Bioinorganic Reaction Centres on Electrodes. Modified Electrodes possessing Amino Acid, Peptide and Ferredoxin-type Groups on a Poly(pyrrole) Backbone

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Some amino-acid and peptide derivatives of pyrrole such as the *N,N'*-bis{*N*-[3-pyrrol-1-yl)propanoyl]-glycyl}-L-cystine methyl ester have been synthesised and characterised. The functionalised pyrroles undergo facile electropolymerisation to give electrode-bound films which are conducting in the oxidised state. Cystine-derivatised co-polymers can be converted to a cysteinyl state which allows the binding of ferredoxin centres. Activated pentafluorophenyl esters provide a convenient route to the amino-acid and peptide derivatised monomers. Importantly, electropolymerisation of an active ester allows covalent modification of electrode-bound films after polymerisation with, for example, redox-active ferrocenemethanol.

Modification of conducting materials with amino acids, di-, oligo- or poly-peptides attached to a polymer backbone may provide a means of (*i*) creating novel surfaces or matrices capable of binding redox-active co-factors, (*ii*) facilitating specific interactions with biological molecules or (*iii*) of constructing biocompatible interfaces. Such assemblies could be of interest in areas such as bioelectrocatalysis, sensors and diagnostics.[1]

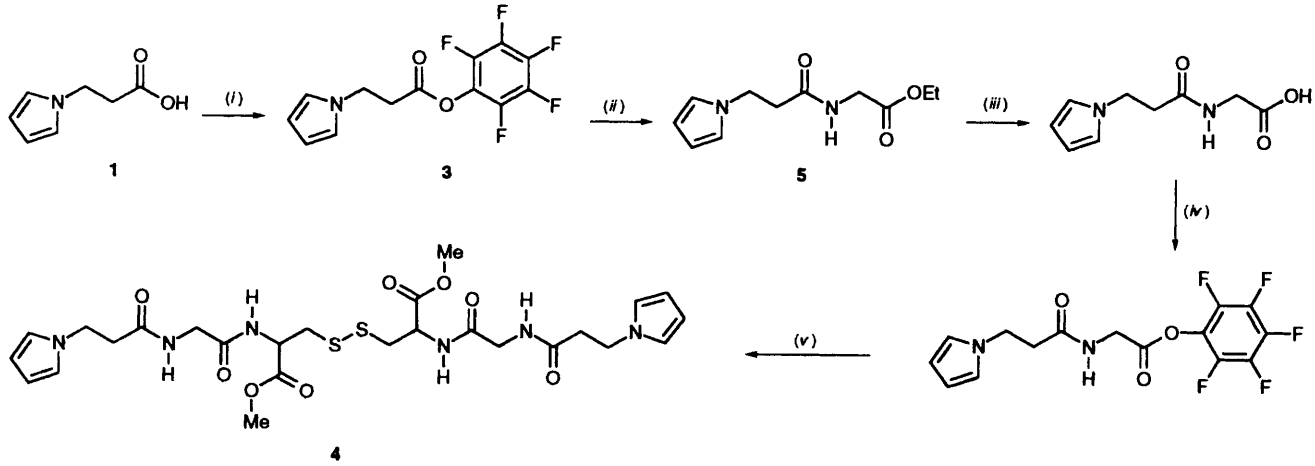
The synthesis and electropolymerisation of pyrrole derivatised with L-phenylglycine, L-serine and L-valine have been described.[2] In addition, we have recently shown that pyrrole can be derivatised with cystyl groups and that electropolymerisation affords a stable adherent poly(pyrrole–cystine) film. Importantly, the film can be reduced to a cysteinyl state which incorporates $\{Fe_4S_4\}^{2+}$ iron–sulfur centres, thereby affording a 'ferredoxin' electrode.[3]

As a first step towards *oligopeptide*-functionalised electrodes capable of binding metal–sulfur cluster centres, we describe herein the construction of poly(pyrrolylglycylcystyl)-modified platinum and vitreous carbon electrodes. We also show the advantage of using activated-ester derivatives of pyrrole in the synthesis of monomers and, uniquely, for the covalent modification of derivatised poly(pyrrole) films.

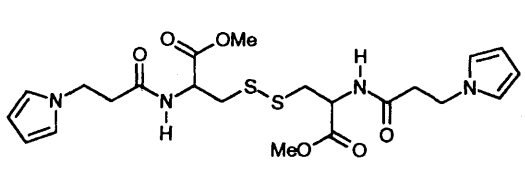
Results and Discussion

Monomer Synthesis.—The starting point for the synthesis of amino acid, peptide and other derivatives of pyrrole described herein was 3-(pyrrol-1-yl)propanoic acid **1**. This material is readily obtained in high yield by hydrolysis of commercially available 3-(pyrrol-1-yl)propiononitrile (see Experimental section).

The general strategy was to couple the carboxylate function of **1** with the NH_2 group of the desired amino acid methyl or ethyl ester. Alkyl esters were employed to avoid undesirable coupling, as is the usual practice in peptide synthesis. Initially, amide-bond formation between **1** and the amino acid esters was achieved in one step by using dicyclohexylcarbodiimide as the coupling reagent. However, yields were generally low, for example *N,N'*-bis[3-(pyrrol-1-yl)propanoyl]-L-cystine methyl ester **2** was obtained in *ca.* 20% yield.[3] A much improved method involved first the conversion of **1** to the activated pentafluorophenyl ester **3** followed by the coupling of this product with the desired amino acid ester. Using this two-step procedure, the yield of **2** was increased to *ca.* 70%. The peptide derivative *N,N'*-bis{*N*-[3-(pyrrol-1-yl)propanoyl]glycyl}-L-cystine methyl ester **4** was synthesised in four steps from **3** as follows. The ester **3** was coupled with glycine ethyl ester, the



Scheme 1 Pathway for the synthesis of the pyrrolylglycyl cystine derivative **4**: (*i*): pentafluorophenol–dicyclohexylcarbodiimide in dioxane stirred for *ca.* 15 h at room temperature; (*ii*) MeCN–NEt_3 stirred for *ca.* 5 h at 60 °C; (*iii*) MeOH–H_2O–NaOH, 60 °C for 1 h; (*iv*) pentafluorophenol–dicyclohexylcarbodiimide in dioxane stirred for *ca.* 15 h at room temperature; (*v*) cystine methyl ester in MeCN–NEt_3 stirred for *ca.* 5 h at 50 °C



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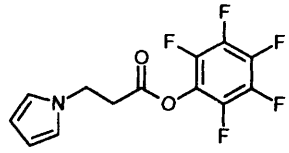

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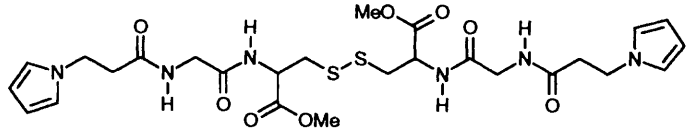

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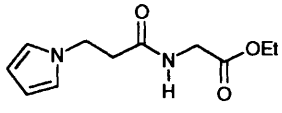

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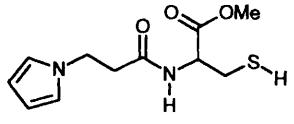

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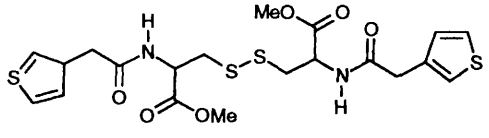

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isolated product *N*-[3-(pyrrol-1-yl)propanoyl]glycine ethyl ester **5** was then deprotected by base hydrolysis to give the free acid which was then converted to the pentafluorophenyl ester. Finally, the activated ester was treated with L-cystine methyl ester to give the desired product **4** in an overall yield of 14%. Scheme 1 summarises the synthetic pathway for **4**.

The cysteinyl derivative **6** was synthesised by reductive cleavage of the S–S bond of **2** using Cleland's reagent, DL-dithiothreitol (1,4-disulfanylbutane-2,3-diol).

The thiophene derivative *N*,*N*′-bis(thiophen-3-ylethanoyl)-L-cystine methyl ester **7** was synthesised from commercially available thiophen-3-ylethanoic acid by an analogous route to that used for the synthesis of **2**.

The structures of the pyrrole and thiophene derivatives were established by ^{1}H, ^{13}C NMR spectroscopy using resolution enhancement and two-dimensional NMR techniques (^{1}H–^{1}H; ^{13}C–^{1}H), FTIR and also by C, H and N microanalysis. Fig. 1 shows the resolution-enhanced ^{1}H NMR spectrum of **4** and Fig. 2 the corresponding ^{13}C–^{1}H chemical shift-correlated two-dimensional NMR spectrum of this compound.

The preparations for all compounds **1** to **7** are given in detail in the Experimental section together with analytical and spectroscopic data.

Electropolymerisation.—Each of the pyrrole derivatives **1**–**6** undergoes facile electropolymerisation under molecular nitrogen in methyl cyanide containing 50–100 mol dm^{-3} $[NBu_4][BF_4]$ at platinum or vitreous carbon electrodes to give stable adherent films. Fig. 3 shows the cyclic voltammetric response for the oxidation and re-reduction of a polymeric film of **4** (*ca.* 0.5–1.0 μm thick) on a Pt substrate. The behaviour is quite typical of poly(pyrroles) which become electronically conducting in the oxidised state. The film thickness was estimated from the electrode area, integration of the charge consumed in reducing the oxidised film and assuming the poly(pyrrole) has a density of *ca.* 1 g cm^{-3}, as described in detail elsewhere.[3–6]

Fourier-transform IR diffuse reflectance spectroscopy of electrodes coated with polymer films of **1**–**5** confirms that the integrity of the substituent group is retained upon oxidation of the monomer. For example, Fig. 4(*a*) and (*b*) show the spectra obtained for polymers of **2** and **4** respectively. [Note that the qualitative difference in intensities of the NH and amide CO vibrations with respect to the ester CO absorption for the two types of polymer is consistent with the 1:1 and 2:1 peptide:ester ratios in the parent molecules **2** and **4**.] Polymeric films can be readily produced by electrooxidation of **6** but FTIR spectroscopy shows that the free SH group is lost, presumably *via* oxidative S–S coupling to give a cystine polymer: the spectrum is indistinguishable from that of polymeric **2**. Films of polymeric **6** possessing free thiol groups can be produced indirectly, as described below.

Covalent Modification of Electrode-bound Polymeric Films.—Covalent modification of pyrrole derivatives *after* electropolymerisation is rarely successful because of poor reagent penetration of the polymeric film.[4] However, a facility for the introduction of groups after polymerisation is often desirable. For example, (*i*) a functional group can be sensitive to oxidation and thus destroyed in the polymerisation step or (*ii*) the group inhibits the electropolymerisation process itself by attacking the pyrrole radical cation.

We have shown that co-polymerisation with alkylammonium pyrroles can allow ingress of anionic reagents and have adopted this strategy to facilitate the cleavage of cystyl S–S bonds and the subsequent binding of anionic iron–sulfur cluster centres within a pyrrole film (see below). We now find that polymers of the activated ester **3** are surprisingly easily modified by neutral reagents. Fig. 5 shows the FTIR spectra obtained for electropolymerised **3** before (*a*) and after exposure to methanol for 5 (*b*) and 15 (*c*) min. The carbonyl stretch of the pentafluorophenolate ester at 1790 cm^{-1} is progressively replaced by that of the methyl ester at 1737, in addition, the band at 990 cm^{-1} corresponding to ν(C–F) of the pentafluorophenolate is diminished in intensity as the reaction proceeds. This esterification method can also be used with larger molecules: $[Fe(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4CH_2OH)]$ reacts with the activated ester polymer to give a redox-active ferrocenyl-modified polymer, Fig. 6. Amide bonds are also readily formed as illustrated by Fig. 7 which shows the reflectance FTIR spectra of electropolymerised **3** before and after reaction with L-cystine methyl ester.

Ferredoxin Centres in Polymers.—The assembly of ferredoxin centres within polymer films requires conversion of the cystyl polymers to a cysteinyl state capable of undergoing thiolate ligand exchange with synthetic clusters of the type $[Fe_4S_4(SR)_4]^{2-}$ (R = alkyl or aryl) and a means of maintaining overall charge neutrality within the film.

Attempts reductively to cleave the S–S bonds in polymers formed from *N*,*N*′-bis[3-(pyrrol-1-yl)propanoyl]-L-cystine methyl ester **2** or of *N*,*N*′-bis{*N*-[3-(pyrrol-1-yl)propanoyl]-glycyl}-L-cystine methyl ester **4** by Cleland's reagent were

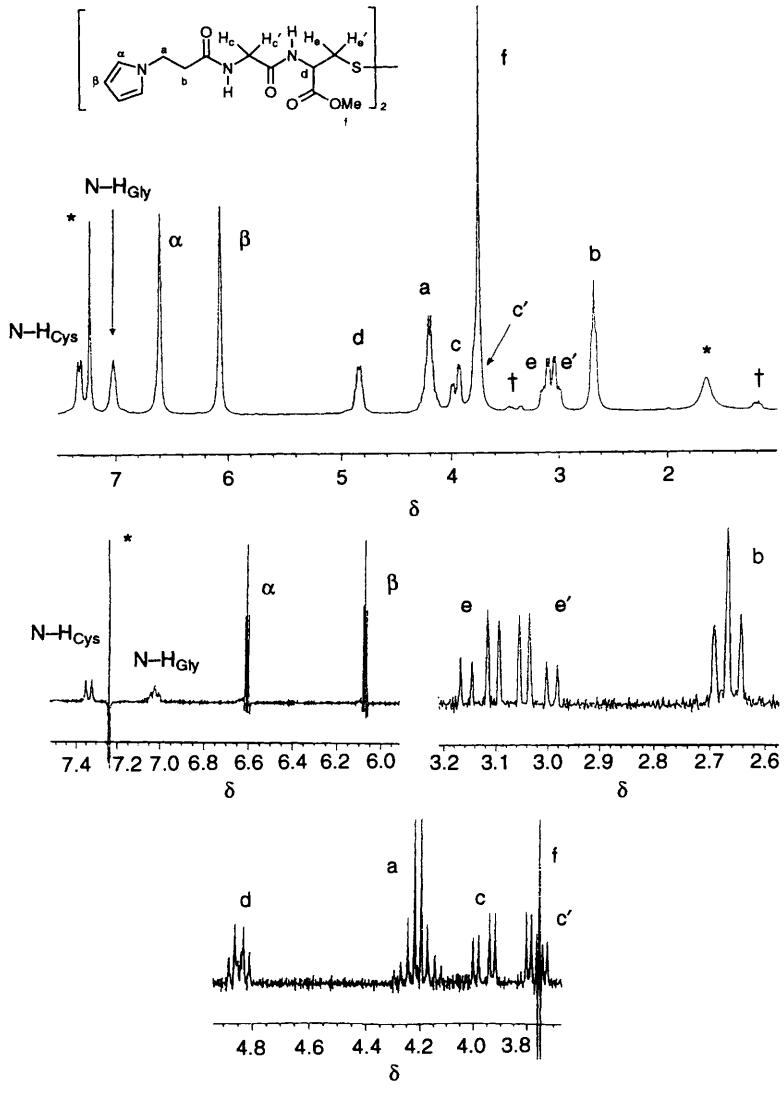
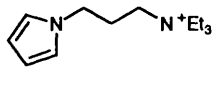


Fig. 1 ^{1}H NMR spectrum of monomer **4** recorded at 270 MHz in $CDCl_3$: resolution-enhanced sections of the spectrum are shown (bottom). Labelling corresponds to the assignments in the structural formula; * = $CDCl_3$ solvent, † = Et_2O contamination

unsuccessful. This was presumably because the reagent was unable to penetrate the film. However, co-polymerisation of **2** or **4** with the pyrrole alkylammonium **8** allowed conversion of the cystyl groups to a cysteinyl state *via* reaction under basic conditions with Cleland's reagent.

Conversion of the S–S groups to SH functions was established by the appearance of ν(SH) in the reflectance FTIR spectra of the co-polymers; the absence of ν(OH) showed that neither the reagent nor its product was retained in the film. An additional advantage of co-polymerisation with the cationic monomer is that it allows ingress of anionic clusters by ion exchange and maintenance of charge neutrality following both ligand exchange and subsequent redox cycling.

So as to provide four cysteinyl groups per cluster (after S–S cleavage) and to accommodate the anionic clusters operating at the $\{Fe_4S_4(CysS)_4\}^{2-/3-}$ redox level, the ideal ratio of cystine : alkylammonium polymer groups is 2:3.[3] Ratios close to this were achieved by empirically varying the relative concentrations of the monomers **8** and **2** or **4** and using intensities of ν(CO, ester) and ν(BF) to monitor the resultant polymer composition. We found that monomer ratios of *ca.* 1:12, **2** or **4**:**8** were necessary to obtain a *ca.* 2:3 ratio in the polymer. Evidently the polymerisation of **8** is slower or less efficient than that of **2** or **4**.



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Before cleavage of the S–S bonds, the electrode-bound films behave as simple ion-exchange polymers.[5,6] Thus exposure to solutions of $[Fe_4S_4(SPh)_4]^{2-}$ led to incorporation of the redox-active dianion by displacement of the $[BF_4]^-$ counter ion. The cyclic voltammetric response of the electrostatically bound cluster was similar to that observed in films of the homopolymer of **8**: the cluster was slowly lost from the film on repetitive cycling and rapidly displaced by increasing the concentration of $[BF_4]^-$ in the electrolyte solution.[5,6]

After cleavage of the S–S bonds (*i.e.* converting the cystine film to a cysteinyl state) exposure to a solution of the cluster leads to displacement of PhSH and incorporation of the cysteinyl-S ligated $[Fe_4S_4]^{2+}$ ferredoxin core. The evidence for this is as follows. Neither repetitive cycling over long periods nor exposure of the cluster-loaded film to high concentrations of $[BF_4]^-$ (*ca.* 1 mol dm^{-3}) leads to a loss of peak intensity in the cyclic voltammetric response for the $[Fe_4S_4]^{2+/1+}$ couple, Fig. 8. This is consistent with the confined cluster being tightly ligated rather than ionically bound. The value of $E^{o'}$ for the ligated cluster in the cysteinyl polymer was shifted by *ca.* 100 mV to potentials more negative than that observed

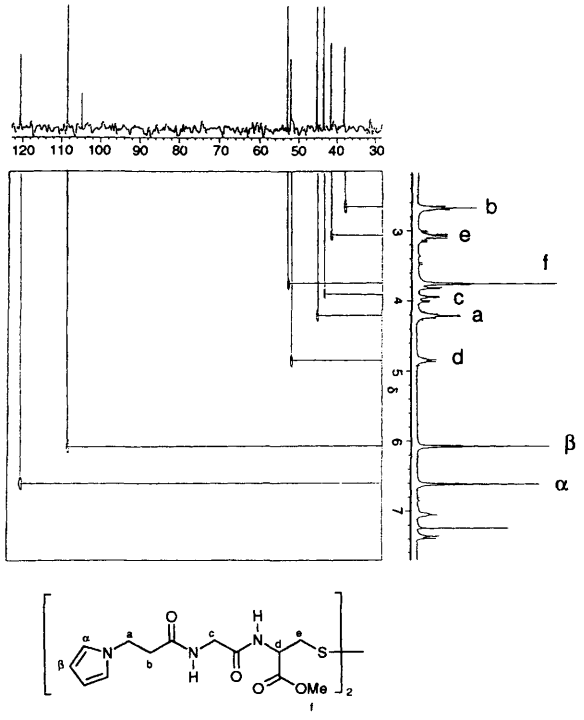


Fig. 2 ^{13}C–^{1}H Chemical-shift correlated two-dimensional NMR spectrum of monomer **4**. The carbonyl carbon resonances were located by conventional ^{13}C NMR spectroscopy

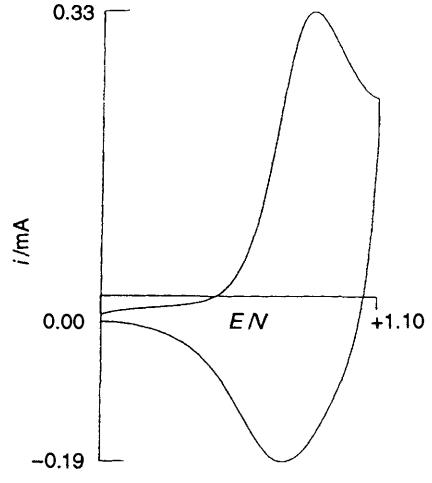


Fig. 3 Cyclic voltammogram of a Pt disc electrode coated with electropolymerised **4** which shows the oxidation of the conjugated poly(pyrrole) backbone to the conducting state and re-reduction to the neutral form. Recorded in MeCN–0.05 mol dm^{-3} $[NBu_4][BF_4]$; potentials are relative to ferrocenium–ferrocene; scan rate 20 mV s^{-1}

for the electrostatically bound thiophenolate cluster in accord with ligand exchange. The FTIR experiments on cysteinyl films containing bound cluster showed the absence of ν(SH) and the absence of ν(CH, aromatic bending modes). This is in accord with the removal of free cysteinyl SH groups by ligand exchange with the thiophenolate cluster and diffusional loss of displaced PhSH from the film into the bulk solution; the low energy absorptions for the aromatic ring are clearly visible in the FTIR spectra of the simple ion-exchange polymer and co-polymer systems. The linear response of peak current *versus* the scan rate for the reduction of the covalently bound clusters is fully in accord with a surface-confined system, as illustrated by Fig. 9.

The loading of the cluster in the polymeric pyrrole–cysteine films was estimated by determining the charge injection for fully reducing the dianion to the trianion and referencing this

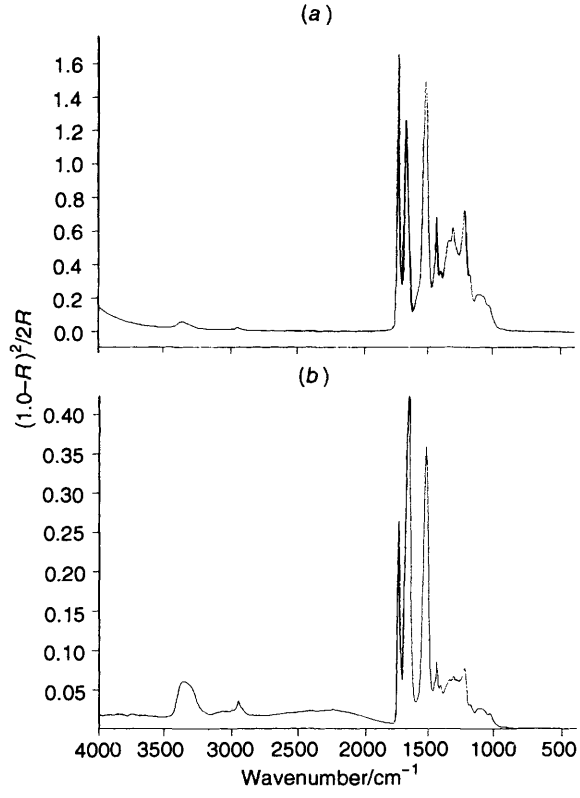


Fig. 4 Reflectance FTIR spectrum of a polished Pt disc electrode coated with homopolymers of electropolymerised **2** (*a*) and **4** (*b*). Polymers were formed by oxidation of *ca.* 5 mmol dm^{-3} solutions of the respective monomers in an MeCN electrolyte and spectra are for the uncharged state. Carbonyl amide and ester bands are clearly evident: ν(NH) at *ca.* 3400 cm^{-1} appear less intense than in spectra of the monomers. Spectra offset

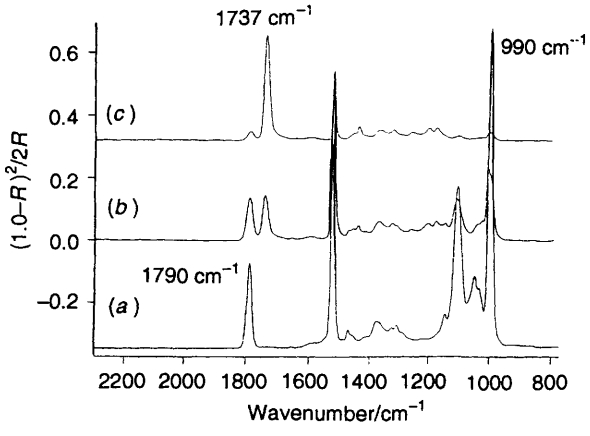


Fig. 5 Reflectance FTIR spectrum of a polished Pt disc electrode coated with (*a*) the electropolymerised pentafluorophenolate ester **3** showing ν(CO) at 1790 cm^{-1} and ν(CF) at 990 cm^{-1}, (*b*) the same electrode after soaking in MeOH for *ca.* 5 min showing the appearance of a new ester band at 1737 cm^{-1} (CO_2Me) and the attenuation of the 1790 and 990 cm^{-1} bands, and (*c*) after further exposure to MeOH for 15 min showing nearly complete conversion to the methyl ester. Spectra offset

to the total number of pyrrole units determined chronocoulometrically from the redox response of the polymer backbone,[3] as described in detail earlier for ionic systems.[6] Reproducible ratios of 1:7 ± 1 cluster: pyrrole units were obtained for films grown from 1:10, **2**:**8**. This is consistent with an approximate electrode composition of [(pyrrole–Cys)$_4$-

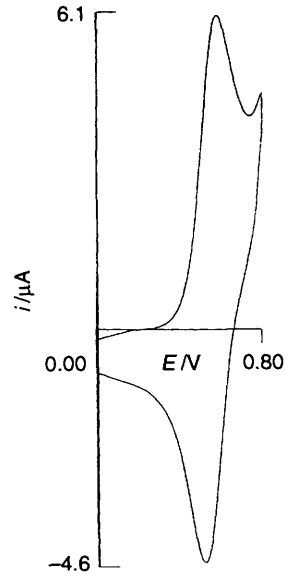


Fig. 6 Cyclic voltammogram of a Pt disc electrode coated with electropolymerised **3** *after* reaction with ferrocenemethanol in MeCN and washing. The voltammetric response shows the reversible oxidation of the immobilised ferrocenyl moieties in MeCN–0.05 mol dm^{-3} $[NBu_4][BF_4]$ at a scan rate of 30 mV s^{-1}. The conductivity of the poly(pyrrole) was destroyed by 'over oxidation' so as to unmask the ferrocenyl response from that of the oxidation of the polymer backbone

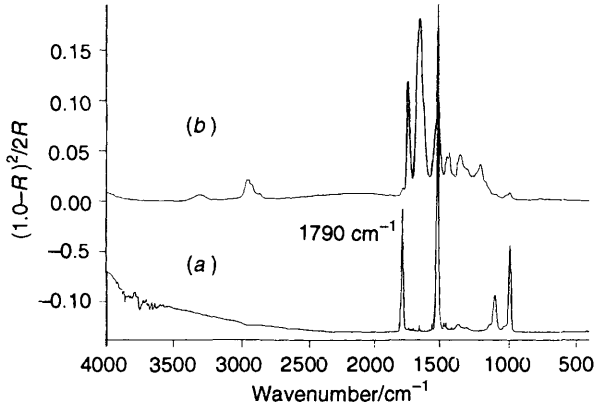


Fig. 7 Reflectance spectrum of a polished Pt disc electrode coated with (*a*) the electropolymerised pentafluorophenolate ester **3** showing ν(CO) at 1790 cm^{-1} and ν(CF) at 990 cm^{-1}, and (*b*) the same electrode after soaking in an MeCN–NEt_3 solution of L-cystine methyl ester for 15 min at 60 °C; the amide and ester bands of the cystinyl-derivatised polymer are clearly evident as is the loss of bands due to the pentafluorophenolate ester. Spectra offset

$Fe_4S_4]^{2-}$·3(pyrrole–NEt_3^+)·$[BF_4]^-$ and a concentration of the cluster in the film of *ca.* 0.6 mol dm^{-3}. This high loading supports charge propagation through the film by electron hopping at the 2−/3− redox levels because electron self-exchange is fast.[6]

Scheme 2 summarises the construction of the Gly-Cys ferredoxin electrode.

The $E^{o'}$ values for the $[Fe_4S_4]^{2+/+}$ couple in the Cys- and Gly-Cys-derivatised co-polymer films are reasonably close to those measured in solution for synthetic peptide derivatives of $[Fe_4S_4]^{2+}$ as listed in Table 1. The $E^{o'}$ values for $[Fe_4S_4(SR)_4]^{2-/3-}$ couples confined within the ion-exchange homopolymer of **8** are positive of those measured for the same species freely diffusing in solution and this has been attributed to strong electrostatic effects: the shift ΔE for R = Ph ($\Delta E = E^{o'}_{polymer} - E^{o'}_{solution}$) in the homopolymer is *ca.* 200 mV.[6] The value of ΔE for the same cluster ionically bound in the copolymer of **2** and **8** is only 80 mV, suggesting that electrostatic

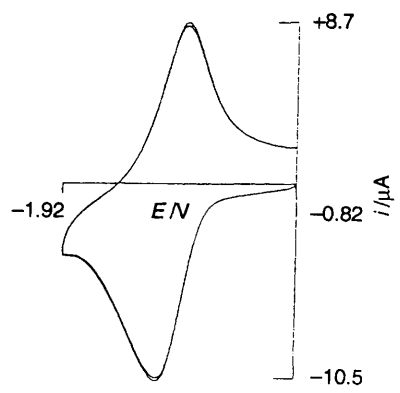


Fig. 8 Reversible reduction of $\{Fe_4S_4\}^{2+}$ centres ligated by S of the co-polymer of **4** and **8** coating a Pt disc electrode. The superimposed 3rd and 11th scans are shown and illustrate the stability of the system. Recorded in MeCN–0.05 mol dm^{-3} $[NBu_4][BF_4]$; potentials are relative to ferrocenium–ferrocene; scan rate 50 m V s^{-1}

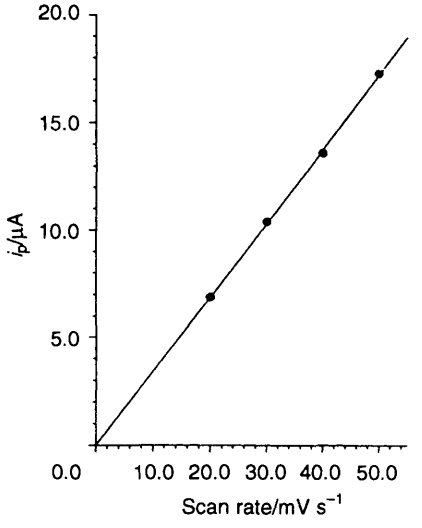


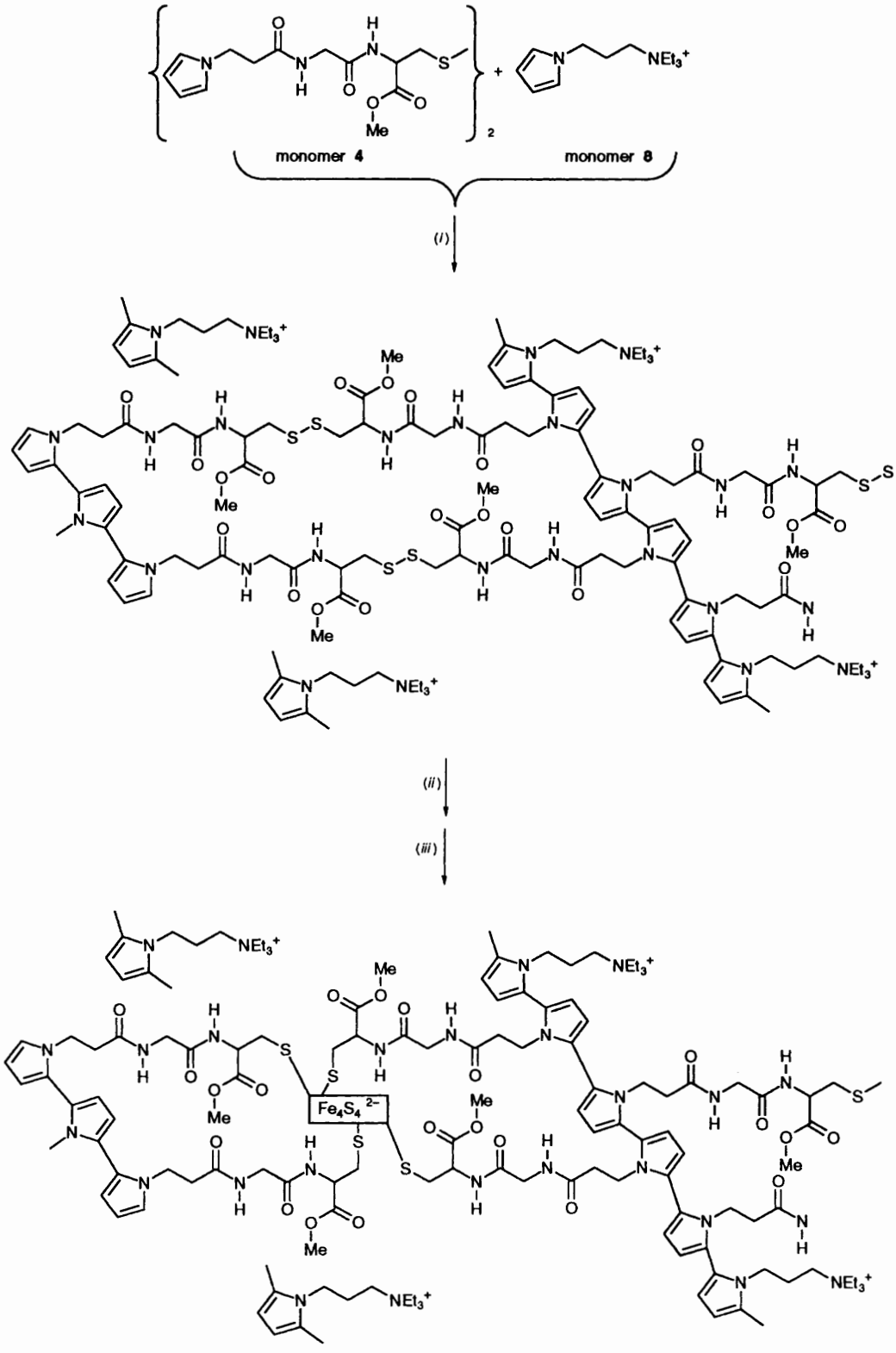
Fig. 9 Dependence of the peak current on scan rate for reduction of $\{Fe_4S_4\}^{2+}$ centres in the co-polymer of **2** and **8** coating a Pt disc electrode of area 0.2 cm^2 in MeCN–0.05 mol dm^{-3} $[NBu_4][BF_4]$. The linear response is typical of that for surface-confined redox centres

Table 1 Values of $E^{o'}$ for the $[Fe_4S_4X_n]^{2-/3-}$ couple with mono- or bi-dentate oligopeptide substituents

X_n	$E^{o'}$ [a]/V	Ref.
$(CH_3CO\text{-Cys-NHMe})_4$ [b]	−0.68	7
(*Z*-Cys-Gly-Ala-Cys-OMe)$_2$ [c]	−0.72	8
(*Z*-Cys-Gly-Ala-OMe)$_4$ [d]	−0.76	9
$\{[C_4H_2N(CH_2)_2C(O)\text{-Gly-Cys-OMe}]_4\}_n$ [e]	−0.76	This work
(*Z*-Cys-Gly-OMe)$_4$ [d]	−0.78	8
(*Z*-Cys-Ile-Ala-Cys-OMe)$_2$ [c]	−0.78	8
$\{[C_4H_2N(CH_2)_2C(O)\text{-Cys-OMe}]_4\}_n$ [f]	−0.80	This work
$\{[C_4H_2N(CH_2)_2C(O)\text{-Cys-OMe}]_4\}_n$ [g]	−0.82	This work

[a] Values *vs.* the normal hydrogen electrode. [b] In dmso–H_2O (4:1) (dmso = dimethyl sulfoxide). [c] In dimethylformamide solution. [d] In CH_2Cl_2 solution. [e] In the reduced co-polymer of **4** and **8** in MeCN. [f] In the reduced co-polymer of **2** and **8** in H_2O. [g] In the reduced co-polymer of **2** and **8** in MeCN.

interactions are somewhat weaker in the diluted ion-exchange film. Presumably $[Fe_4S_4]^{2+}$ centres ligated by cysteinyl sulfur atoms in the reduced co-polymers are also relatively unaffected by electrostatic interactions with the Et_3N^+–polymer groups.



Scheme 2 Pathway for construction of glycine cysteine co-polymer film electrode and incorporation of ferredoxin centres: (*i*) Pt or vitreous carbon anode, MeCN electrolyte; (*ii*) cleavage of S–S bonds with Cleland's reagent in basic MeCN; (*iii*) incorporation of cluster centres by thiolate ligand exchange with $[Fe_4S_4(SPh)_4]^{2-}$ in MeCN

Conclusion

Amino-acid and dipeptide derivatives of pyrrole are readily synthesised using activated pentafluorophenyl ester derivatives.

These pyrroles undergo facile electropolymerisation to give adherent surface-bound films which are conducting in the oxidised state.

Reductive cleavage of the S–S bond in cystyl or glycylcystyl poly(pyrrole) co-polymers to the cysteinyl form allows the construction of ferredoxin-type modified electrodes.

Pentafluorophenyl-derivatised pyrrole can be readily electropolymerised; the activated ester groups provide a means of covalent modification *via* reactions with hydroxyl or amine groups after polymerisation.

Experimental

Syntheses.—3-(*Pyrrol-1-yl*)*propanoic acid* **1**. 3-(Pyrrol-1-yl)-propiononitrile (15 g, 0.125 mol) was added to an aqueous solution (*ca.* 100 cm^3) of NaOH (20 g, 0.5 mol) in a 250 cm^3 round-bottom flask fitted with a water-cooled condenser. The mixture was refluxed until ammonia evolution (detectable at the top of the condenser by moist pH paper) had ceased, *ca.* 6 h, after which time the solution was homogeneous. Cold water (50 cm^3) was run down the condenser and the mixture allowed to cool. The flask and contents were placed in an ice bath and 50% H_2SO_4 (28 cm^3, 0.5 mol) was added. The mixture separated with the product forming an upper oily phase. The product was extracted with Et_2O (4 × 50 cm^3) and the extracts dried overnight with anhydrous Na_2SO_4. Ether was removed under vacuum and the product was obtained as a very pale yellow waxy solid (15.91 g, 0.114 mol), in 92% yield, m.p. 59–60 °C. FTIR: ν(O–H) 3200, ν(C=O) 1700, ν(C–O) 1220 cm^{-1}. ^1H NMR: δ 6.67 [t, $J(H_\alpha H_\beta)$ 2.0, Hα], 6.16 [t, $J(H_\alpha H_\beta)$ 2, H_β], 4.20 [t, $J(H_aH_b)$ 7.3, H_b], 2.83 [t, $J(H_aH_b)$ 6.9 Hz, H_a].

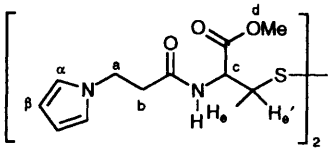
N,N′-*Bis*[3-(*pyrrol-1-yl*)*propanoyl*]-L-*cystine methyl ester* **2**. Pentafluorophenyl 3-(pyrrol-1-yl)propanoate (2.28 g, 7.5 mmol) was added to a 250 cm^3 round-bottom flask containing L-cystine methyl ester dihydrochloride (1.18 g, 3.5 mmol). The solids were dissolved in MeCN (*ca.* 100 cm^3) together with NEt_3 (0.5 cm^3). This mixture was stirred and heated to around 60 °C for 2 h. On cooling a white precipitate formed which was filtered off and washed with Et_2O and hexane (1.61 g, 3.15 mmol, 90% yield, m.p. 146–147 °C) (Found: C, 51.70; H, 5.75; N, 11.00. Calc. for $C_{22}H_{30}N_4O_6$: C, 51.75; H, 5.90; N, 10.95%). FTIR: ν(N–H) 3327, ν(C=O) 1739 (ester), ν(C=O) (amide) 1643 cm^{-1} (S, C=O). ^{13}C NMR: δ 170.59 and 170.06, 120.56 [d, J(C–H) 179.6, C_α], 108.49 [d, J(C–H) 173.4, C_β], 52.84 [q, J(C–H) 148.0, OCH_3], 51.56 [d, J(C–H) 142.0, $(C–H)_c$], 45.19 [t, J(C–H) 141.0, $(CH_2)_b$], 40.37 [t, J(C–H) 147.0 $(CH_2)_c$], 38.45 [t, J(C–H) 129.0 Hz, $(CH_2)_a$]. ^1H NMR data are summarised in Table 2.

Pentafluorophenyl 3-(*pyrrol-1-yl*)*propanoate* **3**. (Pyrrol-1-yl)propanoic acid (3 g, 22 mmol) was added to dioxane (50–60 cm^3) in a 250 cm^3 round-bottom flask and stirred. Pentafluorophenol (4g, 22 mmol) was then added along with dicyclohexylcarbodiimide (4.5 g, 22 mmol). A thick creamy precipitate of dicyclohexylurea developed after stirring overnight, which was then filtered off (4.9 g, 22 mmol) All the solvent was then removed under vacuum to yield a golden oil. This oil was taken up in hexane (*ca.* 150 cm^3) and the solution placed in the freezer. After several hours the product had precipitated as golden needles which were filtered off and washed with cold hexane (5.15 g, 16.9 mmol, 78% yield, m.p. 42–44 °C). FTIR: ν(C=O) at 1790 cm^{-1}. ^{19}F NMR: δ −152.78(m), 157.81(m) and −162.38(m). ^1H NMR: 6.69 [t, $J(H_\alpha H_\beta)$ 1.87, H_α], 6.16 [t, $J(H_\beta H_\alpha)$ 1.93, H_β], 4.32 [t, $J(H_aH_b)$ 6.84, H_b], 3.19 [t, $J(H_bH_a)$ 6.84 Hz, H_a].

N,N′-*Bis*{N-[3-(*pyrrol-1-yl*)*propanoyl*]*glycyl*}-L-*cystine methyl ester* **4**. (*i*) *Hydrolysis of the ester* **5**. *N*-[3-(Pyrrol-1-yl)-propanoyl]glycine ethyl ester (0.86 g, 3.84 mmol) was added to a 100 cm^3 round bottom flask containing NaOH (1 g), MeOH (18 cm^3) and H_2O (3 cm^3). This solution was heated to *ca.* 60 °C for 1 h after which time the methanol was removed under vacuum and the remaining solution acidified with aqueous H_2SO_4. The acid product was extracted with 3 × 20 cm^3 Et_2O and the diethyl ether phase dried over anhydrous $MgSO_4$. After filtration the ether was removed under vacuum to give a waxy solid (0.4 g, 2.04 mmol) in 53% yield.

(*ii*) *Esterification with pentafluorophenol.* The hydrolysis product (0.4 g, 2.04 mmol) was dissolved in a minimum amount of dioxane in a 100 cm^3 round-bottom flask to which were added pentafluorophenol (0.38 g, 2.06 mmol) and dicyclohexyl-carbodiimide (0.42 g, 2.04 mmol). This mixture was stirred overnight after which time a creamy white precipitate of dicyclohexylurea had formed. This was filtered off (0.42 g, 1.87 mmol, 92%) and the dioxane removed from the filtrate under vacuum. The residual solid was washed with copious amounts of hexane. The pentafluorophenyl ester product was isolated as a creamy white solid (0.56 g, 1.54 mmol) in 76% yield, m.p. 101–102 °C.

Table 2 Proton NMR data for compound **2**



2

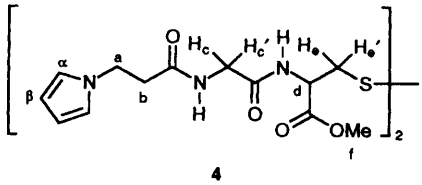
Assignment	δ	Integral	Multiplicity	*J*/Hz
H_α	6.63	1.90(2)	t	2.0 (H_α)
N–H	6.36	0.80(1)	d	7.3 (H_c)
H_β	6.09	1.84(2)	t	2.0 (H_β)
H_c	4.79	1.00(1)	t of d	7.5(N–H), 5.13 (H_e)
H_a	4.21	2.13(2)	d of t	6.7 (H_b), 2.6
H_d	3.73	3.20(3)	s	—
$H_{e'}$	3.10		d of d	5.13 (H_c), 14.5 (H_e)
		2.06(2)		
H_e	3.00		d of d	5.13 (H_c), 14.3 ($H_{e'}$)
H_b	2.67	2.01(2)	t	6.8 (H_a)

(*iii*) *Further amination with* L-*cystine.* The activated ester from step (*ii*) (0.49 g, 1.35 mmol) was added with L-cystine methyl ester dihydrochloride (0.23 g, 0.67 mmol) to a 100 cm^3 round-bottom flask containing MeCN (50 cm^3) and NEt_3 (0.5 cm^3). This mixture was warmed to around 50 °C and stirred for several hours. All the solvent was removed under vacuum, the remaining sticky solid taken up in $CHCl_3$ and washed several times with aqueous Na_2CO_3 solution before being dried over anhydrous Na_2SO_4. Finally all $CHCl_3$ was removed under vacuum and the remaining solid recrystallised from MeCN–Et_2O. The product was isolated as a white solid (0.17 g, 0.27 mmol) in 40% yield (14% overall), m.p. 140–141 °C (Found: C, 49.60; H, 5.80; N, 13.20. Calc. for $C_{26}H_{37}N_6O_8S_2$: C, 50.00; H, 5.80; N, 13.45%). FTIR: ν(N–H) 3307, ν(C=O)(ester) 1744, ν(C=O) (amide) 1654 and 1633 cm^{-1}. ^{13}C-{^1H} NMR: δ 171.3, 170.2, 169.2 (C=O), 120.6 (C_α), 108.5 (C_β), 52.9 (OCH_3), 52.1 [$(CH)_d$], 45.3 $(CH_2)_b$, 43.5 [$(CH_2)_c$ of glycine], 41.6 [$(CH_2)_e$ of cystine] and 38.1 [$(CH_2)_a$]. ^1H NMR data are summarised in Table 3.

N-[3-(*Pyrrol-1-yl*)*propanoyl*]*glycine ethyl ester* **5**. Pentafluorophenyl 3-(pyrrol-1-yl)propanoate (1.79 g, 5.86 mmol) and glycine ethyl ester hydrochloride (0.82 g, 5.88 mmol) were added to a 250 cm^3 round-bottom flask containing MeCN (*ca.* 60 cm^3) and NEt_3 (0.5 cm^3). The mixture was heated gently for several hours after which time all material was in solution. All solvent was removed under vacuum and Et_2O added to the sticky solid, [Et_3NH][Cl] precipitated and was filtered off as a white solid (0.74 g, 5.4 mmol, 92%). The Et_2O phase was washed several times with an aqueous solution of Na_2CO_3 to remove pentafluorophenol and dried over anhydrous $MgSO_4$. The solution was then reduced to dryness under vacuum and the residue washed with hexane to give a white solid (0.95 g, 4.24 mmol) in 72% yield, m.p. 38–39 °C. FTIR: ν(N–H) 3320, ν(C=O) (ester) 1748, ν(C=O) (amide) 1648 cm^{-1}. ^1H NMR data are summarised in Table 4.

N-[3-*Pyrrol-1-yl*)*propanoyl*]-L-*cysteine methyl ester* **6**. *N,N′*-Bis[3-(pyrrol-1-yl)propanoyl]-L-cystine methyl ester **2** (0.55 g, 1.08 mmol) was suspended in degassed MeOH (*ca.* 200 cm^3) to which was added Cleland's reagent (1 g, 6.5 mmol) under an atmosphere of dinitrogen gas. This was warmed for *ca.* 4 h until all the material was in solution. The MeOH was then removed under vacuum to leave a sticky solid, which was washed with small amounts of distilled water to remove excess Cleland's reagent and the ring-closed oxidised by-product. The remaining solid was taken up in $CHCl_3$ which was washed with more

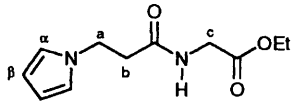
Table 3 Proton NMR data for compound **4**



4

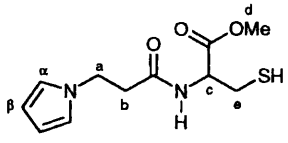
Assignment	δ	Integral	Multiplicity	*J*/Hz
$N-H_{Cys}$	7.35	0.7(1)	d	4.9(H_d)
$N-H_{Gly}$	7.02	0.7(1)	t	5.0(H_c)
H_α	6.61	2.0(2)	t	2.1(H_β)
H_β	6.10	1.9(2)	t	2.1(H_α)
H_d	4.84	1.0(1)	d of t	8.0(H_e), 4.4($N-H_{Cys}$)
H_a	4.20	2.0(1)	m	—
H_c	3.96	(1)	d of d	4.8($N-H_{Gly}$), 16.0($H_{c'}$)
$H_{c'}$	3.75	(1)	d of d	4.8($N-H_{Gly}$), 16.0(H_c)
H_f	3.75	4.7(3)	s	—
H_e	3.13		d of d	6.0(H_d), 14.0($H_{e'}$)
$H_{e'}$	3.02	1.7(2)	d of d	6.0(H_d), 14.0(H_e)
H_b	2.67	1.8(2)	t	4.77 (H_a)

Table 4 Proton NMR data for compound **5**



Assignment	δ	Integral	Multiplicity	*J*/Hz
H_α	6.64	2.00(2)	t	2.0 (H_β)
H_β	6.11	1.90(2)	t	2.0 (H_α)
N–H	5.90	0.60(1)	br s	—
H_a	4.22		t	6.8 (H_b)
Ethyl CH_2	4.19	4.0(4)	q	7.2 (CH_3)
H_c	3.96	2.0(2)	d	5.1 (N–H)
H_b	2.64	2.1(2)	t	6.8 (H_a)
Ethyl CH_3	1.26	3.3(3)	t	7.2 (CH_2)

Table 5 Proton NMR data for compound **6**

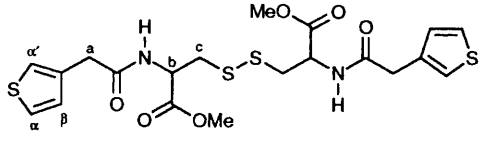


Assignment	δ	Integral	Multiplicity	*J*/Hz
H_α	6.64	2.00(2)	t	2.1 (H_β)
N–H	6.22		d	5.6 (H_c)
H_β	6.12	3.19(3)	t	2.1 (H_α)
H_c	4.83	1.09(1)	d of t	7.4 (N–H), 4.0 (H_e)
H_a	4.24	2.08(2)	m	—
H_d	3.75	3.30(3)	s	—
H_e	2.89	2.20(2)	d of d	9.5 (S–H), 3.9 (H_c)
H_b	2.65	2.20(2)	t	6.7 (H_a)
S–H	1.23	1.11(1)	t	9.2 (H_e)

water before being dried for several hours over anhydrous Na_2SO_4. After filtration all $CHCl_3$ was removed under vacuum and the product washed with pentane to give a white powder (0.39 g, 1.52 mmol) in 70% yield, m.p. 72–73 °C. FTIR: ν(N–H) 3318, ν(S–H) 2554, ν(C=O) (ester) 1743, ν(C=O) (amide) 1640 cm^{-1}. ^1H NMR data are summarised in Table 5.

N,N′-*Bis*(*thiophen*-3-*ylethanoyl*)-L-*cystine methyl ester* **7**. Thiophen-3-ylethanoic acid (2.0 g, 15.6 mmol) was added with 2.6 g (14.1 mmol) pentafluorophenol and dicyclohexylcarbodiimide (3.0 g, 14.5 mmol) to a 250 cm^3, round-bottom flask containing dioxane (*ca.* 50 cm^3). This mixture was stirred overnight after which time a creamy white precipitate of dicyclohexylurea had formed. This was filtered off and the solvent removed from the filtrate under vacuum to leave a gold coloured liquid, pentafluorophenyl (thiophen-3-yl)ethanoate (3.8 g, 12.9 mmol) in 92% yield. This ester (2.5 g, 8.5 mmol) was added with cystine methyl ester dihydrochloride (1.3 g, 3.8 mmol) and NEt_3 (1 cm^3) to a 250 cm^3 round-bottom flask containing MeCN (*ca.* 100 cm^3). This mixture was heated to *ca.* 60 °C and stirred for several hours. The solution was evaporated to dryness under vacuum and the residue taken up in CH_2Cl_2. This solution was washed with H_2O (100 cm^3) and then dried over $MgSO_4$. After filtration the CH_2Cl_2 was removed under vacuum and the residue washed with hexane and Et_2O to give an off-white powder (3.15 g, 6.1 mmol) in 72% yield, m.p. 108–110 °C (Found: C, 46.30; H, 4.50; N, 5.60. Calc. for $C_{20}H_{24}N_2O_6S_4$: C, 46.50; H, 4.70; N, 5.40%). FTIR: ν(N–H) 3320, ν(C=O) (ester) 1740, ν(C=O) (amide) 1640 cm^{-1}. ^1H NMR data are summarised in Table 6.

Table 6 Proton NMR data for compound **7**



Signal	δ	Integral	Multiplicity	*J*/Hz
H_β	7.32	1.04(1)	d of d	4.95 (H_α), 2.89 ($H_{\alpha'}$)
$H_{\alpha'}$	7.17	1.09(1)	d of d	2.95 (H_β), 1.03 (H_α)
H_α	7.02	1.08(1)	d of d	4.95 (H_β), 1.35 ($H_{\alpha'}$)
N–H	6.44	1.00(1)	d	7.20 (H_b)
H_b	4.80	1.30(1)	d of t	7.58 (N–H), 5.14 (H_c)
OCH_3	3.72	3.60(3)	s	—
H_a	3.63	2.50(2)	s	—
H_c	3.08	2.04(2)	d	5.14 (H_b)

Electrochemical Procedures.—Preparation and manipulation of polymer-coated electrodes and cluster incorporation were carried out in an Alvic anaerobic glove box operating with a recirculating atmosphere of dinitrogen (*ca.* 1 ppm O_2 and 5 ppm H_2O). Other operations of air-sensitive materials were carried out using standard Schlenk techniques. All solvents were freshly distilled from appropriate drying agents. Electrochemical measurements were made using an EG and G PAR model 273 potentiostat/waveform generator/integrator controlled by an IBM/PC AT computer running in-house software. Output was

recorded on a Hewlett-Packard HP 7470A plotter or on a Philips PM 8043 X-Y recorder.

NMR Spectroscopy.—The ^1H and ^{13}C NMR spectra were recorded on a JEOL GSX 270 spectrometer (270 MHz) for solutions in $CDCl_3$ and referenced to external $SiMe_4$.

FTIR Spectroscopy.—FTIR spectra of polymeric films were recorded on a Bio-Rad FTS-7 single beam spectrometer continuously purged with dinitrogen. The spectrometer was controlled by a 2340-SPC data station and the resolution was set at 4 cm^{-1}. Measurements were made on polymer films coating a highly polished platinum disc electrode of 1 cm diameter. Freshly washed, thick films of *ca.* 1 μm were generally employed. The spectrometer was operated in the diffuse reflectance mode with the Kubelka–Munk algorithm $(1.0 - R)^2/2R$, where R = reflectance used to resolve multiple internal reflections and thereby relate concentration to intensity. Spectra were referenced against the uncoated polished electrode.

Polymer Electrodes.—Cluster-modified electrodes were constructed in the following way. Co-polymers of the compounds **2** or **4** with the pyrrole alkylammonium **8** were prepared by electroxidation on Pt or vitreous carbon discs of a solution containing *ca.* 2 mmol dm^{-3} **2** or **4** and 20–25 mmol dm^{-3} **8** in MeCN–0.05 mol dm^{-3} $[NBu_4][BF_4]$ following the general procedure described earlier.[6] The electropolymerisation was carried out at *ca.* 600 mV *versus* ferrocenium–ferrocene.

The coated electrodes were removed from the electrolyte and washed copiously with MeCN. A solution containing *ca.* 5 mg cm^{-3} of KOBu^t in MeCN was prepared and 1 cm^3 of this was added to 5 cm^3 MeOH containing 20 mg Cleland's reagent. Freshly prepared co-polymer electrodes were soaked in this solution overnight, washed successively with MeOH and MeCN, soaked for *ca.* 3 min in $HBF_4 \cdot Et_2O$:MeCN (1:10, v/v) and again copiously washed with fresh MeCN.

The cysteinyl co-polymer electrodes were soaked in MeCN saturated with $[NBu_4]_2[Fe_4S_4(SPh)_4]$ for 30 min, and finally washed with MeCN–0.2 mol dm^{-3} $[NBu_4][BF_4]$. The resulting ferredoxin electrodes were subsequently transferred to an electrochemical cell or to the FTIR spectrometer for characterisation.

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