8

Organometallic Chemistry

8.1 What is organometallic chemistry?

Organometallic chemistry is the area of chemistry that deals with compounds containing a metal–carbon bond. As such, this area combines aspects from both organic and inorganic chemistry.

*An **organometallic compound** is characterised by the presence of one or more carbon–metal bonds.*

It is important to note that the metal can either be a member of the s or p block on one hand or a d-block metal (transition metal) on the other. There are no real direct pharmaceutical applications known for group 1 and 2 organometallic compounds, as they are very reactive reagents, except that they are commonly involved in the synthesis of modern medicines. Examples of such synthetic reagents include sodium cyclopentadienide (NaCp, NaC_5H_5) and butyl lithium (BuLi) compounds.

***Cyclopentadienyl** or **Cp**$^-$ ($C_5H_5^-$) is a commonly used ligand in organometallic chemistry, which is versatile in the number of bonds it can form to a metal centre (**M**). There are different ways of representing these interactions* (*see also* Figure 8.1).

NaCp is an organometallic agent that is mainly used to introduce a cyclopentadienyl anion ($C_5H_5^-$) to a metal centre in order to form a so-called metallocene (see Section 8.2 for a definition of metallocenes). NaCp can be synthesised by the reaction of either sodium with cyclopentadiene or from dicyclopentadiene under heating. Sodium hydride (NaH) can also be used as a base, instead of sodium, to deprotonate the acidic CH_2 group of the cyclopentadiene (Figure 8.2).

It is interesting to look at the bonding in this Cp ligand. First of all, it is important to understand the formation of the cyclopentadienyl anion, the Cp^- ligand. Cyclopentadiene is surprisingly acidic, which is

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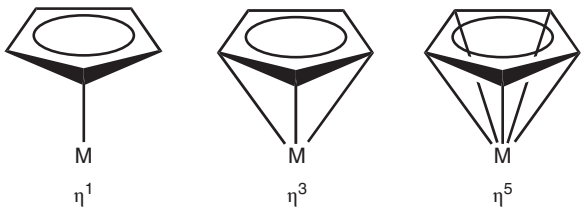

Figure 8.1 *Illustration showing different binding modes of Cp^- ligand to metal centre (M)*

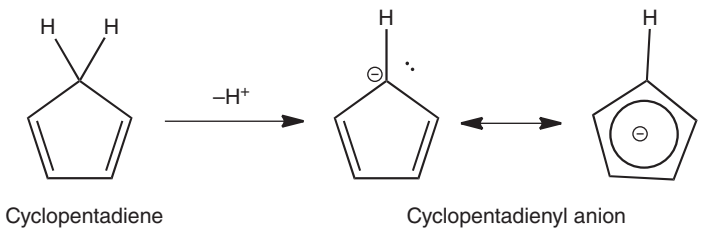

Figure 8.2 *Deprotonation of cyclopentadiene*

a result of the resonance stabilisation of the resulting cyclopentadienyl anion (Cp^-). The cyclopentadienyl anion follows Huckel's rule ($4n + 2$, $n = 0$, 1, 2, etc.), which means that the Cp^- anion is a planar carbocycle of aromatic nature.

*An **aromatic molecule** has to be a cyclic and planar molecule with an uninterrupted network of π electrons. Furthermore, it has to fulfil the Huckel rule, which states it has (4n + 2) π electrons.*

Organolithium compounds (Li—C bond) are probably the best known organometallic agents. In organic synthesis and drug design, they can be used as either an extremely potent base or as nucleophile; in the latter case, the organic moiety will be introduced to the target molecule. They are typically synthesised by reacting an organohalide (RX) with elemental lithium. The best known examples are *n*-butyllithium (*n*-BuLi), *sec*-butyllithium (*s*-BuLi) and *tert*-butyllithium (*tert*-BuLi), with *tert*-BuLi being the most reactive (Figure 8.3).

Alkali metal organometallics are more or less pyrophoric, which means they combust spontaneously on contact with air. They have to be handled under the exclusion of air, humidity and oxygen and are mostly stored in hydrocarbons. The solvent plays an important role and can be responsible for potential decomposition

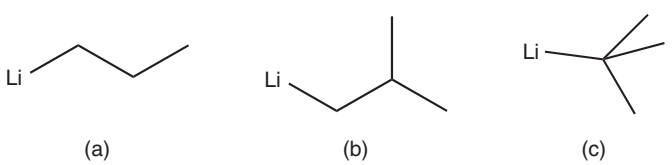

Figure 8.3 *Chemical structures of (a)* n-*BuLi, (b)* s-*BuLi and (c)* t-*BuLi. Note that these compounds can form complex structures in the solid state and in solution*

processes or an increased or reduced reactivity. The heteroatoms of solvents can potentially also coordinate to the alkali metal and therefore influence potential cluster formation of the organometallic compound. Alkali metal organometallics are known to form relatively complex clusters in solution and in the solid state, which influences their reactivity.

Organometallic compounds, containing d-block metals, are currently under intense research within the pharmaceutical chemistry area in order to find new treatment options for cancer and diabetes, amongst others. d-Block organometallics are generally fairly stable complexes, which in contrast to alkali metal organometallics can be handled in the presence of air. Whilst s- and p-block organometallics form σ and π bonds between the metal and the organic group, in d-block organometallics the number of bonds, which is called *hapticity* (see Section 7.1), can be further increased.

The most common ligands for d-block organometallics include carbon monoxide (CO) in the form of the carbonyl group, phosphanes (PR_2H) and derivatives of the cyclopentadienyl (Cp^-) ligand. A characteristic example is the bonding of the metal with the carbonyl ligand, which can be described as one M—CO interaction. A vacant (hybridised) orbital of the metal centre forms a σ bond with the CO ligand, which means that electronic charge is donated from the CO ligand to the metal centre. As CO is also a π-acceptor ligand, a back donation of electronic charge from the metal centre can occur. This donation/back donation interplay results in a strengthening of the metal–carbon bond and a weakening of the carbon–oxygen bond. CO is classified as a σ-donor and π-acceptor molecule.

The chemistry of d-block organometallic chemistry covers a vast amount of material and therefore we will concentrate on the area of so-called metallocenes, which are complexes containing typically a d-block metal and two Cp^- ligands. This area encompasses the most promising drug-like candidates so far.

8.2 What are metallocenes?

Metallocenes are a special class of the so-called sandwich complexes in which the central metal lies between two cyclopentadienyl (Cp^-) ligands. Their chemical formula is typically of the type $(\eta^5\text{-Cp})_2M$, which means each Cp^- ligand forms five bonds with the metal centre (Figure 8.4).

> ***Sandwich complexes*** *are defined as organometallic compounds containing a central metal atom, which is bound by haptic covalent bonds to two arene ligands. The metal is typically placed between the two ring systems, which gives the complex the term sandwich.*

Typically, sandwich structures are classified as parallel or bent. Within a parallel sandwich structure, the two arene ligands are arranged parallel to each other. In a bent sandwich structure, the two ligands are at an angle to each other.

One of the best known metallocenes is ferrocene $(\eta^5\text{-Cp})_2Fe$, which is an iron (Fe^{2+})-based parallel sandwich complex. The metal centre is sandwiched between two cyclopentadienyl ligands, which are coparallel. There are different ways of representing the structure of ferrocene, as shown in Figure 8.5. The structure on (a) gives the best understanding of the bonds present in ferrocene, whilst the structure on (b) is the most commonly used one. The structure on (c) shows the electronic properties of the individual components in ferrocene.

Ferrocene follows the so-called 18-electron rule, which most low-oxidation-state d-block metal organometallic complexes seem to follow. The number 18 is the effective number of electrons that a transition metal can accommodate in its nine valence orbitals (comprising five d orbitals, three p orbitals and one s orbital).

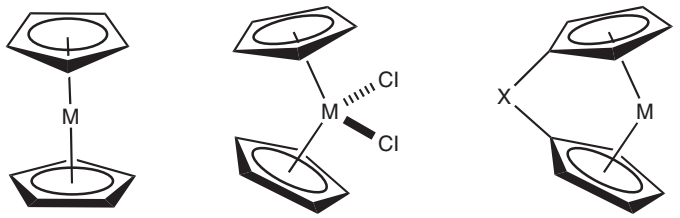

Figure 8.4 *Examples for the generic structure of a metallocenes (M = metal)*

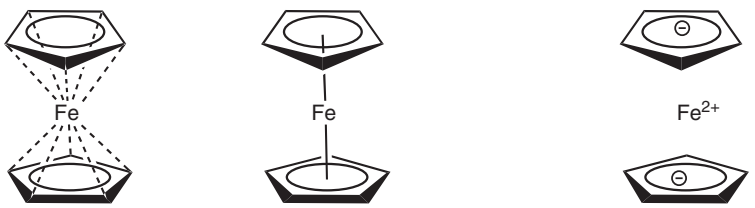

Figure 8.5 *(a–c) Different notations for the chemical structure of ferrocene*

> T*he **18-electron rule** is mainly used to predict the formula for stable transition-metal complexes. The rule is based on the principle that each transition metal has usually nine valence orbitals, one s-type, three p-type and five d-type orbitals. These interact with the ligands and accommodate up to 18 electrons. Transition-metal complexes containing 18 valence electrons (VEs) have the same electronic configuration as the noble gas in this period, similar to the octet rule described for main group metals.*
>
> *Note that there are many examples known that do not follow the 18-electron rule.*

Ferrocene is a good example to explain this rule, and there are two ways of counting the electrons in ferrocene. On one hand, it can be assumed that all partners are neutral, which means that ferrocene consists of an Fe(0) centre and two Cp ligands. That means the Fe(0) centre contributes eight electrons (group 8, eight VEs), whilst each Cp ligand contributes five electrons. This adds up to a total of 18 electrons. On the other hand, the second method of counting is based on the fact that each partner carries a charge, which means an Fe^{2+} centre and two Cp^- ligands. Therefore the iron centre contributes six electrons and each Cp^- ligand also contributes six electrons, which sums up to also 18 electrons (Figure 8.6).

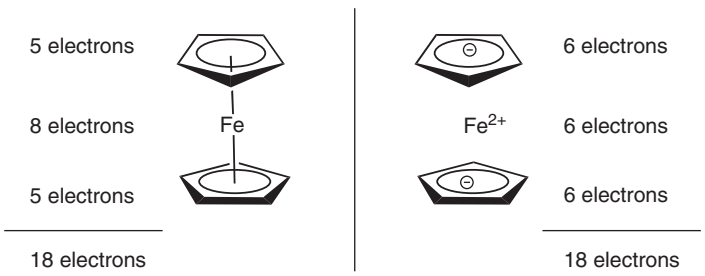

Figure 8.6 *The 18-electron rule exemplified on ferrocene*

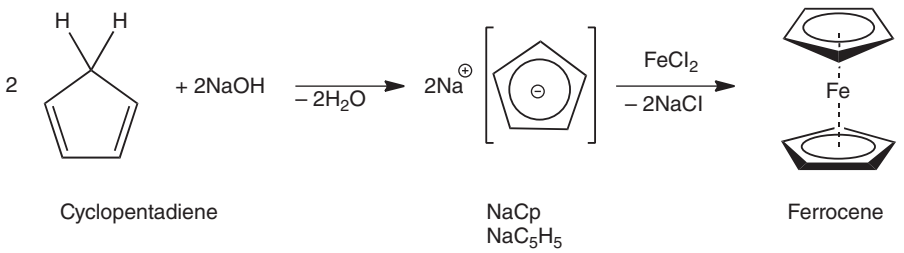

Figure 8.7 *Synthesis of ferrocene*

It is important to note that this rule can be used to predict stable metal complexes, but it is only a formality. There are many examples of stable transition-metal complexes that do not follow the 18-electron rule, such as Pd(0) or Pt(0) complexes, or indeed some of the titanocenes and vanadocenes presented in the following.

8.3 Ferrocene

Ferrocene (or bis(η^5-cyclopentadienyl)iron, $(C_5H_5)_2Fe$) is an orange powder and is probably one of the best studied metallocenes. As previously mentioned, its structure follows the 18-electron rule and it is a very stable complex. Its Cp^- ligands can be easily derivatised to introduce functional groups. Functionalised ferrocene derivatives are currently used as biosensors in blood glucose measuring equipment and they are also under intense research as potential anticancer agents.

Ferrocene was discovered by Paulson and Kealy in 1951. Cyclopentadienyl magnesium bromide and ferric chloride were reacted in a so-called Grignard reaction (reaction involving R-MgBr) in order to create a fulvalene. Instead, they created ferrocene. At that time, it was difficult to identify the correct structure of ferrocene, but Wilkinson, Rosenblum, Whitting and Woodward managed to do this soon after its discovery [1].

$$2C_5H_5MgBr + FeCl_2 \rightarrow Fe(C_5H_5)_2 + MgBr_2 + MgCl_2$$

Nowadays, ferrocene is synthesised via a so-called transmetallation reaction. Typically, commercially available sodium cyclopentadienide is deprotonated with KOH or NaOH, and the obtained anion is reacted with anhydrous ferrous chloride ($FeCl_2$). Instead of purchased sodium cyclopentadienide, freshly cracked cyclopentadiene is often used (Figure 8.7).

Ferrocene is a very stable complex and can be easily functionalised by derivatising its Cp^- ligands. The Cp^- ligands are aromatic, as previously mentioned, and therefore show a chemical behaviour similar to benzene. This means that reactions known for benzene chemistry can be used with ferrocene, such as the Friedel–Crafts acylation reaction. Ferrocene can be acylated by reacting it with the corresponding aluminium halide (AlX_3). Indeed, this chemical behaviour of ferrocene helped in identifying its real structure (Figure 8.8) [2].

Ferrocene and its derivatives are under intense screening for medicinal purposes. Research has shown that especially ferrocene derivatives exhibit very promising effects for a variety of clinical applications, such as antimalarial and anticancer agents as detailed below. Interestingly enough, ferrocene itself is not a particularly toxic compound, as it can be administered orally, injected or inhaled with no serious health concerns. It is believed to be degraded in the liver by cytochrome P_{450}, similar to benzene. Its degradation process involves the enzymatic hydroxylation of the cyclopentadienyl ligand. Animal studies on beagles have shown that treatment with up to 1 g/kg ferrocene did not result in acute toxicity or death, although it did lead to a severe iron overload, which was reversible [2].

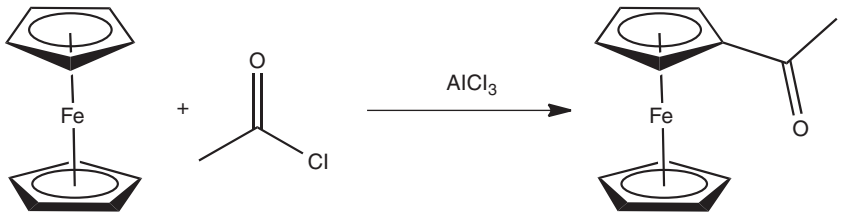

Figure 8.8 *Acylation of ferrocene*

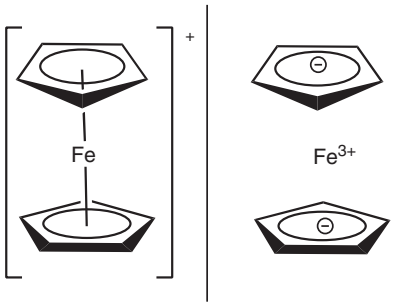

Figure 8.9 *Chemical structure of the ferrocenium cation*

Ferrocene can easily undergo oxidation to the ferrocenium cation in a one-electron oxidation process. The formed cation is fairly stable, and the whole process is reversible. This redox potential, together with a change in lipophilicity, is the main characteristic that makes ferrocene-based compounds interesting for a variety of potential clinical applications, especially the ones outlined in the following (Figure 8.9) [2].

8.3.1 Ferrocene and its derivatives as biosensors

Diabetes is a major health problem with hundreds of millions sufferers worldwide. As part of the illness, diabetic patients have increased glucose levels in their blood due to a lack of insulin or cells not reacting to insulin. Insulin promotes the uptake of glucose into the cells. There are several options to manage diabetes, but it is extremely crucial for the welfare of the patients that the blood glucose levels are closely monitored. In order to facilitate these regular measurements, a significant amount of research has gone into the development of portable and easy-to-use devices. Modern blood glucose monitors benefit from the technical advances of the so-called biosensor research, an area where the majority of the biosensors are used.

Biosensors are based on enzymes that contain redox-active groups. This means that the redox group can change its redox state as a result of a biochemical reaction. In nature, the enzymes glucose oxidase (GOx) or glucose dehydrogenase (GDH) are used as biosensors for blood glucose monitors. Typically, these enzymes accept electrons from the substrate, glucose in this case, and oxidise it. The enzyme changes to its reduced state, which normally deactivates the enzyme. In order to activate the enzyme again, electrons are transferred and the enzyme is oxidised. GOx and GDH in their reduced form transfer electrons to molecular oxygen, and hydrogen peroxide (H_2O_2) is produced. Oxygen or peroxide electrodes can then be used to measure any change of the substrate, which directly relates to the glucose levels present in the sample. Unfortunately, this method has problems, as, for example, molecular oxygen can be a limiting factor and a lack of oxygen can lead to wrong readings (Figure 8.10).

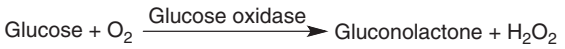

Figure 8.10 *Enzymatic oxidation of glucose*

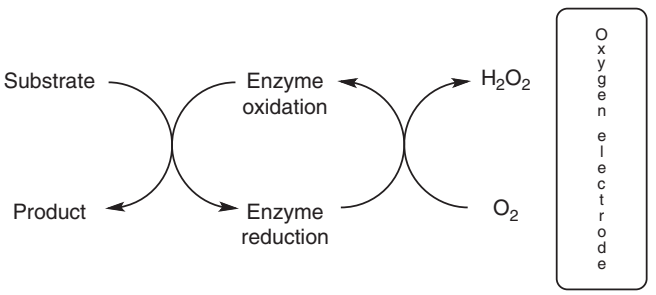

Figure 8.11 *Scheme of a biosensor (Adapted from [3], with permission from Elsevier.)*

Enzymes such as GOx are very specific to the substrate they accept electrons from, that is, the substrates they oxidise, but they are more flexible to the substrate they donate electrons to. Therefore, a variety of inorganic redox-active compounds have been tested as so-called mediators. Mediators function by accepting electrons from the enzyme and thus oxidising the enzyme to its active form. They shuttle electrons from the enzyme to the electrode and are also called *electron sinks*. Electrodes can measure any changes in the redox potential of these mediators, and these changes can directly be related to the amount of glucose present in the sample. This technology excludes the need for molecular oxygen and problems connected to that (Figure 8.11) [3].

In 1984, the first ferrocene-based mediator in conjunction with GOx was used as a biosensor for glucose. Derivatives of ferrocene are still the most important examples for mediators in biosensors, mainly due to their wide range of redox potential, which is independent of any pH changes. Furthermore, the chemistry involved in synthesising these ferrocene derivatives is well explored and fairly straight forward. Additionally, the mediator must successfully compete with the natural mediator (molecular oxygen) in order to ensure accurate readings. From the point of its application as biosensor for blood glucose measurement, it is clear that ferrocene-based mediators can be used only once. This is due to the fact that, whilst ferrocene is relatively insoluble, the reduced form, the ferrocenium ion, is fairly soluble. Mediators should be insoluble in order to lead to reproducible results or, as in this case, can only be used once (Figure 8.12) [3, 4].

$$[Cp_2Fe] \rightarrow [Cp_2Fe]^+ + e^- \tag{8.1}$$

Equation 8.1: Oxidation of ferrocene to the paramagnetic ferrocenium ion.

Initially, ferrocene itself was used as mediator, but later on a variety of its derivatives were tested for their redox potential in biosensors. Some of these examples are shown below. This research has led to development of the modern blood glucose analysers, which are only the size of a pen and highly mobile devices. These devices use disposable strips and are simple to use (Figure 8.13) [4].

8.3.2 Ferrocene derivatives as potential antimalarial agent

A variety of compounds containing the ferrocene group have been synthesised and tested for their clinical properties, especially as antimalarial and anticancer agents. In this context, especially the ferrocene-based

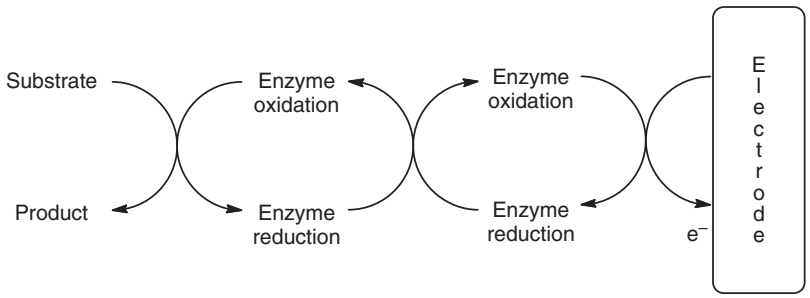

Figure 8.12 *Scheme for mediated biosensor (Reproduced from [3], with permission from Elsevier.)*

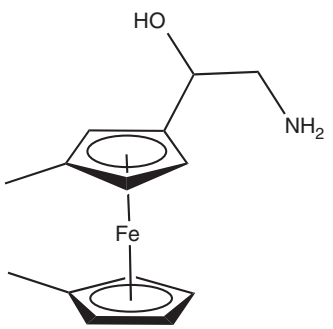

Figure 8.13 *Chemical structure of a simple example of a ferrocene-based biosensor*

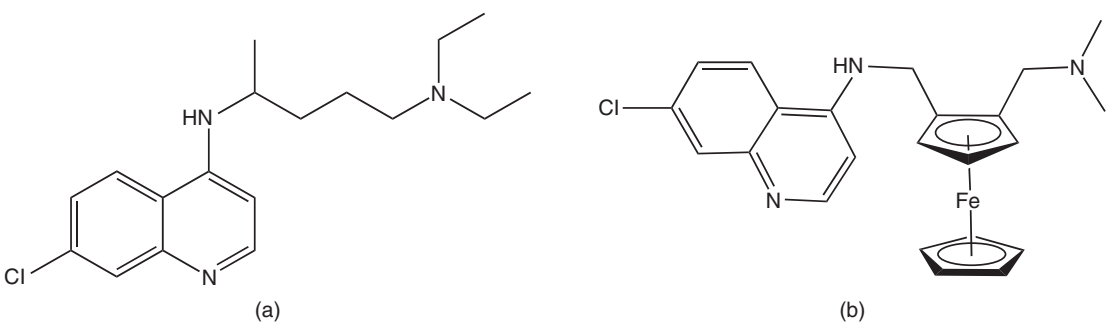

Figure 8.14 *Chemical structures of chloroquinone (a) and ferroquine (b)*

analogue of chloroquine, ferroquine, has shown significant promise. It successfully passed phase II clinical trials and is awaiting results from field testing. Chloroquine is a well-known drug used in the treatment of malaria caused by the parasite *Plasmodium falciparum.* Ferroquine is active against this parasite as well. Even more exciting is the fact that it is also active against the chloroquine-resistant strain of *P. falciparum.* The changed biological activity might be due to the changed lipophilicity and/or the redox action that is present after the introduction of the ferrocene group (Figure 8.14) [2].

8.3.3 Ferrocifen – a new promising agent against breast cancer?

Ferrocene and its derivatives were, and still are, under intense scrutiny as potential anticancer agents. Initially, a range of ferrocenium salts was tested for their cytotoxic activity. The mode of action is still unclear, but DNA, cell membrane and enzymes have been proposed as potential targets. Ferrocenium salts are believed to generate hydroxyl radicals under physiological conditions. These may damage the DNA, possibly by oxidising the DNA. Furthermore, it is believed that the cell membrane might be a target. Research has shown that the counter-ion is crucial for the cytotoxic activity as well as their aqueous solubility. Ferrocenium salts such as the picrate and trichloroacetate derivates display good aqueous solubility and high cytotoxic activity. As part of this research, ferrocene was also successfully bound to polymers in order to improve their water solubility and therefore cytotoxic activity [2].

Jaouen and coworkers substituted phenyl rings in existing drugs and natural products by ferrocene groups in order to introduce a redox-active metal group into these molecules and to change their lipophilicity. A breakthrough was achieved when a phenyl group in tamoxifen, a selective oestrogen receptor modulator (SERM) used as front-line treatment of hormone-dependent breast cancer, was replaced by ferrocene. The active metabolite of tamoxifen is actually the hydroxylated form 4-hydroxytamoxifen, which is highly active in the fight against oestrogen-dependent breast cancer. Breast cancer can be divided into hormone-dependent (also called *oestrogen-dependent*, ER(+)), which is characterised by the presence of an oestrogen receptor, and hormone-independent (also called *oestrogen-independent*, ER(−)) [2, 5].

> ***Selective oestrogen receptor modulators*** *are defined as a class of compounds* that *interact with the oestrogen receptor. This interaction can happen in various tissues leading to different actions.*

The combination of tamoxifen derivatives with ferrocene was a very successful approach, and has led to the development of a class of compounds called *ferrocifens*. Whilst around two-thirds of patients are diagnosed with ER(+) breast cancer and can be treated with hormone therapy such as with SERMs, there is still an urgent need to develop drugs to be used against ER(−) breast cancer. Preclinical studies have shown that ferrocifen is active against the latter type of breast cancer which is not susceptible to the treatment with tamoxifen (Figure 8.15) [2, 5].

The cytotoxic effect of tamoxifen results from the competitive binding to the oestrogen receptor and repressing DNA transcription, which is mediated by oestradiol. It is believed that ferrocifen follows the same mode of action. Research has shown that the replacement of the phenyl group in tamoxifen by ferrocene results in a reduced binding affinity to the receptor (RBA, receptor binding affinity). Variation, and especially increase, of the chain length has a negative effect on the RBA and also on the bioavailability. The optimum chain length seems to be when $n = 4$. It is also important to note that the *Z*-isomer binds more strongly to the receptor. Very surprisingly, ferrocifen (with $n = 4$) showed also an antiproliferative effect when tested on the oestrogen-independent cell line MDA-MB231, which does not have oestrogen receptors and is not accessible for treatment with tamoxifen. This means that there must be an additional mode of action that is independent of the oestrogen receptor.

Replacing the ferrocenyl group by a ruthenium group resulted in a drop of cytotoxic activity, indicating that the iron group is important. It has been proposed that the additional mode of action of ferrocifen could rely on the redox activation of the ferrocenyl group and the presence of reactive oxygen species (ROS) [2].

These extremely promising results stimulated further research in this area. Tamoxifen was coupled to a variety of known metal-based compounds with potential anticancer activity, such as oxaliplatin, titanocene dichloride and others. Oxaliplatin contains the so-called DACH–Pt group (DACH, 1,2-diaminocyclohexane),

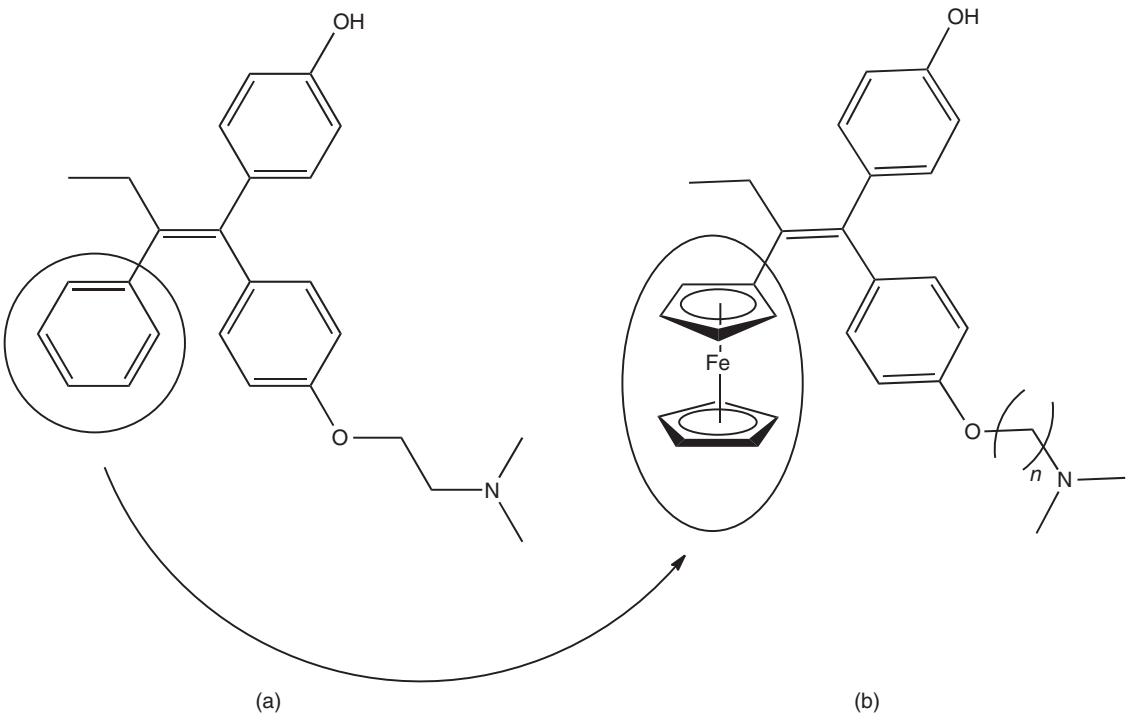

Figure 8.15 *Chemical structure of 4-hydroxytamoxifen (a) and ferrocifen (b)*

which has been used as a basis for a new DACH–Pt–tamoxifen derivative. Oxaliplatin showed a cytotoxic effect of 6.3 μM when tested on the oestrogen-dependent human breast cancer cell line MCF-7, whilst the tamoxifen-vectorised derivatives (see Figure 8.16; R = H, 14 μM and R = OH, 4 μM) also presented an antiproliferative effect at a similar magnitude. Looking in more detail, research shows that the derivative that contains the hydroxyl group displays a higher RBA and also a better IC_{50} value. This shows that the hydroxyl group (also present in the active metabolite of tamoxifen) is important for the recognition by the oestrogen receptor. Nevertheless, the vectorisation of DACH–Pt does not really result in a significant improvement in comparison to oxaliplatin itself, and therefore this combination is not really beneficial as an SERM for the fight against breast cancer [5].

The titanocene-tamoxifen derivative has an RBA value of 8.5%, which means it should recognise the oestrogen receptor well. The results of the cytotoxicity tests were very unexpected, where a proliferative effect was observed. The estrogenic effect was comparable to that of oestrogen itself. It is believed that this estrogenic effect is due to the titanium moiety and/or its hydrolysis products (Figure 8.17) [5].

In order to bring ferrocifen into clinical studies, it was important to find a suitable formulation. This is an area notoriously difficult for metal-based drugs, and OH-ferrocifen finally entered phase II clinical trials. A variety of pharmaceutical approaches have been researched, including the use of nanoparticles, cyclodextrins and lipid nanocapsules [2].

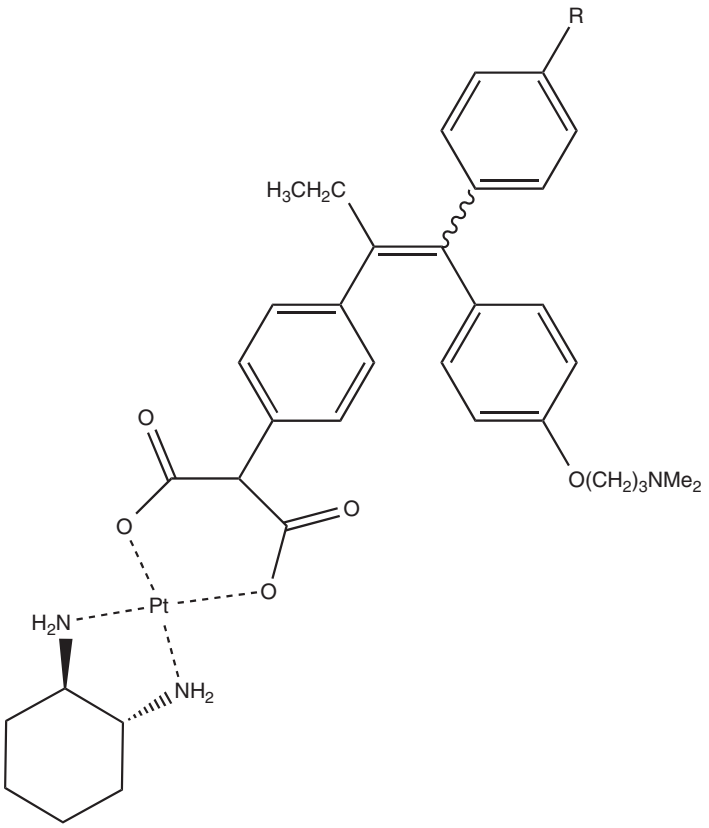

Figure 8.16 *Chemical structure of the DACH–Pt–tamoxifen derivative (R = H, OH)*

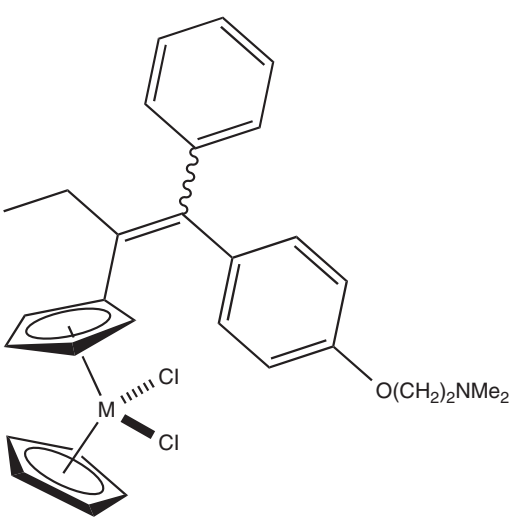

Figure 8.17 *Chemical structure of titanocifen*

8.4 Titanocenes

Titanium, with chemical symbol Ti and atomic number 22, is a member of the d-block metals and belongs to group 4 of the periodic table of elements (Figure 8.18).

Titanium is a metal, which puzzles the bioinorganic scientists, as it has no known native role in the human body (or any organism) despite it is the ninth most abundant element in the earth's crust [6]. The metal titanium is extremely corrosion-resistant and has a remarkable biocompatibility, which has led to its clinical applications. These include endoprostheses of knees and hips, dental implants, heart pacemakers and many more. Titanium complexes are used for their catalytic properties, and there are complexes that interact with biomolecules (this will be shown below). Nevertheless, there is no known function of titanium as a native essential metal [6, 7].

The most stable oxidation state is Ti(IV), and most titanium compounds with lower oxidation states are easily oxidised to Ti(IV). It is very characteristic that these Ti(IV) species easily undergo hydrolysis and form Ti—O bonds and fairly often polymeric structures [7]. Titanium oxides and many Ti(IV) compounds do not show high solubility in water and therefore have only limited bioavailability. This might explain why titanium has no known biological role despite its high natural abundance, and is not an essential metal for any life form [6].

Titanium dioxide (TiO_2) is a white solid with a low aqueous solubility and is the natural occurring oxide of titanium present in minerals such as rutile (composed mainly of TiO_2). TiO_2 is used as a white pigment in paints, food colouring, sunscreens and toothpaste. For the purification of crude TiO_2, the raw material is reduced with carbon and then oxidised in the presence of chlorine. The resulting titanium tetrachloride ($TiCl_4$) is purified by distillation and reacted with oxygen at around 2000 K to form TiO_2 in its pure form.

Current research into the medicinal applications of titanium complexes mainly focusses on their use as anticancer agents. It is important to note that these complexes are different from the above-described titanium alloys/metals or TiO_2, which are highly insoluble in aqueous media. The titanium complexes described below contain functional groups that can be easily exchanged and/or interact with biomolecules in order to fulfil their medicinal role.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La-Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac-Lr	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Uub						

Figure 8.18 *Periodic table of elements; the element titanium is highlighted*

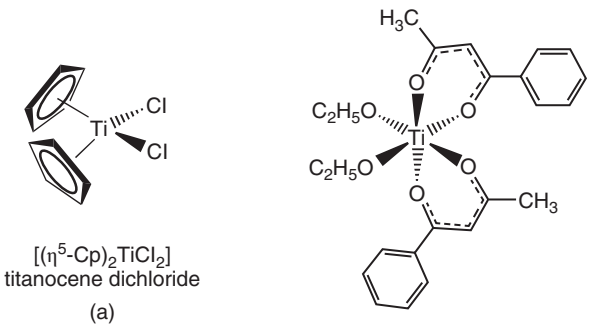

Figure 8.19 *Chemical structures of Cp_2TiCl_2 (a) and budotitane (b) [9]*

8.4.1 History of titanium-based anticancer agents: titanocene dichloride and budotitane

The success of cisplatin stimulated the search for further metal-based anticancer drugs. Two titanium-based complexes, namely titanocene dichloride (Cp_2TiCl_2) and budotitane ($Ti(IV)(bzac)_2(OEt)_2$, bzac, 1-phenylbutane-1,3-dionate), were identified as promising drug candidates and entered clinical trials [7]. Both compounds contain labile groups in the cis position analogous to cisplatin. Unfortunately, hydrolysis in titanium compounds is faster than in cisplatin, and in these cases can lead to the formation of hydroxo-bridge species and potentially to TiO_2, which is insoluble in water. This represents the main challenge for the clinical application of this class of compounds (Figure 8.19) [8].

Cp_2TiCl_2 was first synthesised in 1954 and is a red crystalline solid in its pure form. The Ti(IV) centre is coordinated by two η^5-Cp ligands and two chlorine ligands. Cp_2TiCl_2 can be synthesised by reacting titanium tetrachloride ($TiCl_4$) with NaCp [1]. Compared to those of ferrocene, the planar cyclopentadienyl ligands are coordinated to the titanium centre in a bent sandwich configuration [7]. Furthermore, Cp_2TiCl_2 is an exception to the 18 VEs rule, as it has only 16 VEs, but it is still a stable complex. Each Cp^- ligand contributes six electrons, whilst each Cl^- ligand contributes two electrons. Ti(IV) has no VEs left, which makes a total of 16 VEs.

$$2NaC_5H_5 + TiCl_4 \rightarrow (C_5H_5)_2TiCl_2 + 2NaCl\rangle$$

Compared to cisplatin, much less is known on the mode of action of titanium-based drug candidates. In fact, the active species responsible for the anticancer activity of Cp_2TiCl_2 *in vivo* has yet not been identified, nor has the coordination mechanism to DNA or eventual repair processes. Studies have shown that titanium will accumulate near phosphorus-rich areas, indicating a titanium–DNA interaction takes place. Crystallographic studies suggest that, within Cp_2TiCl_2, the Cp groups sterically prevent the cross-linking of DNA. A direct comparison between Cp_2TiCl_2 and cisplatin shows that their spectrum of activity differs, leading to the conclusion that their mode of action must also differ [7].

Current studies have confirmed the uptake of Ti(IV) by transferrin and transport to the cancer cells. Within the cell, it is believed that Ti(IV) may bind, in contrast to cisplatin, to the DNA backbone, which means coordinating to the negatively charged phosphates. Generally, it is believed that this coordination is not very strong [7].

Cp_2TiCl_2 showed very promising results in preclinical studies. *In vitro* studies have been carried out by Köpf-Maier on a variety of human cancers including human lung and renal cell cancer carcinomas xenografted into mice. The results were very encouraging, as a significant response of the tumour to the chemotherapeutic agent was observed and Cp_2TiCl_2 seemed to be an interesting candidate to bring into the clinic [7]. The major challenge for a clinical application of Cp_2TiCl_2 as an anticancer agent is presented by its hydrolytic instability at pH below 5.5, where both chlorides are lost. Even at neutral pH, research has shown that the Cp rings are lost quickly, resulting in the formation of hydrolysed products which are water insoluble [6]. Therefore, it was necessary to develop a formulation before this compound could be brought into clinical trials. For its use in clinical trials, Cp_2TiCl_2 was formulated as a lyophilised powder in malate buffer (pH = 3.2) or malic acid (pH = 3.5). Cp_2TiCl_2, formulated as a water-soluble powder, has entered phase I and phase II clinical trials [7]. Results from phase I clinical trials were used to establish the maximum tolerable dose and to define the pharmacokinetic properties. Dose-limiting toxicities have been identified, as reversible damage to the liver and kidneys. Phase II clinical trials concentrated on the effect of Cp_2TiCl_2 treatment of renal cell cancer and metastatic breast cancer. Unfortunately, the encouraging effect seen in preclinical studies could not be repeated in the clinical studies. The tumours did not show a significant response to Cp_2TiCl_2, and therefore clinical trials were discontinued [6].

Budotitane is an octahedral Ti(IV) complex that was developed as a potential anticancer agent around the same time as the anticancer properties of Cp_2TiCl_2 were discovered. Budotitane can be synthesised by reacting $TiCl_4$ with the appropriate diketonate in an anhydrous solvent. Important structural features are the unsubstituted aromatic rings, which positively contribute to the cytotoxic activity, and the hydrolysable group. The latter group seems to be less important in regard to the cytotoxicity of budotitane but crucial for formulation purposes as it seems to determine the aqueous stability. Research has shown that the aqueous stability increases with the hydrolysable group in the order: $I^- < Br^- < Cl^- < F^- < OR^-$ (Figure 8.20) [7].

Another major drawback for the clinical use of budotitane is the presence of isomers. There is the possibility of a cis or trans arrangement of the $—OC_2H_5$ group, with the cis arrangement believed to be the more stable one. Furthermore, the β-diketonato ligand is not symmetrical. This means that there are eight possible arrangements in this octahedral scheme. In solution, there is a mixture of isomers and it is difficult to isolate the isomers. So far, it is not known which isomer exhibits the anticancer activity (Figure 8.21) [7].

Preclinical studies using human xenografts in nude mice highlighted the potential of budotitane as novel chemotherapeutic agent in 1984. Budotitane showed cytotoxic activity comparable to cisplatin. Keppler *et al.* intensively studied its antitumour activity in a variety of animal models and found specific activity against Ehrlich ascetic tumour and colon cancer. Budotitane, similar to Cp_2TiCl_2, is prone to hydrolysis in conjunction with a low aqueous solubility. Therefore, significant research went into the development of an appropriate formulation [7]. Several clinical studies were performed using cremophor EL as a nonionic surfactant, which has been used as a vehicle for the solubilisation of a wide variety of hydrophobic drugs (cremophor). Furthermore, 1,2-polyethyleneglycol has been added to encourage the coprecipitation of the drug. All three ingredients (budotitane, cremophor EL and 1,2-polyethyleneglycol) are dissolved in anhydrous ethanol and mixed, and the solvent is then evaporated off. Using this procedure, micelles containing 100–200 mg/100 ml of active pharmaceutical ingredient are formed, which are stable for a few hours. Budotitane went through several phase I and phase II clinical trials. Budotitane was administered as intravenous (IV) infusion twice a week to patients with solid tumours refractory to previous treatments. Cardiac arrhythmia was identified as the dose-limiting

$$2Hbzac + TiCl_4 \rightarrow [Ti(bzac)_2(OEt)_2] + 4HCl$$

Figure 8.20 *Synthesis of budotitane*

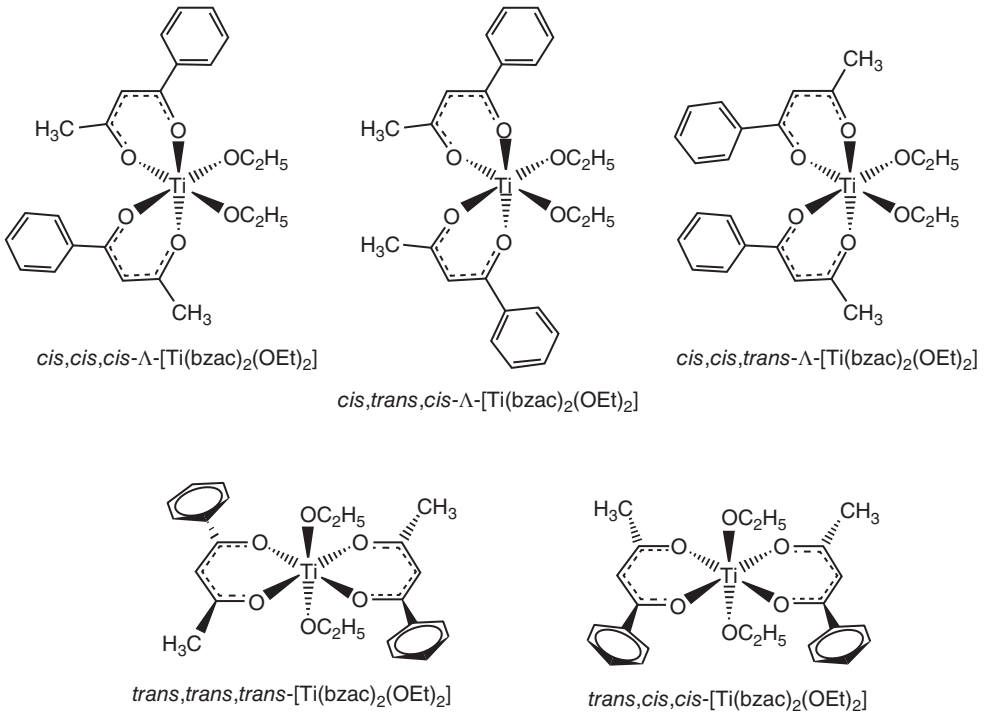

Figure 8.21 *Isomers of budotitane [9]*

side effect. Unfortunately, problems with the formulation, such as the existence of isomers and the difficulty in analysing and characterising the loaded micelles, led to a discontinuation of the clinical trials [7].

Poor water solubility and fast hydrolysis are the main problems for the use of titanocene or titanium-based compounds as anticancer agents. Nevertheless, this intensive research in the 1980s and 1990s has encouraged further research as described in the following.

8.4.2 Further developments of titanocenes as potential anticancer agents

As mentioned, poor aqueous solubility and instability in water are the main problems that restrict titanocenes in clinical applications. McGowan and coworkers renewed the interest in research in this area with their elegant synthesis of ring-substituted cationic titanocene dichloride derivatives. The idea was based on the introduction of charges in order to improve the aqueous solubility. Indeed, the resulting cationic titanocenes are more water-soluble and show a significant cytotoxic activity especially against cisplatin-resistant ovarian cancer (Figure 8.22) [10].

Following on from these results, Tacke and coworkers based their research on novel synthetic methods starting from substituted fulvenes. A fulvenes is an organic molecule with the chemical formula C_6H_6, which consists of a five-membered ring system and can be used to easily introduce functional groups into titanocenes (Figure 8.23) [11].

Reaction of substituted fulvenes with titanium dihalides via a reductive dimerisation process leads to the so-called *ansa*-titanocenes, which are normally used as catalysts. These *ansa*-titanocenes are characterised by a carbon–carbon bridge between the cyclopentadienyl (Cp) rings, which restricts the geometry of the Cp

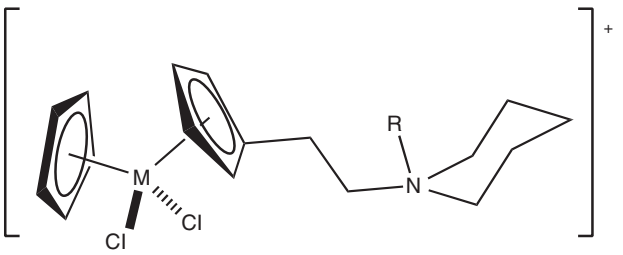

Figure 8.22 *Examples of substituted titanocenes*

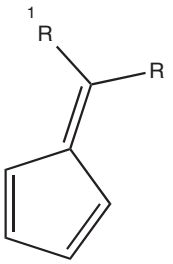

Figure 8.23 *Chemical structure of fulvene*

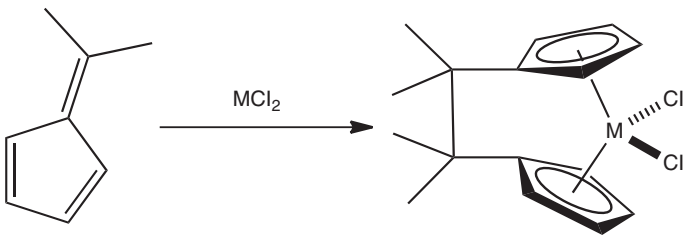

Figure 8.24 *Synthesis of* ansa*-titanocenes using reductive dimerisation of fulvenes*

rings. *In vitro* testing showed that they exhibit a moderate cytotoxic activity, which is similar or better than Cp_2TiCl_2 when tested against a model of renal cell cancer (Figure 8.24) [11].

The reaction of substituted fulvenes with superhydride ($LiBEt_3H$) in a so-called hydridolithiation reaction or the reaction with an organolithium compound in a carbolithiation reaction followed by transmetallation with titanium tetrachloride allows access to a variety of substituted titanocenes. Hydridolithiation allows access to benzyl-bridged titanocenes, whilst titanocenes obtained via carbolithiation typically contain more functional groups. This can be of advantage, for example, for the introduction of groups that can be easily ionised in order to improve the water solubility of these titanocenes, but can also be a disadvantage as additional stereocentres are potentially introduced (Figures 8.25 and 8.26).

These titanocenes have been intensively studied with regard to their potential anticancer activity. It has been shown that some of these compounds are active against a variety of human cancer types. The so-called titanocene Y has been the most intensively studied titanocene of this series. It has shown good activity against

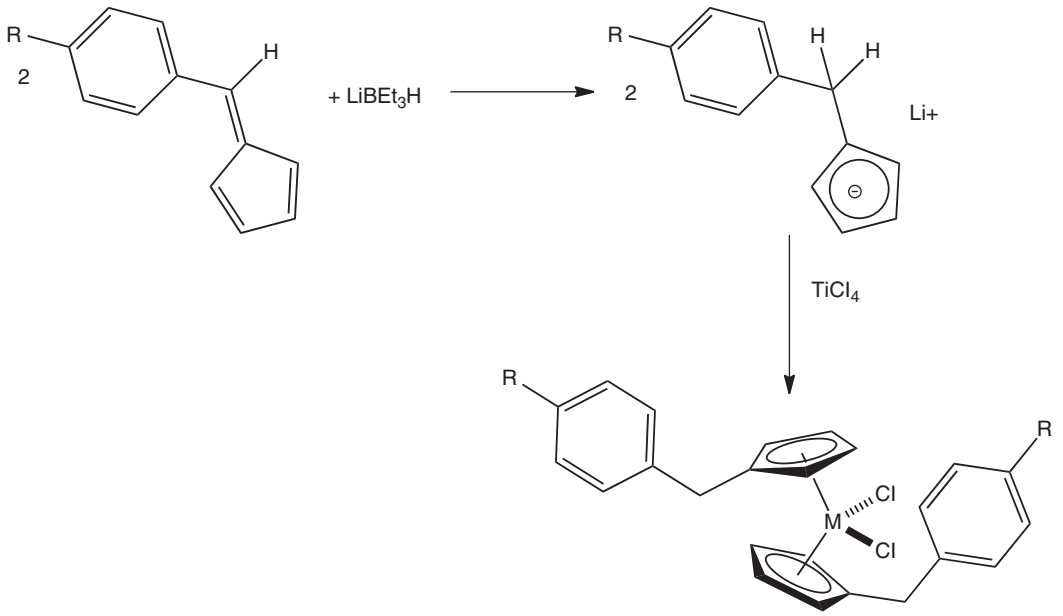

Figure 8.25 *Synthesis of benzyl-substituted titanocenes using hydridolithiation (Adapted from [11].)*

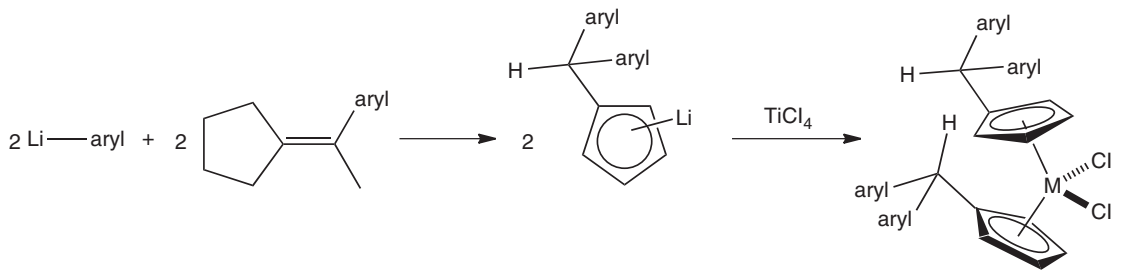

Figure 8.26 *Synthesis of substituted titanocenes using carbolithiation*

a model of renal cell cancer as well as other human cancer cell lines in a variety of *in vitro* experiments (Figure 8.27).

In general, titanocenes obtained via these methods reached IC_{50} values in the low micromolar range when tested against a renal cell cancer model. This represents an up to 2000-fold improvement compared to Cp_2TiCl_2. Nevertheless, the main problems are the potential presence of stereocentres and still a poor aqueous solubility. Further research has been carried out with the aim to replace the chloride ligands by other groups. This includes the incorporation of chelating ligands similar to the research undertaken for cisplatin. Nevertheless, this research did not result in any major improvement with regard to the cytotoxic activity. Also, some initial formulation studies have been carried out with only limited success. The mode of action of these titanocenes is so far not clear. It is believed that they coordinate to DNA via its phosphate backbone. It has also been shown that they can use transferrin as a transporter molecule into the cancer cell [11].

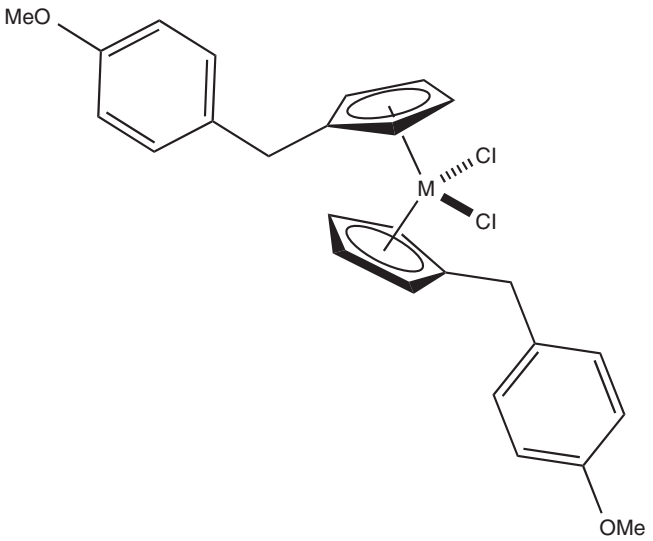

Figure 8.27 *Chemical structure of titanocene Y*

8.5 Vanadocenes

Vanadium, with chemical symbol V and atomic number 23, is a member of the d-block metals and belongs to group 5 of the periodic table of elements (Figure 8.28).

Vanadium can be found in the earth's crust in numerous minerals and is isolated from ores mostly as a by-product. Its main application is in the steel industry, where it is used as an alloy in combination with iron. Vanadium pentaoxide is also being used as a catalyst for the production of sulfuric acid. The metal vanadium has very similar properties to titanium. Therefore, it is not surprising that its metallocene, vanadium dichloride, was also subjected to research as a potential anticancer agent.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La-Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac-Lr	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Uub						

Figure 8.28 *Periodic table of elements; the element vanadium is highlighted*

$$[VO_4]^{3-} \rightarrow [VO_3 \times OH]^{2-} \rightarrow \cdots \rightarrow [V_3O_9]^{3-} \rightarrow V_2O_5 \times (H_2O)_n \rightarrow [V_{10}O_{28}]^{6-} \rightarrow [VO_2]^{+}$$

pH 12 pH 9 pH 6.5 pH 2.2 pH < 1

Figure 8.29 *Vanadium oxide formation depending on pH [12]*

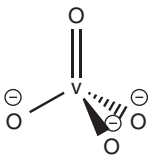

Figure 8.30 *Chemical structure of vanadate*

Vanadium is easily passivated by an oxide film, and the metal is insoluble in nonoxidising acids. Typical oxidation states are +II, +III, +IV and +V, whilst the biologically active oxidation states are +IV and +V. Vanadium reacts to vanadium halide by reacting the metal with the corresponding halogen under heating, whilst it also reacts with oxygen with the formation of V_2O_5. Vanadium (+V) oxides are amphoteric and, as a result, vanadates (VO_4^{3-}) and dioxovanadium ions (VO_2^{+}) are formed in aqueous solutions depending on the pH (Figure 8.29) [4].

Vanadium is an essential trace metal in the human body, but still very little is known about its biological function. Vanadium is mainly found in its ionic state bound to proteins. As mentioned, the metal mostly occupies oxidation states +V and +IV in biological systems, resulting in electron configurations of $[Ar]3d^0$ for V^{+5} and $[Ar]3d^1$ for V^{+4}. The chemical formula for the tetrahedral ion vanadate is written as VO_4^{3+}; whereas the diatomic oxovanadium(+IV) ion, also called *vanadyl*, has the chemical formula VO^{2+} (Figure 8.30).

Vanadium compounds are well known for their toxicity. The most famous example is the poisonous mushroom toadstool, *Amanita muscaria*. *A. muscaria* contains the toxic compound amavadin, which is a toxic octahedral vanadium complex (see Figure 8.31) [13].

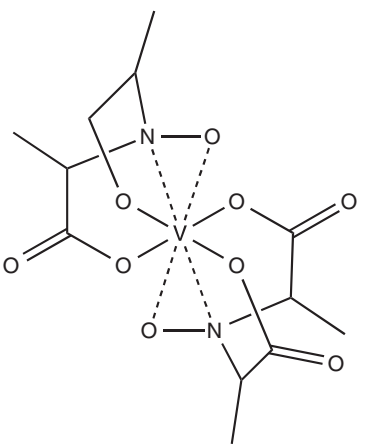

Figure 8.31 *Chemical structure of amavadin*

Vanadate and vanadyl are known to cause adverse effects in mammals, including loss of body weight, gastrointestinal problems, reproductive toxicity and morbidity. However, their toxicity depends on a variety of factors such as the chemical form, oxidation state, route of administration and duration of exposure. Nevertheless, toxic effects of vanadate or vanadyl are observed only at dose levels significantly greater than usual uptake through diet [14]. Nevertheless, it is important to improve the understanding of the adverse and toxic effects of vanadium compounds before any compound can be successfully developed for clinical use.

8.5.1 Vanadocene dichloride as anticancer agents

Vanadocene dichloride [$(\eta^5\text{-}C_5H_5)_2VCl_2$, dichloro bis($\eta^5$-cyclopentadienyl)vanadium(IV)] is structurally very similar to Cp_2TiCl_2. It also consists of a metal centre with an oxidation number of +IV, in this case vanadium, and two Cp^- and two chloride ligands. Vanadocene dichloride is a 17-electron complex containing an unpaired electron and is therefore paramagnetic (Figure 8.32).

Vanadocene dichloride has found application as a catalyst for polymerisation reactions, but was also intensively studied as an anticancer agent in parallel to Cp_2TiCl_2 because of their structural similarities. Vanadocene dichloride has proven to be even more effective than its titanium analogue as an antiproliferative agent against both animal and human cell lines in preclinical testing. The main problems are the difficult characterisation of the active vanadium compounds and their fast hydrolysis. Because of their paramagnetic character, it is difficult to apply standard classical analysis techniques such as NMR (nuclear magnetic resonance) to identify the antiproliferative vanadium species. Furthermore, vanadocene dichloride undergoes fast hydrolytic processes and is even more prone to hydrolysis than titanocene dichloride. This poses even more challenges for its potential clinical application [15].

In recent years, researchers have shown renewed interest in the use of substituted vanadocene dichlorides as potential anticancer agents. A selection of substituted vanadocenes have been synthesised and tested for their cytotoxic activity against testicular cancer. Examples of these compounds include vanadocenes containing substituted cyclopentadienyl ligands and/or replacement groups for the chloride ligands – similar to the research being undertaken for cisplatin analogues. Results of *in vitro* studies show that these compounds exhibit good but variable cytotoxic activity depending on the substitution pattern and induce apoptosis (cell-induced cell death). Interestingly, only organometallic vanadium(+IV) complexes showed cytotoxic activity against testicular cancer. When the purely inorganic compound vanadyl(IV) sulfate was tested in the same study, no cytotoxic effect was observed at the same concentrations. It is also important to note that titanocene dichloride and other metallocenes had no cytotoxic effect against testicular cancer. It was concluded that the mode of action of vanadium-induced cytotoxicity must be different from that of titanocene dichloride and other metallocenes (Figure 8.33) [16].

In parallel to the research undertaken with substituted titanocene dichlorides as potential chemotherapeutic agents, some of their vanadocene analogues have been synthesised. Some examples include the hydrolithiation of fulvenes (see Section 8.4.2) and subsequent transmetallation with vanadium tetrachloride. The resulting

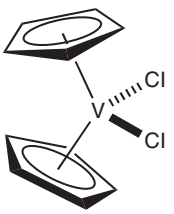

Figure 8.32 *Chemical structure of vanadocene dichloride*

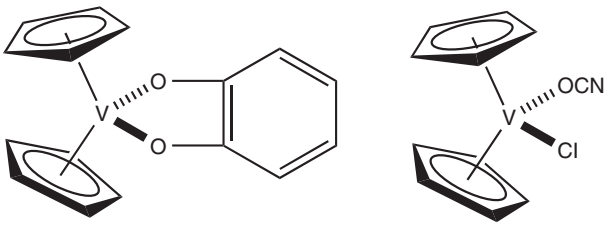

Figure 8.33 *Examples of substituted vanadocenes dichlorides*

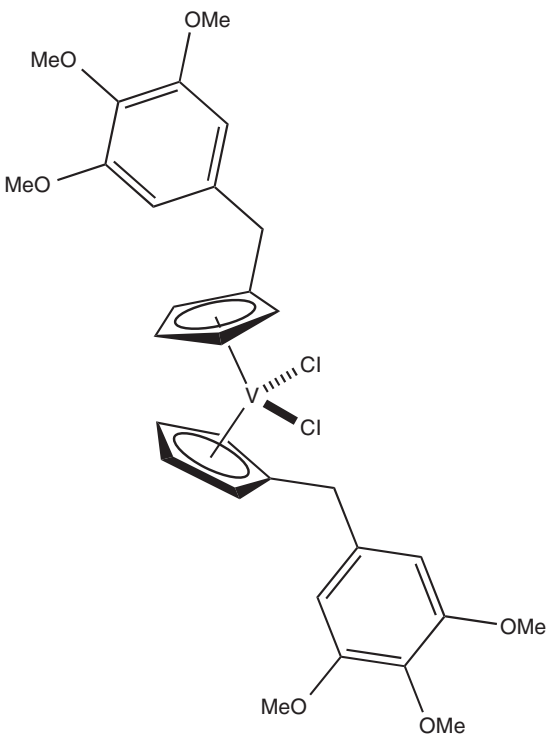

Figure 8.34 *Example of a substituted vanadocenes synthesised via transmetallation*

substituted vanadocene dichlorides were found to be highly toxic compounds when tested *in vitro* against a model of renal cell cancer and more potent than the corresponding titanocene. Further preclinical studies are still needed (Figure 8.34) [15].

8.5.2 Further vanadium-based drugs: insulin mimetics

Towards the end of the nineteenth century, inorganic vanadium compounds were under evaluation as potential treatment options for Diabetes Mellitus (DM) as so-called insulin mimetics. Sodium vanadate ($Na_3V(+V)O_4$) was tested for its ability to lower glucose levels in the blood of candidates with and without DM. The inorganic vanadium compound showed mild effects in some of the patients suffering from DM, whilst no severe

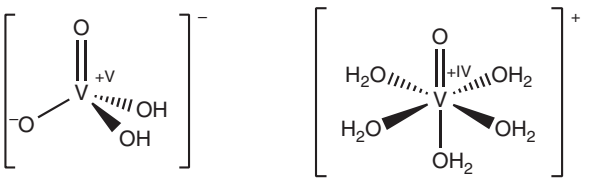

Figure 8.35 *Chemical structures of vanadate, VO_4^{3-}, (a) and vanadyl oxycation (b)*

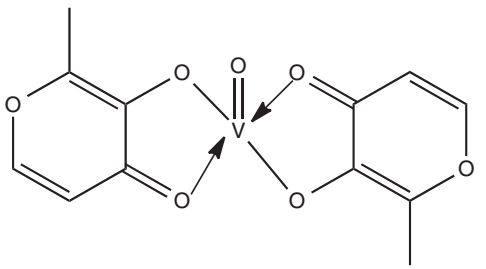

Figure 8.36 *Chemical structure of bis(maltolato)oxovanadium*

side effects were reported for the dose applied. However, research focused more on the less toxic inorganic vanadium compounds, such as vanadyl sulfate ($V(+IV)OSO_4$) which is significantly less toxic than sodium vanadate. Nevertheless, with the development of insulin in 1922, the interest in vanadium compounds as antidiabetic drugs diminished (Figure 8.35) [9].

In more recent years, metal complexes have become of interest for a variety of clinical applications. This also renewed the interest for vanadium complexes to be examined for the treatment of diabetes. The vanadium complexes bis(maltolato)oxovanadium (BMOV) and bis(ethylmaltolato)oxovanadium (BEOV) have shown to be unique insulin mimetics when tested in diabetic rats [9, 17]. An increase in uptake and tolerability compared to the inorganic form was noted. Studies have also shown that there is a difference in distribution between the inorganic and the complexed form of vanadyl in *in vivo* experiments, which might relate to the differences in uptake and tolerability. Animal experiments with vanadyl sulfate have shown accumulation of vanadium mainly in the kidneys and liver, whilst experiments with the vanadyl complexes BMOV and BEOV resulted in a high accumulation on the bones followed by kidneys (Figure 8.36) [14, 18].

BMOV has proven itself as a successful antidiabetic agent when tested in animal models. Nevertheless, only very little is known about its mode of action. It is believed that BMOV acts as a competitive and reversible inhibitor of the enzyme protein tyrosine phosphatase (PTP). Other vanadium complexes are also known to inhibit PTP, but mostly inhibiting it irreversibly [17a].

PTPs belong a family of enzymes that remove phosphate groups from phosphorylated tyrosine residues on proteins. Its member protein tyrosine phosphatase 1B (PTP1B), which is located in the cytosol, has been identified as a negative regulator of insulin signal transduction. Resistance to insulin can be observed in different tissues such as muscles, liver and fat, which are all crucial for the homeostasis of glucose levels in the human body. In the healthy human body, the transport of glucose into the cell occurs through the activation of the insulin receptor including the phosphorylation of the tyrosine residue. As a result, the so-called insulin receptor substrate (IRS) is recruited, followed by the activation of several enzymes. Finally, the glucose transporter GLUT4 is translocated, which mediates the transport of glucose into the cell.

PTP1B seems to be a key regulator for the activity of the insulin receptor, including all downstream signalling processes [19]. It works by the dephosphorylation of the phosphotyrosine residues at the activated

insulin receptor kinase and therefore ultimately hinders the uptake of glucose. PTP1B has been identified as a promising target for new drugs treating DM Type 2. Blocking the PTP1B-mediated dephosphorylation of insulin receptor kinase by an inhibitor of PTB1B is believed to lead to an increase in insulin sensitivity.

As previously mentioned, BMOV is believed to be a potent and competitive inhibitor of the PTP1B activity and additionally seem to support the autophosphorylation of the insulin receptor leading to an increased sensitivity towards insulin. Research has shown that varying the organic ligand has an influence on the effectiveness and bioavailability of the resulting vanadium compound. It is believed that factors such as absorption, tissue uptake and distribution are affected most. Interestingly enough, X-ray crystal data of PTP1B soaked with BMOV showed only vanadate [$V(+V)O_4^{3-}$] at the active site. This would emphasise that the organic ligands are only carriers of the active compound and play no role in the enzyme inhibition itself. Furthermore, in aqueous solution, V(IV) is rapidly and reversibly oxidised to V(V), supporting the possible formation of vanadate [17a].

BEOV entered clinical trials and successfully finished phase IIa trials for the treatment of DM Type 2. In phase I trials, doses of 10–90 mg were given to healthy nondiabetic volunteers and no adverse side effects were seen. In the phase IIa clinical trial, seven diabetic patients were treated with 20 mg/day of BEOV and showed a reduction of around 15% of their blood glucose levels. Two patients were treated with a placebo, and no reduction in blood glucose levels was observed. It was also interesting to note that the glucose level reduction lasted for 1 week after finishing the treatment [9].

8.6 Exercises

8.6.1 Write the electronic configuration for the following elements or ions:

(a) Ti^{4+}
(b) Fe^{2+}
(c) Fe^{3+}
(d) Ru^{2+}

8.6.2 What is the oxidation state of the central metal atom in the following complexes?

(a) $[Fe(C_5H_5)_2]^+$
(b) $TiCl_4$
(c) $Ti(C_5H_5)_2Cl_2$

8.6.3 Draw the energy diagrams displaying the d-orbital splitting for the low and high-spin complexes of the following examples assuming an octahedral complex.

(a) Fe^{3+}
(b) Fe^{2+}
(c) Ti^{4+}

8.6.4 Predict the geometry of the following complexes:

(a) Platinum tetrachloride
(b) Vanadium hexacarbonyl

8.7 Case study – titanium dioxide

Integrated sun protection in cosmetics is becoming increasingly important. Titanium dioxide nanoparticles are a commonly used as an inorganic UV filter. New formulation techniques allow its integration without the previously known whitening effect. Sunscreens contain typically between 5% and 20% w/v nanosized titanium dioxide.

There are a variety of different methods to analyse the quantity of titanium dioxide in cosmetic formulations. One method is based on the reduction of Ti(+IV) and subsequent re-oxidation with a ferric solution. Titanium dioxide is typically dissolved in hot sulfuric acid and reduced by adding metallic aluminium. The resulting Ti(+III) is then titrated against a standard solution of ammonium iron(III) sulfate in the presence of potassium thiocyanate as indicator.

(a) Research the type of titration described.
(b) Describe the chemical structure and mode of action of the indicator.
(c) Formulate all relevant reaction equations.
(d) The package states that the sunscreen contains 10% w/v titanium dioxide. For the analysis, a volume containing the theoretical value of 0.5 g of titanium dioxide is dissolved in sulfuric acid and reacted with metallic aluminium. The resulting solution is titrated against a 0.5 M solution of ammonium iron(III) sulfate using potassium thiocyanate as indicator.

For each titration, the following volume of ammonium iron(III) sulfate has been used:

12.55 ml	12.50 ml	12.60 ml

Calculate the real amount of titanium dioxide present in your sample. Express your answer in grams and moles.

(e) How many millilitres of sunscreen have been used for the analysis?
(f) Discuss the result in relation to the typically accepted error values.

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