Selective Protection of Cysteine with Ferrocene Derivatives. Part 2.[1] Synthesis of Glutathione

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Acid-catalysed reaction of ferrocenylmethanol with L-cysteine gives the corresponding coloured crystalline derivative which is selectively cleaved by trifluoroacetic acid when incorporated into peptide derivatives such as encountered in the synthesis of glutathione.

The use of organometallic reagents, namely *N*-alkylferrocenyl derivatives, in peptide chemistry has been pioneered and exemplified in the 'four component condensation reaction' of Ugi.[2] Our own work stemmed from that of Ratajczak and co-workers[3] who studied the cleavage reactions of ferrocenylalkylamines and ferrocenylalkanethiols with acids and metal ions respectively. Following on from their observations, we were prompted to examine ferrocenylalkyl-protected imidazole and histidine derivatives[4] from which the present work is derived. A recent communication[5] reports on the use of *N*-ferrocenylmethyl-protected amino acids in conventional peptide synthesis.

$R^1NHCH(CH_2SR^2)CO_2R^3$

(1) $R^1 = R^3 = H$, $R^2 = CH_2Fc$
(2) $R^1 = R^3 = H$, $R^2 = CMe_2Fc$
(3) R^1 = Fmoc, $R^2 = CH_2Fc$, $R^3 = H$
(4) R^1 = Fmoc, $R^2 = CMe_2Fc$, $R^3 = H$
(5) R^1 = tBoc, $R^2 = CH_2Fc$, $R^3 = H$

R^1–Cys(FcM)–Gly–OR^2

(6) R^1 = Fmoc, $R^2 = Bu^t$
(7) R^1 = tBoc, R^2 = Et

tBoc–Glu(αOBu^t)–Cys(FcM)–GlyOR

(8) R = Bu^t
(9) R = Et
(10) R = H

Fc = Ferrocenyl

In the present work,[1] we report on the application of the *S*-ferrocenylmethyl group for the *S*-protection of cysteine which is illustrated by the synthesis of γ-L-glutamyl-L-cysteinylglycine-glutathione.

We also report the synthesis of *S*-ferrocenylmethyl-L-cysteine (**1**), *S*-(2-ferrocenylpropan-2-yl)-L-cysteine (**2**), and derivatives, together with a comparison of the conditions required for removal of the ferrocenylalkyl groups.

The new protecting groups are readily accessible, introduced in high yield, lipophilic in character, selectively cleaved by mild acidolytic conditions or mercuric ions, stable to alkaline solution, and are coloured, providing a useful visual monitor.

L-Cysteine on treatment with ferrocenylmethanol[6] in a degassed solution of aqueous acetone in the presence of a catalytic amount of trifluoroacetic acid afforded a quantitative yield of *S*-ferrocenylmethyl-L-cysteine (**1**) which was recovered by filtration.

The propan-2-yl derivative (**2**) could be prepared by a similar procedure using 2-ferrocenylpropan-2-ol.[7] A comparison of the conditions required for the removal of the *S*-ferrocenylalkyl groups from cysteine and derivatives is detailed in the Table. Acidolytic studies demonstrated that both the *S*-ferrocenylalkyl derivatives (**1**) and (**2**) were rapidly cleaved by trifluoroacetic acid but only the propan-2-yl derivative (**2**) was susceptible to removal by formic acid. The favoured conditions for removal of the *S*-ferrocenylmethyl group require the use of a scavenger such as thiophenol to provide reducing conditions and to remove ferrocenylcarbonium ions formed during the cleavage reaction. Treatment of (**1**) and (**2**) with an excess of toluene-*p*-sulphonic acid in acetic acid for 2 h at room temperature only led to removal of the propan-2-ylferrocenyl group from (**2**).

Table. Reactions of *S*-ferrocenylalkylcysteine derivative

Reagent	Time	(1)	(2)	(3)	(4)	Conditions for cleavage and monitoring
TFA	1 h	C	C	C	C	A
TFA/anisole[a]	2 h	C				A
TFA/thioglycollic acid[a]	24 h	C				A
TFA/PhSH[a]	24 h	C				A
TFA/MeOH[b]	24 h	N				A
HCO_2H	24 h	N	C			A
TSOH/HAC	2 h	N	C			A
TSOH/HAC	24 h	S	C			A
HAC	24 h	N	S	N	S	A, (1–4) recovered
NaOH	24 h	N	N			PC
NaOMe	24 h	N	N			PC
NH_2NH_2	24 h	N	S			TLC
$NaBH_4$	24 h			N	N	TLC
Hg^{2+}	5 min	C	C			H_2S passed; PC
Ag^+	5 min	C	C			Silver cysteinate recovered
I_2/HAC	24 h			S	S	TLC
$(SCN)_2$	5 min			C	C	TLC
CmsCl	1 h			C	C	TLC
Zn/HAC	5 h	N	S			TLC; (1), (2) recovered
BBr_3	24 h			S	C	(3) recovered
PhSH	24 h			N	N	TLC

N = No reaction, S = slight reaction, C = complete reaction, A = Reaction monitored by paper chromatography (PC); work-up by addition of water and neutralisation with excess sodium hydrogencarbonate followed by ascorbic acid to reduce the ferricinium ion: the extent of reaction determined by partition with ether, complete reaction, inferred if no colour remained in the aqueous layer.

[a] 5%. [b] 1:1.

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Prolonged exposure of the methyl derivative (**1**) for 1 day under the same conditions gave rise to some cleavage.

Both groups were stable towards bases including sodium hydroxide, sodium methoxide, and hydrazine, although the propan-2-yl derivative (**2**) did give rise to slight decomposition in the presence of hydrazine.

The ferrocenylmethyl derivative (**1**) was stable to reducing agents such as sodium borohydride and zinc in acetic acid, whereas under these conditions the propan-2-yl compound (**2**) was, not surprisingly, removed. Metal ions gave rapid cleavage of both derivatives (**1**) and (**2**) as had been expected.

Electrophilic reagents such as thiocyanogen and carboxymethylsulphenyl chloride lead to rapid cleavage of both derivatives (**1**) and (**2**) whereas iodine when used in accordance with conditions for removal of the trityl group only led to slight decomposition.[8]

In the light of the experimental evidence we found that the propan-2-ylferrocene protecting group was probably too labile for routine use. The Table also details the comparative cleavage properties of the *N*-9-fluorenylmethoxycarbonyl (Fmoc)-derivatives of (**1**) and (**2**), namely (**3**) and (**4**).

Compound (**1**) could be converted into *N*-Fmoc-*S*-ferrocenylmethyl-L-cysteine (**3**) and *N*-t-butoxycarbonyl-*S*-ferrocenylmethyl-L-cysteine (**5**) in high yields using standard procedures. Selective removal of *N*-protecting groups from the *S*-FcM cysteine derivatives was readily achieved. Thus the Fmoc derivative (**3**) gave high yields of FcM cysteine (**1**) under standard conditions for cleavage with piperidine, whilst the t-Boc derivative (**5**) yielded similar results on treatment with toluene-*p*-sulphonic acid. Coupling of t-butyl glycinate and ethyl glycinate with (**3**) and (**5**) respectively using dicyclohexylcarbodi-imide and *N*-hydroxybenzotriazole provided the fully protected dipeptides (**6**) and (**7**) in 97 and 80% yields respectively.

N-Terminal deprotection of (**6**) and (**7**)* followed by coupling to α-t-butyl-*N*-t-butoxycarbonyl-L-glutamate using the foregoing coupling method gave the fully protected tripeptides (**8**) and (**9**) in yields of 76 and 75% respectively.

Hydrolysis of the ethyl ester (**9**) with methanolic sodium hydroxide followed by acidification with citric acid provided the tripeptide acid (**10**) which was taken up in trifluoroacetic acid containing 5% thiophenol. Pure glutathione (**48%**) was recovered after precipitation by ether and repeated ethanol washing and centrifugation.

The tripeptide t-butyl ester (**8**) was subject to simultaneous *C* and *N*-terminal cleavage using trifluoroacetic acid containing 5% of thiophenol. Pure glutathione (52%) was retrieved by the procedure described above.

In conclusion, we have shown that the ferrocenylmethyl group may be introduced into cysteine in high yield, that the properties of the group are congruent with a variety of typical peptide reaction methodologies, and that it can be usefully employed as a selective protecting group in the synthesis of peptides as exemplified by glutathione.

Experimental

M.p.s were determined on Gallenkamp or Electrothermal Apparatus. Mass spectra were obtained using the PCMU Service. Solvents were purified and dried before use according to published methods. Neutral compounds were isolated by partitioning between an organic solvent and water, washing successively with aqueous citric acid (10%), aqueous sodium hydrogencarbonate (5%), and water. The extracts were dried with anhydrous magnesium sulphate and solvent removed under reduced pressure on a rotary film evaporator. ^{1}H NMR spectra were recorded on a Perkin-Elmer R12B (60 MHz) or a Varian FT80A (80 MHz). Deuteriochloroform was employed as a solvent unless otherwise stated with tetramethylsilane as an internal standard except where D_2O was used in conjunction with the water-soluble sodium 3-trimethylsilylpropane-1-sulphonate standard. Chemical shifts are quoted relative to the internal standard. Elemental analyses of new compounds were carried out by C,H,N Analysis Ltd., Leicester. Optical rotations were measured on a NPL type 243 automatic polarimeter. Standard procedures for esterifications, deprotection, hydrolyses, and other routine procedures are described. TLC chromatography used MN-Kieselgel G/UV_{254} plates. Ascending paper chromatography used Whatman No. 1 paper. Column chromatography employed Merck Kieselgel 100 (20–230 mesh). The following solvent systems were used for chromatography (v/v): (A) butanol–acetic acid–water (4:5:1), (B) ethyl acetate, (C) chloroform–methanol (4:1), (D) chloroform, (E) toluene–ethyl acetate (3:1).

Light petroleum refers to that fraction with b.p. 40–60 °C; ether refers to diethyl ether throughout.

Preparation of S-*Ferrocenylmethyl*-L-*cysteine* (**1**).—L-Cysteine (2.0 g, 16.5 mmol) was added to water (20 ml) that had been degassed for 15 min with stirring and passage of nitrogen. Ferrocenylmethanol[6] (3.54 g, 16.5 mmol) dissolved in acetone (25 ml) was added and stirring continued for 5 min. TFA (0.5 ml) was added and the mass of material became freely mobile until a precipitate began to form after 30 min. A solution of acetone (50%) in water (15 ml) was added and the reaction left overnight. Evaporation of the solvent was followed by dissolution in ethanol and repeated evaporation (× 3). The resulting solid was suspended in ether, filtered and washed with ether and ice-cold water and dried *in vacuo*. Recrystallisation from hot water gave S-*ferrocenylmethyl*-L-*cysteine* (**1**) (5.35 g, 15.88 mmol, 96%), m.p. 186 °C, R_{FA} 0.86, $[\alpha]_D^{23}$ + 14.3° (*c* 0.5 in dioxane/5% aq. Na_2CO_3, 1:1) (Found: C, 50.2; H, 5.2; N, 4.1. $C_{14}H_{17}FeNO_2S{\cdot}0.75H_2O$ requires C, 50.5; H, 5.6; N, 4.2%).

Synthesis of S-(2-*Ferrocenylpropan*-2-*yl*)-L-*cysteine* (**2**).—L-Cysteine (4.0 g, 33.0 mmol) was dissolved in water (40 ml) and the solution degassed by stirring and passage of nitrogen for 15 min. Crude 2-ferrocenylpropan-2-ol[7] (8.82 g, 36.1 mmol) dissolved in acetone (25 ml) was added and stirring continued for 5 min. TFA (0.5 ml) was added and stirring continued overnight. Ethanol (100 ml × 5) was added and solvent removed under reduced pressure. The oily residue was triturated with ether and left for 1 h at 0 °C and the precipitate filtered off and suspended in water (50 ml).

After occasional shaking for 2 h, the solid was filtered off washed with water, acetone, and ether, and then dried. The *product* (**2**) a yellow amorphous solid was obtained (4.53 g, 13.05 mmol, 40%), m.p. 194–202 °C, R_{FA} 0.90, $[\alpha]_D^{23}$ − 7.6° (*c* 1.1 in 50% acetic acid) (Found: C, 55.0; H, 6.2; N, 4.0. $C_{16}H_{21}FeNO_2S$ requires C, 55.3; H, 6.1; N, 4.0%).

Preparation N-(*Fluoren*-9-*ylmethoxycarbonyl*)-S-*ferrocenylmethyl*-L-*cysteine* (**3**) *Dicyclohexylamine Salt*.—*S*-Ferrocenylmethylcysteine (**1**) (1.60 g, 5.0 mmol) was dissolved in aqueous sodium carbonate (10%; 10 ml) with stirring at 0 °C. Dioxane (5 ml) was added followed by a solution of fluoren-9-ylmethyl chloroformate (1.3 g, 5.0 mmol) in dioxane (7.5 ml). The reaction mixture was stirred for 1 h at 0 °C and at room temperature overnight. It was then poured into ice–water (100 ml) and extracted with ethyl acetate. Normal work-up gave (**3**) as a yellow foam (2.71 g, 5.0 mmol, 100%) which could not be crystallised, R_{FB} 0.32, R_{FC} 0.70, $[\alpha]_D^{23}$ − 16.1° (*c* 1.1 in

*Selective cleavage achieved with 2 equiv. of toluene-*p*-sulphonic acid at room temperature overnight.

methanol). The acid (0.1 g, 0.18 mmol) was dissolved in ether (2 ml) and dicyclohexylamine (36 μl, 0.18 mmol) added followed by light petroleum (2 ml). After 3 days at −10 °C the *salt* of (**3**) crystallised from the solution and was collected and washed with light petroleum (0.1 g, 0.15 mmol, 83%), m.p. 136–137 °C, $[\alpha]_D^{23}$ − 20.9° (*c* 1.1 in chloroform) (Found: C, 67.8; H, 6.9; N, 3.7. $C_{41}H_{50}FeN_2O_4S{\cdot}0.25H_2O$ requires C, 67.7; H, 7.0; N, 3.8%).

Synthesis of N-*Fluoren-9-ylmethoxycarbonyl*-S-(*2-ferrocenylpropan-2-yl*)-L-*cysteine* (**4**).—*S*-(2-Ferrocenylpropan-2-yl)-L-cysteine (**2**) (1.60 g, 4.61 mmol) was suspended in aqueous sodium carbonate (10%; 20 ml) at 0 °C and dioxane (10 ml) added followed by dropwise addition of a solution of fluoren-9-ylmethyl chloroformate (1.3 g, 5.0 mmol) in dioxane (10 ml). The reaction mixture was stirred for 1 h at 0 °C and at room temperature overnight. It was then poured into ice–water (100 ml) and washed with light petroleum. The aqueous layer was separated, acidified with citric acid, and extracted with ethyl acetate. The extracts were then dried, filtered, and evaporated to give the *derivative* (**4**) (recrystallised from benzene) (1.84 g, 3.23 mmol, 70%), m.p. 68–72 °C, R_{FC} 0.73, $[\alpha]_D^{23}$ − 6.0° (*c* 1.0 in ethanol) (Found: C, 66.6; H, 5.5; N, 2.4. $C_{31}H_{31}FeNO_4S{\cdot}0.33C_6H_6$ requires C, 66.6; H, 5.6; N, 2.4%).

Preparation of N-*t-Butoxycarbonyl*-S-*ferrocenylmethyl*-L-*cysteine* (**5**).—*S*-Ferrocenylmethyl-L-cysteine (**1**) (1.0 g, 2.97 mmol) was suspended in methanol (20 ml) and aqueous sodium carbonate (5%; 20 ml) added. Di-t-butyl dicarbonate (1.0 g, 4.7 mmol) was added and the solution stirred overnight; it was then poured into water (100 ml) and washed with light petroleum (2 × 50 ml). The aqueous solution was neutralised with citric acid and extracted with ether. The ether extracts were washed with water, dried, and evaporated to give the *cysteine derivative* (**5**) as a yellow oil (1.18 g, 2.82 mmol, 95%) R_{FB} 0.40, R_{FC} 0.70, $[\alpha]_D^{23}$ − 7.7° (*c* 0.5 in methanol) (Found: C, 53.9; H, 6.1; N, 3.2. $C_{19}H_{25}FeNO_4S$ requires C, 54.4; H, 6.0; N, 3.3%); δ 6.4–6.1 (1 H, br s, CO_2H), 5.5–5.1 (1 H, br s, NH), 4.60–4.35 (1 H, m, CH), 4.13 (m), 9.0 (Fc), 3.56 (s), 2.0 (FcCH_2), 2.93 (d, *J* 5.2 Hz), 2.0 (CH_2CH), 1.46 (s), and 9.0 (t-Boc).

Preparation of N-*t-Butoxycarbonyl*-S-*ferrocenylmethyl*-L-*cysteine* (**5**) *Dicyclohexylammonium Salt*.—The foregoing acid (**5**) (0.1 g, 0.24 mmol) was dissolved in ether (2 ml). On adding dicyclohexylamine (0.48 μl, 0.24 mmol) the *product* crystallised. It was separated, washed with ether, and dried (0.14 g, 0.23 mmol, 96%), m.p. 178–180 °C, $[\alpha]_D^{23}$ + 7.0° (*c* 2.1 in methanol) (Found: C, 61.9; H, 8.1; N, 4.4. $C_{31}H_{48}FeN_2O_4S$ requires C, 62.0; H, 8.1; N, 4.7%).

Selective Cleavage of the N-*Fluoren-9-ylmethoxycarbonyl Group From* (**3**).—The Fmoc derivative (**3**) (0.1 g, 0.2 mmol) was dissolved in methylene dichloride (5 ml) and piperidine (0.5 ml, 5.0 mmol) added. Precipitation of the cysteine derivative (**1**) was complete in 5 min and was recovered on addition of ethanol and work-up as above.

Selective Cleavage of the t-Butoxycarbonyl Group From (**5**).—The t-butoxycarbonyl derivative (**5**) (0.28 g, 0.58 mmol) was dissolved in acetonitrile (5 ml) containing toluene-*p*-sulphonic acid (0.28 g, 1.46 mmol). After 30 min crystals started to form. After 24 h these were filtered off and washed with ether to give (**1**) as the toluene-*p*-sulphonate, (0.25 g, 0.51 mmol, 88%), m.p. 198–202 °C (Found: C, 50.8; H, 5.1; N, 2.9. $C_{21}H_{25}FeNO_5S_2{\cdot}0.25H_2O$ requires C, 50.8; H, 5.1; N, 2.8%).

Synthesis of Glutathione: Preparation of (N-*Fluoren-9-ylmethoxycarbonyl*-S-*ferrocenylmethyl*-L-*cysteinyl*)*glycine t-Butyl Ester* (**6**).—To a stirred solution of *N*-fluoren-9-ylmethoxycarbonyl-*S*-ferrocenylmethyl-L-cysteine (**3**) (3.25 g, 6.0 mmol) in methylene dichloride (25 ml) cooled to 0 °C, was added DCCI (1.24 g, 6.0 mmol) and HBT (0.81 g, 6.0 mmol) followed by t-butyl glycinium acetate (1.15 g, 6.0 mmol) and triethylamine (0.84 ml, 6.0 mmol). Stirring was continued at 0 °C for 2 h after which acetic acid (0.5 ml) was added. After 30 min the solution was filtered and the solid washed with methylene dichloride. Solvent was removed under reduced pressure and the residue dissolved in ether. Work-up for a neutral product gave the protected dipeptide which was purified by chromatography on silica using ethyl acetate as eluant. Removal of solvent gave the *product* (**6**) as a foam (3.79 g, 5.79 mmol, 97%), m.p. 58–60 °C, R_{FD} 0.30, $[\alpha]_D^{23}$ − 19.9° (*c* 2.5 in acetonitrile) (Found: C, 64.0; H, 5.9; N, 4.2. $C_{35}H_{38}FeN_2O_5S$ requires C, 64.2; H, 5.9; N, 4.3%).

Synthesis of N-*t-Butoxycarbonyl*-α-*t-butyl*-L-γ-*glutamyl*-S-*ferrocenylmethyl*-L-*cysteinylglycine t-Butyl Ester* (**8**).—(*N*-Fluoren-9-ylmethoxycarbonyl-*S*-ferrocenylmethyl-L-cysteinyl)-glycine t-butyl ester (**6**) (0.50 g, 0.76 mmol) was dissolved in a 25% solution of diethylamine in methylene dichloride (10 ml) and left overnight. Solvent was removed under reduced pressure and the residue dissolved in ether, washed with aqueous sodium hydrogen carbonate and water and then extracted into 10% aqueous citric acid. The acid extracts were neutralised, extracted with ether, and the ether extracts were washed with water, dried, filtered, and evaporated to leave the free base as an oil; this was used below without further purification. To *N*-t-butoxycarbonyl-L-γ-glutamic acid α-t-butyl ester[9] (0.23 g, 0.75 mmol) dissolved in acetonitrile (4 ml) were added with stirring at 0 °C, DCCI (0.165 g, 0.80 mmol) and HBT (0.108 g, 0.80 mmol) followed by the free base above, dissolved in acetonitrile (4 ml). Stirring was continued for 2 h at 0 °C after which acetic acid (0.5 ml) was added. After 30 min the solution was filtered and the solid washed with acetonitrile. Solvent was removed under reduced pressure and the residue dissolved in ether. Normal work-up gave the fully protected tripeptide which was purified by column chromatography on silica using ethyl acetate as eluant. Removal of solvent gave the *product* (**8**) as an oil (0.42 g, 0.58 mmol, 76%) R_{FD} 0.18, $[\alpha]_D^{23}$ − 23° (*c* 3.3 in acetonitrile) (Found: C, 56.8; H, 7.1; N, 6.1%; $M^{+\cdot}$, 717. $C_{34}H_{51}FeN_3O_8S$ requires C, 56.9; H, 7.2; N, 5.9%; $M^{+\cdot}$, 717).

Synthesis of (N-*t-Butoxycarbonyl*-S-*ferrocenylmethyl*-L-*cysteinyl*)*glycine Ethyl Ester* (**7**).—*N*-t-Butoxycarbonyl-*S*-ferrocenylmethyl-L-cysteine (**5**) (1.53 g, 3.65 mmol) was dissolved in acetonitrile (15 ml) at 0 °C. DCCI (0.76 g, 3.70 mmol) and HBT (0.50 g, 3.70 mmol) were added with stirring followed by glycine ethyl ester hydrochloride (0.52 g, 3.70 mmol) and triethylamine (0.51 ml, 3.70 mmol). The reaction was stirred for 24 h at 0 °C and acetic acid (0.5 ml) added. The precipitated DCU was filtered off and washed with acetonitrile. The acetonitrile was evaporated and the residue dissolved in ether. Work-up for a neutral product gave an oil (1.66 g) which was purified by column chromatography over silica using ethyl acetate as eluant. An *oil* (**7**) was obtained (1.49 g, 2.93 mmol, 80%), R_{FB} 0.85, $[\alpha]_D^{23}$ − 13.3° (*c* 3.0 in acetonitrile) (Found: C, 54.9; H, 6.6; N, 5.3%; $M^{+\cdot}$, 504. $C_{23}H_{32}FeN_2O_5S$ requires C, 54.8; H, 6.4; N, 5.5%; $M^{+\cdot}$, 504).

Synthesis of N-*t-Butoxycarbonyl*-α-*t-butyl*-L-γ-*glutamyl*-S-*ferrocenylmethyl*-L-*cysteinylglycine Ethyl Ester* (**9**).—*N*-t-Butoxycarbonyl-*S*-ferrocenylmethyl-L-cysteinylglycine ethyl ester (**7**) (0.53 g, 1.05 mmol) was dissolved in a solution of toluene-*p*-sulphonic acid (4.8 g, 2.53 mmol) in acetonitrile (10 ml) and the mixture was left overnight. It was then neutralised

with saturated aqueous hydrogen carbonate and extracted with ether. The ether extracts were extracted with citric acid (10%) and the aqueous extracts neutralised with solid sodium hydrogen carbonate. The latter was extracted with ether and the extracts were washed with water, dried, and evaporated to give the free base as an oil (0.24 g, 0.59 mmol, 56%) which was used immediately in the coupling stage below. To *N*-t-butoxycarbonyl-L-γ-glutamic acid α-t-butyl ester[9] (0.18 g, 0.59 mmol) dissolved in acetonitrile (5 ml) was added DCCI (0.122 g, 0.59 mmol) and HBT (0.091 g, 0.59 mmol) at 0 °C with stirring followed by the free base (0.24 g, 0.59 mmol), dissolved in acetonitrile (5 ml). Stirring was continued for 2 h at 0 °C and acetic acid (0.5 ml) added. The solution was filtered and the precipitate washed with acetonitrile. The filtrate was evaporated and the residue dissolved in ether. Work-up for a neutral product gave the fully protected glutathione derivative as a yellow oil R_{FE} 0.22, 0.00. Column chromatography on silica with ether as eluant, gave the *product* (**9**) as an oil (0.30 g, 0.44 mmol, 75%), $[\alpha]_D^{23}$ − 22.4° (*c* 2.5 in acetonitrile) (Found: C, 55.4; H, 7.1; N, 6.3%; $M^{+\cdot}$, 689. $C_{32}H_{47}FeN_3O_85$ requires C, 55.7; H, 6.9; N, 6.1%; $M^{+\cdot}$, 689).

Isolation of Glutathione from the Tripeptide t-Butyl Ester (**8**).—The tripeptide ester (**8**) (0.3 g, 0.41 mmol) was dissolved in TFA (10 ml) containing thiophenol (5%). After 3 h, ether (100 ml) was added and the precipitate separated by centrifugation. Ether (100 ml) was added to supernatant and the precipitate removed by centrifugation. The combined solids were washed with ethanol and ether. Drying afforded glutathione hydrate (0.07 g, 0.23 mmol, 52%) as a white solid, m.p. 150–155 °C, R_{FA} 0.5 (glutathione), 0.1 (trace, oxidised glutathione), $[\alpha]_D^{23}$ − 18.9° (*c* 1.0 in water) {lit.,[10] $[\alpha]_D$ − 19.1° (*c* 1.0 water)} (Found: C, 37.3; H, 5.6; N, 12.8. $C_{10}H_{17}N_3O_6S{\cdot}H_2O$ requires C, 36.9; H, 5.9; N, 12.9%).

Isolation of Glutathione from the Tripeptide Ethyl Ester (**9**).—The tripeptide ester (**9**) (0.3 g, 0.44 mmol) was dissolved in methanol (20 ml) at 0 °C and aqueous sodium hydroxide (1.0M; 0.5 ml, 0.5 mmol) added. Stirring was continued for 2 h and the solution neutralised with citric acid and extracted with ethyl acetate. The ethyl acetate extracts were dried and evaporated and the residue dissolved in thiophenol (5%) in TFA (10 ml). After 3 h, ether (30 ml) was added, followed by water (30 ml). The aqueous layer was washed with ether and concentrated under reduced pressure at 40 °C. Trituration with ethanol yielded a white solid which was centrifuged and repeatedly washed with ethanol. The solid was collected and dried (0.056 g, 0.21 mmol, 48%). Paper chromatography R_{FA} 0.5 (glutathione), 0.1 (oxidised glutathione). In all other respects the sample was identical with that isolated in the foregoing description.

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