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Facile Synthesis and Detailed Characterization of a New Ferrocenyl Uracil Peptide Nucleic Acid Monomer

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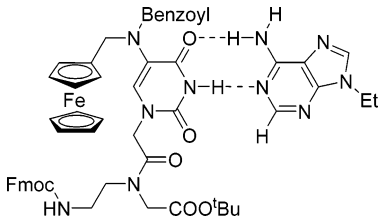

A new ferrocenyl uracil peptide nucleic acid (PNA) monomer, *tert*-butyl-2-(*N*-(2-(((9*H*-floren-9-yl)-methoxy)carbonylamino)ethyl)-2-(5-(*N*-ferrocenylmethylbenzamido)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)acetate (**1**), has been successfully prepared in good yield by a procedure involving the one-pot reaction of the key synthon, 5-(ferrocenylmethylamino)pyrimidine-2,4(1*H*,3*H*)-dione (**4**), itself prepared from the reaction of (ferrocenylmethyl)trimethylammonium iodide and 5-aminouracil, with benzoyl chloride followed by ethyl bromoacetate. After hydrolysis of the ester, the acid was coupled with a protected PNA backbone to generate **1**. NMR spectroscopy showed that **1** hydrogen bonds 9-ethyladenine (EA) in a 1:1 mixture of CD_3CN:$CDCl_3$ with an association constant K_a of 70 M^{-1} at 30 °C. This value is comparable with those observed for model receptors and shows that the ferrocenyl moiety of **1** does not hinder the hydrogen bonding of our new PNA monomer to the complementary DNA base or if it does, not significantly. **1** is oxidized to $\mathbf{1}^+$ with a reversible potential of +538 mV vs the $DMFc^{0/+}$ (decamethylferrocene) couple under voltammetric conditions in a 1:1 mixture of CH_3CN:$CHCl_3$ (0.1 M Bu_4NPF_6). For this reversible process, a slightly larger diffusion coefficient of 4.2×10^{-6} $cm^2 \cdot s^{-1}$ than usually found for these compounds was determined from these electrochemical studies, which should be analytically useful as it will readily afford submicromolar voltammetric detection limits.

Introduction

Since their discovery by Nielsen et al.,[1] there has been considerable interest in the synthesis and application of peptide nucleic acids (PNAs). These oligonucleotide analogues are composed of nucleobase derivatives attached to a polyamide backbone and exhibit favorable properties which include high binding affinity for DNA/RNA strands, high chemical stability, and resistance to nucleases,[2] making them useful for antisense and antigene therapies.[3] A new rapidly developing field of research is their use as biosensors, where their higher specificity, greater discrimination of single-base mismatches, and faster hybridization is particularly attractive.[4] Among the different redox-active supramolecular receptors, the ferrocene group has been the most commonly used because of its stable, reversible electrochemistry.[5] It allows, for example, the detection of single-base mismatches in a DNA strand[6] and neutral biological molecules such as urea derivatives or barbiturates.[7] Surprisingly, little has been published on ferrocene-labeled PNA derivatives.[8] Metzler-Nolte et al. have been the first to report the synthesis

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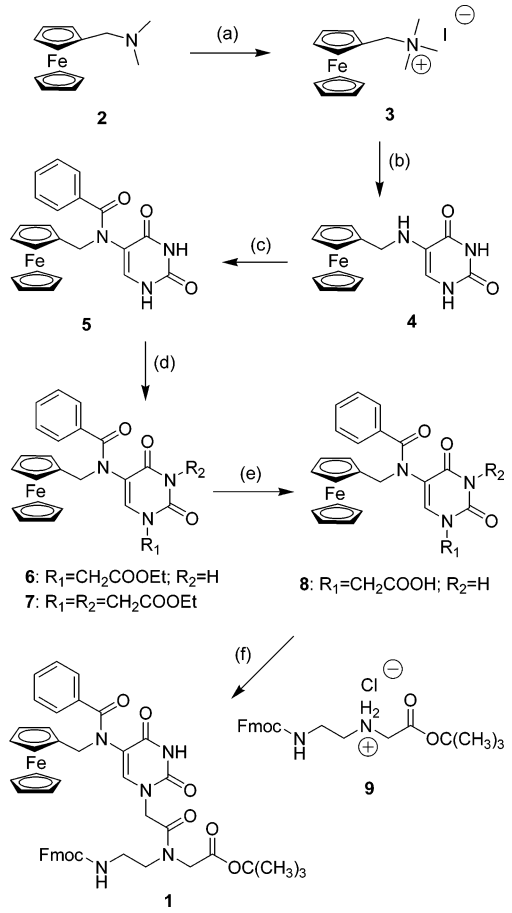
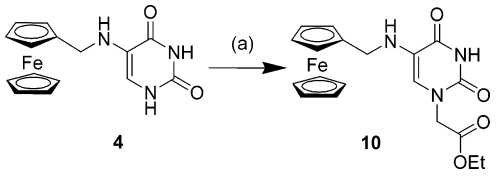
SCHEME 1[a]



[a] Reagents and conditions: (a) CH_3I, MeOH, 100%; (b) 5-aminouracil, H_2O, reflux, 92%; (c) benzoyl chloride, NaH, THF, reflux, 56%; (d) ethyl bromoacetate, NaH, THF, reflux, 48% for **6**, 41% for **7**; (e) NaOH 1 M, MeOH, 100%; (f) (i) sat. $NaHCO_{3(aq)}$, (ii) HBTU, HOBt, NMM, DMF, 60%.

of a thymine PNA monomer with a ferrocenyl group attached to the N-terminus of the PNA backbone[9] before reporting the first automated solid-phase synthesis of ferrocenyl derivatives of PNA oligomer and their interactions with DNA and PNA.[10] Campbell et al. reported a similar system aimed at monitoring seafood safety.[11] Baldoli et al. successfully inserted a ferrocene group on the *N*-(2-aminoethyl)glycine PNA backbone of a thymine monomer.[12] However, to enhance the redox response, an alternative approach has been to prepare a ferrocenyl-modified nucleotide (i.e. compound **8** in Scheme 1) and to attach this to a PNA backbone (i.e. compound **9** in Scheme 1). This strategy allows the modified base to be incorporated into a specific site within the oligonucleotide sequence. Hudson et al. hence reported the synthesis of a PNA monomer bearing a

SCHEME 2[a]



[a] Reagernts and conditions: (a) Ethyl bromoacetate, NaH, THF, reflux, 12%.

ferrocenyl unit directly attached to the uracil base.[13] Moreover, internal labeling of PNA also leaves both the C- and N-termini available for other forms of derivatization.[14] For example, Kraemer et al. used linkers of different lengths to covalently attach bi- and tridentate ligands capable of forming 1:1 complexes with Zn^{II}, Cu^{II}, and Ni^{II} to the N-terminus of PNAs.[15] As these complexes have free coordination sites, they enable direct coordinative interaction of the metal ion with, for example, N-donor atoms of DNA nucleosides or O-atoms of phosphodiester groups of the DNA backbone. Consequently, these metal complex conjugates allow modulation of the PNA-DNA duplex stability.

Due to the wide variety of potential application of redox-labeled PNA conjugates, we decided to investigate a pathway for a facile and cost-effective synthesis of a ferrocenyl PNA monomer derivative of uracil in which the ferrocenyl moiety is bound directly to the uracil base. Herein, we report the synthesis of *tert*-butyl-2-(*N*-(2-(((9*H*-floren-9-yl)methoxy)carbonylamino)-ethyl)-2-(5-(*N*-ferrocenylmethylbenzamido)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)acetate (**1**) (Scheme 1). Furthermore, to demonstrate that **1** can bind its complementary DNA base adenine, NMR studies were undertaken with an adenine derivative, 9-ethyladenine (EA). Structural aspects of the complexation of EA with **1** are also discussed and, in view of possible applications of **1** in the electrochemical detection of nucleic acids, the electrochemical behavior of **1** was investigated.

Results and Discussion

Synthesis of 1. The synthetic route to **1** is described in Scheme 1. An inexpensive commercially available ferrocene derivative, (dimethylaminomethyl)ferrocene (**2**), has been chosen as starting material. **2** is significantly cheaper than the ferrocene methanol[12] and ferrocene carboxylic acid,[13] precursors commonly used for the synthesis of ferrocenyl PNA monomers. **2** was reacted with iodomethane in methanol to quantitatively give the known compound (ferrocenylmethyl)trimethylammonium iodide (**3**).[16] The key synthon 5-(ferrocenylmethylamino)-pyrimidine-2,4(1*H*,3*H*)-dione (**4**) was easily prepared by refluxing **3** in water with 5-aminouracil in 92% yield following a similar procedure to that used by Colbran et al.[17]

Our first approach was to react **4** with ethyl bromoacetate as shown in Scheme 2. However, because of the insolubility of **4**

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(9) Hess, A.; Metzler-Nolte, N. *Chem. Commun.* **1999**, 885−886.
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(12) Baldoli, C.; Falciola, L.; Licandro, E.; Maiorana, S.; Mussini, P.; Ramani, P.; Rigamonti, C.; Zinzalla, G. *J. Organomet. Chem.* **2004**, *689*, 4791−4802.
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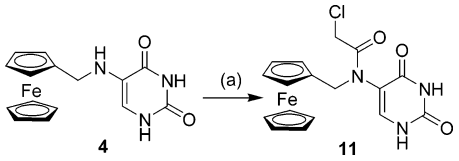
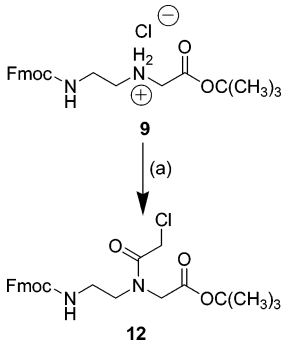
SCHEME 3[a]



[a] Reagents and conditions: (a) chloroacetyl chloride, NaH, THF, reflux, 100%.

SCHEME 4[a]



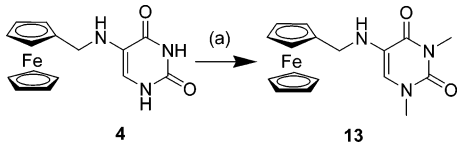
[a] Reagents and conditions: (a) (i) sat. $NaHCO_{3\ (aq)}$, (ii) chloroacetyl chloride, NEt_3, CH_2Cl_2, 0 °C to rt, 100%.

in all common organic solvents, **4** could not be converted in high yield into the desired compound **10**, despite the use of high temperatures and high dilution. Compound **4** remained largely unreacted when treated with the brominated reagent. Furthermore, the small proportion of **4** that did react also generated several undesirable side products.

To overcome this problem, the reaction of **4** with chloroacetyl chloride, a more reactive compound than ethyl bromoacetate, was attempted with the aim of obtaining a product that could then be reacted with the neutralized hydrochloride salt of PNA backbone **9**.[18] It should be noted that this is not strictly a PNA monomer as the PNA backbone would not have been linked to the DNA/RNA base by an amide bond but rather replaced with an amine. Nevertheless, the product formed would still have the required properties for redox applications. However, the only compound to be isolated was **11**, and quantitatively, as shown in Scheme 3! The ^{1}H NMR of **11** showed a splitting of the singlet of the CH_2 linking the ferrocene to the uracil into two distinct doublets with a coupling constant of 14.4 Hz indicating clearly a geminal coupling constant and proving the formation of the unexpected product **11**.

Our second approach was to react the PNA backbone **9** with chloroacetyl chloride after having previously neutralized the hydrochloride salt with a saturated aqueous solution of $NaHCO_3$, as described in Scheme 4. Compound **9** is much easier to handle compared to the neutralized product, which is a sticky oil. Moreover, **9** can be stored at −20 °C indefinitely, unlike the neutralized product as a loss of the Fmoc protecting group can occur.[18] Thus, **12** has been prepared quantitatively and the analytical data confirmed its purity. The aim was then to react **12** with **4**, which would have been first deprotonated with NaH following the approach described by Meltzer et al. for the synthesis of a PNA thymine monomer.[19] Unfortunately, this coupling could not be achieved despite using different bases or solvents.

SCHEME 5[a]



[a] Reagents and conditions: (a) iodomethane, K_2CO_3, DMF, 40 °C, 47%.

To overcome the problems of regioselectivity, which resulted in attack of the different nucleophiles at the secondary amine group in **4**, attempts were made to introduce a substituent at this amine group. In this respect, a methylation reaction was performed by reacting **4** with iodomethane with use of potassium carbonate as a base instead of the usual NaH. However, we found that the methylation occurred predominantly on the uracil base. The dimethylated product **13** shown in Scheme 5 was isolated by chromatography on silica.

To confirm the sites of the dimethylation of **4**, the X-ray crystal structure of **13** was determined. Figures and a complete description of the structure of **13** can be found in the Supporting Information.

Considering these results, it appeared that the secondary amine group of **4** could be converted preferentially into an amide derivative rather than a tertiary amine derivative. To demonstrate this, the reaction of **4** with benzoyl chloride was carried out and found to give **5** in 56% yield. As for **11**, the ^{1}H NMR of **5** showed splitting of the CH_2 signal linking the ferrocene to the uracil into two distinct doublets with a geminal coupling constant of 13.9 Hz confirming the formation of the desired product. The fact that the amine group of **4** reacts so readily and specifically in the presence of an acid chloride to yield an amide as protecting group then allows the nitrogen atom in position 1 of the uracil base to be used in further coupling. Compound **5** was then reacted with ethyl bromoacetate to give **6** in 48% yield. Substitution on the desired nitrogen was confirmed by careful analysis of the NMR spectra, including Heteronuclear Multiple Bond Correlation (HMBC). We observed a correlation of the protons of the CH_2 of the new chain to the carbon of the CH of the uracil. The yield of the monoreacted product has been increased by a factor of 4 in comparison with the similar reaction with **4** to give **10** (Scheme 2). The explanation is the greater solubility of **5** in THF compared with its analogue **4**. The disubstituted product (**7**) has also been isolated during the chromatographic purification of **6** on silica in 41% yield (yield based on the limiting reagent). Notably, an attempt to prepare **6** from **4** via a one-pot reaction was successful and gave a similar yield to that obtained with two successive reactions. The acid **8** was quantitatively obtained by hydrolysis of **6** in a mixture of 1 M NaOH (aq) and MeOH. The best conditions for coupling the neutralized hydrochloride salt of the PNA backbone **9** with **8** were found to be those published by Neuner et al., who used *N*-methylmorpholine (NMM), hydroxybenzotriazole (HOBt), and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in anhydrous DMF for a similar coupling.[20] The final compound **1** was precipitated with water,

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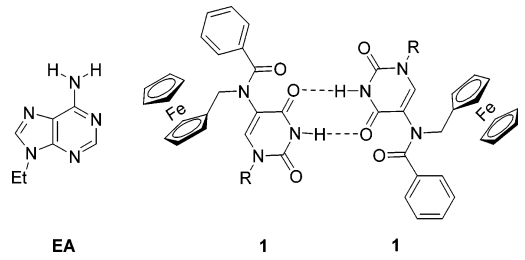
(20) Neuner, P.; Monaci, P. *Bioconj. Chem.* **2002**, *13*, 767−678.

SCHEME 6. Structure of 9-Ethyladenine (EA) and Self-Association of 1



filtered, and purified by chromatography on silica to give a sticky orange solid in 60% yield. The ESI mass spectrum of **1** showed three peaks at *m*/*z* 865, 866, and 888 corresponding respectively to $[M]^+$ (Fe^{II} is oxidized to Fe^{III} in the mass ionizer, which makes the compound positively charged), $[M + H]^+$, and $[M + Na]^+$. The ^1H and ^{13}C NMR signals were particularly hard to assign because of the presence of rotamers. However, this has been achieved with the help of the 2D plots (COSY, HMQC, and HMBC). ^1H and ^{13}C NMR spectra of compounds **1**, **4**, **5**, **6**, and **8** can be found in the Supporting Information.

NMR Studies of the Binding of 9-Ethyladenine (EA) to Receptor 1. To establish that the ferrocenyl PNA monomer can bind its complementary DNA base despite the presence of the bulky ferrocene moiety, ^1H NMR studies were undertaken in a 1:1 CD_3CN:$CDCl_3$ by volume mixture with 9-ethyladenine (EA, Scheme 6) as described in the Supporting Information. The addition of aliquots of EA on a solution of **1** results in the downfield shift of the imide N*H* signal of **1** from 8.97 to 11.15 ppm. However, to determine if self-association of **1** is possible since complementary hydrogen bonds can be formed in imide dimers (Scheme 6),[21−23] a study of the change in chemical shifts of the imide N*H* resonance of receptor **1** with concentration was carried out in the absence of EA. When compared with the change above, only a minor upfield shift of 0.4 ppm of the imide N*H* resonance of **1** was observed when a 0.1 M solution of **1** was diluted by a factor of 1000. Since under the conditions used in our experiments the initial concentrations of **1** were 5 and 10 mM, self-association was concluded to be negligible, as was found previously in related studies.[21]

The first method used to calculate the association constant K_a of **1** with EA was via the ratio of the integrals of the duplex **1**-EA and **1** measured in a 2:1 solution of EA and **1** ($[\mathbf{1}]_{Initial}$ = 5mM). The association constant is defined as K_a = [EA-**1**]/([EA][**1**]), where [EA-**1**], [EA], and [**1**] represent the equilibrium concentrations of each component. To achieve this, low-temperature measurements were carried out as only one signal was observed at 30 °C due to the rapid exchange between the imide protons of the duplex **1**-EA and **1**. The values of K_a did not change significantly with temperature (see Figure 3 in the Supporting Information) and assuming that there is little change between −15 and 30 °C (between −15 and 15 °C, there is an overlap between the two peaks making the reading of the integrals difficult), a value of K_a of 75 ± 2 M^{-1} can be estimated at 30 °C. The experimental error is the standard deviation calculated from the K_a values determined between −50 and −15 °C. However, a larger uncertainty (±10% as suggested in previous studies[21]) should be envisaged from the accuracy of the calculation of the integral ratio and the assumption that there is no measurable self-association of **1**.

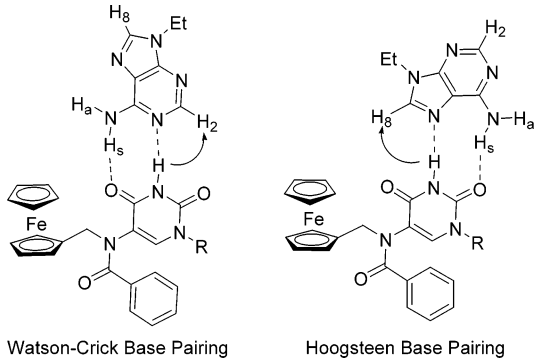


FIGURE 1. Hydrogen bonding of EA to **1** via Watson−Crick (WC) or/and Hoogsteen base (HG) pairing.

A second determination of K_a used the NMR titration method reported by Williams et al.[21] In this case, the shift of the imide N*H* signal of **1** upon the number of equivalents of EA added was found (see Figure 4 in the Supporting Information). By assuming that the maximum chemical shift of the N*H* proton of the **1**-EA duplex was measured at −50 °C during the low-temperature measurements (δ 12.31 ppm, $\Delta\delta$ 3.34 ppm), a value of K_a of 69 ± 2 M^{-1} at 30 °C with a degree of saturation of 65% was found.

The two K_a values determined by the two NMR measurements are in excellent agreement. For comparison, Barawkar et al., using NMR spectroscopy, determined larger values of 330 and 245 M^{-1} for the binding constants for dA:dT and dA:$dUNH_2$, respectively, in $CDCl_3$.[24] However, smaller values in the range of 50 to 125 M^{-1} were found for the interaction of EA with model compounds.[21] These values are in agreement with those reported here and suggest that the bulky ferrocenyl moiety does not hinder the association of **1** with its complementary DNA base, or if it does, not significantly.

Structural Aspect of the Complexation of 1 with EA. Adenine derivatives can hydrogen bond to imide derivatives via Watson−Crick (WC) or/and Hoogsteen base (HG) pairing. This is also possible in our system as described in Figure 1.[25,26] Lancelot[27] and Rao et al.[28] have reported an interesting observation for the binding of carboxylic acids to adenine derivatives: a downfield shift of H(8) is observed when the receptor binds EA through the Hoogsteen site and an upfield shift of H(2) when carboxylic acid is bound to EA through the Watson−Crick site. Interestingly, in our system (imide derivative instead of a carboxylic acid), an upfield shift of H(2) ($\Delta\delta$ 0.09 ppm) and a minor upfield shift of H(8) ($\Delta\delta$ 0.01 ppm) were observed during the titration of **1** by EA, indicating a preference for the WC mode of binding. The $\Delta\delta$ values measured for our system are in accordance with that reported by Lancelot[27] and Rao et al.[28] In the case of WC pairing, Rao et al.[28] proposed that the upfield shift of H(2) results from a

(21) Williams, K.; Askew, B.; Ballester, P.; Buhr, C.; Jeong, K. S.; Jones, S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1090−1094.
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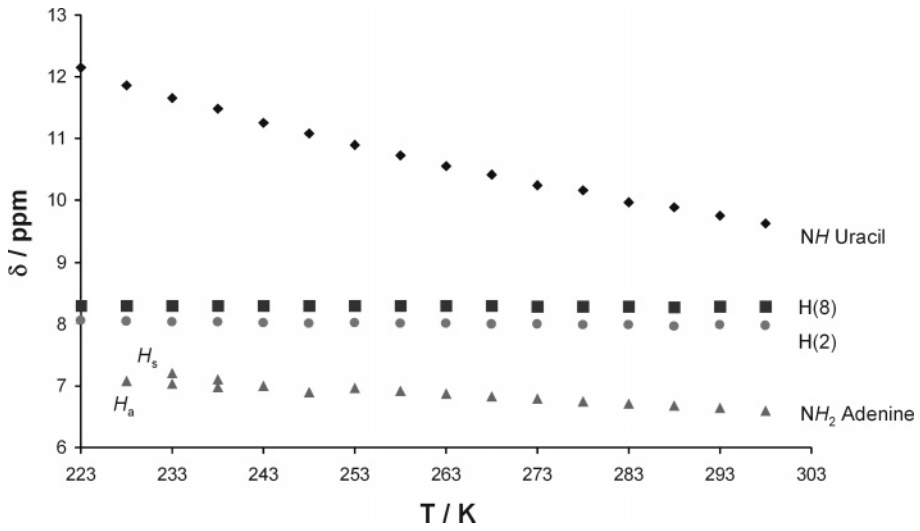

FIGURE 2. Plot of the ^{1}H NMR chemical shifts of different protons in a 2:1 stoichiometric mixture of EA and **1** ($[\mathbf{1}]_{Initial}$ = 5 mM) in a 1:1 CD_3CN:$CDCl_3$ mixture by volume.

binding-promoted π−polarization, inducing a partial negative charge on the adjacent N(1).

Furthermore, it could have been possible for the Fmoc group of **1** to interact with EA through $\pi-\pi$ interactions as was demonstrated by Williams et al. with model receptors.[21] But, in our case, no change in the NMR spectrum was observed for the aromatic protons of the Fmoc group of **1**, suggesting that these kinds of interactions are weak or not present in our system.

Interestingly, similar observations as those described by Barawkar et al. were found during ^{1}H NMR measurements as a function of the temperature in a 2:1 stoechiometric mixture of EA and **1** ($[\mathbf{1}]_{Initial}$ = 5 mM).[24] Upon decreasing the temperature, an important downfield shift of all exchangeable protons (N*H* of the uracil and NH_2 of EA) was observed while the H(2) and H(8) protons did not shift significantly (Figure 2). It is interesting to point out that the protons of the NH_2 group of EA split in two different signals (H_a and H_s) (see Figure 1) below −30 °C due to hindered rotation about the bond between the nitrogen atom of the NH_2 group and the carbon of the pyrimidine ring. However, due to the overlap of the NH_2 protons of EA with those of the benzyl and Fmoc group, it is has been impossible to assign the chemical shifts of the NH_2 protons of EA at temperatures lower than −40 °C. Nevertheless, a linear dependence on temperature of the observed shifts for N*H* of the uracil and NH_2 of EA protons was observed. This is characteristic of complexation by Watson−Crick hydrogen bonding mode.

Askew et al. studied the binding mode of EA to model receptors by nuclear Overhauser effect (NOE) experiments.[29] Irradiation of the imide N*H* proton of the receptor was found to enhance the H(2) or/and H(8) signal of EA and correlated this to a predominance of either WC or HG binding, respectively. If both protons were enhanced, the ratio of the enhancement was considered to reflect the ratio of the WC:HG binding modes present. Such data are not quantitative and only provide a guide to the species present in solution.[29] In our case, no enhancement of either H(2) or H(8) of EA was observed, probably because of the long separation between the N*H* irradiated proton and the protons that could have been enhanced and the absence of $\pi-\pi$ stacking interaction to the receptor (cf. studies of Askew et al.).[29]

UV−Visible Spectral Studies. 1 gave a single band in the visible region of the electronic spectrum (λ_{max} = 435 nm, ϵ_{max} = 101 $M^{-1}\cdot cm^{-1}$) due to the lowest energy spin-allowed d−d transition of the ferrocene unit.[30] Consistent with previous reports, no change in spectrum was observed upon the addition of an excess of the adenine derivative EA to the receptor.[31] However, we note that red shifts in transitions and hypochromism have been observed in the electronic spectrum of a ferrocenyl receptor for thymine derivatives upon the addition of an excess of EA[32] but have been assigned to π-stacking interactions between the receptor and nucleobase, which is lacking in our system.

Electrochemical Studies. To examine the suitability of our PNA monomer for incorporation into PNA oligomers that can be applied as active DNA/RNA probes, voltammetric investigations of monomer **1** were carried out. Cyclic voltammetry was performed at a stationary glassy carbon (GC) electrode at scan rates of 10−1000 $mV\cdot s^{-1}$ in a 1:1 $CHCl_3$:CH_3CN mixture (by volume) with tetrabutylammonium hexafluorophosphate (Bu_4NPF_6, 0.1 M) as the supporting electrolyte. Decamethylferrocene (DMFc) was used as a reference compound instead of the usual ferrocene (Fc) to avoid overlap of **1** and the ferrocene oxidation process. The potential of the $DMFc^{0/+}$ process is close to −500 mV vs $Fc^{0/+}$, in most solvents.[33] A cyclic voltammogram of **1** and DMFc at a scan rate of 50 $mV\cdot s^{-1}$ is presented in Figure 3. The process centered at 0 mV corresponds to the $DMFc^{0/+}$ process and the one in the 500−600 region vs $DMFc^{0/+}$ to the $\mathbf{1}^{0/+}$ process.

Thus, compound **1** exhibits a single reversible one-electron wave with a formal potential E^0_f of +538 mV vs $DMFc^{0/+}$, as determined from the average of the oxidation and reduction peak

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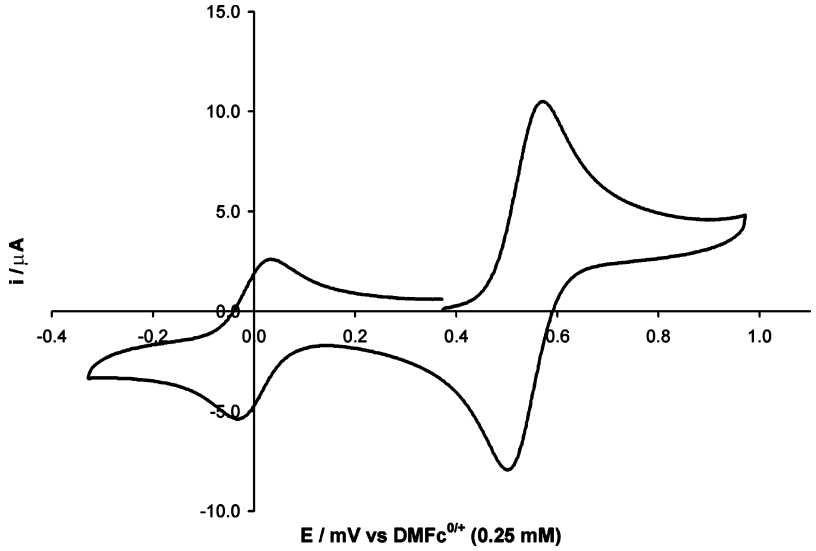

FIGURE 3. A cyclic voltammogram of **1** (1 mM) at a GC electrode, in a 1:1 by volume mixture of CH_3CN:$CHCl_3$ containing Bu_4NPF_6 (0.1 M) as the supporting electrolyte. The experiment was undertaken at a scan rate of 50 mV·s^{-1} and at 20 °C with DMFc as the internal reference (0.25 mM).

TABLE 1. Cyclic Voltammetric Data Obtained at 20 °C for 1 and DMFc at a GC Electrode in a Mixture CH_3CN:$CHCl_3$ 1:1 by Volume (Bu_4NPF_6 (0.1 M) was the Supporting Electrolyte)[a]

compd	scan rate/ mV·s^{-1}	ΔE^0_f vs $DMFc^{0/+}$/mV	ΔE_p/ mV	I_p^{ox}/ μA	I_p^{red}/ μA	I_p^{ox}/I_p^{red}
1	50	+538	70	8.65	9.08	0.95
DMFc	50	0	64	3.37	3.66	0.92
1	250	+538	79	20.61	21.34	0.96
DMFc	250	0	63	8.16	8.90	0.92
1	1000	+542	102	39.78	37.82	1.05
DMFc	1000	0	79	15.45	16.30	0.95

[a] Oxidation peak potential = E_p^{ox}; reduction peak potential = E_p^{red}; formal redox potential $E^0_f = (E_p^{ox} + E_p^{red})/2$; oxidation peak current = I_p^{ox}; reduction peak current = I_p^{red}.

potentials (Table 1). The $\mathbf{1}^{0/+}$ process is assigned to the reversible oxidation/reduction of the ferrocene redox couple of **1** as decribed in the equation below:

$$Fc^0(\mathbf{1}) \rightarrow Fc^+(\mathbf{1}) + e^-$$

Table 1 summarizes data obtained for both the known reversible $DMFc^0/DMFc^+$ reference process and the $\mathbf{1}^{0/+}$ redox couple. The small increase in ΔE_p with scan rate for the known reversible $DMFc^{0/+}$ process implies that a small level of uncompensated IR drop is present, the larger current for oxidation of **1** gives rise to a slightly larger IR drop, and hence even larger ΔE_p for a given scan rate. However, the $\mathbf{1}^{0/+}$ process has essentially the same characteristics as for the $DMFc^{0/+}$ process and is therefore deduced to be also chemically and electrochemically reversible.

The diffusion coefficients (D) of **1** and DMFc in the 1:1 mixture of CH_3CN:$CHCl_3$ were calculated from the linear dependence of the peak current on the square root of the scan rate and the Randles−Sevcik equation.[34] A value of 4.2×10^{-6} cm^2·s^{-1} was found for **1**, which is slighlty larger than values previously reported for ferrocenyl PNA monomers in DMF.[12,35] The lower viscosity of the 1:1 CH_3CN:$CHCl_3$ mixture than that of DMF [η(CH_3CN) at 25 °C = 0.345 cP; η($CHCl_3$) at 25 °C = 0.542 cP; η(DMF) = 0.846 cP][36,37] would explain a significant part of the difference, since according to the Stokes−Einstein relationship,[38] the diffusion coefficient is inversely proportional to the viscosity. A D value of 1.2×10^{-5} cm^2·s^{-1} was found for DMFc. In comparison, a D value of 1.8×10^{-5} cm^2·s^{-1} was reported for DMFc in acetonitrile (5 mM) with $NaCF_3SO_3$ (0.2 M) as the supporting electrolyte at 25 °C.[39] The difference is attributed again to the lower viscosity of CH_3CN compared to the 1:1 CH_3CN:$CHCl_3$ mixture used in our experiment. The relatively large value of 4.2×10^{-6} cm^2·s^{-1} for **1** is promising from the point of view of the voltammetric detection limit in analytical applications since Baldoli et al. showed that they were able to detect submicromolar concentrations using differential pulse voltammetry under conditions where the relevant molecules have smaller diffusion coefficients and hence smaller currents per unit concentration.[12]

Investigations to determine if a shift in the reversible potential of the $\mathbf{1}^{0/+}$ process occurs upon addition of excess EA as found for ferrocene receptors that hydrogen bond to organic molecules such as urea or barbiturates derivatives,[7] were undertaken. No significant shifts were observed in the present case, which is attributed to the lack of significant difference in interaction between the uracil and the ferrocene moiety in **1** and $\mathbf{1}^+$.

Conclusions. A convenient synthetic procedure is reported for a new ferrocenyl uracil PNA monomer. We have demonstrated that the key synthon **4** has an interesting reactivity that will be exploited in further studies. The association constant of **1** with EA measured by NMR titrations corroborates with what was found for other receptors for EA and, importantly, showed that the ferrocenyl group of **1** does not affect, or only slightly

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affects, the binding of the adenine derivative. NMR measurements showed that **1** binds preferentially to EA through the Watson−Crick pairing rather than the Hoogsteen pairing. Electrochemical measurements showed that **1** exhibits a single and reversible one-electron wave with a diffusion coefficient that is slightly larger than that observed for similar ferrocenyl PNA monomers.

Work currently in progress is incorporating the monomer **1** into PNA oligomers for use as redox sensors for specific DNA/RNA sequences.

Experimental Section

Preparation of (Ferrocenylmethyl)trimethylammonium iodide (3). 3 was synthesized following the procedure of Lindsay et al.[16] The analytical data of the product matched those reported previously.[16]

Preparation of 5-(Ferrocenylmethylamino)pyrimidine-2,4-(1*H*,3*H*)-dione (4). 3 (3.50 g, 9.09 mmol) and 5-aminouracil (1.07 g, 8.26 mmol) were refluxed in deoxygenated water (100 mL) for 18 h under nitrogen. The orange precipitate that formed during the reaction was filtered and washed with water, acetone, and CH_2Cl_2 until the filtrate was colorless. A yellow solid was obtained. Yield: 2.48 g (92%). Anal. Found (%): C, 54.0; H, 4.6; N, 12.6. Calcd for $FeC_{15}H_{15}N_3O_2 \cdot {}^1/_2H_2O$ (%): C, 53.9; H, 4.8; N, 12.6. IR bands (KBr; ν, cm^{-1}): 3400, 3169, 3032, 2927, 1713, 1659, 1544, 1520, 1467, 1439, 1414, 1383, 1367, 1247, 1105, 1068, 1036, 868, 797, 699. ^1H NMR spectrum (DMSO-d_6): δ 3.69 (d, $^3J = 5.5$ Hz, 2H), 4.12 (m, 2H), 4.18 (m, 6H), 4.23 (m, 2H), 6.46 (d, 1H), 10.18 (s, 1H), 11.17 (s, 1H). ^{13}C NMR spectrum (DMSO-d_6): δ 42.75, 67.05, 67.99, 68.32, 86.24, 113.49, 123.65, 149.33, 161.37. Electrospray mass spectrum (*m*/*z*) (positive mode): 199 $[Fc - CH_2]^+$ (100%), 325 $[M]^+$ (9%), 348 $[M + Na]^+$ (15%). Electrospray mass spectrum (*m*/*z*) (negative mode): 324 $[M - H]^-$ (100%).

Preparation of *N*-Ferrocenylmethyl-*N*-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)benzamide (5). 4 (0.570 g, 1.75 mmol) and NaH (0.046 g, 1.91 mmol) were heated at reflux in dry THF (200 mL) for 1 h under an atmosphere of nitrogen. Benzoyl chloride (0.246 g, 1.75 mmol), diluted in dry THF (40 mL), was then added dropwise to the refluxing mixture over a period of 15 min. The mixture was left to reflux for another 2 h. The solvent was removed under vacuum and to the solid residue was added ethyl acetate (150 mL) and water (100 mL). The layers were separated and the aqueous phase was extracted with ethyl acetate (100 mL). The combined organic phases were evaporated to dryness to give an orange solid. Purification was performed by chromatography on a silica column with ethyl acetate:hexane 2:1 as eluent (the crude product was preadsorbed on the silica) to give an orange solid (R_f 0.57). Yield: 0.22 g (56%). Anal. Found (%): C, 62.0; H, 4.8; N, 9.3. Calcd for $FeC_{22}H_{19}N_3O_3$ (%): C, 61.6; H, 4.5; N, 9.7. IR bands (KBr; ν, cm^{-1}): 3130, 3060, 2833, 1721, 1679, 1600, 1479, 1421, 1344, 1306, 1206, 1148, 1105, 1026, 986, 817, 742, 699. ^1H NMR spectrum (acetone-d_6): 3.95−4.18 (m, 9H), 4.34 (m, 1H), 5.20 (d, $^2J = 13.9$ Hz, 1H), 6.92 (s, 1H), 7.20−7.40 (m, 5H), 10.03 (s, b). ^{13}C NMR spectrum (DMSO-d_6): δ 46.42, 67.56, 68.09, 68.33, 69.20, 69.65, 82.54, 115.46, 126.95, 127.70, 129.30, 136.61, 144.05, 151.56, 161.51, 170.23. Electrospray mass spectrum (*m*/*z*) (positive mode): 199 $[Fc - CH_2]^+$ (38%), 429 $[M]^+$ (5%), 452 $[M + Na]^+$ (100%). Electrospray mass spectrum (*m*/*z*) (negative mode): 428 $[M - H]^-$ (100%). High-resolution ESI mass determination (acetone:MeOH 1:4): found 429.0771; calcd for $FeC_{22}H_{19}N_3O_3$ 429.0776.

Preparation of Ethyl-2-(5-(*N*-ferrocenylmethylbenzamido)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetate (6). Method A: 5 (0.500 g, 1.16 mmol) and NaH (0.028 g, 1.16 mmol) were heated to reflux in dry THF (50 mL) for 1 h under an atmosphere of nitrogen. Ethyl bromoacetate (0.198 g, 1.16 mmol) diluted in dry THF (50 mL) was then added dropwise to the mixture at room temperature over a period of 30 min. The suspension was refluxed for 15 h. The solvent was evaporated to give an orange solid. To the solid residue was added CH_2Cl_2 (200 mL) and water (100 mL), and the mixture was stirred vigorously. The layers were separated and the organic phase was evaporated to dryness to give an orange solid. Chromatography on a silica column with ethyl acetate:hexane 2:3 as eluent gave an orange solid (R_f 0.16). Yield: 0.32 g (53%). **Method B: 4** (1.20 g, 3.69 mmol) and NaH (0.097 g, 4.06 mmol) were heated to reflux in dry THF (200 mL) for 1 h under an atmosphere of nitrogen. Benzoyl chloride (0.524 g, 3.69 mmol) diluted in dry THF (40 mL) was added dropwise to the refluxing mixture over a period of 25 min. The solution was refluxed for 17 h and was allowed to cool to room temperature. NaH (0.097 g, 4.06 mmol) was added to the mixture, which was then refluxed for 1 h under an atmosphere of nitrogen. The suspension was cooled to 0 °C and ethyl bromoacetate (0.628 g, 3.69 mmol) diluted in dry THF (100 mL) was added over a period of 45 min. The mixture was then refluxed for 19 h. The solvent was removed on a rotary evaporator to give an orange solid. To the solid residue was added CH_2Cl_2 (250 mL) and water (100 mL) and the mixture was stirred vigorously. The layers were separated and the organic phase was evaporated to dryness to give an orange solid. Chromatography on a silica column with ethyl acetate:hexane 2:3 as eluent gave an orange solid (R_f 0.16). Yield: 0.53 g (28% over the 2 steps). IR bands (KBr; ν, cm^{-1}): 3067, 2958, 2926, 1688, 1654, 1544, 1510, 1460, 1378, 1292, 1210, 1104, 1024, 963, 790, 701, 629. ^1H NMR spectrum (acetone-d_6): 1.19 (t, $^3J = 7.1$ Hz, 3H), 4.05−4.27 (m, 11H), 4.36 (m, 3H), 5.09 (d, $^2J = 15.1$ Hz, 1H), 7.25−7.40 (m, 6H), 10.28 (s, 1H). ^{13}C NMR spectrum (acetone-d_6): δ 15.08, 48.62, 50.00, 62.69, 69.33, 69.60, 69.85, 71.29, 71.57, 84.20, 118.90, 128.94, 129.19, 130.91, 138.18, 146.83, 151.44, 162.19, 168.71, 171.81. Electrospray mass spectrum (*m*/*z*): 199 $[Fc - CH_2]^+$ (62%), 515 $[M]^+$ (100%), 538 $[M + Na]^+$ (68%). High-resolution ESI mass determination (CH_2Cl_2:MeOH 1:4): found 538.1030; calcd for $FeC_{26}H_{25}N_3O_5Na$ 538.1041.

Preparation of Diethyl-2,2′-(5-(*N*-ferrocenylmethylbenzamido)-2,4-dioxo-3,4-dihydropyrimidin-1,3(2*H*,4*H*)-diyl)diacetate (7). 7 is a side product from the synthesis of **6** and was obtained during our first attempt to prepare **6**. Compound **5** (0.200 g, 0.559 mmol) and NaH (0.013 g, 0.559 mmol) were refluxed in dry THF (40 mL) for 1 h under an atmosphere of nitrogen. Ethyl bromoacetate (0.095 g, 0.559 mmol) diluted in dry THF (10 mL) was then added dropwise to the refluxing mixture over a period of 10 min. The mixture was refluxed for 1 h. The solvent was evaporated to give an orange solid. To the solid residue was added CH_2Cl_2 (80 mL) and water (40 mL) and the mixture was stirred vigorously. The layers were separated and the organic phase was evaporated to dryness to give an orange solid. Chromatography on a silica column with ethyl acetate:hexane 2:3 as eluent gave an orange solid (R_f 0.27). Yield: 0.070 g (41%). IR bands (KBr; ν, cm^{-1}): 3423 br, 3084, 2926, 2856, 1751, 1720, 1671, 1544, 1525, 1510, 1459, 1378, 1296, 1207, 1104, 1024, 787, 700, 483. ^1H NMR spectrum (acetone-d_6): 1.19 (t, $^3J = 7.1$ Hz, 3H), 1.25 (t, $^3J = 7.1$ Hz, 3H), 4.05−4.22 (m, 15H), 4.38 (m, 3H), 4.57 (s, 2H), 5.20 (d, $^2J = 13.8$ Hz, 1H), 7.23−7.41 (m, 6H). ^{13}C NMR spectrum (acetone-d_6): δ 14.94, 15.06, 43.58, 48.29, 50.01, 50.99, 62.54, 62.74, 69.39, 69.85, 70.00, 70.22, 71.22, 84.06, 118.11, 128.98, 129.33, 131.07, 137.76, 146.32, 149.21, 161.63, 168.38, 168.54, 171.65. Electrospray mass spectrum (*m*/*z*): 199 $[Fc - CH_2]^+$ (63%), 601 $[M]^+$ (50%), 624 $[M + Na]^+$ (100%). High-resolution ESI mass determination (acetone:MeOH 1:4): found 624.1404; calcd for $FeC_{30}H_{31}N_3O_7Na$ 624.1409.

2-(5-(*N*-Ferrocenylmethylbenzamido)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetic Acid (8). 6 (0.080 g, 0.15 mmol) was dissolved in a mixture of MeOH (5 mL) and a 1 M aqueous solution of NaOH (1 mL) and the resulting solution was stirred for 2 h at room temperature. The solvent was evaporated and the resulting residue dissolved in a 10% aqueous solution of citric acid and the pH adjusted to 2. An extraction with ethyl acetate (2 × 10 mL) was carried out and solvent was removed from the combined

organic phases under vacuum to give a yellow solid. Yield: 0.075 g (100%). IR bands (KBr; ν, cm^{-1}): 3450 br, 3214, 3075, 1702, 1688, 1654, 1638, 1544, 1525, 1510, 1460, 1386, 1300, 1233, 1144, 1025, 963, 792, 632, 485. ^{1}H NMR spectrum (acetone-d_6): 4.03−4.40 (m, 12H), 5.10 (d, ^2J = 13.4 Hz, 1H), 7.20−7.43 (m, 6H), 10.27 (s, 1H). ^{13}C NMR spectrum (acetone-d_6): 48.78, 55.95, 69.39, 69.77, 69.78, 71.22, 71.52, 84.16, 118.68, 128.99, 129.12, 130.82, 138.10, 147.13, 151.54, 162.16, 170.45, 171.88. Electrospray mass spectrum (*m*/*z*): 199 [Fc − CH_2]$^+$ (63%), 487 [M]$^+$ (45%), 510 [M + Na]$^+$ (100%). High-resolution ESI mass determination (acetone:MeOH 1:4): found 510.0724; calcd for $FeC_{24}H_{21}N_3O_5$Na 510.0728.

Preparation of *tert*-Butyl-2-(*N*-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)ethyl)-2-(5-(*N*-Ferrocenylmethylbenzamido)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)acetate (1). **8** (0.064 g, 0.147 mmol) was dissolved in CH_2Cl_2 (20 mL), then washed with a saturated aqueous solution of $NaHCO_3$ (10 mL). The organic phase was dried over Na_2SO_4 and filtered and the solvent was removed under vacuum. The oil was dissolved in dry DMF (2 mL). This solution was added to a solution of **7** (0.080 g, 0.164 mmol), *N*-methylmorpholine (NMM) (0.025 g, 0.246 mmol), hydroxybenzotriazole (HOBt) (0.033 g, 0.246 mmol), and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (0.062 g, 0.164 mmol) in dry DMF (3 mL), which had been stirred for 20 min at room temperature under an atmosphere of nitrogen prior to addition. The reaction mixture was stirred for 18 h at room temperature. Water (15 mL) was then added to the mixture until precipitation of a pale orange product was complete. This precipitate was filtered, washed with water, and dried under vacuum. Purification by chromatography on a silica column with ethyl acetate:hexane 5:1 as eluent yielded **1** as a orange solid (R_f 0.70). Yield: 0.077 g (60%). IR bands (KBr; ν, cm^{-1}): 3340 br, 3185, 3067, 2929, 1688 br, 1523, 1450, 1407, 1368, 1290, 1246, 1154, 1104, 1024, 960, 791, 759, 742, 701, 621, 580. ^{1}H NMR spectrum (acetone-d_6): 1.47 (min) and 1.48 (maj) (rotamers, 9H), 3.28−3.43 (m, 2H), 3.45−3.55 (m, 2H), 3.97−4.10 (m, 5H), 4.14 (m, 7H), 4.18−4.40 (m, 5H), 5.07 (d, ^2J = 13.7 Hz, 1H), 6.42 (min) and 6.60 (maj) (rotamers, br s, 1H), 7.11 (min) and 7.19 (maj) (rotamer s, 1H), 7.22−7.45 (m, 9H), 7.67 (m, 2H), 7.86 (m, 2H), 10.13 (s, br, 1H). ^{13}C NMR spectrum (acetone-d_6): δ 28.74 (min) and 28.87 (maj) (rotamers), 40.08 (min) and 40.54 (maj) (rotamers), 48.68 (min) and 48.72 (maj) (rotamers), 49.00 (maj) and 49.41 (min) (rotamers), 49.48 (min) and 49.59 (maj) (rotamers), 50.44, 51.58, 67.55 (min) and 67.71 (maj) (rotamers), 69.63, 69.79, 71.32, 71.70, 82.52 (maj) and 83.62 (min) (rotamers), 84.32, 118.69, 121.34, 126.62, 128.47, 129.05 (b, corresponds to two different carbons), 130.76, 138.12 (min) and 138.17 (maj) (rotamers), 142.64, 145.62 (maj) and 145.68 (min) (rotamers), 147.17 (min) and 147.31 (maj) (rotamers), 151.53, 157.80 (min) and 157.96 (maj) (rotamers), 162.16, 168.13 (maj) and 168.68 (min) (rotamers), 169.73 (maj) and 169.83 (min) (rotamers), 171.76. Electrospray mass spectrum (*m*/*z*): 865 [M]$^+$ (30%), 866 [M + H]$^+$ (18%), 888 [M + Na]$^+$ (100%). High-resolution ESI mass determination (CH_2Cl_2:MeOH 1:4): found 888.2722; calcd for $FeC_{47}H_{47}N_5O_8$Na 888.2672.

Preparation of *tert*-Butyl *N*-[2-(*N*-9-Fluorenylmethoxycarbonyl)aminoethyl)]glycinate Hydrochloride (9). The hydrochloride salt of [2-(9*H*-fluoren-9-ylmethoxycarbonylamino)ethylamino]acetic acid *tert*-butyl ester (**9**) was synthesized following the procedure published by Thomson et al.[18] The analytical data of the obtained product matched that reported previously.[18]

Preparation of Ethyl-2-(5-(ferrocenylmethylamino-2,4-dioxo-3,4-dihydroxyrimidin-1(2*H*)-yl)acetate (10). 4 (0.25 g, 0.77 mmol) and NaH (0.018 g, 0.77 mmol) were heated to reflux in dry THF (50 mL) for 1 h. Ethyl bromoacetate (0.128 g, 0.77 mmol) diluted in dry THF (30 mL) was added dropwise to the refluxing mixture over a period of 4 h and the mixture was refluxed for a further 15 h. The solvent was evaporated to dryness. The solid residue was extracted with CH_2Cl_2 (60 mL) and filtered and the solvents were removed from the organic phase under vacuum. This product was purified by chromatography on a silica column with CH_2Cl_2:MeOH 50:1 as eluent to yield a pale brown solid (R_f 0.22). Yield: 0.04 g (12%). IR bands (KBr; ν, cm^{-1}): 3369 br, 3182, 3086, 2987, 1738, 1686, 1644, 1544, 1510, 1442, 1410, 1378, 1208, 1103, 1024, 817, 555, 482. ^{1}H NMR spectrum (DMSO-d_6): 1.21 (t, ^3J = 7.1 Hz, 3H), 3.68 (m, 2H), 4.10−4.35 (m, 12H), 4.45 (s, 2H), 6.78 (s, 1H), 11.51 (s, 1H). ^{13}C NMR spectrum (DMSO-d_6): δ 13.99, 42.54, 48.59, 60.92, 67.40, 68.19, 68.23, 85.74, 117.58, 123.98, 150.65, 161.44, 168.26. Electrospray mass spectrum (*m*/*z*): 199 [Fc − CH_2]$^+$ (100%), 434 [M + Na]$^+$ (7%). High-resolution ESI mass determination (DMSO:MeOH 1:10): found 434.0771; calcd for $FeC_{19}H_{21}N_3O_4$Na 434.0779.

Preparation of *N*-Ferrocenylmethyl-2-chloro-*N*-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acetamide (11). 4 (0.75 g, 2.31 mmol) and NaH (0.060 g, 2.52 mmol) were heated to 70 °C for 1 h in dry THF (150 mL) under a nitrogen atmosphere. Chloroacetyl chloride (0.26 g, 2.31 mmol) diluted in dry THF (35 mL) was added to the mixture over a period of 15 min. The mixture was left to stir for another 1 h at 70 °C and then the solvent was removed under reduced pressure. The solid was extracted with CH_2Cl_2 (100 mL) to give an orange solid. Yield: 0.92 g (100%). Anal. Found (%): C, 49.8; H, 4.2; N, 9.7. Calcd for $FeC_{17}H_{16}N_3O_3Cl\cdot CH_3OH$ (%): C, 49.9; H, 4.6; N, 9.7. IR bands (KBr; ν, cm^{-1}): 3449, 3090, 3001, 2927, 1737, 1658, 1623, 1512, 1444, 1403, 1325, 1291, 1244, 1199, 1134, 1103, 1025, 996, 918, 836, 812, 710, 651, 632, 613, 588, 565. ^{1}H NMR spectrum (DMSO-d_6): δ 3.89 (d, 1H, ^2J = 14.0 Hz), 4.03 (d, 1H, ^2J = 14.4 Hz), 4.05 (m, 1H), 4.10 (m, 2H), 4.15 (s, 5H), 4.17 (m, 1H), 4.28 (d, 1H, ^2J = 14.4 Hz), 4.85 (d, 1H, ^2J = 14.0 Hz), 7.20 (s, 1H), 10.98 (s, 1H), 11.36 (s, 1H). ^{13}C NMR spectrum (DMSO-d_6): δ 42.75, 46.65, 67.65, 68.13, 63.31, 68.99, 69.35, 82.12, 113.08, 142.20, 150.65, 161.44, 166.08. Electrospray mass spectrum (*m*/*z*) (positive mode): 199 [Fc − CH_2]$^+$ (100%), 401 [M]$^+$ (27%), 424 [M + Na]$^+$ (97%). Electrospray mass spectrum (*m*/*z*) (negative mode): 400 [M − H]$^-$ (100%). High-resolution ESI mass determination (acetone:MeOH 1:4): found 424.0112; calcd for $FeC_{17}H_{16}N_3O_3ClNa$ 424.0127.

Preparation of *tert*-Butyl 2-(*N*-2-(((9*H*-Fluoren-9-yl)methoxycarbonylamino)ethyl)-2-chloroacetamido) acetate (12). 9 (0.20 g, 0.46 mmol) was dissolved in CH_2Cl_2 (50 mL) and washed twice with an aqueous saturated solution of $NaHCO_3$ (50 mL). Triethylamine (0.04 g, 0.46 mmol) was added to the organic phase and the solution was cooled to 0 °C in an ice bath. Chloroacetyl chloride (0.05 g, 0.46 mmol) was dissolved in CH_2Cl_2 (15 mL) and added dropwise over a period of 10 min. The temperature was allowed to warm to room temperature and the solution was stirred for a further 4 h. Water (25 mL) was then added and the aqueous layer was separated from the organic phase. The aqueous solution was extracted with CH_2Cl_2 (50 mL). The organic layer was combined with that from the reaction mixture and washed with an aqueous saturated NaCl solution (50 mL). The solvent was then removed in vacuo to give a pale yellow sticky oil that was used in the next step without further purification. Yield: 0.22 g (100%). IR bands (Nujol; ν, cm^{-1}): 3395 br, 1715, 1652, 1538, 1505, 1153 br, 1016, 848, 668. ^{1}H NMR spectrum ($CDCl_3$): 1.48 (s, 9H), 3.38 (m, 2H), 3.53 (m, 2H), 3.92 (maj) (rotamers, s), 3.99 (min) (rotamers, s), 4.04 (rotamers, m), 4.21 (m, 1H), 4.35 (min) and 4.41 (maj) (rotamers, m, 2H), 5.38 (min) and 5.83 (maj) (rotamers, b s, 1H), 7.24−7.45 (m, 4H), 7.58 (d, ^3J = 7.5 Hz, 2H), 7.76 (d, ^3J = 7.5 Hz, 2H). ^{13}C NMR spectrum ($CDCl_3$): δ 28.22, 39.28 (min) and 39.47 (maj) (rotamers), 40.64 (maj) and 41.19 (min) (rotamers), 50.17 (maj) and 51.69 (min) (rotamers), 47.42, 48.69 (min) and 50.11 (maj) (rotamers), 67.05 (min) and 67.13 (maj) (rotamers), 120.15 (min) and 120.21 (maj) (rotamers), 125.22 (maj) and 125.36 (min) (rotamers), 127.26 (min) and 127.32 (maj) (rotamers), 127.88 (min) and 127.96 (maj) (rotamers), 141.54, 143.98 (maj) and 144.14 (min) (rotamers), 156.79, 167.58 (maj) and 168.11 (min) (rotamers), 168.46 (maj) and 169.08 (min) (rotamers). Electrospray mass spectrum (*m*/*z*): 417 [M − (*tert*-butyl) + H] $^+$ (28%), 473 [M + H]$^+$ (15%), 495 [M + Na]$^+$ (100%). High-resolution ESI mass

determination (CH_2Cl_2:MeOH 1:4): found 495.1659; calcd for $C_{25}H_{29}N_2O_5ClNa$ 495.1663.

Preparation of 5-(Ferrocenylamino)-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (13). 4 (0.20 g, 0.61 mmol), iodomethane (0.087 g, 0.61 mmol), and K_2CO_3 (0.42 g, 3.07 mmol) were mixed in dry DMF (15 mL) and heated to 40 °C for 2 h. The solvent was removed under vacuum, the residual solid extracted with CH_2Cl_2 (50 mL), the solution filtered, and the solvent removed under vacuum. Chromatography on a silica column on the crude product with CH_2Cl_2:MeOH 50:1 as eluent was performed to yield **13** as a brown solid (R_f 0.26). Yield: 0.05 g (47%). Crystals suitable for X-ray crystallography were obtained by allowing a chloroform solution of pure product to evaporate at room temperature over a few days. Anal. Found (%): C, 56.6; H, 5.5; N, 10.9. Calcd for $FeC_{17}H_{19}N_3O_2 \cdot CH_3OH$ (%): C, 56.3; H, 5.8; N, 10.9. IR bands (KBr; ν, cm^{-1}): 3376, 3075, 2949, 1694, 1638, 1544, 1508, 1441, 1416, 1366, 1328, 1193, 1123, 1103, 1000, 814, 771, 738, 567. ^{1}H NMR spectrum (acetone-d_6): δ 3.28, 3.34, 3.78 (d, ^3J = 5.7 Hz, 2H), 4.15 (m, 2H), 4.21 (s, 5H), 4.27 (m, 2H), 6.68 (s, 1H). ^{13}C NMR spectrum (acetone-d_6): δ 28.69, 37.16, 44.50, 69.08, 69.39, 69.88, 87.89, 117.63, 125.57, 149.62, 162.01. Electrospray mass spectrum (*m*/*z*): 199 $[Fc - CH_2]^+$ (100%), 353 $[M]^+$ (25%), 376 $[M + Na]^+$ (7%).

Preparation of 9-Ethyladenine (EA). EA was synthesized following the procedure published by Rasmussen et al.[40] Purification by chromatography on a silica column with CH_2Cl_2:MeOH 19:1 as eluent (R_f 0.37) was performed to obtain a pure compound. The analytical data of the obtained product matched those reported previously.[40]

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Supporting Information Available: Experimental procedures, crystallographic data of **13**, description of the structure **13**, ^{1}H and ^{13}C NMR spectra of compounds **1**, **4**, **5**, **6**, and **8**, plots of the variation in K_a for the binding of EA to **1** with temperature and the shift of the imide N*H* signal of **1** upon the addition of EA. This material is available free of charge via the Internet at http://pubs.acs.org.

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