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Water soluble, hydrolytically stable derivatives of the antitumor drug titanocene dichloride and binding studies with nucleotides[1]

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Abstract

The rate of hydrolysis of the aromatic rings of Cp_2TiX_2 and the dimethylsubstituted derivatives $(MeCp)_2TiX_2$ [X = Cl, $O_2CCH_2NH_3Cl$], in aqueous solutions at pH 2–8 have been studied by ^{1}H-NMR spectroscopy. Rapid hydrolysis of both the halide and cyclopentadienyl ligands in Cp_2TiX_2 [X = Cl, $O_2CCH_2NH_3Cl$] occurs to give predominantly insoluble precipitates at pH 7. In contrast, under the same experimental conditions, the predominant species present in aqueous solutions of $(MeCp)_2TiX_2$ [X = Cl, $O_2CCH_2NH_3Cl$] at pH 2–8 contains both methycyclopentadienyl rings metal bound. At pH < 5, Cp_2TiX_2 and $(MeCp)_2TiX_2$ form similar complex(es) with purine nucleotides. However, at pH > 5, while stable adducts between nucleotides and Cp_2TiX_2 are not formed, in the presence of 1 equiv. of 5′-dAMP or 5′-dGMP, $(MeCp)_2TiX_2$ formed complex(es) which were stable for 24 h. These results suggest that formation of stable chelates between $(MeCp)_2TiX_2$ and nucleic acid constituents in vivo is possible.

Keywords: Titanocene dichloride; Bis(methylcyclopentadienyl) titanocene dichloride; Antitumor; Nucleotide-binding; Cp hydrolysis; NMR spectroscopy

1. Introduction

Titanocene dichloride (Cp_2TiCl_2; $Cp = \eta^5$-C_5H_5) **1** has been shown to exhibit antitumor properties against a range of human and murine tumours [1]. The drug entered phase I clinical trials in late 1991 with initial results showing a lack of cross-reactivity against platinum base drugs [2]. The results of further clinical trials are required in order to assess whether the drug has potential as a viable anticancer drug.

The mechanism of antitumour action of titanocene dichloride **1** has not been determined, but interaction with DNA has been implicated in the mechanism of action [3–7]. Efforts to identify the biologically active species have been largely unsuccessful due to both the poor solubility of **1** in water and the hydrolysis of the Cp rings, a reaction which is accelerated at high pH and results in precipitation of uncharacterised hydrolysis products [8]. Samples have been administered in 9:1 saline:DMSO solutions at low pH [9]; preparation of aqueous solutions at pH > 4.5 is not possible as increasing the pH is accompanied by formation of insoluble, uncharacterised precipitates [7,8].

Prompted by initial reports that administration of buffered solutions of titanocene dichloride **1** reduced the side-effects associated with treatment [10], and in an effort to identify the biologically active species, we set out to prepare titanocene derivatives which were relatively stable at pH 7.0 and demonstrated appreciable solubility in water. This paper reports the synthesis, studies of the solubility and hydrolytic stability, and binding to nucleotides of bis(methylcyclopentadienyl)

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[1] Dedicated to Professor Michael Bruce on the occasion of his 60th birthday.

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titanocene dichloride $(MeCp)_2TiCl_2$ **3** and the bisglycine analogue $(MeCp)_2Ti(O_2CCH_2NH_3Cl)_2$ **4** in comparison to the unsubstituted metallocenes Cp_2TiCl_2 **1** and $Cp_2Ti(O_2CCH_2NH_3Cl)_2$ **2,** respectively. Alkyl substitution of the Cp rings has been reported to improve the hydrolytic stability of titanocene dichloride [11], but introduction of hydrophobic groups generally decreases aqueous solubility. Introduction of glycine ligands [12] gives the highly water soluble complex **4** in which the Cp rings remain metal bound at physiological pH and which forms stable complexes with nucleotides at pH 7.

2. Results and discussion

2.1. Synthesis of complexes

Bis(methylcyclopentadienyl) titanocene dichloride **3** was prepared using a similar procedure to that reported in the literature [13]. Thus, methylcyclopentadiene, generated from freshly cracked dimethylcyclopentadiene, was treated with sodium sand followed by titanium(IV) tetrachloride to give **3** in 25% yield. As reported, considerable difficulty was experienced in obtaining **3** in high purity due to the presence of significant amounts of dicylopentadiene in commercially available methylcyclopentadiene dimer [12]. Careful, multiple distillations were the most effective way of obtaining highly pure methylcyclopentadiene and avoid formation of minor amounts of $Cp(MeCp)TiCl_2$ along with **3** in the reaction.

The glycine analogue **4** was prepared in 75% yield using a similar procedure to that reported for the preparation of titanocene bis(glycine) **2** [12]. Thus, treatment of bis(methylcyclopentadienyl) titanocene dichloride **3** with glycine (2 eq.) in methanol at r.t. afforded the orange complex **4**. The complex was washed with chloroform to remove any unreacted starting material to give **4** which was pure by microanalysis and NMR spectroscopy. In our hands the success of the reaction was highly variable and frequently material was isolated that co-crystallised with unreacted glycine. Attempts to further purify such material was generally unsuccessful.

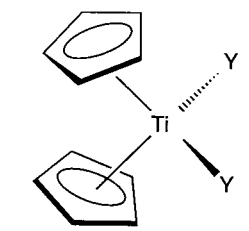
2.2. Solubility properties

Titanocene dichloride **1** has limited solubility in water, but sonication in water results in complete dissolution after 5–10 min to give a clear yellow solution. In comparison, introduction of the two methyl groups to give derivative **3**, not surprisingly, reduces the aqueous solubility significantly and sonication for 2–3 h is required to give a saturated yellow solution that frequently contains undissolved material. In the case of **4**, the charged glycine ligands greatly improve aqueous solubility and the complex dissolves immediately in water and no sonication is required. Replacement of the halide ligands of metallocenes with charged or hydrophilic ligands in order to improve aqueous solubility, including the synthesis and improved solubility of complex **2**, has been reported previously [12,14].

2.3. Hydrolysis studies

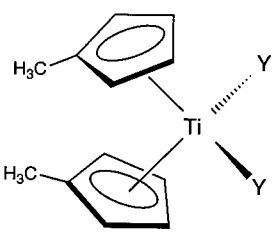
The rate of halide and Cp ring hydrolysis in titanocene dichloride **1** has been well-characterised [8]. Rapid hydrolysis of the two halide ligands occurs in a series of equilibrium reactions to give a solution at pH ca. 2 in which the Cp rings remain metal bound for >24 h. However, at higher pH values protonolysis of the Cp rings occurs to give cyclopentadiene and dicyclopentadiene as well as insoluble, uncharacterised hydrolysis products. The exact nature of the pseudohalide ligands have not been identified with $Cp_2TiCl_{2(aq)}$ likely to contain a number of species such as $Cp_2Ti(H_2O)Cl^+$, or $Cp_2Ti(H_2O)_x(OH)_y^{(2-y)+}$.

While the synthesis of **2** has been reported [13], complete hydrolysis studies in water have not been carried out. In the solid state the complex crystallises from water in an oligomeric form in which the glycine carboxylates are coordinated to the metal [12]. This fact, taken with the downfield chemical shift of the glycine methylene in the ^{1}H-NMR spectrum of **2** (3.38 ppm) compared with unbound glycine (3.68 ppm) was taken as evidence that the glycine ligands are directly coordinated to the metal centre in solution as well as the solid state [12]. The reported changes in chemical shift on coordination of the glycine carboxylate to the metal centre do not appear to have taken into account the change in solution pH. We have carried out careful comparison of the NMR spectra of free glycine at pD 2–8 with a solution of $Cp_2Ti(O_2CCH_2NH_3Cl)_2$ at the same pD values. These experiments showed that there is no change in chemical shift of free glycine and the glycine resonances in complex **2** at the same pD value. While there is no doubt that in the solid state the complex crystallises with the amino acid directly coordinated to the metal [12], our pD titration experiments plus binding and competition studies described below are consistent with only a weak interaction between the glycines and the metal centre in aqueous solution.



1 Y = Cl

2 Y = O–C(=O)–CH$_2$–NH$_3$Cl



3 Y = Cl

4 Y = O–C(=O)–CH$_2$–NH$_3$Cl

Table 1
Hydrolysis of aromatic rings in complexes **1–4** at various pD values over 24 h

Complex	X = Cl				X = gly			
	pD[a]	Time (h)[b]	% Precipitated	% Hydrolysis	pD[a]	Time (h)[b]	% Precipitated[c]	% Hydrolysis
Cp_2TiX_2	2.0–2.1	1	–	0	3.0–3.1	1	0	0
		24	–	<2[d]		24	4	<2[d]
X = Cl	6.1–6.2	0.25	–	15	5.8–6.3	0.25	60	2
1		24	–	>90[e]		24	>90	>90[e]
$X = O_2CCH_2NH_3Cl$	>6.4	–	>95[f]	–	>6.4	–	>95[f]	–
2								
$(CH_3Cp)_2Ti$	1.8–2.0	1	–	0	2.9–3.1	1	0	0
		24	–	<2[d]		24	12	<2[d]
X = Cl	5.9–6.0	0.25	–	0	5.7–6.0	0.25	21	0
3		24	–	<2[d]		24	56	<2[d]
$X = O_2CCH_2NH_3Cl$	7.5–7.9	0.25	–	<2[d]	7.5–7.8	0.25	43	<2[d]
4		24	–	<5[g]		24	88	<5[g]

[a] For pD 1.8–3.1: pD values were monitored with time and did not drop by more than 0.3 units over 24 h. For pD > 5.4: pD values were monitored with time and adjusted to the required pD range prior to recording NMR spectrum; a drop of pD unit over 24 h was typical.
[b] Time = 0 h refers to when all sample had dissolved and pH was adjusted to required value.
[c] Calculated on the ratio of bound Cp signal to CH_2 signal of glycine when compared to the ratio at t = 1 h.
[d] Estimated by integration of the signals from protonated Cp or MeCp dimer/monomer; <2 when the amount of signal was too small to be integrated accurately.
[e] Hydrolysis was estimated to be >90% since the amount of bound Cp signal was negligible.
[f] Above pD values of about 6.4 complexes **1** and **2** were hydrolysed and precipitated completely giving no signal in the NMR and so the % precipitated was estimated at >95%.
[g] Estimated at <5% based on the amount of dimer and monomer peaks.

The rate of Cp hydrolysis in **1–4** was measured using ^{1}H-NMR spectroscopy at different pD values and times in D_2O at 25°C (Table 1). In the case of **1** and **2**, the rate of Cp ring hydrolysis at three different pD values was very similar. While hydrolysis of both **1** and **2** was accompanied by formation of a minor amount of insoluble precipitate at pD ca. 2, precipitation increased at higher pD values with >90% of the metallocene hydrolysed at pD 7.0. In the case of **1**, the amount of precipitate (and hence hydrolysis) could only be estimated visually or by isolation and weighing the precipitate. However, in the case of **2**, a more accurate estimate was possible by integration of the glycine CH_2 peak versus the cyclopentadienyl protons (Table 1). Fully dissolved complex **2** gives by integration a ratio of 10:4 = Cp:CH_2. As precipitation occurred the Cp signal decreased relative to the glycine peak consistent with formation of hydrolysis products derived from the Cp rings.

The effect of the methyl group on the rate of ring hydrolysis was determined by carrying out similar experiments on **3** and **4**. An estimate of the MeCp hydrolysis was calculated from the relative intensities of the new multiplets due to methylcyclopentadiene (6.5 and 6.6 ppm at pD 7.4) which appear downfield of the metal-bound MeCp multiplet (6.4 ppm at pD 7.4) and the new methyl resonances (ca. 2 ppm) of the spectrum. As expected, the halide ligand had little effect on the hydrolysis experiments and similar results were obtained for both complexes **3** and **4** at similar pD values (Table 1). At low pD values a minor amount of precipitate formed with time; the amount of precipitate increased when the solution pD was raised to ca. 7, and was estimated as described above (Table 1). NMR analysis of the supernatant showed that the MeCp rings remained metal bound. The yellow precipitated solid was isolated but due to poor solubility could not be fully characterised.

The hydrolysis studies of both **3** and **4** are consistent with loss of the halide and glycine ligands respectively in water to give the same species, in which both MeCp rings are bound to the metal, i.e. '$(MeCp)_2Ti^{2+}$'. In order to confirm this interpretation, a mixed experiment was carried out. Equimolar amounts of **3** and **4** were dissolved and spectra recorded at different pD values. In all cases only one set of MeCp signals was observed consistent with formation of the same (or very similar) species in solution, presumably aquated species such as $(MeCp_2)Ti(H_2O)_2^{2+}$ or $(MeCp_2)Ti(H_2O)_x(OH)_y^{(2-y)+}$ at pH 7.0. A similar result was obtained on a mixed experiment with **1** and **2**.

2.4. Nucleotide binding studies

The major aim of this work was to prepare water soluble, hydrolytically stable titanocene derivatives that interact with nucleic acid constituents in the same way as titanocene dichloride, and hence may be studied (and administered) in preparations at pH 7.0. To this end, nucleotide binding studies have been carried out with **3** and **4**, and the stabilities, and types of complexes formed with nucleotides compared with those formed by **1** and **2**.

Complexes **3** and **4** were used in separate titration experiments with the nucleotides 5′-dAMP (Figs. 1 and 2) and 5′-dGMP (spectra not shown) at different pD values, using procedures previously described [7]. In each case, new signals were detected, and the percentage complexation was determined by integration of the resonances in the region 7.5–9 ppm corresponding to either the purine or pyrimidine protons at either pD 4 or pD 7 (Figs. 1 and 2, Table 2).

Fig. 1 compares the complexes formed between 5′-dAMP and 1 equiv. of either **1** or **2** (Cp_2TiX_2; Fig. 1b) and 5′-dAMP plus 1 equiv. of either **3** or **4** [$(MeCp)_2TiX_2$; Fig. 1c). In all four spectra, signals assigned to uncomplexed nucleotide (Fig. 1a) are present (indicated by dashed lines), and new signals assigned to the formation of metallocene-nucleotide complex(es) are detected. ^{31}P-NMR spectra of $(MeCp)_2TiCl_2$ (not shown) also showed the appearance of three new signals, consistent with interaction of the metal centre with the phosphate(O) of the nucleotide. While the relative intensities of the signals due to the complex(es) in the ^{1}H-NMR spectra (Fig. 1) are slightly different in each spectrum, when one takes into account the slight variations in solution pD, the spectra are remarkably similar. The effect of the methyl groups on the Cp rings at this pD value (Fig. 1c) appears to slightly reduce the amount of complexation compared with Cp_2TiX_2 (Fig. 1b). However, the presence of the same number of new signals at the same chemical shift values, strongly supports the formation of the same types of complex(es) in each case.

The effect of the X ligand on complexation is apparent by comparison of the pair of spectra in either Fig. 1b or in Fig. 1c. Very similar spectra were obtained at each pD value consistent with the conclusions of our hydrolysis studies that indicate that in solution, both the chloride and glycine ligands dissociate to generate either 'Cp_2Ti^{2+}' in the case of **1** and **2**, and '$(MeCp)_2Ti^{2+}$' in the case of **3** and **4**.

Fig. 2. shows the effect of increasing the solution pD to ca. 7.4 on the relative stabilities of the complexes formed by **3** and **4** at low pD. Spectra are not presented for Cp_2TiX_2, since as reported previously for titanocene dichloride **1**, and also observed by us for **2**, as soon as the pD is raised above 5.4, complete hydrolysis and concomitant precipitation occurs and only signals due to the nucleotide (i.e. Fig. 2a) are detected. In contrast to this result, clear evidence for formation of stable adducts between $(MeCp)_2TiX_2$ at pD ca. 7.5 was obtained (Fig. 2). Fig. 2b shows the spectra obtained on titration of 1 equiv. of 5′-dAMP into a solution of $(MeCp)_2TiX_2$ followed by immediate adjustment of the solution pD to 7.9 (X = Cl) and 7.7 (X = $O_2CCH_2NH_3Cl$). Fig. 2c shows the NMR spectra obtained on the same samples after 24 h. Raising the solution pD from 4 to 7.5 is accompanied by some precipitation, but distinct signals assigned to metallocene complexes are clearly present at pD ca. 7.5 (Fig. 2b). The relative amount of complex is however, reduced to that present at lower pD values (Table 2). After 24 h, the amount of hydrolysis increased, as evidenced by increased precipitation, and the relative amount of complex(es) was reduced (Fig. 2c). As was noted for the spectra in Fig. 1, the slight differences

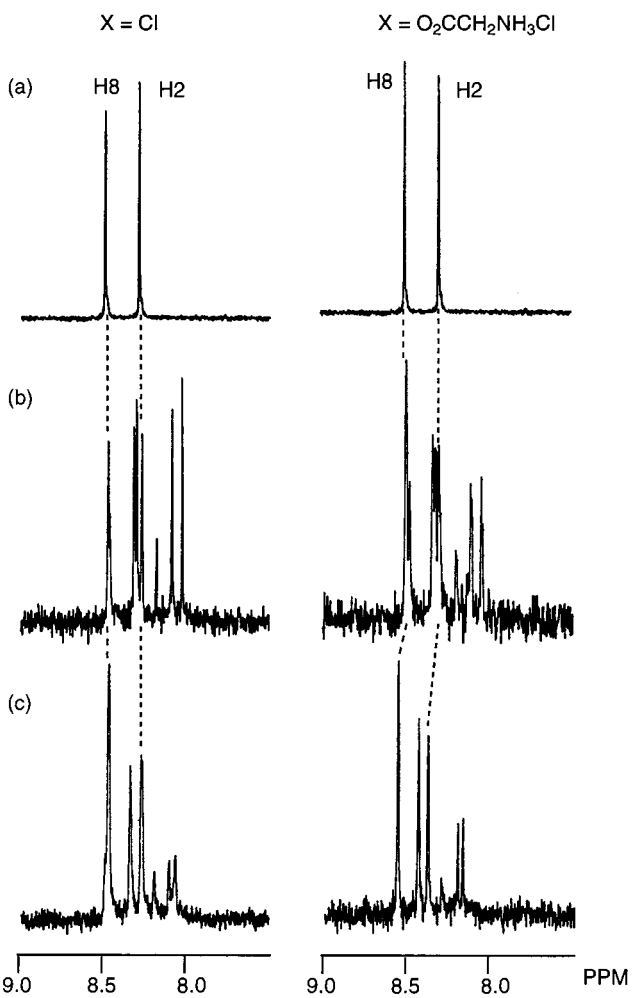


Fig. 1. Aromatic region of ^{1}H-NMR spectra (200 MHz, D_2O) of (a) 5′-dAMP, pD 4.9; (b) 5′-dAMP + 1 equiv. $(MeCp)_2TiX_2$ t = 30 min, pD 4.9 (X = Cl), 4.7 (X = gly); (c) 5′-dAMP + 1 equiv. Cp_2TiX_2, t = 30 min, pD 5.0 (X = Cl), 4.0 (X = gly).

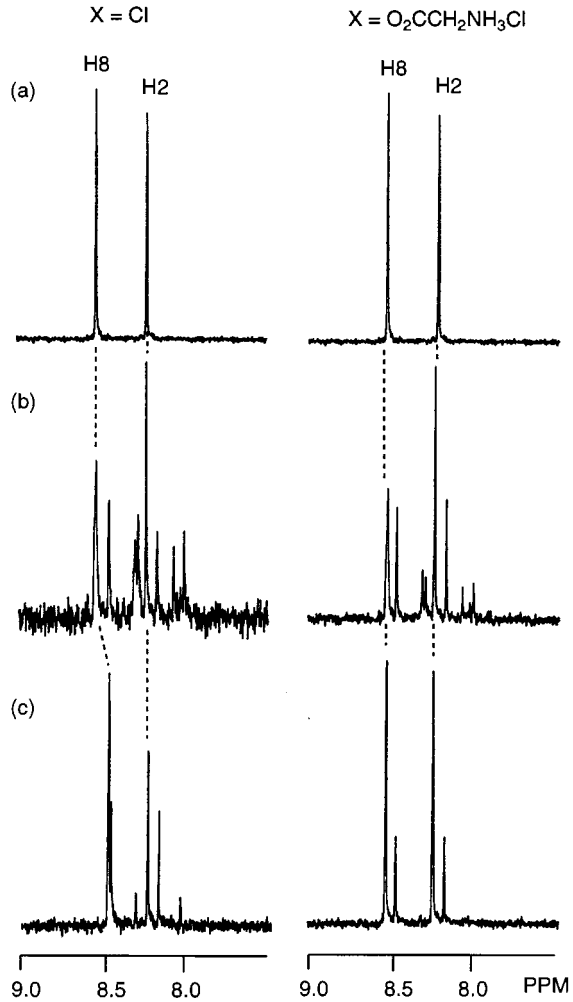


Fig. 2. Aromatic region of ^{1}H-NMR spectra (200 MHz, D_2O) of (a) 5′-dAMP, pD 7.7; (b) 5′-dAMP + 1 equiv. $(MeCp)_2TiX_2$ t = 30 min, pD 7.9 (X = Cl), 7.7 (X = gly); (c) 5′-dAMP + 1 equiv. $(MeCp)_2TiX_2$, t = 24 h, pD 7.8 (X = Cl), 7.5 (X = gly).

between the relative intensities of the signals in the spectra are within the experimental limits of fluctuation of pD with time.

Similar trends were observed with 5′-dGMP (spectra not shown), with the results summarised in Table 2. Analogous studies with 5′-dUMP and 5′-dCMP were carried out, but due to signal overlap between the pyrimidine protons from the complex(es) and unbound nucleotide, accurate integration and assignment of signals was not possible. For this reason, quantitative data for the pyrimidines are not included in Table 2. The exact structures of the species formed in solution by $(MeCp)_2TiX_2$ and the nucleotides 5′-dAMP and 5′-dGMP cannot be unambiguously assigned from the NMR data. However, comparison of the chemical shifts of the new aromatic resonances observed in the ^{1}H-NMR spectra with those observed previously with Cp_2TiX_2 [7], as well as new signals in the ^{31}P-NMR spectra, are consistent with phosphate(*O*) complexation along with base(*N*) coordination and the complexes are most probably analogous to the well-characterized Mo-adducts (e.g. Fig. 3) [7,15]. The methyl groups do not appear to impede complex formation or alter the binding mode compared to titanocene dichloride **1**.

2.5. Conclusions

The rate of hydrolysis of the aromatic rings in $(MeCp)_2TiX_2$ (X = Cl, $O_2CCH_2NH_3Cl$) in water at pH 7 is significantly slower compared to the corresponding unsubstituted metallocenes Cp_2TiX_2. While methyl substitution of the Cp rings decreases aqueous solubility, replacement of the halide ligands with charged glycine ligands gives the highly water soluble complex **4** in which the Cp rings remain metal bound at physiological pH, and which forms complexes with nucleotides which are stable for 24 h at pD 7.4. Substitution of alkyl groups on titanocene dichloride has been reported to generally decrease the antitumour activity of the drug [16]. However, the reduced aqueous solubility of the complexes as well as administration of the compounds in DMSO/saline at low pH needs to be taken into account in the interpretation of biological data. While the glycine ligands present in both **2** and **4** improve aquoues solubility, these ligands may affect the rate of cellular uptake of the metallocenes. Screening of

Table 2
Percentage complexation between Cp_2TiX_2 and $(MeCp)_2TiX_2$ and nucleotides[a]

Complex	+1 eq. Nucleotide	X = Cl			X = gly		
		t (h)	pD	% Complex	t (h)	pD	% Complex
$(MeCp)_2TiX_2$ X = Cl **3**	5′-dAMP	0.5	4.9	70	0.5	4.7	60
		0.5	7.9	62	0.5	7.7	50
		24	7.8	40	24	7.5	30
X = $O_2CCH_2NH_3Cl$ **4**	5′-dGMP	0.5	4.7	60	0.5	3.4	60
		0.5	10.1	14	0.5	7.8	30
		24	7.5	14	24	7.5	20
Cp_2TiX_2 X = Cl **1**	5′-dAMP	0.5	5.0	40	0.5	4.0	50
X = $O_2CCH_2NH_3Cl$ **2**	5′-dGMP	0.5	5.0	45	0.5	4.6	55

[a] Determined by integration of purine aromatic resonances in ^{1}H-NMR spectra.

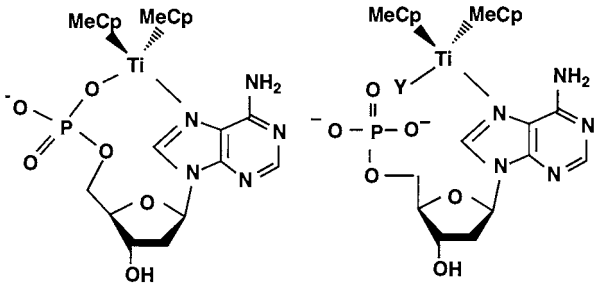


Fig. 3. Tentative assignment of possible complexes formed between $(Cp_2Me)_2TiX_2$ and 5′-dAMP at pH 7.

complex **4**, which may be administered in aqueous solution at pH 7.4, and is capable of formation of stable adducts with nucleotides may provide further insight into the mechanism of antitumour action of titanocene based metallocenes.

3. Experimental

3.1. General

Nucleotides were purchased from Sigma Chemical Company and were used as provided. Titanocene dichloride **1** was obtained from the Aldrich Chemical Company. The bisglycine analogue **2** was prepared from titanocene dichloride **1** according to the literature procedure [12]. Melting points were determined on a Reichert heating stage and are uncorrected. Mass spectra were recorded on an A.E.I. MS-902 spectrometer at 70 eV. Values of m/z are quoted with intensities expressed as percentages of the base peak in parentheses. ^{1}H-NMR spectra were recorded on a Bruker AC200 NMR spectrometer and were referenced to TSP (0.00 ppm). ^{31}P-NMR spectra were recorded on a Bruker AMX400 spectrometer (161.98 MHz) and were referenced to external neat trimethyl phosphite (140.85 ppm)

3.2. Preparation of bis(methylcyclopentadiene) titanocene dichloride *3*

Methylcyclopentadiene dimer (200 ml) was cracked at >200°C over a 20 cm column of glass beads to give methylcyclopentadiene (b.p. 72°C) which was collected at -78°C, recracked and redistilled several times through a 40 cm column of glass beads, and stored at -78°C until used. The initial low boiling fractions (<70°C) were discarded. Sodium sand was generated by refluxing sodium (4.7 g, 0.20 mol) in toluene (100 ml). The toluene was removed, and the sodium sand was washed with THF (3×25 ml) and THF (50 ml) was added. Methylcyclopentadiene (13 ml, 10.4 g, 0.13 mol) was added dropwise over 5 min to the suspension of sodium sand at -10 to -15°C. The reaction mixture was stirred for 5 h at r.t., and filtered through a U-tube containing glass wool. A sample of the filtered reaction mixture containing sodium methylcyclopentadiene (14 ml, 36 mmol, 2.6 M) was added dropwise to titanium(IV) tetrachloride (1 ml, 9 mmol) in THF (40 ml) at -20°C. The reaction mixture was stirred overnight at r.t., the solvents were removed and the brown–red residue was dissolved in chloroform (70 ml) and stirred with flash silica for 40 min. The reaction mixture was filtered and the residual flash silica was further extracted with chloroform (2×100 ml). The solvent was removed from the dark, blood-red filtrate to give a brown–red precipitate which was dried under vacuum. The crude product was recrystallised several times from chloroform to give bis(methylcyclopentadienyl) titanocene dichloride **3** (350 mg, 14%) as a red diamond shaped crystalline solid, m.p. dec. >200°C (lit. [13] dec. 217–218°C). Anal. Calcd for $(MeCp)_2TiCl_2$: C, 52.0; H, 5.1; Cl, 25.6. Found: C, 52.3; H, 5.0; Cl, 25.5. ^{1}H-NMR (D_2O, pD 1.5) δ 6.3–6.4 (m, 8H, Cp), 2.3 (s, 6H, Me). EI MS m/z 276 [$(MeCp)_2TiCl_2^+$, 29%]; 241 [$(MeCp)_2TiCl^+$, 31]; 204 [$(MeCp)_2Ti^+$, 69]; 197 [$(MeCp)TiCl_2^+$, 97]; 161 [$(MeCp)TiCl^+$, 100]; 126 [$(MeCp)Ti^+$, 37].

3.3. Preparation of bis(methylcyclopentadienyl) titanocene bis(glycine) *4*

Bis(methylcyclopentadiene) titanocene dichloride **3** (280 mg, 1.0 mmol) and glycine (150 mg, 2.0 mmol) were stirred in methanol (20 ml) at r.t. under a nitrogen atmosphere for 4 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness. The semicrystalline product solidified after 1 day at -17°C. The solid was washed with chloroform and dried under vacuum to give bis(methylcyclopentadienyl) titanocene bis(glycine) **4** (340 mg, 75%) as an orange crystalline solid, m.p. dec. >200°C. Anal. Calcd for $(MeCp)_2Ti(OOCCH_2NH_3Cl)_2\cdot1.5H_2O$: C, 42.3; H, 6.0; N, 6.2. Found: C, 42.1; H, 5.8; N, 6.4. ^{1}H-NMR (D_2O, pD 2.5) δ 6.4 (m, 8H, MeCp), 3.68 (s, 4H, CH_2), 2.01 (s, 6H, Me).

3.4. Hydrolysis experiments

The general procedure involved dissolving 5–15 μmol of the complex in 500 μl D_2O with TSP added for reference. Dissolution of **3** required sonication for 200 min to give a clear yellow solution. Complex **4** was dissolved by shaking for 2 min. Sonication was carried out with an Elma Transsonic Digital Bath. The solution pD was adjusted with DCl and NaOD. pD values were measured using a Beckman Φ11 meter and a Mettler NMR tube pH probe and are related to the pH meter reading by the formula pD = pH(meter reading) + 0.4 [17]. Measured pD values are ±0.3 due to fluctuations

in sample pH which occurred over 30 min. ^{1}H-NMR spectra were recorded at time intervals with any developing precipitate ignored. The rate of Cp hydrolysis was estimated by integration of one of the two multiplets arising from free cyclopentadiene C_5H_5D (6.5 and 6.6 ppm at pD = 6.2) versus the metal-bound C_5H_5 signals.

Acknowledgements

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