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Bifunctional ferrocene derivatives for molecular recognition of DNA duplexes

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Using 1,1′-bis(chlorocarbonyl)ferrocene as the starting material the novel amide derivative 1,1′-bis(carbamoylmethylcarbamoyl)ferrocene has been isolated. Furthermore, the *in situ* reaction of 1,1′-di(bromomethyl)ferrocene with several amines led to the formation of salt-like and tertiary amine ferrocene compounds. These compounds are all very stable in air and exhibit interesting hydrogen bonding patterns that can complement those of the DNA nucleobases. Differential pulse voltammetry was used to monitor the interactions of the water soluble ferrocene derivatives with DNA oligomers. The three compounds tested bind strongly to the DNA oligomers and the binding constants calculated are similar to those obtained for the $[Fe(phen)_3]^{3+/2+}$ ions. The interaction results from a combination of hydrogen bonding, electrostatic, hydrophobic and π-stacking interactions.

Introduction

During the past forty-five years a wide range of substituted ferrocene compounds have been synthesized and studied. It was, nevertheless, of some topical interest to incorporate substituents on the cyclopentadienyl rings, which would make such substituted ferrocenes capable of recognising molecules of biological importance, such as DNA, through complementary interactions. The well established reversible electrochemical properties of ferrocene derivatives provided a means of detecting and putting on a quantitative basis such interactions.

Metallocenes occur either as regular sandwich compounds, *e.g.* ferrocene and cobaltocene, or bent sandwich compounds, containing Ti, V, Nb, or Mo as the central metal atom.[1,2] Strong antitumour activities have been shown for some of the bent metallocene complexes. Antitumour activities has also been reported for ferrocenium compounds.[3] These ionic, water soluble complexes unlike the bent sandwich compounds do not contain halide ligands bound in *cis* positions and are therefore lacking the *cis*-dihalogenometal moiety characteristic of cisplatin, $[Pt(NH_3)_2Cl_2]$. Antitumour ferrocenium complexes are represented by salt-like, water soluble compounds with a range of anions X^-, such as trichloroacetate ($CCl_3CO_2^-$), picrate ($2,4,6\text{-}(O_2N)_3C_6H_2O^-$), μ-oxo-bis[trichloroferrate(III)] ($\frac{1}{2}[Cl_3FeOFeCl_3]^-$), or tetrachloroferrate(III) ($[FeCl_4]^-$). These compounds have shown marked antitumour activity against several animal and human tumours.[4] Furthermore, iron compounds are less toxic than platinum compounds. This observation opens the possibility of combination therapies with the toxic side effects diminished.

The antitumour activities of bis(diphenylphosphines) and their bis(gold(I)) complexes has also attracted some recent interest.[5] This, together with the finding that ferrocenium salts are active against various tumours, suggested that the incorporation of the essential structural features of the bis(diphenylphosphinogold) complexes with a ferrocene could provide complexes with enhanced antitumour activities. Ferrocenyl derivatives functionalised with nucleobases such as thymine and adenine have also been prepared.[6,7] These compounds are capable of interacting with nucleic acids *via* hydrogen bonding. A series of ferrocene receptors that can bind and electrochemically recognise anions has been reported[8,9] and the recognition of anions in solution has been investigated using ^1H NMR titrations. Ferrocene is a unique functional handle because it does not directly interact with the anion until it is oxidised to the ferrocenium cation, at which stage electrostatic interactions with the guest are "switched on."

Results and discussion

Amide derivatives

Ferrocene amide derivatives can be isolated from 1,1′-bis(chlorocarbonyl)ferrocene, by treatment with different amines. This specific reaction has been utilised extensively by Beer and his group[10] for the formation of appropriately designed charged or neutral redox- and photo-active transition metal organometallic and co-ordination receptor systems that can selectively recognise and sense anionic guest species either by electrochemical or optical measurements.

The new derivative 1,1′-bis(carbamoylmethylcarbamoyl)ferrocene **1** was isolated from the reactions of bis(chlorocarbonyl)ferrocene with glycinamide hydrochloride and characterised using IR and NMR spectroscopy, mass spectrometry and elemental analyses. This derivative was insoluble in all common organic solvents and is only soluble in DMSO and warm water.

For the glycinamide derivative the following peaks were observed in the infrared spectrum: ν(N–H) at 3409, 3319 and 3192 cm^{-1}; ν(C–H) at 2940 cm^{-1}; ν(C=O) at 1663 cm^{-1}; amide I at 1607 cm^{-1}; amide II at 1561 cm^{-1}; ν(C–N) at 1301 cm^{-1} and amide III at 1261 cm^{-1}. Owing to the C_2 symmetry of the complex, the two terminal NH_2 groups are equivalent. However, within each group the two protons are magnetically

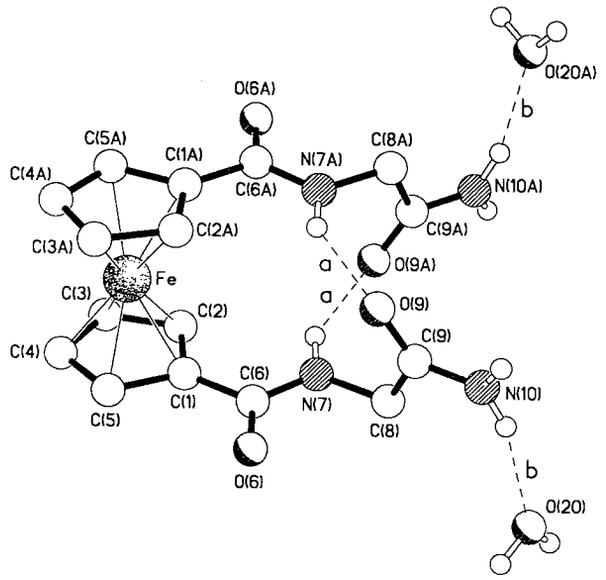


Fig. 1 The molecular structure of complex **1**. The hydrogen bonding geometries are N···O, H···O (Å), N–H···O (°): (a) 2.88, 2.08, 147 and (b) 2.95, 2.08, 165.

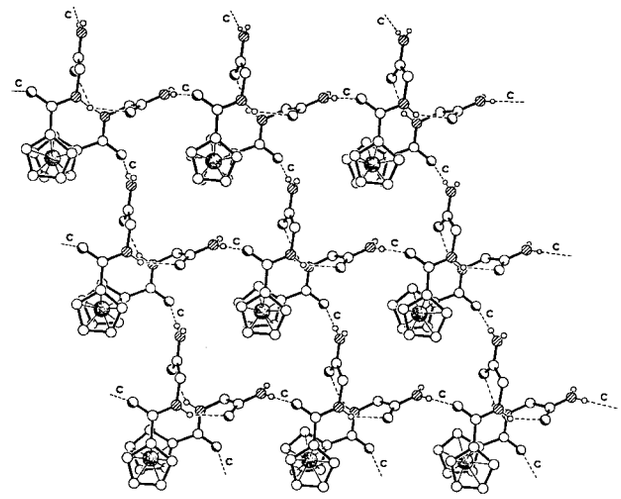


Fig. 2 One of the N–H···O hydrogen bonded sheets of molecules present in the structure of complex **1**. The hydrogen bonding geometry is N···O 2.94, H···O 2.04 Å and N–H···O 177°.

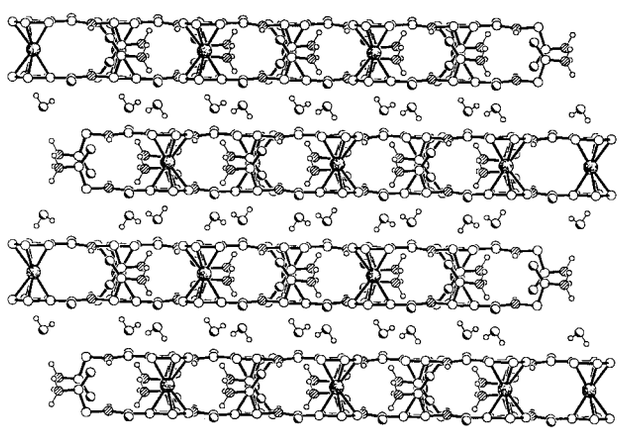


Fig. 3 The intercalation between the hydrogen bonded sheets in the structure of complex **1** by the solvent water molecules. The sheets are cross-linked by O–H···O hydrogen bonds from these included water molecules (see text).

inequivalent due to the orientation of the adjacent carbonyl. Consequently, only two peaks (at δ 7.74 and 7.30) are observed for the NH_2 units in the ^{1}H NMR spectrum of the compound in d_6-DMSO. The remaining peaks observed are as follows: δ 8.64, amide NH; 4.79 and 4.43, Cp H and 3.73, CH_2. In the FAB(+) and FAB(−) mass spectra the molecular ion peaks for $[M]^+$ and $[M - H]^-$ are observed at m/z = 386 and 385 respectively.

Crystal structure of complex 1

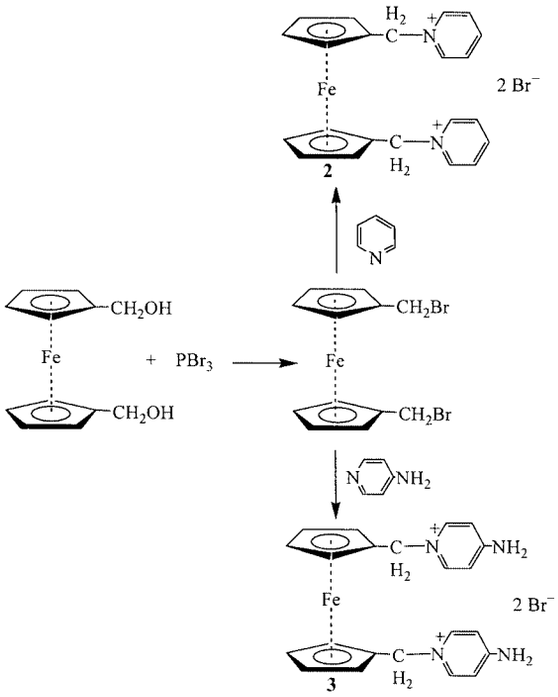
Crystals of complex **1** were obtained from a water–DMSO solution after refrigeration over a period of four days. In the solid state (Fig. 1) the molecule has crystallographic C_2 symmetry about an axis passing through the iron atom and bisecting the vector between the two amide oxygen atoms O(6) and O(6A). The two cyclopentadienyl rings are parallel to each other (inclined by only 0.1°) with the C(6) amide group being rotated by only *ca*. 6° out of the plane of its associated C_5H_4 ring. There is a *ca*. 85° torsional twist about the N(7)–C(8) bond that facilitates the cross-linking of the two substituent sidearms by a pair of intramolecular hydrogen bonds between the N(7) amide hydrogen in one chain and the carbonyl oxygen O(9A) of the other, and *vice versa* (a in Fig. 1). The dihedral angle between the two substituent chains is 64° and the two Cp rings are almost eclipsed, being rotated out of register by *ca*. 9°. The centroid–centroid separation of the Cp rings is 3.29 Å, which is the same as the mean interplanar separation of the two rings, and the Cp–Fe–Cp angle is 178.5(2)°. Adjacent molecules are linked *via* N–H···O hydrogen bonds between one of the terminal amide hydrogen atoms in one molecule and the O(6) carbonyl atom of another (c in Fig. 2) to form a two-dimensional sheet that extends within the 110 plane. These sheets are then cross-linked by N–H···O (b in Fig. 1) and O–H···O hydrogen bonds to the included water molecules that are intercalated between the sheets (Fig. 3). The O–H···O hydrogen bonds from the water molecule are to the carbonyl oxygens O(6) [O–H···O, H···O distances 2.80, 1.93 Å and O–H···O angle 162°] and O(9) [O–H···O, H···O distances 2.99, 2.15 Å and O–H···O angle 154°].

Salt derivatives

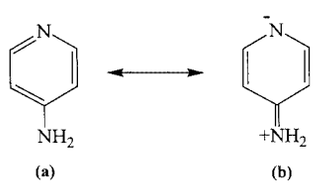
Treatment of 1,1′-ferrocenedimethanol with PBr_3 leads to the formation of 1,1′-di(bromomethyl)ferrocene. The *in situ* reaction of the dibromide with pyridine and 4-aminopyridine led to the formation of the water soluble salts 1,1′-bis(pyridiniomethyl)ferrocene dibromide **2** and [1,1′-bis(4-aminopyridiniomethyl)ferrocene dibromide **3**, shown in Scheme 1. A similar reaction has been reported previously.[11] Treatment of 1,1′-ferrocenedimethanol with *p*-toluenesulfonyl chloride in the presence of pyridine resulted in the isolation of the mixed salt 1,1′-bis(pyridiniomethyl)ferrocene *p*-toluenesulfonate chloride. The pyridine in this mixed salt can be replaced and several simple ferrocene amines, ferrocenophanes and ferrocene cryptands have been synthesized from it.[12]

Both the complexes shown in Scheme 1 were characterised using IR and NMR spectroscopy, mass spectrometry and elemental analyses. In the IR spectrum of **2** the following characteristic peaks were observed: ν(C–H) at 2981 and 2954 cm^{-1}, ν(C=N) at 1629 and 1600 cm^{-1}. For **3** the strongest bands were exhibited for ν(N–H) at 3347, 3309, 3286 and 3112 cm^{-1}, ν(C–H) at 2987 and 2967 cm^{-1} and ν(C=N) at 1650 cm^{-1}. In the ^{1}H NMR spectrum of **2** in d_6-DMSO the peaks for the pyridine protons can be observed at δ 8.16, 8.60 and 9.16, the peak for the methylene protons occurs at δ 5.72 and the peaks for the Cp protons occur at δ 4.68 and 4.40.

In the ^{1}H NMR spectrum of complex **3** in D_2O the peaks of the pyridine protons can be observed at δ 7.96 and 6.76, the peak for the methylene protons at δ 5.07 and the peaks for the Cp protons at 4.44 and 4.39 ppm. The reactivity of 4-aminopyridine can be influenced by its resonance forms. As shown in Scheme 2 two canonical forms are important: (**a**) and (**b**). In the second form there is a partial positive charge on the 4-amino



Scheme 1



Scheme 2

group and this renders this nitrogen atom less nucleophilic. Therefore in reactions involving nucleophilic attack the 4-aminopyridine is expected to react through the 1 position as in the case of the formation of compound **3**.

In the Fast Atom Bombardment FAB(+) spectra of both the salts the characteristic peak for $[M{\cdot}Br]^+$ and $[M{\cdot}Br{\cdot}H]^+$ were respectively observed.

Crystal structure of complex 2

Crystals of the salt compound (**2**) suitable for crystallography were obtained by recrystallisation from CH_2Cl_2. The X-ray analysis shows the complex to have approximate, non-crystallographic C_2 symmetry (Fig. 4). The cyclopentadienyl rings are nearly eclipsed (7° stagger) with their planes inclined by *ca.* 4° to each other; the dihedral angle between the Cp substituent bonds, C(7)–C(12) and C(19)–C(24), is 79°. The torsional twists about these bonds are 81 and 83° respectively, and those about the N(1)–C(7) and N(13)–C(19) bonds are 75 and 74°. The Cp · · · Cp mean interplanar separation is 3.28 Å with a centroid–centroid separation of 3.29 Å; the Cp–Fe–Cp angle is 177.9(4)°.

Adjacent molecules are linked (in the crystallographic *a* direction) to form chains *via* pairs of C–H π interactions between one of the *ortho* Cp hydrogen atoms on each Cp ring of one molecule and the pyridyl rings of the next (a and b in Fig. 5). These interactions are supplemented by a π–π stacking interaction between adjacent Cp rings within the chain (c in Fig. 5). There are no noteworthy interactions involving the Br^- anions.

Tertiary amine derivatives

When 4-nitroaniline reacts with 1,1′-di(bromomethyl)ferrocene the tertiary amine derivative 1,1′-[2-(4-nitrophenyl)-2-aza-propane-1,3-diyl]ferrocene **4** is synthesized. In the IR spectrum

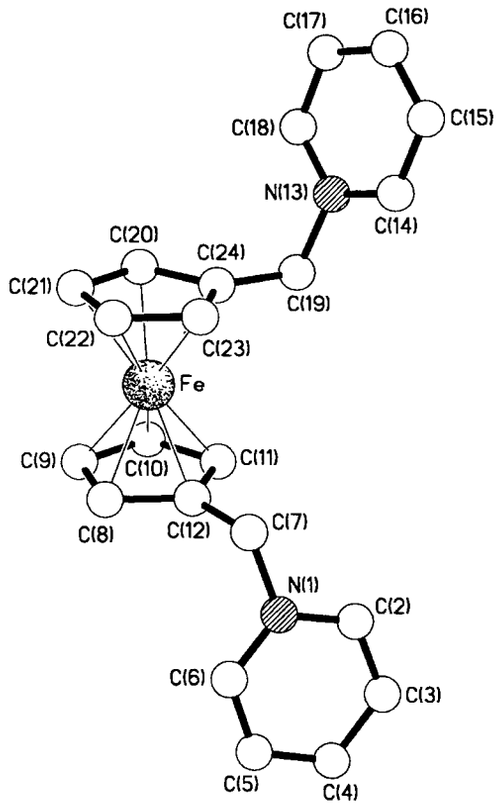


Fig. 4 The molecular structure of complex **2**.

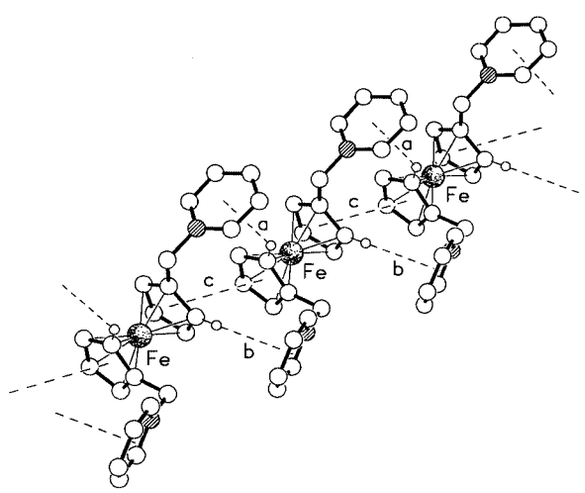


Fig. 5 One of the C–H · · · π and π–π linked chains of molecules present in the structure of complex **2**. The H · · · π distances (Å) and C–H · · · π angles (°) are (a) 2.88, 138 and (b) 2.82, 152. The centroid–centroid and mean interplanar separations (c) are 3.87 and 3.57 Å respectively.

the following characteristic peaks were observed: ν(C–H) at 2893, 2966 and 2927 cm^{-1}, ν(C=N) at 1590 cm^{-1} and $\nu(NO_2)$ at 1382 cm^{-1}. In the ^1H NMR spectrum in $CDCl_3$ there are two doublets at δ 8.14 and 6.88 corresponding to the protons of the aniline, two triplets corresponding to the Cp protons at δ 4.18 and 4.10 and a singlet corresponding to the methylene protons at δ 4.02. In the FAB(+) mass spectra for this derivative the molecular ion peak for $[M]^+$ is observed at m/z = 348.

Crystal structure of complex 4

Crystals of complex **4** were obtained after slow evaporation of a dichloromethane solution. The structure shows the nitro-aniline to have bridged between the two methylene moieties of the dimethylferrocene unit to form the complex illustrated in

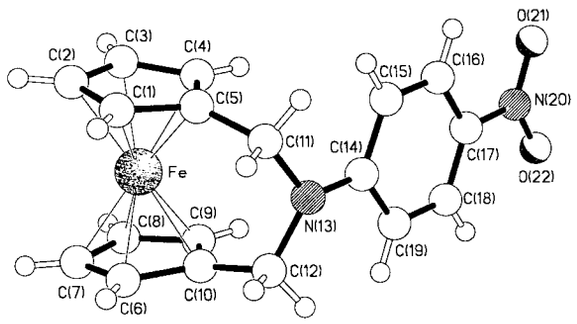


Fig. 6 The molecular structure of complex **4**.

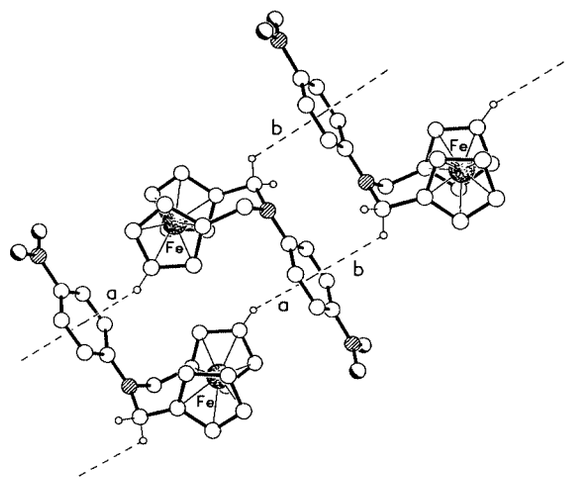


Fig. 7 One of the the C–H · · · π and π–π linked chains of molecules present in the structure of complex **4**. The H · · · π distances (Å) and C–H · · · π angles (°) are (a) 2.92, 151 and (b) 3.05, 123.

Fig. 6. The ferrocenophane component has a geometry very similar to that seen in both 2-*N*,*N*-dimethylammonio-(3)-1,1′-ferrocenophane iodide and 2-*N*-ethylammonio-(3)-1,1′-ferrocenophane thiocyanate,[12] though with the geometry at nitrogen being significantly flattened in the neutral species; the nitrogen atom lies 0.16 Å out of the plane of its substituents. The two Cp rings are only 2° from an eclipsed conformation, but are inclined by *ca*. 10° as a consequence of the CH_2NCH_2 bridge. The Cp · · · Cp centroid–centroid separation is 3.27 Å with a Cp–Fe–Cp angle of 172.9(2)°. The mean torsional twist about the N(13)–C(14) bond is *ca*. 3°.

As was seen in the structure of complex **2**, adjacent molecules are linked to form chains (in the 110 direction) by pairs of C–H π interactions. Here C_i symmetric "dimer" pairs are formed by an interaction between one of the *meta* Cp hydrogen atoms in one molecule and the nitrophenyl ring of the next, and *vice versa* (a in Fig. 7). These pairs are then linked across a neighbouring inversion centre by weak C–H π interactions between one of the methylene hydrogen atoms in one molecule and the opposite face of the nitrophenyl ring in the next, and *vice versa* (b in Fig. 7). The H · · · π vectors a and b subtend an angle of 167°.

Nucleic acid binding studies

Though studies of the binding of compound **4** were not possible due to its insolubility in water, we have investigated the behaviour of the water soluble complexes **1**, **2** and **3**. If one considers the binding of an electroactive metal centre to a given length of duplex DNA,[13] the simplest model of the binding equilibrium (due to Scatchard[7]) can be obtained by assuming each ferrocene complex binds independently and that the number of binding sites is proportional to the concentration of the nucleotide phosphate (NP) as shown in eqn. (1), where *s* is

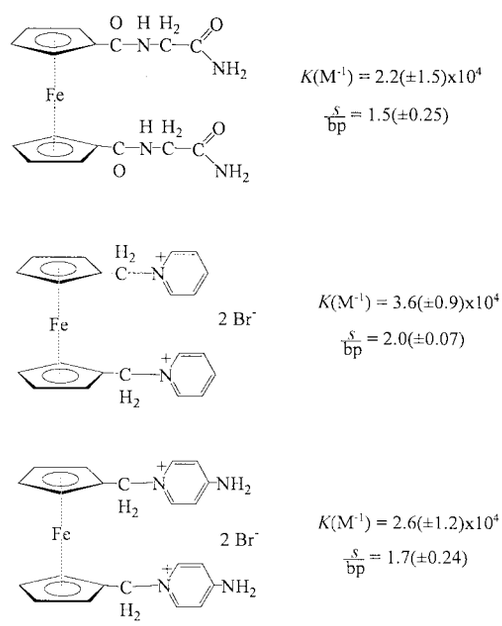
$$[\text{sites}] = [\text{NP}]/2s \quad (1)$$

the binding site size in terms of base pairs.[6] The binding constant *K* can be written as in eqn. (2), where [free] and [bound] are the concentrations of free and bound compound.

$$K = [\text{bound}]/[\text{free}][\text{sites}] \quad (2)$$

Although the Scatchard model is not strictly applicable to DNA, it can be used when the overlap of binding sites is not an important consideration. This is reasonable for small values of *s* in base pairs. Values of current in the absence and presence of nucleic acid were used to calculate the ratio of bound to free compound. Experimental data for the ratio [bound] : [free] were fitted by the model using a standard least-squares method with *K* and *s* the only adjustable parameters.

The water soluble ferrocene derivatives synthesized all proved to bind strongly to DNA. Scheme 3 presents the binding con-



Scheme 3

stant and binding site size values obtained from DPV titrations in the presence and absence of DNA for the metal complexes **1**, **2** and **3**.

Voltammetric methods have been used to probe the interaction of $[Fe(phen)_3]^{2+/3+}$ with DNA. It has been shown that both forms of the couple bind with approximately the same affinity, $K = 7.1(\pm0.2) \times 10^3$ and $1.47(\pm0.04) \times 10^4$ M^{-1} respectively.[13] Using DPV to determine binding constants it has been shown that $K = 6.4(\pm0.4) \times 10^3$ M^{-1} for the ferrocenylnucleobase derivative of thymine.[6]

In the case of the ferrocene compounds **1**, **2** and **3** the binding constants calculated are similar to that obtained for the $[Fe(phen)_3]^{3+}$ complex. For the amide compound **1**, which is not very soluble in water and contains two hydrophobic amide chains, this similarity could be due to hydrogen bonding as well as hydrophobic interactions. For the two salt-like compounds **2** and **3** the binding constants are similar to that of $[Fe(phen)_3]^{3+}$ although the pyridine and aminopyridine moieties are not known to intercalate. Apart from possible π–π stacking interactions, this binding could also be due to the fact that in these compounds the two positive charges are better situated on the nitrogen atoms in order to reach closer to the phosphate backbone of the DNA and therefore interact more strongly than in the case of the $[Fe(phen)_3]^{3+}$ complex, where the positive charge is concentrated on the Fe atom which cannot get very close to the phosphate backbone.

The binding site size is close to 2 for all three compounds, meaning that there is one ferrocene molecule bound to every two base pairs. This is exactly as expected since the distance between the cyclopentadienyl rings in this type of ferrocene derivatives is very similar to the distance between base pairs in DNA.

In conclusion the experimental work described in this paper has established that it is possible to make a series of ferrocenyl derivatives which are capable of binding to DNA oligotides. The derivatives may either be salt like and based on quaternated pyridines or have a carbonyl glycinaimide attached to each cyclopentadienyl ring of the ferrocene rings. Differential pulse voltammetry was used to monitor the interactions of the water soluble ferrocene derivatives with DNA oligomers. The three ferrocene derivatives bind strongly to the DNA oligomers and the bonding constants are comparable to those reported previously for tris(phenanthroline) octahedral co-ordination complexes.

Experimental

Instrumentation

Infrared spectra were recorded on a Perkin-Elmer FTIR1720 spectrometer as KBr discs, NMR spectra on either a JEOL GS 270 MHz or a GS 500 MHz spectrometer. ^{1}H and ^{13}C NMR spectra were referenced internally to the residual ^{1}H impurity and ^{13}C present in the deuteriated solvent. Chemical shifts are reported in parts per million relative to TMS (δ 0). FAB(+) and FAB(−) mass spectra were recorded on a VG Autospec spectrometer using 3-nitrobenzyl alcohol for the sample matrix. The ionising radiation was from a 35 keV Cs^+ primary ion beam.

Differential Pulse Voltammetry (DPV) titrations of nucleic acids with ferrocene complexes in solution were carried out in a standard two compartment electrochemical cell of volume 20 ml. A three electrode system was used comprising of a gold working electrode, a silver wire acting as a reference electrode and a tungsten counter electrode. Prior to each measurement the working electrode surface was polished with alumina paste, then washed with distilled water to achieve a smooth clean surface. Solutions of the complexes studied were prepared as required using 0.1 M Tris buffer (tris(hydroxymethyl)methylamine), pH 7 and 0.1 M potassium chloride as the supporting electrolyte.

Starting materials

1,1′-Ferrocenedicarboxylic acid, 1,1′-ferrocenedimethanol, oxalyl chloride, phosphorus tribromide, glycinamide hydrochloride, 1-phenylbiguanide, 1-(3-chlorophenyl)biguanide hydrochloride, 4-*tert*-butyl-2,6-diaminopyrimidine, 1-(*o*-tolyl)-biguanide, 4-nitroaniline, 4-aminopyridine, 2-amino-4-*tert*-butyl-6-hydroxy-1,3,5-triazine, tris(hydroxymethyl)methylamine and triethylamine were purchased from Sigma-Aldrich Chemicals Co. and used as received. 1,1′-Bis(chlorocarbonyl)-ferrocene was prepared using reported procedures.[14] The solvents used in all preparations were dried and DMSO and pyridine were distilled from BaO just prior to use.

DNA (type III, sodium salt from salmon testes) was purchased from Sigma-Aldrich Chemicals Co. and used without further purification. It was stored at 0 °C. DNA solutions were prepared from stock solutions of 0.1 M Tris buffer, containing 0.1 M potassium chloride. Concentrations of DNA solutions per nucleotide phosphate were determined spectrophotometrically by UV absorbance at 260 nm with $\varepsilon_{260} = 6600$ dm^3 mol^{-1} cm^{-1}. DNA solutions once prepared were discarded after a period of two days.

Titration procedure

The titration experiments were carried out in two steps. First, 20 ml of the Tris buffer solution were placed in the electrochemical cell, followed by an aliquot of the ferrocene complex stock solution. A differential pulse voltammogram was run and the peak current noted. Another aliquot of the ferrocene complex stock solution was added to the cell, followed by another differential pulse voltammogram. The second step involved repeating the titration in exactly the same manner but with 20 ml DNA solution in place of buffer alone. As before, differential pulse voltammograms were recorded for each concentration of metal complex. From these measurements, a graph of peak current against concentration was plotted for both total metal complex and free metal complex.

Preparations

1,1′-Bis(carbamoylmethylcarbamoyl)ferrocene 1. 1,1′-Bis-(chlorocarbonyl)ferrocene (0.20 g, 0.6 mmol) and glycinamide hydrochloride (0.17 g, 1.3 mmol) were refluxed in CH_2Cl_2 (250 ml) and an excess of NEt_3 for 10 hours. A dark yellow solid was obtained which was washed with water (2 × 20 ml) and then treated with MeOH to afford a yellow solution. The solution was filtered and the MeOH evaporated. Yellow crystals of the product were obtained from a DMSO–water solution after refrigerating for a period of four days. Yield: 0.16 g, 64.0% (Found: C, 45.2; H, 5.0; N, 13.0%. Calc. for $C_{16}H_{18}FeN_4O_4\cdot 2H_2O$: C, 45.5; H, 5.2; N, 13.3%). IR (cm^{-1}): 3409s, 3319s, 3192s, 2940m, 1663s, 1607s, 1561s, 1301s and 1261m. ^{1}H NMR [d_6-DMSO, 270 MHz]: δ 8.64 (1 H, t, $^3J_{H\text{-}H}$ 6.2, amide NH), 7.74 [2 H, s, amide NH_2], 7.30 (2 H, s, amide NH_2), 4.79 (2 H, t, $^3J_{H\text{-}H}$ 1.7, Cp H), 4.43 (2 H, t, $^3J_{H\text{-}H}$ 1.7, Cp H), 3.73 (2 H, d, $^3J_{H\text{-}H}$ 6.2 Hz, CH_2), 3.38 (s, H_2O) and 2.50 (s, DMSO). Mass spectrum: FAB(+), *m*/*z* 386 {60, $[M]^+$}; FAB(−): 385 {100%, $[M - H]^-$}.

Crystal data. $C_{16}H_{18}FeN_4O_4\cdot 2H_2O$, $M = 422.2$, monoclinic, space group $C2/c$ (no. 15), $a = 13.617(1)$, $b = 9.886(1)$, $c = 13.195(1)$ Å, $\beta = 92.88(1)°$, $V = 1774.0(3)$ Å^3, $Z = 4$ (the molecule has crystallographic C_2 symmetry), μ(Cu-Kα) = 7.21 mm^{-1}, $T = 293$ K; orange-yellow needles, Siemens P4/PC diffractometer, ω scans, 1324 independent reflections. The structure was solved by the heavy atom method and the non-hydrogen atoms were refined anisotropically using full matrix least squares based on F^2 to give $R1 = 0.045$, $wR2 = 0.100$ for 1082 independent observed absorption corrected reflections [$|F_o| > 4\sigma(|F_o|)$, $2\theta < 120°$] and 144 parameters.

1,1′-Bis(pyridiniomethyl)ferrocene dibromide 2. A solution of freshly distilled PBr_3 (0.07 ml, 0.80 mmol) in THF (5 ml) was added dropwise to a THF (20 ml) solution of 1,1′-ferrocenedimethanol (0.20 g, 0.80 mmol) and pyridine (0.13 ml, 1.6 mmol) at 0 °C. The mixture was left stirring for 1 h at this temperature, the ice bath was removed and the mixture left to stir at room temperature for 3 h. The yellow precipitate formed was filtered off and crystals suitable for X-ray analysis were obtained by recrystallisation from CH_2Cl_2. Yield: 0.31 g, 70.5% (Found: C, 48.0; H, 4.4; N, 4.9%. Calc. for $C_{22}H_{22}Br_2FeN_2\cdot H_2O$: C, 48.2; H, 4.4; N, 5.1%). IR (cm^{-1}): 3469(br), 3403m, 3033m, 2981m, 2954m, 1629s, 1600s, 1525s, 1481s, 1240m, 1052m, 1031m, 750s, 732s and 680s. ^{1}H NMR [d_6-DMSO, 270 MHz]: δ 9.16 (2 H, d, $^3J_{H\text{-}H}$ 5.9, py), 8.60 (1 H, t, $^3J_{H\text{-}H}$ 8.0, py), 8.16 (2 H, t, $^3J_{H\text{-}H}$ 6.8, py), 5.72 (2 H, s, CH_2), 4.68 (2 H, t, $^3J_{H\text{-}H}$ 1.73, Cp H), 4.40 (2 H, t, $^3J_{H\text{-}H}$ 1.73 Hz, Cp H), 3.32 (s, H_2O) and 2.50 (s, DMSO). FAB(+) Mass spectrum: *m*/*z* 449 {50%, $[M\cdot Br]^+$}.

Crystal data. $[C_{22}H_{22}FeN_2][Br]_2\cdot H_2O$, $M = 548.1$, monoclinic, space group $P2_1/n$ (no. 14), $a = 7.002(1)$, $b = 17.849(2)$, $c = 17.560(2)$ Å, $\beta = 91.53(1)°$, $V = 2193.9(5)$ Å^3, $Z = 4$, μ(Cu-Kα) = 9.89 mm^{-1}, $T = 293$ K, orange prismatic needles, 3258 independent reflections. The structure was solved by direct methods, $R1 = 0.058$, $wR2 = 0.110$ for 2063 independent observed absorption corrected reflections and 254 parameters. Other details as for **1**.

1,1′-Bis(4-aminopyridiniomethyl)ferrocene dibromide 3. A solution of freshly distilled PBr_3 (0.04 ml, 0.42 mmol) in THF (5 ml) was added dropwise to a THF (20 ml) solution of 1,1′-ferrocenedimethanol (0.10 g, 0.4 mmol) at 0 °C. After the addition was over stirring was continued for 1 h at 0 °C and 1 h at room temperature. The resulting orange solution was added dropwise to a solution of 4-aminopyridine (0.12 g, 1.2 mmol) in THF (30 ml) and the mixture stirred for 4 h at room temperature. The yellow precipitate obtained was filtered off, washed with acetone and recrystallised three times from a DMSO–$CHCl_3$ mixture, in order to avoid formation of a gum. The final product was dried *in vacuo* at 40 °C for 3 h. Yield: 0.15 g, 64.0% (Found: C, 62.0; H, 4.7; N, 8.1%. Calc. for $C_{18}H_{16}FeN_2O_2$: C, 62.1; H, 4.7; N, 8.1%). IR (cm^{-1}): 3347m, 3309m, 3286m, 3112m, 2987m, 2967m, 1650s, 1536s, 1238w, 1209m, 1162s, 1064m, 1037m and 838m. ^1H NMR [D_2O, 270 MHz]: δ 7.96 (H, d, py), 6.76 (H, d, py), 5.07 (2 H, s, CH_2), 4.80 (s, H_2O), 4.44 (2 H, s, Cp H) and 4.39 (2 H, s, Cp H). FAB(+) Mass spectrum: *m*/*z* 480 {45%, $[M \cdot Br + H]^+$}.

1,1′-[2-(4-Nitrophenyl)-2-azapropane-1,3-diyl]ferrocene 4. A solution of freshly distilled PBr_3 (0.04 ml, 0.42 mmol) in THF (5 ml) was added dropwise to a THF (20 ml) solution of 1,1′-ferrocenedimethanol (0.10 g, 0.4 mmol) at 0 °C. After the addition was over stirring was continued for 1 h at 0 °C and 1 h at room temperature. The resulting orange solution was added dropwise to a solution of 4-nitroaniline (0.27 g, 2.0 mmol) in THF (20 ml) and the mixture stirred for 3 h. When reducing the THF volume an orange precipitate was formed which was recrystallised from CH_2Cl_2. Orange crystals were obtained by slow evaporation of a CH_2Cl_2 solution. Yield: 0.07 g, 50.3% (Found: C, 62.0; H, 4.7; N, 8.1%. Calc. for $C_{18}H_{16}FeN_2O_2$: C, 62.1; H, 4.7; N, 8.1%). IR (cm^{-1}): 3092w, 2966w, 2927w, 2893w, 2663w, 2419w, 1590s, 1509m, 1447m, 1382s, 1247m, 1203m, 1114s, 916s and 823m. ^1H NMR [d-$CDCl_3$, 270 MHz]: δ 8.14 (2 H, d, $^3J_{H\text{-}H}$ 9.6, Ph), 7.25 (s, $CHCl_3$), 6.88 (2 H, d, $^3J_{H\text{-}H}$ 9.6 Hz, Ph), 4.18 (2 H, t, $^3J_{H\text{-}H}$ 1.72, Cp H), 4.10 (2 H, t, $^3J_{H\text{-}H}$ 1.72 Hz, Cp H) and 4.02 (2 H, s, CH_2). FAB(+) Mass spectrum: *m*/*z* 348 {40%, $[M]^+$}.

Crystal data. $C_{18}H_{16}FeN_2O_2$, $M = 348.2$, triclinic, space group $P\bar{1}$ (no. 2), $a = 8.147(1)$, $b = 9.544(1)$, $c = 10.771(1)$ Å, $\alpha = 116.29(1)$, $\beta = 93.36(1)$, $\gamma = 102.47(1)°$, $V = 721.7(1)$ Å^3, $Z = 2$, μ(Cu-Kα) = 8.48 mm^{-1}, $T = 293$ K, orange prisms, 2038 independent reflections. The structure was solved by direct methods, $R1 = 0.044$, $wR2 = 0.105$ for 1772 independent observed reflections and 209 parameters. Other details as for **1**.

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See http://www.rsc.org/suppdata/dt/b0/b004295l/ for crystallographic files in .cif format.

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References

1 N. Farrell, *Transition Metal Complexes as Drugs and Chemotherapeutic Agents*, Kluwer Academic Publishers, Dordrecht, 1989.
2 H. Kopf and P. Kopf-Maier, *Angew. Chem.*, 1979, **91**, 509.
3 P. Kopf-Maier, H. Kopf and E. W. Neuse, *Angew. Chem.*, *Int. Ed. Engl.*, 1984, **23**, 456.
4 P. Kopf-Maier, H. Kopf and E. W. Neuse, *Angew. Chem.*, 1984, **96**, 446.
5 D. T. Hill, G. R. Girard, F. L. McCabe, R. K. Johnson, P. D. Stupik, J. H. Zhang, W. M. Reiff and D. S. Eggleston, *Inorg. Chem.*, 1989, **28**, 3529.
6 C. Price, M. Aslanoglu, C. J. Issac, M. R. J. Elsegood, W. Clegg, B. R. Horrocks and A. Houlton, *J. Chem. Soc.*, *Dalton Trans.*, 1996, 4115.
7 G. Scatchard, *Ann. N. Y. Acad. Sci.*, 1949, **51**, 660; M. T. Carter, M. Rodriguez and A. J. Bard, *J. Am. Chem. Soc.*, 1989, **111**, 8901.
8 P. D. Beer, A. R Graydon, A. O. M. Johnson and D. K. Smith, *Inorg. Chem.*, 1997, **36**, 2112.
9 P. D. Beer, C. Hazlewood, D. Hesek, J. Hodacova and S. E. Stokes, *J. Chem. Soc.*, *Dalton Trans.*, 1993, 1327.
10 P. D. Beer, *Chem. Commun.*, 1996, 689.
11 V. P. Tverdokhlebov, I. V. Tselinski, B. V. Gidaspov and G. Yu. Chikisheva, *J. Org. Chem. USSR*, *Engl. Transl.*, 1976, **12**, 2268.
12 H. Plenio, J. Vang, R. Diodone and J. Heinze, *Inorg. Chem.*, 1994, **33**, 4098; R. Richter, O. Seidelmann, L. Beyer and H. Plenio, *J. Organomet. Chem.*, 1996, **525**, 199.
13 M. T. Carter, M. Rodriguez and A. J. Bard, *J. Am. Chem. Soc.*, 1989, **111**, 8901.
14 J. C. Medina, I. Gay, Z. Chen, L. Echegoyen and G. W. Gokel, *J. Am. Chem. Soc.*, 1991, **113**, 365.