

## Studies Relating to the Ferredoxins. Part 3.<sup>1</sup> The Synthesis of Some Cysteine–Glycine Peptides for Iron–Sulphur Complexing Studies

By Ambikaipakan Balasubramaniam, Roger J. Burt, George Christou, Brian Ridge, and H. N. Rydon,\*  
Department of Chemistry, The University, Exeter EX4 4QD

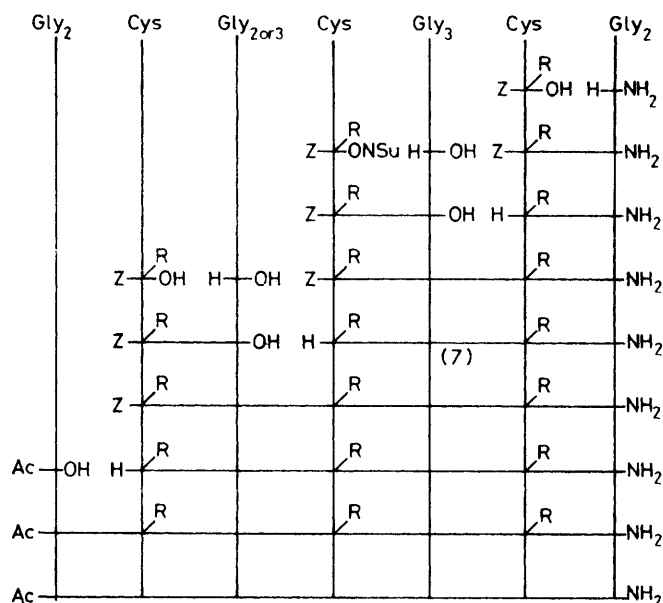
Three peptides with the general structure  $\text{Ac-Gly}_2\text{-(Cys-Gly)}_m\text{-Cys-Gly}_3\text{-Cys-Gly}_2\text{-NH}_2^\dagger$  ( $m = 2$  or  $3$ ;  $n = 0$  or  $1$ ), four with the general structure  $\text{Ac-Gly}_2\text{-Cys}_n\text{-Gly}_2\text{-NH}_2$  ( $n = 1\text{--}4$ ), and three with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

In Part 2 of this series<sup>1</sup> 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 ferredoxins<sup>2</sup> and as components of substitutes for ferredoxin as electron-transfer agents in the *Clostridium pasteurianum* hydrogenase system.<sup>3</sup> 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 In the first group one or two of the diglycyl spacing units between cysteine residues have been replaced by triglycyl units, to simulate more closely the  $\text{-Cys-X}_2\text{-Cys-X}_2\text{-Cys-X}_3\text{-Cys-}$  arrangement found in almost all known 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.<sup>4</sup> 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,<sup>1</sup> 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 fluoride<sup>5</sup> although retaining sufficient selectivity *vis-à-vis* *N*-benzyloxycarbonyl,<sup>6</sup> and gave substantially better overall yields. In addition to  $\text{Ac-Gly}_2\text{-OH}$ ,  $\text{H-Cys(Bzl)-}$

$\text{Gly}_2\text{-NH}_2$ , 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  $\text{Z-Cys(Bzl)-ONSu}$  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

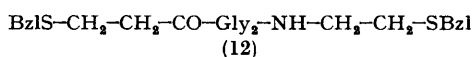
being ca. 80%.  $\text{Z-Cys(BzlOMe)-Gly}_2\text{-OH}$  and  $\text{Z-Cys(BzlOMe)-Gly}_3\text{-OH}$  were prepared similarly from  $\text{Z-Cys(BzlOMe)-ONSu}$  and  $\text{H-Gly}_2\text{-OH}$  or  $\text{H-Gly}_3\text{-OH}$  in 90 and 85% yield, respectively.  $\text{H-Cys(BzlOMe)-Gly}_2\text{-NH}_2$  was prepared in 80% overall yield by mixed carbonic anhydride coupling of  $\text{Z-Cys(BzlOMe)-OH}$  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  $\text{BzlOMe}$ ). 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,<sup>7</sup> except for the coupling of

<sup>†</sup> 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 =  $\text{PhCH}_2$ ; 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 =  $\text{PhCH}_2\text{OCO}$ ; DMF =  $\text{HCONMe}_2$ .



hexylcarbodi-imide-*N*-hydroxysuccinimide procedure<sup>7</sup> with 2-*S*-benzylthioethylamine to give the required protected peptide (12) in 60% overall yield. 2-*S*-



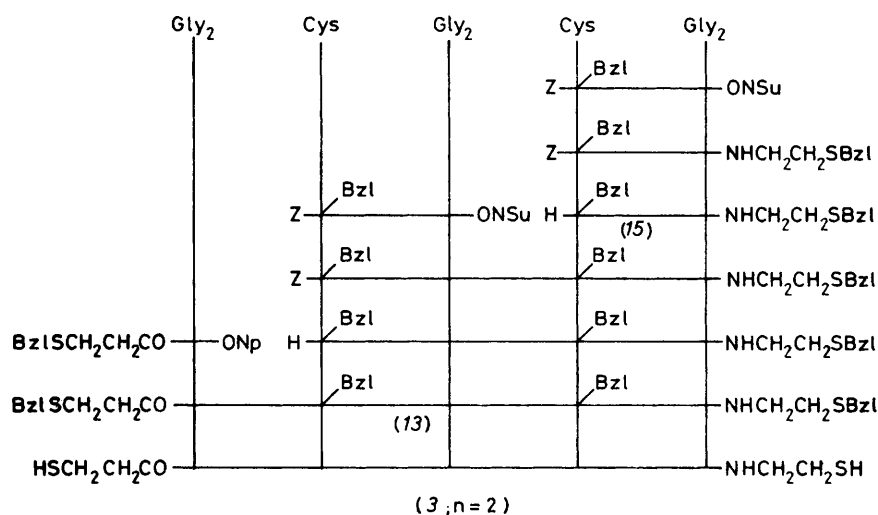
Benzylthioethylamine was conveniently prepared by benzylation of 2-mercaptoethylamine with 1 equiv. benzyl chloride and sodium in liquid ammonia; any excess of benzylating agent leads to the formation of the corresponding amount of the *NS*-dibenzyl derivative, with concomitant reduction in yield of the required compound. This method of preparation, which gives a 94% yield, is more convenient than the other methods described in the literature.<sup>11-13</sup> 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. <sup>1</sup>H N.m.r. spectra were recorded in (CD<sub>3</sub>)<sub>2</sub>SO, unless otherwise stated, at 31 °C on a JEOL MH-100 spectrometer at 100 MHz; chemical shifts (δ) are in p.p.m. from SiMe<sub>4</sub>; bands marked with an asterisk disappeared on treatment with D<sub>2</sub>O.

#### 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-CH<sub>2</sub>-CH<sub>2</sub>-CO-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH-CH<sub>2</sub>CH<sub>2</sub>-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 MgSO<sub>4</sub> 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 <sup>1</sup>H n.m.r. and i.r. spectroscopy. Many of the larger peptides

Me<sub>2</sub>SO 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 increased<sup>14</sup> 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*.<sup>15</sup> 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 –SCH<sub>2</sub>Ph 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 –SCH<sub>2</sub>Ph 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-Gly<sub>2</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub>* (4). *Route 1*.—*Z*-Cys(Bzl)-Gly<sub>2</sub>-ONSu<sup>1</sup> (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)-Gly<sub>3</sub>-OH (3.25 g, 79%), m.p. 200—201 °C, [α]<sub>D</sub><sup>22</sup> –28.2° (*c* 2.0 in DMF) (lit.<sup>16</sup> m.p. 201 °C, [α]<sub>D</sub><sup>18</sup> –28.1°); the same compound was prepared similarly in 84% yield from *Z*-Cys(Bzl)-ONSu<sup>17</sup> 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]<sub>D</sub><sup>25</sup> –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)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr<sup>1</sup> (3.64 g, 9 mmol); recrystallisation from aqueous acetic acid gave *Z*-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub> (4.50 g, 75%), m.p. 207—209 °C, [α]<sub>D</sub><sup>26</sup> –36.1° (*c* 1.0 in DMF) (Found: C, 55.6; H, 5.6; N, 13.8. C<sub>38</sub>H<sub>46</sub>N<sub>8</sub>O<sub>9</sub>S<sub>2</sub> 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)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr as a hygroscopic solid (400 mg, 87%), which without further purification, was coupled by method B, in DMF (20 ml), with *N*-acetylglycylglycine<sup>18</sup> (180 mg, 2 equiv.). Recrystallisation from Me<sub>2</sub>SO-methanol gave *Ac-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub>* (353 mg, 80%), [α]<sub>D</sub><sup>26</sup> –31.0° (*c* 1.0 in Me<sub>2</sub>SO); 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, CONH<sub>2</sub>), 4.55 (2 H, m, Cys-α-CH), 3.7 (18 H, br m, Gly-CH<sub>2</sub> and PhCH<sub>2</sub>), 2.7 (4 H, br m, Cys-β-CH<sub>2</sub>), and 1.85 (3 H, s, COMe) (Found: C, 50.4; H, 5.7; N, 15.7. C<sub>36</sub>H<sub>48</sub>N<sub>10</sub>O<sub>10</sub>S<sub>2</sub>·MeOH requires C, 50.7; H, 6.0; N, 16.0%). Deprotection by method E1 for 90 min gave *Ac-Gly<sub>2</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub>* (4), in 98% yield; δ 8.20\* (9 H, m, CONH), 7.12\* and 7.28\* (2 H, 2 s, CONH<sub>2</sub>), 4.44 (2 H, m, Cys-α-CH), 3.7 (14 H, m, Gly-CH<sub>2</sub>), 2.78 (4 H, m, Cys-β-CH<sub>2</sub>), 2.4\* (2 H, m, SH), and 1.84 (3 H, s, COMe).

*Route 2*. *Z*-Cys(BzlOMe)-OH was liberated from its dicyclohexylamine salt<sup>19</sup> (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]<sub>D</sub><sup>24</sup> –51.2° (*c* 1.0 in Me<sub>2</sub>SO) (Found: C, 58.6; H, 5.2; N, 5.7. C<sub>23</sub>H<sub>24</sub>N<sub>2</sub>O<sub>7</sub>S 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)-Gly<sub>3</sub>-OH (13.94 g, 85%), m.p. 171—172 °C,

$[\alpha]_D^{24} - 12.5^\circ$  ( $c$  0.5 in  $\text{Me}_2\text{SO}$ ) (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 hydrobromide<sup>20</sup> (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)-Gly<sub>2</sub>-NH<sub>2</sub> (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)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr, reprecipitated from ethanol with ether (3.5 g, 88%). This hydrobromide (6.36 g, 14.6 mmol) and Z-Cys(BzlOMe)-Gly<sub>3</sub>-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)-Gly<sub>3</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub> (11.35 g, 88%), m.p. 203–205°,  $[\alpha]_D^{25} - 27.7^\circ$  ( $c$  1.0 in  $\text{Me}_2\text{SO}$ ) (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)-Gly<sub>3</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub>·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  $\text{Me}_2\text{SO}$ –methanol gave Ac-Gly<sub>2</sub>-Cys(BzlOMe)-Gly<sub>3</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub> (1.14 g, 62%),  $[\alpha]_D^{25} - 19.0^\circ$  ( $c$  0.7 in  $\text{Me}_2\text{SO}$ );  $\delta$  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, CONH<sub>2</sub>), 6.85 (4 H, d, Ar-o-H), 4.5 (2 H, m, Cys- $\alpha$ -CH), 3.7 (24 H, br m, Gly-CH<sub>2</sub>, Ar-CH<sub>2</sub>, OMe), 2.7 (4 H, m, Cys- $\beta$ -CH<sub>2</sub>), 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-Gly<sub>2</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub> (4) in 90% yield;  $\delta$  8.20\* (9 H, m, CONH), 7.12\* and 7.30\* (2 H, 2 s, CONH<sub>2</sub>), 4.45 (2 H, m, Cys- $\alpha$ -CH), 3.75 (14 H, m, Gly-CH<sub>2</sub>), 2.78 (4 H, m, Cys- $\beta$ -CH<sub>2</sub>), 2.4\* (2 H, m, SH), 1.80 (3 H, s, COMe).

Ac-Gly<sub>2</sub>-Cys-Gly<sub>2</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub> (5). Route 1.—H-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (3.26 g, 4.2 mmol) and Z-Cys(Bzl)-Gly<sub>2</sub>-OH<sup>1</sup> (3.86 g, 8.4 mmol) were coupled by method B in DMF (168 ml); recrystallisation from  $\text{Me}_2\text{SO}$ –methanol gave Z-Cys(Bzl)-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub> (3.86 g, 81%),  $[\alpha]_D^{24} - 38.0^\circ$  ( $c$  1.0 in  $\text{Me}_2\text{SO}$ ) (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)-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (440 mg, 70%) which was coupled by method B with N-acetylglycylglycine (146 mg, 2 equiv.) in  $\text{Me}_2\text{SO}$ –hexamethylphosphoramide (1 : 1) (16 ml). Reprecipitation from  $\text{Me}_2\text{SO}$ –hexamethylphosphoramide (1 : 1) with methanol gave Ac-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub> (350 mg, 73%),  $[\alpha]_D^{25} - 22.8^\circ$  ( $c$  0.5 in  $\text{CF}_3\text{CO}_2\text{H}$ );  $\delta$  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, CONH<sub>2</sub>), 4.55 (3 H, m, Cys- $\alpha$ -CH), 3.75 (24 H, br m, Gly-CH<sub>2</sub> and Ph-CH<sub>2</sub>), 2.7 (6 H, br m, Cys- $\beta$ -CH<sub>2</sub>), 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-Gly<sub>2</sub>-Cys-Gly<sub>2</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub> (5) in 90% yield;  $\delta$  8.20\* (12 H, m, CONH), 7.12\* and 7.26\* (2 H, 2 s, CONH<sub>2</sub>), 4.44 (3 H, m, Cys- $\alpha$ -CH), 3.7 (18 H, m, Gly-CH<sub>2</sub>), 2.80 (6 H, m, Cys- $\beta$ -CH<sub>2</sub>), 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)-Gly<sub>2</sub>-OH (22.03 g, 90%), m.p. 103–105 °C  $[\alpha]_D^{24} - 23.7^\circ$  ( $c$  1.0 in  $\text{Me}_2\text{SO}$ ) (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)-Gly<sub>3</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (3.25 g, 4.0 mmol) were coupled by method B in DMF (70 ml); recrystallisation from  $\text{Me}_2\text{SO}$ –methanol gave Z-Cys(BzlOMe)-Gly<sub>2</sub>-Cys(BzlOMe)-Gly<sub>3</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub> (3.13 g, 68%),  $[\alpha]_D^{27} - 28.7^\circ$  ( $c$  0.6 in  $\text{Me}_2\text{SO}$ ) (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)-Gly<sub>2</sub>-Cys(BzlOMe)-Gly<sub>3</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub>·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  $\text{Me}_2\text{SO}$ –DMF (5 : 4) (9 ml) using isobutyl chloroformate (0.47 g, 3.4 mmol); recrystallisation from formic acid–ether gave Ac-Gly<sub>2</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-Cys(BzlOMe)-Gly<sub>3</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub> (1.00 g, 93%),  $[\alpha]_D^{25} - 25.1^\circ$  ( $c$  0.6 in  $\text{Me}_2\text{SO}$ );  $\delta$  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, CONH<sub>2</sub>), 6.9 (6 H, d, Ar-o-H), 4.5 (3 H, m, Cys- $\alpha$ -CH), 3.7 (33 H, br m, Gly-CH<sub>2</sub>, Ar-CH<sub>2</sub>, OMe), 2.7 (6 H, m, Cys- $\beta$ -CH<sub>2</sub>), 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-Gly<sub>2</sub>-Cys-Gly<sub>2</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub> (5);  $\delta$  8.20\* (12 H, m, CONH), 7.12\* and 7.24\* (2 H, 2 s, CONH<sub>2</sub>), 4.44 (3 H, m, Cys- $\alpha$ -CH), 3.75 (18 H, m, Gly-CH<sub>2</sub>), 2.66 (6 H, m, Cys- $\beta$ -CH<sub>2</sub>), 2.4\* (3 H, m, SH), and 1.84 (3 H, s, COMe).

Ac-Gly<sub>2</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub> (6).—Z-Cys(Bzl)-Gly<sub>3</sub>-OH (5.17 g, 10 mmol) and H-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (3.85 g, 5 mmol) were coupled by method B in DMF (200 ml); recrystallisation from  $\text{Me}_2\text{SO}$ –methanol gave Z-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub> (4.95 g, 83%),  $[\alpha]_D^{24} - 30.0^\circ$  ( $c$  1.0 in  $\text{Me}_2\text{SO}$ ) (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)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (0.96 g, 94%), which was directly coupled by method B with N-acetylglycylglycine (0.294 g, 2 equiv.) in  $\text{Me}_2\text{SO}$  (35 ml). Reprecipitation of the product from trifluoroacetic acid by pouring into cold water gave Ac-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub> (0.95 g, 91%),  $[\alpha]_D^{25} - 20.9^\circ$  ( $c$  0.5 in  $\text{CF}_3\text{CO}_2\text{H}$ );  $\delta$  (in  $\text{CF}_3\text{CO}_2\text{D}$ ) 7.4\* (15 H, br m, Cys-NH, Gly-NH, CONH<sub>2</sub>), 6.95 (15 H, s, Ph), 4.35 (3 H, m, Cys- $\alpha$ -CH), 3.85 (20 H, m, Gly-CH<sub>2</sub>), 3.45 (6 H, m, Ph-CH<sub>2</sub>), 2.55 (6 H, m, Cys- $\beta$ -CH<sub>2</sub>), 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-Gly<sub>2</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>3</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub> (6);  $\delta$  8.20\* (13 H, m, CONH),

7.14\* and 7.30\* (2 H, 2 s, CONH<sub>2</sub>), 4.48 (3 H, m, Cys- $\alpha$ -CH), 3.7 (20 H, m, Gly-CH<sub>2</sub>), 2.78 (6 H, m, Cys- $\beta$ -CH<sub>2</sub>), 2.4\* (3 H, m, SH), and 1.84 (3 H, s, COMe).

**Attempted Syntheses of Tetracysteine Peptides.**—(a) Z-Cys(Bzl)-Gly<sub>2</sub>-OH (138 mg, 0.3 mmol) and H-Cys(Bzl)-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (160 mg, 0.15 mmol) were coupled by method B in Me<sub>2</sub>SO-hexamethylphosphoramide (1 : 1) (6 ml); reprecipitation of the very insoluble product from hot Me<sub>2</sub>SO with methanol gave Z-Cys(Bzl)-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub> (164 mg, 76%) (Found: C, 54.1; H, 5.4; N, 13.2. C<sub>66</sub>H<sub>80</sub>N<sub>14</sub>O<sub>15</sub>S<sub>4</sub>·2CH<sub>3</sub>OH requires C, 54.4; H, 5.9; N, 13.1%).

(b) Z-Cys(BzlOMe)-Gly<sub>2</sub>-OH (2.65 g, 5.42 mmol) and H-Cys(BzlOMe)-Gly<sub>2</sub>-Cys(BzlOMe)-Gly<sub>3</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (1.58 g, 1.35 mmol) were coupled by method A in DMF-Me<sub>2</sub>SO (2 : 3) (25 ml) using isobutyl chloroformate (0.74 g, 5.42 mmol). Recrystallisation from formic acid-methanol gave Z-Cys(BzlOMe)-Gly<sub>2</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-Cys(BzlOMe)-Gly<sub>3</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub> (1.85 g, 88%), [ $\alpha$ ]<sub>D</sub><sup>25</sup> -29.8° (c 0.6 in Me<sub>2</sub>SO) (Found: C, 52.4; H, 5.5; N, 12.2. C<sub>70</sub>H<sub>88</sub>N<sub>14</sub>O<sub>19</sub>S<sub>4</sub>·2HCO<sub>2</sub>H requires C, 52.4; H, 5.6; N, 11.9%).

(c) Z-Cys(Bzl)-Gly<sub>3</sub>-OH (258 mg, 0.5 mmol) and H-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (284 mg, 0.25 mmol) were coupled by method B in Me<sub>2</sub>SO-hexamethylphosphoramide (3 : 5) (8 ml). Trituration of the very insoluble product with methanol, water, and methanol gave Z-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>3</sub>-Cys(Bzl)-Gly<sub>2</sub>-NH<sub>2</sub> (304 mg, 78%) (Found: C, 53.2; H, 5.6; N, 13.9. C<sub>70</sub>H<sub>88</sub>N<sub>16</sub>O<sub>17</sub>S<sub>4</sub>·H<sub>2</sub>O requires C, 53.5; H, 5.6; N, 14.3%).

#### Peptides of Series 2

*Ac-Gly<sub>2</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub>* (2; *n* = 1).—*N*-Acetylglycylglycine (1.74 g, 10 mmol) and H-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub>·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-Gly<sub>2</sub>-Cys(BzlOMe)-Gly<sub>2</sub>-NH<sub>2</sub>* (4.08 g, 80%), m.p. 182–183 °C, [ $\alpha$ ]<sub>D</sub><sup>26</sup> -18.1° (c 1.0 in MeOH);  $\delta$  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, CONH<sub>2</sub>), 6.8 (2 H, d, Ar-*o*-H), 4.45 (1 H, m, Cys- $\alpha$ -CH), 3.65 (13 H, br m, Gly-CH<sub>2</sub>, Ar-CH<sub>2</sub>, OMe), 2.65 (2 H, m, Cys- $\beta$ -CH<sub>2</sub>), and 1.85 (3 H, s, COMe) (Found: C, 48.4; H, 5.9; N, 15.8. C<sub>21</sub>H<sub>30</sub>N<sub>6</sub>O<sub>5</sub>S·CH<sub>3</sub>OH requires C, 48.7; H, 6.3; N, 15.5%). Deprotection by method E1 for 45 min gave an 89% yield of *Ac-Gly<sub>2</sub>-Cys-Gly<sub>2</sub>-NH<sub>2</sub>*;  $\delta$  8.20\* (5 H, m, CONH), 7.12\* and 7.27\* (2 H, 2s, CONH<sub>2</sub>), 4.40 (1 H, m, Cys- $\alpha$ -CH), 3.7 (8 H, m, Gly-CH<sub>2</sub>), 2.76 (2 H, m, Cys- $\beta$ -CH<sub>2</sub>), 2.4\* (1 H, br, SH), and 1.85 (3 H, s, OMe), identical with material prepared by another route.<sup>1</sup>

*Ac-Gly<sub>2</sub>-Cys<sub>2</sub>-Gly<sub>2</sub>-NH<sub>2</sub>* (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)-Gly<sub>2</sub>-NH<sub>2</sub>·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)]<sub>2</sub>-Gly<sub>2</sub>-NH<sub>2</sub> (1.82 g, 64%), m.p. 195–196 °C, [ $\alpha$ ]<sub>D</sub><sup>24</sup> -11.7° (c 1.0 in AcOH) (Found: C, 57.3; H, 5.9; N, 9.7. C<sub>34</sub>H<sub>41</sub>N<sub>5</sub>O<sub>8</sub>S<sub>2</sub> 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)]<sub>2</sub>-Gly<sub>2</sub>-NH<sub>2</sub>·HBr as a hygroscopic solid (1.54 g, 91%), which was coupled directly with *N*-acetylglycylglycine (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-Gly<sub>2</sub>-[Cys(BzlOMe)]<sub>2</sub>-Gly<sub>2</sub>-NH<sub>2</sub>* (1.30 g, 75%), m.p. 197–199 °C, [ $\alpha$ ]<sub>D</sub><sup>28</sup> -36.5° (c 1.0 in Me<sub>2</sub>SO);  $\delta$  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-NH<sub>2</sub>), 6.8 (4 H, d, Ar-*o*-H), 4.5 (2 H, m, Cys- $\alpha$ -CH), 3.7 (18 H, br m, Gly-CH<sub>2</sub>, Ar-CH<sub>2</sub>, OMe), 2.7 (4 H, m, Cys- $\beta$ -CH<sub>2</sub>), and 1.85 (3 H, s, COMe) (Found: C, 50.6; H, 5.7; N, 12.4. C<sub>32</sub>H<sub>43</sub>N<sub>7</sub>O<sub>9</sub>S<sub>2</sub>·1.5H<sub>2</sub>O requires C, 50.5; H, 6.1; N, 12.9%). Deprotection by method E1 for 90 min gave a 92% yield of *Ac-Gly<sub>2</sub>-Cys<sub>2</sub>-Gly<sub>2</sub>-NH<sub>2</sub>*;  $\delta$  8.20\* (6 H, m, CONH), 7.12\* and 7.28\* (2 H, 2 s, CONH<sub>2</sub>), 4.42 (2 H, m, Cys- $\alpha$ -CH), 3.7 (8 H, m, Gly-CH<sub>2</sub>), 2.76 (4 H, m, Cys- $\beta$ -CH<sub>2</sub>), 2.4\* (2 H, br, SH), and 1.82 (3 H, s, COMe).

*Ac-Gly<sub>2</sub>-Cys<sub>3</sub>-Gly<sub>2</sub>-NH<sub>2</sub>* (2; *n* = 3).—Z-Cys(BzlOMe)-OH (from the dicyclohexylamine salt; 5.01 g, 9 mmol) and H-[Cys(BzlOMe)]<sub>2</sub>-Gly<sub>2</sub>-NH<sub>2</sub> (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)]<sub>3</sub>-Gly<sub>2</sub>-NH<sub>2</sub> (5.09 g, 64%), m.p. 162–164 °C, [ $\alpha$ ]<sub>D</sub><sup>26</sup> -38.3° (c 1.0 in Me<sub>2</sub>SO) (Found: C, 57.7; H, 5.9; N, 8.7. C<sub>45</sub>H<sub>54</sub>N<sub>6</sub>O<sub>10</sub>S<sub>3</sub> 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)]<sub>3</sub>-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (4.24 g, 88%), which was coupled with *N*-acetylglycylglycine (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-Gly<sub>2</sub>-[Cys(BzlOMe)]<sub>3</sub>-Gly<sub>2</sub>-NH<sub>2</sub>* (2.77 g, 60%), m.p. 216–218 °C, [ $\alpha$ ]<sub>D</sub><sup>28</sup> -21.5° (c 1.0 in Me<sub>2</sub>SO);  $\delta$  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, CONH<sub>2</sub>), 6.85 (6 H, d, Ar-*o*-H), 4.55 (3 H, m, Cys- $\alpha$ -CH), 3.8 (23 H, br m, Gly-CH<sub>2</sub>, Ar-CH<sub>2</sub>, OMe), 2.7 (6 H, m, Cys- $\beta$ -CH<sub>2</sub>), and 1.85 (3 H, s, COMe) (Found: C, 51.4; H, 5.6; N, 11.0. C<sub>43</sub>H<sub>56</sub>N<sub>8</sub>O<sub>11</sub>S<sub>3</sub>·2.5H<sub>2</sub>O requires C, 51.5; H, 6.1; N, 11.2%). Deprotection by method E1 for 120 min gave a 96% yield of *Ac-Gly<sub>2</sub>-Cys<sub>3</sub>-Gly<sub>2</sub>-NH<sub>2</sub>*;  $\delta$  8.25\* (7 H, m, CONH), 7.12\* and 7.30\* (2 H, 2 s, CONH<sub>2</sub>), 4.43 (3 H, m, Cys- $\alpha$ -CH), 3.7 (8 H, m, Gly-CH<sub>2</sub>), 2.78 (6 H, m, Cys- $\beta$ -CH<sub>2</sub>), 2.4\* (3 H, br, SH), and 1.82 (3 H, s, COMe).

*Ac-Gly<sub>2</sub>-Cys<sub>4</sub>-Gly<sub>2</sub>-NH<sub>2</sub>* (2; *n* = 4).—Z-Cys(BzlOMe)-OH (from the dicyclohexylamine salt; 1.67 g, 3 mmol) and H-[Cys(BzlOMe)]<sub>3</sub>-Gly<sub>2</sub>-NH<sub>2</sub> (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)]<sub>4</sub>-Gly<sub>2</sub>-NH<sub>2</sub> (1.43 g, 77%), m.p. 183–185 °C, [ $\alpha$ ]<sub>D</sub><sup>28</sup> -33.7° (c 1.0 in Me<sub>2</sub>SO) (Found: C, 57.8; H, 5.8; N, 8.2. C<sub>56</sub>H<sub>67</sub>N<sub>7</sub>O<sub>12</sub>S<sub>4</sub> 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)]<sub>4</sub>-Gly<sub>2</sub>-NH<sub>2</sub>·HBr (1.07 g, 79%), m.p. 204–206 °C, which was directly coupled by method A in DMF (80 ml) with *N*-acetylglycylglycine (0.28 g, 1.7 equiv.) using isobutyl chloroformate (0.23 g, 1.7 equiv.). Recrystallisation from aqueous acetic acid gave *Ac-Gly<sub>2</sub>-[Cys(BzlOMe)]<sub>4</sub>-Gly<sub>2</sub>-NH<sub>2</sub>* (0.73 g, 64%), m.p. 246–248 °C;  $\delta$  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, CONH<sub>2</sub>), 6.75 (8 H, d, Ar-*o*-H), 4.5 (4 H, m, Cys- $\alpha$ -CH), 3.7 (28 H, br m, Gly-CH<sub>2</sub>, Ar-CH<sub>2</sub>, OMe), 2.6 (8 H, m, Cys- $\beta$ -CH<sub>2</sub>), 1.85 (3 H, s, COMe) (Found: C, 53.0; H, 5.6; N, 10.7. C<sub>54</sub>H<sub>69</sub>N<sub>9</sub>O<sub>13</sub>S<sub>4</sub>·2H<sub>2</sub>O requires C, 53.3; H, 6.1; N, 10.4%). Deprotection by method E1 for 150 min gave a 95% yield of *Ac-Gly<sub>2</sub>-Cys<sub>4</sub>-Gly<sub>2</sub>-NH<sub>2</sub>*;  $\delta$

8.25\* (8 H, m, CONH), 7.14\* and 7.32\* (2 H, 2 s, CONH<sub>2</sub>), 4.50 (4 H, m, Cys- $\alpha$ -CH), 3.7 (8 H, m, Gly-CH<sub>2</sub>), 2.80 (8 H, m, Cys- $\beta$ -CH<sub>2</sub>), 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.<sup>12</sup> m.p. 119–120 °C);  $\delta$  (CDCl<sub>3</sub>) 8.34\* (3 H, br, NH<sub>3</sub><sup>+</sup>), 7.32 (5 H, s, Ph), 3.72 (2 H, s, PhCH<sub>2</sub>), 3.14 (2 H, t, S-CH<sub>2</sub>-CH<sub>2</sub>-N), and 2.80 (2 H, t, S-CH<sub>2</sub>-CH<sub>2</sub>-N) (Found: C, 52.8; H, 6.9; N, 6.8. Calc. for C<sub>9</sub>H<sub>14</sub>N<sub>2</sub>S: 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;  $\delta$  (CDCl<sub>3</sub>) 7.0–7.5 (br m, Ph-CH<sub>2</sub>-N) and 7.16 (s, Ph-CH<sub>2</sub>-S) (10 H in all), 3.95 (2 H, s, Ph-CH<sub>2</sub>-N), 3.59 (2 H, s, Ph-CH<sub>2</sub>-S), and 2.79 (4 H, s, S-CH<sub>2</sub>-CH<sub>2</sub>-N) (Found: C, 65.4; H, 6.6; N, 4.6. C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>S requires C, 65.4; H, 6.9; N, 4.8%. *m/e* 257; C<sub>16</sub>H<sub>19</sub>N<sub>2</sub>S requires 257).

**HS-CH<sub>2</sub>-CH<sub>2</sub>-CO-Gly<sub>2</sub>-NH-CH<sub>2</sub>-CH<sub>2</sub>-SH** (3; *n* = 0).—*p*-Nitrophenyl 3-benzylthiopropionate, (10),<sup>10</sup> (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*-CH<sub>2</sub>-CH<sub>2</sub>-CO-Gly<sub>2</sub>-OH, (11) (3.70 g, 93%) had m.p. 164–165 °C (Found: C, 54.2; H, 5.9; N, 9.1. C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S 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*-CH<sub>2</sub>-CH<sub>2</sub>-CO-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>-SBzl (12) (2.80 g, 64%), m.p. 171–172 °C;  $\delta$  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-CH<sub>2</sub>, PhCH<sub>2</sub>), 3.25 (2 H, m, NCH<sub>2</sub>CH<sub>2</sub>S), and 2.55 (6 H, m, other CH<sub>2</sub>) (Found: C, 59.6; H, 6.3; N, 9.0. C<sub>23</sub>H<sub>29</sub>N<sub>3</sub>O<sub>3</sub>S<sub>2</sub> requires C, 60.1; H, 6.4; N, 9.1%). Deprotection by method E2 gave a 53% yield of *HSCH*<sub>2</sub>CH<sub>2</sub>CO-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>SH (3; *n* = 0);  $\delta$  8.3\* and 7.8\* (3 H, 2 m, CONH), 3.6 (4 H, t, Gly-CH<sub>2</sub>), 3.25 (2 H, m, NCH<sub>2</sub>CH<sub>2</sub>S), 2.8\* (2 H, m, SH), and 2.45 (6 H, m, other CH<sub>2</sub>) (Found, corrected for 4% residue: C, 38.8; H, 6.1; N, 14.9. C<sub>9</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>S<sub>2</sub> requires C, 38.7; H, 6.1; N, 15.0%).

**HSCH<sub>2</sub>CH<sub>2</sub>CO-Gly<sub>2</sub>-Cys-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>SH** (3; *n* = 1).—*Z*-Cys(Bzl)-Gly<sub>2</sub>-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)-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>-SBzl (7.14 g, 88%), m.p. 118–119 °C,  $[\alpha]_D^{25}$  –28.0° (*c* 1.0 in COMe<sub>2</sub>) (Found: C, 61.0; H, 5.9; N, 9.1. C<sub>31</sub>H<sub>36</sub>N<sub>4</sub>O<sub>5</sub>S<sub>2</sub> 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)-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>-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. C<sub>23</sub>H<sub>31</sub>BrN<sub>4</sub>O<sub>3</sub>S<sub>2</sub> requires C, 49.7; H, 5.6; N, 10.1%).

*BzIS*-CH<sub>2</sub>CH<sub>2</sub>CO-Gly<sub>2</sub>-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*-CH<sub>2</sub>CH<sub>2</sub>CO-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>-SBzl (14), (1.18 g, 92%), m.p. 219–220 °C,  $[\alpha]_D^{30}$  –9.0° (*c* 1.0 in AcOH);  $\delta$  8.6–8.1\* (6 H, m, CONH), 7.5 (15 H, s, Ph), 4.55 (1 H, m, Cys- $\alpha$ -CH), 3.75 (14 H, br m, Gly-CH<sub>2</sub>, PhCH<sub>2</sub>), 3.2 (2 H, m, NCH<sub>2</sub>-CH<sub>2</sub>S), and 2.55 (8 H, m, other CH<sub>2</sub>) (Found: C, 57.9; H, 6.0; N, 11.1. C<sub>37</sub>H<sub>46</sub>N<sub>6</sub>O<sub>6</sub>S<sub>3</sub> 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 Me<sub>2</sub>SO).

**HSCH<sub>2</sub>CH<sub>2</sub>CO-Gly<sub>2</sub>-Cys-Gly<sub>2</sub>-Cys-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>SH** (3; *n* = 2).—*Z*-Cys(Bzl)-Gly<sub>2</sub>-ONSu (1.73 g, 3.12 mmol) and *H*-Cys(Bzl)-Gly<sub>2</sub>-CH<sub>2</sub>CH<sub>2</sub>-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)-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>-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. C<sub>45</sub>H<sub>53</sub>N<sub>7</sub>O<sub>8</sub>S<sub>3</sub> 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)-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>-SBzl·HBr (1.65 g, 88%).

*BzIS*-CH<sub>2</sub>CH<sub>2</sub>CO-Gly<sub>2</sub>-OH was converted by method C, using *p*-nitrophenol in place of *N*-hydroxysuccinimide, into *BzIS*-CH<sub>2</sub>CH<sub>2</sub>CO-Gly<sub>2</sub>ONp in 50% yield after recrystallisation from ethanol, m.p. 152–153 °C (Found: C, 55.4; H, 4.9; N, 9.3. C<sub>26</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>S 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*-CH<sub>2</sub>CH<sub>2</sub>CO-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>2</sub>-Cys(Bzl)-Gly<sub>2</sub>-NHCH<sub>2</sub>CH<sub>2</sub>-SBzl (13) (380 mg, 64%),  $[\alpha]_D^{26}$  –19.4° (*c* 1.0 in Me<sub>2</sub>SO);  $\delta$  8.6–8.1\* (9 H, m, CONH), 7.4 (20 H, s, Ph), 4.5 (2 H, m, Cys- $\alpha$ -CH), 3.75 (20 H, br m, Gly-CH<sub>2</sub>, PhCH<sub>2</sub>), 3.25 (2 H, m, NCH<sub>2</sub>CH<sub>2</sub>S), and 2.6 (10 H, m, other CH<sub>2</sub>) (Found: C, 56.0; H, 5.9; N, 11.6. C<sub>51</sub>H<sub>63</sub>N<sub>9</sub>O<sub>9</sub>S<sub>4</sub>·H<sub>2</sub>O 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 Me<sub>2</sub>SO).

We thank the S.R.C. (R. J. B. and G. C.) and Bristol Myers Company, International Division, and the University of Exeter (A. B.) for the award of Research Studentships.

[0/409 Received, 13th March, 1980]

#### REFERENCES

- Part 2, R. J. Burt, B. Ridge, and H. N. Rydon, *J.C.S. Dalton*, 1980, 1228.
- G. Christou, B. Ridge, and H. N. Rydon, *J.C.S. Chem. Comm.*, 1977, 908.

- <sup>3</sup> M. W. W. Adams, S. G. Reeves, D. O. Hall, G. Christou, B. Ridge, and H. N. Rydon, *Biochem. Biophys. Res. Comm.*, 1977, **79**, 1184; A. Balasubramaniam, Ph.D. Thesis, Exeter, 1979.
- <sup>4</sup> L. R. Croft, 'Handbook of Protein Sequences,' Joynson-Bruvvers, Oxford, 1973, pp. 66—67; Supplement A, 1974, p. 34a; Supplement B, 1976, p. 28b.
- <sup>5</sup> S. Sakakibara, Y. Shimonishi, M. Okada, and Y. Kishida, 'Peptides: Proceedings Eighth European Peptide Symposium,' eds. H. C. Beyermann, A. van de Linde, W. Muassen van der Brink, North Holland, Amsterdam, 1967, p. 44.
- <sup>6</sup> S. Sakakibara, Y. Nobuhara, Y. Shimonishi, and R. Kiyoi, *Bull. Chem. Soc. Japan*, 1965, **38**, 120.
- <sup>7</sup> F. Weygand, D. Hoffmann, and E. Wünsch, *Z. Naturforsch.*, 1966, **21b**, 426; E. Wünsch and F. Drees, *Chem. Ber.*, 1966, **99**, 110.
- <sup>8</sup> J. R. Vaughan and R. L. Osato, *J. Amer. Chem. Soc.*, 1952, **74**, 676.
- <sup>9</sup> S. Sakakibara in 'Chemistry and Biochemistry of Amino acids, Peptides and Proteins,' ed. B. Weinstein, Marcel-Dekker, New York, 1971, vol. 1, p. 51.
- <sup>10</sup> D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, 1962, **237**, 1563.
- <sup>11</sup> E. Walton, A. N. Wilson, F. W. Holly, and K. Folkers, *J. Amer. Chem. Soc.*, 1954, **76**, 1146; cf. W. Gruber and F. Lynen, *Annalen*, 1962, **659**, 139.
- <sup>12</sup> J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 1952, 800; cf. F. I. Carroll, H. M. Dickson, and M. E. Wall, *J. Org. Chem.*, 1965, **30**, 33.
- <sup>13</sup> J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, 1961, **83**, 663; cf. S.-H. Chu and H. G. Mautner, *J. Org. Chem.*, 1961, **26**, 4498.
- <sup>14</sup> M. A. Tilak, *Tetrahedron Letters*, 1970, 849; H. C. Beyerman in 'Chemistry and Biology of Peptides,' ed. J. Meienhofer, Ann Arbor Science Publishers, Ann Arbor, U.S.A., 1972, p. 351.
- <sup>15</sup> Cf. G. P. Schwartz and P. G. Katsoyannis, *J.C.S. Perkin I*, 1973, 2890.
- <sup>16</sup> P. M. Hardy, B. Ridge, H. N. Rydon, and F. O. dos S. P. Serrao, *J. Chem. Soc. (C)*, 1971, 1722.
- <sup>17</sup> J. Kovacs, G. L. Mayers, R. H. Johnson, R. E. Cover, and U. R. Ghatak, *J. Org. Chem.*, 1970, **35**, 1810.
- <sup>18</sup> A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, 1943, **37**, 79; cf. R. E. Steiger, *Helv. Chim. Acta*, 1934, **17**, 563.
- <sup>19</sup> S. Akabori, S. Sakakibara, Y. Shimonishi, and Y. Nobuhara, *Bull. Chem. Soc. Japan*, 1964, **37**, 433.
- <sup>20</sup> J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, 1942, **145**, 253.