Functionalized Cyclopentadienyl Titanium Organometallic Compounds as New Antitumor Drugs

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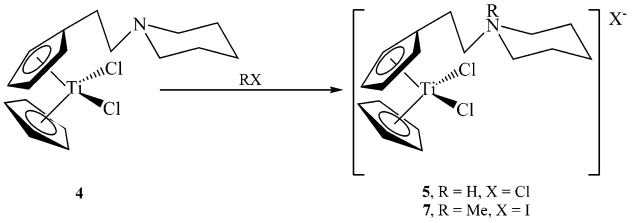
Received May 29, 2003

A number of new ionic titanocene compounds have been isolated and characterized. These exhibit significant cytotoxicity against a number of different human tumor cell lines including a defined cisplatin-resistant cell line. One of the compounds is an order of magnitude more active than its nonfunctionalized equivalent.

Introduction

There has been a vigorous quest to locate effective new anticancer drugs. Preferably these would have fewer side effects and have activity against tumors that are resistant to current drugs. Among the candidates for antitumor agents have been the early transition metal compounds metallocene dichlorides [$(C_5H_5)_2MCl_2$, M = Ti, V, Nb, Mo, Re]. The antitumor activity of both titanocene (bis-cyclopentadienyltitanium) dichloride, [$(C_5H_5)_2TiCl_2$], or Cp_2TiCl_2, and vanadocene (bis-cyclopentadienylvanadium) dichloride, [$(C_5H_5)_2VCl_2$], or Cp_2VCl_2, has been established against various animal and xenografted human tumors.[1,2] Titanocene dichloride is one of the most effective antitumor agents of this type.[3] However, by their very nature, Cp_2TiCl_2 and Cp_2VCl_2 are flawed as drugs because of hydrolysis problems.[4,5] The consequence of this is that the nature of the active species is unknown and the administration of the compound as a drug can be difficult. We have been interested in the synthesis of water-soluble cyclopentadienyl compounds for some time.[6−11] The compounds presented here will help to solve some of the problems normally associated with the water insolubility and instability of this type of compound.

Scheme 1. Synthesis of Compounds 5 and 7



4 **5**, R = H, X = Cl; **7**, R = Me, X = I

Results and Discussion

Addition of an amino pendant arms attached to the Cp ring will allow the ionic functionality to be incorporated onto the metallocene dichloride entity. We were interested in synthesizing a range of different target compounds in order to exploit differences in their biological activity. The syntheses of $[C_5H_4(CH_2)_2N(CH_2)_5]_2TiCl_2 \cdot 2HCl$, **1**, and $[C_5H_4CH(CH_2)_4NMe]_2TiCl_2 \cdot 2HCl$, **2**, have been described by us previously.[12,13] The synthesis of $[C_5H_4(CH_2)_2NMe(CH_2)_5]_2TiCl_2 \cdot 2[I]$, **3**, was achieved by reaction of $[C_5H_4(CH_2)_2N(CH_2)_5]_2TiCl_2$ with MeI. Compound **3** was characterized by microanalysis, ^1H and ^{13}C NMR spectroscopy, and mass spectroscopy.

Changing the structure of the molecule to contain one ionic arm was also achieved synthetically. Reaction of the neutral monoamino-functionalized titanocene dichloride $[\eta\text{-}C_5H_5][\eta\text{-}C_5H_4(CH_2)_2N(CH_2)_5]TiCl_2$, **4**, with hydrogen chloride yields the salt $[\eta\text{-}C_5H_5][\eta\text{-}C_5H_4(CH_2)_2N(CH_2)_5]TiCl_2 \cdot HCl$, **5**, as shown in Scheme 1. The compound $[C_5H_5][C_5H_4CH(CH_2)_4NMe]TiCl_2 \cdot HCl$, **6**, was prepared directly without isolating the neutral analogue. Compounds similar to these have been investigated by Jutzi for their catalytic properties.[14] Compounds **4**, **5**, and **6** have been characterized by microanalysis,

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(1) Köpf-Maier, P. *Complexes in Cancer Chemotherapy*; VCH: Weinheim, 1993.
(2) Kopf-Maier, P.; Kopf, H. *Met. Compd. Cancer Ther.* **1994**, 109−146.
(3) Christodoulou, C.; Eliopoulos, A. G.; Young, L. S.; Hodgkins, L.; Ferry, D. R.; Kerr, D. J. *Br. J. Cancer* **1998**, *77*, 2088−2097.
(4) Toney, J. H.; Marks, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 947−953.
(5) Harding, M. M.; Mokdsi, G. *Curr. Med. Chem.* **2000**, *7*, 1289−1303.
(6) Corradi, M. M.; McGowan, M. A. D.; McGowan, P. C.; Thornton-Pett, M. *Eur. J. Inorg. Chem.* **2002**, 2362−2366.
(7) Bradley, S.; Camm, K.; Liu, X.; McGowan, P. C.; Mumtaz, R.; Oughton, K. A.; Podesta, T.; Thornton-Pett, M.; Walsh, M. E. *Inorg. Chem.* **2002**, *41*, 715−726.
(8) Bradley, S.; Camm, K. D.; Furtado, S. J.; Gott, A. L.; McGowan, P. C.; Podesta, T. J.; Thornton-Pett, M. *Organometallics* **2002**, *21*, 3443−3453.
(9) Bradley, S.; Camm, K. D.; Corradi, M. M.; Furtado, S. J.; McGowan, P. C.; Mumtaz, R.; Thornton-Pett, M. *J. Organomet. Chem.* **2002**, *656*, 49−56.
(10) McGowan, P. C.; McGowan, M. D. (University of Leeds, UK) PCT Int. Appl. WO, 2001.
(11) Bradley, S.; McGowan, P. C.; Oughton, K. A.; Thornton-Pett, M.; Walsh, M. E. *Chem. Commun. (Cambridge)* **1999**, 77−78.
(12) McGowan, M. A. D.; McGowan, P. C. *Inorg. Chem. Commun.* **2000**, 337−341.
(13) McGowan, M. A. D.; McGowan, P. C. International Patent WO 01/42260, 2001.
(14) Jutzi, P.; Redeker, T.; Neumann, B.; Stammler, H. G. *Chem. Ber.-Recl.* **1996**, *129*, 1509−1515.

10.1021/om030403i CCC: $27.50
Publication on Web 12/18/2003

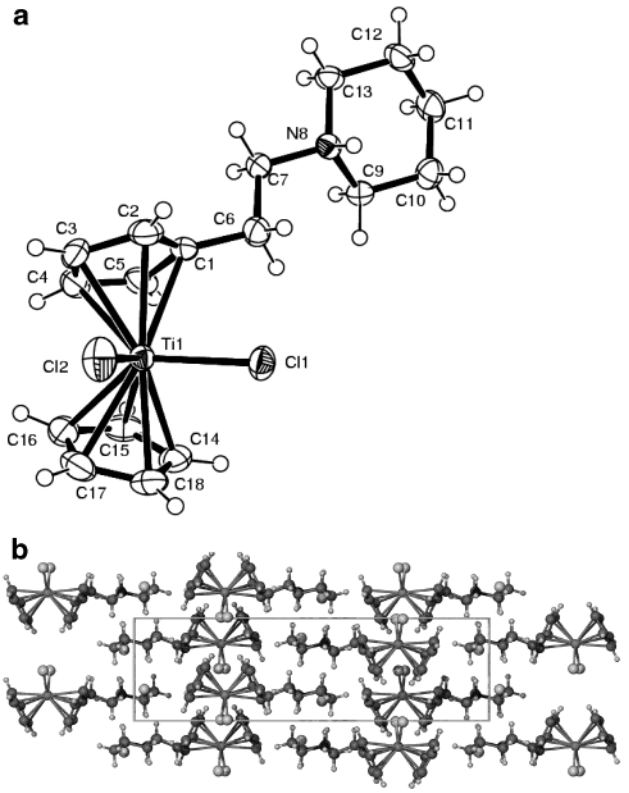

Figure 1. (a) Molecular structure of compound **5**. Chloride counterion omitted for clarity. Probability ellipsoids are at the 50% level. Crystal data for **5**: $C_{17}H_{24}Cl_3NTi$, M = 396.62, monoclinic, space group $P2_1/n$, a = 6.7454(2) Å, b = 23.2486(6) Å, c = 11.7695(4) Å, V = 1845.11(10) Å^3, D_c = 1.428 Mg m^{-3}, Z = 4, μMo = 0.894 mm^{-1}. Crystal size 0.18 × 0.11 × 0.05 mm, min. and max. transmission factors 0.96 and 0.86; 20 440 reflections collected, 2537 observed. Final residuals R, R_w were 0.041, 0.090 for the observed data. (b) Packing diagram of $[C_5H_5][C_5H_4(CH_2)_2N(CH_2)_5]$-$TiCl_2$·HCl.

^{1}H and ^{13}C NMR spectroscopy, and in the case of **5** and **6** X-ray crystallography. The crystal structure of **5** is shown in Figure 1, and the relevant bond lengths and angles are shown in Table 1. It can be seen that there is no interaction between the pendant amino functional group and the titanium center. There is a hydrogen bond between the proton situated on the nitrogen and the Cl^- counterion with a bond length and angle of N(8)−H(8)···Cl(1) = 2.9800 Å and 168.16°. The packing diagram shows layers of the titanium cyclopentadienyl moiety and layers of the piperidyl ligand packing alternately.

The crystal structure of compound **6** is shown in Figure 2, and the relevant bond lengths and angles are shown in Table 2. Like compound **1**, compound **5** is also water soluble and stable. Similar to compound **5**, compound **6** contains a hydrogen bond between the proton situated on the nitrogen and the Cl^- counterion with a bond length and angle of N(8)−H(8)···Cl(1) = 3.0439 Å and 177.54°.

Methylation of the monoamino-functionalized titanocene dichloride $[\eta$-$C_5H_5][\eta$-$C_5H_4(CH_2)_2N(CH_2)_5]TiCl_2$ was achieved in a manner similar to that for compound **3** (Scheme 1). Addition of MeI to a solution of compound **4** resulted in the formation of a red solid, $[C_5H_5]$-$[C_5H_4(CH_2)_2NMe(CH_2)_5]TiCl_2$·[I], **7**, which was washed and dried. The compound $[C_5H_5][C_5H_4CH(CH_2)_4NMe_2$-$TiCl_2$·[I], **8**, was prepared in a similar manner. Compounds **7** and **8** were characterized by NMR spectroscopy and high-resolution mass spectrometry; repeated attempts of obtaining satisfactory microanalyses failed.

Table 1. Selected Bond Lengths (Å) and Angles (deg) for 5[a]

Ti(1)−Cl(1)	2.3582(8)	Ti(1)−Cl(2)	2.3716(9)
C(1)−C(2)	1.397(4)	C(14)−C(15)	1.388(4)
C(2)−C(3)	1.421(4)	C(15)−C(16)	1.405(5)
C(3)−C(4)	1.390(5)	C(16)−C(17)	1.380(5)
C(4)−C(5)	1.408(5)	C(17)−C(18)	1.384(5)
C(5)−C(1)	1.397(4)	C(18)−C(14)	1.396(4)
C(1)−C(6)	1.498(4)	C(6)−C(7)	1.521(4)
C(7)−N(8)	1.502(3)	N(8)−C(9)	1.493(3)
C(9)−C(10)	1.515(4)	C(10)−C(11)	1.527(4)
C(11)−C(12)	1.523(4)	C(12)−C(13)	1.521(4)
N(8)−C(13)	1.507(3)		
Ti(1)−Cp[cent−C(1)−C(5)]	2.07	Ti(1)−Cp[cent−C(14)−C(18)]	2.05
Cl(1)−Ti(1)−Cl(2)	92.68(3)		
C(1)−C(2)−C(3)	108.3(3)	C(14)−C(15)−C(16)	107.8(3)
C(2)−C(3)−C(4)	107.6(3)	C(15)−C(16)−C(17)	107.6(3)
C(3)−C(4)−C(5)	107.9(3)	C(16)−C(17)−C(18)	108.7(3)
C(4)−C(5)−C(1)	108.7(3)	C(17)−C(18)−C(14)	107.9(3)
C(5)−C(1)−C(2)	107.5(3)	C(18)−C(14)−C(15)	107.9(3)
C(5)−C(1)−C(6)	125.3(3)	C(9)−C(10)−C(11)	110.6(2)
C(6)−C(7)−N(8)	111.4(2)	Cp(cent1)−Ti(1)−Cl(1)	107.1
N(8)−C(9)−C(10)	110.8(2)	Cp(cent2)−Ti(1)−Cl(1)	106.2
C(10)−C(11)−C(12)	109.4(2)	Cp(cent1)−Ti(1)−Cp(cent2)	131.2

[a] Estimated standard deviations are in parentheses.

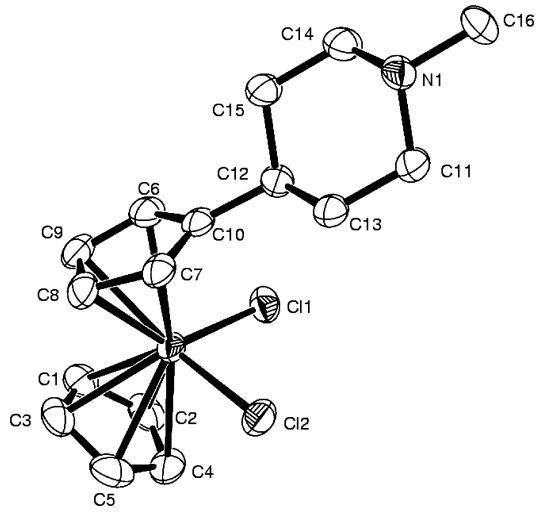

Figure 2. Molecular structure of compound **6**. Chloride counterion omitted for clarity. Probability ellipsoids are at the 50% level. Crystal data for **6**: $C_{16}H_{22}Cl_3NTi$, M = 382.6, monoclinic, space group $P2_1/a$, a = 12.7597(4) Å, b = 6.5512(2) Å, c = 21.2436(7) Å, V = 1729.74(9) Å^3, D_c = 1.469 Mg m^{-3}, Z = 4, μMo = 0.894 mm^{-1}. Crystal size 0.2 × 0.2 × 0.2 mm, min. and max. transmission factors 0.833 and 0.833; 12 224 reflections collected, 2497 observed. Final residuals R, R_w were 0.0437, 0.111 for the observed data.

To evaluate the efficacy of the ionic metallocenes as antitumor agents, many of the compounds were tested against a range of cell lines.[15] Compounds **1** and **2** were cytotoxic against a range of human tumor cell lines. This was deduced using a cytotoxicity assay known as the SRB (sulfur rhodamine buffer) assay that determines the concentration of drug required to inhibit cell growth to 50% of untreated controls (IC_{50}). These values are

(15) Knox, R. J.; McGowan, P. C. (University of Leeds, UK) British Patent Appl. No. 0215593.5, UK, 2002.

Table 2. Selected Bond Lengths (Å) and Angles (deg) for 6[a]

Ti(1)−Cl(1)	2.3578(8)	Ti(1)−Cl(2)	2.3675(8)
Ti(1)−C(1)	2.368(3)	Ti(1)−C(3)	2.375(3)
Ti(1)−C(5)	2.384(3)	Ti(1)−C(4)	2.390(3)
Ti(1)−C(6)	2.401(3)	Ti(1)−C(2)	2.404(3)
Ti(1)−C(7)	2.418(3)	Ti(1)−C(10)	2.463(3)
Ti(1)−C(8)	2.324(3)	Ti(1)−C(9)	2.339(3)
C(6)−C(9)	1.399(4)	C(6)−C(10)	1.418(4)
C(16)−N(1)	1.490(3)	C(7)−C(10)	1.394(4)
C(7)−C(8)	1.411(4)	N(1)−C(14)	1.486(4)
N(1)−C(11)	1.502(3)	C(11)−C(13)	1.513(4)
C(2)−C(4)	1.385(5)	C(2)−C(1)	1.405(5)
C(12)−C(10)	1.509(4)	C(12)−C(13)	1.513(4)
C(12)−C(15)	1.540(4)	C(8)−C(9)	1.405(5)
C(3)−C(5)	1.375(4)	C(3)−C(1)	1.395(5)
C(4)−C(5)	1.403(5)	C(14)−C(15)	1.516(4)
Cl(1)−Ti(1)−Cl(2)	94.63(3)		
C(9)−C(6)−C(10)	108.5(3)	C(10)−C(7)−C(8)	108.9(3)
C(14)−N(1)−C(16)	110.9(2)	C(14)−N(1)−C(11)	112.1(2)
C(16)−N(1)−C(11)	111.1(2)	N(1)−C(11)−C(13)	110.1(2)
C(4)−C(2)−C(1)	108.0(3)	C(10)−C(12)−C(13)	114.0(2)
C(10)−C(12)−C(15)	109.3(2)	C(13)−C(12)−C(15)	108.3(2)
C(9)−C(8)−C(7)	107.6(3)	C(5)−C(3)−C(1)	108.9(3)

[a] Estimated standard deviations are in parentheses.

Table 3. Sensitivity of Cell Lines to Cisplatin and Compounds 1 and 2

cell line	drug	IC_{50} (μM)	differential (μM)
MCF-7	cisplatin	9	
	1	62	6.9
LoVo	cisplatin	0.37	
	1	62	167.6
LS 174T	cisplatin	0.50	
	1	36	72
A2780	cisplatin	0.82	
	1	31	37.8
	2	203	247.6
A2780cis	cisplatin	2.1	
	1	28	13.3
	2	193	91.9
A2780 (24 h)	cisplatin	1	
	1	168	168
A2780cis (24 h)	cisplatin	5.4	

presented in Table 3 and compared with the values obtained with the established antitumor agent cisplatin. Although cisplatin is more potent (on a dose basis) than **1**, there is a wide difference in the sensitivity of the cell lines to cisplatin, while the response to **1** was very similar. Thus MCF-7 cells are 24-fold more resistant to cisplatin than LoVo cells in this assay, but they have the identical sensitivity to **1**. It would appear that **1** is active in tumor cell lines that are resistant to cisplatin. This was further investigated using the ovarian tumor cell line A2780 and its cisplatin-resistant counterpart A2780cis. The A2780cis cell line has been defined, and the resistance is due to an enhanced DNA-repair mechanism. This resistance mechanism is completely ablated by both **1** and **2**, and indeed the resistant cell line appears to be slightly more sensitive to these agents than the parental line The effect was more marked when the length of drug exposure is shortened to 24 h for compound **1** (Table 4).

Of particular significance is that compound **1** appears to be more active when compared to Cp_2TiCl_2: **1** is almost a factor of 10 more potent than Cp_2TiCl_2.[3] It was also noted that the cytotoxicity of **1** decreased significantly when drug exposure time was shortened to 24 h (Table 4). This was not seen with cisplatin. Cisplatin is cytotoxic because it can react with nucleophilic sites

Table 4. Relative Sensitivity of A2780 and A2780cis Cell Lines to Cisplatin and Compounds 1 and 2

cell line	exposure time (h)	cisplatin	**1**	**2**
A2780	144	1	1	1
A2780cis	144	2.56	0.90	0.95
A2780	24	1.22	5.42	
A2780cis	24	6.59	4.97	

Table 5. Sensitivity of A2780 and A2780cis Cell Lines to Compounds 3, 5, 7, 8, and Cisplatin

drug	IC_{50} (μM), A2780 cell line,	IC_{50} (μM), A2780cis cell line
cisplatin	0.54	2.1
3	113.8	5.2
5	796.5	55.5
7	197.7	23.6
8	172.0	17.8

such as those found in DNA. This can only happen after at least one of its chloride ligands is replaced by water to generate an electrophilic aquo species. The rate of aquation of cisplatin is relatively fast such that all the drug will have reacted within 24 h.[16] If **1** reacts by a similar mechanism, the fact that the cytotoxicity increases significantly after 24 h exposure would indicate that **1** is much less labile than cisplatin and more analogous to carboplatin, another established platinum-based antitumor agent.

There is great potential for variation of the complexes' characteristics with changes in both ligand and metal in identifying suitable antitumor reagents with low toxicity. As an example, it can be shown that changing the number of amino functional groups attached to the cyclopentadienyl moieties has a dramatic effect on the efficacy of the drugs, i.e., IC_{50} values for compound **5** as indicated in Table 5. Thus for the A2780 cell line the IC_{50} values for compound **5**, which has only one pendant group, are approximately 20 times greater than those of compound **1** (31 μM vs 796 μM). But interestingly for the cisplatin-resistant cell line of A2870-cis, the IC_{50} values for compounds **3**, **5**, **7**, and **8** are approximately of the same magnitude as compound **1** (Table 5).

It can be envisaged that we will be able to construct some structure−activity relationships in order to determine the factors needed to enhance the anticancer activity. For the ionic metallocenes discussed here, we could vary the (i) transition metal, (ii) counterions, (iii) spacer linking the Cp and the amino group, (iv) substituents attached to the Cp ring (which may affect the hydrophobicity and intercalating ability), or (v) the pH of the metallocene dihalides depending on the substituent on the nitrogen atom and the counterion.

In conclusion, we have synthesized ionic titanocenes and evaluated their cytotoxic properties. They have shown significantly better activity and stability than their nonfunctionalized counterparts and confer a potent cytotoxic effect on cisplatin-resistant ovarian tumor cell lines.

Experimental Details

Unless otherwise stated all manipulations were conducted using standard Schlenk line techniques, under an inert

(16) Knox, R. J.; Friedlos, F.; Lydall, D. A.; Roberts, J. J. *Cancer Res.* **1986**, *46*, 1972−1979.

atmosphere of dinitrogen or argon, using a modified dual vacuum/dinitrogen (or argon) line or in a Braun Labmaster 100 glovebox under an atmosphere of dinitrogen. Diethyl ether, petroleum ether (bp 40−60 °C), THF, and toluene were predried with Na metal and distilled over Na or Na/benzophenone under dinitrogen. CH_2Cl_2 and CH_3CN were predried and distilled over CaH under N_2. All solvents were subsequently stored in ampules under N_2. $TiCpCl_3$ was prepared by literature methods.[17]

^1H and ^{13}C NMR spectra were recorded on Bruker 250, 300, and 500 MHz spectrometers. High-resolution mass spectrometry was performed by the University of Leeds mass spectral service. Elemental analyses were performed by the University of Leeds microanalytical services.

Crystallographic Data Collection and Structure Collection. X-ray Crystallography. Data for compounds **5** and **6** were collected on a Nonius KappaCCD area-detector diffractometer using graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) using 1.0° ϕ-rotation frames. The structure was solved by direct methods using SHELXS 86. Refinement, by full-matrix least squares on F^2 using SHELXL 97, was similar for all four compounds. Hydrogen atoms were constrained to idealized positions using a riding model (with free rotation for methyl groups).

Synthesis of [{η-$C_5H_4(CH_2)_2NMe(CH_2)_5$}$_2$$TiCl_2$]·2I. 3. To a suspension of {η-$C_5H_4(CH_2)_2N(CH_2)_5$}$_2$$TiCl_2$ (2.45 g, 0.005mol) in toluene (100 mL) was added dropwise excess MeI with vigorous stirring. A pale pink precipitate formed immediately. The solid was washed with toluene (2 × 50 mL) and diethyl ether (1 × 50 mL). The solvent was removed under reduced pressure, and the product was isolated as a pale pink powder (2.1 g, 55.6%). Anal. Calcd: C, 41.4; H, 5.6; N, 3.71. Found: C, 44.5; H, 5.95; N, 2.6. ^1H NMR (D_2O, 250 MHz, 300 K): δ 6.57 and 6.47 (2 × vt, 8H, $C_5H_4(CH_2)_2NCH_3(CH_2)_5$), 3.47 (t, 4H, $C_5H_4CH_2CH_2NCH_3(CH_2)_5$), 3.30 (t, 4H, $C_5H_4CH_2CH_2NCH_3(CH_2)_5$), 3.03 (s, 6H, $C_5H_4(CH_2)_2NCH_3(CH_2)_5$), 2.93 (m, 8H, $C_5H_4(CH_2)_2NCH_3(CH_2)_2(CH_2)_2CH_2$), 1.82 (m, 8H, $C_5H_4(CH_2)_2NCH_3(CH_2)_2(CH_2)_2CH_2$), 1.61 (m, 4H, $C_5H_4(CH_2)_2NCH_3(CH_2)_2(CH_2)_2CH_2$). ^{13}C NMR (D_2O, 250 MHz, 300 K): δ 134.3 ($C_5H_4(CH_2)_2NCH_3(CH_2)_5$, quatenary carbon), 118.1 and 116.97 (CH of $C_5H_4(CH_2)_2NCH_3(CH_2)_5$), 117.17 ($C_5H_4(CH_2)_2NCH_3(CH_2)_5$), 61.92 ($C_5H_4(CH_2CH_2NCH_3(CH_2)_5$), 53.85 ($C_5H_4(CH_2CH_2NCH_3(CH_2)_2(CH_2)_2CH_2$), 23.16 ($C_5H_4(CH_2CH_2NCH_3(CH_2)_2(CH_2)_2CH_2$), 20.97 ($C_5H_4(CH_2CH_2NCH_3(CH_2)_2(CH_2)_2CH_2$), 19.95 ($C_5H_4(CH_2CH_2NCH_3(CH_2)_2(CH_2)_2CH_2$).

Synthesis of [η-C_5H_5][η-$C_5H_4(CH_2)_2N(CH_2)_5$]$TiCl_2$, 4. To a solution of $C_5H_5TiCl_3$ (0.9 g, 0.0041mol) in toluene (100 mL) at 0 °C was added $LiCp(CH_2)_2N(CH_2)_5$ (0.82 g, 0.0041mol). A dark red color formed immediately. The solution was allowed to stir for 1 h before being warmed to room temperature and allowed to stir for a further 2 h. The toluene solution was filtered and most of the solvent removed in vacuo. The solution was then refiltered and a dark red crystalline solid formed at −18 °C. Yield: 0.90 g, 61%. Anal. Calcd: C, 56.7; H, 6.4; N, 3.6; Cl, 19.69. Found: C, 56.2; H, 6.7; N, 3.6; Cl, 19.49. ^1H NMR (C_6D_6, 500 MHz, 300 K): δ 6.16, 5.68 (vt, 4H, $C_5H_4(CH_2)_2N(CH_2)_5$), 6.05 (s, 5H, C_5H_5), 3.01 (t, 2H, $C_5H_4CH_2CH_2N(CH_2)_5$), 2.46 (t, 2H, $C_5H_4CH_2CH_2N(CH_2)_5$), 2.31 (bs, 4H, $C_5H_4(CH_2)_2N(CH_2)_2(CH_2)_2CH_2$), 1.52 (m, 2H, ax or eq $C_5H_4(CH_2)_2N(CH_2)_2(CH_2)_2CH_2$), 1.32 (m, 2H, ax or eq $C_5H_4(CH_2)_2N(CH_2)_2(CH_2)_2CH_2$), 0.88 (m, 2H, $C_5H_4(CH_2)_2N(CH_2)_2(CH_2)_2CH_2$)). $^{13}C\{^1H\}$ NMR (C_6D_6, 500 MHz, 300 K): δ 137.26 (s, $C_5H_4(CH_2)_2N(CH_2)_5$] quaternary carbon), 123.87, 114.87 (s, CH of $C_5H_4CH_2CH_2N(CH_2)_5$), 119.35 (s, C_5H_5), 59.47 (s, $C_5H_4CH_2CH_2N(CH_2)_5$), 54.86 (s, $C_5H_4(CH_2)_2N(CH_2)_2(CH_2)_2CH_2$), 29.04 (s, $C_5H_4CH_2CH_2N(CH_2)_5TiCl_2$), 26.69 (s, $C_5H_4(CH_2)_2N(CH_2)_2(CH_2)_2CH_2$), 25.09 (s, $C_5H_4(CH_2)_2N(CH_2)_2(CH_2)_2CH_2$).

Synthesis of [η-C_5H_5][η-$C_5H_4(CH_2)_2N(CH_2)_5$]$TiCl_2$·HCl, 5. To a toluene solution of [C_5H_5][$C_5H_4(CH_2)_2N(CH_2)_5$]$TiCl_2$, **4** (1 g, 2.5 mmol), was added dropwise excess 2 M HCl in diethyl ether with vigorous stirring. To a toluene solution of [C_5H_5]-[$C_5H_4(CH_2)_2N(CH_2)_5$]$TiCl_2$ (1 g, 2.8 mmol) was added dropwise excess 2 M HCl in diethyl ether with vigorous stirring. A pink precipitate formed immediately. The solid was washed with diethyl ether (3 × 30 mL) and recrystallized by vapor diffusion from dichloromethane and diethyl ether to give a red powder (1.05 g, 95.6%). Anal. Calcd: C, 51.4; H, 6.1; N, 3.5; Cl, 26.81. Found: C, 50.7; H, 5.9; N, 3.4; Cl, 27.2. ^1H NMR ($CDCl_3$, 500 MHz, 300 K): δ 12.22 (bs, 1H, $C_5H_4(CH_2)_2NH(CH_2)_5$), 6.59 (s, 5H, C_5H_5), 6.42, 6.32 (2 × vt, 4H, $C_5H_4(CH_2)_2NH(CH_2)_5$), 3.52, 2.66 (m, 4H, ax and eq, $C_5H_4(CH_2)_2NH(CH_2)_2(CH_2)_2CH_2$), 3.42 (m, 2H, $C_5H_4CH_2CH_2N(CH_2)_5$), 3.35 (m, 2H, $C_5H_4CH_2CH_2NH(CH_2)_5$), 2.21, 1.84 (m, 4H, ax and eq, $C_5H_4(CH_2)_2NH(CH_2)_2(CH_2)_2CH_2$), 1.36 (m, 2H, $C_5H_4(CH_2)_2NH(CH_2)_2(CH_2)_2CH_2$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz, 300 K): δ 132.2, (s, $C_5H_4(CH_2)_2NH(CH_2)_5$] quaternary carbon), 123.3, 113.9 (s, CH of $C_5H_4(CH_2)_2NH(CH_2)_5$), 120.2 (s, C_5H_5), 55.4 (s, $C_5H_4CH_2CH_2NH(CH_2)_5$), 53.3 (s, $C_5H_4(CH_2)_2NH(CH_2)_2(CH_2)_2CH_2$), 24.7 (s, $C_5H_4CH_2CH_2N(CH_2)(CH_2)_2$), 22.5 (s, $C_5H_4(CH_2)_2NH(CH_2)_2(CH_2)_2CH_2$), 22.1 (s, $C_5H_4(CH_2)_2NH(CH_2)_2(CH_2)_2CH_2$). MS [FAB; *m*/*z* (%)]: 360 (66) [M^+ − Cl].

Synthesis of [C_5H_5][$C_5H_4CH(CH_2)_4NMe$]$TiCl_2$·HCl, 6. To a toluene solution of [C_5H_5][$C_5H_4CH(CH_2)_4NMe$]$TiCl_2$ (0.9 g, 2.6 mmol) was added excess 2 M HCl. A pink powder immediately precipitated. This was recrystallized from dichloromethane, and single crystals were obtained using vapor diffusion with diethyl ether to leave a pale pink powder (0.9 g, 90.4%). Anal. Calcd: C, 50.23; H, 5.80; N, 3.66. Found: C, 50.1; H, 6.1; N, 3.05. ^1H NMR (CDCl3, 500 MHz, 300 K): δ 12.52 (bs, 1H, $C_5H_4CH(CH_2)_4NHMe$), 6.62 (s, 5H, C_5H_5), 6.45 and 6.39 (2 × t, 4H, $C_5H_4CH(CH_2)_4NHMe$), 3.48 (m, 2H, $C_5H_4CH(CH_2)_2(CH_2)_2NHMe$ ax or eq), 3.21 (t of t, 1H, $C_5H_4CH(CH_2)_4NHMe$), $^3J(^1Hax-^1Heq)$ 4.1 Hz, $^3J(^1Hax-^1Hax)$ 12.1 Hz, 2.85 (m, 2H, $C_5H_4CH(CH_2)_2(CH_2)_2NHMe$ ax or eq), 2.72 (d, 3H, $C_5H_4CH(CH_2)_4NHCH_3$), 2.29 (m, 4H, $C_5H_4CH(CH_2)_2(CH_2)_2NHMe$). ^{13}C NMR ($CDCl_3$, 500 MHz, 300 K): δ 138.9 (s, $C_5H_4CH(CH_2)_4NHMe$ ring quarternary), 122.2, 116.0 (s, CH of $C_5H_4CH(CH_2)_4NHMe$), 120 (s, C_5H_5), 54.7 (s, $C_5H_4CH(CH_2)_2(CH_2)_2NHMe$), 43.8 (s, $C_5H_4CH(CH_2)_4NHCH_3$), 34.4 (s, $C_5H_4CH(CH_2)_4NHMe$), 28.7 (s, $C_5H_4CH(CH_2)_2(CH_2)_2NHMe$). MS [FAB; *m*/*z* (%)]: 345.8 (100) [M^+ − Cl].

Synthesis of [C_5H_5][$C_5H_4(CH_2)_2N(CH_2)_5$]$TiCl_2$·MeI, 7. To a toluene solution of [C_5H_5][$C_5H_4(CH_2)_2N(CH_2)_5$]$TiCl_2$ (0.6 g, 1.6 mmol) was added dropwise excess MeI with vigorous stirring. A red precipitate formed immediately. The solid was washed with diethyl ether (3 × 30 mL) and the solvent removed in vacuo to leave a pink powder (0.75 g, 90.4%). ^1H NMR (D_2O, 250 MHz, 300 K): δ 6.64 (s, 5H, C_5H_5), 6.62, 6.49 (2 × vt, 4H, $C_5H_4(CH_2)_2NMe(CH_2)_5$), 3.54 (t, 2H, $C_5H_4CH_2CH_2NMe(CH_2)_5$), 3.48 (t, 2H, $C_5H_4CH_2CH_2NMe(CH_2)_5$), 3.13 (s, 3H, $C_5H_4CH_2CH_2NMe(CH_2)_5$), 2.98 (m, 4H, ax and eq, $C_5H_4(CH_2)_2NMe(CH_2)_2(CH_2)_2CH_2$), 1.88 (m, 4H, ax and eq, $C_5H_4(CH_2)_2NMe(CH_2)_2(CH_2)_2CH_2$), 1.67 (m, 2H, $C^5H_4(CH_2)_2NMe(CH_2)_2(CH_2)_2CH_2$). $^{13}C\{^1H\}$ NMR, D_2O, 250 MHz, 300 K): δ 135.3 (s,$C_5H_4(CH_2)_2NMe(CH_2)_5$ quaternary carbon), 118.9 (s, C_5H_5), 118.2, 116.3 (s, CH of $C_5H_4(CH_2)_2NMe(CH_2)_5$), 62.0 (s, $C_5H_4(CH_2)_2NMe(CH_2)_2(CH_2)_2CH_2$), 61.9 (s, $C_5H_4CH_2CH_2NMe(CH_2)_5$), 47.8 (s, $C_5H_4(CH_2)_2NMe(CH_2)_5$) 22.5 (s, $C_5H_4CH_2CH_2NMe(CH_2)(CH_2)_2$), 20.9 (s, $C_5H_4(CH_2)_2NMe(CH_2)_2(CH_2)_2CH_2$), 19.9 (s, $C_5H_4(CH_2)_2NMe(CH_2)_2(CH_2)_2CH_2$). MS [FAB; *m*/*z* (%)]: 374.9 (100) [M^+ − I].

Synthesis of [C_5H_5][$C_5H_4CH(CH_2)_4NMeTiCl_2$·MeI, 8. To a toluene solution of [C_5H_5][$C_5H_4CH(CH_2)_4NMeTiCl_2$] (3.15 g, 9.1 mmol) was added excess MeI. A pink powder immediately crashed out; this was washed in diethyl ether to leave a pale pink solid (3.5 g, 78%). ^1H NMR (D_2O, 250 MHz, 300 K): δ 6.57 (s and t, 7H, C_5H_5 and $C_5H_4CH(CH_2)_4N(Me)_2$), 6.52 (t, 2H, $C_5H_4CH(CH_2)_4N(Me)_2$), 3.52 (m, 4H, $C_5H_4CH(CH_2)_2(CH_2)_2N(Me)_2$Ti ax and eq), 3.15 (s, 3H, $C_5H_4CH(CH_2)_4NMeMe$), 3.07 (s, 3H, $C_5H_4CH(CH_2)_4NMeMe$), 2.82 (m, 1H, $C_5H_4CH(CH_2)_4N$-

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$(Me)_2$), 1.97−1.87 (m, 4H, $C_5H_4CH(CH_2)_2(CH_2)_2N(Me)_2$ ax and eq). ^{13}C NMR (D_2O, 250 MHz, 300 K): δ 119.03, 118.6 (s, CH of $C_5H_4CH(CH_2)_4N(Me)_2$), 114.7 (s, C_5H_5), 62.5 (s, $C_5H_4CH(CH_2)_2(CH_2)_2N(Me)_2$), 56.4 (s, $C_5H_4CH(CH_2)_4N(Me)_2$), 47.7, 43.6 (s, $C_5H_4CH(CH_2)_4NCH_3CH_3$), 26.1 (s, $C_5H_4CH(CH_2)_2(CH_2)_2N(Me)_2$). MS [FAB; *m*/*z* (%)]: 360.8(28) [$M^+ - I$].

Typical SRB Assay. The assay is carried out in 96-well plates with an average of 0.5×10^4 cells per mL with 100 μL in each well. The cells were then treated with varying concentrations of drug for 2 h, 24 h, and 6 days and then incubated for 6 days in an atmosphere of 5% CO_2/95% air. After 6 days the cells were removed from the incubator and washed with cold trichloroacetic acid (TCA) to fix the cells to the bottom of the plates. These were then washed with water to ensure the removal of all TCA. The plates were then stained with a solution of 0.02% (w/v) SRB in 1.0% (v/v) acetic acid for 30 min. The plates were washed thoroughly with acetic acid and allowed to dry overnight. A 100 μL portion of 10 mM unbuffered Tris was added to each well to resuspend the SRB dye. The plates were then read using a plate reader, which takes the mean absorbance at 570 nm of each concentration, and using a linear interpolation the IC_{50} is calculated.

Acknowledgment. We gratefully acknowledge the funding of this project by Enact Pharma PLC, Salisbury, U.K.

OM030403I