Chapter 1

Antitumor properties of *cis*platin and titanium(IV) complexes

1.1. Introduction

Biomedical inorganic chemistry is a relative new research field and investigates possible applications of metal containing agents which display biological activities[1,2]. An important part of these studies deals with compounds that display antitumor properties. The most successful anticancer drug to date containing a transition metal is *cis*platin, $[Pt(NH_3)_2Cl_2]$[3]. The bioactivity of organometallic compounds and their anticipated application in medicine is at present a focal point of research. These studies became more relevant after the discovery that certain metallocene derivatives displayed antitumor activity[4]. The first 'International Symposium on Bioorganometallic Chemistry' was held in Paris in July 2002.

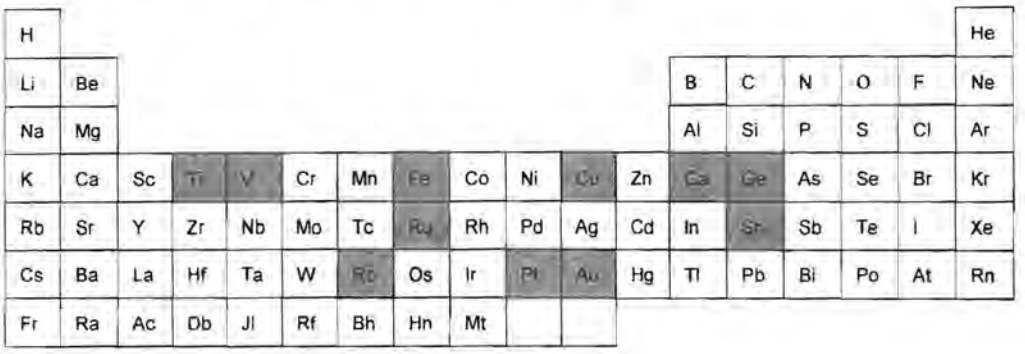
The Periodic Table in Figure 1.1 shows different metals of which some of their compounds display antitumor properties. Many of the complexes have structures, toxic properties and cellular modes of action that differ from those of *cis*platin. Some were found to be effective on *cis*platin resistant tumors.

1. P. J. Sadler, *Adv. Inorg. Chem.*, **1991**, *36*, 1.

2. Z. Guo, P. J. Sadler, *Angew. Chem. Int. Ed.*, **1999**, *38*, 1512.

3. *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, B. Lippert (Ed), Wiley-VCH, Weinheim, **1999**.

4. H. Köpf, P. Köpf-Maier, *Angew. Chem. Int. Ed. Engl.*, **1979**, *18*, 477.



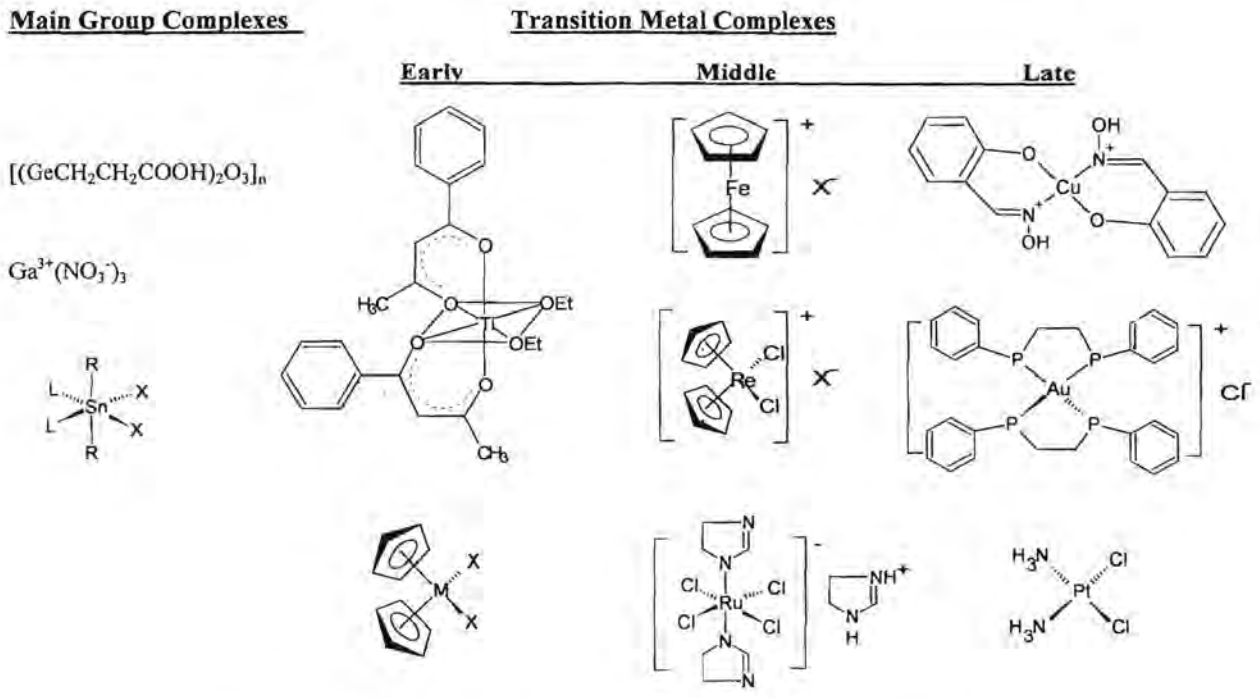


Figure 1.1. A selection of metal complexes that exhibit antitumor properties.

The antitumor active metal complexes range from the early transition metals through to the main group metals. There are basically three groups of metal containing antitumor agents.

- Inorganic complexes with classical heteroatom ligands coordinated to the metal
- Organometallic complexes where some of the ligands are directly linked to metal by carbon bonds
- Complexes with organic substituents or ligands coordinated to the metal via heteroatoms but without carbon-metal bonds

Note that many of the complexes in Figure 1.1 have two labile ligands in a *cis* configuration. It was initially accepted that the presence of readily replaceable *cis* halogen ligands was required for

antitumor activity. Also the comparable bite size of approximately 3.2Å in *cis*platin and in active metallocene dichlorides was seen as a prerequisite for antitumor activity. These sites are vacated and taken up by donor atoms of nucleobases of DNA resulting in cytostatic activities of the compounds. The structure-activity relationship of *cis*platin is well studied and documented[3]. Hence, the mechanism of action is reasonably well understood. The primary attack on DNA and formation of bifunctional intrastrand cross-links between two adjacent guanine bases by *cis*platin was seen as the criterion for the mechanism associated with the antitumor activity.

The first non-platinum complex to enter clinical trials after *cis*platin was budotitane, $[Ti(diketonate)_2X_2]$, and it is presently among the most advanced antitumor complexes[5]. A few other promising complexes (like the metallocenes, germanium complexes and gallium salts) were also studied and are now in pre-clinical phase of testing or in clinical trials[6]. Since DNA is proposed as the target of most metal containing antitumor agents, there is an emphasis on those that interact with nucleic acids. Hence, some analogous derivatives were synthesized and studied to try and improve antitumor activities of the parent compounds.

New investigations in the area of antitumor drugs other than *cis*platin are required, making studies of organometallic complexes such as titanocene derivatives an attractive alternative. Interestingly, all the organotransition metal complexes have cyclopentadienyl ligands which represent strong bonds. After the pioneering work done by Köpf and Köpf-Maier[4] with metallocene dichlorides, this area of research has been neglected. Although the structure-activity relationship of titanocene dichloride has been studied, the mechanism of action responsible for the biological activity remains unclear.

In the short history of inorganic chemistry related to anticancer research, it is evident that the lead compound, *cis*platin, influenced the design of most other metal complexes prepared and studied. The rest of the chapter summarizes the success story of *cis*platin in greater detail. This will serve as an aid to understand DNA-based antitumor activity and the role of the transition metal. The titanium compounds, titanocene dichloride and budotitane are discussed to clarify the present level of understanding of the bioactivities of Ti(IV) compounds. Lastly, important interactions with DNA, such as covalent bond formation and intercalation and their role in antitumor activity, will also be explained.

5. B. K. Keppler, C. Friesen, H. Vorgerichten, E. Vogel in *Metal Complexes in Cancer Chemotherapy*, B. K. Keppler (Ed, VCH, Weinheim, **1993**, 299.

6. B. K. Keppler, M. R. Berger, T. Klenner, M. E. Heim, *Adv. Drug Res.*, **1990**, *19*, 243.

1.2. *Cis*platin

The serendipitous discovery of the antitumor activity of *cis*platin (Figure 1.2) in 1969 by Barnett Rosenberg[7] was a major achievement in bioinorganic chemistry. *Cis*platin was the first metal complex to enter clinical trials in 1972 and after approval was introduced to clinics in 1979. This made it the first important contribution to cancer chemotherapy from the field of inorganic chemistry. Today this drug and its analogue, carboplatin (Figure 1.2), are the only inorganic complexes used on a routine clinical basis in cancer therapy. *Cis*platin (by itself or in combination therapy) is the most extensively used drug in the treatment of certain malignant tumors.

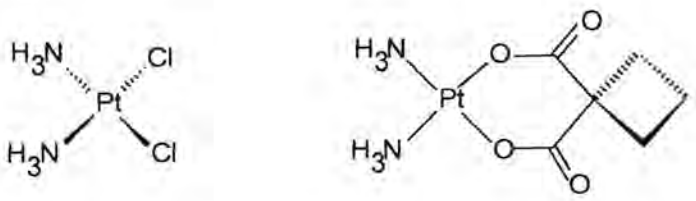


Figure 1.2. *Cis*platin and carboplatin.

Despite the success of *cis*platin as a drug, there are some disadvantages associated with its use. There are severe toxic side effects such as nausea, loss of hair, etc. and the ability of certain tumors to have a natural resistance to cisplatin, while others build up a resistance against *cis*platin after use. In addition the spectrum of tumors inhibited by this drug is very narrow and unfortunately the high activity of *cis*platin is limited to some very rare tumors, while most common tumors are not affected. Finally the drug has limited solubility, which makes it difficult to administer.

To try and minimize negative aspects associated with *cis*platin, researchers synthesized *cis*platin analogues. Although over 3000 second-generation platinum complexes were developed, carboplatin was the only one to receive worldwide registration[8]. The spectrum of activity of the direct derivatives of *cis*platin largely resembles that of the mother complex due to their similar mechanism of action and some show only a limited improvement in drug toxicity.

The interaction of *cis*platin with DNA has been studied extensively by researches using different approaches resulting in a good understanding of factors responsible for its antitumor activity. It is

7. B. Rosenberg, L. Van Camp, *Nature*, **1969**, *222*, 385.

8. R. B. Weiss, M. C. Cristian, *Drugs*, **1993**, *46*, 360.

believed that *cis*platin is not the active species but is converted to the active drug inside the body[9]. *Cis*platin has two chloro ligands (labile) and two ammine ligands (inert to substitution under biological conditions). The two labile chloro ligands are hydrolyzed in a stepwise manner as shown in reactions [1] and [2][10,11].

$$cis\text{-}[Pt(NH_3)_2Cl_2] + H_2O \rightleftharpoons cis\text{-}[Pt(NH_3)_2Cl(OH_2)]^+ + Cl^- \quad ...[1]$$

$$cis\text{-}[Pt(NH_3)_2Cl(OH_2)]^+ + H_2O \rightleftharpoons cis\text{-}[Pt(NH_3)_2(OH_2)_2]^{2+} + Cl^- \quad ...[2]$$

In the blood plasma the chloride concentration is relatively high and this suppresses aquation, so the neutral dichloro complex is predominant outside the cell membrane[12]. After entering the cell, where the chloride ion concentration is low, the hydrolysis products are formed easily. The positively charged, highly active diaquo species are attracted to the nucleophillic sites on DNA first through electrostatic attraction and thereafter by covalent bond formation.

Due to the *cis*-configuration of the complex, DNA is cross-linked as seen in Figure 1.3. (illustrating interstrand and intrastrand cross-links)[9]. It is believed that the 1,2-intrastrand cross-link of guanine bases is the critical lesion that leads to cytotoxicity, because it is specific to the *cis* isomer of the platinum complex[13,14]. These cross-links form due to covalent bonding, preferably to two adjacent guanine bases on the N-7 position, because they are exposed in the major groove and uninvolved in Watson-Crick hydrogen bonding. Once these Pt-N adducts are formed they are very stable under physiological conditions.

9. S. E. Sherman, S. J. Lippard, *Chem. Rev.*, **1987**, *87*, 1153.

10. M. E. Howe-Grant, S. J. Lippard, *Metal Ions Biol. Syst.*, **1980**, *11*, 63.

11. T. G. Appleton, J. R. Hall, S. F. Ralph in *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy.*, M. Nicolini (Ed) Martinas, Nijhoff, Boston, **1988**, 643.

12. M. C. Lim, R. B. Martin, *J. Inorg. Nucl. Chem.*, **1976**, *38*, 1911.

13. J. -P. Macquet, T. Theophanides, *Bioinorg. Chem.*, **1975**, *5*, 59.

14. D. M. L. Goodgame, *Biochim. Biophys Acta*, **1975**, *378*, 153.

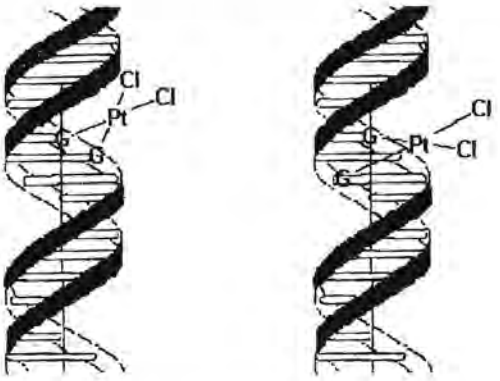


Figure 1.3. Interstrand- and intrastrand cross-linking of *cis*platin to DNA.

The *cis*platin-DNA adduct can be accommodated in the double helix, causing only small localized disruptions of the helix that cannot be recognized by repair enzymes[15]. The double helix can be unwound due to short-range intrastrand cross-links and shortening of the double helix can occur due to long range cross-links[16]. A crystal structure by Lippard and co-workers[17] demonstrates that the one strand of DNA is slightly bent towards the major groove (Figure 1.4) This distortion of the DNA structure by *cis*platin triggers a series of events responsible for its antitumor activity.

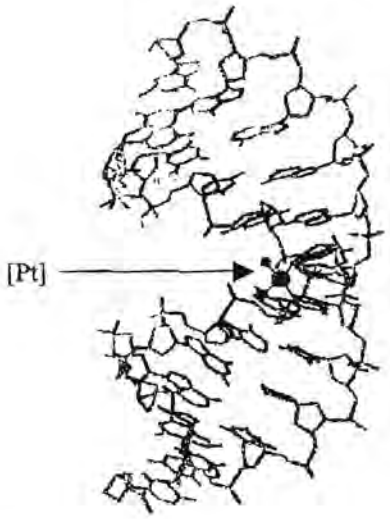


Figure 1.4. Damage done to the DNA structure due to *cis*platin cross-links[17].

15. R. B. Ciccarelli, M. J. Solomon, A. Varshavsky, S. J. Lippard, *Biochemistry*, **1985**, *24*, 7533.

16. S. J. Lippard, *Pure Appl. Chem.*, **1987**, *59*, 731 (and references therein).

17. P. M. Takahara, A. C. Rosenzweig, C. A. Frederick, S. J. Lippard, *Nature*, **1995**, *377*, 649.

The binding of high mobility group domain proteins (which specifically recognize *cis*platin induced DNA 1,2-intrastrand cross-links) shields the adduct from repair[18]. The hydrophobic surface in the minor groove is the target for interaction of the high mobility group proteins. The major biochemical effect is inhibition of replication. Studies into the structure-activity relationships of *cis*platin analogues lead to a set of empirical rules to ensure antitumor activity in second-generation platinum complexes[19,20]. These rules concluded that active complexes should have:

- a *cis* configuration of labile ligands– The *cis* isomer is generally more active because of the chelating ability to biological molecules. Synthesis of new metal complexes that can act against *cis*platin resistant tumors due to different mechanisms of action.
- two labile ligands – The labile groups must be readily replaced by water to be active. Too labile ligands lead to increased toxicity and too stable ligands lead to inactivity.
- two non-labile groups – The nature of the amine ligands has an influence on the antitumor activity and toxicity of the compound. The ligand must have at least one N-H bond.
- a specific oxidation state of platinum – Generally the Pt(II) complexes are more active than Pt(IV) analogues. Pt(0) complexes are too reactive and unstable.
- a neutral charge on complex – Neutral complexes allow for passive diffusion into cells.

The story of *cis*platin shows that it is possible to design and develop new drugs from the field of inorganic chemistry capable of curing specific types of cancers. It has been of interest for inorganic chemists to search for new metal complexes with improved antitumor activity. Basic strategies in the development of new compounds are the following:

- Synthesis of *cis*platin analogues was not very fruitful because of similar mechanism for their antitumor action. The major achievement of this exercise was to reduce toxic side effects.
- Linking tumor-inhibiting platinum complexes to other tumor inhibiting metal complexes with carrier systems to have selective accumulation in specific tissues.
- Using *cis*platin in combination with other chemotherapeutic agents like intercalators, which is often referred to as a cocktail drug.
- Incorporating more than one platinum atom in a molecule.

18. B. A. Donahue, M. Augot, S. F. Bellon, D. K. Treiber, J. H. Toney, S. J. Lippard, J. M. Essigman, *Biochemistry*, **1990**, *29*, 5872.

19. M. J. Cleare, J. D. Hoeschele, *Plat. Met. Rev.*, **1973**, *17*, 3.

20. M. J. Cleare, J. D. Hoeschele, *Bioinorg. Chem.*, **1973**, *2*, 187.

- Using radiosentisizers as ligands. The drug is accumulated in the cancerous tissue and activated *in situ* by irradiation.
- Synthesis of new metal complexes that can act against *cis*platin resistant tumors due to different mechanisms of action.

Eventually scientists realized that an alternative solution would be to find complexes that differ in their mechanisms of DNA attack compared to that of *cis*platin (third generation drugs). Because of the success of *cis*platin, the movement to include other transition metal antitumor agents has been exceptionally slow. Possible advantages of using non-platinum transition metal ions may involve the following:

- Additional coordination sites
- Different oxidation states
- Alterations in ligand and substituent affinities
- Photodynamic approaches to therapy
- Selective targeting

1.3 Metallocene compounds

The metallocene compounds are of interest in this study and they consist of compounds representing various structural types:

- Neutral metallocene diacido complexes $[M(C_5H_5)_2X_2]$ that contain early transition metals like Ti(IV) and V(IV). The acido ligands are favorably positioned and the cyclopentadienyl rings affords a pseudo tetrahedral geometry.
- Ionic metallicenium salts $[M(C_5H_5)_2]^+ X^-$ have improved water solubility and the cyclopentadienyl (Cp) ligands are arranged in a parallel sandwich geometry.
- Uncharged decasubstituted metallocenes $[M(C_5R_5)_2]$, which comprise the main group elements as central atoms, like Sn(II) and Ge(II). The cyclopentadienyl ligands are fully substituted and can be arranged in either sandwich or open sandwich geometry, depending on the substituents.

The titanium complexes were selected for this study, mainly because of titanium's lower toxicity to the body compared to platinum. The main representative of this class of active complexes is titanocene dichloride.

1.3.1. Titanocene dichloride

The titanocene dichloride complex belongs to the class of metallocenes illustrated in Figure 1.5. In 1979 Köpf and Köpf-Maier[4] discovered the cytostatic properties of some metallocenes after it was tested *in vitro* and *in vivo*. Following the discovery of the antitumor properties of titanocene dichloride a series of complexes were synthesized, investigated and tested, but to date titanocene dichloride proved to be the superior compound of its derivatives. Apart from antitumor properties, titanocene dichloride exhibits antiviral[21], antiarthritic and anti-inflammatory activities[22].

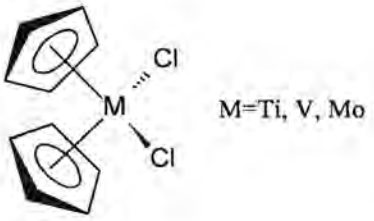


Figure 1.5. Bioactive metallocene dichlorides.

Köpf and Köpf-Maier[23,24] studied the structure activity relationship of the metallocenes. Chemical variation was used to investigate modification at (a) the central atom, (b) the acido ligands and (c) the protons of the cyclopentadienyl rings.

(a) With variation of the central metal (Ti, V, Nb, Mo, Ta, W, Zr and Hf) high cure rates were observed for Ti, V, Nb and Mo at optimal doses. These four metals have almost similar atomic radii, resulting in quite similar intermolecular non-bonding Cl...Cl distances as illustrated in Figure 1.6. This inter-ligand distance of about 3.2Å corresponds approximately to the bite distance of *cis*platin and to the distance between two adjacent DNA base pairs. In spite of this observation it is generally believed that this is coincidental and the mechanism of interaction of titanocene dichloride with DNA differs from that of *cis*platin.

21. E. Tonew, M. Tonew, B. Heyn, H. P. Schroer, *Zentralb. Bakterial. Parasitenskol. Infektionskr. Bakt. Hyg. Abt. Orig. Reihe A.*, **1981**, *250*, 425; *Chem. Abstr.*, **1982**, *96*, 82566.

22. D. P. Fairlie, M. W. Whitehouse, J. A. Broomhead, *Chem. Biol. Interact.*, **1987**, *61*, 277.

23. H. Köpf, P. Köpf-Maier in *Platinum, Gold and Other Metal Chemotherapeutic Agents*, S. J. Lippard (ed.), ACS Symposuim Series, **1983**, 315.

24. I. Haiduc, C. Silvestru, *Organometallics in Cancer Chemotherapy*, Vol II, CRC Press, **1990**, 36.

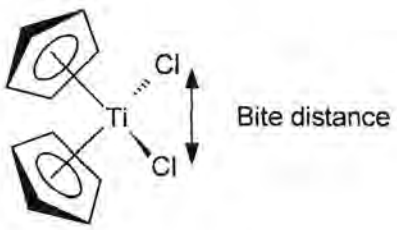


Figure 1.6. The intramolecular non-bonding Cl...Cl distance.

(b) Variation of the acido ligands with halides or pseudohalides (Cl, F, Br, I, NCS and N_3) had no effect on the level of antitumor activity[25]. Titanocene derivatives with certain thiolate ligands, such as $TiCp_2(SR)_2$[26] and $TiCp_2S_5$[27] (Figure 1.7), have low activity against EhAT, which is ascribed to their hydrophobic character and the strength of the Ti-S bonds. This makes the dissociation of the sulfur donor atom ligands and subsequent coordination of the diorgano titanium moiety to biological molecules difficult.

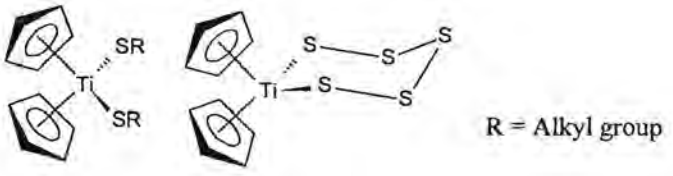


Figure 1.7. Biological inactive titanocene derivatives.

It is interesting to note however that compounds with thiolato and oxo ligands containing fluoro substituted aromatic rings (Figure 1.8) had a 100% cure rate against EhAT at optimal doses[28].

25. P. Köpf-Maier, B. Hesse, R. Voightländer, H. Köpf, *J. Cancer Res. Clin. Oncol.*, **1980**, *97*, 31.

26. H. Köpf, M. Schmidt, *Z. Anorg. Allg. Chem.*, **1965**, *340*, 139.

27. H. Köpf, B. Block, M. Schmidt, *Chem. Ber.*, **1968**, *101*, 272.

28. P. Köpf-Maier, H. Köpf, *J. Organomet. Chem.*, **1988**, *342*, 167.

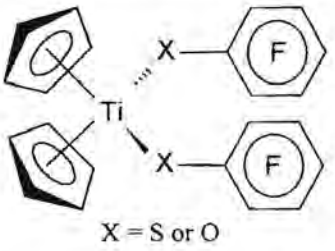


Figure 1.8. Tumor active titanocene derivatives fluorine substituents.

Complexes where one chloro ligand is replaced by an aromatic thiolato ligand displayed much weaker antitumor properties than the parent compound, titanocene dichloride[29]. Note that the ligands in Figure 1.9 consist only of one benzene ring.

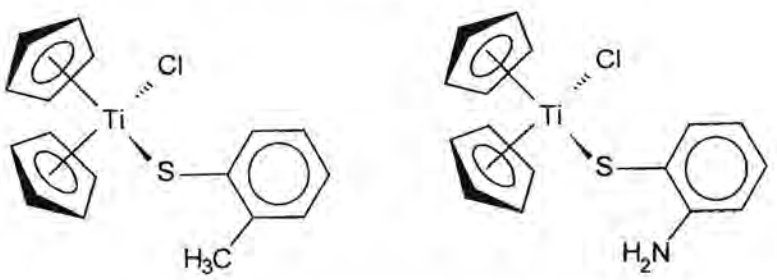


Figure 1.9. Titanocene derivatives with one anionic thioligand.

(c) The modification of the cyclopentadienyl ligands showed that the activity on tumors is strongly influenced by substitution on one of the rings. As more substituents were introduced, the activity against the tumors decreased[30]. When one of the rings was replaced by an acido ligand, a decrease in the antitumor activity was also observed. It was concluded that unsubstituted, small Cp-rings are necessary for antitumor activity because they act as carrier ligands to enable the transport of the active metal centre to intracellular target sites.

DNA is suspected to be the prime target for attack and studies done by Köpf and Köpf-Maier[23] indicated that titanocene dichloride attack the nucleic acids. The interactions of metallocenes with

29. P. Köpf-Maier, S. Grabowski, H. Köpf, *Eur. J. Med. Chem.*, **1984**, *19*, 347.

30. P. Köpf-Maier, W. Kahl, N. Klouras, G. Hermann, H. Köpf, *Eur. J. Med. Chem.*, **1981**, *16*, 275

DNA have recently been reviewed[31]. Initial studies suggested a correlation between DNA binding and antitumor activity. Later studies show that metallocene dihalides do not bond strongly to DNA at neutral pH and do not suppress DNA processing enzymes so it is unlikely that their activity involves nucleic acids.

The cyclopentadienyl ligand can be cleaved to liberate free cyclopentadiene (Fig. 1.10)[32]. The antitumor activity of titanocene derivatives is not due to the cyclopentadienyl and dicyclopentadienyl released, but it should be assumed that the organometallic moiety is involved in the process in a supportive manner[28].

Figure 1.10. Cleavage of the cyclopentadienyl-titanium bond.

Sadler and co-workers[33] studied transferrin as metal ion mediator. The transferrins are a class of iron binding proteins, typified by serum transferrin, the iron transport protein in blood. The transferrin serum involves the specific recognition of Fe^{3+} along with an obligatory synergistic anion. There is potential for use in therapy, because transferrin can also bind strongly to a range of other metals and many metal-transferrin complexes are still recognized by the transferrin receptor. The uptake and release of the metal can be controlled thermodynamically and kinetically. Transferrin can also communicate with other proteins besides blood serum. In agreement with predictions based on metal ion acidity, Ti^{4+} or the hydrated titanium ion forms a strong complex with human apo-transferrin by binding to two specific Fe^{3+} binding sites under physiological conditions[34]. The uptake of titanium by transferrin is slow and requires several hours for completion. Each transferrin molecule binds to two Ti^{4+} ions, one in each lobe. Such binding may be important for the antitumor activity of titanium if

31. L. Y. Kuo, H. Andrew, T. J. Marks in *Metal Ions in Biological Systems*, A. Sigel, H. Sigel (eds.), Marcel Dekker, Inc., New York, **1996**, *33*, 53.

32. J. H. Toney , T. J. Marks, *J. Am. Chem. Soc.*, **1985**, *107*, 947.

33. H. Sun, H. Li, P. J. Sadler, *Chem. Rev.*, **1999**, *99*, 2817.

34. M. Guo, H. Sun, H. J. McArdle, L. Gambling, P. J. Sadler, *Biochem.* **2000**, *39*, 10023.

transferrin delivers Ti^{4+} to the tumor cells[35]. Ti^{4+} binding to transferrin is reversible and Ti is released at low pH. At pH 5.5 Ti^{4+} can bind to DNA. Serum function studies and ^{45}Ti radiolabelling experiments have shown that Ti^{4+} is associated only with transferrin both *in vivo* and *in vitro*[36]. The strong interaction of titanocene dichloride with transferrin may be relevant to its low toxicity and high activity.

Sadler and co-workers[37] also investigated reactions between the anticancer drug titanocene dichloride and various nucleotides and their constituents in aqueous solution or *N,N*-dimethylformamide (DMF) by ^{1}H and ^{31}P NMR spectroscopy and in the solid state by IR spectroscopy. For titanocene dichloride, complexation to nucleobases in water is weak and interactions to nucleobases at pH 2-4 happen with simultaneous binding to the base and the oxygen of the phosphate[38]. At pH > 6, almost no complexation of aqueous titanocene dichloride to nucleotides or nucleobases was observed. In aqueous solution species containing titanocene bound to the phosphate group of deoxynucleoside guanine monophosphate (dGMP), adenine monophosphate (AMP), deoxynucleoside thiamine monophosphate (dTMP) and uracil monophosphate (UMP) are formed. Binding of titanocene dichloride to 5'-deoxynucleoside adenosine monophosphate (5´-dAMP) appears to involve an oxygen of phosphate and a nitrogen atom of the base (N7)[39]. These reactions contrast markedly with those of the drug cisplatin, which binds predominantly to the base nitrogen atoms of nucleotides and only weakly to the phosphate groups. The high affinity of Ti(IV) for phosphate groups may be important for its biological activity.

Titanocene dichloride was considered as model complex, because it proved to be a successful antitumor agent against colon cancer as well as various other tumors *in vivo* and *in vitro*. It can easily be modified to form new complexes, for example, replacing the chloro ligands to accommodate new ligands. The toxic side effects of titanocene dichloride are different and far less than that produced by *cis*platin. *In vivo* studies showed that titanocene dichloride is not selective and all treated cell lines are

35. M. Guo, H. Sun, P. J. Sadler, *J. Inorg. Biochem.* **1999**, *74*, 150.

36. K. Ishiwata, T. Ido, M. Monma, M. Murakami, H. Fukuda, M. Kameyama, K. Yamada, S. Endo, S. Yoshika, T, Sato, T. Matsuzwana, *Appl. Radiat. Isot,*. **1991**, *42*, 707.

37. M. Guo, Z. Guo, P. J. Sadler, *J. Biol. Inorg. Chem.*, **2001**, *6*, 698.

38. J. H. Murray, M. M. Harding, *J. Med. Chem.*, **1994**, *37*, 1936.

39. G. Mokdsi, M. M. Harding, *J. Organomet. Chem.*, **1998**, *565*, 29.

similarly affected by exposure to this antitumor agent[40]. The number of dead cells also increased with an increased concentration of the drug. Inhibition of all growth *in vitro* by titanocene dichloride seems to be due to the cytostatic effect of this compound[23,41]. By modifying the parent compound it may be possible to increase the selectivity of these new complexes.

1.3.2 Budotitane

This complex belongs to the bis-β-diketonate metal complexes as illustrated in Figure 1.11. The antitumor activity of this type of complex was reported as early as 1982[42]. Budotitane shows a high degree of antitumor activity against colon tumors and the toxicity of the complex is less than for *cis*platin. Phase I clinical investigations were started in 1986 and results were promising[43]. Today it is the most advanced antitumor agent in the titanium field.

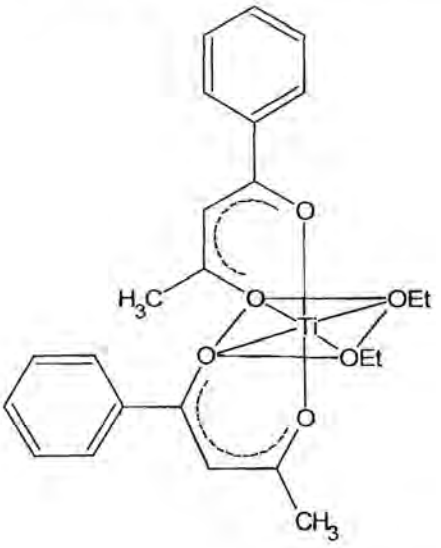


Figure 1.11. Budotitane.

40. P. Köpf-Maier, W. Wagner, H. Köpf, *Cancer Chemother. Pharmacol.*, **1981**, *5*, 237.

41. H. Köpf, P. Köpf-Maier, *Drugs of the Future*, **1986**, *11*, 297.

42. H. J. Keller, B. K. Keppler, D. Schmähl, *Arzneim.-Forsch./Drug Res.* **1982**, *32 (II)*, 806.

43. B. K. Keppler, H. Bischoff, M. R. Berger, M. E. Heim, G. Reznik, D. Schmäl, *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*, M. Nicolini (Ed) Martinas, Nijhoff, Boston, **1988**, 313.

A large number of similar complexes with various β–diketonato ligands were screened and found to have antitumor properties[44]. The structure-activity relation was investigated by variation of the complex at (a) the β–diketonato ligand, (b) central metal and (c) the leaving group.

(a) High antitumor activity was observed when there was a phenyl ring on the β–diketonato ligand (Figure 1.12) in position R'1 or R'3, but decreased when phenyl rings occupied both these positions. Activity increased when a spacer between the diketonato ligand and the second phenyl group was incorporated, for example a benzyl group was more effective. Introduction of water-soluble groups did not increase the antitumor activity.

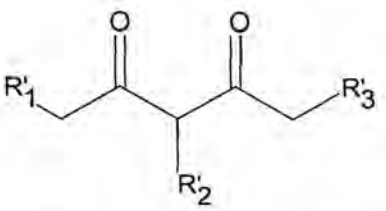


Figure 1.12. β–diketonato ligand.

Substituents on the phenyl group had different effects. When methyl was used, the activity was not altered, but methoxy, chloro or nitro groups decreased the activity. When one of the positions in Figure 1.12 contained a tert-butyl group, the activity decreased, thus supporting the fact that an unsubstituted aromatic ring in the periphery of the molecule is necessary.

(b) Variation of the central metal (Ti, Zr, Hf, Mo, Sn and Ge) showed that the highest antitumor activity was obtained when Ti or Zr was used. The activity of the other metals decreased in the order given above in brackets.

(c) The *cis* configured hydrolysable groups did not seem to play a major role in the antitumor activity. As with titanocene dichloride the leaving group is lost fairly rapidly. The leaving group is important for galenic formulation in the clinic. This galenic formulation is a co-precipitate with chromophor EL and propylene glycol. This is necessary to make the drug more soluble in water and to protect it from hydrolysis. Alkoxy groups proved to be more stable in water than the corresponding halides. Budotitane was chosen for further development, because it displayed the ethoxy group that had the slowest rate of hydrolysis.

44. W. Sun, Y. Ren, *Dalian Gongxueyuan Xuebao*, **1984**, *23*, 138; *Chem. Abstr.*, **1985**, *102*, 105001

Although the complex has a *cis* configuration, the mechanism of action is not yet understood, but believed to be completely different from that of cisplatin[45] as it displays a different structure activity relation. The activity of the complex depends on the β–diketonato ligand and it is believed that the planar phenyl group in the β–diketonato ligand is intercalating with DNA. Comparing the activity of a complex with acetyl acetone with a benzoyl acetone ligand, it was found that the acetyl acetonato complex is almost inactive while the benzoyl acetonato complex is highly active. This tendency is commonly observed with anticancer drugs where an intercalating mechanism is proposed. This result was also observed for certain titanocene complexes studied by Köpf and Köpf-Maier. Since intercalation is an important factor in the design of new complexes for this project, some background information will be presented.

1.4 Modes of action

1.4.1 Covalent bond formation of titanium(IV)

Further studies investigated the binding of metallocenes to the potential donor sites of 2'-deoxynucleoside 5'-monophosphates (Figure 1.13), such as the nitrogen atoms of the nucleobases and the oxygen atoms of the phosphate groups, as was observed for molybdenocene (Figure 1.14[46].), where N⌒N is 5'-dMAP, 5'-dTMP, 5'-deoxynucleoside thiamine monophosphate (5'-dCMP) or Me(5'-dGMP).

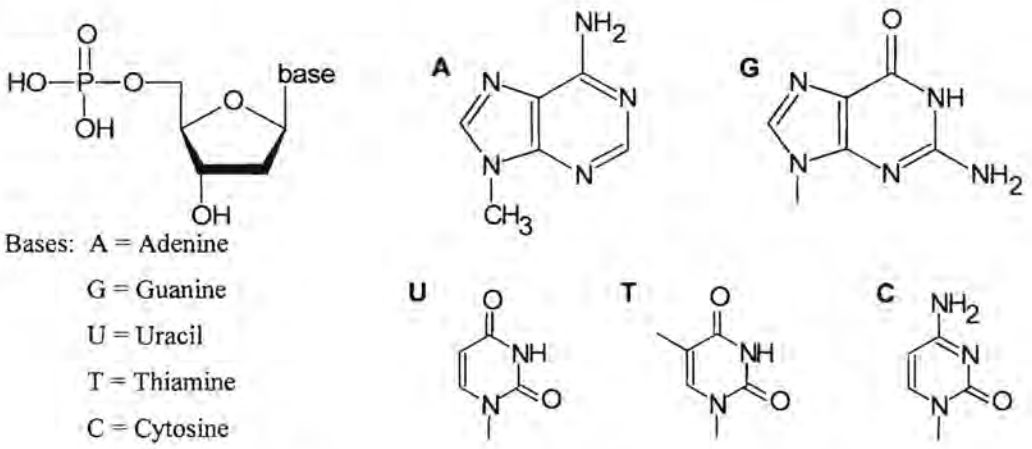


Figure 1.13. Structures of the 2'-deoxynucloside 5'-monophosphates.

45. T. Pieper, K. Borsky, B. K. Keppler, *Top. Biol. Inorg. Chem.*, **1999**, *1*, 171.

46. B. Lippert in *Progress in Inorganic Chemistry*, S. J. Lippard (ed.), John Wiley and Sons, New York, **1989**, 1.

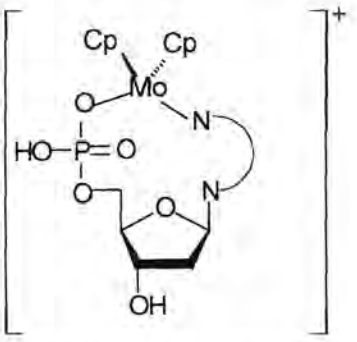


Figure 1.14. Coordination of Cp_2Mo^{2+} to 2'-deoxynucloside 5'-monophosphates.

Also studied was the direct interaction of titanocene dichloride with bases of nucleic acids by formation of covalent bonds. The synthesis of model complexes of the titanocene system with nucleic acid components as ligands has turned out to be difficult under physiological conditions. In literature there are a few examples of purine and uracil complexes that show that the titanocene moiety can be attached to purine and pyrimidine bases[47,48,49,50].

Two titanocene complexes bound to nucleobases were synthesised and characterised in nonaqueous media. In the Ti(IV)Cp_2Cl(purinato) complex[49] (Figure 1.15) the $TiCp_2Cl^+$ moiety binds to the N9 position of the purinato ligand to form a monofunctional bond. In the Ti(III)Cp_2(theophyllinato) complex[47] (Figure 1.15) the Cp_2Ti^+ moiety simultaneously binds to the O6 and the N7 sites of the theophyllinato ligand to form a bifunctional chelate. In both complexes the nucleobase ligand planes are situated in the equatorial plane of the Cp_2Ti^{2+} wedge.

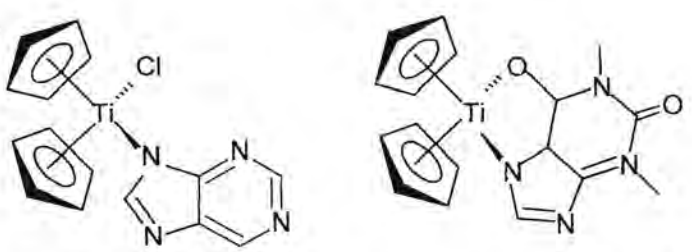


Figure 1.15. Chlorobis(η^5-cyclopentadienyl)purinatotitanium(IV) and bis(η^5-cyclopentadienyl) theophyllinatotitanium(III).

47. D. Cozac, A. Mardhy, A. Morneau, *Can. J. Chem.*, **1986**, *64*, 751.

48. A. L. Beauchamp, F. Bélanger-Garlépy, A. Mardhy, D. Cozac, *Inorg. Chim. Acta*, **1986**, *124*, 23.

49. A. L Beauchamp, D. Cozak, A. Mardhy, *Inorg. Chim. Acta*, **1984**, *92*, 191.

50. D. Cozac, A. Mardhy, M. J. Olievier, A. L. Beauchamp, *Inorg. Chem.*, **1986**, *25*, 2600.

The mechanism of action is also determined by the behaviour of the complex in aqueous media. The antitumor activity of metallocene dichlorides probably depends on hydrolysis of the metal which for Ti, V, Zr and Mo proceeds much faster than for *cis*platin[51]. In aqueous solution most metallocene complexes are not stable but undergo dissociation, aquation and hydrolysis reactions followed by condensation to oxobridged polynuclear species as seen in reactions [1] – [5][32]. These oxobridged and aqua species have a higher affinity for phosphate oxygen atoms than the *cis*platin hydrolysis products[42]

$$(C_5H_5)_2TiCl_2 + H_2O \rightleftharpoons [(C_5H_5)_2TiCl(H_2O)]^+ + Cl^- \quad ...[1]$$

$$[(C_5H_5)_2TiCl(H_2O)]^+ + H_2O \rightleftharpoons [(C_5H_5)_2Ti(H_2O)_2]^{2+} + Cl^- \quad ...[2]$$

$$[(C_5H_5)_2Ti(H_2O)_2]^{2+} \rightleftharpoons [(C_5H_5)_2Ti(H_2O)(OH)]^+ + H_3O^+ \quad ...[3]$$

$$[(C_5H_5)_2Ti(H_2O)(OH)]^+ \rightleftharpoons [(C_5H_5)_2Ti(OH)_2] + H_3O^+ \quad ...[4]$$

$$2[(C_5H_5)_2Ti(H_2O)(OH)]^+ \rightleftharpoons [(H_2O)(C_5H_5)_2Ti\text{-}O\text{-}Ti(C_5H_5)_2(H_2O)]^{2+} + H_2O \quad ...[5]$$

It is known that titanocene dichloride is more stable in acidic or saline solutions than in pure water. The most acidic of the aquated metallocenes is $[TiCp(H_2O)_2]^{2+}$ so that it exists as neutral $[TiCp_2(OH_2)_2]$ at neutral pH. The difference in the aquated forms between the various metallocenes may influence their activity in that those forming neutral species under physiological conditions probably enter the cells easier[52]. The hydrolysed species of titanocene dichloride appear to have high affinity for plasma proteins.

1.4.2 Intercalation

The predominant mode of action of most cytostatic agents with coplanar (heterocyclic) chromophores is intercalation into human DNA. In this study intercalation is understood as the insertion of a chromophoric (planar) part of a molecule between two stacked base pairs as illustrated in Figure 1.16[53]. This causes basically two changes in DNA. Firstly, the DNA tertiary structure (helix) is lengthened and somewhat unwound, while the primary and secondary structures remain intact. With intercalation, the average separation between two stacked base pairs increases from 3.4Å to ~7.0Å. At biochemical level a blockage of the matrix functions occurs. Secondly, there is a significant change in

51. L. Y. Kuo, A. H. Lui, T. J. Marks, *Met. Ion. Biol. Syst.*, **1996**, *33*, 53.

52. M. S. Murthy, L. N. Rao, L. Y. Kuo, J. H. Toney, T. J. Marks, *Inorg. Chim. Acta*, **1988**, *152*, 117.

53. U. Pindur, M. Haber, K. Sattler, *J. Chem. Ed.*, **1993**, *70*, 263.

the torsion angles of the sugar phosphate skeleton. This causes reading errors in the replication process and cell proliferation comes to a standstill. The major contribution to intercalative binding in DNA arises from electrostatic, van der Waals and most importantly hydrophobic forces. Stabilisation is maintained through π,π–stacking and hydrogen bonds. For an optimal intercalation, the planar part of the molecule (the chromophore) should have a minimum surface of 28Å^2 (optimum at three to four condensed five or six membered rings).

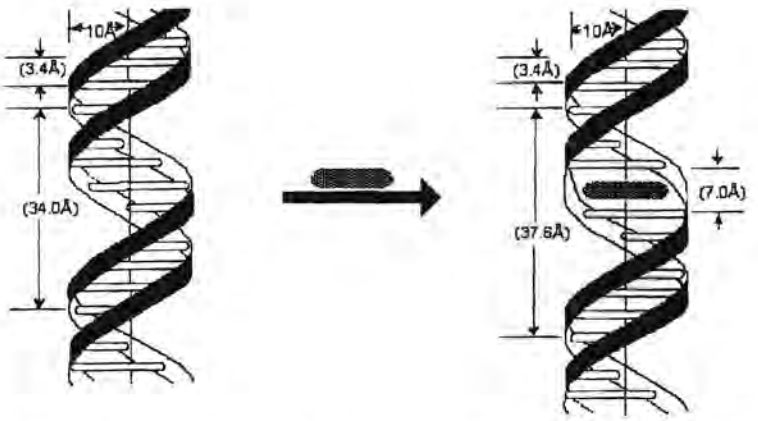


Figure 1.16. Schematic representation of intercalation between stacked base pairs of DNA

Transition metal complexes can also be used as intercalators when they form part of the planar ring system and are referred to as metallointercalators. Lippard *et al* [54] were the first to establish that square planar Pt(II) complexes containing an aromatic heterocyclic ligand bind to DNA by intercalation. Also, three-dimensional octahedral metallointercalators target specific DNA sites by matching the shape, symmetry and functionalities of the metal complex to the DNA target[55]. Different intercalative ligands stack with different orientations in the double helix due to their shape and polarity. Metallointercalators are among few synthetic complexes that target the DNA major groove with specificity. Such site specific targeting can lead to selective inhibition of DNA binding proteins.

In 1988 Palmer[56] studied the chromophore structure-activity relationships for linear, tricyclic carboxamides and concluded that the best antitumor results were obtained when the ligand intercalated. The majority of DNA monointercalating antitumor drugs compose of a planar tri- or tetracyclic chromophore with one or two side chains added, especially on the 1-position. A

54. J. K. Barton, S. J. Lippard, *Biochemistry*, **1979**, *12*, 2661.

55. E. C. Long, J. K. Barton, *Acc. Chem. Res.*, **1990**, *23*, 271.

56. B. D. Palmer, G. W. Rewcastle, G. J. Atwell, B. C. Baguley, W. A. Denny, *J. Med. Chem.*, **1988**, *31*, 707.

heteroatom (preferably nitrogen or oxygen) peri to the side chain is a further prerequisite. It was decided to incorporate these findings in the design of new titanium antitumor complexes for this study.

1.5. Aim of Study

One of the most important problems in the development of new tumor inhibiting complexes and potentially chemotherapeutic drugs is of a strategic nature. The action of existing models must be adequately understood and evaluated in order to improve significantly on their performance. Unless totally unique and selective in its anticancer properties it is unlikely that an improved derivative of an existing compound will succeed to eventually become a drug. This is a result of the enormous costs involved in launching a compound through the clinical trails until it eventually becomes registered. By using titanocene dichloride as model complex and through careful design by incorporating specific ligands it was hoped to synthesize complexes with a different spectrum of antitumor properties. Evaluation of the activity of the new complexes *in vitro* and an understanding of the mechanism of action in the body will direct the design of improved analogous complexes.

In this study new titanium complexes were designed, synthesized, characterized and tested *in vitro*. Certain aspects of the mechanism of their interaction with DNA were investigated.

Design of Complexes

In the design of the complexes it was assumed that the new complex would attack DNA and in the process change the DNA structure. The objective was to incorporate ligands with different functionalities. One ligand should be labile and easily substituted to make available a vacant coordination site for covalent bond formation to DNA. A second, less labile ligand should consist of a planar condensed polycyclic ring system for possible intercalation. To achieve these goals the complexes had to meet certain geometrical requirements based on the metallocene dihalide model (Fig. 1.17).

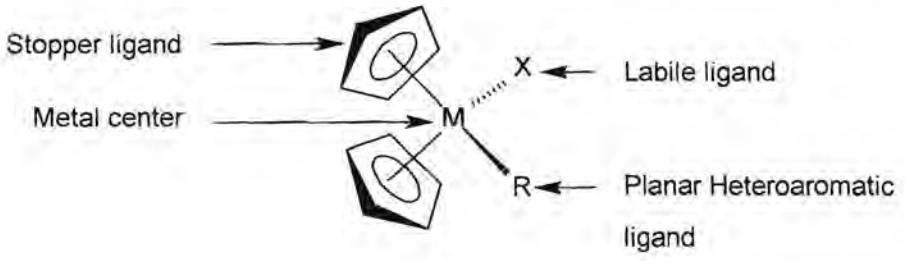


Figure 1.17. A designer model of the features of new antitumor complexes.

The new titanocene complexes have to meet the following requirements:

- The metal centre (**M**) must be in a high oxidation state to prevent oxidation in the body, hence Ti(IV).
- A labile ligand (**X**), which will dissociate readily to leave a vacant coordination site on the metal to allow for covalent bonding to DNA.
- A unique planar heteroaromatic ligand (**R**) with two to four rings to intercalate in the major groove of the DNA helix.
- Stable stopper ligands to direct and align the intercalation process.
- The orientation of the plane of the ring system with respect to the other ligands is important and special attention should be given to obtain the correct structural features, to achieve the above objectives.

Titanocene dichloride was chosen as the starting compound for a number of reasons. It is a very versatile precursor, which can be used to synthesize a large spectrum of derivatives. For this reason it would be possible to combine labile ligands with polycyclic condensed heteroaromatic rings. Alternatively, $[TiCpCl_3]$ could have been used, but for this study $[TiCp_2Cl_2]$ was selected because of its stability and proven success in the field of drug design.

The covalent binding affinities of titanocene dichloride (Ti^{4+} is a hard metal center) to hard heteroatoms are to be combined with the intercalation features of aromatic ring ligands, to synthesize a series of compounds, which could potentially display antitumor properties. The interaction of the complexes with DNA is expected to be different from that of titanocene dichloride, because only one labile ligand will be available for dissociation. Covalent bond formation between the metal and DNA is expected to occur at an oxygen of the phosphate backbone of DNA. No cross-links are expected as in the case of titanocene dichloride.

The unique ligand (**R**) was altered to investigate the effect on antitumor activity in two ways. Firstly, the feature of the intercalator was changed and secondly, the intercalator was positioned differently relative to the metal center to try and determine the ideal geometry for optimized intercalation.

The ligand supposed to intercalate was varied as illustrated in Figure 1.18 to determine the effect of:

- two versus three coplanar rings **(1, 4 vs. 2,5)**
- the type of heteroatom in the ring **(1-5)**
- one versus two heteroatoms in the ring **(2, 5 vs. 3)**
- a substituent on the ring **(6)**

The positioning of the intercalator was investigated by determining the effect of:

- inserting a linker atom between the ring and the metal **(4, 5)**

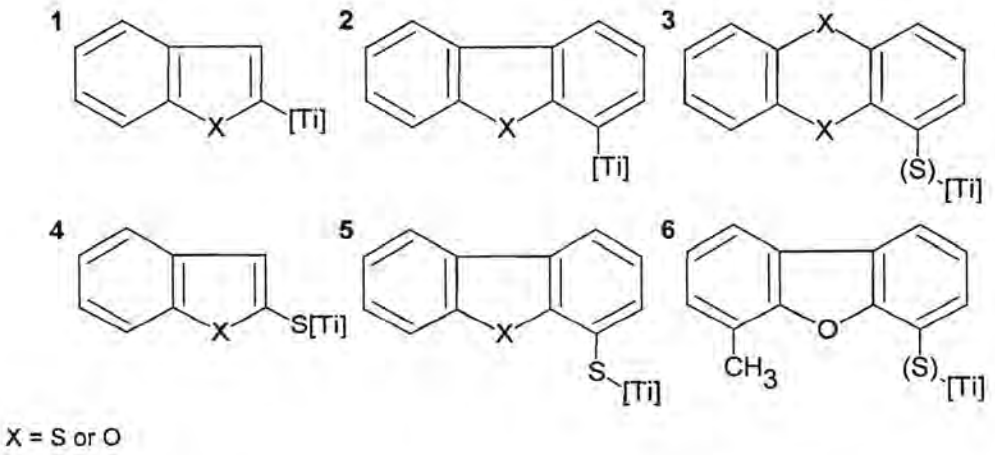


Figure 1.18. The metallated coplanar polycyclic heteroaromatic rings studied.

Synthesis

Titanocene dichloride will, after one chloro ligand (**X**) was displaced, display a unique heteroaryl ligand from a family of heteroaromatic substrates (**R**) illustrated in Figure 1.18. Some of the ligands in Figure 1.18 will be synthesized and others will be prepared from commercially available materials. The synthesis is based on a displacement reaction involving a lithiated heteroaromatic substrate and a chloro ligand of the titanocene dichloride. The site and effectiveness of lithiation of the heteroaromatic rings as well as their subsequent reactivity with titanocene dichloride will also be studied.

Characterization

The complexes will be characterized by using various standard methods. The degree by which the aromatic character of the rings in the new complexes will be affected, due to the metal-carbon or metal-sulphur bond, will be studied with NMR spectroscopy. Structural features and properties will be investigated, using NMR spectroscopy, mass spectrometry and as far as possible, X-ray crystallography.

In Vitro tests

A selection of complexes will be tested *in vitro* against human cervix epitherioid carcinoma (HeLa) and colorectal carcinoma (CoLo) cell cultures to determine possible antitumor activity. One aim will

be to determine whether variations in the heteroaromatic ring ligands have any effect on antitumor activity and if so to determine a pattern for use in future studies.

Aspects of the Mechanism

Tests to confirm or disprove the assumptions about covalent bonding and intercalation by the new complexes will be conducted. A selection of the best complexes will be studied for intercalation by an application of a flow cytometry technique. A further objective was, if intercalation was observed to be relevant, to try and find a correlation between the geometry of the complex and intercalation. The relative labilities of the ligands are to be tested in aqueous medium and monitored by NMR spectroscopy.

Extension to Bi- and Trinuclear Systems

The design of these complexes was based on the mononuclear metallocene model. A further point of interest was to design binuclear titanium complexes that still displayed the features of the parent compound, titanocene dichloride. Another aim was to study the synthesis of multinuclear complexes incorporating cisplatin-like platinum fragments into the titanocene complexes. These will include the following complexes (Figure 1.19):

- Titanocene – titanocene complexes **(1)**
- Titanocene – *cis*platin complexes linked via the cyclopentadienyl ring **(2, 3)**
- Titanocene – *cis*platin complexes linked via the titanium center **(4, 5)**

The aim was to use metal complexes that proved to be successful as antitumor agents (titanocene and *cis*platin) and incorporate two or three of these metal centers into the same molecule. In the first case two titanocene dichloride fragments are to be linked by inserting a hydrocarbon chain between one of the cyclopentadienyl ligands on each metal fragment. This will lead to a complex that should display antitumor properties, but have a different mechanism of action compared to titanocene dichloride. Farrel[57] followed a similar approach in linking *cis*platin units via a bridging amine ligand. The bi- and trinuclear complexes displayed antitumor properties very different from those of *cis*platin.

57. H. Rauter, R. Di Domenico, E. Menta, A. Olivia, Y. Qu, N. Farrel, *Inorg. Chem.*, **1997**,*36*, 3919.

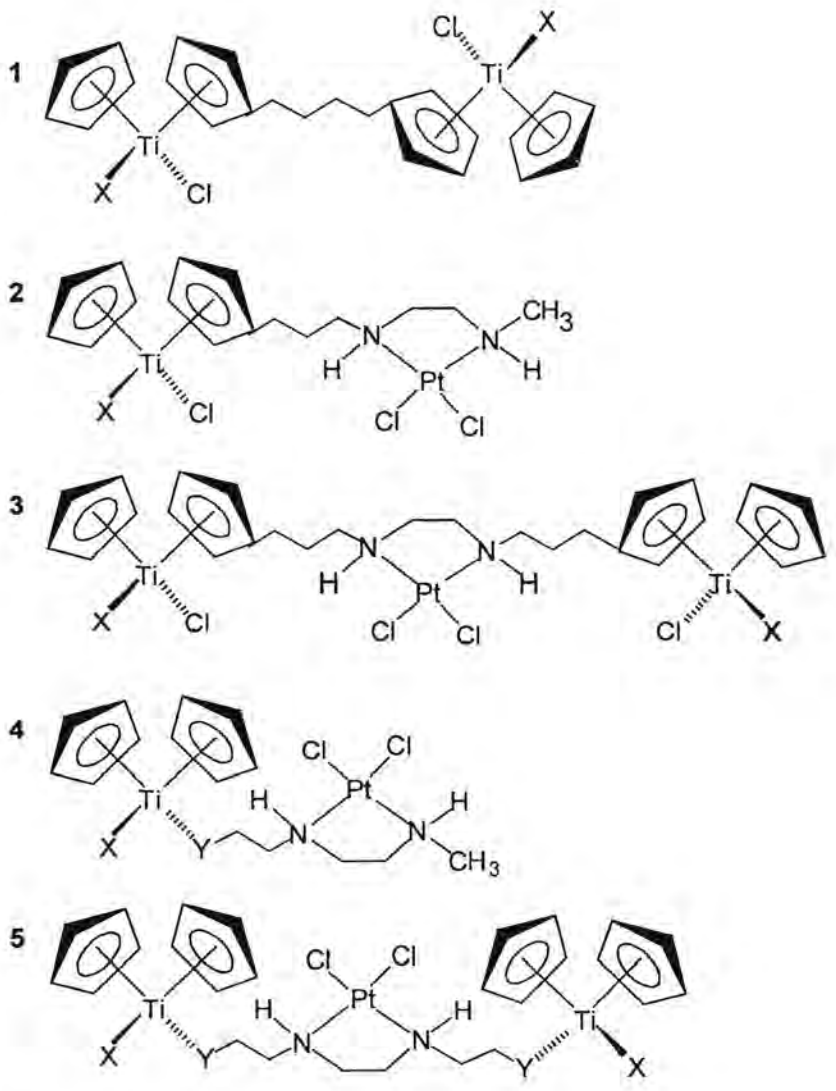


Figure 1.19 Bi- and trinuclear complexes.

Titanocene dichloride is to be combined with a platinum fragment that is analogous to *cis*platin. To accomplish this a linker need to be attached to one of the cyclopentadienyl rings of titanocene dichloride and the nitrogen of the amino group on the platinum fragment. The covalent bond properties of titanocene dichloride can now be combined with the cross-linking properties of the platinum fragment at different sites in the same molecule which could possibly lead to a broader spectrum of antitumor activity. It is also possible that competition between the two metal fragments of the molecule can facilitate a totally different mechanism of action compared to that of the two model

complexes. In this type of Ti-Pt complex there are two chloro ligands on the titanocene fragment available to dissociate and give vacant sites for covalent bonding. These complexes can also be used as starting compounds to incorporate polycyclic condensed heteroaromatic rings by replacing labile chloro ligands as was done with titanocene dichloride as precursor.

A variation of the Ti-Pt type of complexes can be achieved by inserting a linker between the nitrogen of the amino group of the platinum fragment and replacing a chloro ligand on the titanium fragment. The mechanism of this type of complex with DNA is expected to be different from that of the previous type of Ti-Pt complex. In this case only one labile ligand will be available on the titanocene fragment for dissociation to allow for covalent bond formation between the metal and DNA.

1.6 Construction of the thesis

In Chapters 2 - 4 the synthesis and characterisation of the new complexes will be discussed. A scheme of the planned route for the synthesis of the compounds will be given and the results discussed. The purity of the compounds will be determined by chemical analysis and mass spectrometry. Molecular composition and structural features of the products will be derived from techniques of NMR spectroscopy and where possible, single crystal X-ray determinations will be done to confirm the structure of new complexes. In Chapter 6 the experimental procedures for all new compounds are given.

Chapters 2 and 3 deal with titanocene complexes focussing on varying aspects associated with the intercalating ligand. Two classes of complexes will be discussed. Firstly, those where the metal centre is bound directly to the heteroatomic ring ligand and part of the intercalator (Chapter 2) and secondly, those where a sulfur atom is placed between the metal and the ring ligand to distance the metal from the intercalator (Chapter 3). Chapter 4 studies bi- and trinuclear complexes.

In Chapter 5 the antitumor properties of the complexes will be discussed in terms of their *in vitro* test results. Structure-activity correlations will be made. Investigation of the mechanism of bioactivity of these complexes, the results of the intercalation tests will be discussed and the aspect of covalent bonding to DNA will be investigated. The most important results of the ligand substitution studies will be listed and conclusions from this study summarized.

Chapter 2

Titanocene derivatives with a heteroaromatic ligand containing a direct metal-carbon σ-bond

2.1 Introduction

The properties of dibenzothiophene **(L2-01)** and benzo(b)thiophene **(L2-02)** derivatives have been studied extensively and a number of these compounds have shown biological activity[1]. Some of these compounds are illustrated in Figure 2.1. One of the best known benzo(b)thiophene derivatives with antiflammatory properties is Tianafac (**a**), while 3-amino-2-aryl thiophene (**b**) is known as a hypolipidamic compound. The benzothiophene compound (**c**) with an isopropanol substituent has spasmolytic activity, whereas (**d**) is a hydroxytryptamin antagonist. Examples of **L2-01** derivatives with biological activity are RMA 11877 (**e**), a viristaticum agent and (**f**) is a hypolipidamic compound.

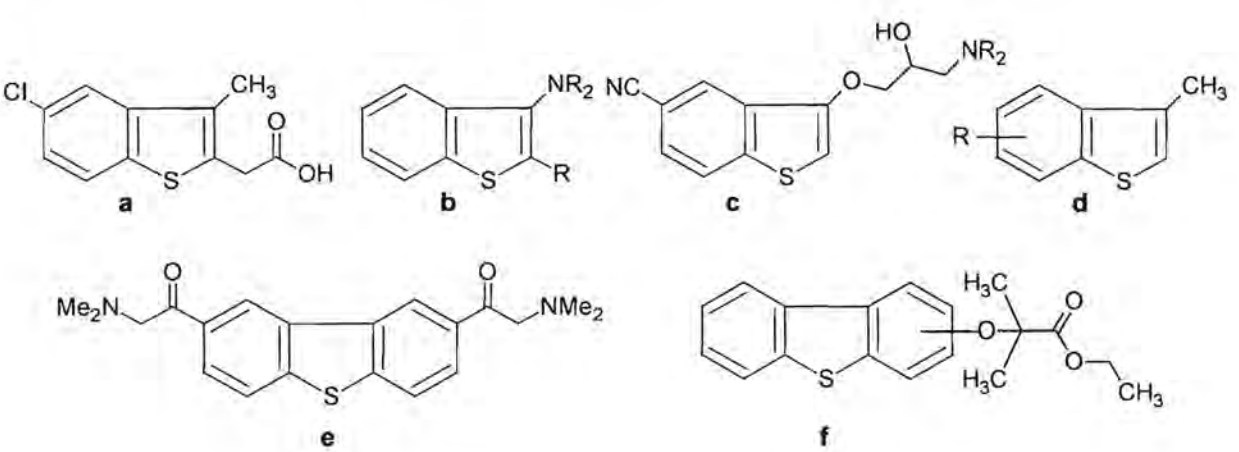


Figure 2.1 Biological active **L2-01** and **L2-02** derivatives: (a) antiflamatory, (b) and (f) hypolipidamic, (c) spasmolytic, (d) hydroxytryptamin and (e) viristaticum.

1. R. Pech, R. Böhm, *Pharmazie*, **1984**, *39*, 4

Osborn and co-workers studied the biological activity of **L2-01** derivatives and they suggested that the activity of these complexes results from their absorption onto adenine-thymine or guanine-cytosine base pairs at the end of DNA strands[2]. Recently McCowan and co-workers[3,4] reported that dibasic benzothiophene derivatives have been discovered and optimised through structural modification to represent a new class of active site-directed thrombin inhibitors of which the complex in Figure 2.2 performed the best.

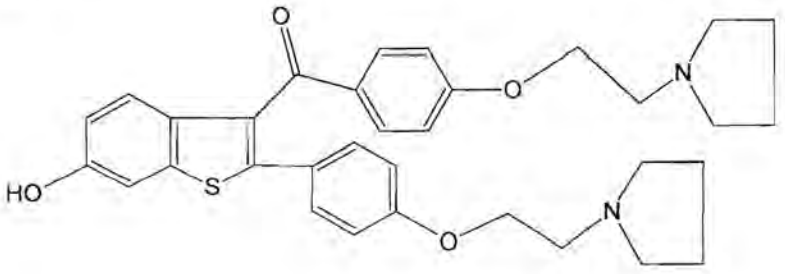


Figure 2.2 A dibasic **L2-02** derivative.

The value of **L2-01** and **L2-02** as ligands in biological active complexes was realized and introducing these ligands to titanocene complexes might lead to antitumor activity. Titanocene derivatives with the general formula $[TiCp_2(R)Cl]$. R = Dbt (**2-08**); Bt; Dbz (**2-05**); Thr (**2-09**); Dbf (**2-01**) and Bf (**2-07**) are shown in Figure 2.3. (DbtH = dibenzothiophene, BtH = benzo(b)thiophene, DbzH = dibenzodioxin, ThrH = thianthrene, DbfH = dibenzofuran and BfH = benzo(b)furan). The heteroatom directs lithiation and ultimately complexation to the titanium in such a manner that the heteroatom is on the same side of the rings as titanium. **L2-01** is easily metallated by butyllithium on the 4-position and a reaction with titanocene dichloride gives $[TiCp_2(Dbt)Cl]$ **2-08** (Figure 2.3). **L2-02** is metallated on the 2-position to produce the titanium complex $[TiCp_2(Bt)Cl]$ which was too unstable for further use in antitumor studies[5].

2. E. Champaigne, J. Ashby, S. W. Osborn, *J. Heterocycl. Chem.*, **1969**, *6*, 885.

3. D. J. Sall, J. A. Bastian, S. L. Briggs, J. A. Buben, N. Y. Chirgadze, D. K. Klawson, M. L. Denny, D. D. Giera, D. S. Gifford-Moore, R. W. Harper, K. L. Hauser, V. J. Klimkovski, T. J. Kohn, H-S. Lin, J. R. McCowan, A. R. Palkovitz, G. F. Smith, K. Takeuchi, K. J. Thrasher, J. M. Tinsley, B. G. Utterback, S-C. B. Yan, M, Zhang, *J. Med. Chem.* **1997**, *40*, 3489.

4. D. J. Sall, D. L. Bailey, J. A. Bastian, J. A. Buben, N. Y. Chirgadze, A. C. Clemmins-Smith, M. L. Denny, M. J. Fisher, D. D. Giera, D. S. Gifford- Moore, R. W. Harper, L. M. Johnson, V. J. Klimkovski, T. J. Kohn, H-S. Lin, J. R. McCowan, A. D. Palkowitz, M. E, Richett, G. F. Smith, D. W. Snyder, K. Takeuchi, J. E. Toth, M. Zhang, *J. Med. Chem.* **2000**, *43*, 649.

5. R. Meyer, *Titanium, Molybdenum and Platinum Complexes with Potential Antitumor Properties*, Ph. D. (Chemistry) Thesis, University of Pretoria, **1998**, 24.

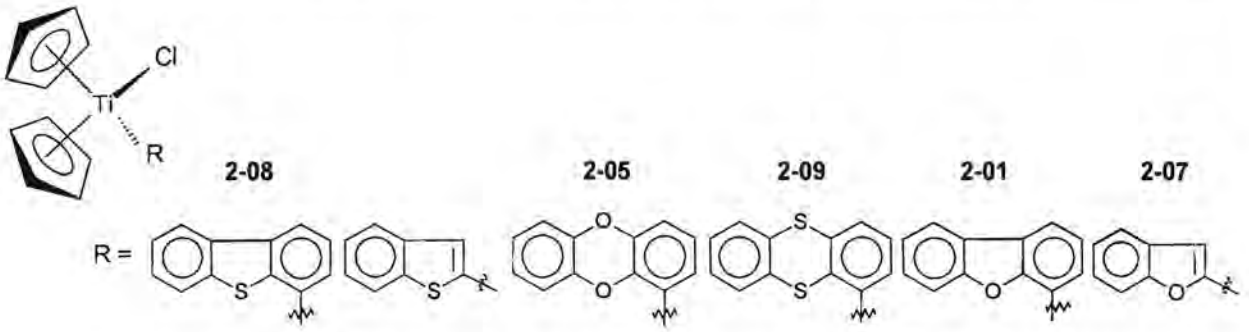


Figure 2.3 Titanocene derivatives with the general formula [$TiCp_2$(R)Cl].

For intercalation it could be advantageous to have a heteroatom on the ring on the opposite side of the metal in the complex. Hence, the next step was to introduce heteroaromatic ligands with two heteroatoms in the ring and dibenzodioxin **(L2-03)** and thianthrene **(L2-04)** were considered. Derivatives of **L2-03** were investigated before and found to be promising biological agents[6]. Palmer[7] reported that the dibenzo[1,4]dioxin-1-carboxamide (Figure 2.4) has significant antitumor activity. Since the surface areas of **L2-03** and **L2-04** are larger than that of **L2-01** it is believed that they would perform better as potential intercalation agents. Metallation of **L2-03** and **L2-04** on the 1-position and subsequent reaction with titanocene dichloride afforded [$TiCp_2$(Dbz)Cl] (**2-05**) and [$TiCp_2$(Thr)Cl] (**2-09**).

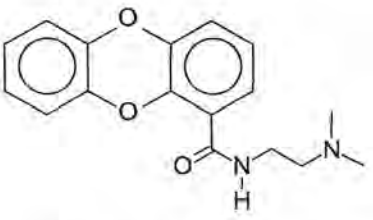


Figure 2.4. Dibenzo[1,4]dioxin-1-carboxamide.

Many substances with pharmacological, therapeutic or toxic properties are found among natural products with a benzofuran (**L2-05**) ring. Of the synthetic **L2-05** derivatives synthesized some are biologically active and their numbers are much larger than the **L2-02** series[8,9]. Examples of these are

6. B. D. Palmer, M. Boyd, W. A. Denny, *J. Org. Chem.*, **1990**, *55*, 438.

7. B. D. Palmer, G. W. Rewcastle, G. J. Atwell, B. C. Baguley, W. A. Denny, *J. Med. Chem.* **1988**, *31*, 707.

8. J.P. Garnier, *Actualitès de Chimie Thérapeutique*, Edifor, Paris, **1971**, 9.

9. P. N. Craig, H. C. Caldwell, W.G. Groves, *J. Med. Chem.* **1970**, *13*, 1079.

nitrobenzofurans, which are bactericidal, and in Figure 2.5 compounds are listed which are esterogenic (**a**), has anti-inflammatory properties (**b**) and anti-fertility properties in animals (**c**).

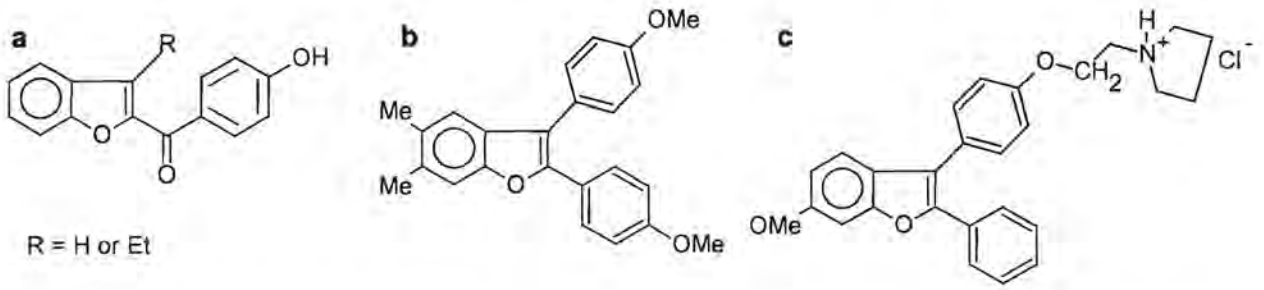


Figure 2.5 Biological active **L2-05** derivatives which display (a) esterogenic, (b) anti-inflammatory and (c) anti-fertility properties.

The ligand **L2-05** and dibenzofuran **(L2-06)** are metallated at slightly higher temperatures than their sulfur analogous and [$TiCp_2$(Dbf)Cl] **(2-01)** and [$TiCp_2$(Bf)Cl] **(2-07)** were isolated. The role of a methyl substituent was tested by introducing it at the 4-position of **L2-06** to give 4-methyl dibenzofuran **(L2-07)**. In all of the above mentioned products the metal fragment was on the outside of the heteroaromatic ligand and not part of the rings. It was decided to investigate a complex where both chloro ligands are replaced by titanium to form a wedge-like complex with the ring oxygen opposite the titanium which is inserted into the central ring of three condensed rings (Figure 2.6).

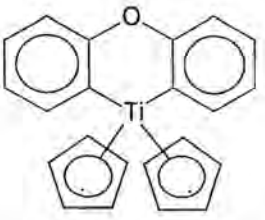
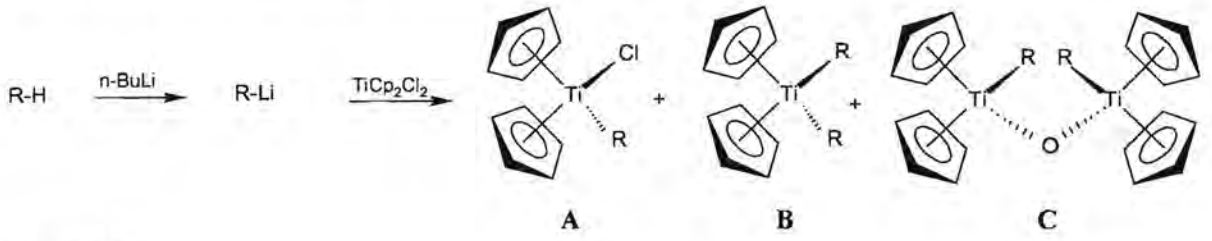


Figure 2.6 Titanocene complex with the metal inserted into the heteroaromatic ring.

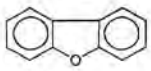
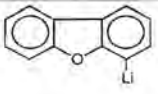
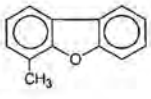
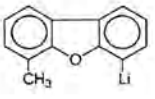
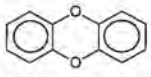
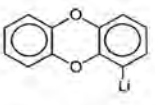
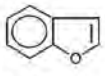
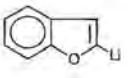
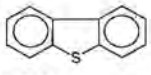
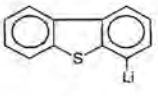
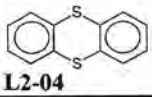
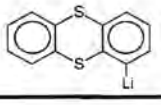
2.2 Synthesis

The complexes were synthesized using the same method for all. The heteroaromatic ring compound was lithiated and added to titanocene dichloride at low temperatures. Lithiation of **L2-01**, **L2-03**, **L2-04**, **L2-05**, **L2-06**, **L2-07** and diphenylether **(L2-08)** proceeded in high yields and complexes were isolated in moderate to high yields. The synthesis route afforded **A**, **B** and **C** type products as is illustrated in Scheme 2.1. Table 2.1 shows the identification numbers of the complexes.



Scheme 2.1

Table 2.1 Identification numbers of the complexes in Scheme 2.1.

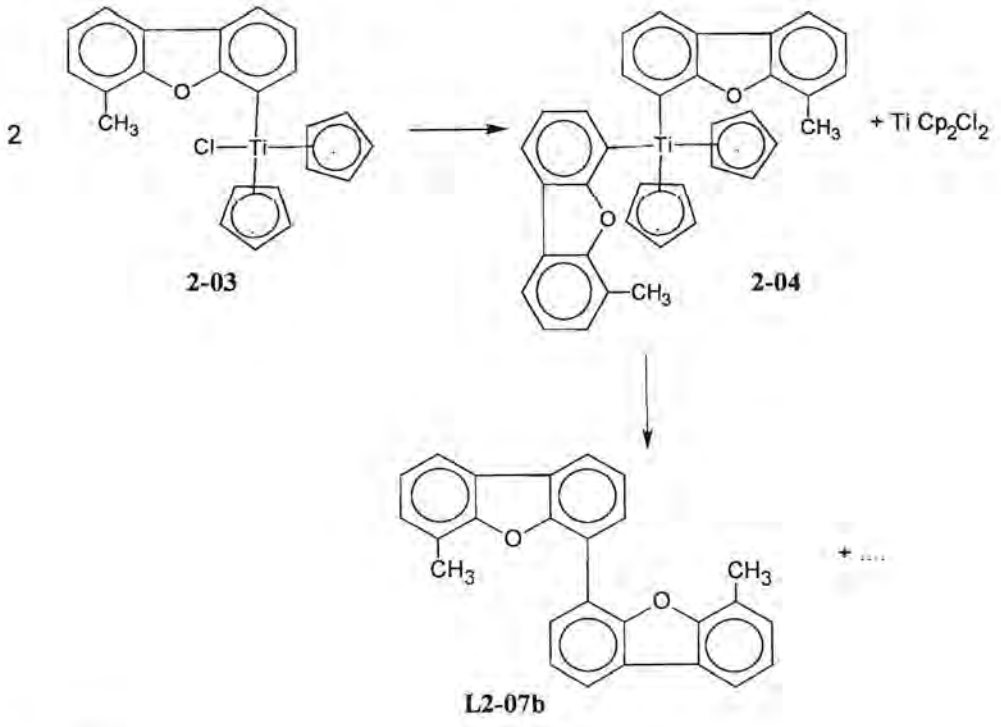
Heteroarene (R-H)	Reagent (R-Li)	Product A	Product B	Product C
 **L2-06**		**2-01**	**2-02**	-
 **L2-07**		**2-03**	**2-04**	-
 **L2-03**		**2-05**	-	**2-06**
 **L2-05**		**2-07**	-	-
 **L2-01**		**2-08**	-	-
 **L2-04**		**2-09**	-	**2-10**

Addition of titanocene dichloride to **L2-06**

L2-06 was metallated by n-BuLi at the 4-position and addition of titanocene dichloride caused a colour change from red to orange. Chromatography on aluminium oxide yielded bis(cyclopentadienyl)bis(dibenzofuran-4-yl)titanium(IV), $[TiCp_2(Dbf)_2]$ **(2-02)** and chlorobis (cyclopentadienyl)(dibenzofuran-4-yl)titanium(IV), $[TiCp_2(Dbf)Cl]$ **(2-01)**. Product **2-02** was crystallized from a dichloromethane-hexane solution that yielded crystals suitable for a single crystal, X-ray diffraction study.

Addition of titanocene dichloride to **L2-07**

Addition of methyl iodide to lithiated **L2-06** afforded a yellow solid that was purified by column chromatography and characterized as **L2-07** after recrystallization from dichloromethane. Lithiation of **L2-07** with n-BuLi deprotonated the unsubstituted ring on the 4-position. On addition of titanocene dichloride the reaction colour turned from red to yellow-brown, which after column chromatography on silica gel, yielded bis(cyclopentadienyl)bis(6-methyl dibenzofuran-4-yl)titanium(IV), [$TiCp_2$(Dbf-Me)$_2$] **(2-04)** and chlorobis(cyclopentadienyl)(6-methyl dibenzofuran-4-yl)titanium(IV), [$TiCp_2$(Dbf-Me)Cl] **(2-03)**. Complex **2-03** is unstable and converts rapidly to **2-04** and titanocene dichloride (Scheme 2.2). Complex **2-04** is also unstable, but more stable than **2-03** and converts slowly to a dimer of **L2-07,** [Me-Dbf-Dbf-Me] **L2-07b**.

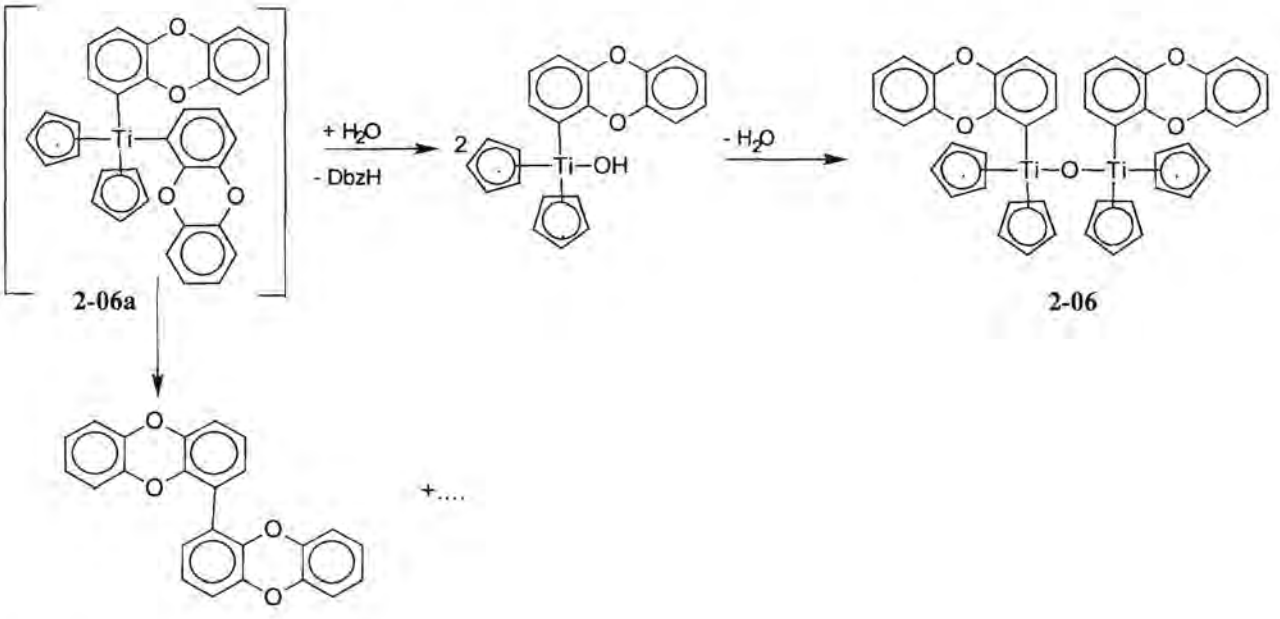


Scheme 2.2

Addition of titanocene dichloride to **L2-03**

L2-03 was metallated by a mixture of n-BuLi/TMEDA at the 1-position. Addition of titanocene dichloride caused a colour change from red to dark orange-brown. Chromatography on silica gel yielded the expected product, chlorobis(cyclopentadienyl)(dibenzodioxin-1-yl)titanium(IV) **2-05** [$TiCp_2$(Dbz)Cl]. Solvent mixtures such as dichloromethane-hexane or toluene-hexane did not produce crystals of a good enough quality for X-ray structural studies. After many attempts, the slow crystallisation from a THF-hexane mixture, afforded crystals suitable for a single crystal X-ray

diffraction study. A second yellow product is believed to be bis(cyclopentadienyl)bis(dibenzodioxin-1-yl)titanium(IV), [$TiCp_2(Dbz)_2$] (**2-06a**). This complex was very unstable and NMR studies revealed decomposition with regeneration of the free ligand. The solution slowly turned black every time the compound was dissolved in a chlorinated solvent. Finally **2-06a** was filtered successfully with benzene. The yellow product **2-06a** converted into a second yellow product, of which the composition was verified by NMR spectral data. The oxygen bridged binuclear complex, (μ-oxo)bis[bis(cyclopentadienyl)(dibenzodioxin-1-yl)titanium(IV)], [(μ-O){$TiCp_2(Dbz)$}$_2$] **(2-06)**, is consistent with the NMR data obtained for this product. The titanocene bis(dibenzodioxin-1-yl) complex, **2-06a** is unstable and slowly reacts with water from the column material. As a result, the water protonates one of the Dbz ligands, leading to the release of **L-03** and the formation of a hydroxo complex (Scheme 2.3). Two hydroxo complexes combine by water elimination to give the oxobridged binuclear complex **2-06**. During the conversion water acts as reactant and catalyst. Complex **2-06a** also decomposes competitively by a reductive elimination reaction of the two heteroarene ligands to give a dimer Dbz-Dbz.



Scheme 2.3

A possible reason for the instability of **2-06a** may result from the presence of two bulky dibenzodioxin ligands. Furthermore, the formation of **2-06** is proof of the fact that in the reaction mixture, **2-05** has a reactive remaining chloro ligand, which in the presence of lithiated **L2-03** can lead to the displacement of the second chloro ligand.

Addition of titanocene dichloride to **L2-05**

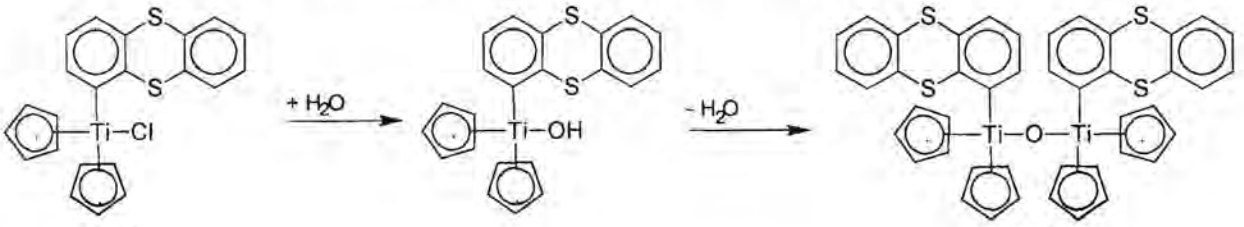
L2-05 was deprotonated on the 2-position using n-BuLi in a THF/hexane solution. Addition of titanocene dichloride to the lithiated product gave an immediate colour change from red to bright orange. Attempts to separate the product on silica gel led to immediate decomposition. Column chromatography on alumina yielded (benzofuran-2-yl)chlorobis(cyclopentadienyl)titanium(IV), [$TiCp_2$(Bf)Cl] **2-07**. This product was very sensitive to oxygen and was highly unstable at room temperature. It could be kept under argon at low temperatures for about one week. Due to the instability it was only possible to characterize **2-07** with ^{1}H NMR spectroscopy.

Addition of titanocene dichloride to **L2-01**

Metallation of **L2-01** with n-BuLi and addition of titanocene dichloride gave the expected product chlorobis(cyclopentadienyl)(dibenzothiophen-4-yl)titanium(IV), [$TiCp_2$(Dbt)Cl] **(2-08)** which was purified by column chromatography on silica gel.

Addition of titanocene dichloride to **L2-04**

Lithiation of **L2-04** proceeded in a high yield and on addition of titanocene dichloride, the colour changed from red to orange-brown. From the silica gel column chlorobis(cyclopentadienyl)(thianthren-1-yl)titanium(IV), [$TiCp_2$(Thr)Cl] **(2-09)** was collected followed by a yellow fraction bis(cyclopentadienyl)bis(thianthren-1-yl)titanium(IV), [$TiCp_2(Thr)_2$] **(2-10a)**, which was too unstable to purify. Complex **2-09** readily converted into a second yellow product, (μ-oxo)bis[bis(cyclopentadienyl)(thianthren-1-yl)titanium(IV)] [(μ-O){$TiCp_2(Thr)$}$_2$] **(2-10)**. Due to the instability of **2-09** in solution, the only way to characterize the complex was through measuring the ^{1}H NMR spectrum as soon as possible. Attempts to obtain a ^{13}C NMR spectrum failed, as the complex already started to decompose to **2-10** during measurement. In comparison with **2-05**, complex **2-09** is far less stable, indicating that the **L2-04** ligand has a stronger activation effect on the remaining chloro ligand, than was the case with the **L2-03** ligand. The yellow fraction in this case originated from **2-09**. The fact that **2-10a** was too unstable to isolate is ascribed to steric constraints of the two ring ligands. Comparing stabilities one could conclude that **2-05** > **2-09** and **2-06a** > **2-10a**. Although **2-10** and **2-06** originate from different precursors, the conversion in both cases is brought about by the presence of water on the column material. The desired complex **2-09** has a highly activated chloro ligand, which in the presence of traces of water converts into the corresponding hydroxo complex. In a subsequent reaction the hydroxo complex forms the dinuclear complex **2-10**, by the elimination of water between two intermediate hydroxo complexes or the elimination of hydrochloric acid between complex **2-09** and a hydroxo intermediate complex (Scheme 2.4).

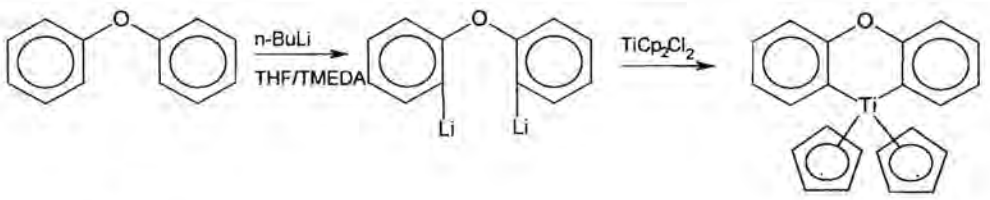


2-09 **2-10**

Scheme 2.4

Addition of titanocene dichloride to **L2-08**

In Scheme 2.5 the ring ligand is bound directly to the metal center by replacing both chloro ligands. Diphenyl ether **(L2-08)** was double lithiated in high yield by a n-BuLi / TMEDA solution and a brown red precipitate settled out. The addition of titanocene dichloride caused an immediate colour change from red to orange affording {bis(cyclopentadienyl)}(diphen-2,2′-ylether)titanium(IV) [$TiCp_2$(Dpe)] ($DpeH_2$ = diphenyl ether) **2-11**.



L2-08 **2-11**

Scheme 2.5

2.3 Characterization

- ***Mass spectrometry***

The mass spectral data for **L2-01**[10], **L2-03**[11], **L2-04**[11], **L2-05**[12] and **L2-06**[13] is reported in literature. The data for complexes **L2-07**, **2-01** – **2-06** and **2-08** – **2-11** is summarized in Table 2.2. It was not possible to record a spectrum for complex **2-07** due to instability.

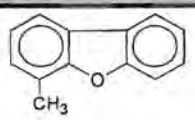
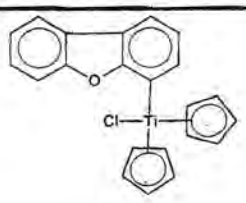
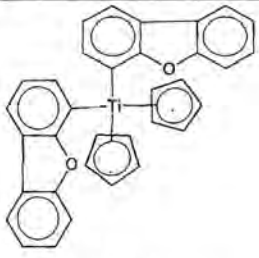
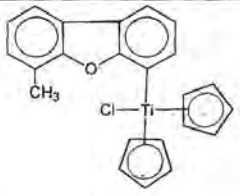
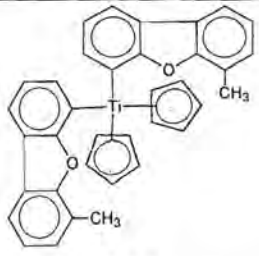
10. W Riepe, M. Zander, *Org. Mass Spectrom.*, **1979**, *14*, 455.

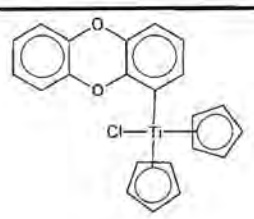
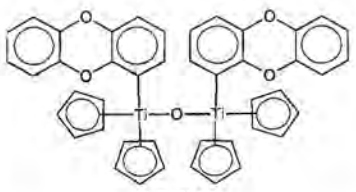
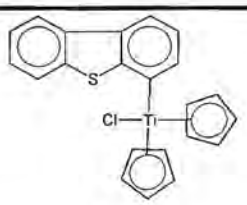
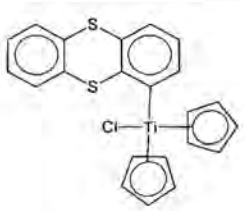
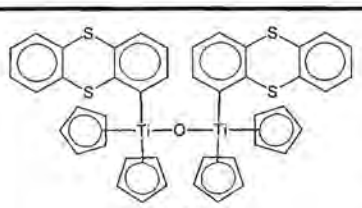
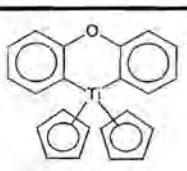
11. N. P. Buu-Hoï, G. Saint-Ruf, M. Mangane, *J. Heterocycl. Chem.*, **1972**, *9*, 691.

12. T. Kuster, H. Mändli, R. Robbiani, J. Seibl, *Helv. Chem. Acta*, **1978**, *61*, 1017.

13. B. G. Pring, N. E. Stjernstrom, *Acta Chem. Scand.*, **1968**, *22*, 549.

Table 2.2 Mass spectral data for ligand **2-07** and complexes **2-01 - 2-06** and **2-08 – 2-11.**

	Mass Peaks, m/z(I%)	
 **L2-07**	182 (100) $[M]^+$	91 (4) $[C_7H_7]^+$
	168 (20) $[M-CH_3]^+$	76 (6) $[C_6H_4]^+$
	140 (5) $[M-CH_3CO]^+$	63 (5) $[C_5H_3]^+$
	127 (5) $[C_{10}H_7]^+$	51 (3) $[C_4H_3]^+$
	115 (3) $[C_9H_7]^+$	39 (2) $[C_3H_3]^+$
	103 (2) $[C_8H_7]^+$	27 (1) $[C_2H_3]^+$
 **2-01**	381 (3) $[M]^+$	215 (25) $[Ti(Dbf)]^+$
	346 (100) $[M^+-Cl]$	178 (5) $[TiCp_2]^+$
	315 (35) $[TiCp(Dbf)Cl]^+$	168 (100) $[DbfH]^+$
	280 (28) $[TiCp(Dbf)]^+$	167 (100) $[Dbf]^+$
	250 (5) $[Ti(Dbf)Cl]^+$	148 (48) $[TiCpCl]^+$
	234 (55) $[Ti(Dbf)O]^+$	113 (15) $[TiCp]^+$
 **2-02**	513 (13) $[M]^+$	280 (27) $[TiCp(Dbf)]^+$
	463 (1) $[TiCp(Dbf)_2O]^+$	234 (20) $[Ti(Dbf)O]^+$
	447 (2) $[TiCp(Dbf)_2]^+$	215 (13) $[Ti(Dbf)]^+$
	398 (2) $[Ti(Dbf)_2O]^+$	178 (12) $[TiCp_2]^+$
	382 (3) $[Ti(Dbf)_2]^+$	168 (100) $[DbfH]^+$
	346 (100) $[M^+-Dbf]$	167 (48) $[Dbf]^+$
	296 (12) $[TiCp(Dbf)O]^+$	113 (8) $[TiCp]^+$
 **2-03**	541 (5) $[M]^+$ (**2-04**)	247 (16) $[Ti(Dbf\text{-}Me)O]^+$
	493 (4) $[TiCp(Dbf\text{-}Me)O]^+$	213 (7) $[TiCp_2Cl]^+$
	395 (4) $[M]^+$	181 (100) $[Dbf\text{-}MeH)]^+$
	359 (48) $[TiCp_2(Dbf\text{-}Me)]^+$	180 (100) $[Dbf\text{-}Me)]^+$
	330 (4) $[TiCp(Dbf\text{-}Me)Cl]^+$	178 (5) $[TiCp_2]^+$
	294 (11) $[TiCp(Dbf\text{-}Me)]^+$	148 (12) $[TiCpCl]^+$
	264 (3) $[Ti(Dbf\text{-}Me)Cl]^+$	113 (5) $[TiCp]^+$
 **2-04**	573 (14) $[M+2O]^+$	330 (5) $[TiCp(Dbf\text{-}Me)Cl]^+$
	557 (9) $[M^+ + O]^+$	294 (3) $[TiCp(Dbf\text{-}Me)]^+$
	541 (85) $[M]^+$	245 (4) $[Ti(Dbf\text{-}Me)O]^+$
	493 (50) $[TiCp(Dbf\text{-}Me)_2O]^+$	181 (100) $[Dbf\text{-}MeH)]^+$
	475 (7) $[TiCp(Dbf\text{-}Me)_2]^+$	180 (100) $[Dbf\text{-}Me)]^+$
	410 (24) $[Ti(Dbf\text{-}Me)_2]^+$	178 (5) $[TiCp_2]^+$
	360 (39) $[TiCp_2(Dbf\text{-}Me)]^+$	113 (3) $[TiCp]^+$

 **2-05**	396 (3) $[M]^+$ 361 (96) $[M^+-Cl]^+$ 366 (13) $[Dbz\text{-}Dbz]^+$ 331 (10) $[TiCp(Dbz)Cl]^{+\cdot}$ 295 (29) $[TiCp(Dbz)]^+$ 248 (23) $[TiCp_2Cl_2]^+$	230 (22) $[Ti(Dbz)]^{+\cdot}$ 213 (12) $[TiCp_2Cl]^+$ 184 (100) $[DbzH]^+$ 183 (100) $[Dbz]^+$ 178 (7) $[TiCp_2]^+$ 148 (32) $[TiCpCl]^+$
 **2-06**	738 $[M^+]$ not observed 430 (53) $[Ti(Dbz)_2O]^+$ 378 (1) $[TiCp_2(Dbz)O]^{+\cdot}$ 366 (3) $[Dbz\text{-}Dbz]^+$ 361 (3) $[TiCp_2(Dbz)]^{+\cdot}$	312 (3) $[TiCp(Dbz)O]^{+\cdot}$ 247 (6) $[Ti(Dbz)O]^+$ 184 (13) $[DbzH]^+$ 183 (13) $[Dbz]^+$
 **2-08**	396 (27) $[M]^+$ 366 (96) $[Dbt\text{-}Dbt]^+$ 331 (29) $[TiCp(Dbt)Cl]^+$	231 (6) $[Ti(Dbt)]^{+\cdot}$ 184 (100) $[Dbt]^+$ 148 (48) $[TiCpCl]^+$
 **2-09**	624 (11) $[TiCp_2(OThr)(Thr)]^{+\cdot}$ 608 (1) $[M^+]$ **(2-10a)** 559 (10) $[TiCp(OThr)(Thr)]^{+\cdot}$ 494 (18) $[Ti(OThr)(Thr)]^+$ 430 (2) $[Thr\text{-}Thr]^+$ 428 (3) $[M^+]$ 409 (28) $[TiCp_2(OThr)]^{+\cdot}$ 393 (11) $[TiCp_2(Thr)]^{+\cdot}$	344 (31) $[TiCp(OThr)]^+$ 280 (59) $[Ti(Thr)OH]^{+\cdot}$ 247 (27) $[TiCp_2Cl_2]$ 216 (21) $[ThrH]^+$ 215 (21) $[Thr]^+$ 178 (15) $[TiCp_2]^+$ 113 (6) $[TiCp]^{+\cdot}$ 64 (9) $[TiO]^+$
 **2-10**	802 $[M^+]$ not observed 624 (2) $[TiCp_2(OThr)(Thr)]^+$ 608 (1) $[M^+]$ **(2-10a)** 559 (1) $[TiCp(OThr)(Thr)]^{+\cdot}$ 543 (1) $[TiCp(Thr)_2]^{+\cdot}$ 494 (7) $[Ti(OThr)(Thr)]^{+\cdot}$ 430 (5) $[Thr\text{-}Thr]^+$ 409 (77) $[TiCp_2(OThr)]^{+\cdot}$ 393 (42) $[TiCp_2(Thr)]^{+\cdot}$	344 (60) $[TiCp(OThr)]^+$ 280 (100) $[Ti(Thr)OH]^{+\cdot}$ 232 (72) $[ThrOH]^+$ 216 (66) $[ThrH]^+$ 215 (66) $[Thr]^+$ 178 (51) $[TiCp_2]^+$ 113 (13) $[TiCp]^{+\cdot}$ 64 (11) $[TiO]^+$
 **2-11**	347 (73) $[M]^+$ 281 (100) $[TiCp(C_{12}H_8O)]^+$ 215 (20) $[Ti(C_{12}H_8O)]^+$ 178 (12) $[TiCp_2]^+$	170 (14) $[DpeH_2]^+$ 168 (10) $[Dpe]^+$ 113 (22) $[TiCp]^+$

L2-07 has the expected fragmentation pattern. The molecular ion has a high intensity and the methyl substituent fragments first. A similar pattern was observed for **L2-06**.

Complexes **2-01** to **2-03** have low intensity signals for the molecular ion. For complex **2-01** two fragmentation pathways can be deducted from the fragment ions in the spectrum. In the first, major route, the chlorine ligand is lost giving the principle ion [M^+-Cl], followed by the cleavage of a Cp or Dbf ligand. There is also a minor pathway where a Cp is fragmented first, followed by a Cp, Cl or ring ligand. For complex **2-02** the dominant pathway represents the fragmentation of the ring ligand to give the principle ion [M^+-Dbf], followed by a Cp or other ring ligand. The major pathway for the fragmentation of complex **2-03** again comprises the cleavage of the chloro ligand (48%), followed by a Dbf or Cp ring. There is a minor route (4%) whereby the ring ligand is fragmented first. Two fragment ions were identified where an oxygen atom was included in the fragment ion. The molecular ion gives a high intensity signal for complex **2-04** and there are different fragmentation pathways whereby the ring ligands or Cp ligands fragments first. Like in the previous case there are some fragment ions with oxygen atoms.

The intensity of the molecular ion peak for **2-05** is low (3%), which means that the compound is relatively unstable under the experimental conditions during the measurement. The dominant pathway corresponds to the initial fragmentation of a chloro ligand from the molecular ion. According to other fragment ions, several possible pathways exist. One pathway shows the initial loss of a chloro ligand, followed by the loss of the ring ligand or one or both Cp ligands. In a second route a Cp ligand is lost first, followed by the ring ligand or the chloro ligand and then a Cp ligand. In another pathway the loss of a chloro or Cp ligand follows the loss of the ring ligand. The intensities of the fragment ions reveal that the importance of initial fragmentation decreases according to Cl > heteroaromatic ring ligand > Cp. Complex **2-06** cleaves into two fragments at the oxygen bridge. The molecular ion peak was not observed.

Although the intensity of the molecular ion peak for **2-08** is high (27%) no fragmentation pathway could be deducted from the spectrum. Fragmentation of the ligands seems to be random. The spectra for **2-09** and **2-10** are almost identical. This is ascribed to the fact that **2-09** readily converts to **2-10**. There is a weak signal for the [M^+] in the spectrum of **2-09** (8%), which indicates the instability of this complex. A fragment indicating thianthrene with an oxygen atom bound to it (most probably an oxidation at a sulfur atom) was detected on many fragments for both **2-09** and **2-10**. The fragmentation pattern of **2-10** shows similarities to that of **2-06**. The molecule is cleaved into two parts and the

fragment ions $[TiCp_2(Thr)O]^+$ and $[TiCp_2(Thr)]^+$ are of highest intensity in the spectrum of **2-10**. A common feature in many of the spectra is the dimerization of the heteroaromatic ring.

Complex **2-11** gives a high intensity signal for the molecular ion and the fragmentation route follows the sequence of losing a Cp ligand first and then the biphenyl ring. Reductive elimination of the ring ligand is manifested in the presence of the fragment ion $[DpeH_2]^+$.

- ***^{1}H NMR and ^{13}C NMR spectroscopy***

The ^{1}H and ^{13}C NMR spectra for **L2-01**[14], **L2-03**[15,16], **L2-04**[15,16], **L2-05**[17,18], **L2-06**[19,20] and **L2-08** were recorded and assignments agree with those reported in literature. A summary is given in Table 2.3 for purposes of comparison with those in the complexes. The ^{1}H and ^{13}C NMR spectral data for **L2-07, L2-07b** and complexes **2-01 – 2-11** are summarized in Table 2.3 and Table 2.4. Due to the instability of **2-07** and **2-09** their ^{13}C NMR spectra could not be obtained.

^{1}H NMR spectroscopy

The proton chemical shifts of the ring of the heteroaromatic ligand that is not attached to the metal were normally quite unaffected by coordination and compared well with the resonances of the heteroaromatic substrate. The greatest changes in shifts were recorded for the ring coordinated to titanium. The more electropositive titanium removes less electron density from the ring compared to a hydrogen of a ring carbon and an upfield shift was observed. The protons closest to the metal fragment were being influenced more (these resonances are shifted upfield).

14. K. D. Bartlet, D. W. Jones, R. S. Matthews, *Tetrahedron*, **1971**, *27*, 5177.

15. N. E. Sharpless, R.B. Bradley, *Org. Magn. Res.*, **1974**, *6*, 115.

16. V. Galasso, K. J. Irgrolic, G.C. Pappalardo, *J. Organomet. Chem.*, **1979**, *181*, 329.

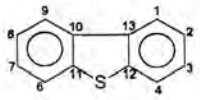
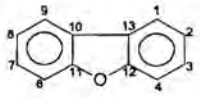
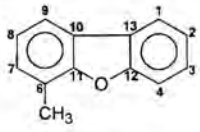
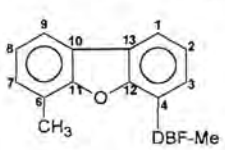
17. M. Audit, P. Demerseman, N. Goasdoue, N. Platzer, *Org. Magn. Reson.*, **1983**, *21*, 698.

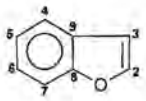
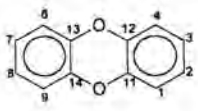
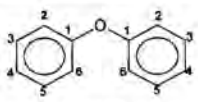
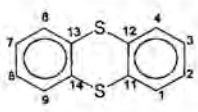
18. R. R. Fraser, T. S. Mansour, S. Savard, *Can. J. Chem.*, **1985**, *63*, 3505.

19. M. E. Amato, A. Grassi, K.J. Irolic, G.C. Pappalardo, L. Radics, *Organometallics*, **1993**, *12*, 775.

20. S. Florea, W. Kimpenhaus, V. Farsacan, *Org. Magn. Reson.*, **1977**, *9*, 133.

Table 2.3 ^{1}H and ^{13}C NMR spectroscopic data[a] for **L2-01** and **L2-03 - L2-08**.

Ligand	C	Chemical Shift (δ ppm)	H	Chemical Shift (δ ppm)	Splitting & Integration		Coupling Constant (J Hz)
 **L2-01**	**1,9**	122.4	**1,9**	8.15	d	2H	8.0/1.2
	2,8	124.0	**2,8**	7.46	t	2H	7.2/8.0/1.0
	3,7	127.1	**3,7**	7.46	t	2H	8.0/7.2/1.2
	4,6	123.3	**4,6**	7.87	d	2H	8.0/1.0
	10,13	136.1					
	11,12	139.1					
 **L2-06**	**1,9**	120.6	**1,9**	7.58	d	2H	8.0
	2,8	122.7	**2,8**	7.46	t	2H	7.2/8.0
	3,7	127.1	**3,7**	7.34	t	2H	7.8/7.2
	4,6	111.6	**4,6**	7.93	d	2H	7.8
	10,13	124.2					
	11,12	156.1					
 **L2-07**	**1**	120.6	**1**	7.58	d	1H	8.0/8.0
	2	122.7	**2**	7.46	t	1H	7.2
	3	127.1	**3**	7.34	t	1H	7.8
	4	111.6	**4**	7.93	d	1H	7.6
	6	120.8	7	7.14	dd	1H	7.2/1.1
	7	127.8	**8**	7.34	t	1H	7.2
	8	122.6	**9**	7.39	dd	1H	7.2/1.1
	9	117.6	**Me**	2.35	s	3H	
	10	134.6					
	11	162.1					
	12	150.9					
	13	114.7					
	Me	15.0					
 **L2-07b**	**1**	120.7	**1**	7.75	dd	2H	6.3/1.5
	2	122.6	**2**	7.56	dd	2H	8.0/8.3
	3	126.9	**3**	7.90-7.93	m	2H	-
	4	118.4	7	7.22	dd	2H	6.2/1.3
	6	111.6	**8**	7.31	dd	2H	7.5/7.5
	7	128.1	**9**	7.43	dd	2H	7.2/1.3
	8	122.5	**Me**	2.59	s	6H	
	9	117.9					
	10/11/12/13	156.3/150.3					
		127.3/113.6					
	Me	15.2					

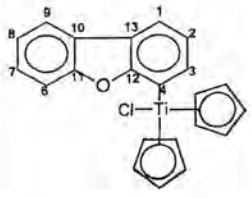
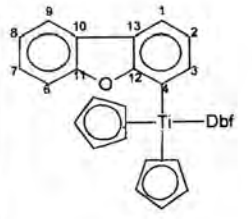
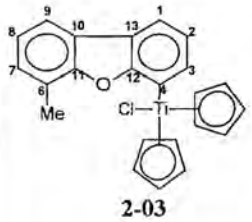
 **L2-05**	**2**	144.8	**2**	7.66	d	1H	2.3
	3	106.5	**3**	6.81	d	1H	2.3
	4	121.1	**4**	7.66	dd	1H	7.2
	5	122.7	**5**	7.36	td	1H	7.2
	6	124.1	**6**	7.30	td	1H	7.8/7.2
	7	111.4	**7**	7.58	d	1H	7.8
	8	154.9					
	9	127.4					
 **L2-03**	**1,9,4,6**	116.2	**1,4,6,9**	6.81	d	4H	8.0/1.5
	2,8,3,7	123.6	**2,3,7,8**	6.85	t	4H	7.5
	11,14,12,13	142.1					
 **L2-08**	**1**	157.3	**2,6**	7.03	d	4H	7.5
	2,6	118.9	**3,5**	7.35	t	4H	7.4/7.5
	3,5	123.2	**4**	7.13	d	2H	7.4
	4	129.8					
 **L2-04**	**1,9,4,6**	128.7	**1,9,4,6**	7.48	d	4H	7.7/1.5
	2,8,3,7	127.7	**2,8,3,7**	7.23	t	4H	7.5/7.7/1.5
	11,14,12,13	135.5					

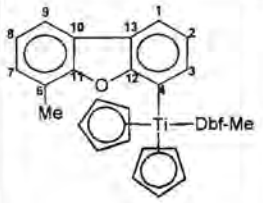
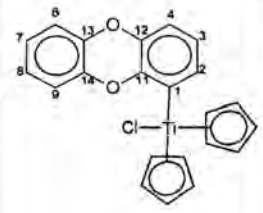
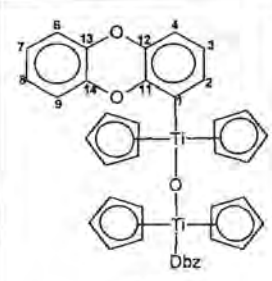
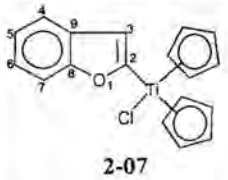
[a] Recorded in $CDCl_3$

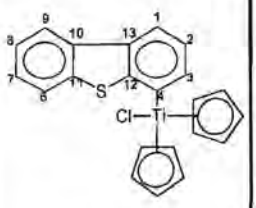
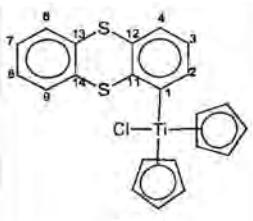
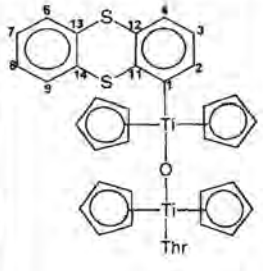
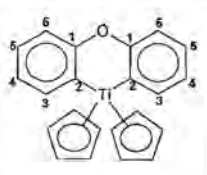
The reason for this shift of electron density can be explained by using different models. Firstly, as a result of an σ-inductive effect whereby the Ti, being the more electropositive element, is part of a polar covalent bond with the carbon the negative pole. This results in the electron density shifting more towards the carbon atom, leaving the metal partially electropositive and the carbon partially electronegative. Secondly, a similar end result is achieved by assuming that the Ti-C bond is an ionic bond; the titanium fragment acts as the cation and the ring carbon as the anion.

The presence of a methyl substituent on a benzene ring, will as a result of the inductive effect of the methyl, shift all the resonances of that particular ring upfield, whereas, the resonances of the unsubstituted ring remain the same and are unaffected. According to Amato, *et al*[19] there are upfield shifts for the H1, H4, H6 and H9 protons in **L2-03**, when compared with the other chalcanthrenes (S, Se, Te), due to the modification of the paramagnetic term by oxygen.

Table 2.4 ^{1}H and ^{13}C NMR spectroscopic data[a] for **2-01 – 2-11**.

Complex	C	Chemical Shift[b] (δ ppm)	H	Chemical Shift (δ ppm)	Splitting & Integration		Coupling Constant (J Hz)
 **2-01**	**1**	116.9	**1**	7.56	dd	1H	7.5/1.3
	2	123.8	**2**	6.98	t	1H	7.2/7.5
	3	136.5	**3**	7.18	dd	1H	7.2/1.3
	4	182.5	**6**	7.94	dd	1H	7.5/1.3
	6	111.7	7	7.28	td	1H	7.2/7.5/1.3
	7	127.1	**8**	7.38	td	1H	8.0/7.2/1.3
	8	122.3	**9**	7.43	tt	1H	8.0/1.3
	9	120.7	**Cp**	6.46	s	10H	
	10	112.7					
	11	157.9					
	12	161.8					
	13	120.6					
	Cp	117.2					
 **2-02**	**1**	111.7	**1**	7.57/7.61	dd	2H	8.3/6.7/1.8
	2	122.0	**2**	7.09/7.11	t	2H	7.2/6.7/8.3
	3	132.4	**3**	7.13	dd	2H	7.2/1.8
	4	169.1	**6**	7.92-7.97	m	2H	-
	6	120.6	7	7.31/7.33	td	2H	7.2/7.8/8.5
	7	122.5	**8**	7.42/7.45	td	2H	8.3/7.2/8.0
	8	127.1	**9**	7.52	dd	2H	8.3
	9	111.0	**Cp**	6.34/6.47	s	10H	
	10	124.2					
	11	155.5					
	12	159.9					
	13	125.1					
	Cp	115.7					
 **2-03**	**1**	118.2	**1**	7.74-7.76	m	1H	-
	2	125.9	**2**	7.56	t	1H	8.3
	3	120.7	**3**	7.91	dd	1H	7.8/1.5
	4	169.6	7	7.21-7.23	m	1H	-
	6	111.6	**8**	7.32	dt	1H	8.3/1.3
	7	136.5	**9**	7.44	dd	1H	7.3/1.3
	8	127.1	**Me**	2.59	s	3H	
	9	122.6	**Cp**	6.44	s	10H	
	10/11/12/13	155.1/156.1/159.8/154.3					
	Me	15.4					
	Cp	117.0					

 **2-04**	**1**	117.9	**1**	7.73-7.75	m	2H	-
	2	126.0	**2**	7.55	td	2H	8.3
	3	120.6	**3**	7.93	dd	2H	7.8/1.5
	4	169.4	**7**	7.18-7.20	m	2H	-
	6	110.9	**8**	7.31	dt	2H	8.3/1.3
	7	136.4	**9**	7.43	dd	2H	7.3/1.33
	8	128.1	**Me**	2.58	s	6H	
	9	122.7	**Cp**	6.44	s	10H	
	10/11/12/13	154.9/156.0/159.6/153.6					
	Me	15.2					
	Cp	117.2					
 **2-05**	**1**	175.1	**2**	6.74	dd	1H	7.4/1.6
	2	133.0	**3**	6.55	dd	1H	7.7/7.4
	3	120.1	**4**	6.48	dd	1H	7.7/1.6
	4	113.4	**6/7/8/9**	6.67-6.70	m	4H	-
	6	116.3	**Cp**	6.43	s	10H	
	7/8	124.0/123.2					
	9	115.1					
	11	150.2					
	12/13/14	142.6/143.0/143.1					
	Cp	117.1					
 **2-06**	**1**	176.0/178.7	**2**	6.34/7.05	dd	2H	8.2/7.5/
	2	132.2/132.4					1.5/1.6
	3	120.8/122.7	**3**	6.72/6.69	t	2H	8.2/7.5
	4	113.1/113.5	**4**	6.29/6.62	dd	2H	8.2/7.5/
	6	116.3/116.5					1.5/1.6
	7/8	123.1/123.5/123.6/123.8	**6/7/8/9**	6.80-6.95	m	8H	-
	9	115.4/116.1	**Cp**	6.05/6.08/	s	20H	
	11	150.1		6.27			
	12/13/14	142.0-145.0					
	Cp	112.6/114.4/114.6					
 **2-07**	**Not recorded**	-	**3**	7.15	d	1H	1.0
			4	7.62	dd	1H	7.1/1.0
			5	7.34	td	1H	7.1/7.2
			6	7.23	dd	1H	7.2/7.2
			7	7.53	d	1H	7.2
			Cp	6.50	s	10H	

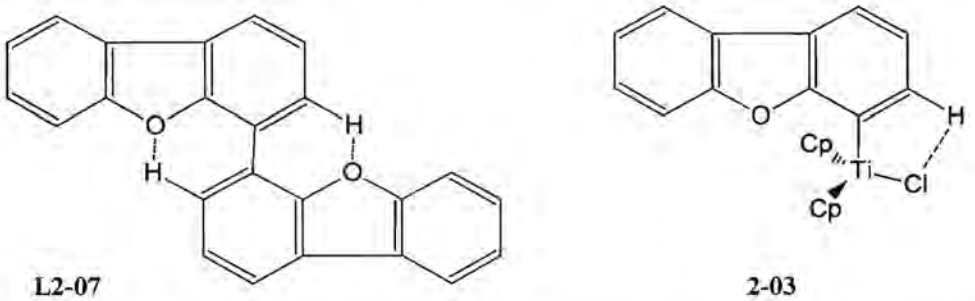
 **2-08**	**1**	117.7	**1**	7.75	dd	1H	7.7/1.1
	2	124.8	**2**	7.07	t	1H	7.6/7.4
	3	134.5	**3**	7.35	dd	1H	7.4/1.1
	4	185.5	**6/9**	7.77-8.09	m	2H	-
	6	121.9	**7/8**	7.38-7.45	m	2H	-
	7	125.9	**Cp**	6.49	s	10H	
	8	124.2					
	9	121.5					
	10	136.2					
	11	138.5					
	12	147.3					
	13	133.2					
	Cp	117.2					
 **2-09**	**Not recorded**	-	**2**	7.14	dd	1H	7.4/1.1
			3	6.86	t	1H	7.5/7.4
			4	6.77	dd	1H	7.5/1.1
			6/9	7.45-7.57	m	2H	-
			7/8	7.20-7.27	m	2H	-
			Cp	6.41	s	10H	
 **2-10**	**1**	207.1/200.9	**2**	6.98/7.68	dd	2H	7.5/1.3
	2	137.5/139.7	**3**	6.93/7.06	t	2H	7.6/7.5
	3	125.7/126.6	**4**	6.38/6.59	dd	2H	7.6/1.3
	4	114.8/116.8	**6/9**	7.46-7.55	m	4H	-
	6/9	128.1/128.3/128.6/128.7	**7/8**	7.22-7.27	m	4H	-
	7/8	127.4/127.5/127.7/127.7	**Cp**	6.38	s	20H	
	11	150.2/150.2					
	12/13/14	135.0-136.0					
	Cp	115.4					
 **2-11**	**1**	no	**3**	6.45	dd	2H	7.8
	2	no	**4**	7.01	td	2H	8.5/7.8
	3	137.2	**5**	6.89	td	2H	8.0/8.5
	4	127.9	**6**	7.69	dd	2H	8.0
	5	124.5	**Cp**	6.06	s	10H	
	6	119.9					
	Cp	115.6					

[a] Recorded in $CDCl_3$ [b] no = not observed

Titanium is a d^0 species and has 16 valence electrons in its coordination sphere in **2-05**, which leaves one available coordination site on the metal. This site can be occupied by an interaction between the lone pair of an oxygen and the metal centre, which in turn could deshield the adjacent protons. Examples of this effect are known in literature and was shown by a crystal structure of

$[TiCp_2(CH_2OCH_3)Cl]$[21]. However for the complexes in this study, the carbon bonded to titanium is sp^2-hybridised and as a result of the orientation of the heteroatom it is less likely to interact with the metal.

The chemical shifts for H6-H9 were difficult to assign in **2-05**, **2-06**, **2-08** and **2-09** due to their overlapping signals, but were not strongly affected. The reason for the high downfield shifts for H3 in **L2-07b** and **2-03** is not easily explainable. A possible option is to ascribe it to an intramolecular hydrogen bond interaction as seen in Figure 2.7. The similar tendency in **2-04** cannot be explained by hydrogen bonding (compare the solid state structure, *vide infra*).



L2-07 **2-03**

Figure 2.7 Possible explanations for the high downfield shift of H3 in **L2-07b** and **2-03**.

The spectra for **2-06** and **2-10** are complex. There are two sets of signals for the heteroaromatic rings. A possible explanation is that the two heteroaromatic rings are caught in two different chemical environments, because of the bulkiness of the heteroaromatic ligands and resulting restriction in rotation. In complexes **2-06** and **2-10** one of the two resonances of H2 displays a significant downfield shift, while the other is shifted upfield, which can only be due to ring current effects.

The singlet observed at 7.15 ppm in the ^{1}H NMR spectrum of **2-07** proves that the titanium fragment is bound to the 2-position of **L2-05**. Since the H3 proton is the closest proton to the coordination site, it is expected that this proton is influenced most as is manifested in a 0.34 ppm upfield shift.

In most complexes the two Cp rings resonate as a singlet due to similar chemical environments. The Cp values are shifted upfield in all the cases compared to titanocene dichloride, which has a Cp value of 6.57 ppm. This is due to the replacement of the electronegative chloro ligand by a carbon atom of the ring in titanocene dichloride and a resulting increase in electron density on the metal fragment. Hence less electron density is transferred from the Cp ligands to the titanium. In most cases the shift is

21. G. Erker, R. Schlund, C. J. Krüger, *J. Organomet. Chem.*, **1988**, *338*, C4.

around 0.10 to 0.50 ppm. The replacement of both chloro ligands caused even more electron density to reside on the metal fragment and the chemical shifts of the Cp rings were observed even further upfield.

There are two different signals for the four Cp rings in **2-06**. One signal displays a strong singlet indicating two Cp rings that are free to rotate while the other two Cp rings afforded two singlets displaying an intensity ratio of 4:1, about 0.2ppm upfield from the first signal. This means that one of the five protons on these Cp rings is in a different electronic environment. The most likely explanation for this shift is that the two oxygen atoms of the heteroaromatic ring ligands caused it. A second possibility is the presence of ring currents of the heteroaromatic rings as a result of two of the Cp ligands being very close to the aromatic rings. Whereas **2-06** displayed two Cp signals, the spectrum of **2-10** revealed only one Cp signal. This means that the Cp rings are all in the same chemical environment in **2-10**, but that the heteroaromatic ligands are in different chemical environments.

Two-dimensional homonuclear shift correlation spectroscopy (COSY) was used to aid in the unambiguous assignment of the different protons in **2-01**, **2-02**, **2-03** and **2-05.**

^{13}C NMR spectroscopy

In the ^{13}C NMR spectra of the complexes the chemical shift for the carbon bound directly to the titanium is shifted far downfield in all the complexes due to attaching a very electropositive metal to the carbon atom. The average is around 30 to 80 ppm compared to the heteroaromatic substrates. The direct neighbouring carbons also have downfield shifts, but much less than that of the *ipso* carbon. The average shift for the neighbouring carbon is about +10 ppm. The rest of the chemical shifts are similar to those of the uncoordinated ligand. The signals for the quaternary carbons C10-C14 were difficult to assign in most of the spectra.

The ^{13}C NMR spectra of complexes **2-05**, **2-06** and **2-10** displayed a duplication of peaks for the two heteroaromatic ligands resulting from different chemical environments for the rings, supporting the ^{1}H NMR spectral data.

In most cases the Cp protons resonate upfield (about 3 - 8 ppm) due to increased electron density on the metal fragment, when compared to titanocene dichloride. The ^{13}C NMR spectrum of **2-06** again displays a 4:1 ratio of Cp ring carbons supporting the ^{1}H NMR data.

HETCOR spectra of **2-02**, **2-03**, **2-05** and **2-08** were used to assign and correlate the specific proton resonances to their corresponding carbons.

- ***X-ray crystallography***

Structure of complex **2-02**

Final confirmation of the structure of **2-02** was obtained from a single crystal X-ray diffraction study. The complex crystallised from a 1:1 dichloromethane:hexane solution by layering the solvents. This method gave bright orange crystals of good quality. In Figure 2.8 the structure of the molecule is given as a ball and stick representation, which also indicates the atom numbering scheme that was used for the structural data. Each unit cell contains two independent molecules with the metal centres labelled Ti(A) and Ti(B) respectively. Only the case for the A molecule is represented in the figure. The most important bond lengths and angles are listed in Table 2.5 and Table 2.6 respectively. Other structural information is given in Chapter 6 and in Appendix A.

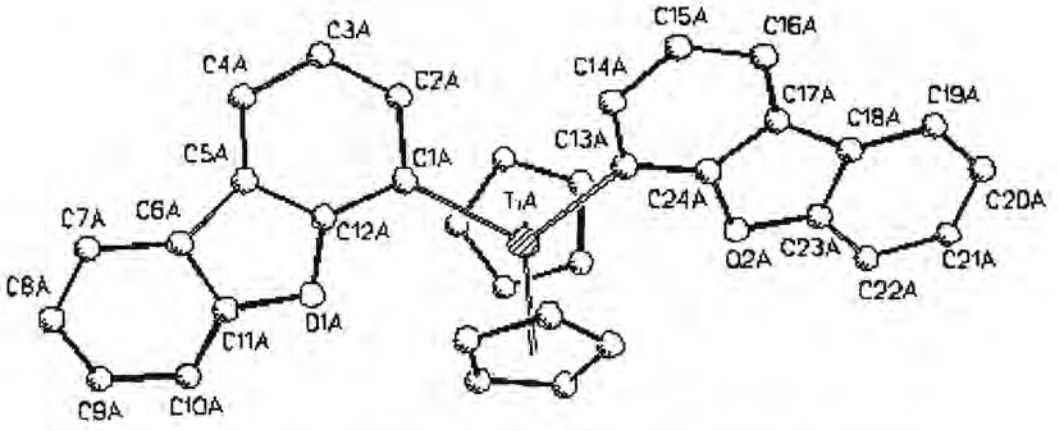


Figure 2.8 Ball and stick representation of the molecular structure A of **2-02**.

Table 2.5. Selected bond lengths for **2-02**.

	Bond length [Å]		Bond length [Å]
TiA-C(13A)	2.210(4)	C(5A)-C(12A)	1.410(6)
TiA-C(1A)	2.218(5)	C(5A)-C(6A)	1.450(7)
O(1A)-C(11A)	1.398(5)	C(6A)-C(11A)	1.395(6)
O(1A)-C(12A)	1.405(5)	C(6A)-C(7A)	1.422(7)
C(1A)-C(12A)	1.410(6)	C(7A)-C(8A)	1.372(8)
C(1A)-C(2A)	1.415(6)	C(8A)-C(9A)	1.363(8)
C(2A)-C(3A)	1.422(6)	C(9A)-C(10A)	1.400(7)
C(3A)-C(4A)	1.382(7)	C(10A)-C(11A)	1.375(7)
C(4A)-C(5A)	1.393(7)		

Table 2.6. Selected bond angles for **2-02**.

	Bond angle [°]		Bond angle [°]
C(13A)-TiA-C(1A)	105.0(2)	C(11A)-C(6A)-C(5A)	106.6(4)
C(11A)-O(1A)-C(12A)	106.3(4)	C(7A)-C(6A)-C(5A)	135.9(5)
C(12A)-C(1A)-C(2A)	110.3(4)	C(8A)-C(7A)-C(6A)	117.8(6)
C(12A)-C(1A)-TiA	126.3(3)	C(9A)-C(8A)-C(7A)	123.2(6)
C(2A)-C(1A)-TiA	123.3(4)	C(8A)-C(9A)-C(10A)	120.7(6)
C(1A)-C(2A)-C(3A)	123.9(5)	C(11A)-C(10A)-C(9A)	116.2(5)
C(4A)-C(3A)-C(2A)	121.5(5)	C(10A)-C(11A)-O(1A)	124.9(5)
C(3A)-C(4A)-C(5A)	118.1(5)	C(10A)-C(11A)-C(6A)	124.4(5)
C(4A)-C(5A)-C(12A)	117.9(5)	O(1A)-C(11A)-C(6A)	110.7(5)
C(4A)-C(5A)-C(6A)	135.6(5)	C(5A)-C(12A)-O(1A)	110.1(4)
C(12A)-C(5A)-C(6A)	106.2(4)	C(5A)-C(12A)-C(1A)	127.9(4)
C(11A)-C(6A)-C(7A)	117.4(5)	O(1A)-C(12A)-C(1A)	122.0(4)

Four ligands surround the titanium centre in a distorted tetrahedral arrangement, with positions defined by the centra of the Cp rings and the carbon connecting the metal to the dibenzofuran ring. The Cp rings and titanium belong to the open clamshell class of Cp-compounds. The bulky ring ligands cause an enlargement of the angle between the two non-Cp ligands. In titanocene dichloride this Cl-Ti-Cl angle[22] is 94.6°, which is smaller than the 105.0° of C(13A)-TiA-C(1A) of **2-02**. This angle in **2-02** is also larger than the values of 96.7 and 98.4° recorded for the two molecules in the unit cell recorded for C-Ti-Cl of $[TiCp_2(Dbt)Cl]$[23] (Dbt = Dibenzothienyl)

The dihedral angle of C(11A)-O(1A)-C(12A)-C(1A) is 2.5(0)° and C(11B)-O(1B)-C(12B)-C(1B) is -0.9(0)°, which shows that the dibenzofuran ring is almost planar. The titanium atom is also in the plane of the furan ligand. If we compare the bond lengths of the dibenzofuran[24,25] to those of **2-02**, we can see that they differ very little and that coordination to titanium has very little effect on the bond distances.

22. A. Clearfield, D. K. Warner, C. H. Salderiaga-Molina, R. Ropal, J. Bernal, *Can. J. Chem.*, **1975**, *53*, 1622.

23. S. Lotz, H. Görls, R. Meyer, unpublished results.

24. O. Dideberg, L. Dupont, J. M. Andre, *Acta Cryst., Sect B*, **1972**, *B28*, 1002.

25. A. Banerjee, *Acta Cryst., Sect B*, **1972**, *B29*, 2070.

The Ti-C(1A) and Ti(C13A) bond distances of 2.210Å and 2.218Å, respectively is significantly shorter than the observed Ti-C (sp^2, phenyl) bond distance of 2.27(1)Å for $TiCp_2Ph_2$[26] and is comparable with the Ti-C(Ph) distance of 2.193(3)Å recorded for the titanacycle $[TiCp_2(C_6H_4\text{-}C_6H_4S)]$[27] and the average value of 2.188(5)Å for $[TiCp_2(C_6H_4\text{-}C_6H_4)]$[28]. Complexes displaying significant π-interaction between titanium and a sp^2-bonded carbon atom, should have Ti-C bond distances of about 2.00-2.10Å [29,30,31,32], which is a little shorter than the corresponding distance in **2-05**.

The shorter Ti-C distance in **2-02** suggests that some multiple bonding interaction exists between the titanium and the dibenzofuran carbon atom. Titanium(IV) has no electrons in the d-orbitals and will therefore, only be able to act like a Lewis acid and accept electron density from the π-cloud of the ring ligand. The σ-bonded ring ligand exhibits some carbene character, which supports observations of the ^{13}C NMR data that the relevant carbon resonances are shifted downfield into the carbene region[28]. If applicable, one would expect that the adjacent bonds of the phenyl bonded to the titanium should be lengthened. Although not significant, these are the longest C-C distances in the ring. The average C-C bond distance for the benzene ring bearing Ti is 1.405 Å compared to 1.378 Å for the other benzene ring. The O-C bond length 1.148Å in **L2-06** [24,25] is shorter than these bond distances in **2-02** which are 1.398(5)Å and 1.405Å for O(1A)-C(11A) and O(1A)-C(12A), respectively, and 1.388(5)Å and 1.418(4)Å for O(2A)-C(11A) and O(2A)-C(12A), respectively. The bond angle of 104.4° for **L2-06** is slightly smaller than that of these angles in **2-02**, which are 106.3(4)° and 105.9(3)° for C(11A)-O(1A)-C(12A) and C(23A)-O(2A)-C(24A), respectively.

26. V. Kocman, J. C. Rucklidge, R. J. O'Brien, W. Santo, *J. Chem. Soc., Chem. Commun*, **1976**, 1340.

27. P. R. Stafford, T. B. Rauchfuss, A. K. Verma, S. R. Wilson, *J. Organomet. Chem.*, **1996**, *526*, 203.

28. C. A. Mike, T. Nelson, J. Graham, A. W. Cordes, N. T. Allison, *Organometallics*, **1988**, *7*, 2573 .

29. P. Binger, P. Müller, P. Phillips, P. Gabor, R. Mynott, A. T. Herrmann, F. Langhauser, C. Krüger, *Chem. Ber.*, **1992**, *125*, 2209.

30. M. M. Francl, W. J. Hehre, *Organometallics*, **1983**, *2*, 457.

31. R. Beckhaus, S. Flatiu, S. Trojanov, P. Hofmann, *Chem. Ber.*, **1992**, *125*, 291.

32. A. K. Rappe, *Organometallics*, **1987**, *6*, 354.

Structure of complex **2-05**

The final confirmation of **2-05** was obtained from a single crystal X-ray diffraction study. The complex crystallised from a 1:1 THF:hexane solution by using the layering technique and gave bright orange crystals. Figure 2.9 gives the structure of the molecule as an ORTEP drawing, which also indicates the atom-labelling scheme that was used. The most important bond lengths and angles are listed in Table 2.7 and Table 2.8, respectively. Other structural information is given in Chapter 6 and in Appendix **B**. In the unit cell of the structure there is also a highly distorted THF molecule and a hexane. Bearing in mind the numerous unsuccessful attempts to obtain crystals of **2-05**, it is believed that the solvent molecules were essential in the packing process of the crystal. High thermal motions in the molecule necessitated low temperature data collections.

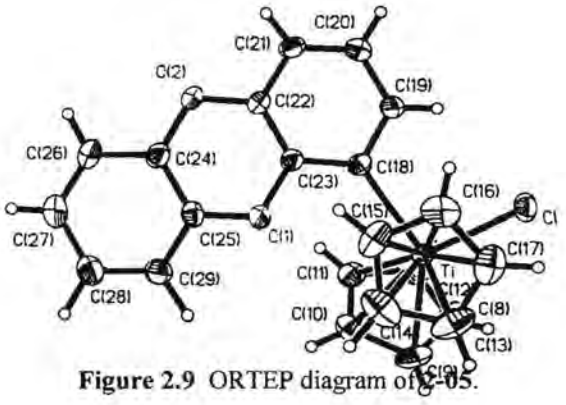


Figure 2.9 ORTEP diagram of **2-05**.

Table 2.7. Selected bond lengths of **2-05**.

	Bond Length [Å]		Bond Length [Å]
Ti-Cl	2.3684(7)	O(2)-C(22)	1.389(3)
Ti-C(18)	2.208(2)	O(2)-C(24)	1.378(3)
C(18)-C(19)	1.397(4)	C(24)-C(25)	1.397(4)
C(19)-C(20)	1.403(4)	C(24)-C(26)	1.387(4)
C(20)-C(21)	1.376(4)	C(26)-C(27)	1.388(4)
C(21)-C(22)	1.383(4)	C(27)-C(28)	1.398(4)
C(22)-C(23)	1.392(3)	C(28)-C(29)	1.383(4)
C(18)-C(23)	1.404(3)	C(29)-C(25)	1.387(4)
O(1)-C(23)	1.389(3)	H(19)-Cl	2.699
O(1)-C(25)	1.379(3)	H(27)-Cl	2.885

Table 2.8. Selected bond angles of **2-05**.

	Bond Angle [°]		Bond Angle [°]
Cl-Ti-C(18)	97.42(7)	C(23)-O(1)-C(25)	116.3(2)
Ti-C(18)-C(19)	118.5(2)	O(1)-C(25)-C(24)	121.5(2)
Ti-C(18)-C(23)	126.2(2)	O(1)-C(25)-C(29)	118.7(2)
C(18)-C(19)-C(20)	122.4(2)	C(21)-C(22)-O(2)	117.1(2)
C(19)-C(20)-C(21)	120.7(2)	C(23)-C(22)-O(2)	122.2(2)
C(20)-C(21)-C(22)	118.4(2)	C(22)-O(2)-C(24)	115.7(2)
C(21)-C(22)-C(23)	120.7(2)	O(2)-C(24)-C(25)	121.3(2)
C(22)-C(23)-C(18)	122.6(2)	O(2)-C(24)-C(26)	118.3(2)
C(23)-C(18)-C(19)	115.2(2)	C(24)-C(26)-C(27)	119.6(3)
C(18)-C(23)-O(1)	117.1(2)	C(26)-C(27)-C(28)	119.6(3)
C(22)-C(23)-O(1)	120.4(2)		

The arrangement of the ligands is like discussed in complex **2-02**. Because only one bulky ring ligand is present a decrease of the angle between the two non-Cp ligands (97.42°) compared to **2-02** (104.96°) with two bulky ligands was observed. The dihedral angle, Cl-Ti-C(18)-C(19) of -6.6(2)° reveals that the chloro ligand is approximately in the plane of the ring ligand. Furthermore, the chloro ligand and oxygen of the ring ligand are on opposite sides of the Ti-C bond. The dihedral angle of C(25)-O(1)-C(23)-C(22) is 12.2(4)°, which shows that the dibenzodioxin ring is not planar. The titanium atom is also in the plane of the dioxin ligand

As a result of the orientation of the ring, the hydrogen on C(19) is forced close to the chloro ligand. In fact, the H(19)...Cl non bonding intramolecular distance is only 2.699Å, which is very short. Comparing the bond lengths of the **L2-03**[33] to those of **2-05**, we can see that they differ very little and that coordination to titanium has very small effect on the bond lengths. Thus the large downfield chemical shift of H2 observed in the NMR spectra is ascribed to the hydrogen bonding. There is also an interatomic hydrogen bond interaction between a Cl of one molecule and the H(27) of the other molecule, which measured 2.885 Å.

The Ti-C(18) bond distance of 2.208Å is slightly shorter than the observed Ti-C(1A) and Ti(C13A) bond distances of **2-02** which are 2.210Å and 2.218Å, respectively. This value is comparable with

33. P. Singh, J. D. McKinney, *Acta Cryst.*, **1978**, *B34*, 2956.

similar complexes in literature as discussed for **2-06** and again suggests that some multiple bonding interactions exists between the titanium and the dibenzodioxin carbon atom.

2.4 Conclusions

The aim was to synthesize a series of titanocene complexes where the heteroaromatic ligand is linked directly to the metal fragment. This was done by lithiation of the heteroaromatic ligand, followed by addition of titanocene dichloride and [$TiCp_2$(Dbf)Cl] **2-01**, [$TiCp_2(Dbf)_2$] **2-02**, [$TiCp_2$(Dbf-Me)Cl] **2-03**, [$TiCp_2(Dbf\text{-}Me)_2$] **2-04**, [$TiCp_2$(Dbz)Cl] **2-05**, [$TiCp_2$(Bf)Cl] **2-07**, [$TiCp_2$(Dbt)Cl] **2-08** and [$TiCp_2$(Thr)Cl] **2-09** were isolated. Due to instability, **2-09** decomposed to yellow [(μ-O){$TiCp_2$(Thr)}$_2$] **2-10** and [$TiCp_2(Dbz)_2$]**2-06a** decomposed to [(μ-O){$TiCp_2$(Dbz)}$_2$] **2-06**. Most complexes were fully characterized using mass spectrometry and NMR spectroscopy, but **2-07** and **2-09** were only characterized by ^{1}H NMR spectroscopy,. The structures of complexes **2-02** and **2-05** were confirmed by X-ray crystallography. To investigate the effect of a heteroaromatic ligand bonding bidentately to the metal center, [$TiCp_2$(Dpe)] **2-11** was synthesized and characterized.

Complexes **2-05** and **2-08** were compared with titanocene dichloride **S-01** for antitumor activity *in vitro* and complexes **2-01**, **2-03**, **2-05**, **2-08** and **2-11** were compared with **S-01** and **3-05** in cell inhibition tests for antitumor activity. It was clear that serious handling problems would eliminate **2-07** and **2-09** as possible candidates for further testing in biological systems. The antitumor properties of these complexes will be investigated in Chapter 5.