Amino Acids and Peptides. Part 31.[1] Total Synthesis of Eglin c. Part 1. Synthesis of a Triacontapeptide corresponding to the *C*-Terminal Sequence 41–70 of Eglin c and Related Peptides and Studies on the Relationship between the Structure and Inhibitory Activity against Human Leukocyte Elastase, Cathepsin G and α-Chymotrypsin

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The peptides eglin c (41–70), eglin c (60–70), eglin c (50–70) and eglin c (45–70) have been synthesized by a conventional solution method in order to allow us to study the relationship between the structure and their inhibitory activity against human leukocyte elastase, cathepsin G and α-chymotrypsin. Six 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 side-chain functional groups of the amino acid residues as much as possible during the peptide synthesis. The protected peptides were treated with 1 mol dm^{-3} trimethylsilyl bromide in trifluoroacetic acid at 0 °C for 3 h in the presence of thioanisole and *m*-cresol, followed by purification by Sephadex G-25 column chromatography and preparative reversed phase HPLC to give the desired peptides, which exhibited a symmetrical, single peak on analytical HPLC. Eglin c (60–70) inhibited human leukocyte elastase (K_i 1.7×10^{-3} mol dm^{-3}) but not cathepsin G or α-chymotrypsin. Eglin c (50–70) and eglin c (45–70) inhibited leukocyte elastase (K_i 2.0×10^{-4} and 7.0×10^{-5} mol dm^{-3}, respectively) and α-chymotripsin (K_i 3.4×10^{-5} and 2.5×10^{-5} mol dm^{-3}, respectively) but not cathepsin G. Eglin c (41–70) inhibited leukocyte elastase, cathepsin G and α-chymotrypsin with K_i-values of 1.2×10^{-5}, 2.1×10^{-4} and 7.0×10^{-6} mol dm^{-3}, respectively, while the K_i-values of acetyleglin c (1–70) for the above enzymes were 5.0×10^{-9}, 1.0×10^{-9} and 2.3×10^{-9} mol dm^{-3}, respectively.

Eglin c isolated from the leech *Hirudo medicinalis*[2] consists of 70 amino acid residues[3],† and effectively inhibits chymotrypsin and subtilisin as well as leukocyte elastase and cathepsin G. The latter two enzymes have attracted our interest due to their possible involvement in connective tissue turnover and diseases such as emphysema, rheumatoid arthritis and inflammation.[4,5] Therefore, eglin c is a candidate therapeutic agent for the treatment of emphysema and inflammation. Rink *et al.* prepared N^{α}-acetyleglin c genetically,[6] although its molecular weight is too large for practical therapeutic use.

Under these circumstances, our studies were directed to the systematic synthesis of eglin c and related peptides with the objectives of studying the structure–inhibitory activity relationship and of obtaining peptide inhibitors, for both leukocyte elastase and cathepsin G, with small molecular size.

Regarding the three-dimensional structure and inhibitory mechanism of eglin c, Bode *et al.*[7] and McPhalen *et al.*[8] reported that the nine residues of the binding loop (40–48) of eglin c are involved in direct contact with subtilisin, as a result of the determination of the crystal structure of the complex formed between eglin c and subtilisin Carlsberg by X-ray analysis. In the eglin c molecule, Thr^{44}, Asp^{46} and Arg^{48} form hydrogen and electrostatic bonds with Arg^{53}, Arg^{51} and Gly^{70}, respectively to stabilize the reactive site. Therefore, although eglin c does not contain a disulphide bond to stabilize the tertiary structure, it is highly resistant to denaturation by acidification and by heat as well as to proteolytic degradation.

Thr-Glu-Phe-Gly-Ser-Glu-Leu-Lys-Ser-Phe[10]-Pro-Glu-Val-Val-Gly-
Lys-Thr-Val-Asp-Gln[20]-Ala-Arg-Glu-Tyr-Phe-Thr-Leu-His-Tyr-Pro[30]-
Gln-Tyr-Asp-Val-Tyr-Phe-Leu-Pro-Glu-Gly[40]-Ser-Pro-Val-Thr-Leu-
Asp-Leu-Arg-Tyr-Asn[50]-Arg-Val-Arg-Val-Phe-Tyr-Asn-Pro-Gly-Thr[60]-
Asn-Val-Val-Asn-His-Val-Pro-His-Val-Gly[70]

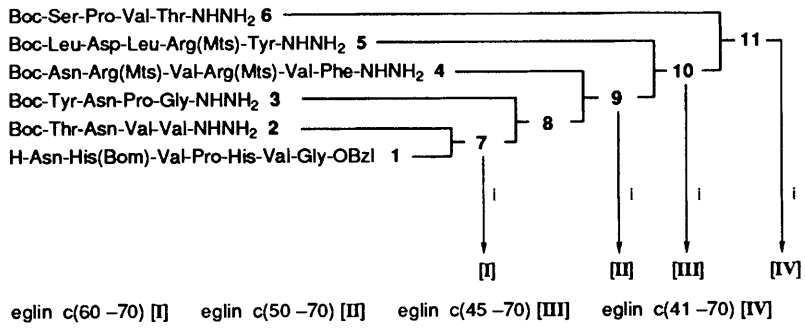
Primary Structure of eglin c

Previously, it was reported that eglin c (41–49) inhibited cathepsin G and α-chymotrypsin, with K_i-values of 4.0×10^{-5} and 2.0×10^{-5} mol dm^{-3}, respectively, but not leukocyte elastase; and that eglin c (60–63) inhibited leukocyte elastase, with a K_i-value of 1.6×10^{-4} mol dm^{-3}.[9,10] Eglin c (41–49) 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 for eglin c (41–49) are some 10^5-times larger than those of eglin c (1–70). Therefore we expected that the potency of the inhibitory activity of eglin c (41–70) might increase upon formation of electrostatic and hydrogen bonds.

This paper deals with the systematic synthesis of a triacontapeptide corresponding to the *C*-terminal sequence 41–70 of eglin c and related peptides and the studies on the relationship between the structure of eglin c and the peptides' inhibitory activity against human leukocyte elastase, cathepsin G and α-chymotrypsin.

Eglin c contains eleven Val residues, including two Val–Val sequences which are known to have affinity for the active centre of leukocyte elastase,[11–14] 11 aromatic amino acid residues

† All amino acid residues have the L-configuration. Abbreviations used throughout this series are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature (*Biochem. J.*, 1967, **102**, 23; 1967, **104**, 17; 1972, **126**, 773). 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. Numbers in parentheses following the name of a particular peptide refer to the two terminal amino acids of the particular molecular fragment under consideration.



Scheme 1 Synthetic scheme for eglin c (41–70) and related peptides. *Reagents and conditions:* i, 1 mol dm^{-3} Me_3SiBr, PhSMe, TFA, *m*-cresol, 0 °C, 3 h.

Table 1 Amino acid analysis of the protected intermediate peptides

	60–70 **7**	56–70 **8**	50–70 **9**	45–70 **10**	41–70 **11**
Asp	2.16(2)	3.33(3)[b]	*4.22*(4)	*4.98*(5)	5.01(5)
Thr	0.99(1)	1.02(1)	1.14(1)	1.01(1)	*1.98*(2)
Ser					*1.03*(1)
Glu					
Gly	1.00(1)	*2.00*(2)	2.00(2)	2.00(2)	2.00(2)
Ala					
Val[a]	3.70(4)	3.62(4)	*5.28*(6)	5.90(6)	*6.82*(7)
Leu				*2.02*(2)	1.97(2)
Tyr		*0.89*(1)	0.91(1)	*2.00*(2)	1.89(2)
Phe			*0.98*(1)	1.13(1)	1.21(1)
Lys					
His	1.93(2)	2.19(2)	1.99(2)	1.80(2)	1.92(2)
Arg			*2.28*(2)	*3.41*(3)	3.11(3)
Pro	1.13(1)	*2.16*(2)	2.13(2)	1.93(2)	*3.22*(3)
	(96.2%)[c]	(76.1%)	(85.1%)	(71.3%)	(64.9%)

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

(five Phe residues and six Tyr residues), which residues are known to have affinity for the active centre of cathepsin G and chymotrypsin,[15–17] eight acidic amino acid residues (five Glu residues and three Asp residues), and nine basic amino acid residues (two Lys residues, four Arg residues and three His residues).

As illustrated in Scheme 1, starting with the *C*-terminal heptapeptide ester **1**, the relatively small four-to-six-peptide fragments **2**–**6** were coupled successively by the azide procedure[18] 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 group. The Bzl protecting group of the β-carboxy function of Asp was removed by catalytic hydrogenation over palladium prior to the synthesis of the corresponding hydrazide **5**. 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[19] or with trimethylsilyl bromide at 0 °C for 3 h,[20] were employed. To introduce the bulky amino acid (Val) in the synthesis of the peptide fragments **1**–**6**, we used a newly developed 6-chloro-2-pyridyl ester.[21] To introduce the Arg residue, the diphenylphosphoryl azide (DPPA) method[22,23] was employed to avoid lactam formation.

According to Scheme 1, peptide intermediates **7**–**11** were obtained after purification at each coupling step by reprecipitation from DMF and MeOH. 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, deprotection of the protected eglin c (60–70), eglin c (50–70), eglin c (45–70) and eglin c (41–70) was performed by treatment with trimethylsilyl bromide (TMSBr). Each peptide was purified by gel filtration on Sephadex G-25 and by preparative HPLC. Homogeneity of the peptides obtained was ascertained by analytical HPLC and amino acid analysis. The results of the amino acid analysis of acid hydrolysates of the eglin c-related peptides are summarized in Table 2.

Each purified peptide exhibited a symmetrical peak on analytical HPLC, as shown in Fig. 1.

Next, inhibitory activity of the synthetic eglin c fragments against human leukocyte elastase, cathepsin G, and α-chymotrypsin was determined and the results are summarized in Table 3 (data given as K_i-values).

As can be seen in Table 3, eglin c (60–70) inhibited only leukocyte elastase, with a K_i-value of 1.7×10^{-3} mol dm^{-3}, which is higher than that of eglin c (60–63) (K_i 1.6×10^{-4} mol dm^{-3}).[9,10] Eglin c (50–70) and eglin c (45–70) inhibited leukocyte elastase and α-chymotrypsin but not cathepsin G, implying that there are some differences in the three dimensional structure of the active centre between cathepsin G and α-chymotrypsin. Eglin c (41–70) inhibited leukocyte elastase, cathepsin G and α-chymotrypsin, with K_i-values of 1.2×10^{-5}, 2.1×10^{-4} and 7.0×10^{-6} mol dm^{-3} respectively. From these results we deduced that, besides the reactive site of eglin C, the Leu^{45}–Asp^{46} bond, a peptide portion corresponding to the sequence 41–44 might be very important for the manifestation of inhibitory activity against cathepsin G. This phenomenon is compatible with that found in the case of eglin c (41–49).[24]

We expected that eglin c (41–70) might inhibit cathepsin G and α-chymotrypsin more potently than eglin c (41–49) due to the formation of electrostatic and hydrogen bonds to maintain the binding loop in a suitable conformation for interaction with enzymes. However, the K_i-values of eglin c (41–70) for cathepsin G and α-chymotrypsin are similar to those of eglin c (41–49), indicating that this peptide fragment cannot form electrostatic and hydrogen bonds. In order to form electrostatic and hydrogen bonds for the manifestation of full inhibitory activity and for stabilization of eglin c, further elongation of the peptide chain to the *N*-terminus is needed.

Experimental

M.p.s were determined with a Yanagimoto micro melting point apparatus and are uncorrected. 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; 18 h) were determined with an amino acid analyser (K-101AS, Kyowa Seimitsu). 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)]. On TLC (Kieselgel G, Merck), R_{f1}-, R_{f2}-, R_{f3}- and R_{f4}-values refer

Table 2 Amino acid analysis of synthetic peptides

	60–70 [I]	50–70 [II]	45–70 [III]	41–70 [IV]
Asp	2.01(2)	4.23(4)	5.11(5)	5.14(5)
Thr	1.12(1)	0.98(1)	1.06(1)	2.16(2)
Ser				0.98(1)
Glu				
Gly	1.00(1)	2.00(2)	2.00(2)	2.00(2)
Ala				
Val[a]	3.36(4)	5.28(6)	5.83(6)	5.86(6)
Leu			1.93(2)	1.91(2)
Tyr		0.90(1)	1.76(2)	1.79(2)
Phe		1.05(1)	1.06(1)	1.19(1)
Lys				
His	2.04(2)	1.89(2)	1.89(2)	1.61(2)
Arg		2.16(2)	2.61(3)	3.22(3)
Pro	0.98(1)	1.94(2)	2.09(2)	3.02(3)
	(73.5%)[b]	(53.5%)	(64.4%)	(50.0%)

[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(M) (mol dm^{-3})		
	Elastase[a]	Cathepsin G[b]	α-Chymotrypsin[b]
Eglin c (60–70) [I]	1.7×10^{-3}	ND[c]	ND
Eglin c (50–70) [II]	2.0×10^{-4}	ND	3.4×10^{-5}
Eglin c (45–70) [III]	7.0×10^{-5}	ND	2.5×10^{-5}
Eglin c (41–70) [IV]	1.2×10^{-5}	2.1×10^{-4}	7.0×10^{-6}
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 (Suc: succinyl; pNA: *p*-nitroanilide). [b] Substrate for cathepsin G and α-chymotrypsin: Suc-Ile-Pro-Phe-pNA. [c] Not detectable.

to the solvent systems (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).

Boc-Thr-Asn-Val-Val-NHNH$_2$ [*Boc*-(60–63)-*NHNH*$_2$ **2**].—Hydrazine hydrate (90%; 1.0 cm^3, 18 mmol) was added to a solution of Boc-Thr-Asn-Val-Val-OMe[10] (1.0 g, 1.8 mmol) in DMF (10 cm^3). The reaction mixture was stored at room temperature overnight. After concentration of the reaction mixture to a small volume, MeOH was added to the residue to afford a precipitate, which was collected by filtration and recrystallized from DMF–MeOH (396 mg, 39.6%), m.p. 249–250 °C (decomp.); $[\alpha]_D^{26}$ −24.0° (*c* 0.2, DMSO) (Found: C, 50.4; H, 7.8; N, 17.7. $C_{23}H_{43}N_7O_8$ requires C, 50.6; H, 7.96; N, 18.0%).

Boc-Tyr-Asn-Pro-Gly-NHNH$_2$ [*Boc*-(56–59)-*NHNH*$_2$ **3**].—Reaction and work-up for Boc-Thr-Asn-Val-Val-NHNH$_2$ with Boc-Tyr-Asn-Pro-Gly-OMe[10] (5.0 g, 8.9 mmol) and hydrazine hydrate (90%; 1.5 cm^3, 27 mmol) gave the *title compound* (3.95 g, 78.9%), m.p. 197–199 °C; $[\alpha]_D^{26}$ −43.9° (*c* 1.0, DMF) (Found: C, 52.5; H, 6.6; N, 17.3. $C_{25}H_{37}N_7O_8 \cdot \frac{1}{2}H_2O$ requires C, 52.5; H, 6.70; N, 17.1%).

Boc-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-NHNH$_2$ [*Boc*-(50–55)-*NHNH*$_2$ **4**].—Reaction and work-up for Boc-Thr-Asn-Val-Val-NHNH$_2$ with Boc-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-OMe[10] (280 mg, 0.22 mmol) and hydrazine hydrate (90%; 0.04 cm^3, 0.66 mmol) gave the *title compound* (185 mg, 66.5%), m.p. 225–228 °C; $[\alpha]_D^{26}$ −15.2° (*c* 0.2, DMSO) (Found: C, 53.5; H, 7.1; N, 16.2. $C_{58}H_{89}N_{15}O_{13}S_2 \cdot 2H_2O$ requires C, 53.4; H, 7.12; N, 16.5%).

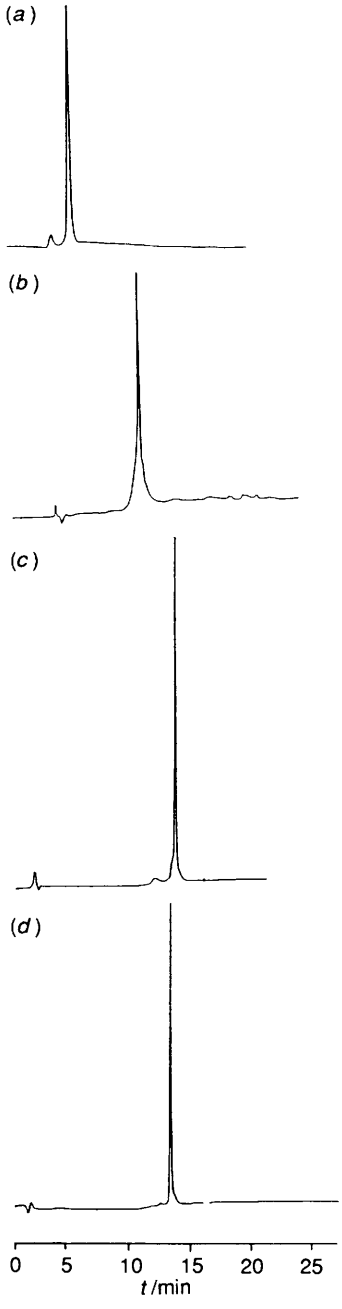


Fig. 1 Analytical HPLC of synthetic eglin c (41–70) and related peptides. (a) Eglin c (60–70), column: YMC-Pack A-302 (6.0 × 150 mm); solvent a = water (0.05% TFA), b = MeCN (0.05% TFA), gradient: 80:20 (a:b) to 60:40 in 20 min, 60:40 for 5 min, and return to 80:20 in 10 min; flow rate 1.0 cm^3 min^{-1}; absorbance 210 nm; (b) eglin c (50–70); (c) eglin c (45–70); (d) eglin c (41–70), column: YMC-Pack A-302 (6.0 × 150 mm); 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.

Boc-Leu-Asp-Leu-Arg(Mts)-Tyr-NHNH$_2$ [*Boc*-(45–49)-*NHNH*$_2$ **5**].—Hydrazine hydrate (90%; 0.16 cm^3, 2.8 mmol) was added to a solution of Boc-Leu-Asp-Leu-Arg(Mts)-Tyr-OMe[10] (550 mg, 0.56 mmol) in MeOH. The reaction mixture was stored at 30 °C for 2 days. After removal of the solvent, 3% AcOH was added to the residue to afford a precipitate, which was collected by filtration and washed with water (410 mg, 74.5%), m.p. 142–147 °C; $[\alpha]_D^{26}$ −35.6° (*c* 1.0, MeOH), R_{f2} 0.16 (Found: C, 54.3; H, 7.42; N, 13.1. $C_{45}H_{72}N_{10}O_{12}S \cdot MeCO_2H \cdot \frac{1}{4}H_2O$ requires C, 54.2; H, 7.24; N, 13.4%).

Boc-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(60–70)-*OBzl* **7**].—Boc-Thr-Asn-Val-Val-N_3 [prepared from Boc-Thr-Asn-Val-Val-$NHNH_2$ (500 mg, 0.92 mmol) and isopentyl nitrite (0.13 cm^3, 0.92 mmol), as usual[25]] in DMF (15 cm^3) cooled in an ice–salt bath was added to a DMF (10 cm^3) solution of H-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl·TFA **1** [prepared from Boc-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl[10] (654 mg, 0.61 mmol), TFA (0.46 cm^3, 6.1 mmol) and anisole (0.14 cm^3, 1.2 mmol), as usual[25]] containing Et_3N (0.085 cm^3, 0.61 mmol). After the reaction mixture had been stirred at 4 °C for 2 days, the solvent was removed by evaporation. Diethyl ether was added to the residue to give the *title compound* (815 mg, 89.9%), m.p. 240.5–242.5 °C; $[\alpha]_D^{26}$ −59.0° (*c* 0.1, DMSO); R_{f2} 0.18, R_{f3} 0.30 (Found: C, 54.4; H, 7.0; N, 15.3. $C_{71}H_{105}N_{17}O_{18}\cdot 5H_2O$ requires C, 54.1; H, 6.73; N, 15.1%).

Boc-Tyr-Asn-Pro-Gly-Thr-Asn-Val-Val-Asn-His(Bom)-Val-Pro-His-Val-Gly-OBzl [*Boc*-(56–70)-*OBzl* **8**].—The *title compound* was prepared from Boc-Tyr-Asn-Pro-Gly-N_3 [prepared from Boc-(56–59)-$NHNH_2$ (500 mg, 0.89 mmol) and isopentyl nitrite (0.12 cm^3, 0.89 mmol), as usual] and H-(60–70)-OBzl·TFA [prepared from Boc-(60–70)-OBzl (659 mg, 0.44 mmol), TFA (0.66 cm^3, 8.8 mmol) and anisole (0.09 cm^3, 0.88 mmol), as usual] (569.8 mg, 67.0%), m.p. 218–222 °C; $[\alpha]_D^{26}$ −27.0° (*c* 0.1, DMSO); R_{f4} 0.75 (Found: C, 51.9; H, 6.9; N, 14.5. $C_{91}H_{130}N_{22}O_{24}\cdot 9H_2O$ requires C, 52.1; H, 7.22; N, 14.7%).

Boc-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*-(50–70)-*OBzl* **9**].—The *title compound* was obtained from Boc-Asn-Arg(Mts)-Val-Arg(Mts)-Val-Phe-N_3 [prepared from Boc-(50–55)-$NHNH_2$ (264 mg, 0.21 mmol) and isopentyl nitrite (0.03 cm^3, 0.21 mmol), as usual] and H-(56–70)-OBzl·TFA [prepared from Boc-(56–70)-OBzl (200 mg, 0.10 mmol) and TFA (0.2 cm^3, 2.7 mmol) containing anisole (0.02 cm^3, 0.18 mmol) and *m*-cresol (0.02 cm^3, 0.18 mmol), as usual], (217 mg, 67.9%), m.p. 259–265 °C (decomp.); $[\alpha]_D^{26}$ −25.0° (*c* 0.1, DMSO) (Found: C, 52.7; H, 6.8; N, 15.0. $C_{144}H_{207}N_{35}O_{36}S_2\cdot 11H_2O$ requires C, 52.9; H, 7.08; N, 15.0%).

Boc-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*-(45–70)-*OBzl* **10**].—The *title compound* was obtained from Boc-Leu-Asp-Leu-Arg(Mts)-Tyr-N_3 [prepared from Boc-(45–49)-$NHNH_2$ (200 mg, 0.21 mmol) and isopentyl nitrite (0.03 cm^3, 0.21 mmol), as usual] and H-(50–70)-OBzl·TFA [prepared from Boc-(50–70)-OBzl (307 mg, 0.10 mmol) and TFA (0.1 cm^3, 1.3 mmol) containing anisole (0.01 cm^3, 0.09 mmol) and *m*-cresol (0.01 cm^3, 0.09 mmol), as usual] (386 mg, 73.6%), m.p. 250–255 °C (decomp.); $[\alpha]_D^{26}$ −38.0° (*c* 0.1, DMSO) (Found: C, 55.2; H, 6.8; N, 15.2. $C_{184}H_{265}N_{43}O_{45}S_3\cdot 5H_2O$ requires C, 55.4; H, 6.97; N, 15.1%).

Boc-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*-(41–70)-*OBzl* **11**].—The *title compound* was obtained from Boc-Ser-Pro-Val-Thr-N_3 [prepared from Boc-(41–44)-$NHNH_2$ (67 mg, 77 μmol) and isopentyl nitrite (0.01 cm^3, 7 μmol), as usual] and H-(45–70)-OBzl·TFA [prepared from Boc-(45–70)-OBzl (100 mg, 26 μmol) and TFA (0.1 cm^3, 1.3 mmol) containing anisole (0.01 cm^3, 0.09 mmol) and *m*-cresol (0.01 cm^3, 0.09 mmol), as usual], (57.5 mg, 53.3%), m.p. 260 °C (decomp.); $[\alpha]_D^{26}$ −69.2° (*c* 0.1, DMSO) (Found: C, 53.5; H, 6.8; N, 14.9. $C_{201}H_{291}N_{47}O_{50}S_3\cdot 14H_2O$ requires C, 53.8; H, 7.18; N, 14.7%).

General Procedure for Deprotection of the Protected Peptides.—A protected peptide **7**–**11** was treated with 1 mol dm^{-3} TMSBr–thioanisole–TFA (350 mol equiv.) in the presence of *m*-cresol (10 mol equiv./Tyr) in an ice-bath for 3 h, and dry diethyl ether was added to the solution. The resulting powder was collected by centrifugation and dissolved in water (10 cm^3). This 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. The crude peptide was purified by gel filtration on Sephadex G-25, followed by reversed-phase HPLC. Each purified peptide exhibited a symmetrical single peak on analytical HPLC (Fig. 1). Amino acid ratios are in good agreement with theoretically expected values, as summarized in Table 2.

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