Amino-acids and Peptides. Part 45.[1] The Protection of the Thiol Function of Cysteine and the Imidazole-N of Histidine by the Diphenyl-4-pyridylmethyl Group

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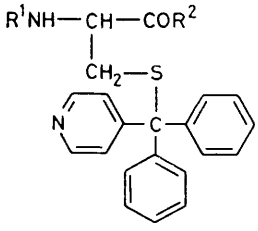
S-(Diphenyl-4-pyridylmethyl)-L-cysteine (1) and its derivatives (2)—(7) have been prepared and used in peptide synthesis. In contrast to the analogous *S*-trityl group, this protection is stable in acid but it is cleaved readily by zinc–acetic acid, by mercury(II) acetate, by iodine, and by electrolytic reduction. *N*(*Im*)-Diphenyl-4-pyridylmethyl-L-histidine derivatives (9)—(13) are also reported; the protecting group is again stable to acid but cleaved by hydrogenolysis, by zinc–acetic acid, and by electrolytic reduction, and it has been used in the synthesis of L-histidyl-L-leucine, -L-phenylalanine, and -glycine.

We have been examining new protecting groups in which the stability to acid has been increased by the introduction of a basic site. In earlier work [2] we noted that 4-picolyl esters are markedly more stable to acid than are benzyl esters, and yet they are removed more readily by reductive methods, *e.g.* by electrolytic reduction [3] and by zinc–acetic acid.[4] We have shown that 4-picolyl provides very stable protection for hydroxy and thiol groups,[3,5] being cleaved in the first case by hydrogenolysis and in both cases by electrolytic reduction. Among other groups examined, 1,4-dimethylpiperidin-4-yloxycarbonyl provides amino-protection rather more stable to acid than is t-butoxycarbonyl stable to hydrogenolysis, and removed by hydrogen bromide in acetic acid.[6] A different type of acid-stable protection arises from the incorporation of dimethylcarbamoyl groups, *e.g.* in the benzyl nucleus; *O*-4-dimethylcarbamoylbenzyltyrosine is very much more stable to acid than is *O*-benzyltyrosine itself.[7]

We describe here the use of the diphenyl-4-pyridylmethyl group for the protection of the thiol group of cysteine and the imidazole-N of histidine. For cysteine, there is still need of additional types of protection for use in conjugation with others such as acetamidomethyl or trityl to allow the selective formation of disulphide bonds. For histidine, there is in our view no entirely satisfactory protection at present available, those in current use being either too unstable during synthesis [*e.g.* *N*(*Im*)-acyl types] or difficult to remove completely (*e.g.* benzyl). Preliminary reports on parts of the present work have appeared.[8]

RESULTS AND DISCUSSION

Diphenyl-4-pyridylmethanol [9] reacted with L-cysteine hydrochloride and boron trifluoride–ether in acetic acid, giving *S*-(diphenyl-4-pyridylmethyl)-L-cysteine (1).† This was completely stable to 45% hydrogen bromide in acetic acid and to trifluoroacetic acid for 48 h at room temperature, the starting material being recovered unchanged in each case in high yield. This contrasts with *S*-tritylcysteine, with which an equilibrium is rapidly established in acid; [10] that no such equilibrium exists in the present case was shown by addition of a trifluoroacetic acid solution to water, when no degradation products were detected.



(1) R^1=H, R^2=OH
(2) R^1=Me_3COCO, R^2=OH
(3) R^1=Me_3COCO, R^2=OMe
(4) R^1=Me_3COCO, R^2=OEt
(5) R^1=Me_3COCO, R^2=OC_6Cl_5
(6) R^1=$PhCH_2OCO$, R^2=OH
(7) R^1=$PhCH_2OCO$, R^2=$OC_6H_2Cl_3$—2,4,5

S-(Diphenyl-4-pyridylmethyl)-L-cysteine was converted into the t-butoxycarbonyl derivative (2) by means of t-butoxycarbonyl azide, thence by diazomethane into

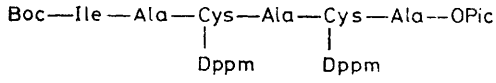
† Abbreviations follow the I.U.P.A.C.-I.U.B. rules, reprinted in the Chemical Society Specialist Periodical Report 'Amino-acids, Peptides, and Proteins,' The Chemical Society, London 1972, vol. 4, p. 441. Chiral amino-acids are of the L-configuration. Dppm = diphenyl-4-pyridylmethyl; Pic = 4-pyridylmethyl.

the methyl ester (3), and by ethanol and dicyclohexylcarbodi-imide to the ethyl ester (4). The pentachlorophenyl ester (5) was prepared using pentachlorophenyl trichloroacetate,[11] and (in lower yield) from pentachlorophenol and dicyclohexylcarbodi-imide. The benzyloxycarbonyl derivative (6) was prepared from (1) by means of benzyl succinimido carbonate,[12] and the 2,4,5-trichlorophenyl ester (7) was prepared using the phenol and dicyclohexylcarbodi-imide. Active esters of cysteine derivatives are abnormally susceptible to racemisation in the presence of base,[13] and we therefore compared the fall in optical rotation of compound (7) in chloroform containing triethylamine with that for the *S*-benzyl analogue; the half-times under the chosen conditions were 35 and 40 min, respectively.

The protecting group was removed from *S*-(diphenyl-4-pyridylmethyl)-L-cysteine by zinc–80% acetic acid within 15 min at room temperature, by mercury(II) acetate in water within 15 min, and by electrolytic reduction [14] in acetic acid–hydrochloric acid–water at a mercury cathode in 45 min; from the last reaction L-cystine was isolated in 91% yield. The t-butoxycarbonyl derivative (2) reacted with iodine–80% acetic acid within 1 h, giving authentic di-t-butoxycarbonyl-L-cystine in 81% yield.

N-t-Butoxycarbonyl-*S*-acetamidomethyl-L-cysteine methyl ester was shown to be stable to zinc–acetic acid during 24 h, and it is also stable to electrolytic reduction.[15] It should therefore be possible to remove the *S*-diphenyl-4-pyridylmethyl group selectively in the presence of *S*-acetamidomethyl by zinc–acetic acid or by electrolytic reduction; alternatively, it should be possible to remove *S*-trityl by acid (in the presence of a competing nucleophile to upset the equilibrium) while the *S*-diphenyl-4-pyridylmethyl group remains.

As an example of the use of the new protected cysteine, we report the synthesis of the protected hexapeptide (8)



(8)

(related to mast-cell degranulating peptide [16]) (see Table 1). The synthesis used the picolyl ester 'handle' procedure [2] and it was noted that the basic nature of the *S*-protection assisted the extraction of the intermediates into aqueous citric acid and their adsorption on the ion-exchanger. The protected peptide (8) was smoothly deprotected by treatment with mercury(II) acetate in

TABLE 1

Synthesis of Boc-Ile-Ala-Cys (Dppm)-Ala-Cys (Dppm)-Ala-OPic: protected peptide intermediates

(14) Boc-Cys (Dppm)-Ala-OPic
(15) Boc-Ala-Cys (Dppm)-Ala-OPic
(16) Boc-Cys (Dppm)-Ala-Cys (Dppm)-Ala-OPic
(17) Boc-Ala-Cys (Dppm)-Ala-Cys (Dppm)-Ala-OPic
(8) Boc-Ile-Ala-Cys (Dppm)-Ala-Cys (Dppm)-Ala-OPic

Compound [a]	Amino component (mmol) [b]	Acylating component [c] (mmol)	Yield (%)	$[\alpha]_D^{22}$ (°) [d]	R_F
(14)	Boc-Ala-OPic (10.0) [e]	Boc Cys(Dppm) (10.5) [f]	99	−7	0.59 (A2), 0.58 (E4)
(15)	Compound (14) (3.95)	Boc-Ala (4.8)	99 [g]	−23	0.53 (A2), 0.47 (E4)
(16)	Compound (15) (3.38)	Boc-Cys(Dppm) (4.2) [f]	93	−13	0.52 (A2), 0.46 (E4)
(17)	Compound (16) (2.47)	Boc-Ala (3.0)	90	−24	0.49 (A2), 0.43 (E4)
(8)	Compound (17) (1.94)	Boc-Ile (3.0)	80 [h]	−25 [i]	0.55 (A2), 0.59 (G3)

Compound	Found (%) C	H	N	S	Formula	Required (%) C	H	N	S
(14)	66.2	6.5	8.55	5.1	$C_{35}H_{38}N_4O_5S\cdot0.5H_2O$	66.1	6.2	8.8	5.05
(15)	63.15	6.15	9.9	4.6	$C_{38}H_{43}N_5O_6S\cdot1.5H_2O$	62.95	6.4	9.65	4.4
(16)	66.45	6.0	9.3	6.05	$C_{59}H_{61}N_7O_7S_2\cdot H_2O$	66.7	6.0	9.25	6.05
(17)	64.7	6.25	9.75	5.45	$C_{62}H_{66}N_8O_8S_2\cdot2H_2O$	64.7	6.15	9.75	5.55
(8)	63.95	6.2	10.15	5.35	$C_{68}H_{77}N_9O_9S_2\cdot2.5H_2O$ [j]	64.15	6.5	9.9	5.05

[a] All compounds are new. [b] The amino-component was prepared by the action of trifluoroacetic acid on the stated t-butoxycarbonyl derivative. [c] Coupling was by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole in tetrahydrofuran for compounds (14) and (16), in dichloromethane for compound (15), and in dimethylformamide for compounds (17) and (8). [d] Optical rotations were measured in dimethylformamide (*c* 1.0). [e] The same product was obtained in 97% yield using L-alanine 4-picolyl ester dihydrobromide (J. G. Warnke and G. T. Young, *J. Chem. Soc., Perkin Trans 1*, in the press) as the amino-component. [f] Liberated from the dicyclohexylammonium salt by 0.7M-citric acid and chloroform. [g] In the citric acid extraction, the organic solvent was ethyl acetate–ether (1:1). The product was extracted from the basified aqueous layer by ethyl acetate. [h] Isolated by the Amberlyst ion-exchange procedure, with dimethylformamide as solvent for application to the resin and 25% pyridine in dimethylformamide as eluant. [i] At 20 °C. [j] Found after acid hydrolysis: Ile + aIle, 0.97; Ala, 3.10; Cys, 1.93

50% acetic acid during 1 h; after removal of the mercury, oxidation by aeration at pH 9.5, followed by saponification, gave a product which may have contained both cyclic monomer and linear disulphides, but ^{1}H n.m.r. evidence showed clearly that the protecting groups had been completely removed. We report also the preparation of *S*-(diphenyl-4-pyridylmethyl)-L-cysteinyl-L-alanine.

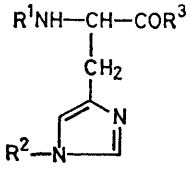
For the protection of histidine, *N*(α)-t-butoxycarbonyl-L-histidine methyl ester [17] was converted into the *N*(*Im*)-diphenyl-4-pyridylmethyl derivative (9) by reaction with diphenyl-4-pyridylmethyl chloride and triethylamine. The free acid (10) and the acid hydrazide

(11) were prepared as usual. The analogous $N(\alpha)$-benzyloxycarbonyl derivatives (12) and (13) were prepared similarly. In each case the product is assumed to be the *tele*-isomer.

removed only the benzyloxycarbonyl group from the same compound during 1 h at room temperature. The *N*-imidazole protection was removed from compound (9) by zinc dust–acetic acid during 1.5 h, authentic

TABLE 2

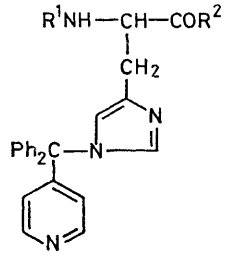
Dipeptides of histidine and protected derivatives [a]



Compound	R^1	R^2 [b]	R^3	$[\alpha]_D^{20}$(°)	R_F(t.l.c.)	Found (%) C	H	N	Formula	Required (%) C	H	N
(18)	Boc-Gly	Dppm	OMe	+6 [c]	0.54 (A2), 0.49 (E4)	67.3	6.4	11.9	$C_{32}H_{35}N_5O_5$	67.5	6.2	12.3
(19)	Boc-Gly	Dppm	OH	+25 [d]	0.43 (A2), 0.28 (G3)	64.1	5.9	11.8	$C_{31}H_{33}N_5O_5 \cdot 1.5H_2O$	63.9	6.2	12.0
(20)	Boc	Dppm	Leu-OMe	+7 [e]	0.6 (E4), 0.60 (Pl)	69.0	7.0	11.0	$C_{36}H_{43}N_5O_5$	69.1	6.9	11.2
(21)	Boc	Dppm	Leu-OH	+32 [e]	0.45 (G3), 0.35 (Pl)	66.6	6.7	10.9	$C_{35}H_{41}N_5O_5 \cdot H_2O$	66.75	6.9	11.1
(22)	Boc	Dppm	Phe-OMe	+30.5 [e]	0.60 (E4), 0.60 (Pl)	70.8	6.4	10.2	$C_{39}H_{41}N_5O_5$	71.0	6.3	10.6
(23)	Boc	Dppm	Phe-OH	+44 [e]	0.40 (G3), 0.40 (Pl)	68.9	6.0	10.2	$C_{38}H_{39}N_5O_5 \cdot H_2O$	68.8	6.2	10.55
(24)	Boc	Dppm	Gly-OMe	+20 [e]	0.45 (E4), 0.55 (Pl)	67.6	6.3	12.1	$C_{32}H_{35}N_5O_5$	67.5	6.2	12.3
(25)	Boc	Dppm	Gly-OH	+26 [e]	0.20 (G3), 0.15 (P1)	66.7	6.05	12.4	$C_{31}H_{33}N_5O_5$	67.0	6.0	12.6
(26)	H	H	Leu-OH	−43 [f]	0.10 (A4), 0.55 (G2)	53.5	7.4	20.9	$C_{12}H_{20}N_4O_3$	53.7	7.5	20.9
(27)	H	H	Phe-OH	+32 [g]	0.10 (A4), 0.55 (G2)	58.5	6.4	18.1	$C_{15}H_{18}N_4O_3 \cdot 0.25H_2O$	58.7	6.1	18.3
(28)	H	H	Gly-OH	+22 [h]	0.05 (A4), 0.13 (G2)	43.5	6.0	25.55	$C_8H_{12}N_4O_3 \cdot 0.5H_2O$	43.3	5.9	25.3

[a] Compounds (18)—(25) inclusive are new. [b] Dppm = diphenyl-4-pyridylmethyl. [c] *c* 1.0 in MeOH. [d] *c* 1.0 in Me_2N·CHO. [e] *c* 0.9—1.1 in $CHCl_3$. [f] *c* 1.0 in 0.1M-NaOH. R. W. Holley and E. Sondheimer (*J. Am. Chem. Soc.*, 1954, **76**, 1326) report $[\alpha]_D$ −43.5°; G. Losse and G. Müller (*Chem. Ber.*, 1961, **94**, 2768) report $[\alpha]_D$ −41.8°. [g] *c* 2.5 in 1M-HCl; G. Losse and G. Müller (*loc. cit.*) report $[\alpha]_D$ +32.9°. [h] *c* 1.0 in H_2O; G. Losse and G. Müller (*loc. cit.*) report $[\alpha]_D$ +24.6°

The stability of the $N(Im)$-diphenyl-4-pyridylmethyl group was shown by treatment of compound (13) with



(9) $R^1 = Me_3COCO$, $R^2 = OMe$
(10) $R^1 = Me_3COCO$, $R^2 = OH$
(11) $R^1 = Me_3COCO$, $R^2 = NHNH_2$
(12) $R^1 = PhCH_2OCO$, $R^2 = OMe$
(13) $R^1 = PhCH_2OCO$, $R^2 = OH$

trifluoroacetic acid for 48 h at room temperature; t.l.c. detected no change, and starting material was recovered in 88% yield. Hydrogen bromide (45%) in acetic acid

$N(\alpha)$-t-butoxycarbonyl-L-histidine methyl ester being recovered in 91% yield, and the removal was also effected by electrolytic reduction (87% yield).

The use of this protection was illustrated by the synthesis of L-histidyl-L-leucine, -L-phenylalanine, and -glycine, using standard methods (see Table 2); removal of the diphenyl-4-pyridylmethyl group in the final stage was by hydrogenolysis in each case.

EXPERIMENTAL

Thin-layer chromatograms were run on Merck silica-60 F-254 plates; the solvents used were: (A2) butanol–acetic acid–water, 10 : 1 : 3; (E3) methanol–chloroform, 1 : 4; (E4) methanol–chloroform, 1 : 9; (G3) ethyl acetate (120 vol.) and pyridine–acetic acid–water, 20 : 6 : 11 (40 vol.); (H) butanol–pyridine–acetic acid–water, 15 : 10 : 3 : 12; (J) acetonitrile–water 3 : 1; (M) methanol–acetic acid–water, 4 : 2 : 1; (Pl) chloroform–methanol–acetic acid, 17 : 2 : 1. Spots were detected by u.v. illumination, by ninhydrin, by iodine vapour, by the Pauly test, and by chlorine and starch-iodide as applicable. M.p.s were determined on a Kofler hot-stage apparatus. Evaporation was by a rotary evaporator below 35 °C; solutions in organic solvents were dried over magnesium sulphate or sodium sulphate. N.m.r. and i.r. spectra are reported only in selected cases.

Diphenyl-4-pyridylmethanol (*with J. M. Maud*).—The method of Tschitschibabin and Benewolenskaja [9] was modified as follows. Phenylmagnesium bromide (from bromobenzene, 78.5 g, 0.5 mol) in ether (200 ml) at 0 °C was added to a solution of 4-benzoylpyridine (45.8 g, 0.25 mol) in ether (800 ml) at 0 °C during 15 min. The solution was heated at reflux temperature for 2 h, stirred at room temperature for 16 h, and then cooled to 0 °C and poured onto ice-water containing concentrated hydrochloric acid (140 ml). The aqueous phase was raised to pH 7 by cautious addition of ammonium hydroxide (*d* 0.88). The precipitate was collected and washed with water and finally with benzene giving an alcohol (57.7 g, 88% yield) of m.p. 240—242 °C (lit.,[18] 238—239 °C); R_F 0.66 (E3), 0.81 (Pl). Recrystallisation from dimethylformamide–water did not raise the m.p.

Diphenyl-4-pyridylmethyl Chloride Hydrochloride.—Diphenyl-4-pyridylmethanol (52.3 g, 0.2 mol) and thionyl chloride (purified by distillation from quinoline and triphenyl phosphite) in chloroform (250 ml) were refluxed for 72 h. The cooled solution was filtered and then evaporated; the residue crystallised from dichloromethane (300 ml)–ether (700 ml) giving the *hydrochloride* (54.2 g, 86%), m.p. 134—135 °C; τ ($CDCl_3$) 1.16 (2 H, d, *J* 6 Hz, pyridyl 2- and 6-H), 2.23 (2 H, d, *J* 6 Hz, pyridyl 3- and 5-H), and 2.55—3.00 (10 H, complex 2 × Ph) (Found: C, 68.3; H, 4.5; Cl, 22.5; N, 4.55. $C_{18}H_{15}Cl_2N$ requires C, 68.35; H, 4.8; Cl, 22.4; N, 4.45%).

S-(*Diphenyl-4-pyridylmethyl*)-L-*cysteine* (1).—L-Cysteine hydrochloride (7.85 g, 50.0 mmol) reacted with diphenyl-4-pyridylmethanol (15.7 g, 60.0 mmol) and boron trifluoride–ether (15 ml, 119.0 mmol) in acetic acid (100 ml) at 60 °C during 48 h. The solvent was evaporated, the residue was dissolved in water and the pH was raised to 7 by addition of solid sodium hydrogencarbonate. The product was taken up from the filtered solution by adsorption on AG1-X2 resin (OH^- form; 150 ml), the resin was washed with 50% aqueous dimethylformamide and then water, and the product was eluted by 20% acetic acid. Evaporation left a foam which was dissolved in a small volume of dimethylformamide; addition of ether gave crystalline *protected amino-acid* (1) (11.7 g, 64%), m.p. 154—157 °C; $[\alpha]_D^{20}$ +51° (*c* 2.1 in 1M-HCl); R_F 0.32 (A2); 0.14 (G3); τ (CF_3CO_2H) 1.22 (2 H, m, pyridyl 2- and 6-H), 1.66 (2 H, d, *J* 6 Hz, pyridyl 3- and 5-H), 2.51 (10 H, s, 2 × Ph), 2.35—2.85 (3 H, br s, $\overset{+}{N}H_3$), and 6.40—6.90 (3 H, complex, $\overset{+}{N}H_3$-$CHCH_2S$) (Found: C, 68.9; H, 5.8; N, 7.45; S, 8.5. $C_{21}H_{20}N_2O_2S$ requires C, 69.2; H, 5.55; N, 7.7; S, 8.8%). For the preparation of the *N*-t-butoxycarbonyl derivative (below) the crude protected amino-acid may be used, avoiding the ion-exchange purification.

A solution of compound (1) (100 mg) in hydrogen bromide–acetic acid (45%; 5 ml) was left at room temperature for 48 h; the solution was then evaporated, and the residue was dissolved in water and the solution passed down a column of Amberlite IR-45 (acetate form) to remove hydrogen bromide; the column was washed with water and the combined eluates were evaporated to dryness; re-precipitation of the residue from dimethylformamide by ether gave compound (1) (91 mg, 91%) identical by t.l.c., i.r., and ^1H n.m.r. with the starting material. A similar experiment using trifluoroacetic acid instead of hydrogen bromide–acetic acid gave a 93% recovery of starting material.

N-t-*Butoxycarbonyl*-S-(*diphenyl-4-pyridylmethyl*)-L-*cysteine* (2).—This was prepared by the reaction of t-butoxycarbonyl azide (25 ml) with *S*-(diphenyl-4-pyridylmethyl)-L-cysteine (7.3 g, 20.0 mmol) in aqueous dioxan (50%, 50 ml) at 23 °C and pH 9.8 during 48 h. The reaction mixture was washed with ether, the pH was brought to 4 by the addition of solid citric acid, and the product was extracted into ethyl acetate as usual, giving the *N-t-butoxycarbonyl derivative* (2) (9.0 g, 97%) as a foam; $[\alpha]_D^{20}$ +17° (*c* 1.0 in MeOH); R_F 0.67 (A2), 0.79 (G3); τ ($CDCl_3$) −1.80 (1 H, br s, CO_2H), 1.53 (2 H, d, *J* 6 Hz, pyridyl 2- and 6-H), 2.50 (2 H, d, *J* 6 Hz, pyridyl 3- and 5-H), 2.69 (10 H, m, 2 × Ph), 4.67 (1 H, br d, *J* 7 Hz, urethane NH), 5.65 (1 H, m, $NHCHCH_2$), 7.00—7.60 (2 H, d, $CHCH_2S$); and 8.58 (9 H, s, Bu^t) (Found: C, 67.0; H, 6.4; N, 5.8; S, 6.6. $C_{26}H_{28}N_2O_4S$ requires C, 67.2; H, 6.1; N, 6.05; S, 6.9%).

The crystalline *dicyclohexylammonium salt* was prepared by the addition of dicyclohexylamine in ether to a solution of compound (2) in ethyl acetate; it had m.p. 202—204 °C, $[\alpha]_D^{20}$ +19° (*c* 1.05 in $CHCl_3$) (Found: C, 70.55; H, 8.05; N, 6.2; S, 4.65. $C_{38}H_{51}N_3O_4S$ requires C, 70.65; H, 7.95; N, 6.5; S, 4.95%). In subsequent preparations the crude *S*-(diphenyl-4-pyridylmethyl)-L-cysteine (omitting the ion-exchange purification) was used, and in this way the overall yield for the conversion of L-cysteine hydrochloride to the dicyclohexylammonium salt of the *N*-t-butoxycarbonyl derivative (2) was raised to 86%.

N-*t-Butoxycarbonyl*-S-(*diphenyl-4-pyridylmethyl*)-L-*cysteine Methyl Ester* (3).—Diazomethane in ether converted *N*-t-butoxycarbonyl-*S*-(diphenyl-4-pyridylmethyl)-L-cysteine in methanol at 2 °C into the methyl ester (90% yield); $[\alpha]_D^{20}$ +17° (*c* 1.02 in $CHCl_3$); R_F 0.67 (E4), 0.26 (Et_2O); τ ($CDCl_3$) 1.46 (2 H, d, *J* 5 Hz, pyridyl 2- and 6-H), 2.50—2.80 (12 H, complex, 2 × Ph and pyridyl 3- and 5-H), 4.93 (1 H, br d, *J* 7 Hz, urethane *NH*), 5.67 (1 H, m, $NHCHCH_2$), 6.30 (3 H, s, OMe), 7.40 (2 H, d, *J* 6 Hz, $CHCH_2S$), and 8.55 (9 H, s, Bu^t) (Found: C, 67.5; H, 6.55; N, 5.7; S, 6.5. $C_{27}H_{30}N_2O_4S$ requires C, 67.75; H, 6.3; N, 5.85; S, 6.7%).

N-*t-Butoxycarbonyl*-S-(*diphenyl-4-pyridylmethyl*)-L-*cysteine Ethyl Ester* (4).—*N*-t-Butoxycarbonyl-*S*-(diphenyl-4-pyridylmethyl)-L-cysteine was liberated from its dicyclohexylammonium salt (663 mg, 1.03 mmol) by partitioning between 0.7M-citric acid and ethyl acetate, and it was then caused to react with dicyclohexylcarbodi-imide (198 mg, 0.96 mol) and ethanol (0.5 ml) in dichloromethane (6 ml) at room temperature during 3 h. The reaction mixture was filtered and then evaporated; the residue was dissolved in ether, the solution was washed (0.7M-citric acid, water, and brine), dried, and evaporated. Traces of dicyclohexylurea were removed by dissolution of the product in benzene, filtration, and evaporation, leaving the *ester* (4) (319 mg, 68%) as a syrup, $[\alpha]_D^{20}$ +15° (*c* 1.0 in EtOH), R_F 0.72 (E4), 0.34 (Et_2O) (Found: C, 68.0; H, 6.7; N, 5.75; S, 6.4. $C_{28}H_{32}N_2O_4S$ requires C, 68.25; H, 6.55; N, 5.7; S, 6.5%).

N-*t-Butoxycarbonyl*-S-(*diphenyl-4-pyridylmethyl*)-L-*cysteine Pentachlorophenyl Ester* (5).—*N*-t-Butoxycarbonyl-*S*-(diphenyl-4-pyridylmethyl)-L-cysteine (217 mg, 0.47 mmol) reacted with pentachlorophenyl trichloroacetate [11] (452 mg, 1.10 mmol) and triethylamine (65 μl, 0.47 mmol) in tetrahydrofuran (8 ml) at room temperature during 28 h. The solution was evaporated, ethyl acetate and water were added, and the organic layer was washed (sodium hydrogencarbonate, water, and brine) and dried; evaporation gave the *ester* (5) (300 mg, 90%), $[\alpha]_D^{20}$ +2° (*c* 1.2 in $CHCl_3$);

R_F 0.80 (E4) (Found: C, 54.05; H, 4.05; Cl, 24.85; N, 3.8; S, 4.45. $C_{32}H_{27}Cl_5N_2O_4S$ requires C, 53.9; H, 3.8; Cl, 24.85; N, 3.95; S, 4.5%). The same product was obtained in 77% yield by reaction of compound (2) with dicyclohexylcarbodi-imide and pentachlorophenol.

N-*Benzyloxycarbonyl*-S-(*diphenyl-4-pyridylmethyl*)-L-*cysteine* (6) *Dicyclohexylammonium Salt*.—*S*-(Diphenyl-4-pyridylmethyl)-L-cysteine (3.64 g, 10.0 mmol) reacted with benzyl succinimido carbonate [12] (4.00 g, 16.0 mmol) added in portions and tetramethylguanidine (2.0 g, 17.4 mmol) in chloroform (30 ml) at 50—60 °C during 2 h. The cooled solution was washed (hydrochloric acid, water, and brine), dried, and evaporated; the residue was dissolved in dioxan and a solution of dicyclohexylamine (1.81 g, 10.0 mmol) in ether was added, giving the *salt* (4.90 g, 72%), m.p. 170—172 °C, $[\alpha]_D^{20}$ +26.5° (*c* 1.0 in $CHCl_3$); R_F 0.68 and 0.37 (G3) (Found: C, 72.25; H, 7.4; N, 6.25; S, 4.7. $C_{41}H_{49}N_3O_4S$ requires C, 72.45; H, 7.25; N, 6.2; S, 4.7%).

N-*Benzyloxycarbonyl*-S-(*diphenyl-4-pyridylmethyl*)-L-*cysteine* 2,4,5-*Trichlorophenyl Ester* (7).—A solution of dicyclohexylcarbodi-imide (176 mg, 0.85 mmol) in dichloromethane (2 ml) was added to a solution of *N*-benzyloxycarbonyl-*S*-(diphenyl-4-pyridylmethyl)-L-cysteine (426 mg, 0.85 mmol, liberated from its dicyclohexylammonium salt by means of 0.7M-citric acid and chloroform) and 2,4,5-trichlorophenol (169 mg, 0.85 mmol) in dichloromethane (10 ml) at 0 °C. After 2 h at 0 °C and 5 h at 24 °C the solution was evaporated, ether was added, and the solution was filtered. Evaporation of the filtrate gave the *ester* (7) (521 mg, 90%) of $[\alpha]_D^{20}$ +16° (*c* 1.0 in $CHCl_3$); R_F 0.72 (E4), 0.34 (Et_2O); $\nu_{max.}$ ($CHCl_3$) 1 777 and 1 720 cm^{-1} (Found: C, 61.75; H, 4.4; N, 4.45; S, 4.5. $C_{35}H_{27}Cl_3N_2O_4S$ requires C, 62.0; H, 4.0; N, 4.15; S, 4.75%). The optical rotation (at 589 nm) of a 0.05M solution of the 2,4,5-trichlorophenyl ester (7) in a 0.4M solution of triethylamine in chloroform at 22 °C fell to half its initial value after 35 min; under the same conditions the corresponding figure for a solution of *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine 4,5-trichlorophenyl ester [19] was 40 min.

Removal of the S-*Diphenyl-4-pyridylmethyl Protecting Group*.—(1) *By electrolytic reduction*.[14] *N*-t-Butoxycarbonyl-*S*-(diphenyl-4-pyridylmethyl)-L-cysteine dicyclohexylammonium salt (654 mg, 1.0 mmol) was treated with trifluoroacetic acid (6 ml) for 10 min and the solution was then evaporated. The residue was dissolved in de-aerated 1M-hydrochloric acid–acetic acid (5 : 1; 12 ml) and electrolysed at a mercury cathode at 0 °C, maintaining a current of 250 mA. Estimation of the thiol group by Ellman's reagent [20] showed that the reduction was 96% complete after 45 min; hydrochloric acid was removed by Amberlite IR-45 (acetate-form) and the solution was then evaporated. The residue was dissolved in methanol–water (1 : 1; 5 ml), the pH was raised to 9 by addition of triethylamine, and air was passed in. Next day the precipitated L-cystine (109 mg, 91%) was collected; it had $[\alpha]_D^{20}$ −212° (*c* 1.0 in 1M-HCl).

(2) *By iodine*. *N*-t-Butoxycarbonyl-*S*-(diphenyl-4-pyridylmethyl)-L-cysteine (116 mg, 0.25 mmol) was oxidised by iodine (640 mg, 2.5 mmol) in 80% acetic acid at room temperature during 1 h. Excess of iodine was removed by sodium thiosulphate, the solution was evaporated, and the residue was taken up in ethyl acetate and 0.05M-sodium thiosulphate; the organic layer was washed (0.05M-sodium thiosulphate, water, and 0.7M-citric acid) and the product was then extracted into 2M-sodium hydrogencarbonate. Solid citric acid was added to bring the pH to 3 and the product was extracted into ethyl acetate, giving product (45 mg, 81%) identical in i.r. and ^1H n.m.r. spectra with authentic di-t-butoxycarbonylcystine. *S*-4-Picolyl-L-cysteine [3] was found to be stable to iodine in methanol–water (1 : 1) at room temperature during 24 h. Under comparable conditions the *S*-trityl, *S*-acetamidomethyl, and *S*-diphenyl-4-pyridylmethyl derivatives of *N*(α)-t-butoxycarbonyl-L-cysteine required 0.25, 0.25, and 1.5 h, respectively, for complete deprotection by iodine in methanol solution at room temperature.

(3) *By mercury*(II) *acetate*. Mercury(II) acetate (50 mg) was added to a solution of *S*-(diphenyl-4-pyridylmethyl)-L-cysteine (12 mg) in water brought to pH 4 by acetic acid. After 15 min hydrogen sulphide was passed into the solution, which was then passed through a millipore filter. T.l.c. of the filtrate [solvents (A2), (M)] showed the presence of only cysteine and diphenyl-4-pyridylmethanol. *S*-4-Picolyl-L-cysteine [3] was found to be stable to mercury(II) acetate under these conditions during 2 h.

(4) *By zinc–acetic acid*. Zinc dust (20 mg) was added to a solution of *S*-(diphenyl-4-pyridylmethyl)-L-cysteine (9 mg) in 80% acetic acid (2 ml). After 15 min t.l.c. [solvent (M)] showed the presence of only cysteine and a ninhydrin-negative co-product of R_F 0.64 (M). No further change was observed during 4 h. The *S*-protection of *N*-t-butoxycarbonyl-*S*-acetamidomethyl-L-cysteine methyl ester was found to be stable to zinc–80% acetic acid at 24 °C during 24 h.

t-Butoxycarbonyl-L-alanine 4-Picolyl Ester.—t-Butoxycarbonyl-L-alanine was esterified with 4-pyridylmethanol by the general procedure described by Pinker *et al.*[21] (yield 88%); the *ester* had m.p. 69—70 °C; $[\alpha]_D^{20}$ −32° (*c* 1.0 in Me_2NCHO); R_F 0.60 (A2), 0.56 (E4) (Found: C, 60.2; H, 7.15; N, 9.95. $C_{14}H_{20}N_2O_4$ requires C, 60.0; H, 7.2; N, 10.0%).

N-*t-Butoxycarbonyl*-S-(*diphenyl-4-pyridylmethyl*)-L-*cysteinyl-L-alanine*.—Hydrolysis of compound (14) (see Table 1) by 1M-sodium hydroxide (1.2 equiv.) in dioxan at room temperature for 30 min gave the *acid* (yield, 95%), m.p. 112—114 °C (Found: C, 64.8; H, 6.4; N, 7.8; S, 5.9. $C_{29}H_{33}N_3O_5S$ requires C, 65.05; H, 6.2; N, 7.85; S, 6.0%).

S-(*Diphenyl-4-pyridylmethyl*)-L-*cysteinyl-L-alanine*.—*N*-t-Butoxycarbonyl-(*S*-diphenyl-4-pyridylmethyl)-L-cysteinyl-L-alanine (1.58 g, 2.9 mmol) was dissolved in trifluoroacetic acid and after 15 min the solution was evaporated; trifluoroacetate was removed by Amberlite IR-45 (acetate form), in 20% acetic acid, giving, after re-precipitation from dimethylformamide with ether, *S*-protected *dipeptide* (1.10 g, 84%), R_F 0.32 (J), 0.81 (M) (Found: C, 63.95; H, 6.0; N, 9.1; S, 7.15. $C_{24}H_{25}N_3O_3S,H_2O$ requires C, 63.55; H, 6.0; N, 9.25; S, 7.05%).

t-Butoxycarbonyl-L-isoleucyl-L-alanyl-S-(*diphenyl-4-pyridylmethyl*)-L-*cysteinyl-L-alanyl*-S-(*diphenyl-4-pyridylmethyl*)-L-*cysteinyl-L-alanine 4-Picolyl Ester* (8).—This was synthesised stepwise from L-alanine 4-picolyl ester by our standard procedures (see Part 42 [22]) using dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole (pre-activation procedure) in dimethylformamide for coupling reactions. Isolation was by the citric acid method,[21] normally with ethyl acetate as solvent, except for compound (8) for which the Amberlyst (3-bromopyridinium form) procedure was used. Details are given in Table 1, where exceptions to the general procedures are noted.

Deprotection of *t-Butoxycarbonyl*-L-*isoleucyl*-L-*alanyl*-S-(*diphenyl*-4-*pyridylmethyl*)-L-*cysteinyl*-L-*alanyl*-S-(*diphenyl*-4-*pyridylmethyl*)-L-*cysteinyl*-L-*alanine* 4-*Picolyl Ester* (8).—Compound (8) (1.26 g, 1.0 mmol) reacted with mercury(II) acetate (956 mg, 3.0 mmol) in 50% acetic acid (25 ml) during 1 h at 25 °C. Hydrogen sulphide was passed in, and the solution was filtered and evaporated. The residue was taken up in water (350 ml) the pH was brought to 9.5 by addition of triethylamine, and the solution was aerated during 12 h, when a negative Ellman's test was given. The solution was evaporated to dryness and the main portion (800 mg) of the crude product was saponified by 1M-sodium hydroxide (2 ml) in dimethylformamide–water (4 : 1; 40 ml). After 1 h at room temperature dithiothreitol (400 mg) was added to cleave disulphide bonds. After 1 h the solution was filtered and then concentrated and the product was precipitated by ether. The precipitated dithiol was re-oxidised in 50% aqueous dimethylformamide (600 ml) by aeration at 23 °C for 16 h in the presence of copper wire catalyst. The solution (negative Ellman's test) was evaporated and a portion (205 mg) was chromatographed on Sephadex LH-20 in dimethylformamide, giving a main fraction [107 mg, 68% from (8)] of the disulphide of t-butoxycarbonyl-L-isoleucyl-L-alanyl-L-cysteinyl-L-alanyl-L-cysteinyl-L-alanine; R_F 0.79 (H) (Found: C, 44.9; H, 6.7; N, 11.7. $C_{26}H_{44}N_6O_9S_2$, 2.5 H_2O requires C, 45.0; H, 7.1; N, 12.1%. Found after acid hydrolysis: Ile, 1.00; Ala, 3.00; Cys, 1.69). The product may have contained both linear disulphide polymer and cyclic monomer. ^1H N.m.r. [$(CD_3)_2SO$ solution] showed no phenyl or pyridyl protons.

N(α)-*t-Butoxycarbonyl*-N(Im)-*diphenyl*-4-*pyridylmethyl*-L-*histidine Methyl Ester* (9).—*N*-(α)-t-Butoxycarbonyl-L-histidine methyl ester [17] (22.9 g, 85 mmol), diphenyl-4-pyridylmethyl chloride hydrochloride (28.2 g, 89 mmol), and triethylamine (25 ml, 178 mmol) in chloroform (500 ml) were refluxed for 3 days. The solution was evaporated and the residue was taken up in ethyl acetate and washed (water, brine), dried, and evaporated. Unreacted chloride was removed by adsorbing the product from an ether solution on a silica column; the chloride was washed through with ether, and then the product was eluted with 5% methanol in ether. Evaporation of the main eluate gave the *methyl ester* (9) (31.5 g, 73%); $[\alpha]_D^{20}$ +2° (*c* 1.2 in MeOH); R_F 0.62 (E4), 0.84 (G3); τ ($CDCl_3$) 1.38 (2 H, d, *J* 6 Hz, pyridyl 2- and 6-H), 2.50—3.05 (13 H, complex, 2 × Ph, pyridyl 3- and 5-H, and NCH=N), 3.49 (1 H, s, NC*H*=C), 4.10 (1 H, br m, CON*H*CH), 5.47 (1 H, m, NHC*H*CH_2), 6.39 (3 H, s, OMe), 6.99 (2 H, d, *J* 6 Hz, CHCH_2C), and 8.58 (9 H, s, Bu^t) (Found: C, 70.4; H, 6.25; N, 11.0. $C_{30}H_{32}N_4O_4$ requires C, 70.3; H, 6.3; N, 10.95%).

N(α)-*t-Butoxycarbonyl*-N(Im)-*diphenyl*-4-*pyridylmethyl*-L-*histidine* (10).—2M-Sodium hydroxide (4.4 ml) was added dropwise during 30 min to a solution of the ester (9) (4.1 g, 8 mmol) in 50% dioxan at 0 °C. After a further 30 min the solution was neutralised with citric acid, evaporated to remove some of the dioxan, then diluted with water and brought to pH 8.5. It was washed with ether and acidified to pH 3.5 by means of citric acid. The product was extracted into ethyl acetate, the solution was washed (water, brine), dried, and evaporated to give *acid* (10) as a foam (3.86 g, 96%); $[\alpha]_D^{20}$ +10° (*c* 1.0 in MeOH); R_F 0.10 (E4), 0.35 (Pl). It retained ether even after drying for 24 h at 60 °C (*cf.* also below) (Found: C, 69.7; H, 6.3; N, 10.8. $C_{29}H_{30}N_4O_4 \cdot 0.25Et_2O$ requires C, 69.7; H, 6.3; N, 10.8%).

N(α)-*t-Butoxycarbonyl*-N(Im)-*diphenyl*-4-*pyridylmethyl*-L-*histidylhydrazide* (11).—Hydrazine hydrate in methanol reacted with methyl ester (9) at room temperature during 2 days, giving *hydrazide* (11) hemi-etherate (70% yield) of m.p. 92—93 °C after recrystallisation from methanol–ether; $[\alpha]_D^{20}$ +5° (*c* 1.0 in MeOH); R_F 0.30 (E4), 0.35 (Pl) (Found: C, 67.3; H, 6.4; N, 15.5. $C_{29}H_{32}N_6O_3 \cdot 0.5Et_2O$ requires C, 67.7; H, 6.8; N, 15.3%). The presence of diethyl ether was confirmed by ^1H n.m.r.

N(α)-*Benzyloxycarbonyl*-N(Im)-*diphenyl*-4-*pyridylmethyl*-L-*histidine Methyl Ester* (12).—This was prepared from *N*(α)-benzyloxycarbonyl-L-histidine methyl ester [17] as described for the *N*(α)-t-butoxycarbonyl analogue; elution from the silica column was by 10% methanol in ether, giving *ester* (12) (50% yield), $[\alpha]_D^{20}$ +4° (*c* 1.0 in MeOH); R_F 0.59 (A2), 0.58 (E4); τ ($CDCl_3$) 1.37 (2 H, d, *J* 5 Hz, pyridyl 2- and 6-H), 2.45—3.05 (18 H, complex, 3 × Ph pyridyl 3- and 5-H, and NCH=N), 3.47 (1 H, s, NCH=C), 3.73 (1 H, br d, *J* 8 Hz, CON*H*CH), 4.89 (2 H, s, PhCH_2O), 5.39 (1 H, m, NHC*H*-CH_2), 6.37 (3 H, s, OMe), and 6.96 (2 H, d, *J* 5 Hz, CHCH_2-C=) (Found: C, 72.2; H, 5.55; N, 10.0. $C_{33}H_{30}N_4O_4$ requires C, 72.5; H, 5.55; N, 10.25%).

N(α)-*Benzyloxycarbonyl*-N(Im)-*diphenyl*-4-*pyridylmethyl*-L-*histidine* (13).—This was prepared by hydrolysis of the methyl ester (12) as described for the *N*(α)-t-butoxycarbonyl analogue (94% yield); the *acid* (13) had m.p. 109—111 °C, $[\alpha]_D^{20}$ +10.5° (*c* 0.9 in MeOH); R_F 0.50 (A2), 0.69 (G3) (Found: C, 71.9; H, 5.4; N, 10.1. $C_{32}H_{28}N_4O_4$ requires C, 72.15; H, 5.3; N, 10.5%).

The Stability of the N(Im)-*Diphenyl*-4-*pyridylmethyl Group towards Acid*.—(1) *Trifluoroacetic acid*. A solution of *N*(α)-benzyloxycarbonyl-*N*(*Im*)-diphenyl-4-pyridylmethyl-L-histidine (13) (120 mg) in trifluoroacetic acid (5 ml) was left at 21 °C for 48 h. The solution was evaporated, and the residue was dissolved in aqueous methanol and was passed down a column of Amberlite IR-45 (OH^- form). Unchanged starting material (t.l.c., ^1H n.m.r. evidence) was recovered in 88% yield.

(2) *Hydrogen bromide in acetic acid*. A solution of compound (13) (133 mg) in 45% hydrogen bromide in acetic acid (4 ml) was left at 21 °C for 1 h. An aqueous solution of the solid, precipitated by the addition of ether, was passed down a column of Amberlite IR-45 (acetate form); evaporation left a white solid shown by ^1H n.m.r. to be hydrated *N*(*Im*)-diphenyl-4-pyridylmethylhistidine.

Removal of the N(Im)-*Diphenyl*-4-*pyridylmethyl group*. (1) *By zinc dust–acetic acid*. *N*(α)-t-Butoxycarbonyl-*N*(*Im*)-diphenyl-4-pyridylmethyl-L-histidine methyl ester (9) (53.5 mg) was reduced with zinc dust (26 mg)–80% acetic acid at room temperature during 1.5 h. The solution was then filtered, the filtrate was evaporated and the residue was partitioned between ethyl acetate and 2M-sodium hydrogencarbonate; the organic layer was washed with water and brine, and was then dried and evaporated. *N*(α)-t-Butoxycarbonyl-L-histidine methyl ester (25.5 mg, 91%) was isolated from the residue by preparative plate chromatography (Kieselgel 254/366) using methanol–chloroform (1 : 9) as eluant (identification by t.l.c., and by i.r. and ^1H n.m.r. spectra); recrystallisation from ethyl acetate–light petroleum gave product (22 mg), m.p. 125.5—126 °C.

(2) *By electrolytic reduction*. A solution of compound (9) (63.7 mg) in 80% acetic acid (4 ml) and 1M-hydrochloric acid (1 ml) at 0 °C was electrolysed at a mercury cathode [14] (current *ca.* 50 mA) during 2.5 h. The solution was concentrated and the product was isolated as described in (1)

above; the preparative plate chromatography gave $N(\alpha)$-t-butoxycarbonyl-L-histidine methyl ester (29 mg, 87%), identified by t.l.c. and by i.r. and ^{1}H n.m.r. spectra; recrystallisation gave product (25 mg) of m.p. 125—126 °C.

Dipeptides of Histidine (*see Table* 2).—Compound (18) was prepared by the condensation of t-butoxycarbonylglycine with the product obtained by the removal of the t-butoxycarbonyl group (by trifluoroacetic acid as usual) from $N(\alpha)$-t-butoxycarbonyl-$N(Im)$-diphenyl-4-pyridylmethyl-L-histidine methyl ester (9), using dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole in dichloromethane; the product was isolated by the citric acid method, giving the *protected dipeptide* (18) (69% yield) with the constants and analysis shown in Table 2. Compounds (20), (22), and (24) were prepared by similar coupling reactions of $N(\alpha)$-t-butoxycarbonyl-$N(Im)$-diphenyl-4-pyridylmethyl-L-histidine (10) with the appropriate amino-ester in dimethylformamide solution (yields, 77, 53, and 64%, respectively). Compound (20) was also prepared by coupling $N(\alpha)$-t-butoxycarbonyl-$N(Im)$-diphenyl-4-pyridylmethyl-L-histidyl azide [prepared from the hydrazide (11)] with L-leucine methyl ester (yield 84.5%). The protected dipeptide esters (20), (22), and (24) were hydrolysed to the corresponding acids by sodium hydroxide (1.1 equiv.) at 0 °C during 1 h (yields 88, 83.5, and 62.5%, respectively). The t-butoxycarbonyl group was removed from the acids by means of trifluoroacetic acid and the $N(Im)$-diphenyl-4-pyridylmethyl group was removed by hydrogenolysis (*ca.* 15 h) over palladium–charcoal (10%) in 80% acetic acid; the filtered reaction mixture was treated with Amberlite IR-45 (acetate form) to remove trifluoroacetate and then evaporated. The residue was taken up in water, the solution washed with ethyl acetate, and then evaporated. The free dipeptide was precipitated from a solution in 5% acetic acid or water by ethanol with a small amount of ether (yields of analytically pure product 71, 95, and 68% respectively). Constants and analyses of all these compounds are given in Table 2.

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