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Organometallic Nucleoside Analogues: Effect of Hydroxylalkyl Linker Length on Cancer Cell Line Toxicity

Jonathan L. Kedge,[a] Huy V. Nguyen,[a] Zahra Khan,[b] Louise Male,[a] Media K. Ismail, Holly V. Roberts, Nikolas J. Hodges,[b] Sarah L. Horswell,[a] Youcef Mehellou,[c] and James H. R. Tucker*[a]

Dedication ((optional))

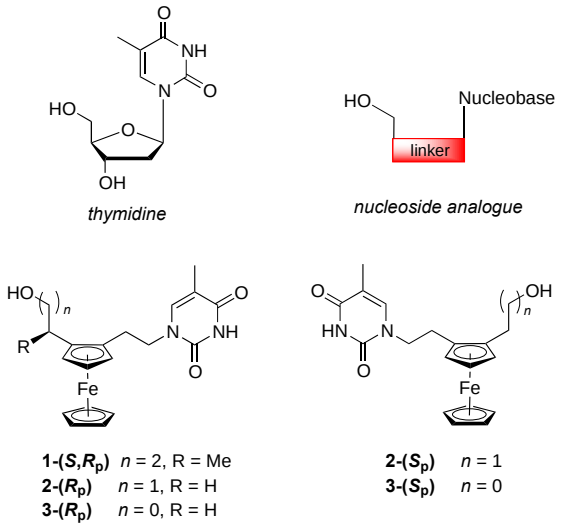
Abstract: A new series of chiral ferrocene derivatives containing both a hydroxyalkyl group and a thyminyl group on one cyclopentadienyl ring have been synthesised in order to probe structure activity relationships in cancer cell-line cytotoxicities. Stereoisomers of enantiomeric pairs of these so-called ferronucleosides have been studied and characterised by a combination of chiral analytical HPLC and single crystal X-Ray diffraction. Biological activity studies reveal that changing the length of the hydroxyalkyl group had marked effects on IC_{50} values, with compounds having shorter arms that more closely resemble endogenous nucleosides exhibiting lower cytotoxicities. Lipophilicities and electrochemical properties of this compound series have been studied in order to rationalise these trends and indicate future directions of study.

Introduction

Nucleoside analogues are a class of compound which interfere with the processes of nucleic acid synthesis through the mimicking of endogenous substrates. The effectiveness of this class of molecules in treating cancer and various viral infections was established a few decades ago.[1],[2] The sugar unit in these drug molecules is frequently modified (e.g. in *AZT* or *gemcitabine*) or completely replaced (e.g. in *abacovir* and *acyclovir*). Fundamentally therefore, for conventional nucleoside mimics, the sugar unit may be viewed as a modifiable or replaceable linker between the active hydroxyl and nucleobase moieties (Figure 1).[3]

Due to its stability, ease of functionalisation and potential for novel modes of action through its redox properties, ferrocene has been at the centre of the now established field of bioorganometallic medicinal chemistry.[4] Complementing the mode of action of the parent compound, the anti-malarial *ferroquine* and the breast cancer drug candidate *ferrocifen* are the most well-known examples in which beneficial secondary mechanisms of action are conferred upon incorporation of the ferrocene moiety.[5] For various ferrocene derivatives, studies indicate that cancer cell cytotoxicity derives from oxidation to the ferrocenium ion, facilitating the generation of reactive-oxygen-species (ROS) which, in turn, inflict damage upon the genetic material, thereby inducing apoptosis.[5c],[6]

Aiming to exploit such apposite biological properties and the potential for novel modes of action, we recently reported the promising anti-cancer activity of organometallic analogues of nucleosides in which the five-membered sugar ring is entirely replaced by a cyclopentadienyl unit in ferrocene.[7] Micro- and sub-micromolar activities against various cancer cell lines were observed for the thymine derivative **1-(S,R_p)** (Figure 1) and its adenine counterpart containing both a hydroxyalkyl linker and a nucleobase. In order to establish the mode of action of these so-called ferronucleosides, we have begun to explore the effect of structure and stereochemistry on cell cytotoxicity. As a starting point, we decided to synthesise enantiomers of two new thymine-containing compounds in which the hydroxyalkyl linker length is reduced from three to two and one carbon atoms respectively. The results demonstrate how small structural changes to these ferronucleosides can have a marked effect on biological activity.



1-(S,R_p) n = 2, R = Me
2-(R_p) n = 1, R = H
3-(R_p) n = 0, R = H

2-(S_p) n = 1
3-(S_p) n = 0

Figure 1. The DNA nucleoside thymidine, a nucleoside analogue and structures of the ferronucleoside target compounds in this study.

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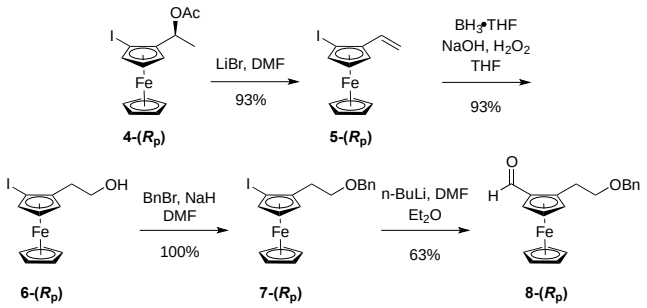
Supporting information for this article is given via a link at the end of the document.

Results and Discussion

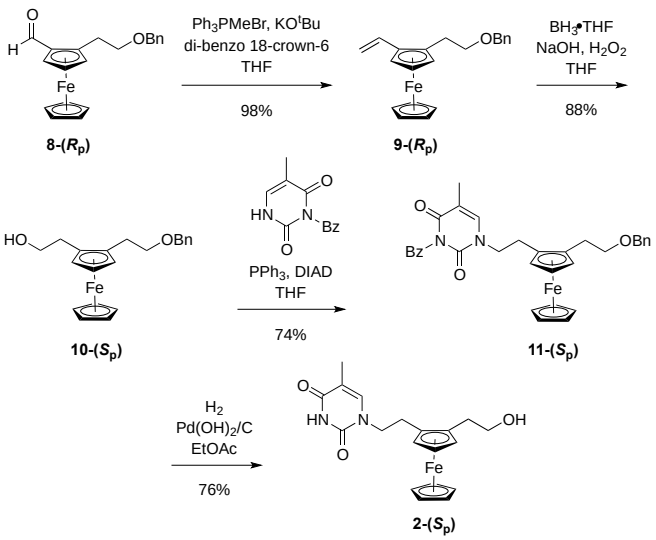
The rationale for varying the linker length between the Cp ring and the hydroxyl group in these ferronucleoside compounds was as follows. Firstly it was considered important to assess the effect on cell toxicity of lipophilicity. Secondly previous cancer cell line studies on mono-functionalised ferrocene derivatives had indicated that the length of an alkyl chain connected to the Cp ring had a marked affect on toxicity,[8] with a possible link to the redox potential of the ferrocene unit. Thirdly, in the spirit of making structural analogues of natural compounds, it was considered that a shorter linker length to the alcohol group would give compounds more closely resembling those of endogenous nucleosides. At the same time, it was also considered opportune to examine the effect of stereochemistry on cancer cell toxicities by synthesizing the enantiomers of these two new compounds.

Conveniently, it was found that the two target compounds **2-(S_p)** and **3-(R_p)** could be made from one common synthon **8-(R_p)**, as was the case for their opposite enantiomers. The synthesis of **8-(R_p)** is outlined in Scheme 1. The chirally pure (S,R_p)-2-α-*O*-acetoxyethyl-1-iodo-ferrocene **4-(R_p)** was made accordingly to our previously published method[7a] and then heated in the presence of LiBr to give the corresponding 1-iodo-2-vinylferrocene **5-(R_p)**. A hydroboration-oxidation reaction gave alcohol **6-(R_p)** which, after benzyl protection, was treated with n-BuLi to affect a halogen-metal exchange before quenching with DMF, to give the desired aldehyde **8-(R_p)**.

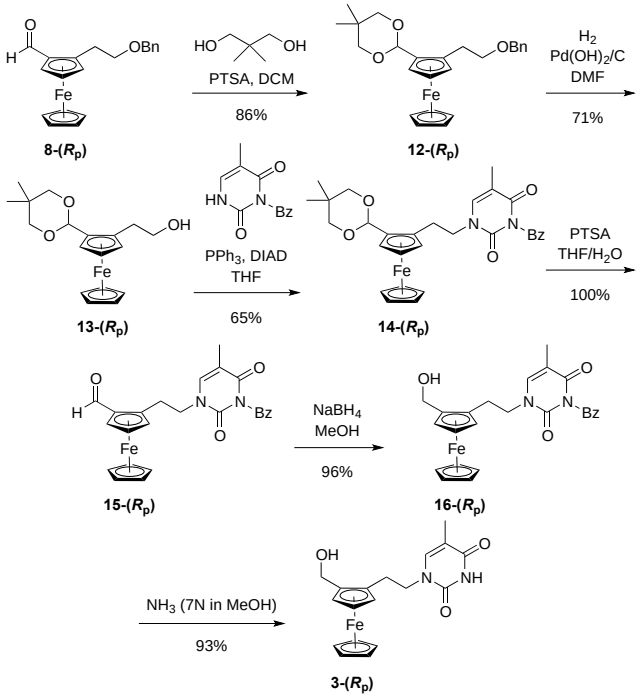


Scheme 1. Synthesis of synthon **8-(R_p)**.

Compound **2-(S_p)** was synthesised (Scheme 2) from **8-(R_p)** by converting the aldehyde to a vinyl group using the Wittig reaction, before hydroboration-oxidation placed a hydroxyl group at the *β*-position. This compound was then reacted with *N*3-benzoyl thymine using the Mitsunobu reaction before removal of the benzyl group by hydrogenolysis and deprotection of the nucleobase gave the desired target compound.



Scheme 2. Synthesis of **2-(S_p)** from chiral synthon **8-(R_p)**.



Scheme 3. Synthesis of **3-(R_p)** from chiral synthon **8-(R_p).**

The route towards the methyl linker target 3-**(R_p)** from the same synthon **8-(R_p)** proceeded by protecting the aldehyde as a cyclic acetal using 2,2-dimethylpropane-1,3-diol (Scheme 3). Benzyl

deprotection was again affected by hydrogenolysis, and the corresponding free alcohol employed in a Mitsunobu reaction with *N*3-benzoyl thymine. The acetal group was removed using PTSA in wet THF and the regenerated aldehyde reduced to the corresponding alcohol using $NaBH_4$. Finally, the nucleobase was deprotected using ammonia in methanol to give the target compound.

Compounds **2-(R_p)** and **3-(S_p)** were synthesised in an identical fashion starting from the opposite enantiomer **8-(S_p)**. The enantiopurities of these four planar chiral targets were assessed by analytical chiral HPLC, giving values as follows: **2-(R_p),** 97%; **2-(S_p);** 97%; **3-(R_p)**, 95%; **3-(S_p)**, 92%. Fortunately, crystals of all four compounds, grown by the slow evaporation of concentrated solutions in ethyl acetate and chloroform for enantiomers of **2** and **3** respectively, were found to be suitable for X-Ray diffraction. The structures of the R_p enantiomers of **2** and **3** are depicted in Figures 2 and 3 respectively, with those for the S_p enantiomers presented in the supplementary information.

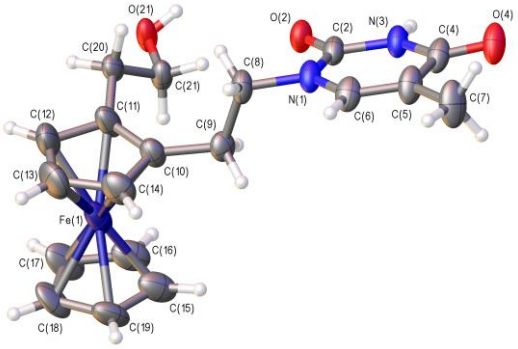


Figure 2. Crystal structure of **2-(R_p)** with ellipsoids drawn at the 50% probability level. The cyclopentadienyl ring C(15)-C(19) / C(15')-C(19') and the group C(20), C(21), O(21) / C(20'), C(21'), O(21') are both disordered over two positions, for clarity only the major positions are shown.

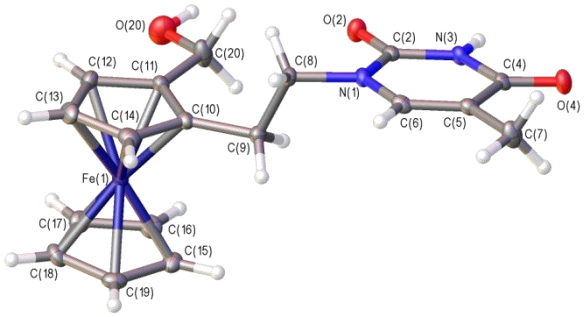


Figure 3. Crystal structure of **3-(R_p)** with ellipsoids drawn at the 50% probability level. The structure contains two crystallographically-independent molecules of which only one is shown.

The R_p isomers of compounds **1**, **2** and **3** were characterised with cyclic voltammetry (CV) in dry acetonitrile (Fig. 4) and the half-wave potentials, $E_{1/2}$, were determined from the peak positions with respect to decamethylferrocene (dmfc) as the internal reference of (Table 1). In each case, the ratio of the peak heights of the oxidation and reduction waves was close to unity, plots of peak current vs the square root of the scan rate were linear and the peak separations were as measured for dmfc (ca. 65–70 mV), indicating reversible one-electron redox behaviour for all three compounds (see supplementary information). It is clear that as the hydroxyalkyl linker length increases, the $E_{1/2}$ value becomes more negative, indicating the progressive stabilisation of the ferrocenium ion. This is consistent with the longer alkyl linker stabilising the ferrocenium cation through increased positive inductive effects, as found previously for related compounds.[9]

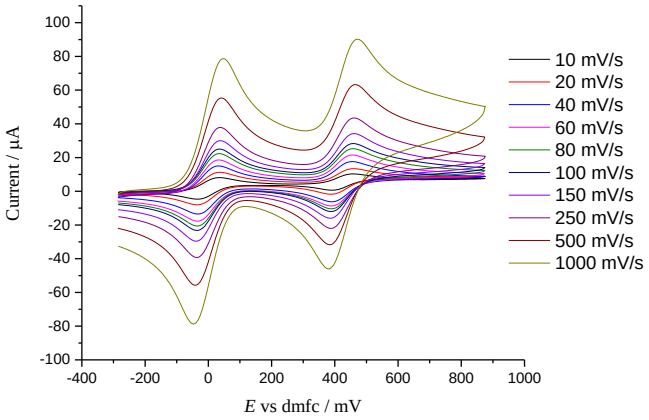


Figure 4. Cyclic voltammograms of **1-(S,R_p)** at various scan rates wrt decamethylferrocene. Concentration 1.0 mM with 0.1 M $TBAPF_6$ in dry acetonitrile at RT.

Previous studies[7] indicated that **1-(S,R_p)** was active in three different human cancer cell lines. For this study, it was decided to probe its activity further in a human osteosarcoma (HOS) cell line and compare these with its enantiomer as well as with compounds **2**, **3** and the established anticancer drug cisplatin. HOS cells were exposed to various concentrations of test agent for four days and the cell number assessed using the crystal violet assay. The resulting IC_{50} values calculated from the inhibition curves are presented in Table 1. In general agreement with our previous studies on other cell lines,[7] **1-(S,R_p)** with its three-carbon spacer displays low micromolar activity with an IC_{50} value of 4.4 µM. However a clear trend between the length of the hydroxyalkyl arm and the IC_{50} values was observed; compounds **2-(R_p)** and **2-(S_p)** with one fewer carbon were found to be approximately one order of magnitude less active than **1-(S,R_p)**, with IC_{50} values of 58.4 and 57.8 µM respectively. Furthermore, **3-(R_p)** and **3-(S_p)** with just a methylene spacer, display lower cytotoxicities still with IC_{50} values of 73.1 and 86.0 µM respectively. Differences in biological activity between the

enantiomers for compounds **2** and **3** do not appear to be significant.

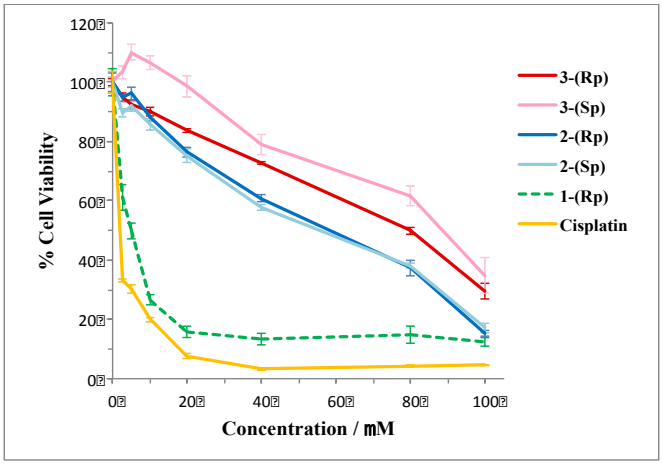


Figure 5. Inhibition of HOS cells after four days incubation with each compound. The results represent the mean of at least three independent experiments ± SEM (n=3).

Table 1. IC_{50}, $E_{1/2}$ and CLogP values for various stereoisomers of ferronucleosides **1**, **2** and **3**.

Compound	IC_{50} (μM)[a]	$E_{1/2}$ (mV)[b]	CLogP[c]
1-(S,R_p)	4.4	424	+ 0.96
2-(R_p)	58.4	467	+ 0.03
2-(S_p)	57.8	nd	+ 0.03
3-(R_p)	73.1	518	– 0.05
3-(S_p)	86.0	nd	– 0.05
Cisplatin	0.31	–	nd

[a] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%, after 4 days incubation. All compounds [b] versus decamethylferrocene, measured at 1 mM concentration in 0.1 M $TBAPF_6$ in dry acetonitrile. Confidence limit ± 5mV [c] Calculated using *ChemBioDraw Ultra 13.0*.[9a] nd = not determined (enantiomers would have identical data).

As previously reported for a series of simple ferrocenyl compounds,[8] cell cytotoxicity is inversely related to the $E_{1/2}$ value, suggesting that biological activity could be linked to the thermodynamic favourability of oxidation to the ferrocenium ion ($E_{1/2}$: **1** < **2** < **3**). However another important consideration is lipophilicity, which would also be expected to change as a function of the alkyl linker length. An increase in drug activity with higher lipophilicity can be explained by better diffusivity across cellular and nuclear membranes, thus producing much higher concentrations at the site of action. Drug lipophilicities are normally quantified through octanol–water partition coefficients (LogP values). Predicted coefficients (as CLogP) values, calculated using a method previously applied to ferrocene compounds,[9a] are also displayed in Table 1. As expected, the trend in these values (CLogP: **1** > **2** > **3**) also correlates with hydroxyalkyl arm linker length. Interestingly, lipophilicity has been previously shown to be linearly correlated with oxidation potentials for a series of structurally related ferrocene derivatives.[9a]

Conclusions

Enantiomers of two new structural variations within a series of ferronucleosides, in which the hydroxyalkyl linker length has been shortened to two and one carbon atoms respectively, have been successfully synthesised and characterised. The biological data for this novel ferrocene series demonstrate a marked structure activity relationship, with their cytotoxicities becoming progressively lower as their structure tends towards that of an endogenous nucleoside (i.e. thymidine). A consideration of their respective $E_{1/2}$ and CLogP values therefore indicates that the thermodynamic ease of oxidation to the ferrocenium ion and lipophilicity may in fact be more central to their biological activity than their structural conformity to the traditional nucleoside analogue pharmacophore. Further studies are now underway to establish whether the mechanism of action of **1-(S,R_p)** and related compounds resembles that of a typical nucleoside analogue, or a new pathway in which the structure and properties of the ferrocene unit play a prominent and distinct role.

Experimental Section

Synthesis

(R_p)-1-iodo-2-vinyl-ferrocene (5-(R_p))
(S,R_p)-2-α-*O*-acetoxyethyl-1-iodo-ferrocene (**4-(S,R_p)**) (4.30 g, 10.81 mmol, 1.0 eq.) was dissolved in dry DMF (20 ml), LiBr (14.0 g, 162 mmol, 15.0 eq.) was added and the resulting mixture heated at 85 °C for 1 hr with stirring. The reaction was quenched by addition of sat. $Na_2S_2O_3$ solution, extracted with DCM, washed with water (2 x 100 ml), dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by flash column chromatography on neutralised (Et_3N) silica gel using an eluent of hexane. The solvent was removed in vacuo to give the title compound as a dark orange oil (3.40 g, 10.09 mmol, 93 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.58 (dd, J = 17.5, 10.9 Hz, 1H (C***H***=CH_2), 5.50 (dd, J = 17.5, 1.4 Hz, 1H (C***H***$_2$=CH)), 5.22 (dd, J = 10.9, 1.4 Hz, 1H (C***H***$_2$=CH)), 4.55 (dd, J = 2.6, 1.4 Hz, 1H (Fc C***H***)), 4.53 (dd, J = 2.6, 1.4 Hz, 1H (Fc C***H***)), 4.34 (dd, J = 2.6, 2.6 Hz, 1H (Fc C***H***)), 4.14 (s, 5H (cp C***H***)). ^{13}C NMR (400 MHz, $CDCl_3$) δ 134.21 (***C***H=CH_2), 113.27 (***C***H$_2$=CH), 84.19 (Fc ***C***–CH), 75.33 (Fc ***C***H), 72.35 (cp ***C***H), 69.54 (Fc ***C***H), 63.80 (Fc ***C***H), 45.01 (Fc ***C***–I). ν_{max}/cm^{-1} = 3087 (Fc CH), 1628 (C=C). HRMS (ESI-TOF) m/z: [M]$^+$ Calcd for $C_{12}H_{11}FeI$ 337.9255; Found 337.9258.

Producing matching ^{1}H and ^{13}C NMR spectra, (S_p)-1-iodo-2-vinyl-ferrocene (**5-(S_p)**) (2.32 g, 6.85 mmol 88 %) was prepared according to the same procedure from (R,S_p)-2-α-*O*-acetoxyethyl-1-iodo-ferrocene (**4-(R,S_p)**) (3.09 g, 7.77 mmol).

(R_p)-2-β-hydroxyethyl-1-iodo-ferrocene (6-(R_p))
(R_p)-1-iodo-2-vinyl-ferrocene (**5-(R_p)**) (3.40 g, 10.09 mmol, 1.0 eq) was dissolved in dry THF (30 ml) in a Schlenk tube under an atmosphere of

argon and cooled to 0 °C in an ice bath. BH_3•THF (1M in THF) (8.87 ml, 8.87 mmol, 1.5 eq.) was added dropwise and stirring continued at 0 °C for 1 hr then the solution was warmed to room temperature and stirring continued for another 1.5 hrs. EtOH (8.87 ml) was carefully added and stirring continued for 5 min, followed by the dropwise addition of NaOH (3M in H_2O) (8.87 ml) and a further 10 min of stirring. H_2O_2 (30 % in H_2O) (16 ml) was then added slowly and stirring continued for 30 min. The reaction was extracted with Et_2O, washed with water (2 x 100 ml) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product purified by flash column chromatography on silica gel using a gradient eluent system of 5 → 10 % EtOAc in hexane. The solvent was removed *in vacuo* to give the title compound as a yellow oil (3.34 g, 9.38 mmol, 93 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.41 (dd, *J* = 2.5, 1.4 Hz, 1H (Fc C***H***)), 4.21 (dd, *J* = 2.5, 1.4 Hz, 1H (Fc C***H***)), 4.18 (dd, *J* = 2.5, 2.5 Hz, 1H (Fc C***H***)), 4.12 (s, 5H (cp C***H***)), 3.85 – 3.62 (m, 2H (C***H***$_2$OH)), 2.83 – 2.55 (m, 2H (C***H***$_2$CH$_2$OH)), 1.61 (s, 2H (O***H***)). ^{13}C NMR (400 MHz, $CDCl_3$) δ 86.22 (Fc ***C***–CH$_2$), 74.49 (Fc ***C***H), 71.73 (cp ***C***H), 68.59 (Fc ***C***H), 67.71 (Fc ***C***H), 63.00 (***C***H$_2$OH), 45.51 (Fc ***C***–I), 33.48 (***C***H$_2$CH$_2$OH). ν_{max}/cm^{-1} = 3303 (OH), 3092 (Fc CH). HRMS (ESI-TOF) m/z: [M]$^+$ Calcd for $C_{12}H_{13}FeIO$ 355.9360; Found 355.9361.

Producing matching ^{1}H and ^{13}C NMR spectra, (*S*$_p$)-2-β-hydroxyethyl-1-iodo-ferrocene (**6-(*S*$_p$)**) (1.37 g, 3.85 mmol, 89 %) was prepared according to the same procedure from (*S*$_p$)-1-iodo-2-vinyl-ferrocene (**5-(*S*$_p$)**) (1.55 g, 4.34 mmol).

(*R*$_p$)-2-β-benzyloxyethyl-1-iodo-ferrocene (**7-(*R*$_p$)**)

(*R*$_p$)-2-β-benzyloxyethyl-1-iodo-ferrocene (**6-(*R*$_p$)**) (1.11 g, 3.13 mmol, 1.0 eq.) was dissolved in dry DMF (25 ml) and stirred in a Schlenk tube under an atmosphere of argon. NaH (0.188 g, 7.83 mmol, 2.5 eq.) was added in small portions, the solution turned from orange to yellow and stirring was continued for 1 hr. BnBr (0.74 ml, 6.26 mmol, 2.0 eq.) was added and stirring continued for 16 hrs. The reaction was quenched by the dropwise addition of water, extracted with Et_2O and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography on silica gel using an eluent of hexane. The solvent was removed *in vacuo* to give the title compound as a yellow oil (1.40 g, 3.13 mmol, 100 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 – 7.12 (m, 5H (Ph C***H***)), 4.43 (s, 2H (Ph C–C***H***$_2$)), 4.28 (dd, *J* = 2.6, 1.3 Hz, 1H (Fc C***H***)), 4.11 (dd, *J* = 2.6, 1.3 Hz, 1H (Fc C***H***)), 4.03 (dd, *J* = 2.6, 2.6 Hz, 1H (Fc C***H***)), 3.99 (s, 5H (cp C***H***)), 3.62 – 3.36 (m, 2H (CH$_2$C***H***$_2$O), 2.73 – 2.53 (m, 2H (C***H***$_2$CH$_2$O)). ^{13}C NMR (400 MHz, $CDCl_3$) δ 138.41 (Ph ***C***–CH$_2$), 128.39 (Ph ***C***H), 127.65 (Ph ***C***H), 127.57 (Ph ***C***H), 86.59 (Fc ***C***–CH$_2$), 74.03 (Fc ***C***H), 72.93 (Ph C–C***H***$_2$), 71.57 (cp ***C***H), 70.24 (CH$_2$C***H***$_2$O), 68.28 (Fc ***C***H), 67.63 (Fc ***C***H), 45.76 (Fc ***C***–I), 30.63 (***C***H$_2$CH$_2$O). ν_{max}/cm^{-1} = 3088 (Fc CH), 3029 (Ph CH). HRMS (ESI-TOF) m/z: [M]$^+$ Calcd for $C_{19}H_{19}FeIO$ 455.9830; Found 455.9827.

Producing matching ^{1}H and ^{13}C NMR spectra, (*S*$_p$)-2-β-benzyloxyethyl-1-iodo-ferrocene (**7-(*S*$_p$)**) (1.47 g, 3.30 mmol, 86 %) was prepared according to the same procedure from (*S*$_p$)-2-β-benzyloxyethyl-1-iodo-ferrocene (**6-(*S*$_p$)**) (1.37 g, 3.85 mmol).

(*R*$_p$)-2-β-benzyloxyethyl-1-formyl-ferrocene (**8-(*R*$_p$)**)

(*R*$_p$)-2-β-benzyloxyethyl-1-iodo-ferrocene (**7-(*R*$_p$)**) (1.47 g, 3.28 mmol, 1.0 eq.) was dissolved in dry Et_2O (20 ml) with stirring and cooled to -78 °C in a Schlenk tube under argon. n-BuLi (2.5 M in hexane) (2.30 ml, 5.74 mmol, 1.75 eq.) was added slowly and stirring continued at -78 °C for 0.5 hrs before the reaction mixture was warmed to room temperature over 0.5 hrs. After cooling again to -78 °C, DMF (0.76 ml, 9.84 mmol, 3.0 eq.) was added slowly and stirring continued for 0.5 hrs, the mixture was then allowed to warm to room temperature and stirring continued for 0.5 hrs. The reaction was quenched by the slow addition of water (10 ml), extracted with Et_2O and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product purified by flash column chromatography on silica gel using a gradient eluent system of 0 → 30 % EtOAc in hexane. The solvent was removed in vacuo to give the title compound as a red oil (0.720 g, 2.07 mmol, 63 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.01 (s, 1H (C***H***O)), 7.33 – 7.18 (m, 6H (Ph C***H***)), 4.67 (dd, *J* = 2.7, 1.5 Hz, 1H (Fc C***H***)), 4.53 (dd, *J* = 2.6, 1.5 Hz, 1H (Fc C***H***)), 4.45 (s, 2H, (Ph C–C***H***$_2$)), 4.44 (dd, *J* = 2.7, 2.6 Hz, 1H (Fc C***H***)), 4.14 (s, 5H (cp C***H***)), 3.63 – 3.47 (m, 2H (CH$_2$C***H***$_2$O)), 3.04 (ddd, *J* = 14.5, 6.5, 6.5 Hz, 1H (Fc C–C***H***$_2$)), 2.80 (ddd, *J* = 14.5, 6.8, 6.8 Hz, 1H (Fc C–C***H***$_2$)). ^{13}C NMR (400 MHz, $CDCl_3$) δ 193.82 (***C***HO), 138.39 (Ph ***C***–CH$_2$), 128.41 (Ph ***C***H), 127.65 (Ph ***C***H), 127.60 (Ph ***C***H), 88.37 (Fc ***C***–CH$_2$), 77.13 (Fc ***C***–CHO), 74.72 (Fc ***C***H), 72.96 (Ph C–***C***H$_2$), 71.50 (Fc ***C***H), 70.79 (CH$_2$***C***H$_2$O), 70.21 (cp ***C***H), 70.17 (Fc ***C***H), 28.86 (***C***H$_2$CH$_2$O). ν_{max}/cm^{-1} = 3087 (Fc CH), 3029 (Ph CH), (CH), 2723 (OC–H), 1669 (C=O). HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{20}H_{20}FeO_2Na$ 371.0710; Found 371.0707.

Producing matching ^{1}H and ^{13}C NMR spectra, (*S*$_p$)-2-β-benzyloxyethyl-1-formyl-ferrocene (**8-(*S*$_p$)**) (1.05 g, 3.02 mmol, 91 %) was prepared according to the same procedure from (*S*$_p$)-2-β-benzyloxyethyl-1-iodo-ferrocene (**7-(*S*$_p$)**) (1.47 g, 3.30 mmol).

(*R*$_p$)-2-β-benzyloxyethyl-1-vinyl-ferrocene (**9-(*R*$_p$)**)

Methyltriphenylphosphonium bromide (2.74 g, 7.67 mmol, 3.0 eq.), KO^{t}Bu (0.86 g, 7.67 mmol, 3.0 eq.) and dibenzo-18-crown-6 (0.005 g, 0.013 mmol, 0.005 eq.) were dissolved in dry THF (20 ml) in a Schlenk tube under an atmosphere of argon and stirred for 30 mins at room temperature to produce a bright yellow solution. (*R*$_p$)-2-β-benzyloxyethyl-1-formyl-ferrocene (**8-(*R*$_p$)**) (0.890 g, 2.56 mmol, 1.0 eq.) was dissolved in dry THF (20 ml) and added slowly, the resulting orange solution was stirred at room temperature for 1 hr. The reaction was quenched by the careful addition of water (20 ml), extracted with Et_2O, dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by flash column chromatography on neutralised (Et_3N) silica gel using an eluent of 10 % EtOAc in hexane. The solvent was removed *in vacuo* to give the title compound as an orange oil (0.869 g, 2.51 mmol, 98 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 – 7.22 (m, 5H (Ph C***H***)), 6.58 (dd, *J* = 17.4, 10.9 Hz, 1H (C***H***=CH$_2$)), 5.37 (dd, *J* = 17.4, 1.6 Hz, 1H (C***H***$_2$=CH)), 5.09 (dd, *J* = 10.9, 1.6 Hz, 1H (C***H***$_2$=CH)), 4.53 (s, 2H (Ph C–C***H***$_2$), 4.43 (dd, *J* = 2.6, 1.4 Hz, 1H (Fc C***H***)), 4.20 (dd, *J* = 2.6, 1.4 Hz, 1H (Fc C***H***)), 4.15 (dd, *J* = 2.6 Hz, 1H (Fc C***H***)), 4.03 (s, *J* = 2.7 Hz, 5H (cp C***H***)), 3.58 (m, 2H (CH$_2$C***H***$_2$O)), 2.81 (m, 2H (C***H***$_2$CH$_2$O)). ^{13}C NMR (400 MHz, $CDCl_3$) δ 138.51 (Ph ***C***–C), 133.17 (***C***H=CH$_2$), 128.48 (Ph ***C***H), 127.76 (Ph ***C***H), 127.66 (Ph ***C***H), 111.78 (***C***H$_2$=CH), 84.12 (Fc ***C***–CH$_2$), 82.42 (Fc ***C***–CH), 73.10 (Ph C–***C***H$_2$), 71.05 (CH$_2$***C***H$_2$O), 69.88 (cp ***C***H), 69.78 (Fc ***C***H), 67.20 (Fc ***C***H), 64.78 (Fc ***C***H), 28.84 (***C***H$_2$CH$_2$O). ν_{max}/cm^{-1} = 3085 (Fc CH), 3029 (Ph CH), 1626 (C=C). HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{21}H_{22}FeONa$ 369.0918; Found 369.0905.

Producing matching ^{1}H and ^{13}C NMR spectra, (*S*$_p$)-2-β-benzyloxyethyl-1-vinyl-ferrocene (**9-(*S*$_p$)**) (0.476 g, 1.37 mmol, 91 %) was prepared according to the same procedure from (*S*$_p$)-2-β-benzyloxyethyl-1-formyl-ferrocene (**8-(*S*$_p$)**) (0.526 g, 1.51 mmol).

(*S*$_p$)-1-β-benzyloxyethyl-2-β-hydroxyethyl-ferrocene (**10-(*S*$_p$)**)

(*R*$_p$)-2-β-benzyloxyethyl-1-vinyl-ferrocene (**9-(*R*$_p$)**) (0.869 g, 2.51 mmol, 1.0 eq) was dissolved in dry THF (15 ml) in a Schlenk tube under an atmosphere of argon and cooled to 0 °C in an ice bath. BH_3•THF (1M in THF) (3.76 ml, 3.76 mmol, 1.5 eq.) was added dropwise and stirring continued at 0 °C for 15 mins before the solution was warmed to room temperature and stirring continued for another 1.5 hrs. The solution was cooled to 0 °C before EtOH (6.0 ml) was carefully added and stirring continued for 5 min, followed by the dropwise addition of NaOH (3M in H_2O) (6.0 ml) and a further 10 min of stirring. H_2O_2 (30 % in H_2O) (6.0 ml) was then added slowly and stirring continued for 30 min and the solution

allowed to warm to room temperature. The reaction was extracted with Et_2O, washed with water (2 x 50 ml) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product purified by flash column chromatography through a short column of neutralised (Et_3N) silica gel using an eluent of 10 % EtOAc in hexane. The solvent was removed *in vacuo* to give the title compound as an orange oil (0.802 g, 2.20 mmol, 88 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.24 (m, 5H (Ph C***H***)), 4.49 (s, 2H (Ph C–C***H***$_2$)), 4.14 – 4.07 (m, 2H (Fc C***H***)), 4.06 – 4.03 (m, 1H (Fc C***H***)), 4.02 (s, 5H (cp C***H***)) 3.76 – 3.45 (m, 4H (CH_2C***H***$_2$OCH$_2$, C***H***$_2$OH)), 2.79 – 2.54 (m, 4H (C***H***$_2$CH$_2$OCH$_2$, C***H***$_2$CH$_2$OH)), 2.18 (t, *J* = 5.9 Hz, 1H (O***H***)). ^{13}C NMR (400 MHz, $CDCl_3$) δ 138.23 (Ph ***C***–CH$_2$), 128.51 (Ph ***C***H), 127.86 (Ph ***C***H), 127.77 (Ph ***C***H), 84.71 (Fc ***C***–CH$_2$CH$_2$OCH$_2$), 84.35 (Fc ***C***–CH$_2$CH$_2$OH), 73.13 (Ph C–***C***H$_2$), 71.03 (CH$_2$***C***H$_2$OCH$_2$), 69.35 (cp ***C***H), 68.31 (Fc ***C***H), 68.21 (Fc ***C***H), 66.32 (Fc ***C***H), 63.67 (CH$_2$***C***H$_2$OH), 31.68 (***C***H$_2$CH$_2$OH), 28.68 (***C***H$_2$CH$_2$OCH$_2$). ν_{max}/cm^{-1} = 3381 (OH), 3089 (Fc CH), 3030 (Ph CH). HRMS (ESI-TOF) m/z: [M]$^+$ Calcd for $C_{21}H_{24}FeO$ 364.1126; Found 364.1120.

Producing matching ^{1}H and ^{13}C NMR spectra, (*R*$_p$)-1-β-benzyloxyethyl-2-β-hydroxyethyl-ferrocene (**10-(*R*$_p$)**) (0.413 g, 1.13 mmol, 82 %) was prepared according to the same procedure from (*S*$_p$)-2-β-benzyloxyethyl-1-vinyl-ferrocene (**9-(*S*$_p$)**) (0.476 g, 1.37 mmol).

(*S*$_p$)-2-β-(*N*3-benzoylthyminyl)ethyl-1-β-benzyloxyethyl-ferrocene (**11-(*S*$_p$)**)

Triphenylphosphine (0.262 g, 1.00 mmol, 2.0 eq.) and *N*3-benzoylthymine (0.173 g, 0.75 mmol, 1.5 eq.) were dissolved in dry THF (5 ml) and stirred under an atmosphere of argon in a schlenk tube wrapped in foil. (*S*$_p$)-1-β-benzyloxyethyl-2-β-hydroxyethyl-ferrocene (**10-(*S*$_p$)**) (0.182 g, 0.50 mmol, 1.0 eq.) dissolved in dry THF (5 ml) was added, followed by diethyl azodicarboxylate (0.20 ml, 1.00 mmol, 2.0 eq.) and the reaction mixture stirred at 75 °C for 1 hr. The reaction was quenched with brine (5 ml), extracted with Et_2O and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product purified by flash column chromatography on neutralised (Et_3N) silica gel using a gradient eluent system of 30 → 50 % EtOAc in hexane. The solvent was removed in vacuo to give the title compound as an orange solid (0.213 g, 0.370 mmol, 74 %). m.p. 57 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 8.3, 1.3 Hz, 2H (Bz C***H***)), 7.63 (tt, *J* = 7.5, 1.3 Hz, 1H ((Bz C***H***)), 7.48 (dd, *J* = 8.3, 7.5 Hz, 2H (Bz C***H***)), 7.36 – 7.25 (m, 5H (Bn C***H***)), 6.67 (d, *J* = 1.2 Hz, 1H (C***H***=C)), 4.49 (s, 1H (Bn C***H***$_2$O)), 4.48 (s, 1H (Bn C***H***$_2$O)), 4.15 (dd, *J* = 2.5, 1.3 Hz, 1H (Fc C***H***)), 4.04 (dd, *J* = 2.5, 2.5 Hz, 1H (Fc C***H***)), 4.03 (s, 5H (cp C***H***)), 3.96 (dd, *J* = 2.5, 1.3 Hz, 1H (Fc C***H***)), 3.76 (dd, *J* = 8.0, 6.6 Hz, 2H (C***H***$_2$N)), 3.70 (ddd, *J* = 8.9, 5.6, 5.5 Hz, 1H (CH$_2$C***H***$_2$O)), 3.57 (ddd, *J* = 8.9, 7.1, 7.1 Hz, 1H (CH$_2$C***H***$_2$O)), 2.88 – 2.58 (m, 4H (C***H***$_2$CH$_2$O, C***H***$_2$CH$_2$N)), 1.78 (d, *J* = 1.2 Hz, 3H (C***H***$_3$)). ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.30 (Bz ***C***O), 163.31 (C=C–***C***O–N), 149.70 (N–***C***O–N), 140.95 (***C***H=C), 138.08 (Bn ***C***–CH$_2$), 134.97 (Bz ***C***H), 131.72 (Bz ***C***–CO), 130.45 (Bz ***C***H), 129.16 (Bz ***C***H), 128.47 (Bn ***C***H), 127.95 (Bn ***C***H), 127.82 (Bn ***C***H), 109.62 (***C***=CH), 84.75 (Fc ***C***–CH$_2$CH$_2$O), 82.58 (Fc ***C***–CH$_2$CH$_2$N), 73.25 (Bn C–***C***H$_2$), 71.40 (CH$_2$***C***H$_2$O), 69.32 (cp ***C***H), 68.45 (Fc ***C***H), 68.38 (Fc ***C***H), 66.41 (Fc ***C***H), 49.54 (***C***H$_2$N), 28.62 (***C***H$_2$CH$_2$O), 27.67 (***C***H$_2$CH$_2$N), 12.26 (***C***H$_3$). ν_{max}/cm^{-1} = 3075 (Fc CH), 1744 (C=O), 1696 (C=O), 1646 (C=O), 1599 (C=C). HRMS (ESI-TOF) m/z: [M]$^+$ Calcd for $C_{33}H_{32}FeN_2O_4$ 576.1711; Found 576.1715.

Producing matching ^{1}H and ^{13}C NMR spectra, (*R*$_p$)-2-β-(*N*3-benzoylthyminyl)ethyl-1-β-benzyloxyethyl-ferrocene (**11-(*R*$_p$)**) (0.241 g, 0.417 mmol, 37 %) was prepared according to the same procedure from (*R*$_p$)-1-β-benzyloxyethyl-2-β-hydroxyethyl-ferrocene (**10-(*R*$_p$)**) (0.413 g, 1.13 mmol).

(*S*$_p$)-1-β-hydroxyethyl-2-β-thyminylethyl-ferrocene (**2-(*S*$_p$)**)

(*S*$_p$)-2-β-(*N*3-benzoylthyminyl)ethyl-1-β-benzyloxyethyl-ferrocene (**11-(*R*$_p$)**) (0.213 g, 0.370 mmol) was dissolved in EtOAc (5 ml) and hydrogen gas bubbled through the solution with stirring using a balloon and needle. $Pd(OH)_2$/C (0.30 g) was added and the resulting mixture stirred at room temperature with bubbling and stirring for 1 hr. The mixture was filtered through a short pad of celite, washed with water (2 x 5 ml) and dried over $MgSO_4$. The crude product was dissolved in minimal hot EtOAc and allowed to cool, the title compound precipitated out as orange crystals which were collected by filtration and dried *in vacuo* (0.108 g, 0.283 mmol, 76 %). m.p. 197 °C. $[\alpha]_D^{25}$ = -4 (±3) (c = 0.375 in methanol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.02 (s, 1H, N***H***), 6.93 (d, *J* = 1.1 Hz, 1H (C***H***=C)), 4.13 (dd, *J* = 2.4, 1.3 Hz, 1H (Fc C***H***)), 4.04 (s, 5H, (cp C***H***)), 4.03 (dd, *J* = 2.4, 2.4 Hz, 1H (Fc C***H***), 3.95 (dd, *J* = 2.4, 1.3 Hz, 1H (Fc C***H***)), 3.85 – 3.60 (m, 4H (C***H***$_2$N, C***H***$_2$OH)), 3.20 (t, *J* = 5.3 Hz, 1H (O***H***)), 2.81 (ddd, *J* = 13.6, 9.1, 4.3 Hz, 1H (C***H***$_2$CH$_2$N)), 2.71 – 261 (m, 3H, (C***H***$_2$CH$_2$OH, C***H***$_2$CH$_2$N)), 1.90 (d, *J* = 1.1 Hz, 3H (C***H***$_3$)). ^{13}C NM R (101 MHz, $CDCl_3$) δ 164.60 (C=C–***C***O–N), 151.57 (N–***C***O–N), 140.63 (***C***H=C), 111.02 (***C***=CH), 84.35 (Fc ***C***–CH$_2$CH$_2$OH), 82.47 (Fc ***C***–CH$_2$CH$_2$N), 69.41 (cp ***C***H), 68.85 (Fc ***C***H), 68.33 (Fc ***C***H), 66.65 (Fc ***C***H), 63.69 (***C***H$_2$OH), 50.09 (***C***H$_2$N), 31.67 (***C***H$_2$CH$_2$OH), 28.20 (***C***H$_2$CH$_2$N), 12.44 (***C***H$_3$). ν_{max}/cm^{-1} = 3477 (OH), 3149 (NH), 3019 (NH), 1735.51 (C=O), 1664.05 (C=C). HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{19}H_{22}FeN_2O_3Na$ 405.0878; Found 405.0874. Anal. Calcd for $C_{19}H_{22}FeN_2O_3$: C, 59.70; H, 5.80; N, 7.33. Anal. Found C, 59.67; H, 5.75; N, 7.52.

(*R*$_p$)-1-β-hydroxyethyl-2-β-thyminylethyl-ferrocene (**2-(*R*$_p$)**)

(*R*$_p$)-2-β-(*N*3-benzoylthyminyl)ethyl-1-β-benzyloxyethyl-ferrocene (**11-(*R*$_p$)**) (0.240 g, 0.417 mmol) was dissolved in EtOAc (5 ml) and hydrogen gas bubbled through the solution using a balloon and needle with stirring. $Pd(OH)_2$/C (0.30 g) was added and the resulting mixture stirred at room temperature with gentle bubbling and stirring for 30 mins. The mixture was filtered through a short pad of celite, washed with water (2 x 5 ml) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the alcohol purified by flash column chromatography on silica gel using an eluent of EtOAc. ^{1}H and ^{13}C NMR confirmed the successful removal of the benzyl group. The residue was then dissolved in NH_3 (7N in MeOH) and stirred at room temperature for 1 hr before the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography on silica gel using an eluent of EtOAc. Recrystalisation from hot EtOAc produced orange crystals which were collected by filtration and dried *in vacuo* to give the title compound (0.111 g, 0.290 mmol, 70 %). $[\alpha]_D^{25}$ = +3 (±3) (c = 0.345 in methanol). ^{1}H and ^{13}C NMR spectra match (*S*$_p$)-1-β-hydroxyethyl-2-β-thyminylethyl-ferrocene (**2-(*S*$_p$)**). Anal. Calcd for $C_{19}H_{22}FeN_2O_3$: C, 59.70; H, 5.80; N, 7.33. Found: C, 59.81; H, 5.99; N, 7.14.

(*R*$_p$)-2-β-benzyloxyethyl-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)-ferrocene (**12-(*R*$_p$)**)

(*R*$_p$)-2-β-benzyloxyethyl-1-formyl-ferrocene (**8-(*R*$_p$)**) (0.720 g, 2.07 mmol, 1.0 eq.) was dissolved in dry DCM (20 ml) in a Schlenk tube under an atmosphere of argon and 4 Å molecular sieves (ca. 0.1 g) were added with stirring. 3,3-dimethylpentane-1,5-diol (0.431 g, 4.14 mmol, 2.0 eq.) was added, followed by PTSA (0.040 g, 0.21 mmol, 0.10 eq.) and the reaction mixture stirred under argon for 4 hrs. The reaction was quenched by the addition of anhydrous K_2CO_3 (0.25 g) and stirring continued for 10 minutes. The mixture was filtered through a small pad of celite, dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by flash column chromatography on neutralised (Et_3N) silica gel using an eluent of 10 % EtOAc in hexane. The solvent was removed *in vacuo* to give the title compound as an orange oil (0.772 g, 1.78 mmol, 86 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.23 (m, 5H Ph C***H***)), 5.27 (s, 1H (C***H***OO)), 4.51 (2 x s, 2H (Ph C–C***H***$_2$)), 4.31 (dd, *J* = 2.5, 1.5 Hz, 1H (Fc C***H***)), 4.09 (s, 6H (cp C***H***, Fc C***H***)), 4.03 (dd, *J* = 2.5

Hz, 2.4 Hz 1H (Fc C***H***)), 3.77 – 3.44 (m, 6H ($CH_2C\boldsymbol{H}_2O$, $OC\boldsymbol{H}_2C(CH_3)_2$)), 2.85 (ddd, *J* = 14.0, 9.1, 5.7 Hz, 1H (Fc C–C$\boldsymbol{H}_2$)), 2.71 (ddd, *J* = 14.0, 9.4, 6.2 Hz, 1H (Fc C–C$\boldsymbol{H}_2$)), 1.23 (s, 3H ($C\boldsymbol{H}_3$)), 0.76 (s, 3H ($C\boldsymbol{H}_3$)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.71 (Ph ***C***–CH_2), 128.47 (Ph ***C***H), 127.86 (Ph ***C***H), 127.63 (Ph ***C***H), 100.68 (***C***HOO), 84.29 (Fc ***C***–CHOO), 83.30 (Fc ***C***–CH_2), 77.88 ($O\boldsymbol{C}H_2C(CH_3)_2$), 77.78 ($O\boldsymbol{C}H_2C(CH_3)_2$), 73.06 (Ph C–***C***$H_2$), 71.17 ($CH_2\boldsymbol{C}H_2O$), 69.53 (Fc ***C***H), 69.50 (cp ***C***H), 66.76 (Fc ***C***H), 66.52 (Fc ***C***H), 30.22 ($\boldsymbol{C}(CH3)_2$), 28.88 ($\boldsymbol{C}H_2CH_2O$), 23.24 ($\boldsymbol{C}H_3$), 22.05 ($\boldsymbol{C}H_3$). ν_{max}/cm^{-1} = 3090 (Fc CH), 3031 (Ph CH). HRMS (ESI-TOF) m/z: $[M]^+$ Calcd for $C_{25}H_{30}FeO_3$ 434.1544; Found 434.1545.

Producing matching ^{1}H and ^{13}C NMR spectra, (S_p)-2-β-benzyloxyethyl-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)-ferrocene (**12-(S_p)**) (0.586 g, 1.35 mmol, 89 %) was prepared according to the same procedure from (S_p)-2-β-benzyloxyethyl-1-formyl-ferrocene (**8-(S_p)**) (0.526 g, 1.51 g).

(R_p)-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)-2-β-hydroxyethyl-ferrocene (**13-(R_p)**)

(R_p)-2-β-benzyloxyethyl-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)-ferrocene (**12-(R_p)**) (0.538 g, 1.24 mmol) was dissolved in dry DMF (10 ml) in a round bottomed flask fitted with a rubber septum. A balloon of hydrogen was fitted with a long needle and the gas bubbled through the solution; a second needle through the septum allowed the pressure to equalise. $Pd(OH)_2$/C (0.30 g) was added and the solution carefully stirred with continued bubbling of hydrogen gas. The reaction was carefully monitored by TLC until, after 20 minutes all starting material was consumed. The hydrogen balloon was removed and the reaction mixture filtered through a small pad of celite. Brine (20 ml) was added, the crude product extracted with Et_2O and dried over $MgSO_4$. The crude product was then purified by flash column chromatography on neutralised (Et_3N) silica gel using an eluent of 30 % EtOAc in hexane. The solvent was removed in vacuo to give the title compound as an orange oil (0.305 g, 0.885 mmol, 71 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (s, 1H (C***H***)), 4.37 (dd, *J* = 2.0, 2.0 Hz, 1H (Fc C***H***)), 4.11 (s, 5H (cp C***H***)), 4.10 – 4.07 (m, 2H (Fc C***H***)), 3.80 – 3.69 (m, 1H ($C\boldsymbol{H}_2$OH)), 3.75 (dd, *J* = 11.1, 2.5 Hz, 1H ($C\boldsymbol{H}_2$OCH)), 3.67 (dd, *J* = 11.0, 2.6 Hz, 1H ($C\boldsymbol{H}_2$OCH)), 3.61 (dd, *J* = 11.1, 0.9 Hz, 1H ($C\boldsymbol{H}_2$OCH)), 3.60 (dd, *J* = 11.0, 0.9 Hz, 1H ($C\boldsymbol{H}_2$OCH)) 3.59 – 3.51 (m, 1H ($C\boldsymbol{H}_2$OH)), 2.76 (ddd, *J* = 14.5, 8.7, 4.7 Hz, 1H ($C\boldsymbol{H}_2CH_2OH$)), 2.75 (s, 1H (O***H***)) 2.63 (ddd, *J* = 14.5, 5.5, 4.2 Hz, 1H ($C\boldsymbol{H}_2CH_2OH$)), 1.23 (s, 3H ($C\boldsymbol{H}_3$), 0.77 (s, 3H ($C\boldsymbol{H}_3$)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 100.55 (***C***H), 84.43 (Fc ***C***–CH), 84.06 (Fc ***C***–CH_2), 77.90 ($\boldsymbol{C}H_2$OCH), 77.71 ($\boldsymbol{C}H_2$OCH), 69.72 (Fc ***C***H), 69.57 (cp ***C***H), 67.08 (Fc ***C***H), 66.91 (Fc ***C***H), 64.14 ($\boldsymbol{C}H_2$OH), 32.11 ($\boldsymbol{C}H_2CH_2OH$), 30.20 ($\boldsymbol{C}(CH_3)_2$), 23.18 ($\boldsymbol{C}H_3$), 21.97 ($\boldsymbol{C}H_3$). ν_{max}/cm^{-1} = 3420 (OH), 3095 (Fc CH). HRMS (ESI-TOF) m/z: $[M]^+$ Calcd for $C_{18}H_{24}FeO_3$ 344.1075; Found 344.1084.

Producing matching ^{1}H and ^{13}C NMR spectra, (S_p)-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)-2-β-hydroxyethyl-ferrocene (**13-(S_p)**) (0.367 g, 1.066 mmol, 79 %) was prepared according to the same procedure from (S_p)-2-β-benzyloxyethyl-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)-ferrocene (**12-(S_p)**) (0.583 g, 1.34 mmol).

(R_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)ferrocene (**14-(R_p)**)

Triphenylphosphine (0.596 g, 2.27 mmol, 1.5 eq.) and *N*3-benzoylthymine (0.454 g, 1.97 mmol, 1.3 eq.) were dissolved in dry THF (10 ml) and stirred under an atmosphere of argon in a schlenk tube wrapped in foil. (R_p)-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)-2-β-hydroxyethyl-ferrocene (**13-(R_p)**) (0.522 g, 1.52 mmol, 1.0 eq.) dissolved in dry THF (10 ml) was added, followed by diethyl azodicarboxylate (0.45 ml, 2.27 mmol, 1.5 eq.) and the reaction mixture stirred at 75 °C for 2 hrs. The reaction was quenched with brine (10 ml), extracted with Et_2O and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product purified by flash column chromatography on neutralised (Et_3N) silica gel using a gradient eluent system of 10 → 40 % EtOAc in hexane. The solvent was removed in vacuo to give the title compound as an yellow solid (0.221 g, 0.398 mmol, 65 %). m.p. 87 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 8.1, 1.2 Hz, 2H (Ph C***H***)), 7.63 (tt, *J* = 7.4, 1.2 Hz, 1H (Ph C***H***)), 7.48 (dd, *J* = 8.1, 7.4 Hz, 2H (Ph C***H***)), 6.78 (d, *J* = 1.0 Hz, 1H (C***H***=C)), 5.30 (s, 1H (C***H***OO)), 4.34 (dd, *J* = 2.4, 1.5 Hz, 1H (Fc C***H***)), 4.11 (s, 5H (cp C***H***)), 4.06 (dd, *J* = 2.4, 2.2 Hz, 1H (Fc C***H***)), 4.05 – 3.96 (m, 1H ($C\boldsymbol{H}_2$N)), 3.95 (dd, *J* = 2.2, 1.5 Hz, 1H (Fc C***H***)), 3.76 – 3.67 (m, 3H ($C\boldsymbol{H}_2$N, $C\boldsymbol{H}_2$O)), 3.61 (d, *J* = 10.7 Hz, 2H ($C\boldsymbol{H}_2$O), 2.94 (ddd, *J* = 14.0, 6.8, 4.4 Hz, 1H ($OC\boldsymbol{H}_2C(CH_3)_2$)), 2.77 (ddd, *J* = 14.0, 8.4, 7.6 Hz, 1H ($OC\boldsymbol{H}_2C(CH_3)_2$)), 1.79 (d, *J* = 1.0 Hz, 3H ($C\boldsymbol{H}_3$C=CH)), 1.24 (s, 3H ($C\boldsymbol{H}_3CCH_3$)), 0.78 (s, 3H ($CH_3C\boldsymbol{H}_3$)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.36 (Bz ***C***O), 163.46 (C–***C***O–N), 149.77 (N–***C***O–N), 141.51 (***C***H=C), 134.94 (Ph ***C***H), 131.83 (Ph ***C***–CO), 130.49 (Ph ***C***H), 129.16 (Ph ***C***H), 109.42 (***C***=CH), 100.95 (***C***HOO), 84.06 (Fc ***C***–CH), 81.97 (Fc ***C***–CH_2), 77.91 ($\boldsymbol{C}H_2$O), 77.79 ($\boldsymbol{C}H_2$O), 70.01 (Fc ***C***H), 69.63 (cp ***C***H), 67.83 (Fc ***C***H), 66.87 (Fc ***C***H), 50.06 ($\boldsymbol{C}H_2$N), 30.16 ($\boldsymbol{C}(CH_3)_2$), 27.92 ($\boldsymbol{C}H_2CH_2$N), 23.32 ($\boldsymbol{C}H_3CCH_3$), 21.97 ($\boldsymbol{C}H_3CCH_3$), 12.21 ($\boldsymbol{C}H_3$C=CH). ν_{max}/cm^{-1} = 3088 (Fc CH), 1745 (C=O), 1697 (C=O), 1647 (C=O), 1599 (C=C). HRMS (ESI-TOF) m/z: $[M]^+$ Calcd for $C_{30}H_{32}FeN_2O_5$ 556.1661; Found 556.1669.

Producing matching ^{1}H and ^{13}C NMR spectra, (S_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)ferrocene (**14-(S_p)**) (0.456 g, 0.819 mmol, 77 %) was prepared according to the same procedure from (S_p)-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)-2-β-hydroxyethyl-ferrocene (**13-(S_p)**) (0.367 g, 1.07 mmol).

(R_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-formyl-ferrocene (**15-(R_p)**)

(R_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)ferrocene (**14-(R_p)**) (0.329 g, 0.591 mmol, 1.0 eq.) was dissolved in a mixture of THF (8 ml) and water (2ml) to produce a yellow solution. PTSA (0.056 g, 0.296 mmol, 0.5 eq.) was added and the resulting mixture stirred at room temperature for 45 min to produce a red solution. The reaction was quenched by the slow addition of sat. $NaHCO_3$ solution, extracted with EtO_2, dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by flash column chromatography using an eluent of 50 % EtOAc in hexane and the solvent was removed in vacuo to give the title compound as an red solid (0.277 g, 0.589 mmol, 100 %). m.p. 69 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.01 (s, 1H (C***H***O)), 7.94 (dd, *J* = 8.4, 1.3 Hz, 2H (Ph C***H***)), 7.64 (tt, *J* = 7.4, 1.3 Hz, 1H (Ph C***H***)), 7.50 (dd, *J* = 8.4, 1.3 Hz, 2H (Ph C***H***)), 7.02 (d, *J* = 1.2 Hz, 1H (C***H***=C)), 4.70 (dd, *J* = 2.7, 1.4 Hz, 1H (Fc C***H***)), 4.53 (dd, *J* = 2.6, 1.5 Hz, 1H (Fc C***H***)), 4.50 (dd, *J* = 2.6 Hz, 1H (Fc C***H***)), 4.24 (s, *J* = 4.0 Hz, 5H (cp C***H***)), 3.98 (ddd, *J* = 13.4, 8.4, 7.0 Hz, 1H ($C\boldsymbol{H}_2$N)), 3.69 (ddd, *J* = 13.6, 8.7, 5.1 Hz, 1H ($C\boldsymbol{H}_2$N)), 3.21 (ddd, *J* = 13.5, 8.4, 5.1 Hz, 1H ($C\boldsymbol{H}_2$)), 2.93 (ddd, *J* = 13.6, 8.5, 7.1 Hz, 1H ($C\boldsymbol{H}_2$)), 1.89 (d, *J* = 1.0 Hz, 3H ($C\boldsymbol{H}_3$)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.49 (***C***HO), 169.34 (Bz ***C***O), 163.41 (C–***C***O–N), 149.89 (N–***C***O–N), 140.82 (***C***H=C), 135.04 (Ph ***C***H), 131.84 (Ph ***C***–CO), 130.62 (Ph ***C***H), 129.22 (Ph ***C***H), 110.36 (***C***=CH), 85.16 (Fc ***C***–CH_2), 75.47 (Fc ***C***H), 73.03 (Fc ***C***H), 72.03 (Fc ***C***H), 70.49 (cp ***C***H), 50.01 ($\boldsymbol{C}H_2$N), 29.80 (Fc ***C***–CO), 28.58 ($\boldsymbol{C}H_2CH_2$N), 12.37 ($\boldsymbol{C}H_3$). ν_{max}/cm^{-1} = 3068 (Ph CH), 1741 (C=O), 1694 (C=O), 1641 (C=O), 1598 (C=C). HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{25}H_{22}FeN_2O_4Na$ 493.0827; Found 493.0825.

Producing matching ^{1}H and ^{13}C NMR spectra, (S_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-formyl-ferrocene (**15-(S_p)**) (0.333 g, 0.707 mmol, 86 %) was prepared according to the same procedure from (S_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-formyl-(3,3-dimethyl-1,5-pentanediol-acetal)ferrocene (**14-(S_p)**) (0.454 g, 0.819 mmol).

(R_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-hydroxymethyl-ferrocene (**16-(R_p)**)

(R_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-formyl-ferrocene (**15-(R_p)**) (0.055 g, 0.117 mmol, 1.0 eq.) was dissolved in MeOH (5 ml) and cooled to 0 °C with stirring. $NaBH_4$ (0.008 g, 0.233 mmol, 2.0 eq.) was added to the red solution and stirring continued for 15 min at 0 °C. The resulting yellow solution was quenched with brine, extracted with DCM and dried over $MgSO_4$. The crude product was purified by flash column chromatography using an eluent of 40 % hexane in EtOAc, the solvent was removed in vacuo to give the title compound as a yellow oil (0.053 g, 0.113 mmol, 96 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 8.2, 1.3 Hz, 2H (Ph C***H***)), 7.71 – 7.58 (m, *J* = 7.5, 1.3 Hz, 1H (Ph C***H***)), 7.48 (dd, *J* = 8.2, 7.5 Hz, 2H (Ph C***H***)), 6.93 (d, *J* = 1.2 Hz, 1H (C***H***=C)), 4.47 (d, *J* = 11.9 Hz, 1H (C***H***$_2$OH)), 4.31 (d, *J* = 11.9 Hz, 1H (C***H***$_2$OH)), 4.19 (s, 1H (Fc C***H***)), 4.11 (t, *J* = 2.1 Hz, 1H (Fc C***H***)), 4.08 (s, 5H (cp C***H***)), 4.04 (s, 1H (Fc C***H***)), 3.91 (ddd, *J* = 13.7, 8.5, 7.4 Hz, 1H (CH$_2$C***H***$_2$N)), 3.76 (ddd, *J* = 13.6, 7.8, 4.4 Hz, 1H (CH$_2$C***H***$_2$N)), 2.85 (ddd, *J* = 14.2, 7.3, 4.4 Hz, 1H (C***H***$_2$CH$_2$N)), 2.74 (dt, J = 14.2, 8.2 Hz, 1H (C***H***$_2$CH$_2$N)), 2.40 (s, 1H (O***H***)), 1.86 (d, *J* = 1.2 Hz, 3H (C***H***$_3$)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.27 (Bz ***C***O), 163.34 (C–***C***O–N), 150.06 (N–***C***O–N), 140.69 (***C***H=C), 135.09 (Ph ***C***H), 131.67(Ph ***C***–CO), 130.62 (Ph ***C***H), 129.20 (Ph ***C***H), 110.43 (***C***=CH), 85.57 (Fc ***C***–CH$_2$CH$_2$), 83.04 (Fc ***C***–CH$_2$OH), 70.04 (Fc ***C***H), 69.93 (Fc ***C***H), 69.22 (cp ***C***H), 67.19 (Fc ***C***H), 59.45 (***C***H$_2$OH), 49.90 (***C***H$_2$N), 27.85 (***C***H$_2$CH$_2$N), 12.33 (***C***H$_3$). ν_{max}/cm^{-1} = 3474 (OH), 3072 (Ph CH), 1743 (C=O), 1693 (C=O), 1639 (C=O), 1599 (C=C). HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{25}H_{24}FeN_2O_4Na$ 495.0983; Found 495.0972.

Producing matching ^{1}H and ^{13}C NMR spectra, (S_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-hydroxymethyl-ferrocene (**16-(S_p)**) (0.329 g, 0.697 mmol, 99 %) was prepared according to the same procedure from (S_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-formyl-ferrocene (**15-(S_p)**) (0.333 g, 0.707 mmol).

(R_p)-1-hydroxymethyl-2-β-thyminylethyl-ferrocene (**3-(R_p)**)

(R_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-hydroxymethyl-ferrocene (**16-(R_p)**) (0.0327 g, 0.069 mmol) was dissolved in MeOH (2 ml) before excess $MeNH_2$ (30 % in MeOH) (0.2 ml) was added and the resulting mixture was stirred at room temperature for 5 min. Brine (5 ml) was added, the crude product extracted with DCM, dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by flash column chromatography on silica gel using an eluent of 20 % hexane in EtOAc. Recrystallisation by the slow evaporation of a concentrated solution in chloroform produced orange crystals and the solvent was removed *in vacuo* to give an orange solid. Recrystallisation from a concentrated solution in chloroform produced single orange crystals of the title compound (0.025 g, 0.067 mmol, 93 %). Decomposes above 159 °C. $[\alpha]_D^{25}$ = -9 (±3) (c = 0.25 in methanol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H (N***H***)), 6.82 (d, *J* = 1.2 Hz, 1H (C***H***=C)), 4.52 (d, *J* = 12.0 Hz, 1H (C***H***$_2$OH)), 4.37 (d, *J* = 12.0 Hz, 1H (C***H***$_2$OH)), 4.20 (dd, *J* = 2.5, 1.4 Hz, 1H (Fc C***H***)), 4.17 – 4.10 (m, 1H (Fc C***H***)), 4.08 (s, 5H (cp C***H***)) 4.00 (dd, *J* = 2.3, 1.4 Hz, 1H (Fc C***H***)), 3.81 (dd, *J* = 8.3, 1.3 Hz, 1H (C***H***$_2$N)), 3.79 (d, *J* = 8.3 Hz, 1H (C***H***$_2$N)), 2.89 – 2.66 (m, 2H (C***H***$_2$CH$_2$N)), 2.17 (s, 1H (O***H***)), 1.85 (d, *J* = 1.2 Hz, 3H (C***H***$_3$)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.12 (C–***C***O–N), 150.99 (N–***C***O–N), 140.89 (***C***H=C), 110.47 (***C***=CH), 85.65 (Fc ***C***–CH$_2$CH$_2$), 83.11 (Fc ***C***–CH$_2$OH), 70.01 (Fc ***C***H), 69.73 (Fc ***C***H), 69.11 (cp ***C***H), 67.14 (Fc ***C***H), 59.56 (***C***H$_2$OH), 49.97 (***C***H$_2$N), 28.05 (***C***H$_2$CH$_2$N), 12.32 (***C***H$_3$). ν_{max}/cm^{-1} = 3411 (OH), 1658 (C=O), 1627 (C=C). HRMS (ESI-TOF) m/z: [M]$^+$ Calcd for $C_{18}H_{20}FeN_2O_3$ 368.0823; Found 368.0811. Anal. Calcd for $C_{18}H_{20}FeN_2O_3$: C, 58.72; H, 5.48; N, 7.61. Found: C, 58.91; H, 5.45; N, 7.74.

Producing matching ^{1}H and ^{13}C NMR spectra, (S_p)-1-hydroxymethyl-2-β-thyminylethyl-ferrocene (**3-(S_p)**) (0.245 g, 0.665 mmol, 95 %) was prepared according to the same procedure from (S_p)-2-β-(*N*3-benzoylthyminyl)ethyl-1-hydroxymethyl-ferrocene (**16-(S_p)**) (0.329 g, 0.697 mmol). $[\alpha]_D^{25}$ = +3 (±3) (c = 0.28 in methanol). Anal. Calcd for $C_{18}H_{20}FeN_2O_3$: C, 58.72; H, 5.48; N, 7.61Anal. Found C, 58.80; H, 5.55; N, 7.47.

Electrochemistry

Electrochemical measurements were performed in dry and de-oxygenated acetonitrile solutions. The base electrolyte was 0.1 M tetrabutylammonium hexafluorophosphate ($TBAPF_6$, ≥ 99%, Fluka). Solutions of compounds **1-(R_p)**, **2-(R_p)**, and **3-(R_p)** were made in this electrolyte at concentrations of 1.0 mM and 1 mM decamethylferrocene (dmfc) was added to serve as an internal reference. (The measured $E_{1/2}$ of dmfc *vs* ferrocene in this electrolyte was −0.509 V, in good agreement with the literature.[10] The measurements were performed on a BioAnalytical Systems Inc. (West Lafayette, IN) EC epsilon potentiostat using a C3 cell stand and a traditional 3-electrode set-up. Measurements were carried out at room temperature. All electrodes were purchased from IJ Cambria (Llanelli, Wales). The working electrode was a glassy carbon electrode of 3 mm diameter, the counter electrode was a platinum wire and the reference electrode was a Ag|AgCl|3 M KCl electrode (BASi), housed in a compartment connected to the cell *via* a frit.

Prior to use, all glassware was cleaned by immersing in a 1:1 mixture of ammonia (35%) and hydrogen peroxide (30%) for several hours and then rinsed with copious quantities of ultrapure water (purified with a Millipore tandem Elix-A10 system, resistivity > 18 MΩ cm, TOC < 5 ppb). The glassware was soaked overnight in ultrapure water and dried carefully before use. The counter electrode was prepared by flame annealing and the reference electrode was rinsed with acetonitrile before each use. The working electrode was prepared by polishing in successively finer grades of alumina slurry (1.0 μm, 0.3 μm, 0.05 μm), rinsed with ultrapure water and then acetonitrile. It was carefully dried under a stream of argon and placed in the electrochemical cell containing the test solution. Molecular sieves were used to maintain dryness of the solvent and an argon blanket was maintained in the cell during the measurements.

Biological Studies

Cell culture: HOS osteosarcoma cells (87070202) were obtained from the European General Cell culture collection and routinely cultured in T75 cell culture flasks using DMEM supplemented with 10% v/v foetal calf serum, 2 mM glutamine, 100U/ml penicillin and 100μg/ml streptomycin. Cells were passaged twice weekly using a trypsin-EDTA solution and reseeded in a fresh T75 flask at an approximate dilution of 1:3.

Crystal violet assay: Cells were seeded in a 96-well plate at a density of 6250 cells per well in 100 μL of media and left to attach overnight. The next day the media was removed and replaced with fresh media containing the appropriate concentration of test compound prepared from a 10 mM stock solution of test compound dissolved in DMSO (storage of **1-(S,R_p)** in this solvent over a period of 12 months at 4 °C gave no evidence of degradation, as monitored by 1-H NMR). All compounds were fully soluble in PBS buffer at the concentrations used; the final concentration of DMSO in all wells was 1% v/v. After 4 days incubation the media was removed and wells washed with 100μl of phosphate buffered saline solution (PBS). Next, 4% paraformaldehyde (100 μL per well) was added and the plates incubated at room temperature for 15 minutes to allow fixation of the cells. The paraformaldehyde was removed and crystal violet (100 μl of 0.5% w/v, dissolved in 10% ethanol, 90% distilled water) added and plates left at room temperature for 20

minutes to allow staining of the cells. The crystal violet solution was removed and wells were washed three times with 100 µl of distilled water and allowed to air dry for approximately 20 minutes. Once the cells were dry, 100 µl of 10% v/v acetic acid was added to each well and the plates were left at room temperature for 20 minutes to allow the stain to dissolve. Absorbance was read at 590 nm wavelength against a blank of acetic acid solution alone in a plate reader.

X-Ray Crystallography

Table 2. Selected crystal data for **2-(R_p)**, **2-(S_p)**, **3-(R_p)** and **3-(S_p)**

	2-(R_p)	**2-(S_p)**	**3-(R_p)**	**3-(S_p)**
Empirical Formula	$C_{19}H_{22}FeN_2O_3$	$C_{19}H_{22}FeN_2O_3$	$C_{18}H_{20}FeN_2O_3$	$C_{18}H_{20}FeN_2O_3$
Formula Weight	382.23	382.23	368.21	368.21
Temperature (K)	100.01(10)	100.0(3)	99.98(12)	100.00(10)
Crystal System	Tetragonal	Orthorhombic	Orthorhombic	Orthorhombic
Space Group	$P4_32_12$ (no. 96)	$P2_12_12_1$ (no. 19)	$P2_12_12_1$ (no. 19)	$P2_12_12_1$ (no. 19)
a ; *b* ; *c* (Å)	11.96948(10) ; 11.96948(10) ; 24.1571(3)	11.8672(3) ; 12.1205(3) ; 24.1747(5)	11.3617(3) ; 11.8847(3) ; 23.7138(6)	11.3958(6) ; 11.8730(6) ; 23.7314(10)
V (Å^3)	3460.95(7)	3477.19(13)	3202.07(14)	3210.9(3)
Z ; Z'	8 ; 1	8 ; 2	8 ; 2	8 ; 2
ρ_{calc} (g/cm^3)	1.467	1.460	1.528	1.523
µ(MoKα) (mm^{-1})	0.892	0.888	0.961	0.958
λ (Å)	0.71073	0.71073	0.71073	0.71073
F(000)	1600.0	1600.0	1536.0	1536.0
2θ Range for Data Collection (°)	4.792 – 58.954	4.76 – 52.74	6.856 – 54.962	4.954 – 56.56
Index Ranges	-16 ≤ h ≤ 15, -15 ≤ k ≤ 16, -31 ≤ l ≤ 33	-14 ≤ h ≤ 14, -15 ≤ k ≤ 15, -30 ≤ l ≤ 29	-14 ≤ h ≤ 14, -14 ≤ k ≤ 15, -30 ≤ l ≤ 30	-14 ≤ h ≤ 10, -14 ≤ k ≤ 15, -29 ≤ l ≤ 31
Reflections Collected	71538	35875	34740	24084
Independent Reflections	4700 [R_{int} = 0.0401]	7092 [R_{int} = 0.0418]	7237 [R_{int} = 0.0381]	7488 [R_{int} = 0.0481]
Goodness-of-Fit on F^2	1.085	1.053	1.097	1.132
Final *R* Indices [I>=2σ (I)]	R_1 = 0.0410, wR_2 = 0.0948	R_1 = 0.0571, wR_2 = 0.1389	R_1 = 0.0318, wR_2 = 0.0743	R_1 = 0.0495, wR_2 = 0.1091
Final *R* Indices (All Data)	R_1 = 0.0542, wR_2 = 0.1030	R_1 = 0.0632, wR_2 = 0.1442	R_1 = 0.0332, wR_2 = 0.0751	R_1 = 0.543, wR_2 = 0.1112
Largest Diff. Peak ; Hole (e Å^{-3})	0.39; -0.40	0.73 ; -0.69	1.11 ; -0.28	1.03 ; -0.40
Flack Parameter	-0.003(5)	0.018(6)	0.003(5)	0.018(7)
CCDC	1492565	1492566	1492567	1492568

The datasets for **2-(R_p)**, **2-(S_p)**, **3-(R_p)** and **3-(S_p)** were measured on an Agilent SuperNova diffractometer using an Atlas detector. The data collections were driven and processed and numerical absorption corrections based on gaussian integration over a multifaceted crystal model were applied using CrysAlisPro.[11] The structures were solved using ShelXS [12] and refined by a full-matrix least-squares procedure on F^2 in ShelXL.[12] Figures and reports were produced using OLEX2.[13] The structures of **2-(S_p)**, **3-(R_p)** and **3-(S_p)** all contain two crystallographically-independent molecules. All non-hydrogen atoms in all four structures were refined with anisotropic displacement parameters. In the structures of **2-(R_p)**, **3-(R_p)** and **3-(S_p)** the hydrogen atoms bonded to nitrogen and oxygen atoms were located in the electron density and the positions refined, with the remaining hydrogen atoms being fixed as riding models. The U_{iso} of all hydrogen atoms were based on the U_{eq} of the parent atoms. In **2-(S_p)** the hydrogen atoms bonded to N(3) and N(103) were located in the electron density and the positions and thermal parameters refined while the remaining hydrogen atoms were fixed as riding models with the U_{iso} of the hydrogen atoms being based on the U_{eq} of the parent atoms. **2-(R_p)**: The cyclopentadienyl ring C(15)-C(19) / C(15')-C(19') is disordered over two positions with the refined occupancy ratio being 0.591(19):0.409(19). The group C(20), C(21), O(21) / C(20'), C(21'), O(21') is disordered over two positions with the occupancy ratio fixed due to symmetry constraints at 0.5:0.5. **2-(S_p)**: The cyclopentadienyl ring C(15)-C(19) / C(15')-C(19') is disordered over two positions with the refined occupancy ratio being 0.58(3):0.42(3). The group C(20), C(21), O(21) / C(0'), C(1'), O(1') is disordered over two positions with the refined occupancy ratio being 0.581(14):0.419(14). The group C(120), C(121), O(121) / C(20'), C(21'), O(21') is disordered over two positions with the refined occupancy ratio being 0.525(11):0.475(11). **3-(S_p)**: The group C(120)-O(120)/C(20')-O(20') is disordered over two positions with the refined occupancy ratio being 0.729(11):0.271(11).

CCDC-1492565 – CCDC-1492568 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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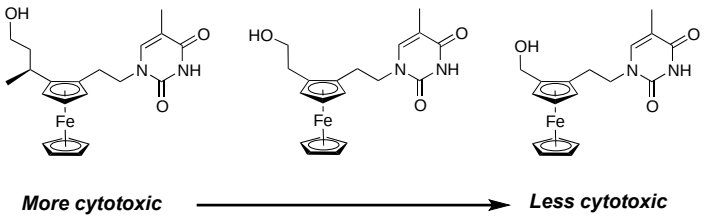

Bioorganometallc Chemistry*

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Organometallic Nucleoside Analogues: Effect Hydroxylalkyl Linker Length on Cancer Cell Line Toxicity

Text for Table of Contents:

Chiral ferrocene derivatives show distinct structure-activity relationships in their cancer cell line activities.