Metallocene – DNA: Synthesis, Molecular and Electronic Structure and DNA Incorporation of C5-Ferrocenylthymidine Derivatives

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Abstract: Ferrocenylthymidine derivatives have been prepared by Pd-catalysed cross-coupling between ethynylferrocene or vinylferrocene and 5-iodo-2′-deoxyuridine. In the latter case a mixture of *trans* (**2a**) and *gem* (**2b**) isomers was obtained. The *cis*-vinylferrocenyl (**2c**), and ethylferrocenyl (**3**) derivatives were obtained by catalytic hydrogenation of ethynylferrocenyl-dT (**1a**), and **2c** respectively. Single-crystal X-ray data for **1a**, the ferrocenyl-2′furano-pyrimidone **1b**, and **2a** show that the nucleobase is essentially co-planar with the substituted Cp ring of the metallocene. The selective reduction of the linkage between the ferrocenyl and thymidine moieties, from –C≡C- to -CH_2CH_2-, causes a shift in the reduction potential of − 124 mV. DFT calculations for the one-electron oxidised species indicate that the diminished conjugation reduces the spin transfer onto the bridging C_2 group, but has less effect on the extent transferred to the nucleobase from the ferrocenyl group. Compound **1a** was incorporated site-specifically into DNA oligonucleotides by using automated solid-phase methods. However, some interconversion of **1a** → **1b** occurs, even under rapid mild conditions of deprotection.

Keywords: DNA · ferrocene · ferrocenyl-nucleosides · redox chemistry · thymidine

Introduction

Recently there have been increasing efforts to attach metal-containing groups to sites on DNA strands.[1–7] Of particular interest are redox-active metal complexes, since these may induce oxidative cleavage for use in the design of artificial nucleases,[1, 8–10] act as electrochemical probes for sensing applications,[11–13] and are also important in studies on DNA-mediated electron transfer.[4, 14–28]

The preparation of DNA oligonucleotides bearing metal-containing groups has typically relied upon either post-synthetic conjugation of a preformed complex at the terminal position of an oligonucleotide[5, 10, 27, 29–32] or addition of metal ions to ligand-modified oligonucleotides.[4, 6, 8, 9, 33] An alternative strategy is the synthesis of metal-modified nucleosides as phosphoramidite monomers for use in automated solid-phase synthesis.[34] This approach has been well developed in organic chemistry but has only rather recently been explored for metal-containing groups.[7, 35–40] The advantages of this approach include the ease of synthesis and the ability to incorporate the modified base into the oligonucleotide sequence site-specifically.

The ferrocenyl group appears to be well suited for the development of redox-active oligonucleotides given its stability, diverse substitution chemistry and amenable stereochemistry. There have been some reports of the terminal attachment of ferrocenyl groups to oligonucleotides using simple derivatives[41, 42] including ferrocenylhexanol as a phosphoramidite.[12] More recently, Grinstaff and Beilstein have reported the on-column attachment of ferrocenylpropargylamide to 5-iododeoxyuridine.[38] This method involves interrupting normal solid-phase synthesis to perform site-specific covalent modification on the reactive nucleoside derivative.

An alternative approach is to attach the ferrocenyl group prior to oligonucleotide synthesis. A range of ferrocenyl-nucleosides has been described,[43] including C5-pyrimidine and C8-adenosine derivatives. These appear highly suitable for incorporation into DNA; however, a very recent report has highlighted possible problems.[36] Here we have reinvesti-

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gated the synthesis of the ferrocenyl C5-modified thymidine derivatives, and report on the redox chemistry and the molecular and electronic structure of the compounds. Also investigated are modified methods for the incorporation of C5-ethynylferrocenylthymidine site-specifically into DNA oligonucleotides.

Results and Discussion

Synthesis, spectroscopy and structure of C5-modified ferrocenylthymidine derivatives: Gautheron et al. have previously described a series of ferrocenyl-nucleosides, including dT, containing C_2 linkages; ethynyl, vinyl and ethyl.[43] Several synthetic strategies were explored; 1) Pd-catalysed cross-coupling between ethynylferrocene and the appropriate halonucleoside, 2) the reaction with 5-chloromercuri-(deoxy)uridine and vinylferrocene and 3) reaction of $[Cp_2ZrCl(CH{=}CHFc)]$ with halonucleosides (Fc = ferrocenyl). Of the four nucleosides, thymidine is the most convenient to modify for use in solid-phase DNA synthesis as it does not require protection of the nucleobase. We have therefore investigated modified and alternative routes to the series of C_2-bridged ferrocenyl C5-thymidine derivatives, with particular interest in their redox properties and electronic structure. Scheme 1 summarises the synthetic routes explored.

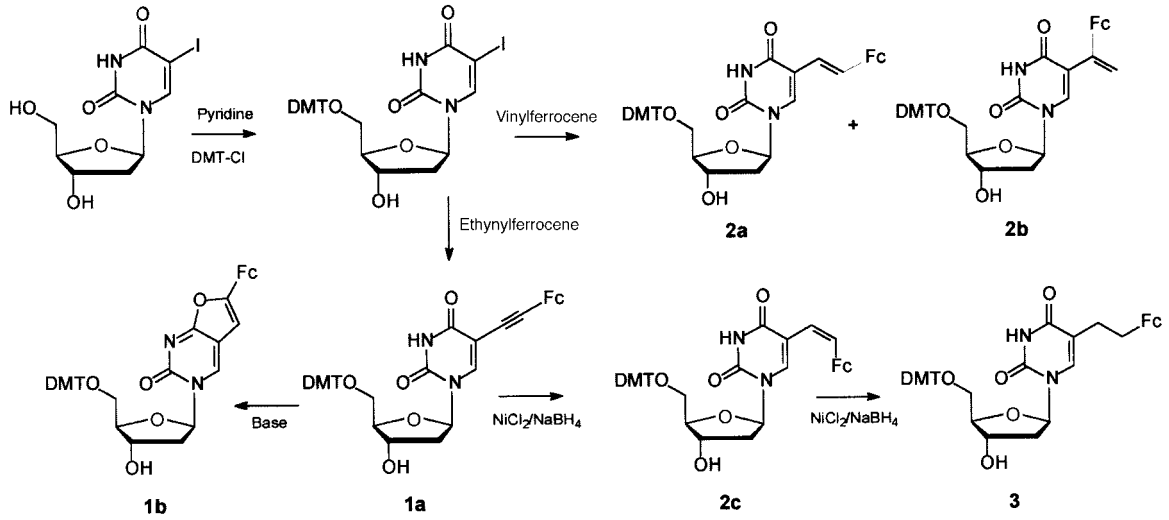
As a general point it was found that protection of the 5′-OH of 5-iododeoxyuridine (5-I-dU) with dimethoxytrityl (DMT) improved the yields due to an enhanced solubility in the solvent systems used. Such protection is of course also necessary for the reagent to be used in solid-phase oligonucleotide synthesis.

It is known that alkynylthymidine derivatives are susceptible to cyclisation to the corresponding furanopyrimidone.[44–46] This type of rearrangement generally occurs under rather forcing conditions, as noted in couplings of *o*-iodoanilines with copper(I) acetylides[47] and by treatment of (*Z*)-5-hexynyl-2′-deoxyuridine with triethylamine under reflux.[45] In reactions with ethynylferrocene as the alkyne, Gautheron et al. used similar conditions (CuI, Et_3N, reflux) and observed similar results.[43] Mixtures containing both the C5-ethynylferrocenylthymidine (**1a**) and ferrocenylfuranopyrimidone (**1b**) compounds were obtained, with 80% of the latter formed with 5-iodouracil, and only 5% with either 5-iodouridine or 5-iodo-2′-deoxyuridine.

In our studies it was found that replacing Et_3N as solvent with a weaker base such as pyridine inhibited the reaction between ethynylferrocene and 5-iododeoxyuridine completely. Addition of small quantities of Et_3N afforded a mixture of **1a** and **1b** in yields of 45 and 30% from reactions at elevated temperatures. Robins and Barr reported that the addition of solid disodium EDTA to reactions moderated the formation of the cyclised furanopyrimid-2-one product in the synthesis of 5-hexenyldeoxyuridine.[45] However, these reaction conditions only slightly reduced the degree of cyclisation in the case of ethynylferrocene. Reducing the reaction temperature to 60 °C decreased the proportion of the **1b** to <10%. The formation of **1b** can however be completely eliminated by careful monitoring of the room temperature reaction over a period of 4 h with typically 75% yield of **1a** obtained. The formation of the isomer **1b** can be identified by the singlet at $\delta = 6.84$ ppm in the ^1H NMR spectrum, attributed to the uncoupled aromatic proton attached to C_7. There is also a significant up-field shift in the H6 resonance ($\delta(CDCl_3) = 8.81$ (**1a**), 7.95 ppm (**1b**)). The two isomers are also distinguished by electronic absorption spectroscopy. The more extended ring system of **1b** gives rise to a red-shifted band in the visible region ($\lambda_{max} = 355$ nm) as has been reported elsewhere.[36]

De-tritylation of **1a** and **1b** aided in the preparation of crystals suitable for analysis by X-ray diffraction and these data confirmed unequivocally the structural assignments based on spectroscopic data. Both structures contain two independent molecules in the asymmetric unit; molecular structures are shown in Figure 1 and Figure 2, respectively. In each case, the two molecules are related by pseudo-inversion inversion symmetry if the sugar groups are ignored, but these sugars have the same chirality for both molecules.

In **1a** the Fe–C bond lengths range from 2.002(8) to 2.064(9) Å with an average of 2.034 Å. The Fe–C_5H_5 perpendicular distances are 1.643 and 1.656 Å in the two molecules,



Scheme 1. Synthetic route to C-5 ferrocenyl thymidine derivatives.

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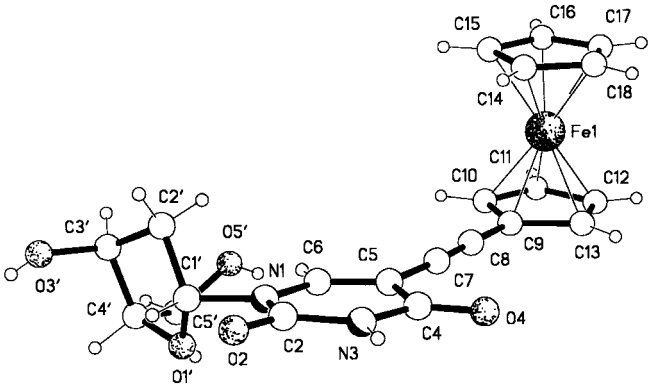

Figure 1. Structure of one independent molecule of **1a**. Selected bond lengths [Å] and angles [°]: Fe–C (av) 2.034, Fe–C_5H_5 1.643, Fe–C_5H_4 1.636, C7–C8 1.175(6), C8–C9 1.432(6); Cp–Cp 1.4°; Cp_{sub}–pyrimidine 6.9°.

insignificantly different from the values of 1.636 and 1.647 Å for the Fe–C_5H_4 distances. The intramolecular interplanar angles between the cyclopentadienyl rings are 1.4 and 2.3°, and those between the nucleobase and the substituted Cp ring are 6.9° and 5.3°. The two cyclopentadienyl rings within each molecule are virtually eclipsed (mean torsional angle C-X1-X2-C is 5.1°, where X1 and X2 are the centres of the rings). The ethynyl unit linking the nucleobase and the ferrocenyl group deviates only slightly from linearity (C5–C7≡C8 176.7(5), 173.8(6) and C7≡C8–C9 178.5(6), 176.9(6) Å for the two independent molecules), with a triple bond length of 1.175(6) and 1.183(6) Å and adjacent single bonds: C5–C7 1.432(6) and 1.456(6) Å, C8–C9 1.432(6) and 1.408(7) Å.

In **1b** the Fe–C bond lengths range from 2.026(2) to 2.046(2) Å (av 2.036 Å). The Fe–C_5H_5 perpendicular distances are 1.640 and 1.645 Å, compared to 1.637 and 1.639 Å for Fe–C_5H_4. The interplanar angles between the cyclopentadienyl rings are 0.6 and 2.3°, and those between the nucleobase and the substituted Cp rings are 11.4 and 14.9°. The two cylcopentadienyl rings are virtually eclipsed (mean torsional angle C-X1-X2-C is 1.5°). The furanopyrimidone is linked directly to the ferrocene through C8–C9 (1.446(3) and 1.438(3) Å), and C7–C8 is lengthened to a typical C=C double bond (1.340(3) and 1.340(3) Å). As a furanopyrimidone, **1b** has lost the ability to act as a hydrogen-bond donor through N3 and can no longer base-pair effectively with adenine.

The reaction of vinylferrocene with 5′-DMT-5-iodo-2′-deoxyuridine, using $[PdCl_2(PPh_3)_2]$ as catalyst, gave a crude product which contained both the *trans*-vinyl (**2a**) and *gem*-vinyl (**2b**) isomers (see Scheme 1) in an approximate 1:1 ratio (based on integration of the ^{1}H NMR spectra). The isomers are distinguished by the coupling constants of the vinylic protons (*gem* = 1.2 Hz and *trans* = 16.2 Hz). Separation of the isomers was not possible by TLC and HPLC; however, after removal of the DMT group, the *trans* isomer **2a** was isolated by fractional crystallisation from a methanol/chloroform (10:90) solvent system. This method also afforded crystals of suitable quality for X-ray diffraction.

The molecular structure of **2a** is shown in Figure 3 and confirms the stereochemical assignment based upon ^{1}H NMR spectroscopy. The structure contains two independent molecules in the asymmetric unit, and is approximately isomorphous with **1a**. The C–Fe bond lengths vary between

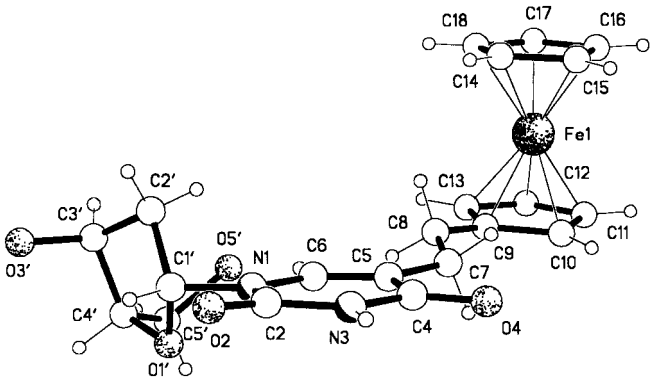

Figure 3. Structure of one independent molecule of **2a**. Selected bond lengths [Å] and angles [°]: Fe–C (av) 2.040, Fe–-C_5H_5 1.652, Fe–C_5H_4 1.649, C7–C8 1.30(2), C8–C9 1.47(2), Cp–Cp 0.6; Cp_{sub}–pyrimidine 3.3.

1.986(19) and 2.100(17) Å with an average of 2.040 Å. The iron atom lies 1.649 or 1.641 Å above the substituted cyclopentadienyl ring and 1.652 or 1.634 Å below the unsubstituted ring in the two molecules. The ferrocenyl rings are virtually co-planar, orientated at an interplanar angle of 0.6 or 2.4°, and are arranged in an almost eclipsed configuration (mean torsional angle C-X1-X2-C is 3.5°, where X1 and X2 are the centres of the cyclopentadienyl rings). The *trans*-vinyl linkage C7=C8 has a bond length (1.30(2) or 1.25(2) Å) consistent with a carbon-carbon double bond and is connected to the ferrocene unit through C8–C9 (1.47(2) or 1.44(2) Å). Figure 3 clearly shows the virtually planar *trans*-vinyl link between the thymine ring and the ferrocene cyclopentadienyl ring. The interplanar angle between the nucleobase and the substituted Cp ring is 3.3° or 5.8°. The dihedral angle between the vinylic bond and the pyrimidine ring is 6.3 or 6.4°, and between the adjoining cyclopentadienyl ring and the vinylic bond it is 3.2 or 11.3°. This geometric alignment looks promising as a rigid and planar delocalised electronic system between the nucleobase and the redox group.

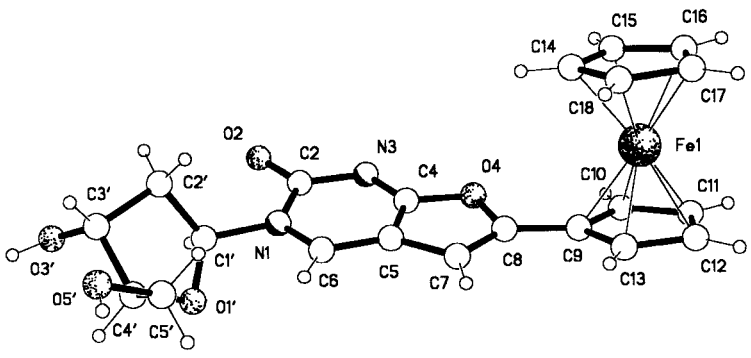

Figure 2. Structure of one independent molecule of **1b**. Selected bond lengths [Å] and angles [°]: Fe–C (av) 2.036, Fe–C_5H_5 1.640, Fe–C_5H_4 1.637, C7–C8 1.340(3), C8–C9 1.446(3), Cp–Cp 0.4°, Cp_{sub}–pyrimidine 11.6°.

The *trans* isomer **2a** has been synthesised previously by two different routes. The reaction of vinylferrocene with C5-HgCl-dU in methanol using $Li_2[PdCl_4]$ as a catalyst followed by treatment with either H_2S or $NaBH_4$ led to a mixture of three products, including the *trans*-vinylferrocenyl derivative.[43] This compound was also isolated in about 15–20% yield by the reaction of $[Cp_2ZrCl(CH{=}CHFc)]$ with 5-iododeoxyuridine. To our knowledge, the *gem* and *cis* isomers of C5-vinylferrocenylthymidine have not previously been reported. The latter compound was prepared as a single isomer in 30% yield by catalytic reduction of **1a** using $NiCl_2$/$NaBH_4$.[48] Distinguishing features of the *cis*-isomer, **2c**, are the ^{1}H NMR vinylic coupling constants ($J = 11$Hz). The electronic absorption spectrum for each C5-vinylferrocenylthymidine isomer is characteristic (Figure 4). The more extended conjugation of the *trans* isomer gives rise to a red-shifted band compared to the *cis* form (298 nm c.f. 275 nm). All three isomers exhibit the weak band at about 450 nm attributed to (d–d) transitions.

The C5-ethylferrocenylthymidine **3** was prepared from **2c** by using an excess of $NiCl_2$/$NaBH_4$ mixture. The reactions were performed at low temperature ($T = -78\,^\circ$C) and monitored by TLC. Yields were typically rather low (~25%) and there were some problems with reproducibility. The previous report on the synthesis of **3** from the *trans*-vinyl compound **2a** employed hydrogenation on Pd/C but did not record a yield.[43]

Redox behavior and electronic structure calculations: Cyclic voltammetry was used to probe the redox properties of the compounds and in particular to assess the effect of the different C_2 linkages, -C≡C-, -C=C-, -C–C-, on the ferrocenyl oxidation. Table 1 summarises the formal electrode potentials of the derivatives measured in MeCN; values for the parent ferrocenyl derivatives are also included. All compounds exhibit reversible one-electron waves with formal potentials, E^o, lying in the -32 to $+226$ mV range versus ferrocenium–ferrocene.

Table 1. Electrode potentials, E^o, of ferrocene–ferrocenium couple of ferrocenylthymidine and related derivatives (from CV measurements in CH_3CN/0.1M $TBAPF_6$, Ag^+ quasi-reference electrode, tungsten counter electrode, gold disc working electrode).

Compound	E^o [mV]
ferrocene	0
FcC≡CH	+226
1a	+140
1b	+131
$FcC{=}CH_2$	+56
2a	+27
2b	+21
2c	+29
$FcCH_2CH_3$	−32
3	+16

The interest in DNA-mediated electron transfer[4, 14–21, 49] has focused attention on methods for incorporating site-specific redox-active centres.[7, 27, 36, 38–40] While nucleobase modification has been used in such studies, very few contain a conjugated pathway with the redox centre.[36] It is therefore worthwhile to consider the extent of interaction between the ferrocenyl group and the nucleobase for the oxidised paramagnetic ferrocenium species. Density functional calculations (DFT) for 1-methyl analogues of **1–3** and their oxidised counterparts provide some insights.

Calculations for the parent ferrocenyl derivatives show a qualitative correlation between the measured redox potentials and ΔE for the neutral and oxidised counterparts (ΔE(a.u.) $= E_{Fc} - E_{Fc+}$: EtFc $= -0.245$; VinylFc $= -0.246$; EthynylFc $= -0.253$ a.u.). This suggests that solvent interactions are consistent for all these compounds upon oxidation.

For the 1-methyl analogues of **1–3** the ethynyl derivative has the largest ΔE value (-0.242 a.u.) however, the vinyl derivatives have a smaller value than that calculated for the ethyl compound **3**. This indicates that for these compounds the correlation between the gas-phase calculations and the solution-phase redox potentials is not good. This may well reflect solvation effects on the nucleobase.

The calculated spin density serves extremely well to illustrate the extent of interaction between the ferrocene moiety and the thymine. The spin density distributions of 1-methyl analogues of the prepared compounds are shown in Figure 5. Table 2 contains the major atomic contributions based on an NAPC (natural atomic populations and charges) analysis as a quantitative guide. As can be seen from the spin density figures there is considerable delocalisation to the nucleobase for all types of C_2 linkage. A simplistic analysis based on a representation of the molecules as comprising of three components; ferrocene-bridge-nucleo-

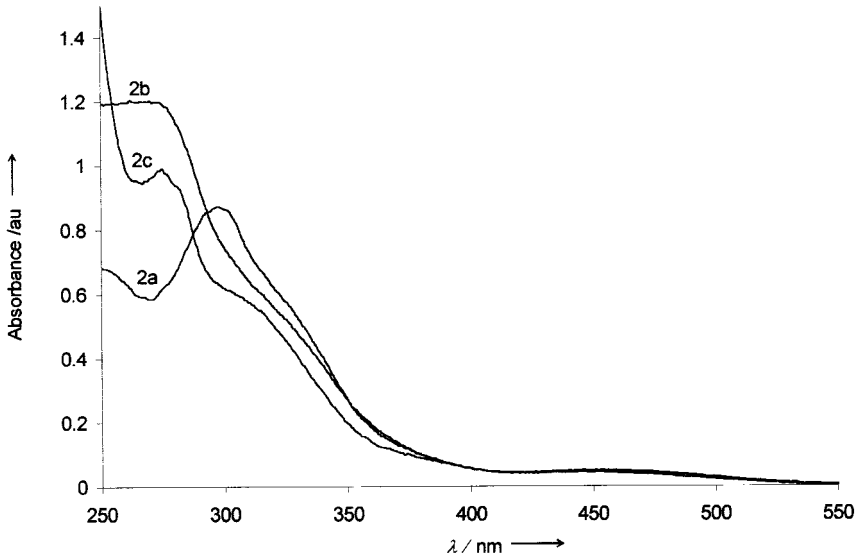

Figure 4. UV/Vis spectra of vinyl isomers of ferrocenyl-dT in acetonitrile.

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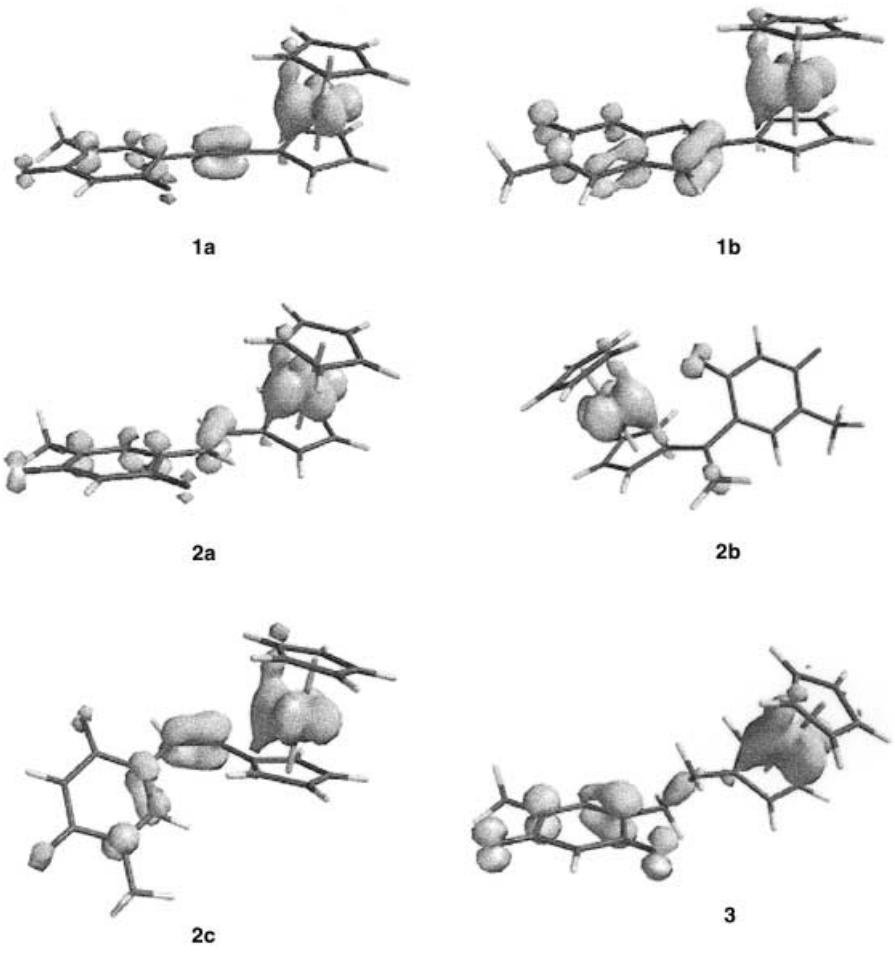

Figure 5. DFT-calculated spin density distribution on **1a**, **1b**, **2a – c**, and **3**.

Table 2. NAPC analysis percentages of spin density on the ferrocenyl-C_2-thymine components.

Derivative	Ferrocene[a]	C2 bridge[b]	Thymine[c]
1a	Fe: 70.4 (78.0)	C7: 3.8, C8: 5.3	12.9
1b	Fe: 61.3 (68.5)	C7: 5.4, C8: 5.2	20.9
2a	Fe: 69.4 (77.3)	C7: 4.0, C8: 6.2	12.5
2b	Fe: 85.5 (92.9)	C7: 0.6, C8: 2.8	3.7
2c	Fe: 63.4 (72.2)	C7: 5.4, C8: 7.0	15.4
3	Fe: 77.5 (83.2)	C7: 0.6, C8: 0.8	15.1

[a] Percentage on iron atom and total ferrocene unit in brackets. [b] Percentages on C7 and C8. [c] Total percentage on thymine ring.

base, reveals that between 72 – 93 % of the spin density is located at the ferrocenyl group and between about 4 – 15 % is transferred to the thymine. This is predominantly located at C5, C6, and the heteroatoms N1, O4 and O2. The conjugated bridging groups, C≡C and C=C, are affected similarly, with 9 – 12 %, while very little spin density resides in the non-conjugated *gem*-bridge (3.4 %) and the saturated C–C bridge (< 2 %).

The extent of ferrocenyl – nucleobase interaction is conformationally dependent as demonstrated for models of **1a**. Calculations of two extreme conformers of **1a**, where the thymine ring was rotated ± 90° to the substituted cyclopentadienyl ring gave spin density values of 84 – 89 %, 7 % and 4 – 9 % for the ferrocene, C_2 bridge and thymine, respectively. These non-planar configurations result in a reduction in conjugation and hence loss of spin density transferred to the thymidine unit.

The 1-methyl analogue for **1b** is quite different as might be expected. The extended aromatic system to which the ferrocene is attached serves to transfer spin density effectively from the metallo group. The NAPC analysis shows about 31 % transferred across the furanopyrimidone ring system.

The electrostatic potential atomic charges derived from these calculations also explain the susceptibility of **1a** to cylcisation. Compared to the results for the model C5-phenylacetylene-dT, C_8 is significantly more positive in **1a** (– 0.134 c.f. – 0.211 eV) accounting for the observed susceptibility to nucleophilic attack which effects ring closure.

Site-specific incorporation of 1a in DNA: Nucleoside derivatives that are highly conjugated to a redox centre have not been widely considered for studies on DNA-mediated charge transport. Compound **1a** is therefore of particular interest as the alkynyl linkage provides an efficient means for delocalisation between the redox group and the nucleobase.

Yu et al. have very recently reported on attempts to incorporate **1a** into synthetic oligonucleotides using solid-phase phosphoramidite methods.[36] They found that during the course of synthesis, using standard reagents and protocols, **1a** → **1b** interconversion occurred. Interestingly, these workers noted that in hybridization studies **1b** forms a more stable interaction with dG compared to dA. Figure 6 shows schematically the two corresponding base pairs. The observed increase in T_m values for the duplex containing the **1b**:dG pair can be easily rationalised, though clearly the pairing is not optimised.[36] In light of these findings we have explored alternative procedures in an effort to retain the structure of **1a** in synthetic oligonucleotides. In particular, given the known susceptibility to base-catalysed cyclisation, prime concern was given to the deprotection step. This is typically treatment with aqueous NH_3 for 12 h at room temperature, or 5 h at 55 °C. As an alternative, we have used ULTRAMILD base phosphoramidites (Glen Research, Va, USA) for all syntheses. These amidites are de-protected in a few hours in 0.05 M methanolic K_2CO_3 or several minutes with anhydrous methylamine.[50]

Compound **1a** was converted to the corresponding cyanoethylphosphoramidite for incorporation into oligonucleotides by reaction with 2-cyanoethoxy-*N,N*-diisopropylaminochloro-

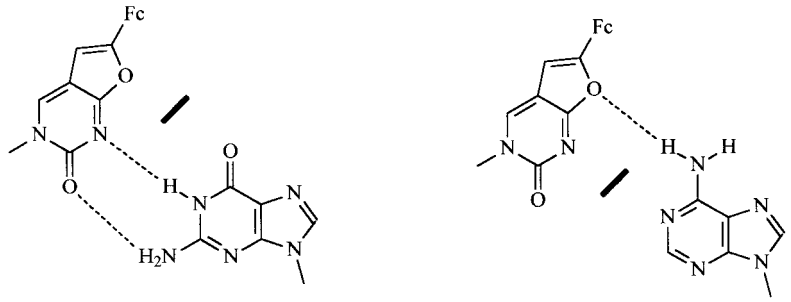

Figure 6. Base-pairing for **1b** with dG (left) and dA (right). The former is preferred after Yu et al.[36]

phosphoramidite as phosphitylating agent. This was preferred to *bis*-(*N,N*-diisopropylamino)-2-cyanoethoxyphosphine which gave poorer yields.

The 20-mer oligonucleotide, TATCGTATCGT$_{Fc}$ATCGTATCG, (where T$_{Fc}$ = **1a**) was prepared on 0.2 μmol CPG columns using standard protocols with the exception of a prolonged coupling reaction time (3 min) for the **1a** phosphoramidite. Deprotection with 0.05 M methanolic K_2CO_3 was monitored by HPLC and it was found that after five hours the oligonucleotide is fully deprotected. Similar data was obtained after deprotection with anhydrous $MeNH_2$ (20 min). The purified ferrocenyl-20mer was characterised by MALDI-TOF mass spectrometry, (calcd: [*M*(−Cp)] 6227.04; found: 6222.4(±5)) and illustrates successful incorporation of ferrocenylthymidine into an oligonucleotide.

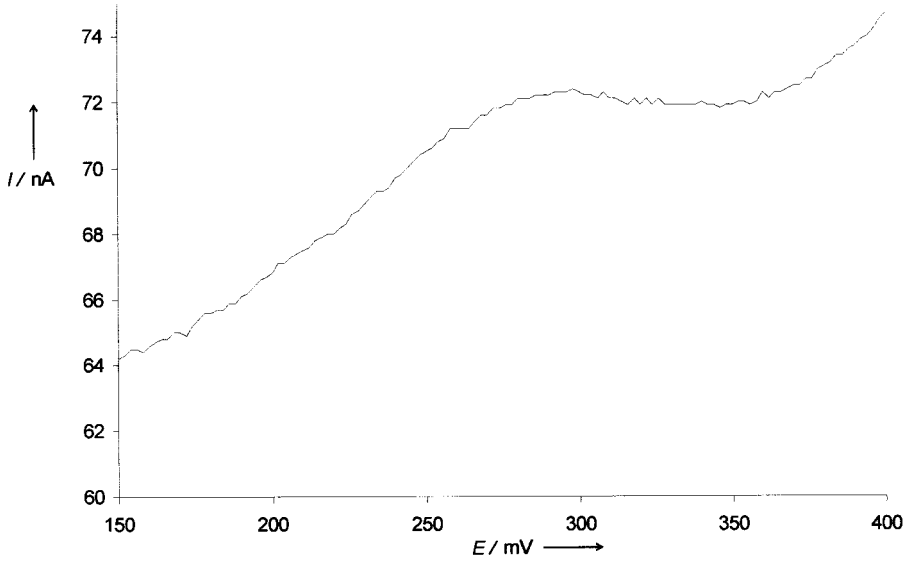

Figure 7. Differential pulse voltammogram of TATCGTATCGT$_{Fc}$ATCGTATCG, E° = 286 mV (Pulse height 25 mV, scan rate 20 mV s^{-1}, Ag^+ quasi-reference electrode, tungsten counter electrode, gold disc working electrode, 0.1 M TEAA buffer pH 6.5).

Re-analysis of the purified 20-mer by HPLC revealed a new peak with a retention time of 16.5 min in addition to the original peak at 21 min. The two species are attributed to the ferrocenyl group in different oxidation states. The latter species can be converted to the former species by addition of the reducing agent dithiothreitol (DTT) to a solution of the 20-mer. The action of DTT on a sample of the oligonucleotide was monitored by HPLC. Treatment showed a decrease in the latter peak concomitant with an increase in the earlier peak. The difference in retention times between the two forms is attributed to the change in overall charge upon oxidation/reduction of the iron centre. As expected the less negatively charged form bearing the ferrocenium cation has a longer retention time on the reverse-phase column. Differential pulse voltammetry confirmed that collected fractions of both peaks were electrochemically active, with a redox potential of 286 mV (Figure 7).

In view of these observations and the known susceptibility of the oxidised ferrocenyl species, ferrocenium, to nucleophilic attack, a precautionary post-synthetic reduction step, using a solution of DTT in acetonitrile, was employed before de-protection and removal from the solid support.

Analysis of the 20-mer oligonucleotide after digestion with snake venom phosphodiesterase and alkaline phosphatase indicated in addition to incorporation of **1a**, the formation of **1b**. The presence of the two isomeric ferrocenyl derivatives was not observed in the HPLC of the 20-mer due to the small difference in the retention times. Figure 8 shows the HPLC of the digested 20-mer. Approximately 50% of the modified nucleoside remains in the ethynylferrocenyl form. This compares with complete conversion to the furanothymidone form in the work of Yu et al.[36] However, despite the ability to retain the ethynylferrocenyl dT moiety intact, the mixture could not be separated.

Conclusion

The conjugated ferrocenyl-nucleoside **1a** can be incorporated into synthetic oligonucleotides to give redox-active DNA strands. However, even with the use of rapid mild methods for de-protection of the oligomers, a considerable fraction of **1a** (ca. 50%) still undergoes cyclisation to **1b**. The difficulties in separating the resulting mixture are non-trivial. DFT calculations show however that delocalisation between the ferrocenyl group and the thymidine occurs for the series of C_2-bridged nucleosides. This indicates that a range of derivatives may be used to prepare site specific redox-active ferrocenyl oligonucleotides in which the electronic structure of the nucleobase is significantly perturbed by redox reactions at the ferrocenyl group.

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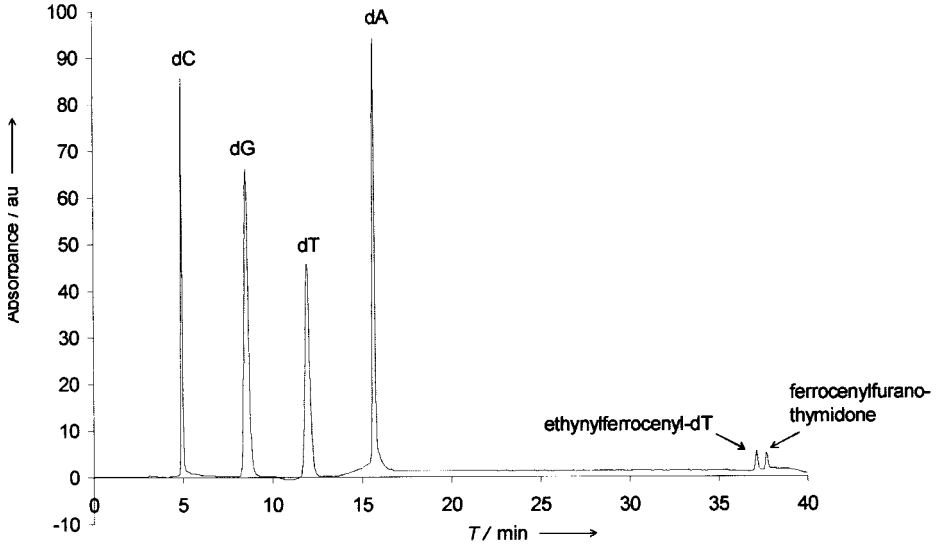

Figure 8. HPLC of digested TATCGTATCGT$_{Fc}$ATCGTATCG.

Experimental Section

Materials: Reagents were purchased from Aldrich and Lancaster and used as received unless otherwise stated. Solvents were dried and distilled under nitrogen prior to use. All reactions were performed under N_2 using standard Schlenk techniques. ^{1}H NMR spectra were performed on a 200 MHz Bruker Spectrospin AC 200E spectrometer and ^{31}P NMR spectra on a 300 MHz Bruker Spectrospin WM 300 WB spectrometer. UV/Vis electronic spectra were recorded on a Shimadzu UV-2101PC Scanning Spectrophotometer. Mass spectra of the ferrocenyl derivatives were obtained at the MS Service Centre, University of Wales, Swansea, whilst the MALDI-TOF spectrum of the modified 20-mer was measured by Nathan Harris at Moredun Research Institute.

Preparation of 5′-Dimethoxytrityl-5-iodo-2′-deoxyuridine: 5-Iodo-2′-deoxyuridine (2.0 g, 5.6 mmol) was dissolved in dry pyridine and then dimethylaminopyridine (DMAP) (70 mg) and dimethoxytrityl chloride (2.3 g, 6.77 mmol) were added. The mixture was stirred at ambient temperature under nitrogen for 16 h. Then 5% (v/w) sodium bicarbonate (5 mL) was added before the reaction mixture was evaporated to dryness. Final traces of pyridine were removed by repeated co-evaporation with toluene. The reaction mixture was then dissolved in chloroform, washed twice with 5% (v/w) sodium bicarbonate and then dried over magnesium sulfate. The crude product was dissolved in chloroform – methanol (98:2) and loaded onto a silica column packed in chloroform—methanol – triethylamine (97:2:1). The product was eluted by using a gradient of 2 – 10% methanol in chloroform and the appropriate fractions collected and solvent removed by rotary evaporation to give a white crystalline powder (2.96 g, 80%). ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): $\delta = 2.20 - 2.54$ (m, 2H: H-2′, H-2“), 3.36 (m, 2H: H-5′, H-5”), 3.76 (s, 6H: OMe), 4.09 (m, 1H: H-4′), 6.31 (t, 1H: H-1′), 6.82 (d, 4H: DMT), 7.22 – 7.41(m, 9H: DMT), 8.13 (s, 1H: H-6); MS: FAB$^+$: 655.8 for $C_{30}H_{29}N_2O_7I$.

5′-Dimethoxytrityl-5-ethynylferrocene-2′-deoxyuridine, 1a-DMT: 5′-Dimethoxytrityl-5-iodo-2′-deoxyuridine (1.5 g, 2.29 mmol) was dissolved in dry acetonitrile. Pyridine (5 mL), triethylamine (2 mL), ethynylferrocene (530 mg, 2.5 mmol), copper(I) iodide (150 mg, 0.79 mmol) and bis(triphenylphosphine)palladium(II) chloride (150 mg, 0.21 mmol) were added sequentially under nitrogen. The reaction mixture was stirred at 35 °C for 5 h. Disodium EDTA 5% (v/w) (5 mL) was added to the resulting suspension before evaporation to dryness. The crude product was redissolved in chloroform (100 mL) and washed twice with disodium EDTA 5% (v/w) and once with water before being dried over sodium sulfate. After filtration and concentration by rotary evaporation the reaction mixture was loaded onto a silica gel column packed in chloroform – methanol – triethylamine (95:4:1) and eluted by using chloroform – methanol (95:5). Fractions containing the product were combined and solvent removed to yield the title compound as a dark orange powder (1.09 g, 65%). ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): $\delta = 2.19 - 2.4$ (m, 2H: H-2′, H-2“), 3.37 (m, 2H: H-5′, H-5”), 3.71 (s, 6H: OMe), 4.07 (t, 1H: H-3′), 4.10 (m, 7H: Fc), 4.19 (m, 2H: Fc), 4.49 (m, 1H: H-4′), 6.30 (t, 1H: H-1′), 6.80 (m, 4H: DMT), 7.27 – 7.44 (m, 9H: DMT), 7.95 (s. 1H: H-6); MS: FAB$^+$: 737.9 for $C_{42}H_{38}N_2O_7Fe$.

The DMT group was removed by treatment with a 2% CCl_3COOH solution in CH_2Cl_2 to give **1a**, which was crystallised from chloroform/methanol (90:10).

Ferrocenyl-2′-furanopyrimidone (**1b**): A sample of **1b** was obtained from the following reaction, which also yielded **1a**. 5-Iodo-2′-deoxyuridine (500 mg, 1.4 mmol) was dissolved in deoxygenated acetonitrile (50 mL). Dry triethylamine (5 mL), ethynylferrocene (550 mg, 2.6 mmol), copper(I) iodide (50 mg) and $[PdCl_2(PPh_3)_2]$ (50 mg) were added and the reaction mixture stirred under nitrogen at 65 °C for 5 h. The reaction mixture was dried by rotary evaporation to a dark foam and then redissolved in chloroform and washed twice with 5% (v/w) disodium ETDA and then once with water before drying over sodium sulfate and concentration to a dark oil. The crude mixture contained both isomers in a ratio of 2:1 (**1a**/**1b**). These were separated by column chromatography on silica using a $CHCl_3$:MeOH gradient 0 – 10% methanol. The cyclised **1b** eluted from the column first and was obtained in 35% yield. ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): $\delta = 2.16 - 2.52$ (m, 2H; H-2′, H-2“), 3.76 (m, 2H; H-5′, H-5”), 4.37 (s, 5H; Fc), 4.57 (d, 2H; Fc), 4.94 (d, 2H; Fc), 5.27 (t, 1H; H-3′), 5.40 (d, 1H; H-4′), 6.29 (t, 1H; H-1′), 6.84 (s, 1H; H-7), 8.81 (s, 1H; H-6); MS: FAB$^+$: 435.9 for $C_{21}H_{20}N_2O_5Fe$.

5′-DMT-5-*trans*-vinylferrocene-2′-deoxythymidine, 2a-DMT and 5′-DMT-5-*gem*-vinylferrocene-2′-deoxythymidine, 2b-DMT: 5-Iodo-2′-deoxyuridine (0.33 g, 0.5 mmol) was dissolved in dry acetonitrile (20 mL). Triethylamine (2 mL), vinylferrocene (100 mg, 0.47 mmol) and $[PdCl_2(PPh_3)_2]$ (50 mg, 0.07 mmol) were added sequentially. The mixture was stirred and heated at 35 °C for 90 h followed by a further 90 h at reflux. After cooling and evaporation to a sticky oil the crude product was dissolved in chloroform and washed twice with disodium EDTA 5% (v/w) and once with water before being dried with sodium sulfate. After filtration and concentration by rotary evaporation the reaction mixture was purified by column chromatography using chloroform – methanol (90:10) as eluent. The appropriate fractions were combined to yield a mixture of the title products as an orange powder (0.21 g, 60%). This was found to be a mixture of the *gem* and *trans* isomers. Attempts to separate the mixture by TLC or HPLC were unsuccessful. Treatment of the mixture with a 2% CCl_3COOH solution in CH_2Cl_2 afforded the de-protected nucleosides. Repeated recrystallisation of the mixture gave sample **2a** in 45% yield. Crystals of **2a** suitable for single-crystal X-ray analysis were obtained by slow evaporation of a saturated solution of **2a** in chloroform – methanol (95:5).

2a: ^{1}H NMR (200 MHz, CD_3OD, 25 °C): $\delta = 2.49$ (t, 2H; H-2′), 4.02 (m, 2H; H-5′), 4.14 (m, 1H; H-3′), 4.30 (s, 5H; Fc), 4.44 (m, 2H; Fc), 4.62 (m, 2H; Fc), 4.64 (m, 1H; H-4′), 6.51 (t, 1H; H-1′), 6.64 (d, ^3J(H,H) = 16.2 Hz, 1H; *trans*-H), 7.36 (d, ^3J(H,H) = 16.2 Hz, 1H; *trans*-H), 8.37 (s, 1H; H-6); UV/Vis (CH_3CN):λ_{max} (ε) = 298 (7660), 325 (5039), 449 nm (408); MS: FAB$^+$: 739.9 for $C_{42}H_{40}N_2O_7Fe$.

2b: ^{1}H NMR (200 MHz, CD_3OD, 25 °C): $\delta = 2.49$ (t, 2H; H-2′), 4.02 (m, 2H; H-5′), 4.14 (m, 1H; H-3′), 4.30 (s, 5H; Fc), 4.44 (m, 2H; Fc), 4.62 (m, 2H; Fc), 4.64 (m, 1H; H-4′), 5.33 (d, ^3J(H,H) = 1.2 Hz, 1H; *gem*-H), 5.79 (d, 1H; *gem*-H, ($J = 1.24$Hz)), 6.51 (t, 1H; H-1′), 8.37 (s, 1H; H-6); UV/Vis ((CH_3CN):λ_{max} (ε) = 264 (10532), 325 (4590), 446 nm (448).

5′-DMT-5-*cis*-vinylferrocene-2′-deoxythymidine, 2c-DMT: Compound **1a-DMT** (1 equiv) was dissolved in distilled, anhydrous MeOH under nitrogen with heating. The orange solution was then cooled to −78 °C and a suspension of $NiCl_2$ (1 equiv) in MeOH was added followed by $NaBH_4$ (1.3 equiv). The reaction mixture was stirred under nitrogen for 1.5 h and then allowed to warm to room temperature. At the point which the mixture

turned black, flash silica was added (equal weight to nucleoside) and solvent was removed. Crude mixture was purified by chromatography (2% MeOH in DCM). Yield 30%. ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): $\delta = 1.9$ (m, 1H; H-2′), 2.3 (m, 1H; H-2′), 3.0 (q, 1H; H-5′), 3.2 (q, 1H; H-5′), 3.8 (m, 8H; OMe, H-4′), 4.0 (m, 3H; Fc, H-3′), 4.05 (s, 5H; Fc), 4.1 (t, 2H; Fc), 6.0 (d, ^3J(H,H) = 11 Hz, 1H; *cis*-H), 6.2 (t, 1H; H-1′), 6.3 (d, ^3J(H,H) = 11 Hz, 1H; *cis*-H), 6.8 (d, 4H; DMT), 7.2 (m, 9H; DMT), 7.4 (s, 1H; H-6); UV/Vis (CH_3CN): λ_{max} (ε) = 275 (14687), 310 (17025), 451 nm(670); MS: EI: 740.22 for $C_{42}H_{40}N_2O_7Fe$.

5′-DMT-5-ethylferrocene-2′-deoxythymidine (**3**): A solution of **2c-DMT** (1 equiv) in distilled anhydrous methanol under nitrogen was cooled to −78 °C. $NiCl_2$ (1 equiv) was added as a solution in methanol followed by $NaBH_4$ (1.3 equiv). Reaction mixture was stirred at −78 °C for one hour then allowed to warm to ambient temperature. At the point at which the solution went black, silica was added and solvent was removed. Crude mixture was separated by column chromatography (1% MeOH in DCM) to give **3** in 25% yield). ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): $\delta = 1.47$ (t, 2H; CH_2), 2.24–2.38 (m, 4H; H-2′, H-2“, CH_2), 3.45 (m, 2H; H-5′, H-5”),3.83 (s, 6H; OMe), 3.87 (t, 1H; H-3′), 3.93(m, 4H; Fc), 4.09 (s, 5H; Fc), 4.54 (m, 1H; H-4′), 6.43 (t, 1H; H-1′), 6.88 (m, 4H; DMT), 7.33–7.48 (m, 10H; DMT, H-6); MS: ES$^+$: 742.0 for $C_{42}H_{52}N_2O_7Fe$.

Crystal Data: **1a:** $C_{21}H_{20}N_2O_5Fe.CHCl_3$, $M_r = 555.6$, monoclinic, space group $P2_1$, $a = 9.9869(7)$, $b = 20.4183(15)$, $c = 12.7998(9)$ Å, $\beta = 109.751(2)°$, $V = 2456.5(3)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.502$ g cm^{-3}; $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, $\mu = 0.976$ mm^{-1}, $T = 160$ K. Of 19517 reflections, corrected for absorption, 10481 were unique ($R_{int} = 0.036$, $\theta = 28.7$ °); $R = 0.062$ (F values, $F^2 > 2\sigma$), $R_w = 0.172$ (F^2 values, all data), GOF = 0.929 for 632 parameters, final difference map extremes = +0.93 and −0.69 e Å^{-3}. The structure was solved by direct methods. All non-H atoms were refined anisotropically. H atoms were refined with a riding model. The asymmetric unit contains two independent molecules of the complex together with one ordered and one disordered chloroform molecule.

1b: $C_{21}H_{20}N_2O_5Fe$, $M_r = 436.2$, monoclinic, space group $P2_1$, $a = 6.5896(3)$, $b = 22.1004(10)$, $c = 12.7405(6)$ Å, $\beta = 96.780(2)°$, $V = 1842.46(15)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.573$ g cm^{-3}; synchrotron radiation (SRS station 9.8), $\lambda = 0.6884$ Å, $\mu = 0.857$ mm^{-1}, $T = 160$ K. Of 13318 reflections, corrected for absorption and incident beam decay, 7290 were unique ($R_{int} = 0.034$, $\theta = 29.4°$); $R = 0.032$ (F values, $F^2 > 2\sigma$), $R_w = 0.078$ (F^2 values, all data), GOF = 0.997 for 528 parameters, final difference map extremes = +0.36 and −0.28 e Å^{-3}. Structure solution and refinement were as for **1a**. The asymmetric unit contains two independent molecules, but there is no solvent present.

2a: $C_{21}H_{22}N_2O_5Fe \cdot CHCl_3$, $M_r = 557.6$, monoclinic, space group $P2_1$, $a = 9.885(2)$, $b = 20.474(4)$, $c = 12.949(3)$ Å, $\beta = 112.156(4)°$, $V = 2427.2(9)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.526$ g cm^{-3}; $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, $\mu = 0.988$ mm^{-1}, $T = 160$ K. Of 9238 reflections, corrected for absorption, 5878 were unique ($R_{int} = 0.043$, $\theta = 22.5°$); $R = 0.116$ (F values, $F^2 > 2\sigma$), $R_w = 0.354$ (F^2 values, all data), GOF = 1.151 for 600 parameters, final difference map extremes = +1.56 and −0.56 e Å^{-3}. Structure solution and refinement were as for **1a**, except that H atoms were not located on the sugar groups. The precision of the structure is limited by the rather weak diffraction data, by pseudo symmetry, and by twinning. Chloroform sites (two in the asymmetric unit, together with two independent molecules of the complex) appear to be only partially occupied. Geometry and displacement parameter restraints were applied to aid refinement.

Programs used: SHELXTL (G. M. Sheldrick, SHEXLTL manual, Bruker AXS Inc., Madison, WI., USA, 1998, version 5.1), together with standard Bruker SMART and SAINT diffractometer control and data integration programs and local software.

CCDC-175814 (**1a**), 175815 (**1b**), and 175816 (**2a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

Electrochemistry: Electrochemical data were recorded on an EG&G Princeton Applied Research Potentiostat, Model 263A (using Echem software 4.11) and using a silver wire reference electrode, a gold disc working electrode and a tungsten wire counter electrode. Cyclic voltammograms were recorded at a scan rate of 100 mV s^{-1} in 0.1M tetrabutylammonium hexafluorophosphate in acetonitrile and $E°$ referenced against Fc/Fc^+ as an external measurement. Differential pulse voltammograms were measured at a pulse height of 25 mV, a pulse width of 50 ms and at a scan rate of 20 mV s^{-1}.

Electronic structure calculations: Density functional calculations (DFT) were performed using the Titan program package (Wavefunction Inc., USA). Geometries were optimised at the Becke-Perdew level of theory using the LA-CVP* basis set.

Oligonucleotide synthesis: An Applied Biosystems 381A DNA synthesizer was used for the preparation of oligonucleotides. The base-phosphoramidites were ULTRAMILD (Glen Research, VA, USA) and the standard Cap A was replaced with phenoxyacetic anhydride. Standard coupling protocols (45 s) were used with the exception of the ferrocenyl-phosphoramidite for which the coupling time was increased to 3 min. On completion of the synthesis the column was washed thoroughly with acetonitrile and then dried with argon. Deprotection of the oligonucleotides involved treatment with either 0.05M methanolic K_2CO_3 for about 5 h or exposure to $MeNH_2$ at a pressure of about 2 bar for 20 min.

HPLC were run using Gilson system 712 controller software. Analytical runs were performed on a Jones APEX ODS 5μ column with an injection loop of 25 µL. The column was incubated at 30 °C. The solvent gradient was made up of solvent A (10% acetonitrile in water containing TEAA buffer pH6.5) and solvent B (65% acetonitrile in water containing TEAA buffer pH 6.5), increasing from 0% B to 40% B over 25 min and returning to 100% A at 30 min.

The 20-mer oligonucleotide was digested into its monodeoxynucleoside constituents using snake venom phosphodiesterase and alkaline phosphatase following literature procedure.[51]

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