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Nishi-ku, Kobe 651-21, Japan

Commencing with a protected *C*-terminal triacontapeptide of eglin c, eglin c (31–70), eglin c (22–30) and eglin c (8–70) and finally eglin c were synthesized by a conventional solution method in order to allow us to study the relationship between their structure and the inhibitory activity against human leukocyte elastase, cathepsin G and α-chymotrypsin. Ten relatively small peptide fragments were coupled successively from the *C*-terminus by the azide method to minimize racemization and to avoid the need for protection of the side-chain functional groups of the amino acid residues as much as possible during the peptide synthesis. The protected peptides were treated with HF at 0 °C for 60 min in the presence of thioanisole and *m*-cresol to give the desired eglin c fragments and eglin c, which exhibited a symmetrical single peak on analytical HPLC. Although the inhibitory activity of eglin c (31–70) and eglin c (22–70) against the aforementioned enzymes did not increase dramatically, eglin c (8–70) exhibited inhibitory activity against the above enzymes with similar or rather lower K_i-values than that of N^{α}-acetyleglin c.

Mass spectrometry of the synthetic eglin c by electrospray ionization exhibited peaks at 1012 $(M + 8H)^{8+}$, 1157 $(M + 7H)^{7+}$ and 1349 $(M + 6H)^{6+}$, supporting the view that the molecular weight of synthetic eglin c [$C_{373}H_{550}N_{96}O_{107}$] is 8090.9 [Calc. for $(M + 8H)/8 = 1012.36$, $(M + 7H)/7 = 1156.84$ and $(M + 6H)/6 = 1349.48$]. Furthermore, the synthetic eglin c exhibited the same inhibitory activity against human leukocyte elastase, cathepsin G and α-chymotrypsin ($K_i = 5.1 \times 10^{-9}$, 1.5×10^{-9}, and 2.2×10^{-9} mol dm^{-3}, respectively) as N^{α}-acetyleglin c synthesized genetically ($K_i = 5.0 \times 10^{-9}$, 1.0×10^{-9}, and 2.3×10^{-9} mol dm^{-3}, respectively).

The synthesis of a *C*-terminal triacontapeptide of eglin c, eglin c (41–70) and related peptides, and studies on the relationship between the structure and inhibitory activity against leukocyte elastase, cathepsin G and α-chymotrypsin were described in Part 1 of this series.[1]

As described previously[1] in connection with the three-dimensional structure and inhibitory mechanism of eglin c† Thr[44], Asp[46], and Arg[48] in eglin c form hydrogen and electrostatic bonds with Arg[53], Arg[51], and Gly[70], respectively, to stabilize the reactive site; in addition, the nine residues of the

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                                   10
Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys-Ser-Phe-Pro-Glu-Val-Val-Gly-
                        20
Lys-Thr-Val-Asp-Gln-Ala-Arg-Glu-Tyr-Phe-Thr-Leu-His-Tyr-
 30                                      40
Pro-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly -Ser-Pro-Val-
                             50
Thr-Leu-Asp-Leu-Arg-Tyr-Asn-Arg-Val-Arg-Val-Phe-Tyr-Asn-
       60                                        70
Pro-Gly-Thr-Asn-Val-Val-Asn-His-Val-Pro-His-Val-Gly
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Primary structure of eglin c

binding loop (40–48) of eglin c are involved in direct contact with subtilisin.[2,3] Synthetic eglin c (41–49)[4,5] does not have the electrostatic and hydrogen bonds needed to maintain the comfortable conformation for interaction with the enzyme. This might be a possible reason why K_i-values of eglin c (41–49) are 10^5-times larger than those of eglin c. Therefore, we expected that the potency of the inhibitory activity of eglin c (41–70) might increase upon formation of electrostatic and hydrogen bonds. However, eglin c (41–70) exhibited inhibitory activity against cathepsin G and α-chymotrypsin with similar K_i-values to those of eglin c (41–49).[1]

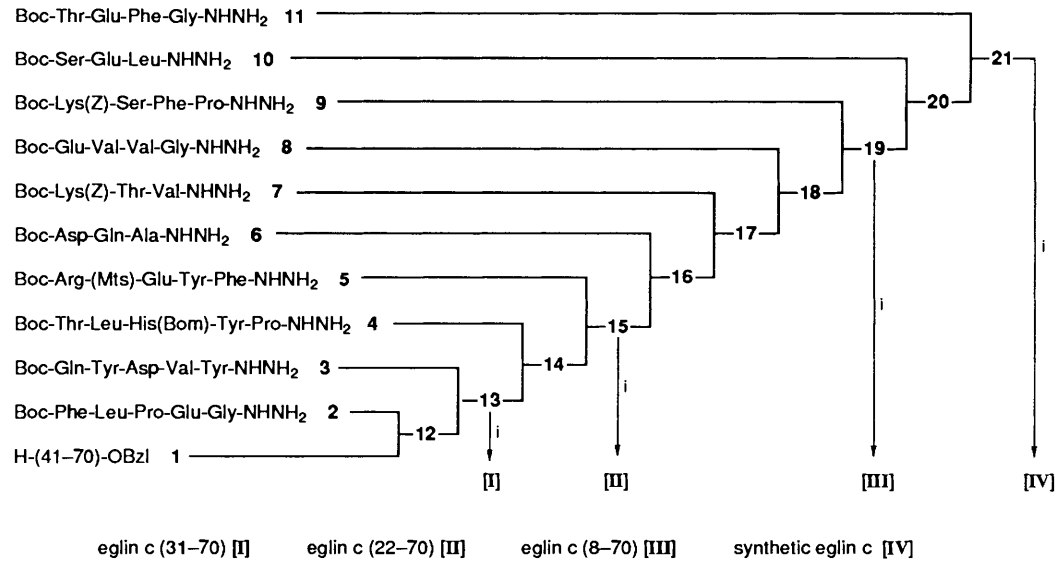
It has also been reported that eglin c, present as a complex with subtilisin, was shortened *N*-terminally by seven amino acid residues[2] and that eglin c (5–70) and eglin c (7–70), which were prepared enzymatically using cathepsin C, did not influence the equilibrium dissociation constant for the interaction of eglin c with chymotrypsin.[6]

Therefore, further chain elongation to a heptacontapeptide (eglin c) and studies on the structure–activity relationship have been performed.

This paper deals with the systematic synthesis of a heptacontapeptide, corresponding to the entire amino acid sequence of eglin c, and related peptides in order to facilitate studies of the relationship between the structure of eglin c and the inhibitory activity against human leukocyte elastase, cathepsin G and α-chymotrypsin.

As illustrated in Scheme 1, starting with the *C*-terminal triacontapeptide [eglin c (41–70)] **1**, ten relatively small peptide fragments (**2**–**11**) were coupled successively by the azide procedure[7] in order to minimize racemization and to avoid the need for protection of side-chain functional groups of the amino acid residues as much as possible during the synthesis. The α-amino functions of the amino acids were protected by the Boc

† The following abbreviations are used: Z, benzyloxycarbonyl; Bzl, benzyl; Boc, *t*-butoxycarbonyl; Bom, benzyloxymethyl; Mts, mesitylenesulphonyl; AcOEt, ethyl acetate; DMF, dimethylformamide; DMSO, dimethyl sulphoxide; TFA, trifluoroacetic acid; AcOH, acetic acid; BuOH, butan-1-ol; OPyCl, 6-chloro-2-pyridyl ester; Suc, succinyl; pNA, *p*-nitroanilide.



Scheme 1 Synthetic scheme for eglin c. *Reagents and conditions:* i, HF–PhSMe–*m*-cresol, 0 °C, 90 min

Table 1 Amino acid analysis of the protected intermediate peptides

	36–70 **12**	31–70 **13**	26–70 **14**	22–70 **15**	19–70 **16**	16–70 **17**	12–70 **18**	8–70 **19**	5–70 **20**	1–70 **21**
Asp	5.05(5)	*6.22*(6)	5.95(6)	6.01(6)	*7.34*(7)	7.11(7)	7.32(7)	7.29(7)	7.05(7)	6.99(7)
Thr	1.97(2)	1.97(2)	*2.76*(3)	3.06(3)	2.93(3)	*3.95*(4)	3.93(4)	4.23(4)	3.97(4)	*4.53*(5)
Ser	0.80(1)	0.92(1)	0.89(1)	0.85(1)	0.98(1)	0.92(1)	0.89(1)	*2.09*(2)	*2.97*(3)	2.93(3)
Glu	*0.98*(1)	*2.19*(2)	2.09(2)	*3.17*(3)	*3.67*(4)	4.03(4)	*4.92*(5)	4.98(5)	*6.65*(6)	*7.26*(7)
Gly	*3.00*(3)[b]	3.00(3)	3.00(3)	3.00(3)	3.00(3)	3.00(3)	*4.00*(4)	4.00(4)	4.00(4)	*5.00*(5)
Ala					*1.03*(1)	1.13(1)	1.12(1)	1.05(1)	1.01(1)	1.19(1)
Val[a]	6.55(7)	*7.58*(8)	7.88(8)	7.76(8)	7.88(8)	*8.83*(9)	*10.9*(11)	10.2(11)	10.1(11)	10.0(11)
Leu	3.25(3)	3.04(3)	*4.43*(4)	4.43(4)	4.17(4)	4.00(4)	4.32(4)	4.18(4)	*5.33*(5)	5.00(5)
Tyr	2.18(2)	*4.00*(4)	*4.90*(5)	*5.92*(6)	5.95(6)	5.89(6)	5.94(6)	5.89(6)	5.92(6)	5.90(6)
Phe	*2.16*(2)	2.18(2)	2.11(2)	*3.29*(3)	3.19(3)	3.07(3)	3.02(3)	*4.05*(4)	3.98(4)	*5.37*(5)
Lys						*1.07*(1)	1.05(1)	*1.98*(2)	2.14(2)	2.14(2)
His	1.70(2)	1.70(2)	*2.78*(3)	2.81(3)	2.80(3)	2.81(3)	2.82(3)	2.75(3)	2.80(3)	2.85(3)
Arg	2.72(3)	3.25(3)	3.26(3)	*4.00*(4)	4.22(4)	4.04(4)	4.24(4)	4.29(4)	4.42(4)	4.18(4)
Pro	*3.92*(4)	4.03(4)	*5.01*(5)	4.91(5)	4.68(5)	5.02(5)	4.92(5)	*5.80*(6)	6.53(6)	5.95(6)
	(72.8%)[c]	(71.2%)	(80.1%)	(71.9%)	(70.3%)	(81.2%)	(72.1%)	(77.7%)	(69.2%)	(79.4%)

[a] Acid hydrolysates (6 mol dm^{-3} HCl; 110 °C; 72 h). [b] Values in italics: Newly introduced amino acid. [c] Average recovery.

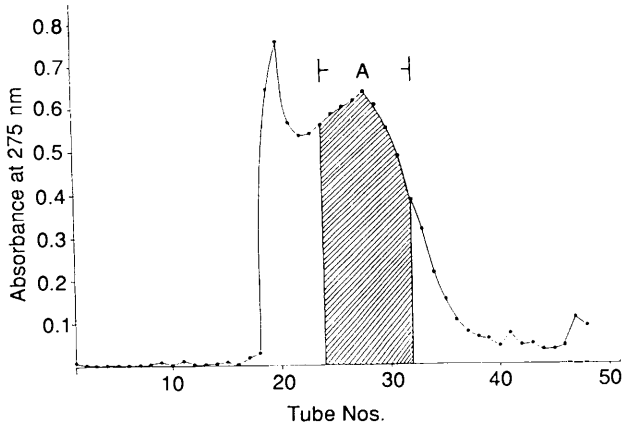


Fig. 1 Purification of synthetic eglin c by Sephadex G-50, with 3% AcOH as eluent

group. The Bzl protecting group of the β- and γ-carboxy functions of Asp and Glu was removed by catalytic hydrogenation over palladium prior to the synthesis of the corresponding hydrazide (**2**, **5**, **6**, **8**, **10** and **11**). The carboxy group of the *C*-terminal Gly residue was protected as its Bzl ester. Arg(Mts), Lys(Z) and His(Bom), which protecting groups can be removed by treatment with HF at 0 °C for 60 min[8] or with trimethylsilyl bromide (TMSBr) at 0 °C for 3 h,[9] were employed. To introduce the bulky amino acid (Val) in the synthesis of the peptide fragments **1**–**11**, we used a newly developed 6-chloro-2-pyridyl ester.[10] To introduce the Arg residue, the diphenylphosphoryl azide (DPPA) method[11,12] was employed to avoid lactam formation.

According to Scheme 1, peptide intermediates **12**–**20** and finally the protected heptacontapeptide **21** were obtained after purification at each coupling step by reprecipitation from DMF and MeOH, and column chromatography on Sephadex LH-60 using DMF as eluent (for peptide **19**). Homogeneity of the intermediates was ascertained by TLC, elemental analysis and amino acid analysis as summarized in Table 1. From the table, it was ascertained that each coupling reaction was successful.

Next, the deprotection of the protected heptacontapeptide and related peptides was performed by treatment with HF at 0 °C in the presence of thioanisole and *m*-cresol. Each peptide was purified by gel filtration on Sephadex G-50 and by preparative HPLC. As illustrated in Fig. 1, synthetic crude eglin c was first purified by Sephadex G-50. Fraction A was further purified by HPLC as shown in Fig. 2. The homogeneity of the peptides eglin c (31–70), eglin c (22–70) and eglin c (8–70) was ascertained by analytical HPLC as shown in Fig. 3.

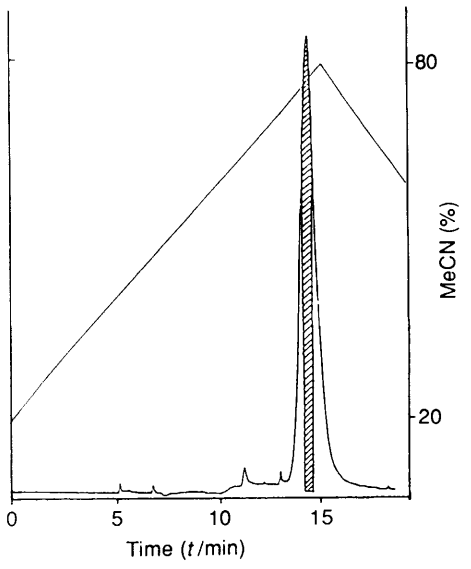


Fig. 2 Purification of partially purified eglin c by reversed-phase HPLC. Column: YMC-Pack R-ODS-5 (4.6 mm × 25.0 cm); solvent: a = water (0.05% TFA), b = MeCN (0.05% TFA); gradient 80:20 (a:b) to 20:80 in 15 min and return to 80:20 in 15 min; flow rate 1.0 cm^3 min^{-1}; absorbance 210 nm.

The results of the amino acid analysis of acid hydrolysates of the synthetic peptides are summarized in Table 2 and compared with those of natural eglin c.[13]

The inhibitory activity of these purified peptides against human leukocyte elastase, cathepsin G and α-chymotrypsin was examined and the results are summarized in Table 3. Although the inhibitory activity of eglin c (31–70) and eglin c (22–70) against the aforementioned enzymes did not increase dramatically, eglin c (8–70) exhibited inhibitory activity with similar to or rather lower K_i-values than those for N^{α}-acetyleglin c, supporting the previous reports by Bode *et al.*[2] and Dodt *et al.*[6] that an *N*-terminal heptapeptide is not required in order to manifest full inhibitory activity. In addition, these results indicate that the *N*-terminal part of eglin c (8–70), positions 8–21, is very important for forming electrostatic and hydrogen bonds (as stated above) to maintain the conformation of eglin c suitable for reaction with these enzymes, whose three-dimensional structures are different.

Finally, synthetic eglin c exhibited a symmetrical peak at the same retention time as the authentic sample derived from N^{α}-acetyleglin c[14] and a different retention time from N^{α}-acetyleglin c on analytical HPLC as shown in Fig. 4.

A synthetic eglin c was digested with trypsin[15] and digested compounds were analysed by HPLC and compared with N^{α}-acetyleglin c.[16] As shown in Fig. 5, both HPLC profiles were identical except for the *N*-terminal octapeptides (T_1 and Ac-T_1). Each fraction was analysed by amino acid analysis, amino acid sequence analysis, and peptide synthesis and the results are summarized in the linear structure. From the amino acid sequencing, Asp and Asn residues were recovered quantitatively, indicating that aspartimide was not formed during the peptide synthesis.

Furthermore, on mass spectrometry by electrospray ionization (see Fig. 6), synthetic eglin c exhibited peaks at 1012 $(M + 8H)^{8+}$, 1157 $(M + 7H)^{7+}$ and 1349 $(M + 6H)^{6+}$, supporting the view that the molecular weight of the synthetic eglin c is 8090.9 [$C_{373}H_{550}N_{96}O_{107}$. Calc. for (M + 8H)/8 = 1012.36; (M + 7H)/7 = 1156.84; and (M + 6H)/6 = 1349.48].

Next, the inhibitory activity of synthetic eglin c against human leukocyte elastase, cathepsin G, α-chymotrypsin and porcine pancreatic elastase was examined and the results are summarized in Table 4 as their K_i-values, in comparison with those of N^{α}-acetyleglin c prepared genetically. As can be seen from Table 4, inhibitory activity of synthetic eglin c against leukocyte elastase, cathepsin G and chymotrypsin was the same as that of N^{α}-acetyleglin c.

Therefore we had systematically synthesized eglin c (31–70), eglin c (22–70), eglin c (8–70) and finally eglin c in pure form and had clarified their structure–activity relationships.

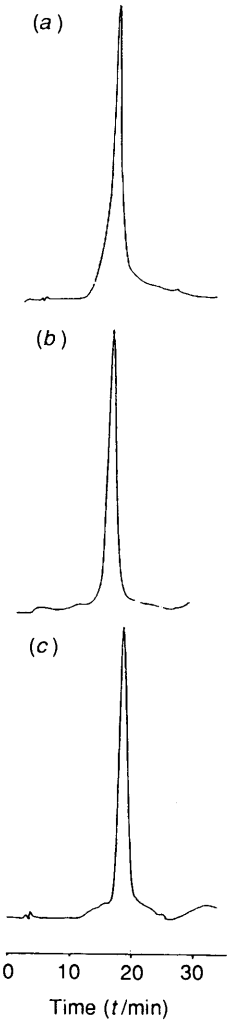


Fig. 3 Analytical HPLC of eglin c derivatives (a) eglin c (31–70), (b) eglin c (22–70). Column: YMC-Pack R-ODS-5 (4.6 × 25 cm^3); solvent: a = water (0.05% TFA), b = MeCN (0.05% TFA); gradient 80:20 (a:b) to 40:60 in 10 min, 40:60 for 5 min and then return to 80:20 in 10 min; flow rate 1.0 cm^3 min^{-1}; absorbance 210 nm, (c) eglin c (8–70). Solvent: a = water (0.05% TFA), b = MeCN (0.05% TFA); gradient 80:20 (a:b) to 20:80 in 15 min and return to 80:20 in 15 min.

Experimental

M.p.s were determined with a Yanagimoto micro melting point apparatus. Optical rotations were measured with an automatic DIP-360 polarimeter (Japan Spectroscopic Co. Ltd). Amino acid compositions of acid hydrolysates (6 mol dm^{-3} HCl; 110 °C; 20 or 72 h) were determined with an amino acid analyser (K-101AS, Kyowa Seimitsu). pH was determined with a pH Meter 26 (Radiometer Copenhagan). HPLC was conducted with a Waters M 600 instrument [column YMC-Pack A-312 ODS (6 × 150 mm), YMC-Pack A-302 ODS (4.6 × 150 mm) or YMC-Pack D-ODS-5 (29 × 250 mm)]. Mass spectra (MS) by electrospray ionization were determined on an Hitachi

Table 2 Amino acid analysis of synthetic peptides

	31–70 [I]	22–70 [II]	8–70 [III]	1–70 [IV] 20 h	1–70 [IV] 72 h	Ref. 13 20 h	Ref. 13 72 h
Asp	5.95(6)	6.25(6)	7.19(7)	7.02	6.98(7)	6.76	6.70(7)
Thr	1.98(2)	2.54(3)	3.98(4)	4.83	3.99(5)	4.52	4.08(5)
Ser	0.90(1)	0.91(1)	1.81(2)	2.90	1.46(3)	2.93	2.31(3)
Glu	2.11(2)	2.98(3)	5.21(5)	6.96	6.90(7)	7.16	7.11(7)
Gly	3.00(3)	3.00(3)	4.00(4)	5.00	5.00(5)	5.18	5.27(5)
Ala			0.95(1)	1.05	0.90(1)	1.16	1.17(1)
Val[a]	7.66(8)	7.80(8)	10.3(11)	9.23	11.1(11)	10.1	10.8(11)
Leu	2.90(3)	4.21(4)	4.21(4)	5.02	5.12(5)	4.83	4.83(5)
Tyr	3.87(4)	5.78(6)	5.55(6)	5.67	4.98(6)	5.12	4.61(6)
Phe	2.02(2)	3.32(3)	4.12(4)	5.03	5.09(5)	4.93	4.93(5)
Lys			2.12(2)	2.08	1.91(2)	2.18	2.23(2)
His	1.68(2)	2.89(3)	2.77(3)	2.80	2.21(3)	2.99	3.26(3)
Arg	3.11(3)	4.01(4)	4.17(4)	4.05	4.03(4)	2.60	3.84(4)
Pro	4.12(4)	5.21(5)	6.30(6)	5.96	5.91(6)	5.90	6.22(6)
	(73.5%)[b]	(53.5%)	(64.4%)	(74.8%)	(67.9%)		

[a] Acid hydrolysates (6 mol dm^{-3} HCl; 110 °C; 72 h). [b] Average recovery.

Table 3 K_i-Values of eglin c derivatives

	K_i (mol dm^{-3}) Elastase[a]	Cathepsin G[b]	α-Chymotrypsin[b]
Eglin c (31–70) [I]	2.6×10^{-6}	3.5×10^{-6}	1.0×10^{-5}
Eglin c (22–70) [II]	2.8×10^{-6}	1.7×10^{-4}	1.3×10^{-5}
Eglin c ((8–70) [III]	2.2×10^{-9}	1.0×10^{-9}	1.4×10^{-9}
N^{α}-Ac-eglin c	5.0×10^{-9}	1.0×10^{-9}	2.3×10^{-9}

[a] Substrate for elastase: Suc-Ala-Tyr-Leu-Val-pNA. [b] Substrate for cathepsin G and α-chymotrypsin: Suc-Ile-Pro-Phe-pNA.

Table 4 Comparison with K_i-values between synthetic eglin c and N^{α}-Ac-eglin c

Proteinase	K_i (mol dm^{-3}) Synthetic eglin c [IV]	N^{α}-Ac-eglin c
Leukocyte elastase[a]	5.1×10^{-9}	5.0×10^{-9}
Cathepsin G[b]	1.5×10^{-9}	1.0×10^{-9}
α-Chymotrypsin[b]	2.2×10^{-9}	2.3×10^{-9}
Pancreatic elastase[c]	2.9×10^{-8}	2.5×10^{-8}

[a] Substrate: Suc-Ala-Tyr-Leu-Val-pNA. [b] Substrate: Suc-Ile-Pro-Phe-pNA. [c] Substrate: Suc-$(Ala)_3$-pNA.

M-2500 mass spectrometer. On TLC (Kieselgel G, Merck), R_{f1}-, R_{f2}-, R_f[3]- and R_f[4]-values refer to the solvent systems of (1) $CHCl_3$–MeOH–ACOH (90:8:2), $CHCl_3$–MeOH–water (8:3:1, lower phase), BuOH–AcOH–water (4:1:5, upper phase) and BuOH–pyridine–AcOH–water (4:1:1:2). Trypsin (Lot. 38F-8140) was purchased from Sigma Chemical Co.

Boc-Phe-Leu-Pro-Glu-Gly-NHNH$_2$ [*Boc*-(36–40)-*NHNH$_2$* **2**].—Hydrazine hydrate (98%; 0.89 cm^3, 18 mmol) was added to a solution of Boc-Phe-Leu-Pro-Glu-Gly-OMe[5] (2.0 g, 2.9 mmol) in MeOH (50 cm^3). The reaction mixture was stored at room temperature overnight. The pH of the solution was adjusted to 6 by addition of AcOH (pH indicator paper). After concentration of the reaction mixture to a small volume, the residue was applied to a column of Sephadex LH-20 (3.5 × 126 cm), equilibrated and eluted with MeOH. Individual fractions (5 g each) were collected. The solvent of the desired fraction (tube Nos. 65–85) was removed by evaporation and diethyl ether was added to the residue to give *crystals*, which were collected by filtration (1.2 g, 60%), m.p. 124–129 °C; $[\alpha]_D^{26}$ −65.2° (*c* 1.0, MeOH) (Found: C, 55.7; H, 7.4; N, 13.3. $C_{32}H_{49}N_7O_9$·$MeCO_2H$ requires C, 55.5; H, 7.26; N, 13.3%).

Boc-Thr-Leu-His(Bom)-Tyr-Pro-NHNH$_2$ [*Boc*-(26–30)-*NHNH$_2$* **4**].—The *title compound* was prepared from Boc-Thr-Leu-His(Bom)-Tyr-Pro-OMe[5] (100 mg, 0.12 mmol) and hydrazine hydrate (90%; 0.06 cm^3, 1.2 mmol) (45 mg, 44.4%), m.p. 114 °C (decomp.); $[\alpha]_D^{26}$ −54.0° (*c* 1.0, MeOH) (Found: C, 58.5; H, 7.0; N, 14.2. $C_{42}H_{61}N_9O_{10}$·$\frac{1}{2}H_2O$ requires C, 58.6; H, 7.26; N, 14.6%).

Boc-Arg(Mts)-Glu-Tyr-Phe-NHNH$_2$ [*Boc*-(22–25)-*NHNH$_2$* **5**].—The title compound was prepared from Boc-Arg(Mts)-Glu-Tyr-Phe-OMe[5] (2.0 g, 2.2 mmol) and hydrazine hydrate (90%; 1.1 cm^3, 22 mmol) (1.8 g, 90.3%), m.p. 179–182 °C (decomp.); $[\alpha]_D^{26}$ −2.2° (*c* 1.0, DMF); R_{f2} 0.22 (Found: C, 55.6; H, 6.45; N, 13.6. $C_{43}H_{59}N_9O_{11}S$·H_2O requires C, 55.6; H, 6.61; N, 13.8%).

Boc-Asp-Gln-Ala-NHNH$_2$ [*Boc*-(19–21)-*NHNH$_2$* **6**].—Hydrazine hydrate (90%; 0.39 cm^3, 6.9 mmol) was added to a solution of Boc-Asp-Gln-Ala-OMe[5] (1.0 g, 2.3 mmol) in MeOH (10 cm^3). The solution was kept at room temperature overnight. After removal of the solvent, the residue, as a solution in 3% AcOH (5 cm^3), was applied to a column of Sephadex G-25 (2.4 × 115 cm), equilibrated, and eluted with 3% AcOH. Individual fractions (10 cm^3 each) were collected. The desired fractions (tube Nos. 30–34) were combined and lyophilized (784 mg, 78.4%), m.p. 88–95 °C; $[\alpha]_D^{26}$ −28.5° (*c* 1.0, MeOH) (Found: C, 45.4; H, 7.0; N, 19.2. $C_{17}H_{30}N_6O_8$ requires C, 45.7; H, 6.78; N, 18.9%).

Boc-Lys(Z)-Ser-Phe-Pro-NHNH$_2$ [*Boc*-(8–11)-*NHNH$_2$* **9**].—The title compound was prepared from Boc-Lys(Z)-Ser-Phe-Pro-OMe[5] (1.0 g, 1.4 mmol) and hydrazine hydrate (90%; 1.4 cm^3, 27.6 mmol) in the same way as described previously[17] (0.9 g, 90.0%), m.p. 79–84 °C; $[\alpha]_D^{26}$ −52.5° (*c* 1.0, MeOH) (Found: C, 58.5; H, 7.0; N, 14.1. Calc. for $C_{43}H_{61}N_9O_{10}$·H_2O: C, 58.5; H, 7.21; N, 14.3%).

Boc-Phe-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(36–70)-*OBzl* **12**].—The *title compound* was prepared from Boc-Phe-Leu-Pro-Glu-Gly-N_3 [prepared from

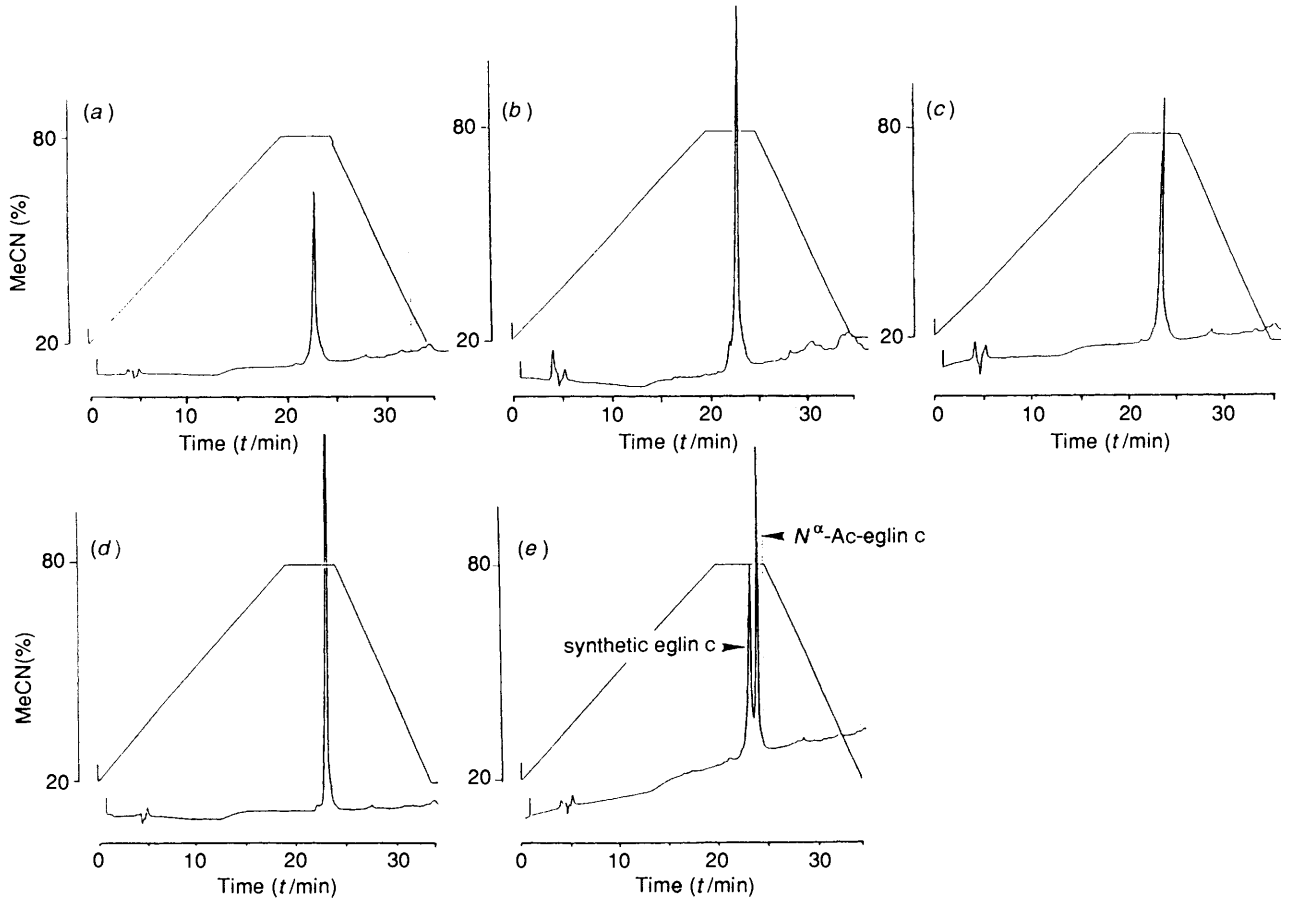


Fig. 4 Analytical HPLC of synthetic eglin c: (a) synthetic eglin, (b) authentic eglin c, (c) synthetic eglin c + authentic eglin c, (d) N^{α}-acetyleglin c, (e) synthetic eglin c + N^{α}-acetyleglin c. Column: YMC-Pack R-ODS-5 (4.6 mm × 25.0 cm); solvent: a = water (0.05% TFA), b = MeCN (0.05% TFA); gradient 80:20 (a:b) to 35:65 in 20 min, 35:65 for 5 min and then return to 80:20 in 10 min; flow rate 1.0 cm^3 min^{-1}; absorbance 210 nm.

Boc-(36–40)-$NHNH_2$ (251 mg, 0.37 mmol) and isopentyl nitrite (52 mm^3, 0.37 mmol)] and H-(41–70)-OBzl·TFA [prepared from Boc-(41–70)-OBzl (523 mg, 0.13 mmol), TFA (1.0 cm^3, 12 mmol), anisole (0.1 cm^3, 0.92 mmol) and *m*-cresol (0.1 cm^3, 0.96 mmol), as usual][17] (368 mg, 62.3%), m.p. 235–242 °C; $[\alpha]_D^{26}$ −34.0° (*c* 0.1, DMSO) (Found: C, 54.7; H, 7.1; N, 14.5. $C_{228}H_{328}N_{52}O_{57}S_3 \cdot 11H_2O$ requires C, 55.0; H, 7.11; N, 14.6%).

Boc-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg-(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His-(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(31–70)-*OBzl* **13**].—The *title compound* was prepared from Boc-Gln-Tyr-Asp-Val-Tyr-N_3 [prepared from Boc-(31–35)-$NHNH_2$ (150 mg, 0.19 mmol) and isopentyl nitrite (27 mm^3, 0.19 mmol)] and H-(36–70)-OBzl·TFA [prepared from Boc-(36–70)-OBzl (310 mg, 65 μmol), TFA (1.0 cm^3, 12 mmol), anisole (0.1 cm^3, 0.92 mmol) and *m*-cresol (0.1 cm^3, 0.96 mmol)] (289 mg, 82.3%), m.p. 218–225 °C; $[\alpha]_D^{26}$ −31.2° (*c* 0.1, DMSO) (Found: C, 54.7; H, 6.85; N, 14.1. $C_{260}H_{368}N_{58}O_{65}S_3 \cdot 15H_2O$ requires C, 55.0; H, 7.08; N, 14.3%).

Boc-Thr-Leu-His(Bom)-Tyr-Pro-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(26–70)-*OBzl* **14**].—The *title compound* was prepared from Boc-Thr-Leu-His(Bom)-Tyr-Pro-N_3 [prepared from Boc-(26–30)-$NHNH_2$ (119 mg, 0.14 mmol) and isopentyl nitrite (19 mm^3, 0.14 mmol)] and H-(31–70)-OBzl·TFA [prepared from Boc-(31–70)-OBzl (248 mg, 46 μmol), TFA (1.0 cm^3, 12 mmol), anisole (0.1 cm^3, 0.92 mmol) and *m*-cresol (0.1 cm^3, 0.96 mmol)] (150 mg, 52.5%), m.p. 220–227 °C; $[\alpha]_D^{26}$ −47.3° (*c* 0.1, DMSO) (Found: C, 56.0; H, 6.9; N, 14.1. $C_{298}H_{417}N_{65}O_{73}S_3 \cdot 10H_2O$ requires C, 56.3; H, 6.87; N, 14.3%).

Boc-Arg(Mts)-Glu-Tyr-Phe-Thr-Leu-His(Bom)-Tyr-Pro-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(22–70)-*OBzl* **15**].—The *title compound* was prepared from Boc-Arg(Mts)-Glu-Tyr-Phe-N_3 [prepared from Boc-(22–25)-$NHNH_2$ (46 mg, 52 μmol) and isopentyl nitrite (7.3 mm^3, 52 μmol)] and H-(26–70)-OBzl·TFA [prepared from Boc-(26–70)-OBzl (106 mg, 17.2 μmol), TFA (0.5 cm^3, 6.4 mmol), anisole (0.05 cm^3, 0.46 mmol) and *m*-cresol (0.05 cm^3, 0.48 mmol)] (78 mg, 65.5%), m.p. 208–217 °C; $[\alpha]_D^{26}$ −32.1° (*c* 0.1, DMSO) (Found: C, 55.4; H, 6.7; N, 13.8. $C_{336}H_{464}N_{72}O_{81}S_4 \cdot 18H_2O$ requires C, 55.6; H, 6.96; N, 13.9%).

Boc-Asp-Gln-Ala-Arg(Mts)-Glu-Tyr-Phe-Thr-Leu-His(Bom)-Tyr-Pro-Gln-Tyr-Asp-Val-Tyr-Phr-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg-(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His-(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(19–70)-*OBzl* **16**].—The *title compound* was prepared from Boc-Asp-Gln-Ala-N_3 [prepared from Boc-(19–21)-$NHNH_2$ (65 mg, 0.15 mmol) and isopentyl nitrite (30 mm^3, 0.15 mmol)] and H-(22–70)-OBzl·TFA [prepared from Boc-(22–70)-OBzl (200 mg, 29

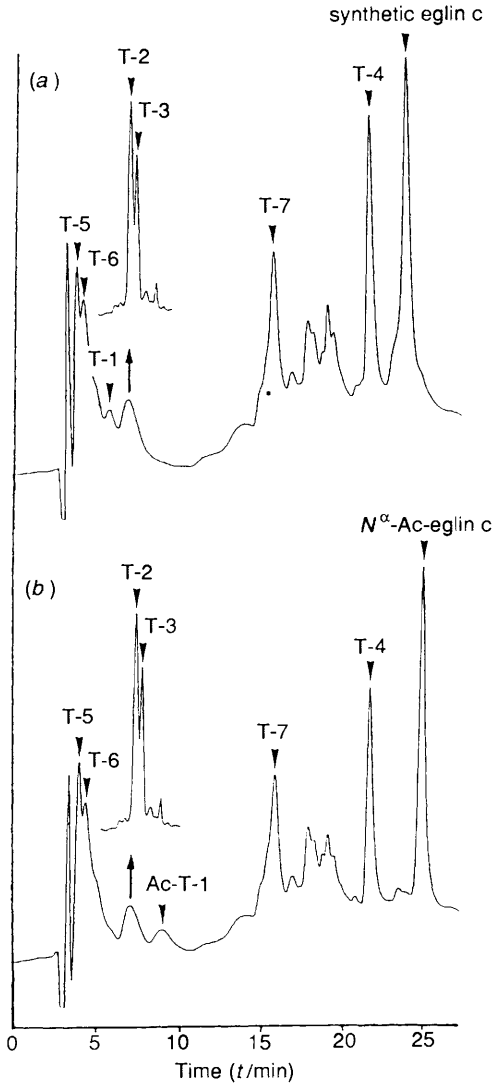


Fig. 5 Reversed-phase HPLC of trypsin digests of (a) synthetic eglin c, (b) N^{α}-acetyleglin c Column: YMC-Pack R-ODS-5 (4.6 mm × 25.0 cm); solvent: a = water (0.05% TFA), b = MeCN (0.05% TFA); gradient 80:20 (a:b) to 35:65 in 20 min, 35:65 for 5 min and then return to 80:20 in 10 min; flow rate 1.0 cm^3 min^{-1}: absorbance 210 nm.

μmol), TFA (1.0 cm^3, 12 mmol), anisole (0.1 cm^3, 0.92 mmol) and *m*-cresol (0.1 cm^3, 0.96 mmol)] (175 mg, 84.1%), m.p. 225–240 °C; $[\alpha]_D^{26}$ −34.4° (*c* 0.1, DMSO) (Found: C, 54.7; H, 6.6; N, 13.9. $C_{348}H_{482}N_{76}O_{87}S_4 \cdot 20H_2O$ requires C, 54.9; H, 6.91; N, 14.0%).

Boc-Lys(Z)-Thr-Val-Asp-Gln-Ala-Arg(Mts)-Glu-Tyr-Phe-Thr-Leu-His(Bom)-Tyr-Pro-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(1–70)-*OBzl* **16**].—The *title compound* was prepared from Boc-Lys(Z)-Thr-Val-N_3 [prepared from Boc-(16–18)-$NHNH_2$ (49 mg, 83 μmol) and isopentyl nitrite (11 mm^3, 83 μmol)] and H-(19–70)-OBzl·TFA [prepared from Boc-(19–70)-OBzl (120 mg, 17 μmol), TFA (0.5 cm^3, 6.4 mmol), anisole (0.5 cm^3, 0.46 mmol) and *m*-cresol (0.05 cm^3, 0.48 mmol)] (120 mg, 95.2%), m.p. 208–222 °C; $[\alpha]_D^{26}$ −27.2° (*c* 0.1, DMSO) (Found: C, 54.0; H, 6.8; N, 13.3. $C_{371}H_{516}N_{80}O_{91}S_4 \cdot 30H_2O$ requires C, 54.2; H, 7.07; N, 13.6%).

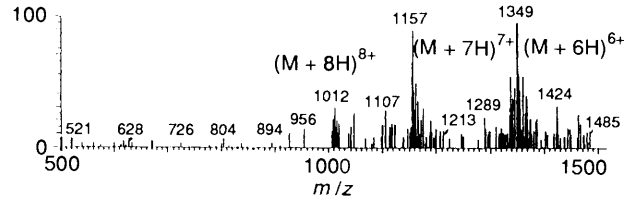
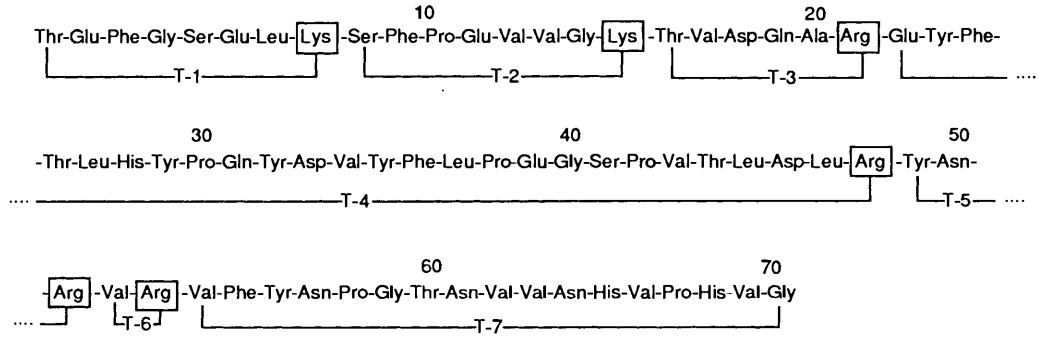


Fig. 6 Mass spectrum of synthetic eglin c by electrospray ionization. Eglin c: $C_{373}H_{550}N_{96}O_{107}$, molecular weight 8090.9. Calc. for (M + 8H)/8: 1012.36; (M + 7H)/7: 1156.84; (M + 6H)/6: 1349.48.

Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(16–70)-*OBzl* **17**].—The *title compound* was prepared from Boc-Lys(Z)-Thr-Val-N_3 [prepared from Boc-(16–18)-$NHNH_2$ (49 mg, 83 μmol) and isopentyl nitrite (11 mm^3, 83 μmol)] and H-(19–70)-OBzl·TFA [prepared from Boc-(19–70)-OBzl (120 mg, 17 μmol), TFA (0.5 cm^3, 6.4 mmol), anisole (0.5 cm^3, 0.46 mmol) and *m*-cresol (0.05 cm^3, 0.48 mmol)] (120 mg, 95.2%), m.p. 208–222 °C; $[\alpha]_D^{26}$ −27.2° (*c* 0.1, DMSO) (Found: C, 54.0; H, 6.8; N, 13.3. $C_{371}H_{516}N_{80}O_{91}S_4 \cdot 30H_2O$ requires C, 54.2; H, 7.07; N, 13.6%).

Boc-Glu-Val-Val-Gly-Lys(Z)-Thr-Val-Asp-Gln-Ala-Arg-(Mts)-Glu-Tyr-Phe-Thr-Leu-His(Bom)-Tyr-Pro-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(12–70)-*OBzl* **18**].—The *title compound* was prepared from Boc-Glu-Val-Val-Gly-N_3 [prepared from Boc-(12–15)-$NHNH_2$ (36 mg, 71 μmol) and isopentyl nitrite (9 mm^3, 71 μmol)] and H-(16–70)-OBzl·TFA [prepared from Boc-(16–70)-OBzl (108 mg, 14 μmol), TFA (0.5 cm^3, 6.4 mmol), anisole (0.05 cm^3, 0.46 mmol) and *m*-cresol (0.05 cm^3, 0.48 mmol)] (100 mg, 82.6%), m.p. 235–243 °C; $[\alpha]_D^{26}$ −26.5° (*c* 0.1, DMSO) (Found: C, 56.4; H, 6.7; N, 14.1. $C_{388}H_{544}N_{84}O_{97}S_4 \cdot 9H_2O$ requires C, 56.6; H, 6.90; N, 14.3%).

Boc-Lys(Z)-Ser-Phe-Pro-Glu-Val-Val-Gly-Lys(Z)-Thr-Val-Asp-Gln-Ala-Arg(Mts)-Glu-Tyr-Phe-Thr-Leu-His(Bom)-Tyr-Pro-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(8–70)-*OBzl* **19**].—The *title compound* was prepared from Boc-Lys(Z)-Ser-Phe-Pro-N_3 [prepared from Boc-(8–11)-$NHNH_2$ (170 mg, 0.24 mmol) and isopentyl nitrite (33 mm^3, 0.24 mmol)] and H-(12–70)-OBzl·TFA [prepared from Boc-(12–70)-OBzl (379 mg, 47 μmol), TFA (1.5 cm^3, 2.0 mmol), anisole (0.15 cm^3, 1.4 mmol) and *m*-cresol (0.15 cm^3, 1.4 mmol)] (289 mg, 71.1%), m.p. 218–225 °C; $[\alpha]_D^{26}$ −39.6° (*c* 0.1, DMSO) (Found: C, 57.3; H, 6.5; N, 14.5. $C_{419}H_{583}N_{89}O_{104}S_4 \cdot 5H_2O$ requires C, 57.5; H, 6.84; N, 14.2%).



Amino acid sequences of tryptic peptide fragments of eglin c

Boc-Ser-Glu-Leu-Lys(Z)-Ser-Phe-Pro-Glu-Val-Val-Gly-Lys(Z)-Thr-Val-Asp-Gln-Ala-Arg(Mts)-Glu-Tyr-Phe-Thr-Leu-His(Bom)-Tyr-Pro-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc-(5–70)-OBzl* **20**].—The *title compound* was prepared from Boc-Ser-Glu-Leu-N_3 [prepared from Boc-(5–7)-$NHNH_2$ (60 mg, 0.13 mmol) and isopentyl nitrite (18 mm^3, 0.13 mmol)] and H-(8–70)-OBzl·TFA [prepared from Boc-(8–70)-OBzl (142 mg, 16 μmol), TFA (1 cm^3, 12 mmol), anisole (0.1 cm^3, 0.92 mmol) and *m*-cresol (0.1 cm^3, 0.96 mmol)] (134 mg, 91.1%), m.p. 220–230 °C; $[\alpha]_D^{26}$ −34.3° (*c* 0.1, in DMSO) (Found: C, 56.9; H, 6.5; N, 13.9. $C_{433}H_{606}N_{92}O_{110}S_4 \cdot 6H_2O$ requires C, 57.2; H, 6.86; N, 14.2%).

Boc-Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys(Z)-Ser-Phe-Pro-Glu-Val-Val-Gly-Lys(Z)-Thr-Val-Asp-Gln-Ala-Arg(Mts)-Glu-Tyr-Phe-Thr-Leu-His(Bom)-Tyr-Pro-Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly-Ser-Pro-Val-Thr-Leu-Asp-Leu-Arg(Mts)-Tyr-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc-(1–70)-OBzl, Protected Eglin c* **21**].—The *title compound* was prepared from Boc-Thr-Glu-Phe-Gly-N_3 [prepared from Boc-(1–4)-$NHNH_2$ (58 mg, 0.10 mmol) and isopentyl nitrite (0.014 cm^3, 0.10 mmol)] and H-(5–70)-OBzl·TFA [prepared from Boc-(5–70)-OBzl (115 mg, 13 μmol), TFA (1.0 cm^3, 12 mmol), anisole (0.1 cm^3, 0.92 mmol) and *m*-cresol (0.1 cm^3, 0.96 mmol)] (104 mg, 81.2%), m.p. 229–239 °C; $[\alpha]_D^{26}$ −42.7° (*c* 0.1, DMSO) (Found: C, 56.4; H, 6.5; N, 13.7. $C_{453}H_{632}N_{96}O_{117}S_4 \cdot 9H_2O$ requires C, 56.7; H, 6.85; N, 14.0%).

General Procedure for Deprotection of the Protected Peptides.—A protected peptide (**13**, **15**, **19** or **21**) (50 mg) was treated with HF (1.5 cm^3) containing thioanisole (0.7 cm^3, 6.7 mmol) and *m*-cresol (0.7 cm^3, 6.7 mmol) at 0 °C for 90 min. After removal of HF (by evaporation at reduced pressure), dry ether was added to the residue. The resulting powder was collected by filtration and dried over KOH pellets *in vacuo*. This product was again treated with HF in the same way as described above in order to complete the deprotection. The resulting powder was dissolved in water (10 cm^3) and the solution was treated with Amberlite IRA-45 (acetate form) for 30 min. The pH of the filtrate was adjusted to 8 with 1 mol dm^{-3} NH_4OH. After 30 min, the pH of the solution was adjusted to 6.5 with 1 mol dm^{-3} AcOH and the solvent was removed by lyophilization to give a crude hygroscopic powder. This crude peptide was purified by gel filtration on Sephadex G-50, followed by reversed-phase HPLC. Each peptide obtained exhibited a symmetrical single peak on analytical HPLC. HPLC profiles of synthetic eglin c are illustrated in Fig. 4. Amino acid ratios of synthetic peptides are summarized in Table 2.

Trypsin Digestion of Synthetic Eglin c and N^{α}*-Acetyleglin c.*—Synthetic eglin c (100 μg, 12 nmol) and N^{α}-acetyleglin c (100 μg, 12 nmol) were digested with trypsin (enzyme:substrate ratio 1:16) in 0.1 mol dm^{-3} Tris–HCl buffer (pH 7.4) at 37 °C for 4 h according to the method described previously.[15] Each digested mixture was analysed by reversed-phase HPLC (Fig. 5). The structure of the tryptic fragments ($\mathbf{T_1}$–$\mathbf{T_7}$) was determined by sequence analysis, amino acid analysis, and peptide synthesis as shown in the line diagram under Fig. 5.

Boc-Ser-Glu-Leu-Lys(Z)-OBzl.—The *title compound* was prepared from Boc-Ser-Glu-Leu-N_3 [prepared from Boc-Ser-Glu-Leu-$NHNH_2$ (287 mg, 0.62 mmol) and isopentyl nitrite (0.1 cm^3, 0.62 mmol)] and H-Lys(Z)-OBzl·Tos-OH (500 mg, 0.93 mmol) (627 mg, 78.5%), m.p. 102–105 °C; $[\alpha]_D^{26}$ −13.0° (*c* 0.1, DMF), R_{f1} 0.48 (Found: C, 58.8; H, 7.2; N, 8.8. $C_{40}H_{57}N_5O_{12} \cdot H_2O$ requires C, 58.7; H, 7.28; N, 8.55%).

Boc-Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys(Z)-OBzl.—The *title compound* was prepared from Boc-Thr-Glu-Phe-Gly-N_3 [prepared from Boc-Thr-Glu-Phe-Gly-$NHNH_2$ (119 mg, 0.21 mmol) and isopentyl nitrite (0.03 cm^3, 0.21 mmol)] and H-Ser-Glu-Leu-Lys(Z)-OBzl·TFA [prepared from Boc-Ser-Glu-Leu-Lys-(Z)-OBzl (140 mg, 0.18 mmol) and TFA (0.13 cm^3, 1.75 mmol)] (71.4 mg, 33.0%), m.p. 121–123 °C; $[\alpha]_D^{26}$ −18.0° (*c* 0.1, DMF); R_{f5} 0.58 (Found: C, 58.8; H, 7.1; N, 10.1. $C_{60}H_{83}N_9O_{19}$ requires C, 58.5; H, 6.80; N, 10.2%).

Ac-Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys-OH (*Ac-*T_1).—The title compound was prepared by coupling of (4-acetoxyphenyl)-dimethylsulphonium methyl sulphate (11.5 mg, 37 μmol) and H-Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys(Z)-OBzl [prepared from Boc-Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys(Z)-OBzl (30 mg, 24 μmol) and TFA (0.1 cm^3, 1.4 mmol)], followed by hydrogenation over Pd catalyst (5 mg, 16%). Amino acid proportions in an acid hydrolysate: Thr 0.90; Glu 1.91; Phe 1.01; Gly 0.98; Ser 0.84; Leu 1.03; Lys 1.00 (average recovery 78%).

H-Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys-OH (T_1).—The title compound was prepared from Boc-Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys(Z)-OBzl (9.3 mg, 7.55 μmol) by treatment with TFA (0.1 cm^3, 1.4 mmol), followed by hydrogenation (2 mg, 32%). Amino acid proportions in an acid hydrolysate: Thr 0.92; Glu 2.03; Phe 0.96; Gly 1.00; Ser 0.86; Leu 0.98; Lys 1.00 (average recovery 80%).

Z-Val-Arg(Mts)-OBzl.—The *title compound* was prepared from Z-Val-OPyCl (228 mg, 0.68 mmol) and H-Arg(Mts)-OBzl·Tos-OH (324 mg, 0.52 mmol) (138 mg, 32.3%), m.p. 62–66 °C; $[\alpha]_D^{26}$ −16.0° (*c* 0.1, MeOH); R_{f1} 0.62, R_{f2} 0.83 (Found: C, 62.0; H, 6.7; N, 10.1. $C_{35}H_{45}N_5O_7S$ requires C, 61.8; H, 6.68; N, 10.3%).

H-Val-Arg-OH (T_6)—Z-Val-Arg(Mts)-OBzl (15 mg, 21 mmol) was treated with HF (10 cm^3) in the presence of thioanisole (0.5 cm^3), as usual (3 mg, 12%). Amino acid proportions in an acid hydrolysate: Val 1.00; Arg 0.97 (average recovery 76.3%).

Boc-Asn-Arg(Mts)-OBzl.—The *title compound* was prepared from Boc-Asn-ONp (362 mg, 1.02 mmol) and H-Arg(Mts)-OBzl·Tos-OH (528 mg, 0.85 mmol) (185 mg, 27.5%), m.p. 82–85 °C; $[\alpha]_D^{26}$ −8.0° (*c* 0.1, MeOH); R_{f1} 0.37, R_{f2} 0.86 (Found: C, 55.7; H, 6.7; N, 12.4. $C_{31}H_{44}N_6O_8S \cdot 0.5H_2O$ requires C, 55.4; H, 6.79; N, 12.5%).

Z-Tyr-Asn-Arg(Mts)-OBzl.—The *title compound* was prepared from Z-Tyr-N_3 [prepared from Z-Tyr-$NHNH_2$ (80 mg, 0.24 mmol) and isopentyl nitrite (0.04 cm^3, 0.24 mmol)] and H-Asn-Arg(Mts)-OBzl·TFA [prepared from Boc-Asn-Arg(Mts)-OBzl (130 mg, 0.20 mmol) and TFA (0.2 cm^3, 1.97 mmol)] (80 mg, 50%), m.p. 96–99 °C; $[\alpha]_D^{26}$ +1.0° (*c* 1.0, MeOH) (Found: C, 59.8; H, 6.1; N, 10.9. $C_{43}H_{51}N_7O_{10}S \cdot 0.5H_2O$ requires C, 59.6; H, 6.06; N, 10.9%).

H-Tyr-Asn-Arg-OH (T_5).—Z-Tyr-Asn-Arg(Mts)-OBzl (15 mg, 18 mmol) was treated with HF (10 cm^3) in the presence of thioanisole (0.5 cm^3) and *m*-cresol (0.2 cm^3), as usual (2 mg, 9.2%). Amino acid proportions in an acid hydrolysate: Tyr 0.90; Asp 1.01; Arg 1.00 (average recovery 80%).

Acknowledgements

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