

Studies Relating to the Ferredoxins. Part 3.¹ The Synthesis of Some Cysteine–Glycine Peptides for Iron–Sulphur Complexing Studies

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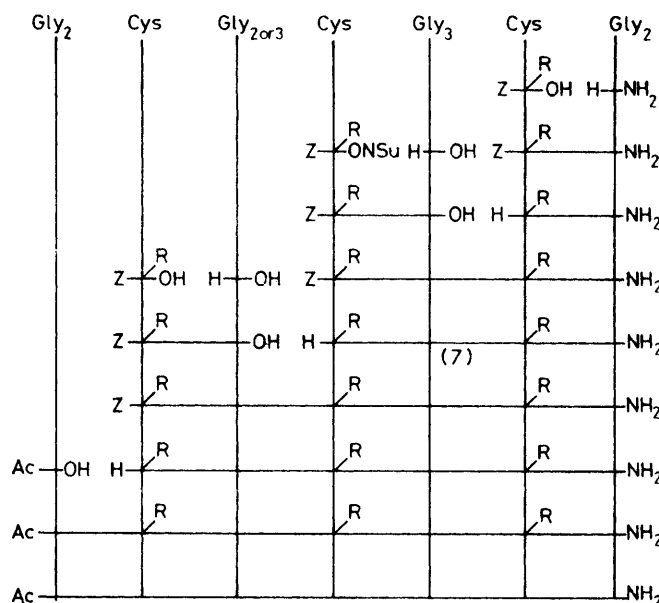
Three peptides with the general structure $\text{Ac-Gly}_2\text{-(Cys-Gly)}_m\text{-Cys-Gly}_3\text{-Cys-Gly}_2\text{-NH}_2^\dagger$ (1; $m = 2$ or 3 ; $n = 0$ or 1), four with the general structure $\text{Ac-Gly}_2\text{-Cys}_n\text{-Gly}_2\text{-NH}_2$ (2; $n = 1\text{--}4$), and three with the general structure $\text{HS-CH}_2\text{-CH}_2\text{-CO-Gly}_2\text{-(Cys-Gly)}_n\text{-NH-CH}_2\text{-CH}_2\text{-SH}$ (3; $n = 0\text{--}2$) have been synthesised for iron–sulphur complexing studies.

In Part 2 of this series¹ we described the synthesis of four cysteine–glycine peptides, $\text{Ac-Gly}_2\text{-(Cys-Gly)}_n\text{-Cys-Gly}_2\text{-NH}_2^\dagger$ ($n = 0\text{--}3$), and their conversion by ligand exchange with $[\text{Fe}_4\text{S}_4(\text{SBU})_4]^{2-}$ into iron–sulphur cluster compounds analogue to the four-iron ferredoxins; these peptides were also used in the direct formation of peptide analogous of rubredoxins and four-iron ferredoxins² and as components of substitutes for ferredoxin as electron-transfer agents in the *Clostridium pasteurianum* hydrogenase system.³ We now describe the synthesis of a further ten cysteine–glycine peptides for use in similar studies, which will be reported elsewhere. All the new peptides are modifications of the original series, designed to throw light on the relationship between structure and, *inter alia*, the ease of formation of iron–sulphur cluster compounds, and fall into three groups. In the first group one or two of the diglycyl spacing units between cysteine residues have been replaced by triglycyl units, to simulate more closely the $\text{-Cys-X}_2\text{-Cys-X}_2\text{-Cys-X}_3\text{-Cys-}$ arrangement found in almost all known $[\text{4Fe-4S}]$ and $[\text{8Fe-8S}]$ ferredoxins.⁴ In the second group the cysteine residues are directly joined, and in the third the *N*-terminal $\text{Ac-Gly}_2\text{-NH-}$ and *C*-terminal $\text{-CO-Gly}_2\text{-NH}_2$ portions of the molecules have been removed.

RESULTS AND DISCUSSION

The first group comprises the three peptides $\text{Ac-Gly}_2\text{-Cys-Gly}_3\text{-Cys-Gly}_2\text{-NH}_2$ (4), $\text{Ac-Gly}_2\text{-Cys-Gly}_2\text{-Cys-Gly}_3\text{-Cys-Gly}_2\text{-NH}_2$ (5), and $\text{Ac-Gly}_2\text{-Cys-Gly}_3\text{-Cys-Gly}_3\text{-Cys-Gly}_2\text{-NH}_2$ (6); the synthetic route is summarised in Scheme 1. The benzyloxycarbonyl group was used throughout for *N*-protection and removed at intermediate stages by the action of hydrogen bromide in acetic acid. As in the earlier work,¹ the benzyl group was used for the protection of cysteine side-chains in syntheses of all three peptides; in alternative syntheses of (4) and (5) the *p*-methoxybenzyl group was used, since it is more easily removed by hydrogen fluoride⁵ although retaining sufficient selectivity *vis-à-vis* *N*-benzyloxycarbonyl,⁶ and gave substantially better overall yields. In addition to $\text{Ac-Gly}_2\text{-OH}$, H-Cys(Bzl)-

$\text{Gly}_2\text{-NH}_2$, and $\text{Z-Cys(Bzl)-Gly}_2\text{-OH}$ and its *N*-succinimidyl ester, the preparation of which is described in Part 2, four further key intermediates were required. $\text{Z-Cys(Bzl)-Gly}_3\text{-OH}$ was prepared by coupling either Z-Cys(Bzl)-ONSu and $\text{H-Gly}_3\text{-OH}$, or $\text{Z-Cys(Bzl)-Gly}_2\text{-ONSu}$ and glycine, in aqueous dioxan in the presence of sodium hydrogencarbonate, the yield by either route



SCHEME 1

being *ca.* 80%. $\text{Z-Cys(BzlOMe)-Gly}_2\text{-OH}$ and $\text{Z-Cys(BzlOMe)-Gly}_3\text{-OH}$ were prepared similarly from $\text{Z-Cys(BzlOMe)-ONSu}$ and $\text{H-Gly}_2\text{-OH}$ or $\text{H-Gly}_3\text{-OH}$ in 90 and 85% yield, respectively. $\text{H-Cys(BzlOMe)-Gly}_2\text{-NH}_2$ was prepared in 80% overall yield by mixed carbonic anhydride coupling of Z-Cys(BzlOMe)-OH and $\text{H-Gly}_2\text{-NH}_2$ followed by removal of the *N*-protecting group.

The fully protected derivatives of (4), (5), and (6) were built up from these intermediates by the fragment condensation strategy outlined in Scheme 1, the first-mentioned by coupling $\text{Ac-Gly}_2\text{-OH}$ with (7; $\text{R} = \text{Bzl}$ or BzlOMe). In the three syntheses using the *S*-benzyl protecting group all the coupling reactions were carried out using *NN'*-dicyclohexylcarbodi-imide in the presence of *N*-hydroxysuccinimide,⁷ except for the coupling of

[†] Abbreviations for amino-acid residues are those recommended by I.U.P.A.C.–I.U.B. (*Biochem. J.*, 1972, **126**, 773); Cys indicates the *L*-enantiomer. Other abbreviations: Bzl = PhCH_2 ; BzlOMe = $p\text{-MeOC}_6\text{H}_4\text{CH}_2$; Np = $p\text{-NO}_2\text{C}_6\text{H}_4$; NSu = $\text{CO-CH}_2\text{-CH}_2\text{-CO-N}$; Z = PhCH_2OCO ; DMF = HCONMe_2 .

¹H N.m.r. spectra of protected peptides of series 1 and 2 [chemical shifts in (CD₃)₂SO; ±0.05 p.p.m.]

(A) Protected peptides

CONH		Ar			CONH ₂	Cys-α-CH	Gly-CH ₂ , ArCH ₂ , ArOMe br m	Cys-β-CH ₂	COMe
Cys m	Gly m	Ph s	Ar- <i>m</i> -H d	Ar- <i>o</i> -H d	2 s	m		m	s
8.35	8.1	7.3	7.25	6.8	7.15, 7.05	4.5	3.7	2.7	1.85

(B) Deprotected peptides

CONH m	CONH ₂ 2 s	Cys-α-CH m	Gly-CH ₂ m	Cys-β-CH ₂ m	SH m	COMe s
8.21	7.28, 7.12	4.44	3.7	2.76	2.4	1.83

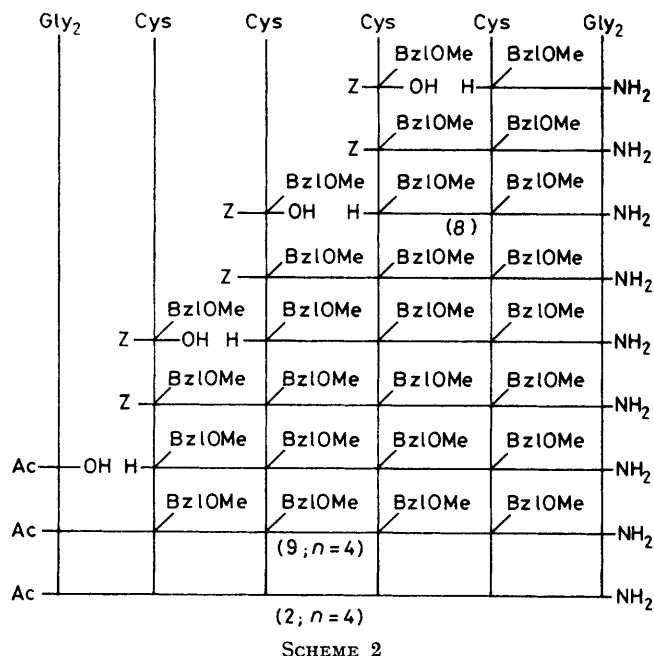
Z-Cys(Bzl)-Gly₃-OH with H-Cys(Bzl)-Gly₂-NH₂ in which the isolated *N*-succinimidyl ester was used. In the two syntheses using the *p*-methoxybenzyl group for *S*-protection, the couplings were all effected by the mixed carbonic anhydride procedure using isobutyl chloroformate⁸ in dimethylformamide; in the coupling of Ac-Gly₂-OH with H-Cys(BzlOMe)-Gly₂-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂ the yield was greatly improved by using four equivalents of the mixed anhydride. In general this method gave satisfactory yields (60–90%) except in the coupling of Z-Cys(BzlOMe)-Gly₂-OH with H-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂ where the use of the dicyclohexylcarbodi-imide-*N*-hydroxysuccinimide method increased the yield from 36 to 68%.

The following fully protected peptides were prepared in this way in the overall yields (from the key intermediates) indicated: Ac-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (42%), Ac-Gly₂-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂ (59%), Ac-Gly₂-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (22%), Ac-Gly₂-Cys(BzlOMe)-Gly₂-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂ (44%), and Ac-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (37%), and characterised by their ¹H n.m.r. spectra (see Table). The side-chain benzyl or *p*-methoxybenzyl protecting groups were removed from these five protected peptides by the action of hydrogen fluoride containing anisole as carbocation scavenger,⁹ followed by treatment with 2-mercaptoethanol to remove adventitious disulphide as far as possible, to give the three required cysteine-glycine peptides (4)–(6), the ¹H n.m.r. spectra of which are summarised in the Table; in these, and in the protected peptides, the amide protons are magnetically non-equivalent, appearing as two singlets.

It was hoped to extend the series by synthesising analogous tetracysteine peptides. To this end the three fully-protected peptides, Z-Cys(R)-Gly₂-Cys(R)-Gly₂-Cys(R)-Gly₃-Cys(R)-Gly₂-NH₂ (R = Bzl or BzlOMe) and Z-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂, were prepared by the obvious extension of the route outlined in Scheme 1, but their *N*-deprotected derivatives were too insoluble to be coupled with Ac-Gly₂-OH to complete the syntheses.

The second group comprises the four peptides (2; *n* = 1–4). Their syntheses are exemplified by that of (2; *n* = 4) which is set out in Scheme 2; the lower members of the series were prepared by coupling the appropriate intermediate, *e.g.* (8) for the synthesis of (2; *n* = 2), with

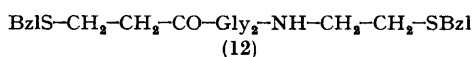
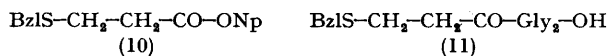
Ac-Gly₂-OH. The *p*-methoxybenzyl group was used for *S*-protection in view of its superiority to the unsubstituted benzyl group in the syntheses of (4) and (5). All the couplings were brought about by the mixed carbonic anhydride method;⁸ in the last two couplings in the synthesis of (9; *n* = 4) it was found advantageous to use 2 equiv. of the mixed



anhydride. The four fully protected peptides, Ac-Gly₂-[Cys(BzlOMe)]_{*n*}-Gly₂-NH₂ (9), were obtained in the following overall yields from the three starting materials, Ac-Gly₂-OH, Z-Cys(BzlOMe)-OH, and H-Cys(BzlOMe)-Gly₂-NH₂: (9; *n* = 1), 80%; (9; *n* = 2), 44%; (9; *n* = 3), 20%; (9; *n* = 4), 13%. They were characterised by their ¹H n.m.r. spectra (see Table), and finally deprotected by the procedure used for the first group of peptides. The ¹H n.m.r. spectra of the resulting four peptides (2; *n* = 1–4) are summarised in the Table; in this series, too, the amide protons are magnetically non-equivalent.

The third group comprises the three peptides (3; *n* = 0–2). For the synthesis of the simplest member of this group, the *p*-nitrophenyl ester (10)¹⁰ was coupled with glycylglycine in aqueous dimethylformamide to give (11), which was in turn coupled by the dicyclo-

hexylcarbodi-imide-*N*-hydroxysuccinimide procedure⁷ with 2-*S*-benzylthioethylamine to give the required protected peptide (12) in 60% overall yield. 2-*S*-



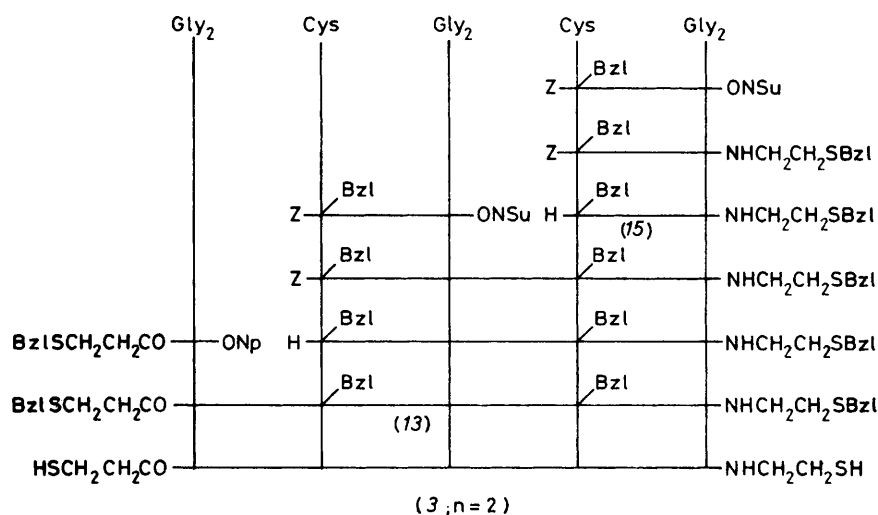
Benzylthioethylamine was conveniently prepared by benzylation of 2-mercaptoethylamine with 1 equiv. benzyl chloride and sodium in liquid ammonia; any excess of benzylating agent leads to the formation of the corresponding amount of the *NS*-dibenzyl derivative, with concomitant reduction in yield of the required compound. This method of preparation, which gives a 94% yield, is more convenient than the other methods described in the literature.¹¹⁻¹³ The synthesis of the

and derivatives separated from the 'recrystallisation' solvent as bulky gels from which it was not possible to remove the last traces of solvent. Decomposition points are not reported.

Optical rotations were measured with a Bendix-N.P.L. 143A polarimeter and i.r. spectra on a Perkin-Elmer 237 recording spectrophotometer. ¹H N.m.r. spectra were recorded in (CD₃)₂SO, unless otherwise stated, at 31 °C on a JEOL MH-100 spectrometer at 100 MHz; chemical shifts (δ) are in p.p.m. from SiMe₄; bands marked with an asterisk disappeared on treatment with D₂O.

General Synthetic Methods

(A) *Mixed Anhydride Couplings*.—Triethylamine (1 equiv.) was added to a stirred solution of the carboxy-component (1 equiv.) in anhydrous DMF and the mixture cooled to -15 °C before adding ethyl or isobutyl chloroformate (1 equiv.). After 15 min a solution of the hydrobromide of the amino-component (1 equiv.) in DMF or



SCHEME 3

fully-protected peptide (13), corresponding to (3; *n* = 2), is outlined in Scheme 3 and calls for no special comment; the overall yield was 25%. The third protected peptide, BzIS-CH₂-CH₂-CO-Gly₂-Cys(Bzl)-Gly₂-NH-CH₂CH₂-SBzl, (14), was prepared (59% overall yield) by coupling the intermediate (15) with the *N*-succinimidyl ester of (11). The three protected peptides, (12)–(14), were finally deprotected with sodium in liquid ammonia to give the required products (3; *n* = 0–2); this method was chosen in preference to the use of liquid hydrogen fluoride since these products are insoluble in cold water and can readily be freed from sodium chloride by washing with water.

EXPERIMENTAL

All solvents were dried and purified by appropriate methods. Organic solutions were dried over MgSO₄ and all solutions evaporated or concentrated under reduced pressure at 40 °C or below using a rotary evaporator. The purity of all peptides and peptide derivatives was confirmed by t.l.c. in at least two solvent systems and their structures by ¹H n.m.r. and i.r. spectroscopy. Many of the larger peptides

Me₂SO containing triethylamine (1 equiv.) was added. The mixture was stirred for 1 h at -10 °C and then at room temperature overnight. The solvent was removed and the residue triturated with water or other suitable solvent, washed, dried *in vacuo*, and recrystallised. The yields of some of the larger peptides were greatly increased¹⁴ by using an excess (up to 4 equiv.) of the mixed anhydride, which was destroyed at the end of the reaction by adding 2*M*-potassium hydrogencarbonate at 0 °C and precipitating the coupling product after 30 min by adding water.

(B) *Carbodi-imide Couplings*.—The amino-component (1 equiv.) was liberated from its hydrobromide by adding triethylamine (1 equiv.) to a solution in DMF or other suitable solvent, the volume of which was such that, at completion of the reaction, the concentration of residual carboxy-component would be *ca.* 0.05*M*.¹⁵ The carboxy-component (2 equiv.) and *N*-hydroxysuccinimide (2 equiv.) were added and the stirred solution cooled to -10 °C. *NN'*-Dicyclohexylcarbodi-imide (2 equiv.) was then added and the mixture stirred at -10 °C for 1 h and then at room temperature until reaction was complete (t.l.c.). The product was precipitated, usually as a gelatinous solid, by pouring into methanol at -5 °C, collected by filtration, washed, dried, and recrystallised.

(C) *Active Ester Couplings*.—*N*-Succinimidyl esters were prepared by adding *NN'*-dicyclohexylcarbodi-imide (1—1.1 equiv.) to a solution of the carboxy-component (1 equiv.) and *N*-hydroxysuccinimide (1—1.1 equiv.) in ethyl acetate or DMF at 0 °C. The mixture was stirred at 0 °C for 1—2 h and then overnight at room temperature. *NN'*-Dicyclohexylurea was then filtered off and washed with a little acetone. The combined filtrate and washings were evaporated to dryness and the residue taken up in a little acetone. The solution was kept at –10 °C for at least 4 h and then filtered. The ester obtained by evaporation to dryness was either recrystallised or used directly in one of the following procedures.

(C1) *Coupling in anhydrous solution*. The amino-component (1 equiv.) was liberated from its hydrobromide by adding triethylamine (1 equiv.) to a solution in anhydrous DMF. After stirring for 5 min, the active ester (1 equiv.) was added and the mixture stirred for 24 h or until reaction was complete (t.l.c.). The product was isolated by precipitation with water, dried, and recrystallised.

(C2) *Coupling in aqueous dioxan*. The active ester (1 equiv.), in dioxan, was added to a solution of the amino-component or its hydrochloride (1 equiv.) in aqueous 1*M*-sodium hydrogencarbonate (1 or 2 equiv., respectively). After 24 h at room temperature, the mixture was concentrated to half-volume, acidified (pH 2—2.5) with hydrochloric acid and the product collected by filtration, dried, and recrystallised.

(D) *Selective Removal of N-Benzzyloxycarbonyl Groups*.—The derivative was kept at room temperature for 40 min in 10—45% w/v hydrogen bromide in acetic acid. The hydrobromide of the deprotected peptide was then precipitated, usually by adding a large volume of anhydrous ether, washed, and dried *in vacuo* over potassium hydroxide pellets.

(E) *Final Deprotection*.—(E1) *With hydrogen fluoride*. The protected peptide (ca. 100 mg) and anisole (3 mol per –SCH₂Ph group) were dissolved at –70 °C in anhydrous hydrogen fluoride. The reaction vessel was closed, the temperature allowed to rise to ambient, and the mixture stirred until reaction was complete. The mixture was then cooled to 0 °C and the hydrogen fluoride evaporated under reduced pressure. The residue was kept overnight at 0.01 mmHg over sodium hydroxide pellets, dissolved in water (8 ml), and the solution extracted with ether (3 × 5 ml). The residual aqueous solution was concentrated to half-volume and 2-mercaptoethanol (4—5 g) added. The mixture was stirred for 30 min and evaporated to dryness and the residue triturated successively with 5% 2-mercaptoethanol in methanol (5 ml) and 5% 2-mercaptoethanol in ethyl acetate (15 ml); these and all subsequent operations were carried out anaerobically. The flocculent solid was collected by filtration, washed with 5% 2-mercaptoethanol in ethyl acetate, and dried at 0.025 mmHg/40 °C. The final products could not be recrystallised and were used directly for complexing experiments, being stored, if necessary, in a dry-box in an atmosphere of oxygen-free nitrogen.

(E2) *With sodium in liquid ammonia*. A stirred solution of the protected peptide (100 mg) in liquid ammonia (50 ml; distilled from sodium) at –33 °C was treated with small pieces of sodium until the blue colour persisted. The colour was discharged by adding a few crystals of ammonium chloride; more ammonium chloride (2 mol per –SCH₂Ph group) was added and the mixture stirred for

5 min. The ammonia was evaporated in a stream of nitrogen and the last traces removed under reduced pressure. The residue was triturated with hexamethylphosphoramide (water cannot be used at this stage since the peptides, although insoluble in water, are soluble in sodium chloride solution) and the product precipitated from the extract with ether, filtered off, washed with water and dried *in vacuo*. The crude product was dissolved in hot water (20 ml) and 2-mercaptoethanol (1 ml) added to the solution; evaporation to dryness and trituration with ethyl acetate or ether gave a white solid, which was collected by filtration, washed with ether, and dried *in vacuo*; these final operations were carried out anaerobically.

Peptides of Series 1

Ac-Gly₂-Cys-Gly₃-Cys-Gly₂-NH₂ (4). *Route 1*.—*Z*-Cys(Bzl)-Gly₂-ONSu¹ (4.45 g, 9 mmol) and glycine (0.60 g, 8 mmol) were coupled by method C2 in dioxan (80 ml) and 0.1*M* sodium hydrogencarbonate (80 ml). Recrystallisation from aqueous ethanol gave *Z*-Cys(Bzl)-Gly₃-OH (3.25 g, 79%), m.p. 200—201 °C, [α]_D²² –28.2° (*c* 2.0 in DMF) (lit.¹⁶ m.p. 201 °C, [α]_D¹⁸ –28.1°); the same compound was prepared similarly in 84% yield from *Z*-Cys(Bzl)-ONSu¹⁷ and diglycylglycine. This tetrapeptide (4.14 g, 8 mmol), in DMF (80 ml), was converted by method C into its *N*-succinimidyl ester (4.00 g, 83%), m.p. 127—129 °C (from ethyl acetate), [α]_D²⁵ –22.4° (*c* 1.0 in dioxan); the unrecrystallised ester (4.56 g, 7.43 mmol) was coupled by method C1, in DMF (100 ml), with *H*-Cys(Bzl)-Gly₂-NH₂·HBr¹ (3.64 g, 9 mmol); recrystallisation from aqueous acetic acid gave *Z*-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (4.50 g, 75%), m.p. 207—209 °C, [α]_D²⁶ –36.1° (*c* 1.0 in DMF) (Found: C, 55.6; H, 5.6; N, 13.8. C₃₈H₄₆N₈O₉S₂ requires C, 55.5; H, 5.6; N, 13.7%). Treatment of this protected heptapeptide (500 mg, 0.60 mmol) with 20% hydrogen bromide in acetic acid (12 ml) (method D) and reprecipitation from ethanol with ether gave *H*-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂·HBr as a hygroscopic solid (400 mg, 87%), which without further purification, was coupled by method B, in DMF (20 ml), with *N*-acetylglycylglycine¹⁸ (180 mg, 2 equiv.). Recrystallisation from Me₂SO–methanol gave *Ac-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂* (353 mg, 80%), [α]_D²⁶ –31.0° (*c* 1.0 in Me₂SO); δ 8.4* (2 H, m, Cys-NH), 8.1* (7 H, m, Gly-NH), 7.3 (10 H, s, Ph), 7.2* and 7.1* (2 H, 2 s, CONH₂), 4.55 (2 H, m, Cys-α-CH), 3.7 (18 H, br m, Gly-CH₂ and PhCH₂), 2.7 (4 H, br m, Cys-β-CH₂), and 1.85 (3 H, s, COMe) (Found: C, 50.4; H, 5.7; N, 15.7. C₃₆H₄₈N₁₀O₁₀S₂·MeOH requires C, 50.7; H, 6.0; N, 16.0%). Deprotection by method E1 for 90 min gave *Ac-Gly₂-Cys-Gly₃-Cys-Gly₂-NH₂* (4), in 98% yield; δ 8.20* (9 H, m, CONH), 7.12* and 7.28* (2 H, 2 s, CONH₂), 4.44 (2 H, m, Cys-α-CH), 3.7 (14 H, m, Gly-CH₂), 2.78 (4 H, m, Cys-β-CH₂), 2.4* (2 H, m, SH), and 1.84 (3 H, s, COMe).

Route 2. *Z*-Cys(BzlOMe)-OH was liberated from its dicyclohexylamine salt¹⁹ (22.3 g, 0.04 mol) by partitioning between ethyl acetate (125 ml) and 1*M*-phosphoric acid (75 ml). The ethyl acetate solution was dried and used directly for the preparation (method C) of *Z*-Cys(BzlOMe)-ONSu (15.7 g, 83%), m.p. 102—103 °C (from ethyl acetate), [α]_D²⁴ –51.2° (*c* 1.0 in Me₂SO) (Found: C, 58.6; H, 5.2; N, 5.7. C₂₃H₂₄N₂O₇S requires C, 58.5; H, 5.1; N, 5.9%). This ester (14.2 g, 30 mmol) was coupled by method C2 with diglycylglycine (5.7 g, 30 mmol) in 50% aqueous dioxan (150 ml); recrystallisation from aqueous ethanol gave *Z*-Cys(BzlOMe)-Gly₃-OH (13.94 g, 85%), m.p. 171—172 °C,

$[\alpha]_D^{24} - 12.5^\circ$ (c 0.5 in Me_2SO) (Found: C, 54.9; H, 5.5; N, 10.0. $\text{C}_{25}\text{H}_{30}\text{N}_4\text{O}_8\text{S}$ requires C, 54.9; H, 5.5; N, 10.2%).

Z-Cys(BzlOMe)-OH, liberated as above from its dicyclohexylamine salt (5.56 g, 0.01 mol), was coupled by method A with glycylglycine amide hydrobromide²⁰ (2.21 g, 0.01 mol) using ethyl chloroformate (1.09 g, 0.01 mol) in DMF (120 ml). Recrystallisation from aqueous methanol gave Z-Cys(BzlOMe)-Gly₂-NH₂ (4.50 g, 92%), m.p. 148–149 °C, $[\alpha]_D^{26} - 16.9^\circ$ (c 1.0 in MeOH) (Found: C, 56.3; H, 5.7; N, 11.3. $\text{C}_{23}\text{H}_{28}\text{N}_4\text{O}_6\text{S}$ requires C, 56.5; H, 5.8; N, 11.5%) which was converted by method D in 22.5% hydrogen bromide in acetic acid (18 ml) into the hygroscopic H-Cys(BzlOMe)-Gly₂-NH₂·HBr, reprecipitated from ethanol with ether (3.5 g, 88%). This hydrobromide (6.36 g, 14.6 mmol) and Z-Cys(BzlOMe)-Gly₃-OH (9.02 g, 16.5 mmol) were coupled by method A in DMF (280 ml) using isobutyl chloroformate (2.25 g, 16.5 mmol); recrystallisation from acetic acid–ether gave Z-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂ (11.35 g, 88%), m.p. 203–205°, $[\alpha]_D^{25} - 27.7^\circ$ (c 1.0 in Me_2SO) (Found: C, 54.2; H, 5.6; N, 12.3. $\text{C}_{40}\text{H}_{50}\text{N}_8\text{O}_{11}\text{S}_2$ requires C, 54.4; H, 5.7; N, 12.7%). Treatment (method D) with 22.5% hydrogen bromide in acetic acid (27 ml) gave H-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂·HBr (10.13 g, 95%) after reprecipitation from methanol with ether. This hydrobromide (1.67 g, 2.04 mmol) was coupled with N-acetylglycylglycine (0.54 g, 3.1 mmol) by method A in DMF (50 ml) using isobutyl chloroformate (0.42 g, 3.1 mmol). Recrystallisation from Me_2SO –methanol gave Ac-Gly₂-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂ (1.14 g, 62%), $[\alpha]_D^{25} - 19.0^\circ$ (c 0.7 in Me_2SO); δ 8.35* (2 H, m, Cys-NH), 8.1* (7 H, m, Gly-NH), 7.75 (4 H, d, Ar-*m*-H), 7.15* and 7.05* (2 H, 2 s, CONH₂), 6.85 (4 H, d, Ar-*o*-H), 4.5 (2 H, m, Cys- α -CH), 3.7 (24 H, br m, Gly-CH₂, Ar-CH₂, OMe), 2.7 (4 H, m, Cys- β -CH₂), and 1.85 (3 H, s, COMe) (Found: C, 49.7; H, 5.7; N, 15.1. $\text{C}_{38}\text{H}_{52}\text{N}_{10}\text{O}_{12}\text{S}_2\cdot\text{MeOH}$ requires C, 50.0; H, 6.0; N, 15.0%). Deprotection by method E1 for 90 min gave Ac-Gly₂-Cys-Gly₃-Cys-Gly₂-NH₂ (4) in 90% yield; δ 8.20* (9 H, m, CONH), 7.12* and 7.30* (2 H, 2 s, CONH₂), 4.45 (2 H, m, Cys- α -CH), 3.75 (14 H, m, Gly-CH₂), 2.78 (4 H, m, Cys- β -CH₂), 2.4* (2 H, m, SH), 1.80 (3 H, s, COMe).

Ac-Gly₂-Cys-Gly₂-Cys-Gly₃-Cys-Gly₂-NH₂ (5). Route 1.—H-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂·HBr (3.26 g, 4.2 mmol) and Z-Cys(Bzl)-Gly₂-OH¹ (3.86 g, 8.4 mmol) were coupled by method B in DMF (168 ml); recrystallisation from Me_2SO –methanol gave Z-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (3.86 g, 81%), $[\alpha]_D^{24} - 38.0^\circ$ (c 1.0 in Me_2SO) (Found: C, 54.6; H, 5.5; N, 13.4. $\text{C}_{52}\text{H}_{68}\text{N}_{11}\text{O}_{12}\text{S}_3\cdot\text{MeOH}$ requires C, 54.8; H, 5.8; N, 13.3%). This decapeptide (678 mg, 0.6 mmol) was treated (method D) with 45% hydrogen bromide in acetic acid (1.2 ml); precipitation with water and trituration with water, ethanol, and ether gave H-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂·HBr (440 mg, 70%) which was coupled by method B with N-acetylglycylglycine (146 mg, 2 equiv.) in Me_2SO –hexamethylphosphoramide (1 : 1) (16 ml). Reprecipitation from Me_2SO –hexamethylphosphoramide (1 : 1) with methanol gave Ac-Gly₂-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (350 mg, 73%), $[\alpha]_D^{25} - 22.8^\circ$ (c 0.5 in $\text{CF}_3\text{CO}_2\text{H}$); δ 8.35* (3 H, m, Cys-NH), 8.1* (9 H, m, Gly-NH), 7.3 (15 H, s, Ph), 7.15* and 7.05* (2 H, 2 s, CONH₂), 4.55 (3 H, m, Cys- α -CH), 3.75 (24 H, br m, Gly-CH₂ and Ph-CH₂), 2.7 (6 H, br m, Cys- β -CH₂), and 1.85 (3 H, s, COMe) (Found, corrected for 2% inorganic residue: C, 51.8; H, 5.6; N, 15.8. $\text{C}_{50}\text{H}_{66}\text{N}_{13}\text{O}_{13}\text{S}_3$ requires C, 52.1; H, 5.7; N, 15.8%).

Deprotection by method E1 for 120 min gave Ac-Gly₂-Cys-Gly₂-Cys-Gly₃-Cys-Gly₂-NH₂ (5) in 90% yield; δ 8.20* (12 H, m, CONH), 7.12* and 7.26* (2 H, 2 s, CONH₂), 4.44 (3 H, m, Cys- α -CH), 3.7 (18 H, m, Gly-CH₂), 2.80 (6 H, m, Cys- β -CH₂), 2.4* (3 H, m, SH), and 1.82 (3 H, s, COMe).

Route 2. Z-Cys(BzlOMe)-ONSu (23.63 g, 50 mmol) was coupled by method C2 with glycylglycine hydrochloride (9.33 g, 50 mmol) in aqueous dioxan (200 ml); recrystallisation from aqueous methanol gave Z-Cys(BzlOMe)-Gly₂-OH (22.03 g, 90%), m.p. 103–105 °C $[\alpha]_D^{24} - 23.7^\circ$ (c 1.0 in Me_2SO) (Found: C, 55.5; H, 5.5; N, 8.2. $\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_7\text{S}\cdot 0.5\text{H}_2\text{O}$ requires C, 55.4; H, 5.7; N, 8.4%). This tripeptide (2.03 g, 4.17 mmol) and H-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂·HBr (3.25 g, 4.0 mmol) were coupled by method B in DMF (70 ml); recrystallisation from Me_2SO –methanol gave Z-Cys(BzlOMe)-Gly₂-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂ (3.13 g, 68%), $[\alpha]_D^{27} - 28.7^\circ$ (c 0.6 in Me_2SO) (Found: C, 53.7; H, 5.7; N, 12.3. $\text{C}_{55}\text{H}_{69}\text{N}_{11}\text{O}_{15}\text{S}_3$ requires C, 54.1; H, 5.7; N, 12.6%). Treatment with 22.5% hydrogen bromide in acetic acid (20 ml) (method D) gave H-Cys(BzlOMe)-Gly₂-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂·HBr (2.64 g, 83%), m.p. 194–196 °C after reprecipitation from methanol with ether. This hydrobromide (1.00 g, 0.86 mmol) was coupled by method A with N-acetylglycylglycine (0.60 g, 3.4 mmol) in Me_2SO –DMF (5 : 4) (9 ml) using isobutyl chloroformate (0.47 g, 3.4 mmol); recrystallisation from formic acid–ether gave Ac-Gly₂-Cys(BzlOMe)-Gly₂-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂ (1.00 g, 93%), $[\alpha]_D^{25} - 25.1^\circ$ (c 0.6 in Me_2SO); δ 8.4* (3 H, m, Cys-NH), 8.2* (9 H, m, Gly-NH), 7.3 (6 H, d, Ar-*m*-H), 7.2* and 7.1* (2 H, 2 s, CONH₂), 6.9 (6 H, d, Ar-*o*-H), 4.5 (3 H, m, Cys- α -CH), 3.7 (33 H, br m, Gly-CH₂, Ar-CH₂, OMe), 2.7 (6 H, m, Cys- β -CH₂), and 1.85 (3 H, s, COMe) (Found: C, 50.6; H, 5.7; N, 14.4. $\text{C}_{53}\text{H}_{71}\text{N}_{13}\text{O}_{16}\text{S}_3\cdot\text{HCO}_2\text{H}$ requires C, 50.3; H, 5.7; N, 14.1%). Deprotection by method E1 for 120 min gave a 90% yield of Ac-Gly₂-Cys-Gly₂-Cys-Gly₃-Cys-Gly₂-NH₂ (5); δ 8.20* (12 H, m, CONH), 7.12* and 7.24* (2 H, 2 s, CONH₂), 4.44 (3 H, m, Cys- α -CH), 3.75 (18 H, m, Gly-CH₂), 2.66 (6 H, m, Cys- β -CH₂), 2.4* (3 H, m, SH), and 1.84 (3 H, s, COMe).

Ac-Gly₂-Cys-Gly₃-Cys-Gly₃-Cys-Gly₂-NH₂ (6).—Z-Cys(Bzl)-Gly₃-OH (5.17 g, 10 mmol) and H-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂·HBr (3.85 g, 5 mmol) were coupled by method B in DMF (200 ml); recrystallisation from Me_2SO –methanol gave Z-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (4.95 g, 83%), $[\alpha]_D^{24} - 30.0^\circ$ (c 1.0 in Me_2SO) (Found: C, 53.7; H, 5.5; N, 13.2. $\text{C}_{54}\text{H}_{68}\text{N}_{12}\text{O}_{13}\text{S}_3\cdot 2\text{CH}_3\text{OH}$ requires C, 53.7; H, 6.0; N, 13.4%). This (1.06 g, 0.9 mmol) was treated (method D) with 45% hydrogen bromide in acetic acid (1.8 ml); precipitation with water and trituration with water, ethanol, and ether gave H-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂·HBr (0.96 g, 94%), which was directly coupled by method B with N-acetylglycylglycine (0.294 g, 2 equiv.) in Me_2SO (35 ml). Reprecipitation of the product from trifluoroacetic acid by pouring into cold water gave Ac-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (0.95 g, 91%), $[\alpha]_D^{25} - 20.9^\circ$ (c 0.5 in $\text{CF}_3\text{CO}_2\text{H}$); δ (in $\text{CF}_3\text{CO}_2\text{D}$) 7.4* (15 H, br m, Cys-NH, Gly-NH, CONH₂), 6.95 (15 H, s, Ph), 4.35 (3 H, m, Cys- α -CH), 3.85 (20 H, m, Gly-CH₂), 3.45 (6 H, m, Ph-CH₂), 2.55 (6 H, m, Cys- β -CH₂), and 1.95 (3 H, s, COMe) (Found: C, 49.6; H, 5.3; N, 14.8. $\text{C}_{52}\text{H}_{68}\text{N}_{14}\text{O}_{14}\text{S}_3\cdot\text{CF}_3\text{CO}_2\text{H}$ requires C, 49.7; H, 5.4; N, 15.3%). Deprotection by method E1 for 120 min gave a 90% yield of Ac-Gly₂-Cys-Gly₃-Cys-Gly₃-Cys-Gly₂-NH₂ (6); δ 8.20* (13 H, m, CONH),

7.14* and 7.30* (2 H, 2 s, CONH₂), 4.48 (3 H, m, Cys- α -CH), 3.7 (20 H, m, Gly-CH₂), 2.78 (6 H, m, Cys- β -CH₂), 2.4* (3 H, m, SH), and 1.84 (3 H, s, COMe).

Attempted Syntheses of Tetracysteine Peptides.—(a) Z-Cys(Bzl)-Gly₂-OH (138 mg, 0.3 mmol) and H-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂·HBr (160 mg, 0.15 mmol) were coupled by method B in Me₂SO-hexamethylphosphoramide (1 : 1) (6 ml); reprecipitation of the very insoluble product from hot Me₂SO with methanol gave Z-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (164 mg, 76%) (Found: C, 54.1; H, 5.4; N, 13.2. C₆₆H₈₀N₁₄O₁₅S₄·2CH₃OH requires C, 54.4; H, 5.9; N, 13.1%).

(b) Z-Cys(BzlOMe)-Gly₂OH (2.65 g, 5.42 mmol) and H-Cys(BzlOMe)-Gly₂-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂·HBr (1.58 g, 1.35 mmol) were coupled by method A in DMF-Me₂SO (2 : 3) (25 ml) using isobutyl chloroformate (0.74 g, 5.42 mmol). Recrystallisation from formic acid-methanol gave Z-Cys(BzlOMe)-Gly₂-Cys(BzlOMe)-Gly₂-Cys(BzlOMe)-Gly₃-Cys(BzlOMe)-Gly₂-NH₂ (1.85 g, 88%), [α]_D²⁵ -29.8° (c 0.6 in Me₂SO) (Found: C, 52.4; H, 5.5; N, 12.2. C₇₀H₈₈N₁₄O₁₉S₄·2HCO₂H requires C, 52.4; H, 5.6; N, 11.9%).

(c) Z-Cys(Bzl)-Gly₃-OH (258 mg, 0.5 mmol) and H-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂·HBr (284 mg, 0.25 mmol) were coupled by method B in Me₂SO-hexamethylphosphoramide (3 : 5) (8 ml). Trituration of the very insoluble product with methanol, water, and methanol gave Z-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₃-Cys(Bzl)-Gly₂-NH₂ (304 mg, 78%) (Found: C, 53.2; H, 5.6; N, 13.9. C₇₀H₈₆N₁₆O₁₇S₄·H₂O requires C, 53.5; H, 5.6; N, 14.3%).

Peptides of Series 2

Ac-Gly₂-Cys-Gly₂-NH₂ (2; *n* = 1).—*N*-Acetylglucylglycine (1.74 g, 10 mmol) and H-Cys(BzlOMe)-Gly₂-NH₂·HBr (4.35 g, 10 mmol) were coupled by method A in DMF (60 ml) using ethyl chloroformate (1.08 g, 10 mmol); recrystallisation from methanol-ether gave *Ac-Gly₂-Cys(BzlOMe)-Gly₂-NH₂* (4.08 g, 80%), m.p. 182–183 °C, [α]_D²⁶ -18.1° (c 1.0 in MeOH); δ 8.3* (1 H, m, Cys-NH), 8.05* (4 H, m, Gly-NH), 7.2 (2 H, d, Ar-*m*-H), 7.1* and 7.0* (2 H, 2 s, CONH₂), 6.8 (2 H, d, Ar-*o*-H), 4.45 (1 H, m, Cys- α -CH), 3.65 (13 H, br m, Gly-CH₂, Ar-CH₂, OMe), 2.65 (2 H, m, Cys- β -CH₂), and 1.85 (3 H, s, COMe) (Found: C, 48.4; H, 5.9; N, 15.8. C₂₁H₃₀N₆O₅S·CH₃OH requires C, 48.7; H, 6.3; N, 15.5%). Deprotection by method E1 for 45 min gave an 89% yield of *Ac-Gly₂-Cys-Gly₂-NH₂*; δ 8.20* (5 H, m, CONH), 7.12* and 7.27* (2 H, 2s, CONH₂), 4.40 (1 H, m, Cys- α -CH), 3.7 (8 H, m, Gly-CH₂), 2.76 (2 H, m, Cys- β -CH₂), 2.4* (1 H, br, SH), and 1.85 (3 H, s, OMe), identical with material prepared by another route.¹

Ac-Gly₂-Cys₂-Gly₂-NH₂ (2; *n* = 2).—Z-Cys(BzlOMe)-OH (from the dicyclohexylamine salt; 2.23 g, 4 mmol) was coupled by method A with H-Cys(BzlOMe)-Gly₂-NH₂·HBr (1.47 g, 4 mmol) in DMF (65 ml), using isobutyl chloroformate (0.55 g, 4 mmol); recrystallisation from aqueous ethanol gave Z-[Cys(BzlOMe)]₂-Gly₂-NH₂ (1.82 g, 64%), m.p. 195–196 °C, [α]_D²⁴ -11.7° (c 1.0 in AcOH) (Found: C, 57.3; H, 5.9; N, 9.7. C₃₄H₄₁N₅O₈S₂ requires C, 57.3; H, 5.8; N, 9.8%). Treatment of this (method D) with 15% hydrogen bromide in acetic acid (20 ml) and re-precipitation from methanol with ether gave H-[Cys(BzlOMe)]₂-Gly₂-NH₂·HBr as a hygroscopic solid (1.54 g, 91%), which was coupled directly with *N*-acetylglucylglycine (0.41 g, 1 equiv.) by method A in DMF (60 ml) using isobutyl chloroformate (0.31 g, 1 equiv.). Recrystallisation from aqueous

acetic acid gave *Ac-Gly₂-[Cys(BzlOMe)]₂-Gly₂-NH₂* (1.30 g, 75%), m.p. 197–199 °C, [α]_D²⁸ -36.5° (c 1.0 in Me₂SO); δ 8.25* (2 H, m, Cys-NH), 8.05* (4 H, m, Gly-NH), 7.2 (4 H, d, Ar-*m*-H), 7.1* and 7.0* (2 H, 2 s, CO-NH₂), 6.8 (4 H, d, Ar-*o*-H), 4.5 (2 H, m, Cys- α -CH), 3.7 (18 H, br m, Gly-CH₂, Ar-CH₂, OMe), 2.7 (4 H, m, Cys- β -CH₂), and 1.85 (3 H, s, COMe) (Found: C, 50.6; H, 5.7; N, 12.4. C₃₂H₄₃N₇O₉S₂·1.5H₂O requires C, 50.5; H, 6.1; N, 12.9%). Deprotection by method E1 for 90 min gave a 92% yield of *Ac-Gly₂-Cys₂-Gly₂-NH₂*; δ 8.20* (6 H, m, CONH), 7.12* and 7.28* (2 H, 2 s, CONH₂), 4.42 (2 H, m, Cys- α -CH), 3.7 (8 H, m, Gly-CH₂), 2.76 (4 H, m, Cys- β -CH₂), 2.4* (2 H, br, SH), and 1.82 (3 H, s, COMe).

Ac-Gly₂-Cys₃-Gly₂-NH₂ (2; *n* = 3).—Z-Cys(BzlOMe)-OH (from the dicyclohexylamine salt; 5.01 g, 9 mmol) and H-[Cys(BzlOMe)]₂-Gly₂-NH₂ (from the hydrobromide; 5.59 g, 8.52 mmol) were coupled by method A in DMF (80 ml) using isobutyl chloroformate (1.23 g, 9 mmol); recrystallisation from aqueous acetic acid gave Z-[Cys(BzlOMe)]₃-Gly₂-NH₂ (5.09 g, 64%), m.p. 162–164 °C, [α]_D²⁶ -38.3° (c 1.0 in Me₂SO) (Found: C, 57.7; H, 5.9; N, 8.7. C₄₅H₅₄N₆O₁₀S₃ requires C, 57.8; H, 5.8; N, 9.0%). Treatment (method D) with 15% hydrogen bromide in acetic acid (25 ml) and re-precipitation from methanol with ether gave H-[Cys(BzlOMe)]₃-Gly₂-NH₂·HBr (4.24 g, 88%), which was coupled with *N*-acetylglucylglycine (1.13 g, 1.35 equiv.) by method A in DMF (65 ml) using isobutyl chloroformate (0.88 g, 1.35 equiv.). Recrystallisation from aqueous methanol gave *Ac-Gly₂-[Cys(BzlOMe)]₃-Gly₂-NH₂* (2.77 g, 60%), m.p. 216–218 °C, [α]_D²⁸ -21.5° (c 1.0 in Me₂SO); δ 8.4* (3 H, m, Cys-NH), 8.2* (4 H, m, Gly-NH), 7.25 (6 H, d, Ar-*m*-H), 7.15* and 7.05* (2 H, 2 s, CONH₂), 6.85 (6 H, d, Ar-*o*-H), 4.55 (3 H, m, Cys- α -CH), 3.8 (23 H, br m, Gly-CH₂, Ar-CH₂, OMe), 2.7 (6 H, m, Cys- β -CH₂), and 1.85 (3 H, s, COMe) (Found: C, 51.4; H, 5.6; N, 11.0. C₄₃H₅₆N₈O₁₁S₃·2.5H₂O requires C, 51.5; H, 6.1; N, 11.2%). Deprotection by method E1 for 120 min gave a 96% yield of *Ac-Gly₂-Cys₃-Gly₂-NH₂*; δ 8.25* (7 H, m, CONH), 7.12* and 7.30* (2 H, 2 s, CONH₂), 4.43 (3 H, m, Cys- α -CH), 3.7 (8 H, m, Gly-CH₂), 2.78 (6 H, m, Cys- β -CH₂), 2.4* (3 H, br, SH), and 1.82 (3 H, s, COMe).

Ac-Gly₂-Cys₄-Gly₂-NH₂ (2; *n* = 4).—Z-Cys(BzlOMe)-OH (from the dicyclohexylamine salt; 1.67 g, 3 mmol) and H-[Cys(BzlOMe)]₃-Gly₂-NH₂ (from the hydrobromide; 1.41 g, 1.6 mmol) were coupled by method A in DMF (60 ml) using isobutyl chloroformate (0.41 g, 3 mmol); recrystallisation from aqueous acetic acid gave Z-[Cys(BzlOMe)]₄-Gly₂-NH₂ (1.43 g, 77%), m.p. 183–185 °C, [α]_D²⁸ -33.7° (c 1.0 in Me₂SO) (Found: C, 57.8; H, 5.8; N, 8.2. C₅₆H₆₇N₇O₁₂S₄ requires C, 58.1; H, 5.8; N, 8.5%). Treatment of this (method D) with 22.5% hydrogen bromide in acetic acid (5.7 ml) and re-precipitation from methanol with ether gave H-[Cys(BzlOMe)]₄-Gly₂-NH₂·HBr (1.07 g, 79%), m.p. 204–206 °C, which was directly coupled by method A in DMF (80 ml) with *N*-acetylglucylglycine (0.28 g, 1.7 equiv.) using isobutyl chloroformate (0.23 g, 1.7 equiv.). Recrystallisation from aqueous acetic acid gave *Ac-Gly₂-[Cys(BzlOMe)]₄-Gly₂-NH₂* (0.73 g, 64%), m.p. 246–248 °C; δ 8.3* (4 H, m, Cys-NH), 8.05* (4 H, m, Gly-NH), 7.15 (8 H, d, Ar-*m*-H), 7.05* and 6.95* (2 H, 2 s, CONH₂), 6.75 (8 H, d, Ar-*o*-H), 4.5 (4 H, m, Cys- α -CH), 3.7 (28 H, br m, Gly-CH₂, Ar-CH₂, OMe), 2.6 (8 H, m, Cys- β -CH₂), 1.85 (3 H, s, COMe) (Found: C, 53.0; H, 5.6; N, 10.7. C₅₄H₆₉N₉O₁₃S₄·2H₂O requires C, 53.3; H, 6.1; N, 10.4%). Deprotection by method E1 for 150 min gave a 95% yield of *Ac-Gly₂-Cys₄-Gly₂-NH₂*; δ

8.25* (8 H, m, CONH), 7.14* and 7.32* (2 H, 2 s, CONH₂), 4.50 (4 H, m, Cys- α -CH), 3.7 (8 H, m, Gly-CH₂), 2.80 (8 H, m, Cys- β -CH₂), 2.4* (4 H, br, SH), and 1.85 (3 H, s, COMe).

Peptides of Series 3

2-Benzylthioethylamine.—Sodium metal (6 g, 0.26 mol) was added in small pieces to a solution of 2-mercaptoethylamine hydrochloride (14.8 g, 0.13 mol) in liquid ammonia (500 ml) until the blue colour persisted. The colour was discharged by the addition of a little ammonium chloride and benzyl chloride (15 ml, 0.13 mol) added dropwise to the clear solution. The ammonia was then allowed to evaporate through a soda-lime guard tube and the residue dissolved in a mixture of ether (100 ml) and water (100 ml). The ethereal layer was separated, dried, and acidified at 0 °C with anhydrous ethereal hydrogen chloride. The precipitate was collected by filtration and recrystallised from chloroform–light petroleum (b.p. 60–80 °C). The amine hydrochloride (24.8 g, 94%) formed needles, m.p. 117–118 °C (lit.¹² m.p. 119–120 °C); δ (CDCl₃) 8.34* (3 H, br, NH₃⁺), 7.32 (5 H, s, Ph), 3.72 (2 H, s, PhCH₂), 3.14 (2 H, t, S-CH₂-CH₂-N), and 2.80 (2 H, t, S-CH₂-CH₂-N) (Found: C, 52.8; H, 6.9; N, 6.8. Calc. for C₉H₁₄N₂S: C, 53.1; H, 6.9; N, 6.9%). When a 20% excess of benzyl chloride was used the product was contaminated with a water-insoluble impurity (19% yield), identified as *N*-benzyl-2-S-benzylthioethylamine hydrochloride, needles, m.p. 159–161 °C, from water or chloroform–light petroleum; δ (CDCl₃) 7.0–7.5 (br m, Ph-CH₂-N) and 7.16 (s, Ph-CH₂-S) (10 H in all), 3.95 (2 H, s, Ph-CH₂-N), 3.59 (2 H, s, Ph-CH₂-S), and 2.79 (4 H, s, S-CH₂-CH₂-N) (Found: C, 65.4; H, 6.6; N, 4.6. C₁₆H₂₀N₂S requires C, 65.4; H, 6.9; N, 4.8%. *m/e* 257; C₁₆H₁₉N₂S requires 257).

HS-CH₂-CH₂-CO-Gly₂-NH-CH₂-CH₂-SH (3; *n* = 0).—*p*-Nitrophenyl 3-benzylthiopropionate, (10),¹⁰ (4.0 g, 12.6 mmol) in DMF (70 ml) and ethanol (15 ml) was added to a stirred solution of glycylglycine (1.65 g, 12.6 mmol) and sodium hydrogencarbonate (1.06 g, 12.6 mmol) in water (40 ml). After stirring for 24 h the solution was washed successively with ethyl acetate (100 ml) and ether (100 ml), and acidified (pH 2.5) with 2M-hydrochloric acid. After 18 h at 4 °C, the crystals were collected, washed with water, dried, and recrystallised from aqueous ethanol; *BzIS*-CH₂-CH₂-CO-Gly₂-OH, (11) (3.70 g, 93%) had m.p. 164–165 °C (Found: C, 54.2; H, 5.9; N, 9.1. C₁₄H₁₈N₂O₄S requires C, 54.2; H, 5.8; N, 9.0%). This peptide (3.0 g, 9.68 mmol) was coupled by method B with 2-benzylthioethylamine (from the hydrochloride; 1.97 g, 9.68 mmol) in DMF (200 ml) using *NN'*-dicyclohexylcarbodi-imide (2.49 g, 12.1 mmol) and *N*-hydroxysuccinimide (2.22 g, 19.4 mmol). Precipitation with water followed by recrystallisation from ethanol gave *BzIS*-CH₂-CH₂-CO-Gly₂-NHCH₂CH₂-SBzl (12) (2.80 g, 64%), m.p. 171–172 °C; δ 8.35*, 8.2*, and 8.0* (3 H, 3 t, CONH), 7.4 (10 H, s, Ph), 3.8 (8 H, br m, Gly-CH₂, PhCH₂), 3.25 (2 H, m, NCH₂CH₂S), and 2.55 (6 H, m, other CH₂) (Found: C, 59.6; H, 6.3; N, 9.0. C₂₃H₂₉N₃O₃S₂ requires C, 60.1; H, 6.4; N, 9.1%). Deprotection by method E2 gave a 53% yield of *HSCH*₂CH₂CO-Gly₂-NHCH₂CH₂SH (3; *n* = 0); δ 8.3* and 7.8* (3 H, 2 m, CONH), 3.6 (4 H, t, Gly-CH₂), 3.25 (2 H, m, NCH₂CH₂S), 2.8* (2 H, m, SH), and 2.45 (6 H, m, other CH₂) (Found, corrected for 4% residue: C, 38.8; H, 6.1; N, 14.9. C₉H₁₇N₃O₃S₂ requires C, 38.7; H, 6.1; N, 15.0%).

HSCH₂CH₂CO-Gly₂-Cys-Gly₂-NHCH₂CH₂SH (3; *n* = 1).—*Z*-Cys(Bzl)-Gly₂-ONSu (7.42 g, 13.33 mmol) and 2-benzyl-

thioethylamine (from the hydrochloride; 2.95 g, 14.5 mmol) were coupled by method C1 in DMF (100 ml). Recrystallisation from methanol gave *Z*-Cys(Bzl)-Gly₂-NHCH₂CH₂-SBzl (7.14 g, 88%), m.p. 118–119 °C, $[\alpha]_D^{25}$ –28.0° (*c* 1.0 in COMe₂) (Found: C, 61.0; H, 5.9; N, 9.1. C₃₁H₃₆N₄O₅S₂ requires C, 61.2; H, 6.0; N, 9.2%) which was converted by method D, using 22.5% hydrogen bromide in acetic acid (42 ml), into *H*-Cys(Bzl)-Gly₂-NHCH₂CH₂-SBzl·HBr, (4.76 g; 73%) after recrystallisation from ethanol–ether, m.p. 163–164 °C, $[\alpha]_D^{25}$ +26.5° (*c* 2.0 in EtOH) (Found: C, 49.0; H, 5.6; N, 10.0. C₂₃H₃₁BrN₄O₃S₂ requires C, 49.7; H, 5.6; N, 10.1%).

BzIS-CH₂CH₂CO-Gly₂-OH was converted into its *N*-succinimidyl ester by method C in 90% yield, after recrystallisation from ethyl acetate. This ester (0.68 g, 1.67 mmol) and the above hydrobromide (1.0 g, 1.80 mmol) were coupled in DMF (50 ml) by method C1; recrystallisation from aqueous acetic acid gave *BzIS*-CH₂CH₂CO-Gly₂-Cys(Bzl)-Gly₂-NHCH₂CH₂-SBzl (14), (1.18 g, 92%), m.p. 219–220 °C, $[\alpha]_D^{30}$ –9.0° (*c* 1.0 in AcOH); δ 8.6–8.1* (6 H, m, CONH), 7.5 (15 H, s, Ph), 4.55 (1 H, m, Cys- α -CH), 3.75 (14 H, br m, Gly-CH₂, PhCH₂), 3.2 (2 H, m, NCH₂-CH₂S), and 2.55 (8 H, m, other CH₂) (Found: C, 57.9; H, 6.0; N, 11.1. C₃₇H₄₆N₆O₆S₃ requires C, 57.9; H, 6.0; N, 11.0%). Deprotection by method E2 gave a 64% yield of (3; *n* = 1), $[\alpha]_D^{27}$ –10.5° (*c* 1.0 in Me₂SO).

HSCH₂CH₂CO-Gly₂-Cys-Gly₂-Cys-Gly₂-NHCH₂CH₂SH (3; *n* = 2).—*Z*-Cys(Bzl)-Gly₂-ONSu (1.73 g, 3.12 mmol) and *H*-Cys(Bzl)-Gly₂-CH₂CH₂-SBzl (from the hydrobromide; 1.73 g, 3.12 mmol) were coupled in DMF (100 ml) by method C1; recrystallisation from ethanol gave *Z*-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₂-NHCH₂CH₂-SBzl (2.00 g, 70%), m.p. 186.5–187.5 °C, $[\alpha]_D^{27}$ –26.7° (*c* 1.0 in DMF) (Found: C, 58.8; H, 5.8; N, 10.7. C₄₅H₅₃N₇O₈S₃ requires C, 59.0; H, 5.8; N, 10.7%). Treatment with 11% hydrogen bromide in acetic acid (16 ml) (method D) gave, after re-precipitation from ethanol with ether, the hygroscopic *H*-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₂-NHCH₂CH₂-SBzl·HBr (1.65 g, 88%).

BzIS-CH₂CH₂CO-Gly₂-OH was converted by method C, using *p*-nitrophenol in place of *N*-hydroxysuccinimide, into *BzIS*-CH₂CH₂CO-Gly₂ONp in 50% yield after recrystallisation from ethanol, m.p. 152–153 °C (Found: C, 55.4; H, 4.9; N, 9.3. C₂₆H₂₁N₃O₆S requires C, 55.7; H, 4.9; N, 9.7%). This ester (225 mg, 0.52 mmol) was coupled in DMF (20 ml) by method C1 with the above hydrobromide (450 mg, 0.52 mmol); reprecipitation from acetic acid with water gave *BzIS*-CH₂CH₂CO-Gly₂-Cys(Bzl)-Gly₂-Cys(Bzl)-Gly₂-NHCH₂CH₂-SBzl (13) (380 mg, 64%), $[\alpha]_D^{26}$ –19.4° (*c* 1.0 in Me₂SO); δ 8.6–8.1* (9 H, m, CONH), 7.4 (20 H, s, Ph), 4.5 (2 H, m, Cys- α -CH), 3.75 (20 H, br m, Gly-CH₂, PhCH₂), 3.25 (2 H, m, NCH₂CH₂S), and 2.6 (10 H, m, other CH₂) (Found: C, 56.0; H, 5.9; N, 11.6. C₅₁H₆₃N₉O₉S₄·H₂O requires C, 56.0; H, 6.0; N, 11.5%). Deprotection by method E2 gave an 89% yield of (3; *n* = 2), $[\alpha]_D^{27}$ –17.7° (*c* 1.0 in Me₂SO).

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