Synthesis, Characterization, and Assessment of Cytotoxic Properties of a Series of Titanocene Dichloride Derivatives

Patrick W. Causey and Michael C. Baird*

Department of Chemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6

Susan P. C. Cole

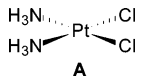
Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada K7L 3N6

Received May 5, 2004

A series of 15 water-soluble titanocene dichloride derivatives containing alkylammonium groups pendant to one (monocationic complexes) or both (dicationic complexes) cyclopentadienyl rings has been synthesized and characterized. The in vitro cytotoxicities of this small library of potential anticancer drugs have been assessed against human lung cancer (H209, A549, H209/CP) and ovarian cancer (A2780, A2780/CP) cell lines, and the results are compared with the cytotoxicities of both cisplatin (*cis*-$PtCl_2(NH_3)_2$) and a clinical formulation of titanocene dichloride that is commonly used against cisplatin-resistant tumors. While none of the compounds exhibit potency greater than that of cisplatin, several are clearly superior to the clinical formulation. In particular dicationic complexes generally exhibit greater potency than do the corresponding monocationic analogues, and derivatives containing protonated piperidinyl rings exhibit greater potency than do compounds containing protonated 2-aminoethyl or 3-aminopropyl groups. Eight of the compounds, representing a wide range of potencies, were characterized crystallographically but no correlations between effectiveness and structures were obvious.

Introduction

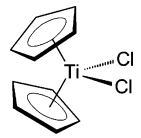
Since the discovery of the anticancer activity of *cis*-diaminodichloroplatinum(II) (cisplatin, **A**),[1] this compound has become one of the most widely used drugs for the treatment of cancer. Indeed, this and similar platinum-based drugs have over the past 30 years dominated the treatment of solid tumors, notably ovarian, lung, bladder, and head and neck carcinomas and are among the world's most broadly used chemotherapeutic agents.[1]



A

Unfortunately, the remarkable anticancer properties of cisplatin can be accompanied by marked toxic effects which include neurotoxicity, nephrotoxicity, and severe emesis. In addition, tumors often develop resistance to cisplatin and related platinum-based drugs, limiting significantly their clinical use, and thus there is a need for new compounds exhibiting high activity coupled with lower toxicity and non-cross-resistance.[1]

The search for other transition metal complexes that also possess cytotoxic activities has yielded a number of promising leads. Since the mode of action of cisplatin involves substitution of the mutually cis chloride ligands by water followed by covalent modification of DNA by the cationic moiety [*cis*-$Pt(NH_3)_2]^+$, thereby interfering with transcription and/or replication,[1] a great deal of research has focused on the possible applications of a variety of metal compounds containing cis chloride and other halide ligands. Pseudotetrahedral metallocene compounds such as $(\eta^5\text{-}C_5H_5)_2TiCl_2$ (titanocene dichloride, **B**) therefore represented a seemingly logical extension of cisplatin, and they have also received considerable attention.[2,3]



B

Heavily investigated since about 1980, titanocene dichloride (henceforth TDC) has been found to be very effective against, for example, Ehrlich ascites tumor, B16 melanoma, colon B adenocarcinoma, Lewis lung carcinoma, and sarcoma 180. It is currently undergoing phase II clinical trials,[2] but, despite its structural similarity to cisplatin, its effectiveness against cisplatin-resistant cancer cell lines strongly suggests a different mechanism of action.[2,3]

* Corresponding author. E-mail: bairdmc@chem.queensu.ca. Fax: 613-533-6669.

(1) (a) Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. *Nature (London)* **1969**, *222*, 385. For recent reviews, see: (b) Reedijk, J. *Chem. Commun.* **1996**, 801. (c) Sharma, V.; Piwnica-Worms, D. *Chem. Rev.* **1999**, *99*, 2545. (d) Natile, G.; Coluccia, M. *Coord. Chem. Rev.* **2001**, *216−217*, 383. (e) Wheate, N. J.; Collins, J. G. *Coord. Chem. Rev.* **2003**, *241*, 133. (f) Fuertes, M. A.; Alonso, C.; Pérez, J. M. *Chem. Rev.* **2003**, *103*, 645.

10.1021/om049679w CCC: $27.50
Publication on Web 08/20/2004

Derivatives of TDC can be generated either by substitution of the chloride ligands or through modification of the cyclopentadienyl ring. A review of the literature,[2c] however, reveals that incorporation of other inorganic anions and organic ligands such amino acid anions has resulted in little improvement, perhaps to be anticipated if rapid hydrolytic substitution of the anions is a preliminary step in the drug action. Consistent with this scenario, it has been shown that TDC undergoes rapid chloride substitution in aqueous media, resulting in a complex, pH-dependent equilibrium involving a series of soluble ionic species such as $[(\eta^5\text{-}C_5H_5)_2Ti(H_2O)_2]^{2+}$ and $[(\eta^5\text{-}C_5H_5)_2Ti(H_2O)(OH)]^{+}$ (Figure 1).[4]

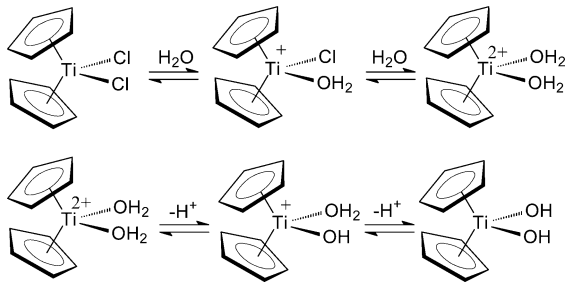
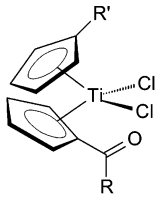


Figure 1. Hydrolysis of TDC in water.

In addition, proton-induced dissociation of the cyclopentadienyl rings (as cyclopentadiene) ultimately ensues, yielding insoluble materials of uncertain composition. Clearly the drug action of TDC somehow circumvents formation of the insoluble materials, an issue that needs to be addressed if the mechanism of drug action is to be understood.

Modification through functionalization of the cyclopentadienyl ring has previously shown that, in general, electron-donating functional groups diminish the cytotoxicity of the drug.[2c] These results are reasonable if a $[(\eta^5\text{-}C_5H_5)_2Ti]^{2+}$ cation binds to Lewis base sites in DNA, since electron-donating groups would presumably render the metal center a poorer Lewis acid. This line of thinking prompted our earlier research, which involved the incorporation of electron-withdrawing ester groups on the rings in the compounds $(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4CO_2Me)TiCl_2$ (**C**; R = Me, R′ = H) and $(\eta^5\text{-}C_5H_4CO_2Me)_2TiCl_2$ (**C**; R = Me, R′ = CO_2Me). We found these to exhibit significant toxicity against a human small cell lung cancer cell line,[5a] but a subsequent extension of these and similar compounds containing butyl and phenyl ester substituents to a variety of other human tumor cell lines has found little cytotoxic activity in general,[5b] and this approach has been abandoned.



C; R = Me, *t*-Bu, Ph; R' = H, CO_2Me, CO_2Bu, CO_2Ph

A limiting property of TDC is its very low aqueous solubility, generally necessitating considerable manipulation when being administered as a drug. Currently, water-soluble formulations in which the chlorides are substituted by chelating polyol ligands constitute a patented approach to the generation of water-soluble TDC analogues,[6a] one that has been widely employed in clinical practice.[6b−g] The partially insoluble TDC is dissolved in a hot aqueous sodium chloride solution containing a simple polyol such as mannitol. The mixture is allowed to equilibrate and is then freeze-dried (lyophilized) to give a powder that can be stored in air for prolonged periods. The procedure results in air-stable, water-soluble materials, of uncertain composition but suitable for administration following reconstitution in an appropriate aqueous medium.

Our earlier work on ester-functionalized TDC derivatives was initiated in part as an attempt to increase water solubility by incorporation of the polar ester group.[5a] As mentioned above, this approach was not successful, and we opted to work with a series of water-soluble TDC analogues containing alkylammonium substituents such as $-NH_3^+$, $-NH_2R^+$, $-NHR_2^+$, and $-NR_3^+$. A number of such species have been reported over the years,[7] and we have accordingly synthesized and characterized, spectroscopically and by elemental analyses, a small library of 15 water-soluble TDC derivatives containing various alkylammonium substit-

(2) For reviews, see: (a) Clarke, M. J., Zhu, F.; Frasca, D. R. *Chem. Rev.* **1999**, *99*, 2511. (b) Köpf-Maier, P.; Köpf, H. *Chem. Rev.* **1987**, *87*, 1137. (c) Köpf-Maier, P.; Köpf, H. *Struct. Bonding* **1988**, *70*, 103. For recent work, see: (d) Fairlie, D. P.; Whitehouse, M. W.; Broomhead, J. A. *Chem.-Biol. Interact.* **1987**, *61*, 277. (e) Harstrick, A.; Schmoll, H. J.; Sass, G.; Poliwoda, H.; Rustum, Y. *Eur. J. Cancer* **1993**, *29A*, 1000. (f) Kröger, N.; Kleeberg, U. R.; Mross, K.; Edler, L.; Sass, G.; Hossfeld, D. K. *Onkologie* **2000**, *23*, 60. (g) Tarillion, P.; Taubert, G.; Nenning, H. *Mutat. Res.* **1997**, *389*, 213. (h) Villena-Heinsen, C.; Friedrich, M.; Ertan, A. K.; Farnhammer, C.; Schmidt, W. *Anticancer Drugs* **1998**, *9*, 557. (i) Christodoulou, C. V.; Eliopoulos, A. G.; Young, L. S.; Hodgkins, L.; Ferry, D. R.; Kerr, D. J. *Br. J. Cancer* **1998**, *77*, 2088. (j) Valadares, M. C.; Klein, S. I.; Zyngier, S.; Queiroz, M. L. S. *Int. J. Immunopharm.* **1998**, *20*, 573. (k) Kopf-Maier, P. *Anticancer Res.* **1999**, *19*, 493. (l) Ghosh P.; D'Cruz, O. J.; Narla, R. K.; Uckun, F. M. *Clin. Cancer Res.* **2000**, *6*, 1536. (m) Mokdsi, G.; Harding, M. M. *J. Inorg. Biochem.* **2001**, *83*, 205.

(3) (a) Köpf-Maier, P. *Eur. J. Clin. Pharm.* **1994**, *47*, 1. (b) Yang, P.; Guo, M. *Coord. Chem. Rev.* **1999**, *185−186* 189. (c) Harding, M. M.; Mokdsi, G. *Curr. Med. Chem.* **2000**, *7*, 1289. (d) Guo, M.; Sun, H.; Bihari, S.; Parkinson, J. A.; Gould, R. O.; Parsons, S.; Sadler, P. J. *Inorg. Chem.* **2000**, *39*, 206. (e) Guo, M.; Sun, H.; McArdle, H. J.; Gambling, L.; Sadler, P. J. *Biochemistry* **2000**, *39*, 10023. (f) Guo, M.; Guo, Z.; Sadler, P. J. *J. Biol. Inorg. Chem.* **2001**, *6*, 698. (g) Moebus, V. J.; Stein, R.; Kieback, D. G.; Runnebaum, I. B.; Sass, G.; Kreienberb, R. *Anticancer Res.* **1997**, *17*, 815.

(4) (a) Toney, J. H.; Marks, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 947. (b) Mokdsi, G.; Harding, M. M. *J. Organomet. Chem.* **1998**, *565*, 29.

(5) (a) Boyles, J. R.; Baird, M. C.; Campling, B. G.; Jain, N. *J. Inorg. Biochem.* **2001**, *84*, 159. (b) Causey, P. W.; Sparks, K.; Cole, S. P. C.; Baird, M. C., unpublished results.

(6) (a) Muller, B. W.; Muller, R.; Lucks, S.; Mohr, W. (Medac Gesellschaft fur Klinische Spezialpraparate mbH, Hamburg) US Patent 5,296,237, 1994. (b) Kurbacher, C. M.; Nagel, W.; Mallmann, P.; Kurbacher, J. A.; Sass, G.; Huebner, H.; Andreotti, P. E.; Krebs, D. *Anticancer Res.* **1994**, *14*, 1529. (c) Kurbacher, C. M.; Bruckner, H. W.; Andreotti, P. E.; Kurbacher, J. A.; Sass, G.; Krebs, D. *Anti-Cancer Drugs* **1995**, *6*, 697. (d) Luemmen, G.; Sperling, H.; Luboldt, H.; Otto, T.; Ruebben, H. *Cancer Chemother. Pharmacol.* **1998**, *42*, 415. (e) Christodoulou, C. V.; Ferry, D. R.; Fyfe, D. W.; Young, A.; Doran, J.; Sheehan, T. M. T.; Eliopoulos, A.; Hale, K.; Baumgart, J.; Sass, G.; Kerr, D. J. *J. Clin. Oncol.* **1998**, *16*, 2761. (f) Korfel, A.; Scheulen, M. E.; Schmoll, H.-J.; Grundel, O.; Harstrick, A.; Knoche, M.; Fels, L. M.; Skorzec, M.; Bach, F.; Baumgart, J.; Sass, G.; Seeber, S.; Thiel, E.; Berdel, W. E. *Clinical Cancer Res.* **1998**, *4*, 2701. (g) Wittrisch, H.; Schroeer, H.-P.; Vogt, J.; Vogt, C. *Electrophoresis* **1998**, *19*, 3012.

(7) (a) Jutzi, P.; Redeker, T.; Neumann, B.; Stammler, H. G. *Organometallics* **1996**, *15*, 4153. (b) Jutzi, P.; Redeker, T.; Neumann, B.; Stammler, H.-G. *Chem. Ber.* **1996**, *129*, 1509. (c) Enders, M.; Köhler, K.; Frosch, W.; Pritzkow, H.; Lang, H. *J. Organomet. Chem.* **1997**, *538*, 163. (d) McGowan, M. A. D.; McGowan, P. C. *Inorg. Chem. Commun.* **2000**, *3*, 337. (e) Hitchcock, P. B.; Leigh, G. J.; Togrou, M. *J. Organomet. Chem.* **2003**, *669*, 101.

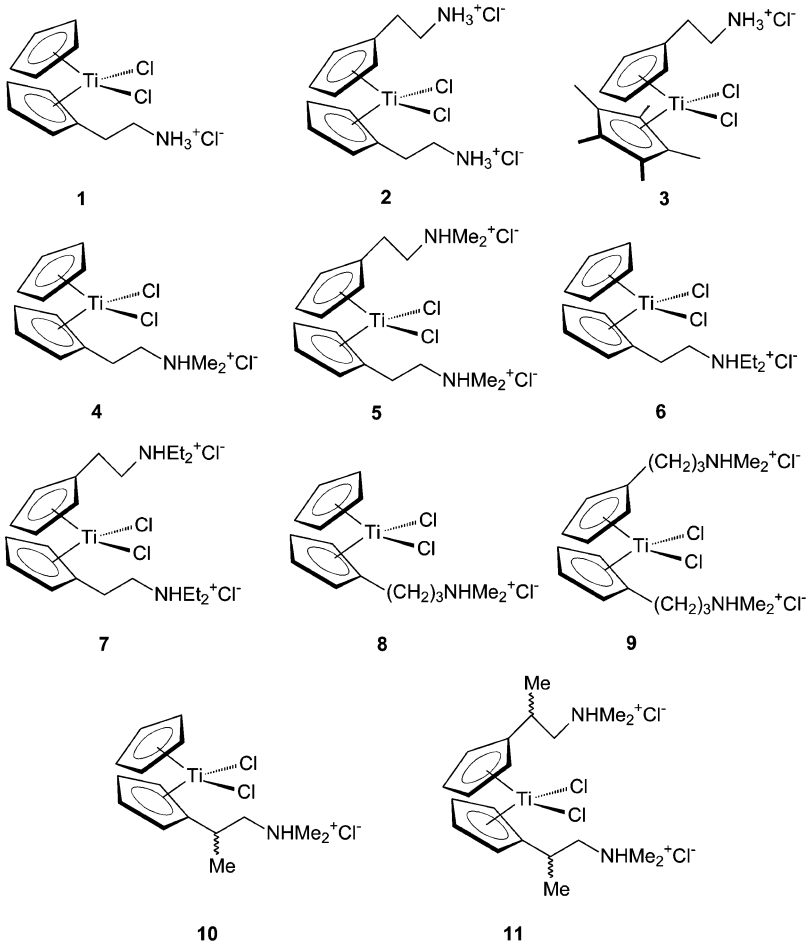


Figure 2. Acyclic titanocene dichloride derivatives assessed.

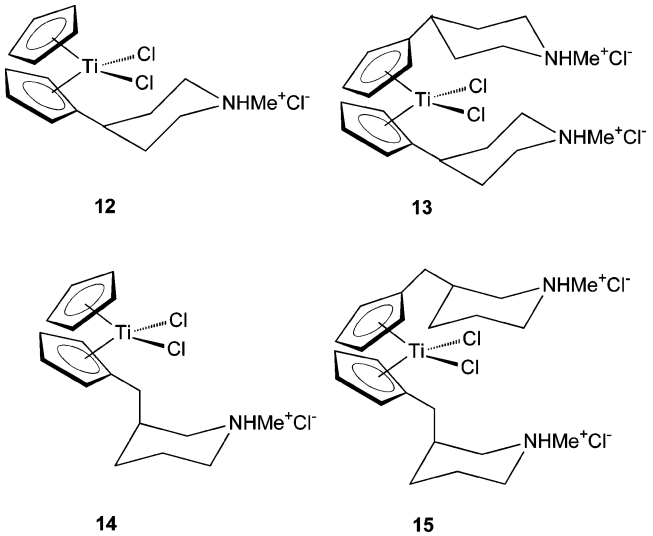


Figure 3. Cyclic titanocene dichloride derivatives assessed.

uents; these are shown in Figures 2 and 3. Twelve of these compounds are new. In addition eight of the compounds have been characterized crystallographically (**2**, **5**, **7**, **8**, **10**, **12**, **13**, **14**); of these, two (**8**[7e] and **12**[8]) have previously been characterized crystallographically.

We have carried out a series of in vitro assays to measure the cytotoxicity of the these and related compounds against human lung cancer (H209, A549, H209/CP) and ovarian cancer (A2780, A2780/CP) cell lines.[9] These cell lines represent a mix of both standard candidates for which many benchmarks of drug efficacies exist and solid tumor types for which new drugs are urgently needed; for purposes of comparison complementary assays were also carried out using cisplatin and the above-mentioned lyophilized clinical formulation of TDC.[6a] While this work was in progress, McGowan et al.[8] reported a largely complementary study of the anticancer properties of six TDC derivatives, two of which (**12** and **13**) are among those investigated here.

Experimental Section

Most synthetic procedures, unless otherwise noted, were carried out under an atmosphere of nitrogen or argon purified by passing through a column of BASF catalyst heated to 140 °C and a column of 5 Å molecular sieves. Manipulation of air-sensitive materials employed standard Schlenk line techniques and an Mbraun Labmaster glovebox. Solvents were either dried and distilled over sodium metal (except dichloromethane, which was dried over calcium hydride) or taken from directly from anhydrous grade solvents from Aldrich after passing through activated alumina columns. All chemicals were purchased from Aldrich or Sigma and were purified as appropriate before use.

The ^{1}H NMR and COSY spectra were run on Bruker AC 200, Avance 300, Avance 400, or Avance 600 NMR spectrometers, the residual proton resonances of the deuterated solvents serving as internal references. Chemical shift

(8) Allen, O. R.; Croll, L.; Gott, A. L.; Knox, R. J.; McGowan, P. C. *Organometallics* **2004**, *23*, 288.
(9) Campling, B. G.; Pym, J.; Baker, H. M.; Cole, S. P. C.; Lam, Y. M. *Br. J. Cancer* **1991**, *63*, 75.

Table 1. ^{1}H NMR Data (δ) for Compounds 1−15

	Cp, −CH═CH−	CH, CH_2 linking rings to N	other
1	6.57 (s, 5H, C_5H_5), 6.58, 6.48 (both t, 2 × 2H, *J* 2.3 Hz, C_5H_4)	3.16 (t, 2H, *J* 7.1 Hz, CH_2N), 2.78 (t, 2H, *J* 7.1 Hz, CH_2CH_2N)	
2	6.55, 6.48 (both t, 2 × 2H, *J* 2.4 Hz, C_5H_4)	3.16 (t, 2H, *J* 7.2 Hz, CH_2N), 2.78 (t, 2H, *J* 7.2 Hz, CH_2CH_2N)	
3	6.09, 5.84 (both t, 2 × 2H, *J* 2.2 Hz, C_5H_4	2.89 (t, 2H, *J* 7.2 Hz, CH_2N), 2.43 (t, 2H, *J* 7.2 Hz, CH_2CH_2N)	1.73 (s, 15H, Cp*)
4	6.53 (s, 5H, Cp), 6.51, 6.38 (both t, 2 × 2H, *J* 2.6 Hz, C_5H_4)	3.25 (t, 2H, *J* 7.6 Hz, CH_2N), 2.81 (t, 2H, *J* 7.6 Hz, CH_2CH_2N)	2.80 (s, 6H, NMe_2)
5	6.51, 6.41 (both t, 2 × 2H, *J* 2.4 Hz, C_5H_4)	3.25 (t, 2H, *J* 7.6 Hz, CH_2CH_2N), 2.81 (t, 2H, *J* 7.6 Hz, CH_2CH_2N)	2.79 (s, 6H, NMe_2)
6	6.50 (s and t, 7H, *J* 1.2 Hz, Cp and C_5H_4), 6.32 (t, 2H, *J* 1.2 Hz, C_5H_4)	3.21 (t, 2H, *J* 7.3 Hz, CH_2N), 2.77 (t, 2H, *J* 7.3 Hz, CH_2CH_2N)	3.09 (q, 4H, *J* 7.4 Hz, NCH_2CH_3, 1.14 (t, 6H, *J* 7.4 Hz, NCH_2CH_3
7	6.56, 6.41 (both t, 2 × 2H, *J* 1.3 Hz, C_5H_4)	3.25 (t, 2H, *J* 7.3 Hz, CH_2N), 2.79 (t, 2H, *J* 7.3 Hz, CH_2CH_2N)	3.10 (q, 4H, *J* 7.4 Hz, NCH_2CH_3, 1.20 (t, 6H, *J* 7.4 Hz, NCH_2CH_3.
8	6.57 (s, 5H, Cp), 6.59, 6.51 (both t, 2 × 2H, *J* 1.4 Hz, C_5H_4)	3.02 (t, 2H, *J* 8.0 Hz, CH_2N), 2.44 (t, 2H, *J* 7.9 Hz, CH_2CH_2CH_2N), 1.83 (tt, 2H, *J* 7.9 Hz, *J* 8.0 Hz, CH_2CH_2CH_2N)	2.79 (s, 6H, NCH_3)
9	6.47, 6.40 (both t, 2 × 2H, *J* 1.2 Hz, C_5H_4)	3.01 (t, 2H, *J* 7.6 Hz, CH_2CH_2CH_2N), 2.46 (t, 2H, *J* 7.3 Hz, CH_2CH_2CH_2N), 1.88 (tt, 2H, *J* 7.3, 7.6 Hz, CH_2CH_2CH_2N).	2.77 (s, 6H, NCH_3)
10	6.58 (s, 5H, Cp), 6.61, 6.39 (both t, 2 × 2H, *J* 1.2 Hz, C_5H_4)	3.58 (m, 1H, C_5H_4C*H*), 3.31, 3.18 (both m, 2 × 1H, CH(CH_3)CH_2), 1.10 (d, 3H, *J* 7.2 Hz, CH(CH_3)CH_2)	2.70, 2.67 (both s, 2 × 3H, NCH_3)
11[a]	6.59, 6.53 (both m, 2 × 2H, C_5H_4)	3.55 (m, 1H, C*H*(CH_3)CH_2), 2.80, 2.62 (both m, 2 × 1H, CH(CH_3)CH_2), 1.14, 1.08 (both d, total 3H, *J* 6.8 Hz, CH(CH_3)CH_2)	2.77, 2.79, 2.70, 2.74 (all s, total 12H, NCH_3)
12	6.50 (s, 5H, Cp), 6.51, 6.40 (2 br s, 2 × 2H, C_5H_4)	3.46 (br d, 2H, ^1J 13.0 Hz, C$H_{2(eq)}$N), 2.98 (dd, 2H, ^3J 13.2 Hz, ^1J 13.0 Hz, C$H_{2(ax)}$N), 2.74 (m, 1H, C_5H_4C*H*) 1.92 (ddt, 2H, ^1J 12.9 Hz, $^3J_{eq-ax}$ 3.9 Hz, $^3J_{CH2-CH2ax}$ 4.0 Hz, CH(C$H_{2(eq)}$), 1.66 (ddt, 2H, ^1J 12.9 Hz, $^3J_{eq-ax}$ 12.0 Hz, $^3J_{CH2-CH2eq}$ 4.0 Hz, CH(C$H_{2(ax)}$)	2.76 (s, 3H, NCH_3)
13	6.49, 6.42 (both t, 2 × 2H, ^3J 1.2 Hz, C_5H_4)	3.45 (ddd, 2H, ^1J 12.9 Hz, $^3J_{ax-eq}$ 3.5 Hz, $^3J_{eq-eq}$ 3.5 Hz, C$H_{2(eq)}$N), 2.98 (ddd, 2H, ^1J 12.9 Hz, $^3J_{ax-ax}$ 12.4 Hz, $^3J_{ax-eq}$ 3.5 Hz, C$H_{2(ax)}$N), 2.76 (m, 1H, C_5H_4C*H*), 1.97 (dddd, 2H, ^1J 12.9 Hz, $^3J_{eq-CH2ax}$ 3.9 Hz, $^3J_{eq-CHax}$ 4.0 Hz, $^3J_{eq-CH2eq}$ 4.0 Hz, CH(C$H_{2(eq)}$), 1.69 (dddd, 2H, ^1J 12.9 Hz, $^3J_{ax-CHax}$ 12.5 Hz, $^3J_{ax-CH2ax}$ 12.4 Hz, $^3J_{ax-CH2eq}$ 4.0 Hz, CH(C$H_{2(ax)}$)	2.78 (s, 3H, NCH_3)
14	6.52 (s, 5H, Cp), 6.48 (t, 2H, ^3J 2.1 Hz, C_5H_4), 6.39 (tt, 2H, ^3J 2.1 Hz, ^4J 3.9 Hz, C_5H_4)	3.38 (d, 1H, ^1J 12.0 Hz, −CH_2CH_{eq2}N), 3.23 (dd, 1H, ^1J 12.3 Hz, ^3J 2.0 Hz, −CH_2N(Me)CH_{eq2}−), 2.81 (dd, 1H, ^1J 12.0 Hz, ^3J 12.1 Hz, CH_2CH_{ax2}N(Me)CH_2), 2.59 (dd, 1H, ^1J 12.3 Hz, ^3J 12.2 Hz, CH_2N(Me)CH_{ax2}), 2.39 (dd, 2H, ^3J 6.8 Hz, ^4J 3.9 Hz, C_5H_4CH_2CH), 1.88 (m, 2H, ^3J 6.8 Hz for CH_2C*H*(CH_2−)(CH_2) and ^1J 14.1 Hz, $^3J_{eq-ax}$ 3.7 Hz for CH_2C$H_{eq2}$$CH_2$N(Me)$CH_2$), 1.75 (d, 1H, ^1J 13.0 Hz, CH(C$H_{eq2}$$CH_2$$CH_2$)($CH_2$)N), 1.62 (d, 1H, ^1J 14.1 Hz, CH_2C$H_{ax2}$$CH_2$N), 1.11 (m, 1H, ^1J 13.0 Hz, $^3J_{ax-eq}$ 3.7 Hz)	2.72 (s, 3H, NCH_3)
15	6.48, 6.44 (both t, 2 × 2H, ^3J 2.1 Hz, C_5H_4)	3.38 (d, 1H, ^1J 12.3 Hz, −CH_2CH_{eq2}N(Me)CH_2-), 3.17 (d, 1H, ^1J 12.2 Hz, −CH_2N(Me)CH_{eq2}−), 2.81 (dd, 1H, ^1J 12.3 Hz, ^3J 12.1 Hz, CH_2CH_{ax2}N(Me)CH_2), 2.53 (dd, 1H, ^1J 12.2 Hz, ^3J 12.2 Hz, CH_2N(Me)CH_{ax2}), 2.34 (br s, 2H, C_5H_4CH_2CH), 1.84 (m, 1H, $^3J_{ax-ax}$ 12.2 Hz, CH_2C*H*(CH_2−)(CH_2)), 1.79 (d, 1H, ^1J 13.9 Hz, ^3J 3.8 Hz, CH_2C$H_{eq2}$$CH_2$N(Me)), 1.63 (d, 1H, ^1J 13.4 Hz, CH(C$H_{eq2}$$CH_2$$CH_2$)N), 1.52 (m, 1H, ^1J 13.9 Hz, $^3J_{ax-ax}$ 12.1 Hz, CH_2C$H_{ax2}$$CH_2$N(Me)), 1.03 (m, 1H, ^1J 13.4 Hz, $^3J_{ax-eq}$ 3.8 Hz, CH(C$H_{ax2}$$CH_2$$CH_2$)N)	2.68 (s, 3H, NCH_3)

[a] Ratio of two diastereomers; 3.8:1.

and coupling constant data for all of the titanium compounds prepared are presented in Table 1. Mass spectra were obtained on a Quatro Fisons Pro Quadrupole mass spectrometer in either EI+ mode or ES+ mode with either nitromethane or methanol used as solvent. FT-IR spectra were obtained on a Perkin-Elmer Spectrum One FT-IR spectrometer and were processed using Spectrum v5.01 software; all samples were prepared as Nujol mulls. Canadian Microanalytical Services of Delta, B.C., performed elemental analyses; data for all new compounds are presented in Table 2.

X-ray crystallographic structure determinations was carried out using a Bruker SMART CCD 1000 X-ray diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 A) controlled with Crysostream Controller 700. Typically a crystal was mounted on a glass fiber with epoxy glue. No significant decay was observed during data collection. Data were processed on a Pentium PC using the Bruker AXS Windows NT SHELXTL software package (version 5.10).[10a] Neutral atom scattering factors were taken from Cromer and Waber.[10b] The raw intensity data were converted (including

(10) (a) *SHELXTL NT: Crystal Structure Analysis Package, version 5.10*; Bruker AXS Inc.: Madison, WI, 1999. (b) Cromer, D. T.; Waber, J. T. *International Tables for X-ray Crystallography*; Kynoch Press: Birmingham, UK, 1974; Vol. 4, Table 2.2 A.

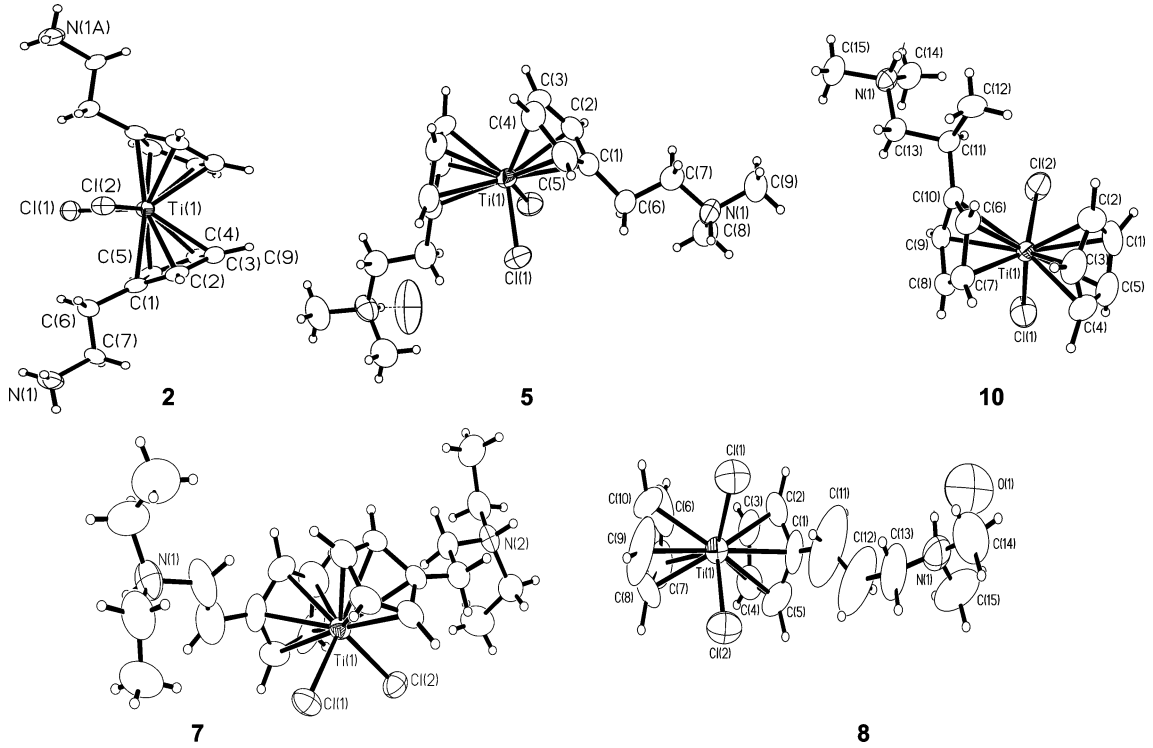


Figure 4. Molecular structures of the acyclic titanocene dichloride derivatives assessed.

Table 2. Elemental Analytical Data

	calculated			found		
compound	C	H	N	C	H	N
1	41.59	5.24	4.04	41.96	5.09	4.48
2	39.47	5.68	6.58	39.22	5.56	6.03
3	49.01	6.77	3.36	49.49	6.82	3.76
4	46.00	5.79	3.83	45.72	5.37	4.03
5	44.84	6.69	5.81	44.18	6.63	5.62
6	49.97	6.29	3.64	49.53	6.32	3.68
7	50.79	7.36	5.38	50.14	7.48	5.41
8	47.46	6.11	3.69	47.82	6.11	4.24
9	47.08	7.11	5.49	46.92	6.77	5.31
10	48.62	5.98	3.78	48.64	5.63	3.50
11	48.81	6.96	5.69	48.62	7.25	5.26
12	50.23	5.80	3.66	49.75	5.95	3.58
13	51.19	6.64	5.43	51.02	7.06	4.92
14	49.36	6.09	3.39	48.98	6.07	3.34
15	52.96	7.04	5.15	52.64	7.42	4.99

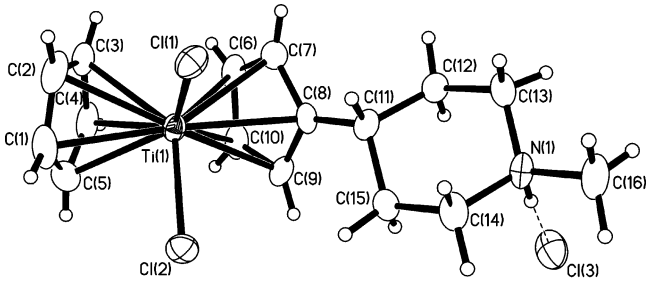
corrections for scan speed, background, and Lorentz and polarization effects) to structure amplitudes and their esds using the program SAINT, which corrects for *Lp* and decay. Absorption corrections were applied using the program SADABS. All non-hydrogen atoms were refined anisotropically. The positions for all hydrogen atoms were calculated, and their contributions were included in the structure factor calculations. Pertinent crystallographic data are given in the Supporting Information, and selected bond distances and angles in Table 3. Molecular structures are shown in Figures 4 and 5.

Syntheses of [(η^5-Cp)(η^5-$C_5H_4CH_2CH_2NHEt_2$)$TiCl_2$]Cl (compound 6 of Figure 1) and [(η^5-$C_5H_4CH_2CH_2NHEt_2$)$_2$Ti-Cl_2]Cl_2 (compound 7 of Figure 1). The syntheses of these two new compounds are outlined in detail as examples of the general methodology for the syntheses of all compounds used here. Synthetic details for all other compounds are available in the Supporting Information. The starting material, C_5H_5-$CH_2CH_2NEt_2$, was prepared by treating a rapidly stirred suspension of [$NHEt_2CH_2CH_2Cl$]Cl (4.60 g, 26.7 mmol) in 100 mL of THF at 0 °C under argon with a THF solution of NaCp (2 M, 27 mL, 54 mmol). A fine white precipitate formed, and the solution was refluxed for 4 h. The solvent was then removed under reduced pressure followed by the addition of 100 mL of water. The aqueous layer was extracted with ether (1 × 50 mL) and hexanes (3 × 75 mL), and the combined extracts were dried ($MgSO_4$) and the solvent was removed to give an amber oil, shown by ^{1}H NMR to be a mixture of three isomers. The crude product was purified by distillation (~45 °C, 8 × 10^{-2} Torr) to give a clear, colorless oil (1.62 g, 36.7%). ^{1}H NMR ($CDCl_3$): δ 6.37−6.42, 6.18, 6.11, 5.97, 5.89, 5.41 (m, CH=CH of rings), 2.93 (d, J = 2.5 Hz, ring CH_2), 2.31−2.74 (m, $C_5H_5CH_2CH_2NEt_2$), 2.47 (m, $C_5H_5CH_2CH_2N(CH_2CH_3)_2$ 2.05−2.47 (m, $C_5H_5CH_2CH_2NEt_2$), 0.98 (m, $C_5H_5CH_2CH_2N$-$(CH_2CH_3)_2$). TLC: R_f (1:1 ethyl acetate/MeOH) 0.31. This was

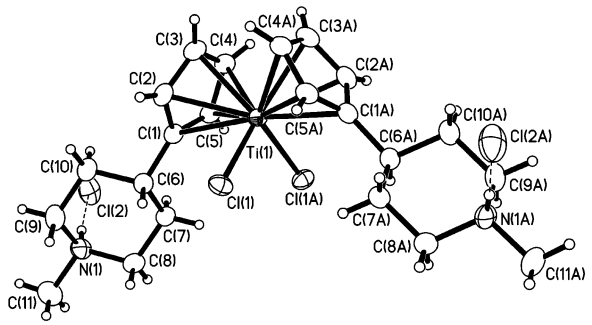
Table 3. Selected Bond Distances and Angles

compound	Ti−Cl (Å)	Ti−Cp(cent[a]) (Å)	Ti−Cp′(cent)[b,c] (Å)	Cl−Ti−Cl (deg)	cent−Ti−cent (deg)[c]
8	2.331(3), 2.358(3)	2.050	2.072	93.93(12)	131.4
10	2.3465(12), 2.3506(12)	2.060	2.082	94.88(5)	132.2
2	2.371(2), 2.355(3), 2.370(2), 2.374(3)		2.053, 2.065	93.00(9), 91.68(8)	132.2, 131.4
5	2.3533(12), 2.3533(12)		2.048	93.98(6),	132.1
7	2.3602(14), 2.3672(14)		2.070, 2.065	93.52(4)	129.7
12	2.3533(12), 2.3670(11)	2.068	2.069	94.60(4)	131.5
13	2.3638(7), 2.3638(7)		2.079	93.87(4),	130.4
14	2.351(2), 2.362(3)	2.062	2.069	93.78(8)	131.9
TDC[12]	2.367(2), 2.361(1), 2.363(1), 2.365(2)	2.058		94.43(6), 94.62(6)	131.6

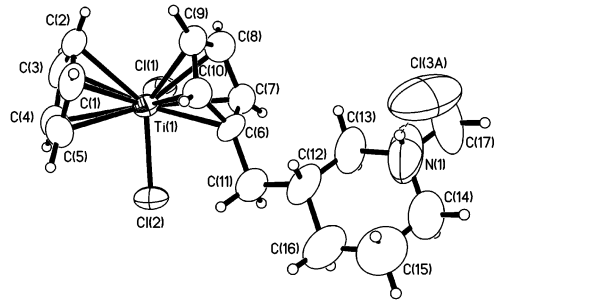
[a] cent = ring centroid. [b] Cp′ = substituted cyclopentadienyl. [c] Calculated using PLATON; Spek, A. L. *J. Appl. Crystallogr.* **2003**, *36*, 7.



12



13



14

Figure 5. Molecular structures of the cyclic titanocene dichloride derivatives assessed.

converted to Li[$C_5H_4CH_2CH_2NEt_2$] via the dropwise addition of n-BuLi (17 mL, 1.6 M, 50 mmol) in hexanes to a cooled (0 °C), stirred solution of $C_5H_5CH_2CH_2NEt_2$ (3.64 g, 22.0 mmol) in 100 mL of hexanes under argon. Gas evolved and a white precipitate formed. The mixture was stirred for 30 min, and the precipitate was then filtered, washed with cold hexanes (3 × 100 mL), and dried under vacuum to give the desired product as a white, air- and moisture-sensitive solid (3.50 g, 93.1%). ^{1}H NMR (d_6-DMSO, 400 MHz): δ 5.14 (br s, 4H, C_5H_4-$CH_2CH_2NEt_2$), 2.47 (m, 8H, $C_5H_4CH_2CH_2NEt_2$, $C_5H_4CH_2CH_2$-NEt_2, $C_5H_4CH_2CH_2N(CH_2CH_3)_2$), 0.96 (t, 6 H, J = 7.2 Hz, $C_5H_4CH_2CH_2N(CH_2CH_3)_2$).

The compound [(η^5-Cp)(η^5-$C_5H_4CH_2CH_2NHEt_2$)$TiCl_2$]Cl was then prepared by dropwise addition of a freshly prepared solution of 1.98 g of $CpTiCl_3$ (9.03 mmol, 1 equiv) in 30 mL of toluene to a stirred suspension of 1.53 g of Li[$C_5H_4CH_2CH_2$-NEt_2] (9.04 mmol) in 75 mL of toluene at 0 °C. The solution turned dark red immediately, and an off-white precipitate formed. The reaction mixture was stirred for 1 h, after which it was warmed to room temperature and stirred for another 1 h. The mixture was filtered, and an excess of HCl (6 mL, 2 M) in diethyl ether was added dropwise with stirring. A dark greenish red precipitate formed immediately and was filtered, washed with toluene (1 × 50 mL), hexanes (1 × 50 mL), and diethyl ether (3 × 60 mL), and dried. The dark solid product so formed was air- and moisture-stable and was subsequently handled under air. A solution in 25 mL of a 1:2 mixture of methanol and ethanol was added dropwise to 250 mL of diethyl ether, and the compound immediately precipitated as an orange-red powder. This was filtered, washed with ether, and dried under vacuum. The crude product was recrystallized under air from dichloromethane by slow diffusion with hexanes to give dark red crystals of [(η^5-Cp)(η^5-$C_5H_4CH_2CH_2NHEt_2$)-$TiCl_2$]Cl, suitable for elemental analysis and X-ray crystallographic determination (2.23 g, 64.2%). ^{1}H NMR (D_2O, 400 MHz): δ 6.50 (s and t, 7H, J = 1.2 Hz, C_5H_5 and $C_5H_4CH_2$-CH_2NEt_2), 6.32 (t, 2H, J = 1.2 Hz, $C_5H_4CH_2CH_2NEt_2$), 3.21 (t, 2H, J = 7.3 Hz, $C_5H_4CH_2CH_2NEt_2$), 3.09 (q, 4H, J = 7.4 Hz, $C_5H_4CH_2CH_2N(CH_2CH_3)_2$), 2.77 (t, 2H, J = 7.3 Hz, $C_5H_4CH_2$-CH_2NEt_2), 1.14 (t, 6H, J = 7.4 Hz, $C_5H_4CH_2CH_2N(CH_2CH_3)_2$). Anal. Calcd for $C_{16}H_{24}Cl_3NTi$: C, 49.97; H, 6.29; N, 3.64. Found: C, 49.53; H, 6.32; N, 3.68. MS [ES$^+$ in methanol; m/z (%)]: 348 (24) [M − Cl], 344 (100) [M − HCl − Cl + MeOH].

The compound [(η^5-$C_5H_4CH_2CH_2NHEt_2$)$_2TiCl_2$]Cl_2 was prepared by adding dropwise a solution of $TiCl_4$ in toluene (4 mmol, 4 mL of a 1 M solution) to a stirred suspension of 1.36 g of Li[$C_5H_4CH_2CH_2NEt_2$] (8.04 mmol, 2 equiv) in 75 mL of toluene at 0 °C. A dark red color and an off-white precipitate formed immediately, and the reaction mixture was stirred for 1 h. The reaction mixture was then warmed to room temperature and stirred for another 1 h. The mixture was then filtered, and an excess of HCl (6 mL, 2 M) in diethyl ether was added dropwise to the stirred, dark red solution. A dark red precipitate formed immediately while the solution changed from dark red to light yellow. The supernatant was discarded, and the solid was washed with toluene (1 × 100 mL) and diethyl ether (3 × 75 mL) and then dissolved in 20 mL of ethanol. This solution was added dropwise to 250 mL of diethyl ether to precipitate the crude product as a red powder that was dried under vacuum overnight. The compound was recrystallized from dichloromethane by slow diffusion with hexanes to give dark red crystals suitable for elemental and X-ray crystallographic analysis (1.56 g, 74.8%). ^{1}H NMR (D_2O, 400 MHz): δ 6.56 (t, 2H, J = 1.3 Hz, $C_5H_4CH_2CH_2NEt_2$), 6.41 (t, 2H, J = 1.3 Hz, $C_5H_4CH_2CH_2NEt_2$), 3.25 (t, 2H, J = 7.3 Hz, $C_5H_4CH_2CH_2NEt_2$), 3.10 (q, 4H, J = 7.4 Hz, $C_5H_4CH_2$-$CH_2N(CH_2CH_3)_2$), 2.79 (t, 2H, J = 7.3 Hz, $C_5H_4CH_2CH_2NEt_2$), 1.20 (t, 6H, J = 7.4 Hz, $C_5H_4CH_2CH_2N(CH_2CH_3)_2$). Anal. Calcd for $C_{22}H_{38}Cl_4N_2Ti$: C, 50.79; H, 7.36; N, 5.38. Found: C, 50.14; H, 7.48; N, 5.41. MS [ES$^+$ in nitromethane; m/z (%)]: 447 (100) [M − HCl − Cl], 411 (22) [M − 2HCl − Cl].

The analogous synthetic procedures and spectroscopic data for the other compounds shown in Figure 2, new and previously reported, are given in the Supporting Information. The molecular structures of all compounds for which crystallographic information is available are shown in Figures 4 and 5, while selected bond distances and angles are shown in Table 3.

Synthesis of "Lyophilized" TDC Containing Mannitol as a Solubilizing Ligand. This material was prepared as described in a patent.[6a] A mixture of 40 mg of TDC (0.16 mmol) in 10 mL of an aqueous solution of mannitol (200 mg, 1.10 mmol) and sodium chloride (180 mg, 3.10 mmol) was stirred near boiling until all of the TDC had dissolved. The orange-red solution was then filtered, frozen at −78 °C, and freeze-dried at −50 °C, $<10^{-3}$ Torr. The resulting air-stable lyophilizate could be completely reconstituted in water to form a stable orange solution. ^{1}H NMR (D_2O, 300 MHz): δ 6.65 (s, Cp), 3.60−4.01 (m, mannitiol).

Cell Culture and Chemosensitivity Testing. The human tumor cell lines used to measure the cytotoxicity of the titanocene derivatives have been described previously.[9] The H209, H209/CP, and A549 lung tumor cell lines were cultured in RPMI 1640 medium containing 5% calf serum, while the A2780 and A2780/CP ovarian tumor cell lines were cultured

in DMEM medium with 7.5% fetal bovine serum. All cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

The cytotoxic potencies of the various compounds were determined using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as developed by Mosmann[11a] and adapted for chemosensitivity testing of human tumor cells.[11b,c] Cells (>90% viable as determined by trypan blue exclusion) were suspended in culture medium and dispensed into 96-well microtiter plates in a volume of 100 μL. H209 cells were plated at 2.5×10^4 cells per well, A549 cells at 1.0×10^4 cells per well, and A2780 cells at 0.5×10^4 cells per well, based on preliminary experiments indicating these to be optimal cell densities for the MTT assay conditions chosen. After incubation of the cells for 24 h, compounds were added in a volume of 100 μL to bring the total volume of culture medium in the wells to 200 μL.

All solutions and dilutions of the titanocene derivatives were prepared immediately before addition to the cultured cells to limit the possibility of precipitation upon standing. The compounds were first dissolved in approximately 5 mL of medium, and the volume was adjusted such that the final drug concentration was 2 mM. The stock solutions were then diluted as required and added to the wells. Final drug concentrations ranged from 0.001 to 100 uM in initial screening assays and from 0.1 to 200 μM in subsequent assays. Each drug concentration was added to four replicate wells. Cisplatin (Sigma) was tested in each set of assays as a positive control.

After addition of the drugs, the microtiter plates were returned to the incubator for 4 days. Three hours before completion of the incubation time, 100 μL of medium was removed from each well, and 25 μL of MTT (Sigma M2128) solution (2 mg/mL in phosphate-buffered saline) was added. The plates were then returned to the 37 °C incubator for 3 h to allow reduction of the tetrazolium salt by viable cells. Subsequently, 100 μL of 1 M HCl/2-propanol (1:24) was added to each well followed by vigorous mixing with a multichannel pipet to dissolve any dark blue formazan crystals formed by MTT reduction. Absorbance values at 570 nm were then measured using a EL_X800 UV spectrophotometer. Controls consisted of wells with untreated cells and provided the baseline absorbance. Mean values (±SD) of the quadruplicate determinations were calculated and results expressed as a percentage of the baseline absorbance at 570 nm. Using GraphPAD Prism v3.02 software, IC_{50} values (defined as the drug concentration that reduced the absorbance to 50% of control values) were obtained from the best fit of the data to a sigmoidal curve.

Results and Discussion

Syntheses and Characterization. We describe in the Experimental Section the syntheses of compounds **6** and **7** as typical of the synthetic routes used; the individual reaction steps are indicated in eqs 1−6.

$$[NHEt_2CH_2CH_2Cl]Cl + 2NaC_5H_5 \rightarrow C_5H_5CH_2CH_2NEt_2 \quad (1)$$

$$C_5H_5CH_2CH_2NEt_2 + \text{n-BuLi} \rightarrow Li[C_5H_4CH_2CH_2NEt_2] \quad (2)$$

$$Li[C_5H_4CH_2CH_2NEt_2] + CpTiCl_3 \rightarrow (\eta^5\text{-Cp})(\eta^5\text{-}C_5H_4CH_2CH_2NEt_2)TiCl_2 \quad (3)$$

$$2Li[C_5H_4CH_2CH_2NEt_2] + TiCl_4 \rightarrow (\eta^5\text{-}C_5H_4CH_2CH_2NEt_2)_2TiCl_2 \quad (4)$$

$$(\eta^5\text{-Cp})(\eta^5\text{-}C_5H_4CH_2CH_2NEt_2)TiCl_2 + HCl \rightarrow [(\eta^5\text{-Cp})(\eta^5\text{-}C_5H_4CH_2CH_2NHEt_2)TiCl_2]Cl \quad (5)$$

$$(\eta^5\text{-}C_5H_4CH_2CH_2NEt_2)_2TiCl_2 + 2HCl \rightarrow [(\eta^5\text{-}C_5H_4CH_2CH_2NHEt_2)_2TiCl_2]Cl_2 \quad (6)$$

Procedures for the syntheses of all of the other compounds utilized here were unexceptional and may be found in the Supporting Information. Overall yields were quite good in all cases.

The neutral amino products of eqs 3 and 4 were not normally isolated, as several were found to be unstable, converting to unidentified, insoluble products. Similar observations have been made previously for these types of compounds,[7a−d] and it has been suggested that the pendant amino groups take part in intermolecular bridging or possibly induce ring hydrolysis by altering the pH. In any case, protonation in ethyl ether, as in eqs 5 and 6, resulted in all cases in precipitation of the corresponding deep red alkylammonium salts, which were readily isolated and purified. These ionic compounds are air- and water-stable, although some were found (IR spectroscopy, X-ray crystallography, elemental analyses) to form hydrates during crystal growth. In general, the presence of water was indicated by broad OH stretching bands in the region 3340−3460 cm^{-1}. All of the compounds shown in Figures 2 and 3 have been obtained analytically pure, some (**1**−**5**, **8**, **9**, **14**) as monohydrates, and have been characterized by ^1H NMR spectroscopy; all are new compounds except for **4**,[7e] **8**,[7e] **12**,[8] and **13**.[7d,8]

As can be seen in Table 1, the ^1H NMR spectra of the monocyclopentadienyl compounds **1**, **4**, **6**, **8**, **10**, **12**, and **14** all exhibit a strong singlet in the region δ 6.50−6.58 attributable to the η^5-cyclopentadienyl group, while the ^1H NMR spectra of all of the compounds exhibit AA′BB′ multiplets in the region δ 6.32−6.61, attributable to the substituted η^5-cyclopentadienyl groups. The similarities in the chemical shifts throughout the series are consistent with the anticipated similarities in structures. The remaining resonances in all of the spectra are attributable to hydrogens on the pendant alkylammonium groups and were assigned on the basis of chemical shift, coupling constant, and COSY data. All of the data are presented in the Supporting Information and will not be discussed further as none seem exceptional.

The crystallographically determined molecular structures are shown in Figures 4 and 5; relevant bond length and bond angle data, in Table 3. Details of the structures are presented in the Supporting Information and will not be discussed here. As can be seen, all of the structures are similar to that of the parent compound TDC,[12] with pseudotetrahedral arrangements of two chloride ligands and two η^5-bonded ring ligands. Such a large number of crystal structures were determined because we anticipated the possibility of finding relationships between structure features and relative drug

(11) (a) Mosmann, T. *J. Immun. Methods* **1983**, *65*, 55. (b) Cole, S. P. C. *Cancer Chemother. Pharmacol.* **1986**, *17*, 259. (c) Cole, S. P. C. *Cancer Chemother. Pharmacol.* **1990**, *26*, 250.

(12) Clearfield, A.; Warner, D. K.; Saldarriaga-Molina, C. H.; Ropal, R.; Bernal, I. *Can. J. Chem.* **1975**, *53*, 1622.

Table 4. Effects of Titanium-Based Drugs on Human Solid Cancer Cell Viability (IC_{50}, μM)

	cell lines				
compound	H209	H209/CP	A549	A2780	A2780/CP
1	>200, >200		>200, >200	>200, >200	
2	>200, 110		>200, >200	>200, 83	
3	198, >200		>200, >200	>200, >200	
4	>200, >200		>200, >200	>200, >200	
5	100, 107	>200	>200, >200	>200, 173	>200
6	>200, >200	>200	>200, >200	>200, >200	>200
7	110, 78	170	>200, >200	>200, 123	>200
8	>200, >200		>200, >200	>200, >200	
9	49 ± 10	51, 74	>200, >200, >200	33, 46	97, 35
10	>200, >200		>200, >200	>200, 170	
11	48 ±16		94 ± 69	55 ± 35	
12	129, 158		>200, >200	106, 125	
13	103, 26	169	170, 66	106, 17	112
14	38 ± 19	58, 23	91 ± 22	28, 21	63, 20
15	50, 25	19	70, 38	21	56
MKT-4	89, 151		200, >200	109, 131	
cisplatin	0.03 ± 0.01		9.8 ± 4.0	0.86 ± 0.9	

potencies. Further thoughts on this possibility are discussed below.

The lyophilized, water-soluble TDC formulation in which the chlorides were substituted by mannitol was synthesized using the above-mentioned, patented methodology.[6a] The orange, powdery product was air-stable and quite soluble in water. The procedure results in materials of uncertain composition, but only a single Cp resonance, at δ 6.65, was observed in the NMR spectrum of a D_2O solution. This result probably implies the presence of a single species rather than a rapidly exchanging mixture since ^{1}H NMR spectra of mixtures of titanocene complexes generally exhibit well-resolved Cp singlet resonances.

Assessments of Cytotoxic Properties. We have assessed the cytotoxic activities of the small library of water-soluble, TDC derivatives **1**−**15**, in addition to the water-soluble, patented formulation of TDC, which has been much used elsewhere.[6] The cell lines used to this point include A549, H209, and A2780, representing a mix both of standard candidates for which many benchmarks of drug potencies exist and of cell lines that represent solid tumor types for which new drugs are urgently needed. Furthermore, selected compounds were also evaluated against the resistant variants of cell lines H209 and A2780, H209/CP and A2780CP. The in vitro assay results for all compounds are discussed, with similar mono- and disubstituted analogues considered together, followed by a summary of the relative potencies for the entire series of compounds.

In all cases, an MTT assay was used to obtain IC_{50} data, i.e., the concentration of a compound required to kill 50% of a cell population during the chosen time period. The results are shown in Table 4, where the potencies of compounds **1**−**15** are compared with those of cisplatin and of the lyophilized clinical formulation of TDC, MKT-4. Where esd data are given, the data are the results of 3−5 replications.

As is apparent from Table 4, the three cancer cell lines H209, A549, and A2780 were all highly sensitive to cisplatin. Average values of IC_{50} were <0.1 μM for the H209 and A2780 cell lines and <10 μM for the A549 cell line. In contrast, MKT-4 was not very active against A549, with IC_{50} values ≥ 200 μM, but it was moderately active against H209 and A2780. The biological activities of all analogues of titanocene dichloride that have been synthesized are compared with MKT-4 and cisplatin in the following sections. The data for cisplatin and MKT-4 thus provide useful benchmarks by which to assess the potencies of compounds **1**−**15**.

Cytotoxicity Results for 1−11. Compounds **1**−**11** form a series of drug candidates in which protonated 2-aminoethyl or 3-aminopropyl groups substitute a hydrogen on a cyclopentadienyl ligand. Within the series are subsets in which mono- and dicationic compounds contain the same pendant alkylammonium groups, e.g., **1** and **2**, **4** and **5**, **6** and **7**, **8** and **9**, and **10** and **11**. Compound **3** is a monocationic compound very similar to **1** but with a pentamethylcyclopentadienyl rather than a cyclopentadienyl ligand. Compounds **10** and **11** are analogous to **4** and **5**, but contain chiral centers on the pendant alkylammonium chains. Compound **10** was used as an unresolved racemic mixture of enantiomers, while **11** was a more complex mixture of diastereoisomers because of the presence of the two stereogenic centers.

Readily evident from Table 4 is the almost total lack of cytotoxicity of all of the monocationic 2-aminoethyl and 3-aminopropyl compounds (**1**, **3**, **4**, **6**, **8**, **10**) against the three cancer cell lines H209, A549, and A2780, with IC_{50} values comparable to or above the upper limit of the concentration range studied (0−200 μM). Only compound **10** showed any degree of potency (against A2780), and that was marginal.

In contrast, the dicationic analogues demonstrated quite significant potency in several cases. Thus **2**, **5**, and **7** were at least moderately effective against H209 and A2780, while **9** was very effective against H209 and A2780 and **11** was very effective against H209, H549, and A2780. In view of the promising effectiveness of compounds **5**, **7**, and **9**, these were also tested against the cisplatin-resistant cell lines A2780/CP and H209/CP. As is seen in Table 4, **9** is also effective against A2780/CP and H209/CP.

Although the potency of none of the compounds studied here approached that of cisplatin, several of the compounds compare well with MKT-4. Thus compounds **5**, **7**, **9**, and **11** are all comparable to or better than MKT-4 against H209, A549, and A2780, with **9** and **11** being significantly better in some cases.

The series of compounds **12**−**15** also contain pairs of mono- and dicationic compounds related in the sense

that each pair contains the same pendant alkylammonium groups. Compounds **12** and **13** have been reported previously, and indeed **13** has been examined for anticancer activity against cell lines A2780 and A2780/CP.[8] Compounds **12** and **13** differ from those discussed above in that they incorporate protonated piperidinyl rings rather than protonated aminoethyl or -propyl groups (see Figure 3). In contrast to the monocationic compounds discussed above, **12** exhibited moderate potency against H209 and A2780, although again the activity of the corresponding disubstituted analogue **13** was greater. Compound **12** was inactive against A549 in the concentration range observed, but the disubstituted complex **13** did exhibit significant activity against this cell line. It was further assayed against the cisplatin-resistant cell lines H209CP and A2780CP and exhibited significant potency.

As mentioned above, the cytotoxicity of **13** has previously been reported against a range of tumor cell lines, including A2780 and a cisplatin-resistant variant of A2780.[8] The potency against A2780 was reported to be 203 μM, while activity against the cisplatin-resistant variant was similar at 193 μM. Although a somewhat greater potency to A2780 and A2780CP tumor cells is reported here, again a similarity between cisplatin-sensitive and cisplatin-resistant tumor cell lines is observed.

Compounds **14** and **15** are similar to **12** and **13** but contain methylene bridges linking the cyclopentadienyl and piperidinyl rings. These compounds exhibited significant activities against all five cancer cell lines H209, A549, A2780, H209/CP, and A2780/CP. Furthermore, unlike other mono/disubstituted analogue pairs, the potency of the disubstituted complex was not significantly greater than that of the corresponding monosubstituted derivative against either H209 or A2780. Only against the non-small cell lung cancer A549 did the dicationic compound **15** have a lower IC_{50} value than **14**.

Cytotoxicity Trends for TDC Derivatives. Although not all of the TDC derivatives tested exhibited pronounced anticancer activities against H209, A549, and A2780, some general trends regarding the effects of substitution onto the cyclopentadienyl ring on potency are evident. As shown above, these three cancer cell lines were much more sensitive to the dicationic complexes than the corresponding monocationic analogues. Furthermore, derivatives substituted with a piperidinyl ring were more potent.

It was not clear at this point why these trends should prevail, and attempts were made to obtain crystal structures of as many compounds as possible in an attempt to correlate biological activities with structural parameters. Structures were eventually obtained for eight of the 15 compounds studied, and pertinent structural data are given in Table 3. These include data for two relatively inactive compounds, **8** and **10**, four (**2**, **5**, **7**, **12**) of marginal activity, and two (**13**, **14**) that were highly active. Comparable data for the parent compound, TDC,[12] are also given.

As can be seen, however, the ranges of structural parameters for TDC, for the relatively inactive, for the marginally active, and for the highly active compounds all overlap considerably, with no discernible differences of import. Thus rationalization for the very significant differences in potencies of these compounds lies not in their ground state structures, but in their in vivo chemistry.

However, the mechanism by which TDC and its analogues exert their cytotoxicities is unclear. Previous studies have shown that TDC is toxic to cisplatin-selected drug-resistant cell lines, suggesting that there is a lack of cross-resistance between the two compounds both in vitro and in vivo. From this it has been concluded that their modes of action differ.[2e,i] Moreover, unlike cisplatin, titanium(IV) apparently does not bind strongly to the nitrogens of DNA bases at physiological pH, although it appears to target phosphoesters of isolated nucleotides.[3e,f] Thus it has been suggested that nucleic acids may not be the predominant target of this agent in intact cells.[3c]

Intriguingly, titanium(IV) derived from TDC has been shown to bind strongly to transferrin (Tf),[3e,14] and it has been suggested that Tf mediates uptake of Ti from the drug into cells via the Tf receptor (TfR), which is abundantly expressed on the surface of many cancer cells. If this hypothesis is correct, we anticipate that the titanocene analogues currently being explored will release their heavy metal ion in the same way and the differences in potencies may arise as a result of differing rates of cleavage of the Ti-η^5-ring cleavage reactions. Examination of the structural data in Table 3 suggests that the more potent drugs contain slightly longer Ti−C bonds, possibly a result of both electronic and steric factors. However, it remains to be seen whether these rings in particular are more readily removed in the aqueous medium used for drug testing. Experiments to this end are under way.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada and the Canadian Institutes of Health Research (MOP-10519) for the funding that made this research possible, and Kathy Sparks for expert technical assistance.

Supporting Information Available: Synthetic details for all compounds and crystallographic details for compounds **2**, **5**, **7**, **8**, **10**, **12**, **13**, and **14**, including figures showing complete numbering schemes and thermal ellipsoids and tables of positional and thermal parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

OM049679W

(13) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; et al. *J. Nat. Cancer Inst.* **1991**, *83*, 757. For a general reference for clinical use of cisplatin and Pt-containing drugs, see: *Cancer Chemotherapy and Biotherapy: Principles and Practice*, 2nd ed.; Chabner, B. A., Longo, D. L., Eds.; 1996; Chapter 14, pp 357−378.

(14) Messori, L.; Orioli, P.; Banholzer, V.; Pais, I.; Zatta, P. *FEBS Lett.* **1999**, *442*, 157.