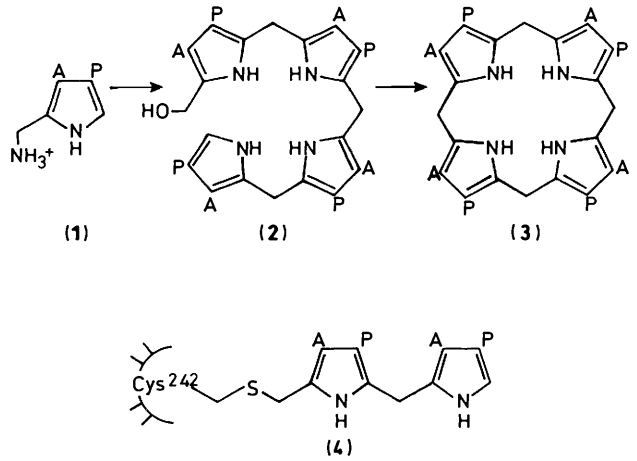
Biosynthesis of Porphyrins and Related Macrocycles. Part 34.[1] Synthesis and Properties of *S*-Pyrrolylmethylcysteinyl and ε-*N*-Pyrrolylmethyllysyl Peptides

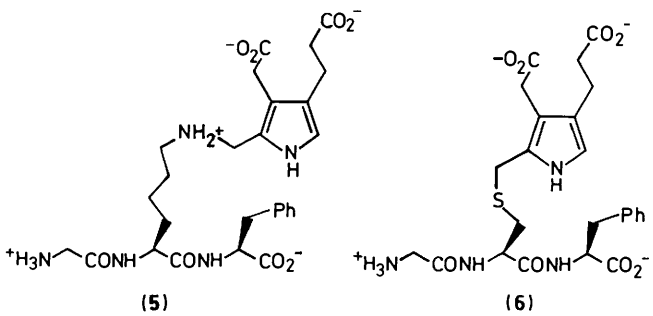
Andrew D. Miller, Finian J. Leeper, and Alan R. Battersby *
University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW

Syntheses are described of two compounds having a pyrrolylmethyl group attached respectively to the sulphur of cysteine (**6**) and the ε-nitrogen of lysine (**5**). These compounds were built to act as model systems for two possible ways in which pyrrolylmethyl groups could be bound to the protein of the enzyme hydroxymethylbilane synthase (HMBS), also known as porphobilinogen deaminase. We show how results from these studies were important in the discovery and characterisation of the novel pyrromethane cofactor (**4**) of HMBS.

Hydroxymethylbilane synthase (HMBS), EC 4.3.1.8 [also known as porphobilinogen (PBG) deaminase], together with uroporphyrinogen III synthase, EC 4.2.1.75, catalyse the conversion of PBG (**1**) into uroporphyrinogen III (**3**) the precursor for haem, chlorophyll, and vitamin B_{12}.[2] HMBS is an assembly enzyme which constructs hydroxymethylbilane (HMB) (**2**) from PBG (**1**)[3] and binds the growing oligopyrrolic chain covalently through a group X.[4,5] Initially, lysine or cysteine were thought to be possible candidates for the point of covalent attachment.[5,6] Consequently we undertook the synthesis of model pyrrolylmethyl tri-peptides (**5**) and (**6**) to investigate their spectral and physical properties. The results from this work were later to play an important role in the studies leading to the discovery[7] that the first molecule of PBG becomes covalently bound to a novel pyrromethane cofactor (**4**) in the enzymic active site. This cofactor (**4**) has been proved to be linked to the protein through the sulphur atom[8] of cysteine-242.[9] It was later reported that the cofactor had been identified independently[10] and confirmation of its attachment at cysteine-242 has come from yet more recent papers.[11]



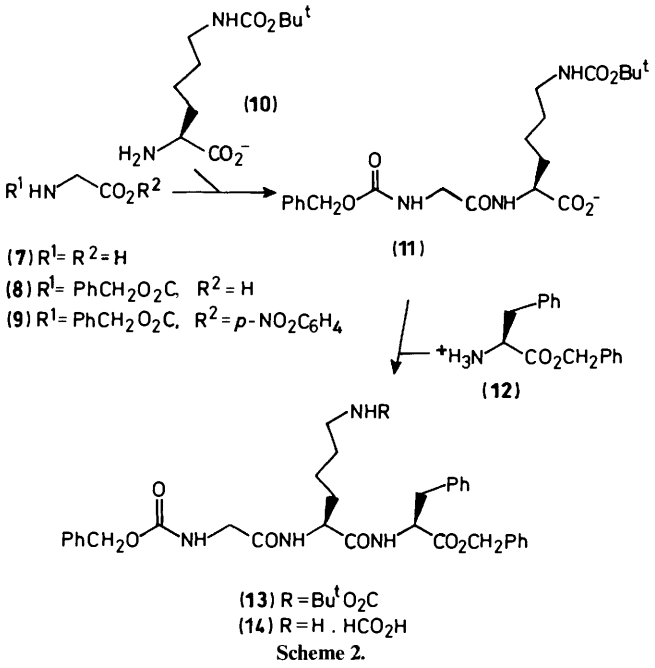
Scheme 1. A = CH_2CO_2H, P = $CH_2CH_2CO_2H$



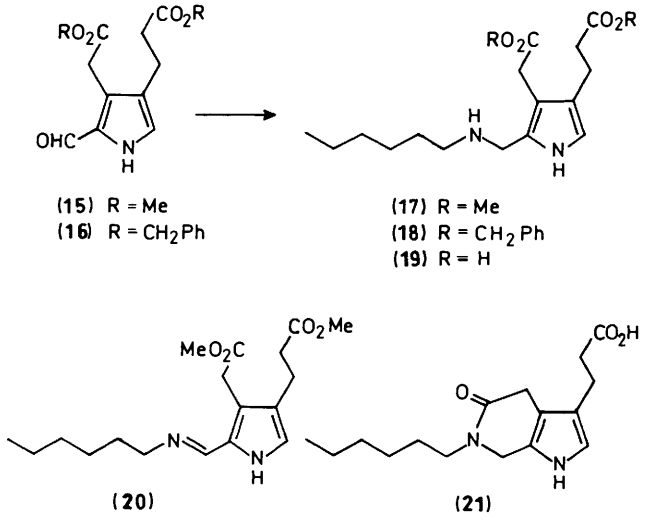
Discussion

Synthesis of the ε-N-*Pyrrolylmethyl-lysyl Peptide* (**5**).—The plan was to synthesise a lysyl tripeptide (**14**) (Scheme 2) and attach a pyrrole to the ε-nitrogen of lysine by forming an imine with the known[12] formylpyrrole (**15**) (Scheme 3) followed by reduction. Removal of the protecting groups would then give the desired product (**5**). It has been customary to synthesize peptides working from the *C* to the *N* termini to minimise racemisation during the coupling reactions arising from the azlactone mechanism.[13] However attempts to synthesize (**14**) were troubled by facile dioxopiperazine formation at the dipeptide stage. In consequence a less orthodox synthesis (*N* to *C* direction) was developed (Scheme 2) using minimal protection to maximise the final yield of (**14**). Glycine (**7**) was converted[14] into its *N*-benzyloxycarbonyl derivative (**8**) which, as its *p*-nitrophenyl ester[15] (**9**), was coupled with ε-*N*-t-butoxycarbonyl-L-lysine (**10**) giving the dipeptide acid (**11**) in 99% yield. Coupling of (**11**) with phenylalanine benzyl ester hydrochloride (**12**), using the reagents of König and Geiger,[16,17] gave the fully protected lysyl tripeptide (**13**) with essentially no racemisation in 76% yield. Treatment of (**13**) with 98% formic acid resulted in selective removal[18] of the t-butoxycarbonyl group and gave the desired lysyl tripeptide (**14**) as its formate salt.

Before attempting to synthesize the peptide (**5**), model studies were carried out with hexylamine to determine the best way to make aminomethylpyrroles by the imine strategy. A typical reductive amination procedure[19] with the aldehyde (**15**), hexylamine, and sodium cyanoborohydride resulted in a very low yield of the desired product (**17**) (Scheme 3), presumably because of the poor electrophilicity of the formyl group (vinylogous amide). Hence the imine was preformed by heating the formylpyrrole (**15**) and 1 mol equiv. of hexylamine in tetrahydrofuran in the presence of pre-activated 3 Å-molecular sieves.[20] The aldimine (**20**) was reduced by sodium borohydride[21] to give the almost pure amine (**17**). However, attempted saponification of the methyl esters of (**17**) gave not the desired di-acid (**19**) but the lactam (**21**). Accordingly, the dibenzyl ester (**16**) was synthesized from the dimethyl ester (**15**) by transesterification using sodium benzyl oxide in benzyl



Scheme 2.

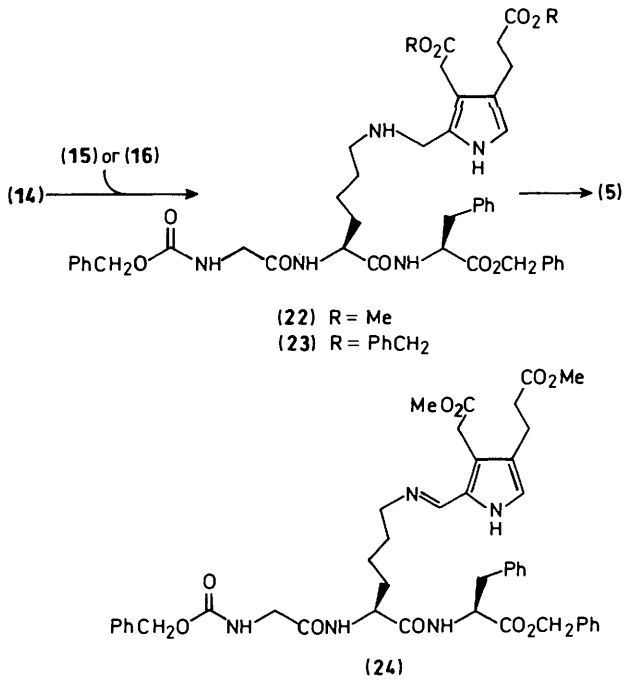


Scheme 3.

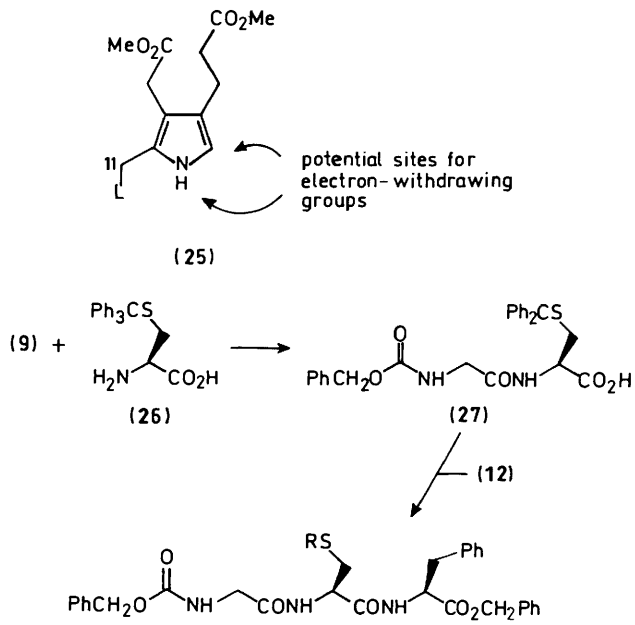
alcohol. This pyrrole (**16**) was converted as above into the aminomethylpyrrole (**18**) which, by catalytic transfer hydrogenolysis,[22] gave the diacid (**19**) in 98% yield as its monoformate salt.

Application of this chemistry to the synthesis of the peptide (**5**) (Scheme 4) proved more difficult than had been anticipated because of solubility problems. However, by condensation of the tripeptide (**14**) with the formylpyrrole dimethyl ester (**15**), using dimethylformamide as co-solvent and triethylamine, an imine (**24**) was formed. Reduction of the imine (**24**) as before gave the crude product (**22**) which was isolated in only 36% yield due to decomposition during purification. The tri-benzyl ester (**23**) was synthesized similarly in 45% yield after rapid purification [56% yield based upon unrecovered formylpyrrole (**16**)]. Both the *N*-benzyloxycarbonyl and benzyl ester protecting groups were removed by hydrogenolysis, as above, followed by a short saponification step and reverse-phase chromatography to give the lysyl peptide (**5**) in 43% yield.

Synthesis of the S-*Pyrrolylmethylcysteinyl Peptide* (**6**).—The



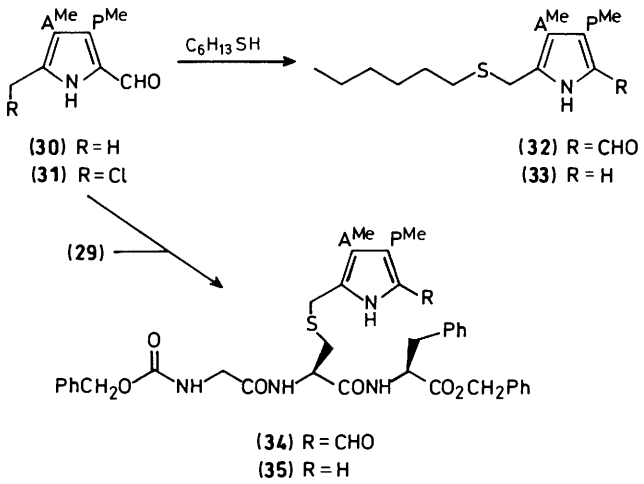
Scheme 4.



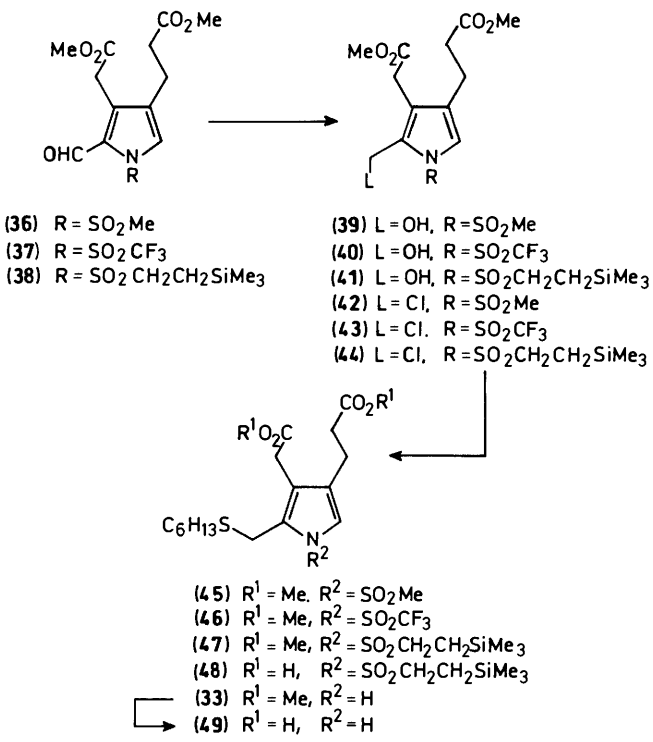
Scheme 5.

plan was to use the sulphur atom of a cysteinyl peptide to displace a suitable leaving group from the pyrrole (**25**). However pyrroles of this type are extremely reactive[23] and polymerise in solution; hence it was necessary to deactivate the pyrrole using a subsequently removable electron-withdrawing group either at the α-position or on the nitrogen. The approach involving deactivation by an α-formyl group was explored first.

Synthesis of the tripeptide (**29**) was accomplished as shown in Scheme 5. The active ester (**9**) reacted with *S*-trityl-L-cysteine (**26**) to give the dipeptide acid (**27**) in 99% yield which was coupled with phenylalanine benzyl ester hydrochloride (**12**) as



Scheme 6. $A^{Me} = CH_2CO_2Me$, $P^{Me} = CH_2CH_2CO_2Me$



Scheme 7.

above to give the protected cysteinyl tripeptide (**28**) in 79% yield. Finally, selective removal of the *S*-trityl group was accomplished[24] with silver(I) nitrate followed by hydrochloric acid to give the desired tripeptide (**29**). In the crystalline state, this peptide proved stable to oxidation for up to several months.

Model studies were carried out using hexanethiol, which coupled with the chloromethylpyrrole (**31**) in the presence of Hünig's base to give the sulphide (**32**); the pyrrole (**31**) was made by treating the methylpyrrole (**30**) with t-butyl hypochlorite.[25] The formyl group was removed with a stoicheiometric amount of Wilkinson's catalyst[26] to yield the sulphide (**33**), which was a very sensitive compound and decomposed even on storage under argon at low temperatures.

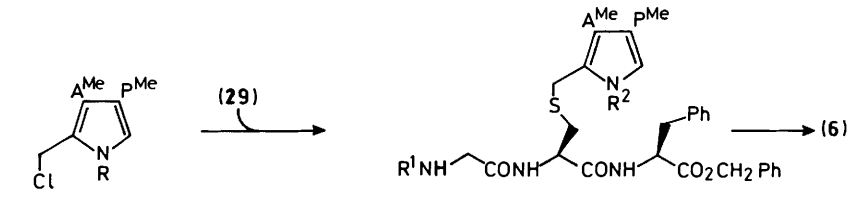
The foregoing displacement reaction was found to work well with the tripeptide (**29**) to give the pyrrolylpeptide (**34**) in good yield. However, final removal of the formyl group, under conditions used for the model compound (**32**), resulted in rapid decomposition of the product and the optimised yield was only 4%. Clearly the electron-withdrawing group needs to be removable under very mild conditions, see Scheme 6.

Accordingly, studies were made of *N*-sulphonylpyrroles, *e.g.* the *N*-mesylchloromethylpyrrole (**42**) (Scheme 7). The formylpyrrole (**15**) was *N*-mesylated[27] by successive treatment with an excess of sodium hydride and mesyl chloride to give the pyrrole (**36**) which was reduced with sodium borohydride to the alcohol (**39**). This was transformed into the chloromethylpyrrole (**42**) by treatment with mesyl chloride and triethylamine;[28] it proved unstable to chromatography and was therefore used directly without purification. Hexanethiol alone was unable to displace chloride from (**42**) but in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) the desired sulphide (**45**) was formed in 69% yield. Slow removal of the *N*-mesyl group with sodium methoxide to give the sulphide (**33**) occurred at 50 °C, but the lability of the product (**33**) resulted in many by-products. It was reasoned that exchanging the *N*-mesyl group for the *N*-trifluoromethanesulphonyl (triflyl) group should increase the rate of methanolysis.

Accordingly the *N*-triflylpyrrole (**37**) was synthesized from the formylpyrrole (**15**) using trifluoromethanesulphonic anhydride in the presence of Hünig's base. Yields were always low (*ca.* 33%) and extensive experimentation failed to improve this reaction. The sulphide (**46**) was then synthesized essentially as described above (Scheme 7) and upon treatment with sodium methoxide was cleanly converted into the deprotected sulphide (**33**). Using these procedures, the tripeptide (**29**) was coupled with the *N*-mesyl- and *N*-triflyl-chloromethylpyrroles (**42**) and (**43**) to give the pyrrolylpeptides (**50**) and (**51**) in good yields (Scheme 8). Deprotection of the pyrrolylpeptide (**51**) to give (**6**), however, proved impossible. Boron tribromide[29] or sodium in liquid ammonia[30] completely destroyed the starting material and whilst trimethylsilyl (TMS) iodide[31] removed the benzyloxycarbonyl and benzyl ester groups of the pyrrolyl peptide (**51**), subsequent methanolysis of the *N*-triflyl group with sodium methoxide proved prohibitively slow and many by-products were formed; this approach to (**6**) was therefore abandoned.

This *impasse* was overcome by use of the trimethylsilylethylsulphonyl (SES) group.[32] Reaction of the formylpyrrole (**15**) with SES chloride converted it into (**38**), using the reaction conditions developed earlier for *N*-mesylation. The alcohol (**41**) was then obtained from (**38**) by reduction with sodium borohydride, buffered[33] to prevent methanolysis of the *N*-protecting group, and following formation of chloromethylpyrrole (**44**), the *N*-SES sulphide (**47**) was obtained by displacement of chloride by hexanethiol. Virtually instantaneous removal of the *N*-protecting group from pyrrole (**47**) by tetrabutyl ammonium fluoride (TBAF)[32] was observed, giving the labile thiomethylpyrrole (**33**) in 39% yield. Hydrolysis of the methyl ester groups of (**33**) in aqueous methanolic potassium hydroxide yielded the sulphide (**49**) which was stable under the reaction conditions for up to 48 h. Interestingly, hydrolysis of the methyl esters of (**47**) to give the dianion of (**48**) made subsequent removal of the *N*-SES group extremely difficult. Thus, successful removal of the *N*-SES group depended upon the acetate and propionate side chains of the pyrrole being uncharged, *i.e.* esterified.

Building on this experience, the cysteinyl tripeptide (**29**) and the chloromethyl pyrrole (**44**) were combined (Scheme 8) under the usual reaction conditions to give the protected *N*-SES cysteinyl pyrrolyl peptide (**52**) in 75% yield. In this case, TMS iodide failed to remove the benzyloxycarbonyl and benzyl ester protecting groups of (**52**) without causing extensive hydrolysis of the methyl esters. Hydrolysis of these ester groups on the acetate and propionate side chains of the pyrrole was found to prevent removal of the *N*-SES group, as observed in the model



Scheme 8.

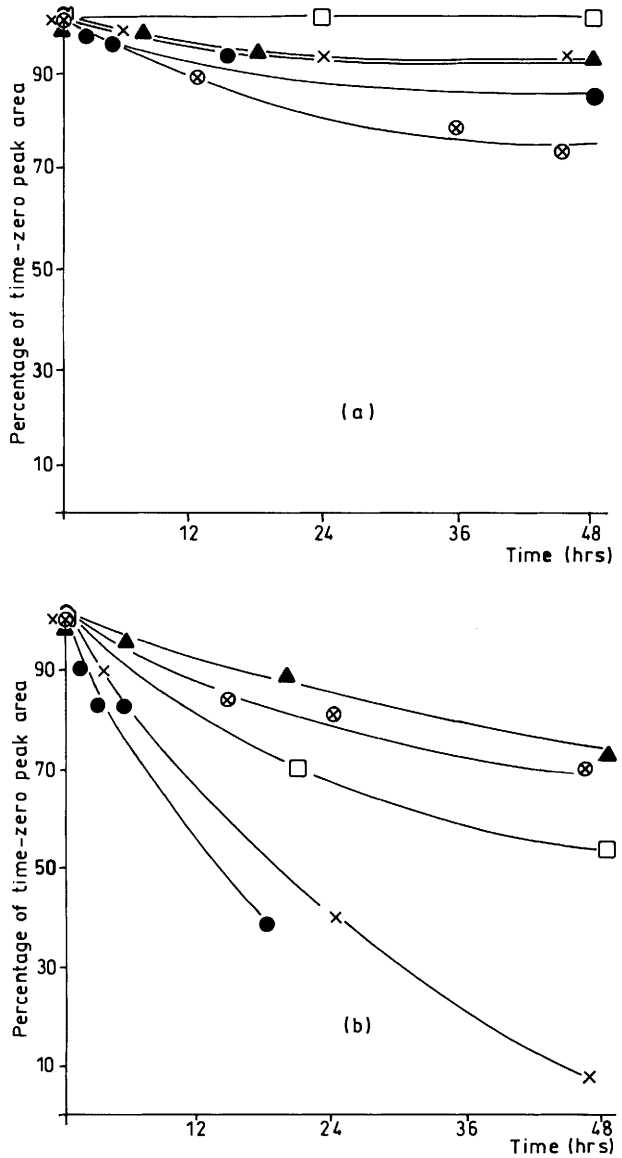


Figure. (*a*) Effect of pH on the stability of the lysyl pyrrolylpeptide (**5**). Percentage of residual pyrrolyl peptide is plotted versus time; additional details given in experimental section. The key to the conditions is as follows: □ = pH 12, ▲ = pH 8, × = pH 5, ● = pH 1, all at ambient temperature, and ⊗ = pH 8 at 37 °C. (*b*) Effect of pH on the stability of cysteinyl pyrrolylpeptide (**6**); symbols as above.

series. However, by using a combination of sodium iodide and TMS chloride,[34] chemoselective removal of the benzyloxycarbonyl protecting group was possible. The *N*-SES group was then removed from the purified pyrrolyl peptide (**53**) with TBAF to give the pyrrolylpeptide triester (**54**).

Hydrolysis of the ester groups of (**54**) in aqueous methanolic potassium hydroxide gave the desired cysteinyl tripeptide (**6**) in 18% overall yield from (**52**) after final purification using preparative reverse phase h.p.l.c. The smooth removal of the *N*-SES group from pyrrole (**47**) and pyrrolyl peptide (**53**) highlights the synthetic potential of the *N*-SES protecting group in pyrrole chemistry, especially when attempted deprotection of pyrroles lacking electron-withdrawing groups can often cause difficulties.[35]

Properties of the Pyrrolyl Peptides.—^{13}C N.m.r. experiments were central to our investigations[7,8] on the pyrromethane cofactor of HMBS. The model pyrrolyl peptides (**5**) and (**6**) have been essential both for determining suitable pH conditions for n.m.r. experiments on the intact enzyme and as a source of accurate reference data on chemical shifts.

The stability of the model compounds in aqueous solution at different pH values was assessed by taking fixed aliquots from standard solutions of both pyrrolyl peptides and diluting into a set of buffers covering the pH range 1—12. These solutions were kept for up to 43 h during which time fixed aliquots were removed and analysed by reverse-phase h.p.l.c. The percentage of residual pyrrolyl peptide was plotted *versus* time (Figures 1a and 1b). The lysyl pyrrolylpeptide (**5**) was largely stable over the whole pH range used, whilst the cysteinyl counterpart (**6**) proved very acid labile and also showed some tendency to decompose under strongly alkaline conditions (pH 12). The most appropriate conditions to maximise the stability of both pyrrolyl peptides was therefore pH 9, but this proved inappropriate for ^{13}C n.m.r. spectral studies[7] on the intact enzyme due to relaxation problems (slow tumbling of buried ^{13}C-pyrrole residues). However, drawing on results from the stability studies, we felt confident that ^{13}C n.m.r. analysis under strongly basic conditions (pH 12) to denature the protein would give discrete ^{13}C n.m.r. signals without seriously decomposing the enzyme-bound pyrrolic system.

In the event, ^{13}C n.m.r. analysis of HMBS at pH 12 gave sharp signals and led to the unambiguous conclusion that the first molecule of PBG bound to the enzyme is covalently linked to the pyrromethane cofactor (**4**) in the enzyme active site.[7]

Chemical shift data obtained from the model pyrrolyl peptides were used to provide very strong evidence for the nature of the heteroatom, and hence the amino acid residue by which the novel cofactor was bound to the enzyme polypeptide. ^{13}C N.m.r. spectra of (**5**) and (**6**) were recorded and the signals from the bridging methylene group between heteroatom and pyrrole (*i.e.* C-11) were assigned by selective ^1H-decoupling of the attached protons. The Table shows the results of an investigation into the pH-dependence of the ^{13}C chemical shifts of this methylene group. It is well known[36] that when groups,

Table. Effect of pH on ^{13}C chemical shifts (δ p.p.m.) of C-11 of the pyrrolyl peptides (**5**) and (**6**) in H_2O–D_2O

	pH				
	11.1	8.2	6.8	5.7	3.1
^{13}C-Nucleus					
NH–CH_2–pyrr	45.44	45.02	45.12	45.13	44.96
S–CH_2–pyrr	29.52	29.54	29.62	29.62	29.40

such as amino or carboxy groups, acquire a charge as a result of changes in solvent environment, the resonance positions of ^{13}C nuclei in close proximity are shifted. No significant variation in the position of the S–$^{13}CH_2$–pyrrole resonance was observed over the pH range of the experiment. By contrast, an upfield shift of *ca.* 0.5 p.p.m. was observed for the N–$^{13}CH_2$–pyrrole resonance for (**5**) as a result of protonation of the amino group.[37]

We were able to obtain ^1H-decoupled ^{13}C n.m.r. spectra of HMBS whose pyrromethane cofactor had been specifically ^{13}C-labelled using *in vitro* and *in vivo* labelling methods.[8] In both cases, difference spectra for ^{13}C-labelled enzyme minus [^{12}C]HMBS (*i.e.* native enzyme) showed a clear signal at δ 29.5 which, under the pH conditions of the n.m.r. experiments, matched exactly the chemical shift observed for the S–CH_2–pyrrole methylene in the model cysteinyl pyrrolyl peptide (**6**). This established that the cofactor (**4**) of HMBS is bound through a methylene to a cysteine residue. In further experiments, knowledge of the stability properties of the pyrrolyl peptides (**5**) and (**6**) was invaluable in designing conditions for isolation of a peptide carrying the bound cofactor (**4**), which allowed rigorous identification of cysteine-242 as the residue to which the cofactor is bound.[9]

Experimental

General Directions.—M.p.s were determined on a Reichert Kofler hot-stage apparatus and are uncorrected. U.v.–visible spectra were recorded on a Uvikon 810P recording spectrophotometer in 1 cm cells using methanol as solvent unless otherwise stated. I.r. spectra were recorded on a Perkin-Elmer 297 instrument as solutions in 0.5 mm sodium chloride cells using ethanol-free chloroform unless otherwise stated. ^1H N.m.r. spectra were recorded on Bruker WM 250 (250 MHz) and AM 400 (400 MHz) Fourier transform spectrometers. Chemical shifts are given on the δ scale with the solvent deuterium signal as internal reference. ^{13}C N.m.r. spectra were recorded, using ^1H decoupling, at 62.9 MHz on the Bruker WM 250 spectrometer and at 100.6 MHz on the AM 400 spectrometer. All n.m.r. spectra were recorded on solutions in $CDCl_3$ unless otherwise stated. Mass spectra were recorded on Kratos MS 30, MS 902, and MS 50 machines; field desorption (f.d.) and fast atom bombardment (f.a.b.) mass spectra were recorded on the MS 50, whilst electron impact spectra were recorded on the MS 30 and MS 902 instruments. Optical rotations at the sodium D line were recorded on a Perkin-Elmer 241 polarimeter at ambient temperature.

Analytical thin layer chromatography (t.l.c.) was performed on commercial Merck plates coated to a thickness of 0.25 mm with Kieselgel 60 F_{254} silica or 150 F_{254} (type T) alumina. Preparative t.l.c. (p.l.c.) was carried out on 200 × 200 mm plates coated to a thickness of 0.25 or 1.0 mm with Merck Kieselgel 60 F_{254} silica. The 1.0 mm plates were prepared 'in house'. P.l.c. was also carried out using 200 × 200 mm plates coated to a thickness of 1.5 mm with 150 F_{254} (type T) alumina. For normal column chromatography Merck Kieselgel 60 PF_{254} silica (70—230 mesh) was used. For flash column chromatography Merck Kieselgel 60 PF_{254} silica (230—400 mesh) was employed and all columns were prepared for use according to Still *et al.*[38] Alumina columns were run using Fluka UG I Type 507 C neutral alumina deactivated according to Keese *et al.*[39] to give UG II and UG III grades. Reverse-phase high performance liquid chromatography (h.p.l.c.) was performed on a PEP RPC HR 10/10 C_{18} reverse-phase column attached to a Pharmacia fast protein liquid chromatography (f.p.l.c.) system.

Removal of solvents was carried out under reduced pressure (water pump pressure) on a Buchi rotary evaporator or at high vacuum (*ca.* 0.1 mmHg) on a Buchi RE III rotary evaporator. Freeze-drying was carried out on an Edwards Modulyo freeze-dryer at *ca.* 0.2 mmHg with the pump trap at −56 °C.

All solvents were redistilled and, where necessary, were dried by the standard methods,[40] and then stored under an inert atmosphere over pre-activated 3 Å molecular sieves. Organic solutions which had been in contact with water were dried over AnalaR grade sodium sulphate prior to solvent removal. Ether refers to diethyl ether; Hünig's base is *N*-ethyldi-isopropylamine; Wilkinson's catalyst refers to tris(triphenylphosphine)-rhodium(I) chloride.

N-*Benzyloxycarbonylglycyl*-ε-N-(*t-butoxycarbonyl*)-L-*lysine* (**11**).—A suspension of ε-*N*-(t-butoxycarbonyl)-L-lysine (**10**) (4.99 g, 20.3 mmol) in tetrahydrofuran (60 ml) was made homogeneous with 1M aqueous sodium hydroxide (20.3 ml, 20.3 mmol) and combined with the active ester (**9**)[15] (7.36 g, 22.3 mmol). The mixture was heated under reflux for 16 h and the tetrahydrofuran was then evaporated. The residue was diluted with water (300 ml), acidified with 2% hydrochloric acid, and extracted with ethyl acetate (4 × 60 ml). The organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with chloroform followed by chloroform–methanol–acetic acid (89:10:1 v/v/v) as eluants, removal of acetic acid by azeotroping with toluene, and freeze-drying yielded the *dipeptide acid* (**11**) as a foam (8.75 g, 99%) (Found: M^+, 437.2167. $C_{21}H_{31}N_3O_7$ requires *M*, 437.2170); $[\alpha]_D$ +14.1° (*c* 1.0 in THF); $\lambda_{max.}$(EtOH) 255 nm; $\nu_{max.}$ 2 920 (CH), 2 880 (CH), 1 700 (acid and amide I), and 1 500 cm^{-1} (amide II); δ_H(400 MHz; CD_3SOCD_3) 1.68—1.78 (6 H, m, $CH_2CH_2CH_2$), 1.36 (9 H, s, Bu^t), 2.87 (2 H, br q, *J* 6.3 Hz, $CH_2NHCO_2Bu^t$), 3.63 and 3.68 (each 1 H, dd, *J* 16.9 and 6.2 Hz, $NHCH_2CO$), 4.16 (1 H, q, *J* 8.0 Hz, α-H), 5.03 (2 H, s, OCH_2Ph), 6.76 (1 H, br t, *J* 5.2 Hz, $NHCO_2Bu^t$), 7.31—7.38 (5 H, m, Ph), 7.41 (1 H, t, *J* 6.2 Hz, $NHCO_2CH_2Ph$), and 8.05 (1 H, d, *J* 7.7 Hz, CONH); δ_C(62.9 MHz) 22.1 (γ-CH_2), 28.4 (CMe_3), 29.5 (δ-CH_2), 31.3 (β-CH_2), 40.1 ($CH_2NHCO_2Bu^t$), 44.4 ($NHCH_2CO$), 52.2 (α-C), 67.2 (OCH_2Ph), 79.0 (CMe_3), 127.9—128.9 (phenyl CH), 136.2 (phenyl C), 156.9 (2 × $NHCO_2$), 169.7 (CONH), and 174.8 (CO_2H); *m/z* (f.d.) 438 (MH^+, 100%), 394 (MH^+ − 44), and 338 (MH^+ − 100).

N-*Benzyloxycarbonylglycyl*-ε-N-(*t-butoxycarbonyl*)-L-*lysyl*-L-*phenylalanine Benzyl Ester* (**13**).—A solution of the dipeptide acid (**11**) (8.36 g, 19.1 mmol), L-phenylalanine benzyl ester hydrochloride (**12**) (5.58 g, 19.1 mmol), and triethylamine (2.67 ml, 19.1 mmol) in dichloromethane (150 ml) was stirred for 1 h at 0 °C. The mixture was treated with 1-hydroxybenzotriazole (2.59 g, 19.1 mmol) and stirred for a further 1 h at 0 °C before dicyclohexylcarbodi-imide (4.34 g, 21.0 mmol) was added. After being stirred for 2 h at 0 °C, the mixture was left at −10 °C for 48 h with occasional agitation. The dicyclohexylurea was filtered off (Celite) and the residue was washed with dichloromethane. The combined filtrate and washings were washed with 10% aqueous sodium hydrogen carbonate (3 × 100 ml), 2% hydrochloric acid (3 × 100 ml), and brine (3 × 100 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was dispersed in acetone (500 ml) and stirred until the peptide had dissolved, whereupon residual

dicyclohexylurea was filtered off (Celite) and the filtrate evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate–hexane (85:15, v/v) as eluant, yielded the desired *tripeptide* (**13**) as an amorphous solid (9.81 g, 76%), m.p. 118—121 °C (from dichloromethane–ether–hexane) (Found: C, 66.15; H, 6.9; N, 8.4. $C_{37}H_{46}N_4O_8$ requires C, 65.9; H, 6.80; N, 8.31%); $[\alpha]_D$ −9.1° (*c* 1.0 in THF); $\lambda_{max.}$ 276 nm; $\nu_{max.}$(Nujol) 3 300br (NH), 1 720 (C=O), 1 680 (amide I), and 1 530 cm^{-1} (amide II); δ_H(250 MHz; CD_3SOCD_3) 1.06—1.75 (6 H, m, $CH_2CH_2CH_2$), 1.37 (9 H, s, Bu^t), 2.96 (1 H, dd, *J* 13.9 and 8.5 Hz, C*H*HPh), 3.05 (1 H, dd, *J* 13.9 and 6.5 Hz, CH*H*Ph), 3.63 (2 H, br d, *J* 5.9 Hz, NHCH_2CO), 4.29 (1 H, q, *J* 5.0 Hz, α-H), 4.50 (1 H, q, *J* 7.2 Hz, α-H), 5.03 (2 H, s, OCH_2Ph), 5.03 and 5.07 (each 1 H, *J* 12.5 Hz, OCH_2Ph), 6.73 (1 H, t, *J* 5.1 Hz, O_2CNH), 7.19—7.36 (15 H, m, 3 × Ph), 7.42 (1 H, t, *J* 6.0 Hz, O_2CNH), 7.87 (1 H, d, *J* 8.3 Hz, CONH), and 8.46 (1 H, d, *J* 7.3 Hz, CONH); δ_C(100 MHz) 22.3 (γ-CH_2), 28.4 (Bu^t), 29.4 (δ-CH_2), 31.8 (β-CH_2), 37.7 (*C*H_2Ph), 40.0 (*C*H_2NHCO$_2$Bu^t), 44.5 (NH*C*H_2CO), 52.9 and 53.4 (2 × α-C), 67.1 and 67.2 (2 × O*C*H_2Ph), 79.0 (*C*Me$_3$), 127.0—129.2 (phenyl CH), 135.1, 135.7, and 136.3 (3 × phenyl C), 156.1 and 156.9 (2 × $NHCO_2$), and 169.1 and 171.1 (2 × CONH); *m/z* (f.d.) 675 (*M*H$^+$, 100%).

N-*Benzyloxycarbonylglycyl*-L-*lysyl*-L-*phenylalanine Benzyl Ester* (**14**).—A solution of the foregoing lysyl tripeptide (**13**) (10.0 g, 14.8 mmol) in 98—100% formic acid (200 ml) was stirred at room temperature for 3.5 h. Following careful evaporation of the solvent, the residue was triturated with anhydrous ether to yield the desired *peptide monoformate salt* (**14**) as an amorphous solid (7.58 g, 83%), m.p. 121.5—123 °C (from methanol–ether) (Found: C, 63.65; H, 6.45; N, 9.0. $C_{33}H_{40}N_4O_8$ requires C, 63.9; H, 6.45; N, 9.03%); $[\alpha]_D$ −21.4° (*c* 1.0 in MeOH); $\nu_{max.}$(Nujol) 3 400 (NH), 3 250br (OH and NH), 1 720 (esters), 1 640 (amide I), and 1 530 cm^{-1} (amide II); δ_H(250 MHz; CD_3SOCD_3) 1.00—1.54 (6 H, m, $CH_2CH_2CH_2$), 2.51—2.67 (2 H, br m, C$H_2NH_3^+$), 2.97 (1 H, dd, *J* 12.5 and 8.1 Hz, C*H*HPh), 3.06 (1 H, dd, *J* 12.5 and 6.1 Hz, CH*H*Ph), 3.65 (2 H, br d, *J* 4.3 Hz, NHCH_2CO), 4.25—4.35 (1 H, m, α-H), 4.50 (1 H, q, *J* 7.3 Hz, α-H), 5.04 (2 H, s, OCH_2Ph), 5.03 and 5.07 (each 1 H, d, *J* 13.2 Hz, OCH_2Ph), 7.19—7.39 (15 H, m, 3 × Ph), 7.53 (1 H, t, *J* 5.8 Hz, O_2CNH), 8.06 (1 H, d, *J* 8.2 Hz, CONH), 8.49 (1 H, s, HCO_2^-), and 8.64 (1 H, d, *J* 7.1 Hz, CONH); δ_C(62.9 MHz; CD_3OD) 26.0 (γ-CH_2), 30.8 (β-CH_2), 35.1 (δ-CH_2), 40.8 (*C*H_2Ph), 43.1 (NH*C*H_2CO), 47.7 ($CH_2NH_3^+$), 56.5 and 58.0 (2 × α-C), 70.5 and 70.7 (2 × O*C*H_2Ph), 130.5—132.9 (phenyl CH), 139.6, 140.5, and 140.7 (3 × phenyl C), 162.0 (O_2CNH), 173.2, 174.5, and 176.3 (2 × CONH and *C*O_2-CH_2Ph), and 175.2 (HCO_2^-); *m/z* (f.d) 575 (*M*H$^+$, 100%).

4-(2-*Benzyloxycarbonylethyl*)-3-*benzyloxycarbonylmethyl*-2-*formylpyrrole* (**16**).—Sodium metal (363 mg, 15.8 mmol) and dry benzyl alcohol (5 ml) were stirred vigorously at room temperature, under an atmosphere of argon, until the sodium had dissolved. A solution of the formylpyrrole (**15**)[12] (1.00 g, 3.95 mmol) in dry benzyl alcohol (5 ml) was then added dropwise (transferred under argon by cannula). The mixture was stirred for a further 3 h at room temperature and then diluted with dichloromethane (200 ml), washed with water (50 ml) and brine (50 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Residual benzyl alcohol was removed by bulb-to-bulb distillation (70 °C, 0.1 mmHg) and the residue purified by flash chromatography on silica eluting with ethyl acetate (10—40%, v/v) in hexane, followed by trituration with ether–hexane to give the desired *pyrrole dibenzyl ester* (**16**) as long needles (746 mg, 47%), m.p. 51.5—53 °C (Found: C, 70.92; H, 5.76; N, 3.42. $C_{24}H_{23}NO_5$ requires C, 71.1; H, 5.7; H, 3.5%); $\lambda_{max.}$ 298 nm; $\nu_{max.}$ 3 430m (NH), 3 250br (NH), 3 000 (CH), 1 720s (esters), 1 640s (CHO), 1 180 (C–O), and 705 cm^{-1} (pyrrole CH); δ_H(250 MHz) 2.58 (2 H, t, *J* 7.2 Hz, $CH_2CH_2CO_2$), 2.78 (2 H, t, *J* 7.2 Hz, $CH_2CH_2CO_2$), 3.77 (2 H, s, CH_2CO_2), 5.09 and 5.11 (each 2 H, s, OCH_2Ph), 6.82 (1 H, d, *J* 2.6 Hz, pyrr-H), 7.25—7.36 (10 H, m, 2 × Ph), 9.24 (1 H, br s, NH), and 9.60 (1 H, s, CHO); *m/z* (f.d.) 406 (*M*$^+$, 100%).

2-*Hexylaminomethyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrole* (**17**).—A solution of the formylpyrrole (**15**)[12] (100 mg, 0.395 mmol) and distilled hexylamine (52 μl, 0.395 mmol) in dry tetrahydrofuran (7 ml) was heated under reflux, in the presence of pre-activated 3 Å molecular sieves, under an atmosphere of argon for 16 h. The mixture was cooled to 0 °C, diluted with dry methanol (2 ml), treated with sodium borohydride (45 mg, 1.19 mmol), and stirred for 0.5 h under an atmosphere of argon. It was then diluted with water (10 ml), saturated with solid sodium chloride, decanted free of the molecular sieves, and extracted with dichloromethane (3 × 15 ml). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to yield the *secondary amine* (**17**) as needles (100 mg, 75%), m.p. 58—60 °C (from dichloromethane–ether–hexane) (Found: C, 63.7; H, 8.65; N, 8.45. $C_{18}H_{30}N_2O_4$ requires C, 63.9; H, 8.9; N, 8.3%); $\nu_{max.}$(CH_2Cl_2) 3 450 and 3 300br (NH), 2 950, 2 920, and 2 850 (CH), 1 720s (ester), and 1 180 cm^{-1} (C–O); δ_H(250 MHz; CD_2Cl_2) 0.88 (3 H, t, *J* 6.8 Hz, Me), 1.21—1.52 (8 H, m, $CH_2CH_2CH_2CH_2$), 1.76 (1 H, br s, NH), 2.49—2.64 (4 H, m, $CH_2CH_2CO_2$ and CH_2NH), 2.70 (2 H, t, *J* 8.3 Hz, $CH_2CH_2CO_2$), 3.42 (2 H, s, CH_2CO_2), 3.64 (6 H, s, 2 × OMe), 3.69 (2 H, s, NHCH_2-pyrr), 6.46 (1 H, d, *J* 1.9 Hz, pyrr-H), and 8.56 (1 H, br s, pyrr-NH); δ_C(100 MHz) 14.0 (Me), 20.7 ($CH_2CH_2CO_2$), 22.6 (CH_2Me), 27.0, 29.9, and 30.0 (3 × CH_2), 31.7 (CH_2CO_2), 34.9 ($CH_2CH_2CO_2$), 44.9 (NH*C*H_2-pyrr), 49.8 (*C*H_2NHCH$_2$-pyrr), 51.5 and 51.8 (2 × OMe), 110.8, 113.8, 121.4, and 128.4 (4 × pyrrole C), and 172.9 and 173.9 (2 × *C*O_2Me); *m/z* (f.d.) 339 (*M*$^+$, 100%).

4-(2-*Benzyloxycarbonylethyl*)-3-*benzyloxycarbonylmethyl*-2-*hexylaminomethylpyrrole* (**18**).—A solution of the formylpyrrole (**16**) (193 mg, 0.477 mmol) and distilled hexylamine (63 μl, 0.477 mmol) in dry tetrahydrofuran (8 ml) was heated under reflux, in the presence of pre-activated 3 Å molecular sieves, under an atmosphere of argon for 16 h. The mixture was cooled to 0 °C, diluted with dry methanol (3 ml), and, following the addition of ammonium chloride (84 mg, 1.57 mmol), treated with sodium borohydride (54 mg, 1.43 mmol). It was then stirred at 0 °C for 0.5 h, diluted with water (10 ml), saturated with sodium chloride, decanted free of the molecular sieves, and extracted with dichloromethane (3 × 15 ml). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to give the *secondary amine* (**18**) as needles (177 mg, 76%), m.p. 73—75 °C (from dichloromethane–ether–hexane) (Found: C, 73.1; H, 7.7; N, 5.6%; *M*$^+$, 490.2831. $C_{30}H_{38}N_2O_4$ requires C, 73.5; H, 7.76; N, 5.71%; *M*, 490.2831); $\nu_{max.}$ 3 450 (NH), 3 300br (NH), 3 000, 2 930, and 2 850 (CH), 1 720s (C=O), 1 500 (aromatic), 1 450 (CH), and 1 120 and 1 170 cm^{-1} (C–O and CH); δ_H(250 MHz; CD_2Cl_2) 0.89 (3 H, t, *J* 6.9 Hz, Me), 1.22—1.46 (8 H, m, $CH_2CH_2CH_2CH_2$), 1.71 (1 H, br s, NH), 2.52—2.61 (4 H, m, $CH_2CH_2CO_2$ and CH_2NH), 2.73 (2 H, t, *J* 8.0 Hz, $CH_2CH_2CO_2$), 3.47 (2 H, s, CH_2CO_2), 3.68 (2 H, s, NHCH_2-pyrr), 4.95 and 4.96 (each 2 H, s, 2 × OCH_2Ph), 6.45 (1 H, d, *J* 2.0 Hz, pyrr-H), 7.28—7.38 (10 H, m, 2 × Ph), and 8.54 (1 H, br s, pyrr-NH); *m/z* (f.d.) 491 (*M*H$^+$, 100%).

4-(2-*Carboxyethyl*)-3-*carboxymethyl*-2-*hexylaminomethylpyrrole* (**19**).—A solution of the secondary amine (**18**) (80 mg, 0.163 mmol) and ammonium formate (62 mg, 0.978 mmol) in propan-2-ol–water (5:1, v/v; 6.25 ml) was stirred under an atmosphere of hydrogen, at room temperature and pressure, for

5 h in the presence of 10% palladium-on-charcoal (32 mg). The catalyst was filtered off (Celite) and the residue washed with propan-2-ol–water (1:1, v/v; 3 ml). The filtrate and washings were diluted with water (5 ml) and freeze-dried, yielding the *di-acid* (**19**) as a white powdery formate salt (57 mg, 98%) (Found: MH^+, 311.1976. $C_{16}H_{27}N_2O_4$ requires MH, 311.1971); δ_H(400 MHz; D_2O at pD 7) 0.82 (3 H, t, *J* 6.6 Hz, Me), 1.25—1.33 and 1.58—1.62 (8 H, both m, $CH_2CH_2CH_2CH_2$), 2.38 (2 H, t, *J* 7.3 Hz, $CH_2CH_2CO_2$), 2.64 (2 H, t, *J* 7.3 Hz, $CH_2CH_2CO_2$), 2.93 (2 H, t, *J* 7.6 Hz, $CH_2{}^+NH_2CH_2$-pyrr), 3.67 (2 H, s, CH_2CO_2), 4.14 (2 H, s, $^+NH_2CH_2$-pyrr), 6.66 (1 H, s, pyrr-H), and 8.41 (1 H, s, HCO_2^-); δ_C(100 MHz; D_2O at pD 7) 16.1 (Me), 24.3 ($CH_2CH_2CO_2$), 24.5 (CH_2Me), 28.2, 28.3, and 33.6 (3 × CH_2), 35.9 (CH_2CO_2), 41.0 ($CH_2CH_2CO_2$), 44.8 ($^+NH_2CH_2$-pyrr), 49.0 ($CH_2NH_2^+$), 119.5, 120.0, 122.0, and 125.6 (4 × pyrrole-C), and 183.8 and 185.6 (2 × CO_2); *m/z* (f.d.) 310 (M^+, 100%).

N-*Benzyloxycarbonylglycyl*-ε-N-[4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-L-*lysyl*-L-*phenylalanine Benzyl Ester* (**22**).—A solution of the tripeptide formate salt (**14**) (300 mg, 0.484 mmol), the formylpyrrole (**15**) (122 mg, 0.484 mmol), and triethylamine (74 μl, 0.532 mmol) in dry dimethylformamide–tetrahydrofuran (1:6, v/v; 21 ml) was heated under reflux, in the presence of pre-activated 3 Å molecular sieves, under an atmosphere of nitrogen for 16 h. The mixture was then cooled to room temperature, treated with sodium borohydride (45 mg, 1.19 mmol) dissolved in a minimum volume of dry methanol, and stirred for 0.5 h under an atmosphere of nitrogen. The molecular sieves were filtered off (Celite) and the residue washed with methanol. The combined filtrate and washings were diluted with an equal volume of water, the organic solvents were evaporated, and the remaining aqueous residue was extracted with dichloromethane (3 × 30 ml). The combined organic extracts were washed with 10% aqueous sodium carbonate (20 ml) and water (3 × 20 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Chromatography on UG III alumina, with methanol (1—3%, v/v) in dichloromethane as eluant followed by further chromatography of the mixed fractions on UG II alumina, also with methanol (1—3%, v/v) in dichloromethane as eluant, yielded the *pyrrolyl peptide* (**22**) as an oil (141 mg, 36%) (Found: MH^+, 812.3882. $C_{44}H_{54}N_5O_{10}$ requires MH, 812.3870); $[\alpha]_D$ −6.0° (*c* 1.0 in THF); $\nu_{max.}$ 3 350 (NH), 1 720s (ester and carbamate), 1 660 (amide I), 1 500 (amide II), and 1 180 cm^{-1} (C–O ester); δ_H(400 MHz) 1.25—1.75 (6 H, m, $CH_2CH_2CH_2$), 2.20 (1 H, br s, NH), 2.47—2.53 (4 H, m, CH_2NHCH_2-pyrr and $CH_2CH_2CO_2$), 2.71 (2 H, t, *J* 7.1 Hz, $CH_2CH_2CO_2$), 2.97 (1 H, dd, *J* 13.8 and 6.5 Hz, CHHPh), 3.03 (1 H, dd, *J* 13.8 and 6.1 Hz, CHHPh), 3.39 (2 H, s, CH_2CO_2), 3.60 and 3.63 (each 3 H, s, OMe), 3.61—3.65 (2 H, m, $NHCH_2$-pyrr), 3.68—3.78 (2 H, br m, $NHCH_2CO$), 4.45 (1 H, br q, *J* 6.7 Hz, α-H), 4.83 (1 H, q, *J* 7.4 Hz, α-H), 5.03 and 5.09 (each 1 H, d, *J* 12.2 Hz, OCH_2Ph), 5.07 (2 H, s, OCH_2Ph), 5.77 (1 H, br s, O_2CNH), 6.39 (1 H, d, *J* 1.9 Hz, pyrr-H), 6.98—7.34 (15 H, m, 3 × Ph), and 9.22 (1 H, br s, pyrr-NH); δ_C(62.9 MHz) 20.7 ($CH_2CH_2CO_2$), 22.7 (γ-CH_2), 28.8 (β-CH_2), 30.0 (CH_2CO_2), 31.8 (δ-CH_2), 35.0 ($CH_2CH_2CO_2$), 37.8 (CH_2Ph), 44.6 ($NHCH_2CO$ and $NHCH_2$-pyrr), 48.6 (CH_2NHCH_2-pyrr), 51.3 and 51.8 (2 × OMe), 53.1 and 53.5 (2 × α-C), 67.1 (2 × OCH_2Ph), 111.1, 114.0, and 121.5 (pyrrole C), 127.0—129.3 (phenyl CH), 135.2, 135.8, and 136.3 (3 × phenyl C), 156.6 (O_2CNH), and 169.2, 171.2, 171.4, 172.9, and 173.8 (CO_2CH_2Ph, 2 × CONH, and 2 × CO_2Me); *m/z* (f.d.) 812 (MH^+, 100%).

N-*Benzyloxycarbonylglycyl*-ε-N-[4-(2-*benzyloxycarbonylethyl*)-3-*benzyloxycarbonylmethylpyrrol*-2-*ylmethyl*]-L-*lysyl*-L-*phenylalanine Benzyl Ester* (**23**).—A solution of the formate salt (**14**) (76.5 mg, 0.120 mmol), the formylpyrrole (**16**) (50 mg, 0.120 mmol), and triethylamine (19 μl, 0.132 mmol), in dry degassed dimethylformamide–tetrahydrofuran (1:6, v/v; 3.5 ml), was heated under reflux, in the presence of pre-activated 3 Å molecular sieves, under an atmosphere of argon for 18 h. The mixture was then cooled to 0 °C, treated with sodium borohydride (14 mg, 0.360 mmol) dissolved in a minimum volume of dry methanol, and stirred for 0.25 h under an atmosphere of argon. The reaction was quenched with water (10 ml) and the aqueous supernatant decanted and extracted with dichloromethane (60 ml, 2 × 20 ml). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (20 ml) and water (4 × 20 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Preparative t.l.c. on alumina (1.5 mm thick plates), with methanol (3%) in dichloromethane as eluant, gave the *pyrrolyl peptide* (**23**) as an oil (53 mg, 45%) [56% based on unrecovered pyrrole (**16**)] (Found: MH^+, 964.4480. $C_{56}H_{62}N_5H_{10}$ requires MH, 964.4496); $[\alpha]_D$ −4.2° (*c* 1.0 in THF); $\nu_{max.}$ 3 350w (NH), 2 950w (CH), 1 720s (ester and carbamate), 1 660 (amide I), 1 500 (amide II), and 1 180 cm^{-1} (C–O ester); δ_H(400 MHz; CD_2Cl_2) 1.28—1.74 (6 H, m, $CH_2CH_2CH_2$), 2.48—2.55 (2 H, br m, CH_2NHCH_2-pyrr), 2.54 (2 H, t, *J* 7.2 Hz, $CH_2CH_2CO_2$), 2.72 (2 H, t, *J* 7.2 Hz, $CH_2CH_2CO_2$), 2.99 (1 H, dd, *J* 13.7 and 6.7 Hz, CHHPh), 3.07 (1 H, dd, *J* 13.7 and 6.0 Hz, CHHPh), 3.47 (2 H, s, CH_2CO_2), 3.65 (2 H, s, $NHCH_2$-pyrr), 3.69—3.79 (2 H, br m, $NHCH_2CO$), 4.43 (1 H, q, *J* 6.1 Hz, α-H), 4.80 (1 H, q, *J* 7.2 Hz, α-H), 5.03—5.10 (8 H, m, 4 × OCH_2Ph), 5.86 (1 H, br s, O_2CNH), 6.42 (1 H, s, pyrr-H), 7.05—7.37 (25 H, m, 5 × Ph), and 9.42 (1 H, br s, pyrr-NH); δ_C(62.9 MHz; CD_2Cl_2) 21.0 ($CH_2CH_2CO_2$), 23.0 (γ-CH_2), 28.6 (β-CH_2), 30.5 (CH_2CO_2), 31.8 (δ-CH_2), 35.5 ($CH_2CH_2CO_2$), 38.1 (CH_2Ph), 44.7 and 44.9 ($NHCH_2CO$ and $NHCH_2$-pyrr), 48.5 (CH_2NHCH_2-pyrr), 66.3, 66.9, 67.4, and 67.5 (4 × OCH_2Ph), 112.1, 114.9, and 121.9 (pyrrole C), 127.3—129.8 (phenyl CH), 135.8, 136.5, 136.6, 136.9, and 137.0 (5 × phenyl C), 156.9 (O_2CNH), and 169.5, 171.6, 171.7, 172.8, and 173.3 (3 × CO_2CH_2Ph and 2 × CONH); *m/z* (f.d.) 964 (MH^+, 100%).

Glycyl-ε-N-[4-(2-*carboxyethyl*)-3-*carboxymethylpyrrol*-2-*ylmethyl*]-L-*lysyl*-L-*phenylalanine* (**5**).—A solution of pyrrolyl-peptide (**23**) (72 mg, 75 μmol) and ammonium formate (57 mg, 900 μmol) in propan-2-ol–water (4:1, v/v; 3.75 ml) was stirred vigorously under an atmosphere of hydrogen, at room temperature and pressure, for 4.5 h in the presence of 10% palladium-on-charcoal (29 mg). The catalyst was filtered off (Celite) and the residue washed with propan-2-ol–water (1:1, v/v; 5 ml) followed by water (2 ml). The combined filtrate and washings were treated with 1M aqueous potassium hydroxide (1.5 ml) and stirred vigorously for 0.5 h before being neutralised by careful addition of conc. hydrochloric acid followed by 0.5M hydrochloric acid and then freeze-dried. The residue was purified on a reverse-phase h.p.l.c. column equilibrated with 50mM aqueous ammonium hydrogen carbonate and eluted at 5.6 ml/min with a gradient of acetonitrile (0—15%, v/v) in the same buffer (total volume, 142 ml). The product was collected on ice with manual peak cutting and following two rounds of freeze-drying the *pyrrolyl peptide* (**5**) was obtained as a solid (18 mg, 43%). The unstable product was stored under anhydrous argon, in the cold and excluded from light (Found: MH^+, 560.2730. $C_{27}H_{38}N_5O_8$ requires MH, 560.2720); δ_H(400 MHz; D_2O at pD 4) 1.17—1.62 (6 H, m, $CH_2CH_2CH_2$), 2.46 (2 H, t, *J* 7.4 Hz, $CH_2CH_2CO_2$), 2.71 (2 H, t, *J* 7.4 Hz, $CH_2CH_2CO_2$), 2.89—2.94 (3 H, m, ε-$CH_2NH_2{}^+$ and CHHPh), 3.20 (1 H, dd, *J* 13.9 and 5.0 Hz, CHHPh), 3.43 (2 H, s, CH_2CO_2), 3.83 (2 H, s, $^+H_3NCH_2CO$), 4.16 and 4.22 (each 1 H, d, *J* 14.4 Hz, $^+H_2NCH_2$-pyrr), 4.49 (1 H, dd, *J* 8.9 and 5.0 Hz, α-H), 6.72 (1 H, s, pyrr-H), and 7.21—7.33 (5 H, m, Ph); δ_C(100 MHz; D_2O at pD 11) 24.5 ($CH_2CH_2CO_2$), 25.5 (γ-CH_2), 30.0 (β-CH_2), 33.5 (δ-CH_2),

35.5 (CH_2CO_2), 40.5 ($CH_2CH_2CO_2$), 40.9 (CH_2Ph), 45.4 ($^+H_2NCH_2$-pyrr), 46.6 ($^+H_3NCH_2CO$), 49.7 (ε-CH_2), 56.7 and 58.8 (2 × α-C), 117.7, 118.5, 125.2, and 127.7 (pyrrole C), 129.6, 131.3, and 132.0 (phenyl CH), 140.3 (phenyl C), and 175.0, 178.5, 180.5, 184.2, and 186.0 (3 × CO_2^- and 2 × CONH); *m/z* [f.a.b.; thioglycerol–glycerol (1 : 1, v/v)–1M HCl] 560 (MH^+, 100%), 545 (24, MH^+ – NH), 516 (14, MH^+ – CO_2), 503 (5, MH^+ – $NHCH_2CO$), 488 (6, MH^+ – $NHCH_2CONH$), 412 (9, MH^+ – $CHCH_2PhCO_2$), 395 (6, MH^+ – $NHCHCH_2PhCO_2$), 351 (100, MH^+ – pyrrole), 336 (45, MH^+ – pyrrole – NH), and 166 (8, $^+NH_3CHCH_2PhCO_2H$); *m/z* [f.a.b.; thioglycerol–glycerol (1 : 1, v/v)–18-crown-6] 558 (M^- – H. 100%), 543 (6, M^- – NH_2), 514 (6, M^- – H – CO_2), 501 (2, M^- – H – $NHCH_2CO$), 410 (3, M^- – H – $CHCH_2PhCO_2$), 349 (63, M^- – H – pyrrole), and 164 (15, $^-NHCHCH_2PhCO_2H$).

N-*Benzyloxycarbonylglycyl*-S-*trityl*-L-*cysteine* (**27**).—A suspension of *S*-trityl-L-cysteine (**26**) (5.00 g, 13.8 mmol) in tetrahydrofuran (40 ml) was made homogeneous with 1M aqueous sodium hydroxide (13.8 ml, 13.8 mmol), combined with the active ester (**9**) (5.00 g, 15.2 mmol), and heated under reflux for 20 h, after which the tetrahydrofuran was evaporated. The residue was diluted with water (300 ml), acidified with 2% hydrochloric acid, and extracted with ethyl acetate (4 × 60 ml). The organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with chloroform followed by chloroform–methanol–acetic acid (89 : 10 : 1, v/v/v) as eluants, removal of the acetic acid by azeotroping with toluene, and freeze-drying yielded the *dipeptide acid* (**27**) as a foam (7.55 g, 99%) (Found: M^+, 554.1891. $C_{32}H_{30}N_2O_5S$ requires *M*, 554.1882); $[\alpha]_D$ +19.1° (*c* 1.0 in THF); $\lambda_{max.}$ 256 nm; $\nu_{max.}$ 2 900s (CH), 1 705 (C=O), and 1 670 cm^{-1} (amide I); δ_H(250 MHz; CD_3SOCD_3) 2.40 and 2.52 (each 1 H, dd, *J* 15.0 and 5.2 Hz, CH_2S), 3.66 (2 H, br d, *J* 6.1 Hz, $NHCH_2CO$), 4.22 (1 H, q, *J* 5.3 Hz, α-H), 5.03 (2 H, s, OCH_2Ph), 7.22—7.37 (20 H, m, 4 × Ph), 7.47 (1 H, t, *J* 6.2 Hz, O_2CNH), and 8.23 (1 H, d, *J* 7.8 Hz, CONH); δ_C(100 MHz) 33.1 (CH_2S), 44.0 ($NHCH_2CO$), 51.3 (α-C), 67.3 (OCH_2Ph), 126.9, 128.0, 128.5, and 129.4 (phenyl CH), 144.1 (phenyl C), 157.0 (O_2CNH), and 169.6 and 172.9 (CONH and CO_2H); *m/z* (f.d.) 554 (M^+, 100%).

N-*Benzyloxycarbonylglycyl*-S-*trityl*-L-*cysteinyl*-L-*phenylalanine Benzyl Ester* (**28**).—A solution of the dipeptide acid (**27**) (7.45 g, 13.4 mmol), L-phenylalanine hydrochloride benzyl ester (**12**) (3.92 g, 13.4 mmol), and triethylamine (1.87 ml, 13.4 mmol) in dichloromethane (150 ml) was stirred for 1 h at 0 °C, then treated with 1-hydroxybenzotriazole (1.82 g, 13.4 mmol) and stirred for a further 1 h at 0 °C before dicyclohexylcarbodiimide (3.05 g, 14.7 mmol) was added. After being stirred for a further 2 h at 0 °C, the mixture was left at −10 °C for 48 h and then stirred for a final 2 h at 0 °C. The dicyclohexylurea was filtered off (Celite) and the residue washed with dichloromethane. The combined filtrate and washings were washed with 10% aqueous sodium hydrogen carbonate (3 × 50 ml), 2% aqueous hydrochloric acid (3 × 50 ml), and brine (2 × 50 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was resuspended in acetone (200 ml) and stirred until the peptide had dissolved, whereupon residual dicyclohexylurea was filtered off (Celite) and the filtrate evaporated to dryness. Flash chromatography on silica gel with ethyl acetate–hexane (1 : 1, v/v) as eluant yielded the *tripeptide* (**28**) (8.36 g, 79%), m.p. 118—120 °C (from dichloromethane–ether–hexane) (Found: C, 72.95; H, 5.65; N, 5.3. $C_{48}H_{45}N_3O_6S$ requires C, 72.8; H, 5.7; N, 5.3%); $[\alpha]_D$ −11.6° (*c* 1.0 in THF); $\lambda_{max.}$ 276 nm; $\nu_{max.}$(Nujol) 3 250 (NH), 3 020 (CH), 2 995 (CH), 1 720 (ester), 1 700 (urethane), 1 640 (amide I), and 1 520 cm^{-1} (amide II); δ_H(400 MHz; CD_3SOCD_3) 2.28 (1 H, dd, *J* 11.7 and 8.3 Hz, CHHS), 2.33 (1 H, dd, *J* 11.7 and 5.7 Hz, CHHS), 2.92 (1 H, dd, *J* 13.7 and 8.3 Hz, CHHPh), 3.01 (1 H, dd, *J* 13.7 and 6.3 Hz, CHHPh), 3.60 and 3.67 (each 1 H, dd, *J* 16.9 and 6.1 Hz, $NHCH_2CO$), 4.42 (1 H, q, *J* 7.1 Hz, α-H), 4.46 (1 H, q, *J* 7.3 Hz, α-H), 4.97 and 5.02 (each 1 H, d, *J* 12.6 Hz, OCH_2Ph), 5.02 (2 H, s, OCH_2Ph), 7.12—7.42 (30 H, m, 6 × Ph), 7.46 (1 H, t, *J* 6.1 Hz, O_2CNH), 8.10 (1 H, d, *J* 8.4 Hz, CONH), and 8.42 (1 H, d, *J* 8.4 Hz, CONH); δ_C (62.9 MHz) 33.1 (CH_2S), 37.6 (CH_2Ph), 44.3 ($NHCH_2CO$), 52.1 and 53.3 (2 × α-C), 67.2 (2 × OCH_2Ph), 126.0—129.6 (phenyl CH), 135.1, 135.6, 136.2, and 144.3 (phenyl C), 157.0 (O_2CNH), and 168.7, 169.3, and 170.6 (2 × CONH and CO_2CH_2Ph); *m/z* (f.d.) 791 (M^+, 100%).

N-*Benzyloxycarbonylglycyl*-L-*cysteinyl*-L-*phenylalanine Benzyl Ester* (**29**).—A solution of the tripeptide (**28**) (8.00 g, 10.1 mmol) in hot methanol (100 ml) was treated with silver(I) nitrate (1.72 g, 10.1 mmol) and dry pyridine (818 μl, 10.1 mmol). The insoluble sulphide was allowed to settle at room temperature in the dark under an atmosphere of argon, and then filtered off, suspended in dimethylformamide–conc. hydrochloric acid (15 : 1, v/v; 64 ml), and agitated vigorously for 3 h in the dark. Silver chloride was filtered off (Celite) and the residue was washed with chloroform (50 ml). The combined filtrate and washings were diluted with chloroform (200 ml) and vigorously washed with water (300 ml, 9 × 150 ml). Residual silver chloride was filtered off (Celite) and the filtrate was dried (Na_2SO_4), filtered, and evaporated to dryness to yield the *free-thiol tripeptide* (**29**) (3.80 g, 69%), m.p. 120.5—123.5 °C (from dichloromethane–ether–hexane) (Found: C, 63.10; H, 5.64; N, 7.39. $C_{29}H_{31}N_3O_6S$ requires C, 63.4; H, 5.65; N, 7.65%); $[\alpha]_D$ −19.4° (*c* 0.5 in THF); $\lambda_{max.}$ 276 nm; $\nu_{max.}$(Nujol) 3 260 (NH), 3 020 (CH), 2 995 (CH), 1 720 (ester), 1 630 (amide I), and 1 540 cm^{-1} (amide II); δ_H (400 MHz; CD_3SOCD_3) 2.12 (1 H, t, *J* 8.4 Hz, SH), 2.50—2.73 (2 H, m, CH_2S), 2.98 (1 H, dd, *J* 13.7 and 8.6 Hz, CHHPh), 3.06 (1 H, dd, *J* 13.7 and 6.2 Hz, CHHPh), 3.67 (2 H, br d, *J* 5.8 Hz, $NHCH_2CO$), 4.45 (1 H, q, *J* 6.9 Hz, α-H), 4.53 (1 H, q, *J* 7.4 Hz, α-H), 5.03 (2 H, s, OCH_2Ph), 5.04 and 5.08 (each 1 H, d, *J* 12.6 Hz, OCH_2Ph), 7.19—7.38 (15 H, m, 3 × Ph), 7.49 (1 H, t, *J* 5.8 Hz, O_2CNH), 8.02 (1 H, d, *J* 8.2 Hz, CONH), and 8.57 (1 H, d, *J* 7.4 Hz, CONH); δ_C(62.9 MHz), 26.6 (CH_2S), 37.6 (CH_2Ph), 44.5 ($NHCH_2CO$), 53.5, 54.2 (2 × α-C), 67.2 and 67.3 (2 × OCH_2Ph), 127.0—129.2 (phenyl CH), 135.0, 135.6, and 136.2 (3 × phenyl C), 157.0 (O_2CNH), and 169.2, 169.3, and 170.9 (2 × CONH and CO_2CH_2Ph); *m/z* (f.d.) 550 (MH^+, 100%).

2-*Formyl*-5-*hexylthiomethyl*-3-(2-*methoxycarbonylethyl*)-4-*methoxycarbonylmethylpyrrole* (**32**).—A solution of the formylpyrrole[25] (**30**) (250 mg, 0.936 mmol) in dry tetrahydrofuran (5 ml) was treated with a solution of t-butyl hypochlorite[25] (141 μl, 1.24 mmol) in dry ether (5 ml) and stirred for 0.25 h at 0 °C under an atmosphere of argon. The mixture was warmed to room temperature, treated with hexanethiol (132 μl, 0.936 mmol) and Hünig's base (163 μl, 0.936 mmol) in dry tetrahydrofuran (1 ml), and stirred for a further 1 h at room temperature under argon. It was then diluted with ether (50 ml), washed with water (50 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate–hexane (1 : 1, v/v) as eluant, gave the *sulphide* (**32**) as needles (172 mg, 48%), m.p. 62.5—64.5 °C (from ether–hexane) (Found: C, 59.74; H, 7.57; N, 3.63. $C_{19}H_{29}NO_5S$ requires C, 59.5; H, 7.57; N, 3.66%); $\lambda_{max.}$ 307 nm; $\nu_{max.}$(Nujol) 3 220br (NH), 1 730 and 1 720s (esters), and 1 635 cm^{-1} (CHO); δ_H(250 MHz) 0.86 (3 H, t, *J* 6.5 Hz, Me), 1.20—1.53 (8 H, m, $CH_2CH_2CH_2CH_2$), 2.41 (2 H, t, *J* 7.2 Hz, CH_2S), 2.59 (2 H, t, *J* 7.8 Hz, $CH_2CH_2CO_2$), 3.03 (2 H, t, *J* 7.8 Hz, $CH_2CH_2CO_2$), 3.47 (2 H, s, CH_2CO_2), 3.65 and 3.68 (each 3 H,

s, OMe), 3.70 (2 H, s, SCH_2-pyrr), 9.45 (1 H, br s, NH), and 9.58 (1 H, s, CHO); δ_C(100 MHz) 14.0 (Me), 19.0 ($CH_2CH_2CO_2$), 22.5 (CH_2Me), 26.9 (SCH_2-pyrr), 28.4, 29.2, and 29.4 (3 × CH_2), 31.3 (CH_2CO_2), 32.2 (CH_2SCH_2-pyrr), 35.9 ($CH_2CH_2CO_2$), 51.7 and 52.9 (OMe), 115.4, 128.0, 134.2, and 136.0 (pyrrole C), 171.5 and 172.8 (CO_2Me), and 177.0 (CHO); *m/z* (f.d.) 383 (M^+, 100%).

2-*Hexylthiomethyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrole* (**33**).—A solution of the sulphide (**32**) (100 mg, 0.261 mmol) and Wilkinson's catalyst (241 mg, 0.261 mmol) in dry toluene (20 ml) was vigorously stirred and heated under reflux for 2 h under an atmosphere of argon. The mixture was evaporated and the residue was treated with ether (30 ml) and filtered (Celite) to remove the insoluble yellow rhodium(I) monocarbonyl by-product, the residue being washed with more ether. The combined filtrate and washings were evaporated to dryness and the residue purified by preparative t.l.c. on silica, with ethyl acetate-hexane (1:1, v/v) as the eluant, yielding the *sulphide* (**33**) as a viscous oil (52 mg, 56%) (Found: M^+, 355.1817. $C_{18}H_{29}NO_4S$ requires *M*, 355.1817); $\nu_{max.}$(CH_2Cl_2) 3 420 (NH), 2 950, 2 920, and 2 850 (CH), 1 720s (esters), 1 440 (CH), and 1 180 cm^{-1} (C–O); δ_H(250 MHz; CD_3COCD_3) 0.87 (3 H, t, *J* 6.9 Hz, Me), 1.19—1.55 (8 H, m, $CH_2CH_2CH_2CH_2$), 2.40 (2 H, t, *J* 7.1 Hz, CH_2S), 2.48—2.72 (4 H, m, $CH_2CH_2CO_2$), 3.46 (2 H, s, CH_2CO_2), 3.88 (6 H, s, OMe), 3.73 (2 H, s, SCH_2-pyrr), 6.50 (1 H, d, *J* 2.7 Hz, pyrr-H), and 9.67 (1 H, br s, NH); δ_C(100 MHz) 14.0 (Me), 20.7 ($CH_2CH_2CO_2$), 22.5 (CH_2Me), 27.0 (SCH_2-pyrr), 28.5, 29.3, and 29.9 (3 × CH_2), 31.4 (CH_2CO_2), 31.7 (CH_2SCH_2-pyrr), 34.8 ($CH_2CH_2CO_2$), 51.4 and 51.8 (2 × OMe), 112.0, 114.1, 121.9, and 125.9 (4 × pyrrole C), and 172.3 and 173.8 (2 × CO_2Me); *m/z* (f.d.) 355 (M^+, 100%).

N-*Benzyloxycarbonylglycyl*-S-[5-*formyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-L-*cysteinyl*-L-*phenylalanine Benzyl Ester* (**34**).—A solution of the formylpyrrole (**30**) (340 mg, 1.28 mmol) in dry tetrahydrofuran–ether (1:1, v/v), under an atmosphere of nitrogen, was treated with fresh t-butyl hypochlorite (185 μl, 1.64 mmol) and stirred at 0 °C for 0.25 h. It was then treated with a solution of the tripeptide (**29**) (700 mg, 1.28 mmol) and Hünig's base (222 μl, 1.28 mmol) in dry dichloromethane–tetrahydrofuran (3:7, v/v) (transfered under nitrogen by cannula) and was stirred at room temperature under an atmosphere of nitrogen for 1 h. The solvent was evaporated and the residue purified by flash chromatography on silica, with methyl acetate–hexane (6:4, v/v) as eluant, yielding the *pyrrolyl peptide* (**34**) as a foam (811 mg, 78%) (Found: M^+, 814.2856. $C_{42}H_{46}N_4O_{11}S$ requires *M*, 814.2884); $[\alpha]_D$ −15.8° (*c* 1.0 in THF); $\lambda_{max.}$ 307 and 270 nm; $\nu_{max.}$ 3 200 (NH), 3 000 (CH), 2 950 (CH aldehyde), 1 720s (ester and carbamate), 1 650s (CHO and amide I), 1 500 (amide II), 1 440 (CH), and 1 210 and 1 180 cm^{-1} (C–O ester); δ_H(400 MHz) 2.56—2.63 (4 H, m, $CH_2CH_2CO_2$ and CH_2S), 2.98—3.04 (2 H, m, $CH_2CH_2CO_2$), 3.06 (1 H, dd, *J* 14.1 and 6.7 Hz, CHHPh), 3.14 (1 H, dd, *J* 14.1 and 5.7 Hz, CHHPh), 3.48 (2 H, s, CH_2CO_2), 3.63 and 3.64 (each 3 H, s, OMe), 3.68 and 3.72 (each 1 H, d, *J* 14.7 Hz, SCH_2-pyrr), 3.84 (1 H, dd, *J* 12.2 and 5.7 Hz, NHCHHCO$_2$), 3.92 (1 H, dd, *J* 12.2 and 5.3 Hz, NHCHHCO$_2$), 4.53 (1 H, q, *J* 5.3 Hz, α-H), 4.84 (1 H, q, *J* 5.6 Hz, α-H), 5.06—5.17 (4 H, m, 2 × OCH_2Ph), 5.87 (1 H, br s, O_2CNH), 7.02—7.34 (15 H, m, 3 × Ph), 9.55 (1 H, s, CHO), and 10.52 (1 H, br s, pyrr-NH); δ_C(100 MHz) 19.1 ($CH_2CH_2CO_2$), 26.1 (SCH_2-pyrr), 29.2 (CH_2CO_2), 33.6 (CH_2S), 35.7 ($CH_2CH_2CO_2$), 37.4 (CH_2Ph), 44.3 ($NHCH_2$-CO), 51.6, 52.0, 52.1, and 53.7 (2 × OMe and 2 × α-C), 67.1 and 67.2 (2 × OCH_2Ph), 115.4 (pyrrole C), 126.9—129.1 (aromatic C), 134.9, 135.2, 135.6, and 136.2 (pyrrole C and 3 × phenyl C), 156.8 (O_2CNH), 169.8, 169.9, 170.8, 171.9, and 172.8 (2 × CONH and 3 × CO_2), and 177.3 (CHO); *m/z* (f.d.) 814 (M^+, 100%).

N-*Benzyloxycarbonylglycyl*-S-[4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-L-*cysteinyl*-L-*phenylalanine Benzyl Ester* (**35**).—A solution of the pyrrolyl-peptide (**34**) (400 mg, 0.491 mmol) and Wilkinson's catalyst (455 mg, 0.491 mmol) in dry acetonitrile (20 ml) was heated under reflux for 5 h under an atmosphere of nitrogen. The solvent was evaporated and the residue treated with methyl acetate (5 ml). The insoluble inorganic material was filtered off (Celite) and the residue washed with methyl acetate. The filtrate and washings were evaporated to dryness and the residue (773 mg) purified by chromatography on silica gel (70 g), with methyl acetate (50—55%, v/v) in hexane as eluant, and then by preparative t.l.c. on silica gel, with methyl acetate–hexane (7:3, v/v) as eluant, to yield the desired *pyrrolyl peptide* (**35**) as an oil (15 mg, 4%) (Found: M^+, 786.2934. $C_{41}H_{46}N_4O_{10}S$ requires *M*, 786.2935); δ_H(400 MHz) 2.54 (2 H, t, *J* 7.1 Hz, $CH_2CH_2CO_2$), 2.71 (2 H, t, *J* 7.1 Hz, $CH_2CH_2CO_2$), 2.56—2.76 (2 H, m, CH_2S), 3.06 (1 H, dd, *J* 13.9 and 7.0 Hz, CHHPh), 3.12 (1 H, dd, *J* 13.9 and 5.9 Hz, CHHPh), 3.42 (2 H, s, CH_2CO_2), 3.59 and 3.65 (each 3 H, s, OMe), 3.62—3.71 (2 H, m, SCH_2-pyrr), 3.83 (2 H, br s, $NHCH_2CO_2$), 4.27 (1 H, q, *J* 6.3 Hz, α-H), 4.83 (1 H, q, *J* 6.7 Hz, α-H), 5.06—5.15 (4 H, m, OCH_2Ph), 5.45 (1 H, br s, O_2CNH), 6.41 (1 H, d, *J* 1.9 Hz, pyrr-H), 6.94—7.47 (15 H, m, 3 × Ph), and 9.09 (1 H, br s, pyrr-NH); δ_C(100 MHz) 20.6 ($CH_2CH_2CO_2$), 26.8 (SCH_2pyrr), 29.7 (CH_2CO_2), 33.4 (CH_2S), 34.8 ($CH_2CH_2CO_2$), 37.5 (CH_2Ph), 44.5 ($NHCH_2CO$), 51.5 and 51.9 (2 × OMe), 52.0 and 53.8 (2 × α-C), 67.3 (2 × OCH_2Ph), 112.5, 114.7, 122.0, and 125.5 (4 × pyrrole C), 126.9—129.6 (phenyl CH), 135.0, 136.0, and 136.2 (3 × phenyl C), 156.6 (O_2CNH), and 169.8, 169.9, 170.9, 173.0, and 173.8 (2 × CONH and 3 × CO_2); *m/z* (f.d.) 786 (M^+, 100%).

2-*Formyl*-1-*methylsulphonyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrole* (**36**).—To a stirred suspension of sodium hydride (60% dispersion in oil; 1.00 g, 23.8 mmol) in dry tetrahydrofuran (200 ml), at room temperature under an atmosphere of argon, the formylpyrrole (**15**)[12] (3.00 g, 11.9 mmol) was added as a solid. The mixture was stirred for 1 h at room temperature in the dark, treated with mesyl chloride (4.60 ml, 59.5 mmol), and stirred for a further 1.5 h at room temperature. The reaction was quenched with saturated aqueous sodium hydrogen carbonate (10 ml) and the tetrahydrofuran evaporated. The residue was partitioned between dichloromethane (200 ml) and water (50 ml) and the organic layer was separated, washed with 10% aqueous sodium hydrogen carbonate (100 ml) and water (2 × 100 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate–hexane (1:9 then from 1:1 to 3:2, v/v) as eluant, yielded the N-*mesylpyrrole* (**36**) as needles (3.12 g, 79%), m.p. 64—65 °C (from dichloromethane–ether–hexane) (Found: C, 47.0; H, 5.05; N, 4.0. $C_{13}H_{17}NO_7S$ requires C, 47.1; H, 5.14; H, 4.23%); $\lambda_{max.}$ 291 and 259 nm; $\nu_{max.}$ 3 000 (CH), 2 950 and 2 750 (CH aldehyde), 1 730s (ester), 1 640 (CHO), 1 370s (SO_2), and 1 180 cm^{-1} (SO_2 and C–O ester); δ_H(400 MHz) 2.59 (2 H, t, *J* 7.2 Hz, $CH_2CH_2CO_2$), 2.75 (2 H, t, *J* 7.2 Hz, $CH_2CH_2CO_2$), 3.51 (3 H, s, SO_2Me), 3.68 and 3.72 (each 3 H, s, OMe), 3.83 (2 H, s, CH_2CO_2), 7.32 (1 H, s, pyrr-H), and 9.95 (1 H, s, CHO); *m/z* (f.d.) 331 (M^+, 100%).

2-*Hydroxymethyl*-1-*methylsulphonyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrole* (**39**).—A stirred solution of the *N*-mesylpyrrole (**36**) (250 mg, 0.755 mmol) in dichloromethane–methanol (2:1, v/v), at 0 °C under an

atmosphere of nitrogen, was treated with sodium borohydride (43 mg, 1.13 mmol) over 5 min, and then stirred for a further 40 min at 0 °C before being diluted with dichloromethane (75 ml), washed with 5% aqueous oxalic acid (10 ml) and water (10 ml), dried (Na_2SO_4), filtered, and evaporated to yield the *hydroxymethylpyrrole* (**39**) as needles (209 mg, 83%), m.p. 97.5—98.5 °C (from dichloromethane–ether–hexane) (Found: C, 46.7; H, 5.7; N, 4.25. $C_{13}H_{19}NO_7S$ requires C, 46.8; H, 5.71; N, 4.20%); $\nu_{max.}$ 3 350br (OH), 3 000 and 2 950 (CH), 1 710s (ester), 1 370s (SO_2), 1 180 (SO_2 and C–O ester), and 1 010 cm^{-1} (C–O alcohol); $\lambda_{max.}$ 229 nm; δ_H(250 MHz) 2.52—2.74 (4 H, m, $CH_2CH_2CO_2$), 3.36 (3 H, s, SO_2Me), 3.49 (2 H, s, CH_2CO_2), 3.67 and 3.73 (each 3 H, s, OMe), 4.76 (2 H, s, CH_2OH), and 6.94 (1 H, s, pyrr-H); *m/z* (f.d.) 333 (M^+, 100%).

2-*Hexylthiomethyl*-1-*methylsulphonyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrole* (**45**).—A solution of the hydroxymethylpyrrole (**39**) (300 mg, 0.900 mmol), mesyl chloride (139 µl, 1.80 mmol), and triethylamine (251 µl, 1.80 mmol) in dry dichloromethane (18 ml) was stirred at 0 °C, under an atmosphere of nitrogen, for 0.5 h and then warmed to room temperature and stirred for a further 0.75 h. The mixture was diluted with dichloromethane (75 ml), washed with water (30 ml), 2% hydrochloric acid (30 ml), 10% aqueous sodium hydrogen carbonate (30 ml), and brine (30 ml), and then dried (Na_2SO_4), filtered, and evaporated at high vacuum (0.01 mmHg, 4 h) to give the crude *chloromethylpyrrole* (**42**) as a red oil (315 mg, quantitative) (Found: M^+, 351.0538. $C_{13}H_{19}ClNO_7S$ requires *M*, 351.0544); δ_H(250 MHz; CD_2Cl_2) 2.54—2.75 (4 H, m, $CH_2CH_2CO_2$), 3.32 (3 H, s, SO_2Me), 3.51 (2 H, s, CH_2CO_2), 3.66 and 3.68 (each 3 H, s, OMe), 4.97 (2 H, s, CH_2Cl), and 6.99 (1 H, s, pyrr-H); *m/z* (f.d.) 354 and 352 (MH^+, 33 and 100%).

A stirred solution of the chloromethylpyrrole (**42**) (315 mg, 0.900 mmol) and hexanethiol (127 µl, 0.900 mmol) in dry and degassed tetrahydrofuran (18 ml), at room temperature under an atmosphere of nitrogen, was treated with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (269 µl, 1.80 mmol) causing a white precipitate to form instantaneously. The mixture was stirred in the dark for 17 h after which the solvent was evaporated. The residue was redissolved in dichloromethane (50 ml), and the solution washed with water (2 × 20 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate–hexane (4:6, v/v) as eluant, yielded the *thiomethylpyrrole* (**45**) as needles (263 mg, 69%), m.p. 53—54.5 °C (from ether–hexane) (Found: C, 52.5; H, 7.35; N, 3.25. $C_{19}H_{31}NO_6S_2$ requires C, 52.7; H, 7.16; N, 3.23%); $\lambda_{max.}$ 233 nm; $\nu_{max.}$ 3 000, 2 920, and 2 850 (CH), 1 720s (ester), 1 440 (CH), 1 370s (SO_2), and 1 180 cm^{-1} (SO_2 and C–O ester); δ_H(250 MHz; CD_2Cl_2) 0.88 (3 H, t, *J* 6.6 Hz, Me), 1.24—1.60 (8 H, m, $CH_2CH_2CH_2CH_2$), 2.48—2.67 (6 H, m, $CH_2CH_2CO_2$ and CH_2S), 3.40 (3 H, s, SO_2Me), 3.45 (2 H, s, CH_2CO_2), 3.65 and 3.67 (each 3 H, s, OMe), 4.00 (2 H, s, SCH_2-pyrr), and 6.90 (1 H, s, pyrr-H); δ_C(100 MHz; CD_2Cl_2) 14.2 (Me), 20.8 ($CH_2CH_2CO_2$), 23.0 (CH_2Me), 25.8 (SCH_2-pyrr), 29.0, 29.9, and 30.3 (3 × CH_2), 31.8 (CH_2CO_2), 32.7 (CH_2S), 33.8 ($CH_2CH_2CO_2$), 43.4 (SO_2Me), 51.9 and 52.4 (2 × OMe), 119.2, 120.5, 125.9, and 129.0 (4 × pyrrole C), and 171.5 and 173.4 (2 × CO_2Me); *m/z* (f.d.) 434 (MH^+, 100%).

N-*Benzyloxycarbonylglycyl*-S-[1-*methylsulphonyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-L-*cysteinyl*-L-*phenylalanine Benzyl Ester* (**50**).—A stirred solution of the crude chloromethylpyrrole (**42**) (523 mg, 1.50 mmol) and the thiol tripeptide (**29**) (824 mg, 1.50 mmol) in dry and degassed tetrahydrofuran (20 ml), at room temperature under an atmosphere of argon, was treated with DBU (449 ml, 3.00 mmol) and stirred for 16.5 h in the dark. The solvent was evaporated and the residue partitioned between dichloromethane (50 ml) and water (20 ml). The organic solution was separated, washed with water (4 × 20 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate–hexane (8:2, v/v) as eluant, yielded the *pyrrolyl peptide* (**50**) as an amorphous solid (829 mg, 64%), m.p. 154—157 °C (from dichloromethane–ether–hexane) (Found: C, 58.0; H, 5.75; N, 6.2. $C_{42}H_{48}N_4O_{12}S_2$ requires C, 58.3; H, 5.56; N, 6.48%); $[\alpha]_D$ −12.9° (*c* 1.0 in THF); $\nu_{max.}$ 3 350br (NH), 3 000 and 2 950 (CH), 1 720s (ester and carbamate), 1 660 (amide I), 1 500 (amide II), 1 370s (SO_2), and 1 220 and 1 180 cm^{-1} (C–O ester and SO_2); δ_H(400 MHz; CD_2Cl_2) 2.55—2.68 (4 H, m, $CH_2CH_2CO_2$), 2.75—2.89 (2 H, m, CH_2S), 3.06 (1 H, dd, *J* 13.9 and 6.8 Hz, CHHPh), 3.15 (1 H, dd, *J* 13.9 and 5.7 Hz, CHHPh), 3.22 (3 H, s, SO_2Me), 3.46 (2 H, s, CH_2CO_2), 3.64 and 3.65 (each 3 H, s, OMe), 3.80 and 3.85 (each 1 H, dd, *J* 14.0 and 2.7 Hz, $NHCH_2CO$), 4.02 (2 H, s, SCH_2-pyrr), 4.58 (1 H, q, *J* 6.2 Hz, α-H), 4.80 (1 H, q, *J* 6.7 Hz, α-H), 5.08—5.15 (4 H, m, OCH_2Ph), 5.48 (1 H, br s, O_2CNH), 6.90 (1 H, s, pyrr-H), and 6.87—7.36 (15 H, m, 3 × Ph); δ_C(100 MHz; CD_2Cl_2) 20.7 ($CH_2CH_2CO_2$), 26.7 (SCH_2-pyrr), 30.3 (CH_2CO_2), 33.7 (CH_2S), 34.4 ($CH_2CH_2CO_2$), 38.0 (CH_2Ph), 43.3 ($NHCH_2CO$), 44.8 (SO_2Me) 51.9, 52.6, and 52.8 (2 × OMe and 2 × α-C), 67.4 and 67.6 (2 × OCH_2Ph), 119.6, 121.2 and 126.2 (pyrrole C), 127.4—129.7 (phenyl CH), 135.8, 136.4, and 136.9 (3 × phenyl C), 157.0 (O_2CNH), and 169.6, 170.0, 171.3, 172.0, and 173.4 (2 × CONH and 3 × CO_2); *m/z* (f.d.) 865 (MH^+, 100%).

2-*Formyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethyl*-1-*trifluoromethanesulphonylpyrrole* (**37**).—A stirred solution of the formylpyrrole (**15**) (500 mg, 1.98 mmol) and Hünig's base (689 µl, 3.96 mmol) in dichloromethane (40 ml), at −78 °C under an atmosphere of argon, was treated dropwise with trifluoromethanesulphonic anhydride (598 µl, 3.56 mmol). The solution was stirred for 3 min, quenched with 10% aqueous sodium hydrogen carbonate (10 ml), and rapidly warmed to room temperature. The mixture was washed with further 10% aqueous sodium hydrogen carbonate (20 ml then 30 ml) and water (30 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate–hexane (4:6, v/v) as eluant, yielded the N-*triflylpyrrole* (**37**) as an oil (251 mg, 33%) which crystallised from dichloromethane–ether–hexane, with some difficulty, to give needles, m.p. 38—40 °C (Found: C, 40.2; H, 3.7; N, 3.6. $C_{13}H_{14}F_3NO_7S$ requires C, 40.5; H, 3.64; N, 3.64%); $\lambda_{max.}$ 282 and 256 nm; $\nu_{max.}$(NaCl discs) 2 995 (CH), 1 730s (ester), 1 670 (CHO), 1 440 and 1 420 (CH and SO_2), and 1 220s and 1 160 cm^{-1} (C–F, SO_2, and C–O ester); δ_H(250 MHz; CD_2Cl_2) 2.62 (2 H, t, *J* 7.0 Hz, $CH_2CH_2CO_2$), 2.78 (2 H, t, *J* 7.0 Hz, $CH_2CH_2CO_2$), 3.68 and 3.82 (each 3 H, s, OMe), 3.94 (2 H, s, CH_2CO_2), 7.20 (1 H, s, pyrr-H), and 10.10 (1 H, s, CHO); *m/z* (f.d.) 386 (MH^+, 100%).

2-*Hydroxymethyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethyl*-1-*trifluoromethylsulphonylpyrrole* (**40**).—A stirred solution of the *N*-triflylpyrrole (**37**) (400 mg, 1.03 mmol) in dichloromethane–methanol (2:1, v/v; 18 ml) at 0 °C, under an atmosphere of nitrogen, was treated with sodium borohydride (59 mg, 1.55 mmol) over 5 min and then stirred for a further 10 min at 0 °C. The mixture was diluted with dichloromethane (50 ml), washed with 5% aqueous oxalic acid (20 ml) and brine (20 ml), dried (Na_2SO_4), filtered, and evaporated to dryness to yield the *hydroxymethylpyrrole* (**40**) as an oil (386 mg, 97%) (Found: M^+, 387.0602. $C_{13}H_{16}F_3NO_7S$ requires *M*, 387.0599); $\lambda_{max.}$ 228 nm; $\nu_{max.}$(NaCl discs) 3 400br (OH), 2 995 (CH), 1 730s (ester), 1 440 and 1 420s (CH and SO_2), 1 220 and 1 160 (CF, SO_2, and C–O ester), and 1 010 cm^{-1} (C–O alcohol); δ_H(250 MHz) 2.54—2.76 (5 H, m, $CH_2CH_2CO_2$ and CH_2OH),

3.54 (2 H, s, CH_2CO_2), 3.65 and 3.71 (each 3 H, s, OMe), 4.65 (2 H, d, *J* 6.9 Hz, CH_2OH), and 6.92 (1 H, s, pyrr-H); *m/z* 387 (M^+, 4%), 338 (47), 222 (27), 194 (100), 166 (37), and 152 (32).

2-*Hexylthiomethyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethyl*-1-*trifluoromethylsulphonylpyrrole* (**46**).—A solution of the hydroxymethylpyrrole (**40**) (90 mg, 0.233 mmol), triethylamine (110 μl, 0.792 mmol), and mesyl chloride (61 μl, 0.792 mmol) in dry dichloromethane (4 ml) was stirred at 0 °C, under an atmosphere of nitrogen, for 1 h and then warmed to room temperature and stirred for a further 1 h. The mixture was diluted with dichloromethane (5 ml), washed with water (5 ml), 2% aqueous hydrochloric acid (5 ml), 10% aqueous sodium hydrogen carbonate (5 ml), and brine (5 ml), dried (Na_2SO_4), filtered, and evaporated at high vacuum (0.1 mmHg, 3 h) to give the crude *chloromethylpyrrole* (**43**) as an oil (88 mg, 93%) (Found: M^+, 405.0247. $C_{13}H_{15}ClF_3NO_6S$ requires *M*, 405.0260); δ_H(250 MHz; CD_2Cl_2) 2.56—2.76 (4 H, m, $CH_2CH_2CO_2$), 3.55 (2 H, s, CH_2CO_2), 3.65 and 3.69 (each 3 H, s, OMe), 4.81 (2 H, s, CH_2Cl), and 6.97 (1 H, s, pyrr-H); *m/z* 407 and 405 (M^+, 7 and 20%), 370 (68), 346 (62), 338 (87), 272 (67), 240 (32), and 212 (100).

A stirred solution of the chloromethylpyrrole (**43**) (88 mg, 0.217 mmol) and hexanethiol (31 μl, 0.217 mmol) in dry and degassed tetrahydrofuran (2 ml) at room temperature under an atmosphere of argon was treated with DBU (65 μl, 0.434 mmol), causing a white precipitate to form instantaneously, and stirred in the dark for 16 h. The solvent was evaporated and the residue was taken up in dichloromethane (10 ml), washed with 10% aqueous sodium hydrogen carbonate (5 ml), water (5 ml), and brine (5 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate (10—20%, v/v) in hexane as eluant, yielded the *thiomethylpyrrole* (**46**) as an oil (70 mg, 66%) (Found: M^+, 487.1314. $C_{19}H_{28}F_3NO_6S_2$ requires *M*, 487.1310); $\lambda_{max.}$ 232 nm; $\nu_{max.}$(NaCl discs) 2 950 and 2 930 (CH), 1 730s (ester), 1 440 and 1 420 (CF, CH, and SO_2), and 1 220br, 1 180, and 1 160 cm^{-1} (SO_2 and C–O ester); δ_H(250 MHz; CD_2Cl_2) 0.82 (3 H, t, *J* 6.6 Hz, Me), 1.18—1.51 (8 H, m, $CH_2CH_2CH_2CH_2$), 2.44 (2 H, t, *J* 7.4 Hz, CH_2S), 2.51 (2 H, t, *J* 6.8 Hz, $CH_2CH_2CO_2$), 2.62 (2 H, t, *J* 6.8 Hz, $CH_2CH_2CO_2$), 3.44 (2 H, s, CH_2CO_2), 3.58 and 3.61 (each 3 H, s, OMe), 3.82 (2 H, s, SCH_2-pyrr), and 6.81 (1 H, s, pyrr-H); *m/z* (f.d.) 487 (M^+, 100%).

N-*Benzyloxycarbonylglycyl*-S-[4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethyl*-1-*trifluoromethylsulphonylpyrrol*-2-*ylmethyl*]-L-*cysteinyl*-L-*phenylalanine Benzyl Ester* (**51**).—A solution of the crude chloromethylpyrrole (**43**) (374 mg, 0.922 mmol) and the free-thiol peptide (**29**) (506 mg, 0.922 mmol) in dry and degassed tetrahydrofuran (18 ml) was treated with DBU (276 μl, 1.84 mmol) causing a white precipitate to form instantaneously. The mixture was stirred at room temperature in the dark for 16 h and then evaporated. The residue was redissolved in dichloromethane (50 ml), washed with water (5 × 20 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate–hexane (6:4, v/v) as eluant, yielded the *pyrrolyl peptide* (**51**) as an oil (600 mg, 72%) which was crystallised from dichloromethane–ether–hexane as an amorphous white solid, m.p. 77—80 °C (Found: C, 54.8; H, 4.95; N, 6.1. $C_{42}H_{45}F_3N_4O_{12}S_2$ requires C, 54.9; H, 4.90; N, 6.10%); $[\alpha]_D$ −10.8° (*c* 1.0 in THF); $\nu_{max.}$ 3 350br (NH), 3 000 (CH), 1 720s (ester and carbamate), 1 660 (amide I), 1 500 (amide II), 1 410s (CF and SO_2), and 1 220s and 1 180 cm^{-1} (C–O ester, CF, and SO_2); δ_H(250 MHz; CD_2Cl_2) 2.54—2.72 (4 H, m, $CH_2CH_2CO_2$), 2.74 (1 H, dd, *J* 15.6 and 6.2 Hz, CHHS), 2.92 (1 H, dd, *J* 15.6 and 4.2 Hz, CHHS), 3.05 (1 H, dd, *J* 15.4 and 6.8 Hz, CHHPh), 3.16 (1 H, dd, *J* 15.4 and 6.0 Hz, CHHPh), 3.52 (2 H, s, CH_2CO_2), 3.64 and 3.65 (each 3 H, s, OMe), 3.83 (2 H, br d, *J* 5.6 Hz, $NHCH_2CO$), 3.91 (2 H, s, SCH_2-pyrr), 4.52 (1 H, q, *J* 4.6 Hz, α-H), 4.80 (1 H, q, *J* 6.4 Hz, α-H), 5.08—5.12 (4 H, m, 2 × OCH_2Ph), 5.44 (1 H, br s, O_2CNH), 6.89 (1 H, s, pyrr-H), and 6.78—7.35 (15 H, m, 3 × Ph); δ_C(100 MHz) 20.2 ($CH_2CH_2CO_2$), 25.7 (SCH_2-pyrr), 30.0 (CH_2CO_2), 32.8 (CH_2S), 34.2 ($CH_2CH_2CO_2$), 37.5 (CH_2Ph), 44.3 ($NHCH_2CO$), 51.8, 51.9, 52.5, and 53.7 (2 × α-C and 2 × OMe), 67.2 (2 × OCH_2Ph), 120.3, 123.7, and 127.0 (pyrrole C), 127.1—130.3 (phenyl CH), 135.0, 135.5, and 136.0 (3 × phenyl C), 157.0 (O_2CNH), and 168.9—170.7 (2 × CONH and 3 × CO_2); *m/z* (f.a.b.) 919 (MH^+, 100%).

2-*Formyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethyl*-1-(2-*trimethylsilylethylsulphonyl*)*pyrrole* (**38**).—The formylpyrrole (**15**)[12] (1.00 g, 3.95 mmol) was added as a solid to a stirred suspension of sodium hydride (60% dispersion in oil; 320 mg, 7.90 mmol) in dry tetrahydrofuran (50 ml) at room temperature, under an atmosphere of argon. The mixture was stirred for 0.75 h and, then treated dropwise over 5 min with a solution of the 2-trimethylsilylethanesulphonyl chloride[32] (1.82 g, 9.09 mmol) in dry tetrahydrofuran (10 ml). The mixture was stirred for a further 1.5 h and then quenched with saturated 10% aqueous sodium hydrogen carbonate (90 ml). The organic solvent was evaporated and the aqueous residue partitioned between water (50 ml) and dichloromethane (50 ml). The organic phase was separated, washed with 10% aqueous sodium hydrogen carbonate (50 ml) and brine (25 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Chromatography on silica gel, with ether (60—70%, v/v) in hexane as eluant, gave the *sulphonylpyrrole* (**38**) as an oil (1.20 g, 73%) which could be crystallised from dichloromethane–ether–hexane, with some difficulty, to give needles, m.p. 98—99.5 °C (Found: C, 48.6; H, 6.5; N, 3.15. $C_{17}H_{27}NO_7SiS$ requires C, 48.9; H, 6.50; N, 3.40%) (Found: M^+, 417.1264. $C_{17}H_{27}NO_7SiS$ requires *M*, 417.1277); $\lambda_{max.}$ 288 and 260 nm; $\nu_{max.}$(CH_2Cl_2) 2 950 (CH), 1 720s (ester), 1 660 (CHO), 1 360 (SO_2), and 1 180s and 1 160 cm^{-1} (SO_2 and C–O ester); δ_H(250 MHz) 0.03 (9 H, s, $SiMe_3$), 0.87—0.95 (2 H, m, CH_2Si), 2.59 (2 H, t, *J* 7.1 Hz, $CH_2CH_2CO_2$), 2.75 (2 H, t, *J* 7.1 Hz, $CH_2CH_2CO_2$), 3.45—3.52 (2 H, m, CH_2SO_2), 3.67 and 3.71 (each 3 H, s, OMe), 3.86 (2 H, s, CH_2CO_2), 7.25 (1 H, s, pyrr-H), and 10.01 (1 H, s, CHO); *m/z* 417 (M^+, 8%), 386 (10), 327 (29), 310 (100), 266 (20), and 252 (53).

2-*Hydroxymethyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethyl*-1-(2-*trimethylsilylethylsulphonyl*)*pyrrole* (**41**).—A stirred solution of the sulphonylpyrrole (**38**) (300 mg, 0.719 mmol) and ammonium chloride (64 mg, 1.19 mmol) in dry methanol (15 ml) at 0 °C, under an atmosphere of nitrogen, was treated with sodium borohydride (41 mg, 1.09 mmol) over 5 min. The mixture was stirred for 0.5 h then poured into water (50 ml), saturated with solid sodium chloride, and extracted with dichloromethane (3 × 25 ml). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate (30—40%, v/v) in hexane as eluant, yielded the *hydroxymethylpyrrole* (**41**) as an oil (279 mg, 93%) (Found: M^+, 419.1409. $C_{17}H_{29}NO_7SiS$ requires *M*, 419.1433); $\lambda_{max.}$ 279 and 227 nm; $\nu_{max.}$(CH_2Cl_2) 3 450br (OH), 2 950 (CH), 1 720s (ester), 1 360 (SO_2), 1 180 and 1 160 (SO_2 and C–O ester), and 1 010 cm^{-1} (C–O alcohol); δ_H(400 MHz; CD_2Cl_2) 0.04 (9 H, s, $SiMe_3$), 0.79—0.83 (2 H, m, CH_2Si), 2.55 (2 H, t, *J* 7.9 Hz, $CH_2CH_2CO_2$), 2.70 (2 H, t, *J* 7.9 Hz, $CH_2CH_2CO_2$), 3.44—3.49 (2 H, m, CH_2SO_2), 3.50 (2 H, s, CH_2CO_2), 3.64 and 3.70 (each 3 H, s, OMe), 4.69 (2 H, s, CH_2OH), and 6.93 (1 H, s, pyrr-H); *m/z* 419 (M^+, 4%), 404 (10), 388 (8), 327 (37), 296 (45), 268 (57), 254 (100), 238 (48), 222 (85), and 194 (52).

2-*Hexylthiomethyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxy-*

carbonylmethyl-1-(2-*trimethylsilylethylsulphonyl*)*pyrrole* (**47**).—A solution of the foregoing hydroxymethylpyrrole (**41**) (258 mg, 0.615 mmol), mesyl chloride (162 μl, 2.09 mmol), and triethylamine (292 μl, 2.09 mmol) in dry dichloromethane (10 ml), at 0 °C under an atmosphere of argon, was stirred for 1 h and then warmed to room temperature and stirred for a further 1 h. The mixture was washed with water (5 ml), 2% hydrochloric acid (5 ml), 10% aqueous sodium hydrogen carbonate (5 ml), and brine (5 ml), dried (Na_2SO_4), filtered, and evaporated at high vacuum (0.05 mmHg, 4 h) to give the crude *chloromethylpyrrole* (**44**) as an oil which crystallised as needles (272 mg, quantitative) (Found: M^+, 437.1103. $C_{17}H_{28}NO_6SiSCl$ requires *M*, 437.1095); δ_H(250 MHz; CD_2Cl_2) 0.02 (9 H, s, $SiMe_3$), 0.86—0.93 (2 H, m, CH_2Si), 2.57—2.79 (4 H, m, $CH_2CH_2CO_2$), 3.39—3.47 (2 H, m, CH_2SO_2), 3.51 (2 H, s, CH_2CO_2), 3.65 and 3.67 (each 3 H, s, OMe), 4.97 (2 H, s, CH_2Cl), and 6.97 (1 H, s, pyrr-H); *m*/*z* (f.d.) 440 and 438 (M^+, 33 and 100%).

A stirred solution of the crude chloromethylpyrrole (**44**) (272 mg, 0.615 mmol) and hexanethiol (88 μl, 0.615 mmol) in dry, degassed tetrahydrofuran (4 ml), under an atmosphere of argon, was treated with DBU (186 μl, 1.23 mmol) causing a white precipitate to form instantaneously. The mixture was stirred at room temperature for 16.5 h in the dark and then evaporated. The residue was redissolved in dichloromethane (50 ml), washed with 10% aqueous sodium hydrogen carbonate (25 ml) and water (25 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate (0—20%, v/v) in hexane as eluant, yielded the *thiomethylpyrrole* (**47**) as an oil (218 mg, 68%) (Found: M^+, 519.2130. $C_{23}H_{41}NO_6SiS_2$ requires *M*, 519.2113); $\lambda_{max.}$ 230 nm; $\nu_{max.}$(CH_2Cl_2) 2 995 (CH), 1 720s (ester), 1 410 (CH and SO_2), and 1 180 and 1 160 cm^{-1} (SO_2 and C–O ester); δ_H(250 MHz; CD_2Cl_2) 0.03 (9 H, s, $SiMe_3$), 0.86—0.93 (5 H, m, Me and CH_2Si), 1.27—1.54 (8 H, m, $CH_2CH_2CH_2CH_2$), 2.49—2.67 (6 H, m, $CH_2CH_2CO_2$ and CH_2S), 3.46 (2 H, s, CH_2CO_2), 3.54—3.61 (2 H, m, CH_2SO_2), 3.65 and 3.67 (each 3 H, s, OMe), 4.01 (2 H, s, SCH_2-pyrr), and 6.88 (1 H, s, pyrr-H); *m*/*z* 519 (M^+, 10%), 402 (33), 354 (8), 338 (45), 310 (100), and 178 (40).

2-*Hexylthiomethyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrole* (**33**).—A solution of the foregoing sulphonylpyrrole (**47**) (95 mg, 0.183 mmol) and tetrabutylammonium fluoride (TBAF) trihydrate (173 mg, 0.549 mmol) in double-distilled dimethylformamide (5 ml), under an atmosphere of nitrogen, was stirred at room temperature for 0.5 h. The mixture was diluted with ether (40 ml), washed vigorously with water (5 × 30 ml), dried (Na_2SO_4), filtered, and evaporated to dryness to give the *thiomethylpyrrole* (**33**) as an oil (56 mg, 89%). Analytical data as above.

4-(2-*Carboxyethyl*)-3-*carboxymethyl*-2-*hexylthiomethylpyrrole* (**49**).—A solution of the hexylthiomethylpyrrole (**33**) (56 mg, 0.163 mmol) and 1M aqueous potassium hydroxide (360 μl, 0.359 mmol) in methanol (3 ml) was stirred, under an atmosphere of nitrogen, for 6 h during which time two further aliquots of 1M potassium hydroxide (360 μl, 0.359 mmol) were added after 2 and 4 h respectively. The solution was neutralised with concentrated hydrochloric acid followed by 2% hydrochloric acid and then freeze-dried to give the *disodium salt* of (**49**) mixed with inorganic residues; no further purification was undertaken (Found: M^+, 327.1502. $C_{16}H_{25}NO_4S$ requires *M*, 327.1504); δ_H(400 MHz; D_2O at pD 7) 0.83 (3 H, t, *J* 6.5 Hz, Me), 1.22—1.49 (8 H, m, $CH_2CH_2CH_2CH_2$), 2.43 (4 H, t, *J* 7.0 Hz, $CH_2CH_2CO_2$ and CH_2S), 2.60 (2 H, t, *J* 7.0 Hz, $CH_2CH_2CO_2$), 3.37 (2 H, s, CH_2CO_2), and 3.70 (2 H, s, SCH_2-pyrr); δ_C(100 MHz; D_2O at pD 7) 16.2 (Me), 23.9 ($CH_2CH_2CO_2$), 24.8 (CH_2Me), 28.9 (SCH_2-pyrr), 30.9, 31.7, and 33.7 ($CH_2CH_2CH_2$), 33.9 (CH_2CO_2), 34.2 (CH_2SCH_2-pyrr), 39.5 ($CH_2CH_2CO_2$), 116.3, 124.3, and 128.0 (pyrrole C), and 182.3 and 184.3 (2 × CO_2); *m*/*z* (f.d.) 328 (MH^+, 100%).

4-(2-*Carboxyethyl*)-3-*carboxymethyl*-2-*hexylthiomethyl*-1-(2-*trimethylsilylethanesulphonyl*)*pyrrole* (**48**).—A solution of the thiomethylpyrrole (**47**) (81 mg, 0.156 mmol) and 1M aqueous sodium hydroxide (624 μl, 0.624 mmol) in methanol (2 ml) was stirred for 3 h at room temperature. A further portion of 1M aqueous sodium hydroxide (312 μl, 0.312 mmol) was added. The mixture was stirred for a further 2 h, diluted with water (10 ml), cooled to 0 °C, and acidified to below pH 1 with a few drops of concentrated hydrochloric acid. The resulting precipitate was extracted into ethyl acetate (5 × 5 ml). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. The residue (73 mg, 95%) was recrystallised from ethyl acetate–hexane to give the *diacid pyrrole* (**48**) as needles (53 mg, 70%), m.p. 132—134 °C (Found: C, 51.1; H, 7.6; N, 2.65%; M^+, 491.1828. $C_{21}H_{37}NO_6SiS_2$ requires C, 51.3; H, 7.54; N, 2.85%; *M*, 491.1831); $\lambda_{max.}$ 231 nm; $\nu_{max.}$(CH_2Cl_2) 3 600 and 3 400br (OH), 2 900 and 2 850 (CH), 1 700s (ester), 1 360 (CH and SO_2), and 1 180 cm^{-1} (SO_2); δ_H(250 MHz; CD_3COCD_3) 0.05 (9 H, s, $SiMe_3$), 0.86—0.94 (5 H, m, Me and CH_2Si), 1.27—1.65 (8 H, m, $CH_2CH_2CH_2CH_2$), 2.55—2.77 (6 H, m, $CH_2CH_2CO_2$ and CH_2S), 3.56 (2 H, s, CH_2CO_2), 3.62—3.72 (2 H, m, CH_2SO_2), 4.10 (2 H, s, SCH_2-pyrr), 6.99 (1 H, s, pyrr-H), and 10.74 (2 H, br s, CO_2H); *m*/*z* (f.d.) 491 (M^+, 100%).

N-*Benzyloxycarbonylglycyl*-S-[4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethyl*-1-(2-*trimethylsilylethanesulphonyl*)-*pyrrol*-2-*ylmethyl*]-L-*cysteinyl*-L-*phenylalanine Benzyl Ester* (**52**).—A stirred solution of the crude chloromethylpyrrole (**44**) (281 mg, 0.642 mmol) and the free-thiol tripeptide (**29**) (353 mg, 0.642 mmol) in dry, degassed tetrahydrofuran (10 ml), under an atmosphere of argon, was treated with DBU (192 μl, 1.28 mmol) and stirred at room temperature in the dark for 16 h by which time a white precipitate was observed. The solvent was evaporated and the residue redissolved in dichloromethane (10 ml), washed with 10% aqueous sodium hydrogen carbonate (5 ml), 2% hydrochloric acid, and brine (5 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Flash chromatography on silica gel, with ethyl acetate (40—60%, v/v) in hexane as eluant, yielded the *pyrrolyl peptide* (**52**) as a foam (456 mg, 75%) (Found: MH^+, 951.3289. $C_{46}H_{59}N_4O_{12}SiS_2$ requires *M*H, 951.3339); $[\alpha]_D$ −11.5° (*c* 1.0 in THF); $\nu_{max.}$(CH_2Cl_2) 3 400br (NH), 2 995br (CH), 1 710s (ester and carbamate), 1 670 (amide I), 1 500m (amide II), 1 360 (SO_2 and CH), and 1 180s cm^{-1} (C–O ester and SO_2); δ_H(400 MHz; CD_3COCD_3) 0.03 (9 H, s, $SiMe_3$), 0.83—0.86 (2 H, m, CH_2Si), 2.56—2.66 (4 H, m, $CH_2CH_2CO_2$), 2.74 (1 H, dd, *J* 14.2 and 8.0 Hz, C*H*HS), 2.99 (1 H, dd, *J* 14.2 and 5.6 Hz, CH*H*S), 3.05 (1 H, dd, *J* 13.8 and 7.8 Hz, C*H*HPh), 3.16 (1 H, dd, *J* 13.8 and 6.0 Hz, CH*H*Ph), 3.57 (2 H, s, CH_2CO_2), 3.61 and 3.62 (each 3 H, s, OMe), 3.61—3.65 (2 H, m, CH_2SO_2), 3.88 (2 H, br d, *J* 5.7 Hz, $NHCH_2CO$), 4.09 and 4.13 (each 1 H, d, *J* 13.7 Hz, SCH_2-pyrr), 4.72—4.76 (2 H, m, 2 × α-H), 5.09 and 5.13 (each 2 H, s, OCH_2Ph), 6.66 (1 H, t, *J* 4.0 Hz, O_2CNH), 6.96 (1 H, s, pyrr-H), 7.18—7.40 (15 H, m, 3 × Ph), 7.56 (1 H, d, *J* 8.3 Hz, CONH), and 7.80 (1 H, d, *J* 7.7 Hz, CONH); δ_C(100 MHz) 9.80 (CH_2Si), 20.1 ($CH_2CH_2CO_2$), 25.9 (SCH_2-pyrr), 29.9 (CH_2CO_2), 33.2 ($CH_2CH_2CO_2$), 34.1 (CH_2S), 37.5 (CH_2Ph), 44.1 ($NHCH_2CO$), 51.6, 52.0, 52.2, and 52.4 (2 × OMe and 2 × α-C), 53.6 (CH_2SO_2), 66.9 and 67.2 (2 × OCH_2Ph), 120.1, 120.6, and 124.4 (pyrrole C), 126.9—129.1 (phenyl CH), 135.0, 135.5, and 136.0 (3 × phenyl C), 156.7 (O_2CNH), and 169.1, 169.6, 170.7, 171.5, and 173.0 (2 × CONH and 3 × CO_2); *m*/*z* (f.d.) 950 (M^+, 100%).

Glycyl-S-[4-(2-*carboxyethyl*)-3-*carboxymethylpyrrol*-2-*yl*-

methyl]-L-*cysteinyl*-L-*phenylalanine* (**6**).—A solution of the pyrrolyl peptide (**52**) (250 mg, 0.265 mmol) and sodium iodide (318 mg, 2.12 mmol) in dry acetonitrile (5 ml), under an atmosphere of argon, was treated with freshly distilled trimethylsilyl chloride (269 μl, 2.12 mmol) and stirred for 30 h in the dark. The mixture was carefully poured into a vigorously stirred two-phase system of saturated aqueous sodium hydrogen carbonate (12.5 ml) and ethyl acetate (20 ml). The organic layer was separated and the aqueous layer washed with further ethyl acetate (4 × 10 ml). The combined organic extracts were washed with 10% aqueous sodium thiosulphate (20 ml) and water (20 ml), dried (Na_2SO_4), filtered, and evaporated to a minimum volume. The residue was purified by preparative t.l.c. on alumina (1.5 mm thick plates), with methanol–dichloromethane (2:98, v/v) as eluant, giving the crude benzyloxycarbonyl-deprotected product (**53**) (91 mg) as a white solid.

A solution of (**53**) in distilled dimethylformamide (2.5 ml) was treated with tetrabutylammonium fluoride (TBAF) trihydrate (106 mg, 3.36 mmol) and stirred, under an atmosphere of argon, for 1 h. The mixture was diluted with ethyl acetate (15 ml), washed vigorously with water (10 × 5 ml), dried (Na_2SO_4), filtered, and evaporated to give the crude deprotected product (**54**) as a pale yellow oil (39 mg).

A solution of (**54**) (39 mg) in methanol (600 μl) was treated with 1M aqueous potassium hydroxide (600 μl) followed by a further volume of methanol (600 μl) and then stirred at room temperature, under an atmosphere of argon, for 4 h. The mixture was made neutral with 0.5M hydrochloric acid and then freeze-dried. The aqueous dimethylformamide solution obtained above was freeze-dried and partitioned between water (2 ml) and ethyl acetate (3 × 3 ml). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to give more crude (**54**) as a pale yellow oil (31 mg). A methanolic solution of recovered (**54**) (31 mg) was treated with 1M potassium hydroxide, neutralised, and freeze-dried in the same way as before.

Both freeze-dried residues were purified by reverse-phase h.p.l.c. The column was pre-equilibriated with 50 mM aqueous ammonium hydrogen carbonate and was eluted at 5.6 ml/min with a gradient of acetonitrile (0—15%, v/v) in the same buffer; gradient volume 142 ml. Elution was continuously monitored at 214 nm. Appropriate fractions, collected on ice, were freeze-dried twice to give the *pyrrolyl peptide* (**6**) as a solid (25 mg, 18%). The product was stored in the cold, under anhydrous argon, and with the exclusion of light (Found: MH^+, 535.1836. $C_{24}H_{31}N_4O_8S$ requires MH, 535.1862); δ_H(400 MHz; D_2O at pD 8) 2.33 (2 H, t, *J* 6.9 Hz, $CH_2CH_2CO_2$), 2.54 (2 H, t, *J* 6.9 Hz, $CH_2CH_2CO_2$), 2.62 (1 H, dd, *J* 14.0 and 8.8 Hz, CHHS), 2.79 (1 H, dd, *J* 14.0 and 5.5 Hz, CHHS), 2.89 (1 H, dd, *J* 13.9 and 8.2 Hz, CHHPh), 3.14 (1 H, dd, *J* 13.9 and 4.8 Hz, CHHPh), 3.25 (2 H, s, CH_2CO_2), 3.62 and 3.70 (each 1 H, d, *J* 14.5 Hz, SCH_2-pyrr), 3.68 (2 H, s, $NHCH_2CO$), 4.24 and 4.41 (each 1 H, m, α-H), 6.50 (1 H, s, pyrr-H), and 7.17—7.30 (5 H, m, Ph); δ_C(100 MHz; D_2O at pD 6) 24.6 ($CH_2CH_2CO_2$), 29.6 (SCH_2-pyrr), 35.3 (CH_2CO_2), 35.6 (CH_2S), 40.5 ($CH_2CH_2CO_2$), 40.7 (CH_2Ph), 43.6 ($^+NH_2CH_2CO$), 56.3 and 59.1 (2 × α-C), 117.5, 117.9, 125.5, and 127.9 (4 × pyrrole C), 129.7, 131.4, and 132.3 (phenyl CH), 140.3 (phenyl C), and 163.2, 170.6, 173.9, 180.4, and 184.1 (2 × CONH and 3 × CO_2); *m/z* [f.a.b.; thioglycerol–glycerol (1:1, v/v)–H_2SO_4] 535 (MH^+, 100%), 520 (2, $MH^+ - NH$), 491 (4, $MH^+ - CO_2$), 478 (1, $MH^+ - NHCH_2CO$), 463 (1, $MH^+ - NHCH_2CONH$), 387 (2, $MH^+ - CHCH_2PhCO_2$), 326 (45, MH^+ – pyrrole), and 166 (76, $^+H_3NCHCH_2PhCO_2H$); *m/z* [f.a.b.; aminoglycerol] 533 ($M^- - H$, 100%), 518 (12, $M^- - NH_2$), 489 (18, $M^- - H - CO_2$), 476 (8, $M^- - H - NHCH_2CO$), 324 (100, $M^- - H$ – pyrrole), 309 (28, $M^- - NH_2$ – pyrrole), and 164 (92, $^-NHCHCH_2PhCO_2H$).

Stability of Pyrrolyl Peptides in Aqueous Solution at Different pH *Values*.—(a) Lysyl pyrrolyl peptide (**5**): a standard solution of (**5**) (5 mg, 8.9 μmol) in 50 mM aqueous ammonium hydrogen carbonate (500 μl) was prepared and aliquots (50 μl) were diluted into a series of solutions (850 μl) at different pH values. The solutions used were 0.1M aqueous sodium hydroxide (pH 12.20), 0.1M aqueous sodium phosphate (pH 8.09), 0.1M aqueous sodium phosphate (pH 8.09, for incubation at 37 °C for 48 h), 0.1M aqueous sodium acetate (pH 5.03), and 0.1M hydrochloric acid (pH 1.20). All pH values were measured at room temperature.

Immediately after mixing, an aliquot (100 μl) was removed from each mixture and diluted into 50 mM aqueous ammonium hydrogen carbonate (500 μl) prior to application onto a PEP RPC HR 5/5 reverse-phase column attached to a Pharmacia fast protein liquid chromatography (f.p.l.c.) system. This column had been equilibriated with 50 mM aqueous ammonium hydrogen carbonate and was eluted at 0.7 ml/min with a linear gradient of acetonitrile (0—15%, v/v) in the same buffer; the gradient volume was 18 ml. The eluant was continuously monitored at 214 nm.

Each mixture above was left at room temperature or incubated at 37 °C and periodically analysed by reverse-phase h.p.l.c. (as described above) for up to 48 h from the initial time of mixing. The area under the peak due to pyrrolyl peptide (**5**) was determined as a percentage of the peak area at time zero.

(b) Cysteinyl pyrrolyl peptide (**6**): the experiment was repeated in essentially the same way as above.

^{13}C *N.m.r. Chemical Shift Variation with* pD *of the Pyrrole* 11-*Methylene Group of Pyrrolyl Peptides* (**5**) *and* (**6**).—(a) Lysyl pyrrolyl peptide (**5**): a solution of the lysyl pyrrolyl peptide (**5**) (*ca.* 7 mg, 12.5 μmol) and 50 mM sodium phosphate buffer (pH 8.0) in water (400 μl) was prepared for ^{13}C n.m.r. spectroscopy by the addition of deuterium oxide (100 μl) and sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate (TSP-d_4) (*ca.* 2 mg). The ^1H-decoupled (Waltz decoupling) 100.6 MHz ^{13}C n.m.r. spectrum was acquired at ambient temperature with pulse width = 90°, acquisition time = 0.655 s, size of data table = 32 K, and number of transients = 5 952. Different pH conditions were created *in situ* by the sequential addition of solution aliquots, detailed below, directly to the n.m.r. tube. After thorough mixing, the next ^1H-decoupled ^{13}C n.m.r. spectrum was acquired directly. These further spectra were acquired using essentially the same acquisition parameters as above; each further spectrum was accumulated over *ca.* 1 h resulting in *ca.* 4 000—7 000 transients.

The pH conditions used were: (1) pH 8.0, (2) pH 6.8 [addition of 0.5M aqueous phosphoric acid (10 μl) to (1)], (3) pH 5.7 [addition of 0.5M aqueous phosphoric acid (10 μl) to (2)], (4) pH 3.1 [addition of 0.5M aqueous phosphoric acid (30 μl) to (3)], (5) pH 11.1 [addition of 4M aqueous sodium hydroxide (15 μl) to (4)]. The pH values were measured *in situ* using an Aldrich 3.5 mm diameter (x-long) pH-electrode designed for pH measurements in n.m.r. tubes.

Where there were assignment ambiguities between the 11-methylene group ($NHCH_2$-pyrr) and the glycine ($NHCH_2CO$) resonances, these were resolved by recording a selective ^1H-decoupled 100.6 MHz ^{13}C n.m.r. spectrum at the appropriate pH using the same acquisition parameters as above. Selective decoupling of the protons corresponding to multiplets centred at δ_H 4.19 p.p.m. ($NHCH_2$-pyrr and α-H) gave the 11-methylene group as a singlet in the ^{13}C n.m.r. spectrum whilst the glycine methylene group appeared as a triplet with reduced coupling constant.

(b) Cysteinyl pyrrolyl peptide (**6**): the experiment was repeated essentially as above with cysteinyl pyrrolyl peptide (**6**) (*ca.* 7 mg, 13 μmol). In this case, no assignment ambiguities concerning the

pyrrole 11-methylene group (SCH_2-pyrr) needed to be resolved by selective ^{1}H-decoupled ^{13}C n.m.r. spectroscopy.

Acknowledgements

Grateful acknowledgement is made to Dr. G. J. Hart for his help with the chromatographic work and to the S.E.R.C. and Roche Products Ltd. for financial support.

References

1 Part 33, A. D. Miller, L. C. Packman, G. J. Hart, P. R. Alefounder, C. Abell, and A. R. Battersby, *Biochem. J.*, in the press.
2 F. J. Leeper, *Nat. Prod. Rep.*, 1985, **2**, 19 and 561; *ibid.*, 1987, **4**, 441; A. R. Battersby, C. J. R. Fookes, G. W. J. Matcham, and E. McDonald, *Nature (London)*, 1980, **285**, 17.
3 A. R. Battersby, C. J. R. Fookes, K. E. Gustafson-Potter, E. McDonald, and G. W. J. Matcham, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2427.
4 P. M. Jordon and A. Berry, *Biochem. J.*, 1981, **195**, 177.
5 A. R. Battersby, C. J. R. Fookes, G. W. J. Matcham, E. McDonald, and R. Hollenstein, *J. Chem. Soc., Perkin Trans. 1*, 1983, 3031; A. R. Battersby, C. J. R. Fookes, G. J. Hart, G. W. J. Matcham, and P. S. Pandey, *J. Chem. Soc., Perkin Trans. 1*, 1983, 3041.
6 J. N. S. Evans, G. Burton, P. E. Fagerness, N. E. Mackenzie, and A. I. Scott, *Biochemistry*, 1986, **25**, 905.
7 G. J. Hart, A. D. Miller, F. J. Leeper, and A. R. Battersby, *J. Chem. Soc., Chem. Commun.*, 1987, 1762.
8 G. J. Hart, A. D. Miller, and A. R. Battersby, *Biochem. J.*, 1988, **252**, 909; U. Beifuss, G. J. Hart, A. D. Miller, and A. R. Battersby, *Tetrahedron Lett.*, 1988, **29**, 2591.
9 A. D. Miller, G. J. Hart, L. C. Packman, and A. R. Battersby, *Biochem. J.*, 1988, **254**, 915.
10 P. M. Jordan and M. J. Warren, *FEBS Lett.*, 1987, **225**, 87.
11 P. M. Jordan, M. J. Warren, H. J. Williams, N. J. Stolowich, C. A. Roessner, S. K. Grant, and A. I. Scott, *FEBS Lett.*, 1988, **235**, 189; A. I. Scott, N. J. Stolowich, H. J. Williams, M. D. Gonzalez, C. A. Roessner, S. K. Grant, and P. Pichon, *J. Am. Chem. Soc.*, 1988, **110**, 5898.
12 J.-H. Fuhrhop and K. M. Smith in 'Porphyrins and Metalloporphyrins,' ed. K. M. Smith, Elsevier, Amsterdam, 1975, ch. 19; A. R. Battersby, C. J. R. Fookes, M. J. Meegan, E. McDonald, and H. K. W. Wurziger, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2786.
13 M. Bodansky, Y. S. Klausner, and M. A. Ondetti in 'Peptide Synthesis,' John Wiley, New York, 1976.
14 J. P. Greenstein and M. Winitz in 'Chemistry of the Amino Acids,' John Wiley, New York, 1961, vol. 2, p. 891.
15 M. Bodansky and V. du Vigneaud, *J. Am. Chem. Soc.*, 1959, **81**, 5688.
16 W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.
17 J. S. Davies and A. K. Mohammed, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2982.
18 B. Halpern and D. E. Nitecki, *Tetrahedron Lett.*, 1967, 3031.
19 R. F. Borch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, 1971, **93**, 2897; M.-H. Boutigue and R. Jacquesy, *Bull. Soc. Chim. Fr.*, 1973, 750.
20 K. Taguchi and F. H. Westheimer, *J. Org. Chem.*, 1971, **36**, 1570; F. M. F. Chen and T. P. Forrest, *Can. J. Chem.*, 1973, **51**, 881.
21 C. L. Bumgardner, E. L. Lawton, and J. G. Carver, *J. Org. Chem.*, 1972, **37**, 407.
22 M. K. Anwer and A. F. Spatola, *Synthesis*, 1980, 929; S. H. Nakagawa, H. S. H. Lau, F. J. Kezdy, and E. T. Kaiser, *J. Am. Chem. Soc.*, 1985, **107**, 7087.
23 R. A. Jones and G. P. Bean in 'The Chemistry of the Pyrroles,' Academic Press, London, 1977, p. 355.
24 L. Zervas and I. Photaki, *J. Am. Chem. Soc.*, 1962, **84**, 3887.
25 W. M. Stark, M. G. Baker, F. J. Leeper, P. R. Raithby, and A. R. Battersby, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1187.
26 A. R. Battersby, M. Ihara, E. McDonald, J. Saunders, and R. J. Wells, *J. Chem. Soc., Perkin Trans. 1*, 1976, 283; K. Ohno and J. Tsuji, *J. Am. Chem. Soc.*, 1968, **90**, 99.
27 A. Abell, unpublished work, Cambridge, 1986.
28 R. K. Crossland and K. L. Servis, *J. Org. Chem.*, 1970, **35**, 3195.
29 A. M. Felix, *J. Org. Chem.*, 1974, **39**, 1427.
30 C. W. Roberts, *J. Am. Chem. Soc.*, 1954, **76**, 6203.
31 M. E. Jung and M. A. Lyster, *J. Am. Chem. Soc.*, 1977, **99**, 968; G. A. Olah and S. C. Narang, *Tetrahedron*, 1982, **38**, 2225; R. J. Parry, A. E. Mizusawa, I. C. Chiu, M. V. Naidu, and M. Ricciardone, *J. Am. Chem. Soc.*, 1985, **107**, 2512.
32 S. M. Weinreb, D. M. Demko, T. A. Lessen, and J. P. Demers, *Tetrahedron Lett.*, 1986, **27**, 2099.
33 G. W. K. Cavill and R. J. Quinn, *Aust. J. Chem.*, 1973, **26**, 595.
34 G. A. Olah, S. C. Narang, B. G. B. Gupta, and R. Malhotra, *J. Org. Chem.*, 1979, **44**, 1247.
35 C. Gonzalez, R. Greenhouse, R. Tallabs, and J. M. Muchowski, *Can. J. Chem.*, 1983, **61**, 1697; J. M. Muchowski and D. R. Solas, *J. Org. Chem.*, 1984, **49**, 203.
36 R. A. Dwek in 'Nuclear Magnetic Resonance in Biochemistry,' Clarendon Press, Oxford, 1973, p. 149.
37 W. J. Horsley and H. Sternlicht, *J. Am. Chem. Soc.*, 1968, **90**, 3738.
38 W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
39 R. Keese, R. K. Müller, and T. P. Toube in 'Fundamentals of Preparative Organic Chemistry,' Ellis Horwood, Chichester, 1982, p. 35.
40 D. D. Perrin and W. L. F. Armarego in 'Purification of Laboratory Chemicals,' Pergamon Press, Oxford, 3rd Edition, 1988.

Received 6th December, 1988; *Paper* 8/04815K