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Journal of Organometallic Chemistry 690 (2005) 117–125
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

Synthesis, characterization and antitumor properties of titanocene derivatives with thiophene containing ligands

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Received 22 April 2004; accepted 30 August 2004

Abstract

The titanocene complexes $[TiCp_2(Cl)R]$ (**1**), $[TiCp_2(Cl)SR]$ (**2**), $[TiCp_2(SR)_2]$ (**3**) with R = benzothienyl (BT) **A** and dibenzothienyl (DBT) **B**, were synthesized and the molecular structures of $[TiCp_2(Cl)DBT]$ (**1B**) and $[TiCp_2(Cl)SDBT]$ (**2B**) confirmed by single crystal X-ray diffraction studies. The dibenzothiophene rings are planar and for **1B** in the plane of the titanium and chloro ligand. The chloro ligand is in a *trans* position to the sulfur atom with respect to the titanium–carbon bond. The complexes were studied for their electronic and structural features and preliminary tests were conducted for their tumor inhibiting properties against HeLa and COLO 320M tumor cell lines. These antitumor activities were compared against those observed for titanocene dichloride (**S-01**) under similar conditions and the highest antitumor activity was recorded for **2B**.

Keywords: Titanocene complexes; Thiophene derivatives; Antitumor properties; Bioorganometallics; Thiolato complexes

1. Introduction

Bioorganometallic chemistry is an emerging field of interest of which the importance has been highlighted by the first International Symposium on Bioorganometallic Chemistry held in July 2002 in Paris [1] and recent review articles appearing in the *Journal of Organometallic Chemistry* and *Organometallics* [2]. In 1979 the antitumor properties of titanocene dichloride, $[Ti(\eta^5\text{-}C_5H_5)_2Cl_2]$, were reported by Köpf and co-workers [3]. Following this important discovery and pioneering investigations, the antitumor properties of a number of structurally similar neutral and ionic metallocene dihalides have been investigated [4]. Of these, titanocene dichloride was the most promising and as a result has entered clinical trails as a potential anticancer drug [5].

The mode of action resulting in the antitumor activity in biological systems for titanocene dichloride is presently under investigation [6,7]. Some uncertainty exists regarding the composition of the biologically active titanium species responsible for the antitumor activity. Reasons for this are related to the poor solubility of titanocene dichloride in water, the rapid hydrolysis of titanium complexes at neutral pH and the precipitation of polymeric hydrolysis products [8]. Water-soluble, ionic titanocene dichloride derivatives have been prepared and studied recently by the groups of McGowan [9] and Harding [10]. Sadler and co-workers [6] observed that above a pH of 6, titanocene dichloride does not bond strongly to nucleotides and nucleobases. On the basis of model studies, they proposed that a titanium

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doi:10.1016/j.jorganchem.2004.08.046

species, stripped of cyclopentadienyl ligands, once in the cell could complex to phosphates and provide a mechanism whereby Ti is delivered to DNA. The titanium(IV) ion forms a strong complex with the human blood plasma protein transferrin, which has been implicated in the transport and delivery of titanium ions to cancer cells, by binding to specific iron(III) binding sites [11].

For this study, we attached a titanocene chloride fragment to a second biological active heteroarene group by replacing one of the chloro ligands. The titanocene complexes contain the Ti^{4+} metal, labile chloro and heteroaromatic ligands, the latter being bound either directly to titanium or via a sulfur atom as thiolato ligands. It is known that certain planar, condensed heteroaromatic rings display antitumor activity as they can insert or intercalate into the grooves of double stranded DNA, resulting in the distortion or unwinding of the DNA structure [12]. Once the complex disintegrates under physiological conditions, enhanced antitumor activity could result from the combined antitumor properties of titanium(IV), as well as the decomplexed heteroarene substrate. This paper reports the synthesis, structure and preliminary in vitro screening of titanocene complexes of the type shown in Fig. 1.

The planar, heteroaromatic molecules selected for this study were benzothiophene (BTH) and dibenzothiophene (DBTH). The biological activities of thiophene derivatives have previously been investigated extensively and Osborn and co-workers [13] suggested that the antitumor activity of DBTH derivatives could result from their absorption onto either adenine–thymine or guanine–cytosine pairs of bases at the end of DNA molecules. Bioactive derivatives of BTH and DBTH are known and have been studied extensively. The compound 5-chloro-3-methylbenzothiophen-2-yl acetic acid is an example and is commercially available under the trade name Tianafac and being used for its antiinflammatory properties [14].

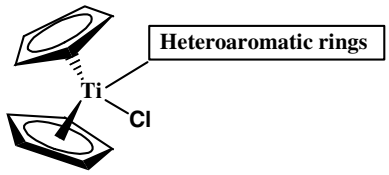


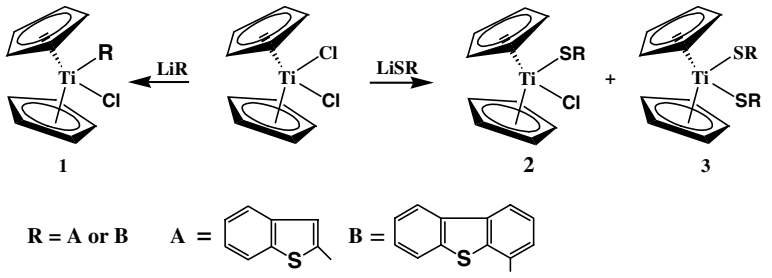
Fig. 1. Titanocene complex with planar heteroaromatic ligands.

2. Results and discussion

Benzo[*b*]thiophene is readily deprotonated in high yields in the 2-position of the thiophene ring to give benzothienyllithium [15]. Direct metallation of DBTH is unsatisfactory, resulting in low yields of the 4-substituted DBTH. Higher yields of mono- and dimetallated species are possible by using a modified procedure or TMEDA-complexed butyllithium [16]. The lithiated precursors were reacted with titanocene dichloride in an exchange reaction to afford organometallic complexes **1A** and **1B** with σ-bonded 2-benzothienyl and 4-dibenzothienyl ligands (Scheme 1). The complexes are soluble in dichloromethane, chloroform, ether, acetone and ethanol, but insoluble in hexane. Interestingly **1B** is more stable than **1A** in the solid state and can be stored at room temperature for several months.

The analogous complex $[Ti(\eta^5\text{-}C_5H_5)_2(2\text{-thienyl})_2]$ was prepared by this method from titanocene dichloride and two equivalents of Li-thienyl [17]. The corresponding arylthiolates were synthesized by reacting the lithiated precursors with flowers of sulfur. The thiolate precursors were added to one equivalent of titanocene dichloride dissolved in THF at low temperature. Purification by column chromatography afforded the complexes $[Ti(\eta^5\text{-}C_5H_5)_2(SR)Cl]$ **2A** or **2B** and $[Ti(\eta^5\text{-}_5H_5)_2(SR)_2]$ **3A** or **3B** in high yields. The thiolate ligand in **2** activates the remaining chloro ligand leading to a competitive reaction with titanocene dichloride and the formation of **3**. Whereas **3** is very stable and can be stored for several months under nitrogen atmosphere, **2** slowly converts to **3** and the well-known oxo-bridged dinuclear titanium complex, $[(\mu\text{-}O)\{Ti(\eta^5\text{-}C_5H_5)_2Cl\}_2]$, in solution [18].

The benzothienyl- and dibenzothiophene protons in the ^1H and ^{13}C NMR spectra of **1A** and **1B** gave resonances that were uniquely assigned by comparing coupling information and applying two-dimensional NMR techniques. Two-dimensional homonuclear shift correla-



Scheme 1.

tion spectroscopy (COSY) was used to distinguish between H4 and H7, as well as between H5 and H6 for **1A**. Long-range proton–proton coupling of H3 to H4 was used to assign H4. Inter-ring coupling observed for BTH was absent in **1A** [19]. Since H3 is the proton closest to the site of coordination to titanium, the chemical shift of this proton is affected most. Two-dimensional heteronuclear shift correlation spectroscopy (HETCOR) was used to uniquely assign the carbon atoms in **1**. In **1A** and **1B**, C2 resonate significantly downfield from the value recorded for BTH [19] and DBTH [22], but the shift value is comparable to the 189.6 ppm recorded for the *ipso*-carbons of the phenyl ligands in $[Ti(\eta^5\text{-}C_5H_5)_2(C_6H_4\text{–}C_6H_4)]$ [24]. This dramatic shift is ascribed to the presence of a strong carbo-cationic atom, resulting in the disruption of the aromaticity of the thienyl ring and downfield shifts for all the thienyl carbon atoms. Interestingly, the chemical shifts of the other carbons of the benzene ring in **1A** are shifted upfield in comparison to the corresponding resonances in BTH. In the ^{1}H NMR spectrum of **1B** H6 and H7 overlap which makes unambiguous assignments impossible. This was also observed in the ^{1}H NMR spectrum of uncoordinated DBTH [19]. Although the synthesis of BTSH has been reported earlier [23], a ^{1}H NMR data set for comparison was recorded, and the chemical shifts are listed in Table 1. The main difference in the spectra of **2A** and **3A** is found for the H3 chemical shifts of the benzothienyl protons. The H3 proton of **2A** is shielded compared to the free thiol, but deshielded compared to **3A**. The phenyl resonances most affected compared to the free dibenzothiophenethiol [23] are those on the phenyl ring bearing the thiolato ligand. Insertion of an sp^3-sulfur atom between the 2-benzothienyl and dibenzothiophene rings and a $TiCp_2Cl$ fragment (**2**) causes a significant upfield shift of the *ipso*-carbon, C2 for **2A** or C1 for **2B** (Table 1). This seems to indicate that bonding between sulfur and titanium is localized and does not involve or affect the phenyl ring. Whereas the cyclopentadienyl ring protons of **1A** resonate upfield from those of **1B** in the ^{1}H NMR spectra, this order is reversed for **2A** and **3A**.

2.1. Structural studies

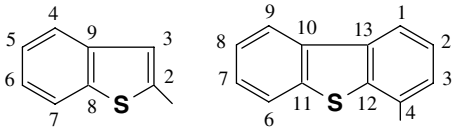
Single crystals of **1B** were isolated from a cold ether–hexane mixture of which a suitable crystal was selected for X-ray diffraction studies. The complex crystallizes in the space group $P2_1/c$ and has two non-identical molecules (A and B) in the unit cell. The molecular structure with atom numbering of **1B** (A-molecule) is shown in Fig. 2 and represents the first example of DBT σ-bonded to a transition metal in the C4-position (C2A in the crystal structure).

Rauchfuss and co-workers [25] reacted titanocene dichloride with a dilithiated C–S cleaved DBTH, and reported the structure of the resulting titanacycle. A similar type of structure of the titanacycle obtained from the reaction of 2,2′-dilithiobiphenyl and titanocene dichloride has also been recorded [24]. In both cases, non-planar rings were obtained. Selected bond lengths and bond angles of **1B** are given in Table 2. Four ligands surround the titanium centre in a distorted tetrahedral arrangement, with positions defined by the centroids of the Cp rings and the chloro and thiolato ligands. The titanium atom is in the plane of the DBT rings and displaced from the plane defined by the arene carbons by 0.0952(6) Å for the molecule A and 0.1144(7) Å for molecule B. The dihedral angle Ti–C(2)–C(1)–S for molecule A is −0.2(4)° and for B it is 0.6(4)°. The Cp rings and titanium belong to the open clamshell class of compounds of which $[TiCp_2Cl_2]$ is the parent molecule and the substitution of a chloro ligand by DBT causes enlargements of the C(4)–Ti–Cl angle, as well as the C(Cp)–Ti–C(Cp) angle (see Table 2) to accommodate the more bulky DBT ligand. The corresponding Cl–Ti–Cl and Cp–Ti–Cp angles in $[TiCp_2Cl_2]$ are 94.62(6)° and 131.04(5)°, respectively [26]. The dihedral angle Cl–Ti–C(2)–C(3) of −4.5(2)° for molecule A or −1.8(2)° for B reveals that the chloro ligand is approximately in the plane of the DBTH ring and in a *trans* position to the sulfur atom with respect to the Ti–C(2) bond. The Ti–C(2) bond distance, 2.209(3) Å, in **1B** (B-molecule) is significantly shorter than the observed Ti–C(sp^2, phenyl) bond distance of 2.27(1) Å for $[TiCp_2Ph_2]$ [27] and a distance of 2.232(3) for the A-molecule of **1B**. The latter distance in **1B** is comparable to 2.193(3) and 2.188(5) Å recorded for the titanacycles $[TiCp_2(C_6H_4\text{–}C_6H_4S)]$ and $[TiCp_2(C_6H_4\text{–}C_6H_4)]$ [24,25].

Confirmation of the structure of **2B** was obtained from a single X-ray diffraction study. The complex was recrystallized from a 1:1 dichloromethane:hexane solution by using the layering technique giving dark red crystals. Fig. 3 shows a ball and stick representation of the structure, also indicating the labeling scheme that was used. The most important bond lengths and angles are listed in Table 3.

The insertion of a sulfur atom between Ti and the DBT heteroarene in **2B** creates more space around the titanium and causes a slight decrease in the angle between the two non-Cp ligands compared to the value in **1B**. Because of the relatively bulky thiolato ligand this value is still significantly larger than the Cl–Ti–Cl angle in titanocene dichloride [26]. The dihedral angle C(1)–C(12)–S(2)–C(11) of −179.2(2)° reveals that the DBT rings are planar and the dihedral angle Ti–S(1)–C(1)–S(2) of 12.2(4)° shows that the DBT rings do not share a plane with the titanium atom. Comparison of the bond lengths of DBTH to those of **2A**, showed that they differ very little and that coordination to titanium has little effect on the bond lengths in the rings of DBT [28].

Table 1
Physical and Spectroscopic data for **1–3** and thiophene derivatives



Complex	Elemental analysis (%)	^{1}H NMR (δ/ppm in $CDCl_3$)	^{13}C NMR (δ/ppm in $CDCl_3$)
1A	Calc.: C, 62.17; H, 4.35	6.64 (s, 1H, H3), 7.56 (d, 1H, H4, $^3J_{HH}$ = 7.9), 7.20 (td, 1H, H5, $^3J_{HH}$ = 7.4, $^4J_{HH}$ = 0.9), 7.09 (td, 1H, H6, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.2), 7.69 (d, 1H, H7, $^3J_{HH}$ = 8.0), 6.42 (s, 10H, Cp)	185.2 (C2), 128.4 (C3), 141.6, 145.8 (C8, C9), 121.4 (C4), 123.2 (C5), 121.5 (C6), 120.5 (C7)
	Found: C, 62.34; H, 4.47		
1B	Calc.: C, 66.59; H, 4.32	67.35 (dd, 1H, H2, $^3J_{HH}$ = 7.4, $^4J_{HH}$ = 1.1), 7.07 (t, 1H, H3, $^3J_{HH}$ = 7.6), 7.75 (dd, 1H, H4, $^3J_{HH}$ = 7.7, $^4J_{HH}$ = 1.1), 8.07 (m, 1H, H5), 7.41 (m, 2H, H6, H7), 7.79 (m, 1H, H8), 6.49 (s, 10H, Cp)	185.5 (C1), 134.5 (C2), 124.8 (C3), 117.7 (C4), 121.5 (C5), 124.2, (C6), 125.9 (C7), 121.9 (C8), 138.5 (C9), 136.2 (C10), 133.2 (C11), 147.3 (C12), 117.2 (Cp)
	Found: C, 66.78; H, 3.98		
2A	Calc.: C, 56.92; H, 3.98	7.16 (s, 1H, H3), 7.69 (d, 1H, H4, $^3J_{HH}$ = 7.5), 7.32 (td, 1H, H5, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.2), 7.24 (td, 1H, H6, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.2), 7.74 (d, 1H, H7, $^3J_{HH}$ = 8.8), 6.35 (s, 10H, Cp)	149.6 (C2), 127.7 (C3), 122.8 (C4), 124.2 (C5), 123.7 (C6), 121.5 (C7), 141.9 (C8), 140.4 (C9), 116.3 (Cp)
	Found: C, 57.22; H, 4.18		
2B	Calc.: C, 61.61; H, 4.00	7.53 (dd, 1H, H2, $^3J_{HH}$ = 7.2, $^4J_{HH}$ = 1.3), 7.46 (t, 1H, H3, $^3J_{HH}$ = 8.3), 8.00 (dd, 1H, H4, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.2), 8.14 (m, 1H, H5), 7.45 (m, 2H, H6, H7), 7.85 (m, 1H, H8), 6.27 (s, 10H, Cp)	143.1 (C1), 131.2 (C2), 124.9 (C3), 119.5 (C4), 121.9 (C5), 124.4 (C6), 126.6 (C7), 122.7 (C8), 139.8 (C9), 136.4 (C10), 134.7 (C11), 142.0 (C12), 115.9 (Cp)
	Found: C, 61.94; H, 4.15		
3A	Calc.: C, 61.16; H, 3.95	7.34 (s, 2H, H3), 7.66 (dd, 2H, H4, $^3J_{HH}$ = 7.4, $^4J_{HH}$ = 0.8), 7.29 (td, 2H, H5, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.1), 7.22 (td, 2H, H6, $^3J_{HH}$ = 7.5, $^4J_{HH}$ = 1.3), 7.72 (d, 2H, H7, $^3J_{HH}$ = 7.9), 6.19 (s, 10H, Cp)	149.9 (C2), 126.8 (C3), 122.5 (C4), 124.1 (C5), 123.5 (C6), 121.5 (C7), 141.6 (C8), 140.6 (C9), 113.6 (Cp)
	Found: C, 61.36; H, 4.08		
3B	Calc.: C, 67.09; H, 3.98	7.96 (dd, 2H, H2, $^3J_{HH}$ = 6.4, $^4J_{HH}$ = 1.1), 7.48 (t, 2H, H3, $^3J_{HH}$ = 7.6), 7.99 (dd, 2H, H4, $^3J_{HH}$ = 6.7, $^4J_{HH}$ = 1.0), 8.16 (m, 2H, H5), 7.45 (m, 4H, H6, H7), 7.86 (m, 2H, H8), 6.09 (s, 10H, Cp)	142.5 (C1), 130.7 (C2), 125.0 (C3), 118.9 (C4), 122.0 (C5), 124.3 (C6), 126.5 (C7), 122.8 (C8), 139.7 (C9), 136.6 (C10), 134.5 (C11), 142.3 (C12), 112.8 (Cp)
	Found: C, 67.25; H, 4.11		
BTH[a]		7.43 (d, 1H, H2), 7.41 (d, 1H, H3), 7.92 (d, 1H, H4), 7.48 (td, 1H, H5), 7.45 (td, 1H, H6), 7.98 (d, 1H, H7)	126.1 (C2), 123.7 (C3), 139.5, 134.6 (C8, C9), 123.5 (C4), 124.0 (C5), 124.1 (C6), 122.3 (C7)
BTSH[b]		7.48 (d, 1H, H2), 7.38 (t, 1H, H3), 8.07 (d, 1H, H4), 8.11 (d, 1H, H5), 7.43 (m, 2H, H6, H7), 7.81 (d, 1H, H8), 2.14 (s, 1H, SH)	142.8 (C2), 130.9 (C3), 122.7 (C4), 125.4 (C5), 123.9 (C6), 122.1 (C7), 141.6 (C8), 139.9 (C9)
DBTH[c]		7.87 (d, 2H, H1, H8), 7.46 (m, 4H, H2, H3, H6, H7), 8.15 (d, 2H, H4, H5)	123 (C1,C8), 127, 124 (C2, C3, C6, C7), 122 (C4, C5), 139 (C9, C12) 136 (C10, C11)
DBTSH[d]		7.36–7.48 (m, 3H, H2, H7, H8), 7.75–7.83 (m, 2H, H3, H6), 8.04–8.14 (m, 2H, H1, H9), 2.14 (s, 1H, SH)	Not recorded

[a] Refs. [19,20].
[b] Ref. [21].
[c] Refs. [19,22].
[d] Ref. [23].

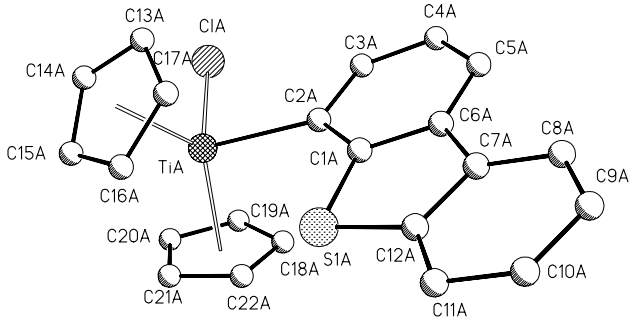


Fig. 2. Ball and stick representation of the molecular structure of **1B**.

2.2. Antitumor activities

Comparison of preliminary antitumor test results for the complexes will give some insight of the relative activities related to the composition of the complexes. For these tests, COLO 320DM and HeLa cell lines were exposed to various concentrations of the complexes for three days. Titanocene dichloride **(S-01)** was selected as a primary standard. The free ligands BTH, DBTH and DBTSH, as well as the complexes **1A**, **1B**, **2A**, **2B** and **3B** were selected for testing and the results are shown in Fig. 4. In the graphs the spectrophotometric

Table 2
Selected bond lengths and angles **1B**[a]

	Ti(A)	Ti(B)
Bond lengths (Å)		
Ti–Cl	2.364(1)	2.351(1)
Ti–C(2)	2.232(3)	2.209(3)
[b]Ti–C(Cp′)	2.377(3)	2.379(4)
[b] Ti–C(Cp″)	2.345(5)	2.362(4)
S(1)–C(12)	1.746(3)	1.753(3)
S1–C(1)	1.758(3)	1.768(3)
C(1)–C(6)	1.411(4)	1.416(4)
C(6)–C(7)	1.459(4)	1.441(5)
C(7)–C(12)	1.397(5)	1.409(4)
Bond angles (°)[c]		
Cl–Ti–C(2)	97.4(1)	98.7(1)
Cp′–Ti–Cl	105.3(2)	105.9(3)
Cp′–Ti–C(2)	105.5(2)	104.2(3)
Cp′–Ti–Cp″	132.6(3)	132.9(3)
Cp″–Ti–Cl	107.2(3)	106.3(3)
Cp″–Ti–C(2)	103.5(2)	104.4(3)
Ti–C(2)–C(1)	129.6(2)	130.2(2)
Ti–C(2)–C(3)	116.9(2)	115.8(2)
C(1)–C(2)–C(3)	113.6(3)	114.0(3)
S1–C(1)–C(6)	110.8(2)	110.4(3)
C(1)–S1–C(12)	92.3(2)	92.3(3)
S1–C(12)–C(7)	112.3(2)	111.6(3)
C(12)–C(7)–C(6)	111.9(3)	112.5(3)
C(7)–C(6)–C(1)	112.7(3)	113.3(3)

[a] Cp′ is described by the ring carbon atoms C(13)–C(17) and Cp″ by C(18)–C(22).
[b] Ti–C(Cp) distances are averaged for all the carbons of the ring.
[c] Angles incorporating Cp-ligands are described by connecting the centroid of the Cp′ and Cp″ rings to Ti.

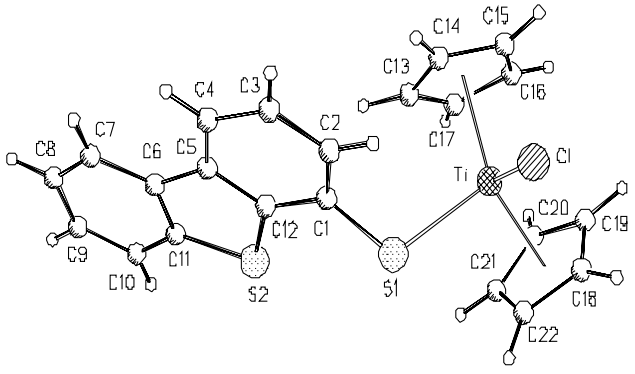


Fig. 3. Ball and stick representation of the molecular structure of **2B**.

reading obtained with the untreated control cells after the three-day incubation period was taken as 100% growth. The reading obtained on day 0 (before the incubation period) was between 40% and 50% relative to the untreated control after the three-day incubation period. A line to indicate data on day 0 is shown on the graphs (Fig. 4) and any increase above this line represent positive growth whereas a reading below this line indicates cell death. The free ligands, as well as the bis-substituted thiolato complexes **3** had a much smaller effect on cell

Table 3
Selected bond lengths and angles for **2B**

Bond lengths (Å)[a]			
Ti–Cl	2.3948(7)	C(5)–C(12)	1.408(3)
Ti–S(1)	2.4068(7)	C(5)–C(6)	1.460(3)
Ti–C(Cp′)	2.388(2)	C(6)–C(11)	1.411(3)
Ti–C(Cp″)	2.393(2)	S(2)–C(12)	1.757(2)
S(1)–C(1)	1.782(2)	S(2)–C(11)	1.762(2)
Bond angles (°)			
Cl–Ti–S(1)	96.13(3)	C(12)–S(2)–C(11)	91.06(12)
C(1)–S(1)–Ti	114.89(8)	C(12)–C(5)–C(6)	111.7(2)
C(2)–C(1)–C(12)	117.6(2)	C(11)–C(6)–C(5)	112.0(2)
C(2)–C(1)–S(1)	124.43(19)	C(6)–C(11)–S(2)	112.36(18)
C(12)–C(1)–S(1)	117.75(18)	C(5)–C(12)–S(2)	112.75(18)

[a] Cp′ is described by the ring carbon atoms C(13)–C(17) and Cp″ by C(18)–C(22). Ti–C(Cp) distances are averaged for all the carbons of the ring.

growth inhibition and to simplify the graphs their results are not shown.

It is clear that the complexes exhibited a concentration dependent suppression of growth of both cell lines tested. The introduction of a heteroaromatic condensed ring ligand in **1B** improved the activity compared to that of the standard titanocene dichloride (**S-01**) and it was further noted that a significant improvement of cell growth inhibition was observed for the thiolato complexes. At concentration levels of 6 mg/l of titanium the values for the inhibition of cell growth compared to the untreated control recorded were for titanocene dichloride 95% (HeLa) and 99% (COLO 320DM) inhibition compared to 70% and 93% for **1B** and 63% and 89% for **2B**, respectively. The corresponding values for HeLa for **2A** was 88% and for **3B** and DBTH above 95%. Furthermore, because **2B** showed a greater inhibition of cell growth compared to **2A** and **1B** it was concluded that three condensed rings (bulkier ligand) are more effective than two rings and that the sulfur donor ligand enhances activity compared to a carbon-bonded heteroarene ligand.

The tumor inhibiting properties of the complexes studied revealed that for both cell lines the activities of both the DBTH and corresponding thiolato complexes the activities were significantly higher compared to **S-01**. In fact, the complex **2B** was ca. 30 times more effective than **S-01** by comparing equivalent masses of these complexes required for 100% suppression of cell growth. It is possible that the test results reflect the rates of formation of a titanium cell growth inhibiting species under physiological conditions, which could be the same for all complexes tested. Hence, high rates of hydrolysis of complexes will correlate with high cell growth suppression. This is supported by the concentration dependency of the cell growth inhibition of the various compounds. Preliminary ^{1}H NMR studies showed that the hydrolysis rate of the cyclopentadienyl ligands was increased significantly compared to titanocene dichloride when **2B** was

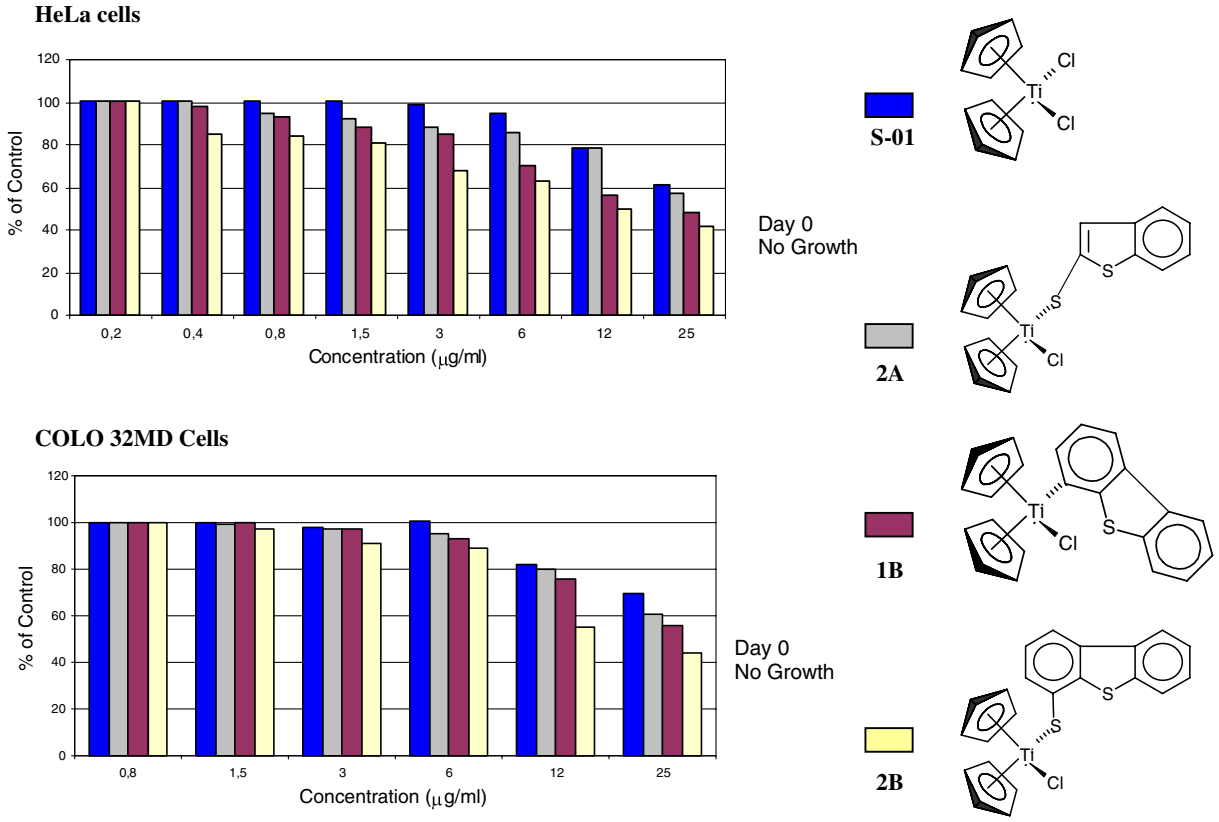


Fig. 4. Inhibition of HeLa and COLO 320DM cell growth by titanocene dichloride, **2A**, **1B** and **2B**.

dissolved in d^6-DMSO and diluted in a D_2O saline (1:3) solution [29]. Based on this data the order for Cp-hydrolysis is $[TiCp_2\{SR\}Cl] > [TiCp_2Cl_2] > [TiCp_2\{SR\}_2]$. Further studies to be undertaken will focus on ligand substitution kinetics and modification of complexes to improve their solubility in aqueous medium.

3. Experimental

3.1. General details

All NMR spectra were recorded in deuterated chloroform, unless stated otherwise, on a Bruker ARX-300 spectrometer. The ^1H and ^{13}C NMR spectra were recorded at 300.135 and 75.469 MHz, respectively. The resonance of the deuterated solvent was used as internal reference for determining the chemical shifts (ppm), i.e. $CDCl_3$ δ 7.24 (^1H) and 77.00 (^{13}C). Mass spectra were recorded at ca. 70 eV on a Finnigan Mat 8200 instrument using the electron impact method. Melting points were determined on a Kofler hot stage microscope and are uncorrected. Only melting points are noted of compounds, which did not decompose during heating.

All reactions were performed in an inert atmosphere of either nitrogen or argon by using standard Schlenk techniques and vacuum-line methods. Solvents were dried and distilled under nitrogen prior to use. Column chromatography was carried out under nitrogen using either silica gel (particle size 0.063–0.200 nm) or alumina (neutral) as stationary phase in jacketed columns at temperatures below 0 °C. Most chemicals were used directly without prior purification.

3.2. Synthesis of complexes

3.2.1. Synthesis of $[TiCp_2(2\text{-}BT)Cl]$ (*1A*)

To a solution of 0.402 g (3.0 mmol) of BTH in 20 ml of THF, which was cooled to −50 °C, was added 2.06 ml (3.30 mmol) of a 1.6 M solution of *n*-butyllithium in hexane. The resulting mixture was stirred at −10 °C for 30 min. The freshly prepared 2-benzothienyllithium was cooled to −60 °C and added to an equimolar solution of titanocene dichloride (0.747 g, 3.0 mmol) in THF (30 ml). After being stirred for 30 min, the reaction mixture was allowed to warm to room temperature and stirred for another hour. Removal of the solvent under reduced pressure gave a dark orange solid, which was subjected to column chromatography on silica gel. Elution with dichloromethane–petroleum ether mixture (1:1) gave a colorless fraction that was identified as unreacted BTH, followed by an orange zone containing $[TiCp_2(2\text{-BT})Cl]$ (**1A**). Yield: 0.49 g (48%); m.p.: 137 °C.

*3.2.2. Synthesis of [$TiCp_2$(2-DBT)Cl] (**1B**)*

A cooled (−20 °C) solution of 0.70 g (3.79 mmol) of DBTH in 30 ml THF was treated dropwise with 2.60 ml (4.18 mmol) of a 1.6 M solution of *n*-butyllithium in hexane. The temperature of the reaction mixture was allowed to rise to 0 °C and stirring was continued for an additional 5 h, during which time the solution changed color from orange to green. The freshly prepared lithiated DBTH solution was added to an equimolar solution of titanocene dichloride (0.95 g, 3.79 mmol) in THF (50 ml) at −60 °C. After stirring for 30 min, the reaction mixture was allowed to rise to room temperature and stirring was continued for 1 h. Removal of the solvent under reduced pressure gave an orange residue, which was subjected to column chromatography on silica gel. Elution with dichloromethane–hexane (2:1) mixture yielded a colorless fraction identified as unreacted DBTH, followed by an orange band containing [$TiCp_2$(2-DBT)Cl] (**1B**). Yield: 0.78 g (52%). After the solvent had been removed, the residue was dissolved in a minimum amount of diethyl ether, and an equal volume of hexane was carefully layered on top of the ether. The Schlenk tube was left undisturbed at 4 °C for several days, after which the mother liquor was decanted to give orange crystals which were suitable for X-ray diffraction studies; m.p.: 173 °C.

*3.2.3. Synthesis of [$TiCp_2$(2-SBT)Cl] (**2A**) and [$TiCp_2(SBT)_2$] (**3A**)*

A solution of 0.6 g (4.47 mmol) BTH in 20 ml THF at −50 °C was treated dropwise with 3.07 ml of a 1.6 mol/dm^3 solution of *n*-butyllithium (4.92 mmol) in hexane. The reaction mixture was stirred at −10 °C for 30 min, after which the solution was cooled to −78 °C and 0.143 g of dry flowers of sulfur was added. Stirring at this temperature was continued until all sulfur dissolved. This freshly prepared 2-benzothienylthiolate was added to an equimolar solution of [$TiCp_2Cl_2$] (1.11 g, 4.47 mmol) in THF, while stirring was continued at this temperature for 15 min. The temperature of the reaction mixture was allowed to rise by intervals of 10 °C every 10 min until room temperature was reached. Removal of the solvent under reduced pressure gave a dark red solid. The crude residue was subjected to purification by column chromatography on silica gel, with a dichloromethane–hexane mixture (1:1) as eluant. The first colorless band was identified as unreacted BTH, while the second purple zone afforded 1.11 g of [$TiCp_2(SBT)_2$] (**3A**) (49%). A third red band gave 0.64 g of [$TiCp_2$(SBT)Cl] (**2A**) (38%).

*3.2.4. Synthesis of [$TiCp_2$(SDBT)Cl] (**2B**) and [$TiCp_2(SDBT)_2$] (**3B**)*

To a solution of 0.90 g (4.88 mmol) DBTH in THF (30 ml) at −30 °C, a hexane solution of 1.6 mol/dm^3 *n*-butyllithium (3.36 ml, 5.37 mmol) was added dropwise. The resulting deep orange solution was left to warm up to 0 °C and stirred continuously for 5 h, during which time the solution changed color from orange to green. The resulting green solution was cooled to −10 °C and 0.156 g of flowers of sulfur (0.608 mmol) was added. Stirring was continued for 1 h at room temperature, during which time the green color of the solution faded and changed into a yellow color. The yellow reaction mixture was cooled to −78 °C and reacted with an equimolar solution of [$TiCp_2Cl_2$] (1.22 g, 4.88 mmol) in 50 ml THF. The resulting red solution was stirred for 30 min at low temperature, whereafter the reaction mixture was allowed to warm to room temperature. Removal of the solvent under reduced pressure gave a red residue, which was subjected to column chromatography on silica gel. Elution with dichloromethane–hexane (1:2) mixture yielded a colorless fraction of DBTH, followed by a purple band containing [$TiCp_2(SDBT)_2$] (**3B**) (1.22 g, 41%) and a red zone affording 0.61 g of [$TiCp_2$(1-SDBT)Cl] (**2B**) (29%). Complex **2B** was recrystallized from a dichloromethane–hexane solution by dissolving the complex in the minimum dichloromethane, while hexane was layered on top. The solution was kept at 4 °C for several weeks and this yielded crystals suitable for a single crystal structure determination.

3.3. Cytotoxic tests

A human cervix epitheliod cancer cell line, HeLa (ATCC CCL-2) and a human colorectal carcinoma cell line, COLO 320DM (ATCC CCL-220) were grown as monolayer cultures at 37 °C in 5% CO_2 using either MEM (in the case of HeLa) or RPMI 1640 (in the case of COLO 320DM) supplemented with 10% heat inactivated serum and 1% penicillin and streptomycin. The assay was performed using a metabolic assay based on the reactivity of MTT (3-[4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Diagnostics Inc). Cells were seeded at 2×10^3/well in 96 well microtiter plates in a final volume of 200 μl of the relevant medium in the presence or absence of different concentrations of the experimental drugs. Appropriate solvent control systems were included. After incubation for three days, MTT was added to each well and the plates processed as described previously [30]. Results are expressed as the percentage of the relevant untreated control systems (100% growth represent the spectrophotometric reading of this control). The reading obtained on day 0, relevant to the control reading obtained on day 3, was 43%. This percentage therefore represents no growth if obtained with other samples after treatment for three days.

Sample preparation: Complexes **1**, **2**, **3** and **S-01** were dissolved in DMSO to give stock concentration of 10 mg/cm^3 and diluted in the appropriate growth medium supplemented with FCS to give final DMSO concentra-

Table 4
Crystal data, collection and refinement details for **1B** and **2B**

	1B	**2B**
Empirical formula	$C_{22}H_{17}ClSTi$	$C_{22}H_{17}ClS_2Ti$
Formula weight	396.77	428.83
Temperature (K)	183(2)	183(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Unit cell dimensions		
a (Å)	14.6975(3)	11.1309(5)
b (Å)	18.0415(6)	7.8238(3)
c (Å)	14.4476(3)	21.946(1)
β (°)	110.502(2)	96.464(2)
Volume (Å^3)	3588.34(16)	1899.0(1)
Z	8	4
D_{calc} (Mg/m^3)	1.469	1.500
Absorption coefficient (mm^{-1})	0.743	0.814
F(0 0 0)	1632	880
Crystal size (mm^3)	$0.06 \times 0.05 \times 0.03$	$0.28 \times 0.22 \times 0.18$
Theta range for data collection (°)	1.88–27.48	3.19–27.48
Index ranges	$-19 \leqslant h \leqslant 19$, $-21 \leqslant k \leqslant 23$, $-18l \leqslant l \leqslant 18$	$-14 \leqslant h \leqslant 14$, $-9 \leqslant k \leqslant 10$, $-28 \leqslant l \leqslant 28$
Reflections collected	14,297	7637
Independent reflections	8189 [R_{int} = 0.0646]	4277 [R_{int} = 0.0424]
Completeness to theta = 27.48°	99.6%	98.2%
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	8189/0/451	4277/0/235
Goodness-of-fit on F^2	0.984	1.022
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0530, wR_2 = 0.1077	R_1 = 0.0424, wR_2 = 0.0877
R indices (all data)	R_1 = 0.1192, wR_2 = 0.1285	R_1 = 0.0705, wR_2 = 0.0967
Largest diff. peak and hole (e Å^{-3})	0.588 and −0.535	0.267 and −0.469

tions not exceeding 0.5% and drug concentration of 1–3000 μg/cm^3 prior to cell experiments.

3.4. Crystal structure determination

Data of **1B** and **2B** were collected at −90 °C on a Nonius-Kappa CCD diffractometer using graphite-monochromated, Mo Kα radiation. Data were corrected for Lorenz polarization effects but not for absorption [31,32]. The structures were solved by direct methods (SHELXS) [33] and refined by full-matrix least squares techniques against F^2 (SHELXL-97) [34]. The hydrogen atoms of the structures were included at calculated positions with fixed thermal parameters. All non-hydrogen atoms were refined anisotropically. XP (SIEMENS Analytical X-ray Instruments, Inc.) was used for structure representations. Complex **1B** crystallizes with two independent molecules in the unit cell. Crystal data and other experimental procedures and refinement parameters are given in Table 4. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with Cambridge Crystallographic Data Centre as supplementary material publication nos. CCDC-246553 (**1B**) and CCDC-246554 (**2B**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: (internal) +44 1223/336 033; e-mail: deposite@ccdc.cam.ac.uk].

Acknowledgments

This investigation was supported by a grant awarded by the Cancer Association of South Africa, as well as a grant from the Technology and Human Resources for Industry Programs of the National Research Foundation and the Department of Trade and Industry. Some of this material is based upon work supported by the National Research Foundation under Grant No. 2047221.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.jorganchem.2004.08.046.

References

[1] ISBOMC'02, First International Symposium on Bio-organometallic Chemistry, 18–20 July, Paris, France.

[2] (a) Special issue on bio-organometallic chemistry, J. Organomet. Chem. 668 (2003) 1;
(b) R.H. Fish, G. Jaouen, Organometallics 22 (2003) 2166.
[3] H. Köpf, P. Köpf-Maier, Angew. Chem. Int., Ed. Engl. 18 (1979) 477.
[4] For review articles see: (a) H. Köpf, P. Köpf-Maier, in: S.J. Lippard (Ed.), Platinum, Gold and Other Metal Chemotherapeutic Agents, ACS Symposium Series, 209 (1983) 315;
(b) P. Köpf-Maier, H. Köpf, Drug. Future 11 (1986) 297;
(c) H. Köpf, P. Köpf-Maier, Chem. Rev. 87 (1987) 1137;
(d) P. Köpf-Maier, H. Köpf, Struct. Bond. 70 (1988) 103;
(e) I. Haiduc, C. Silvestru, Organometallics in Cancer Chemotherapy, vol. II, CRC press, Inc., Boca Raton, FL, 1990, p. 13;
(f) P. Köpf-Maier, in: B.K. Keppler (Ed.), Metal Complexes in Cancer Chemotherapy, VCH Weinheim, 1993, p. 260;
(g) P. Köpf-Maier, H. Köpf, in: S.P. Fickler (Ed.), Metal Compounds in Cancer Therapy, Chapman & Hall, 1994, p. 109.
[5] (a) P.J. Sadler, Adv. Inorg. Chem. 36 (1991) 1;
(b) Z. Guo, P.J. Sadler, Adv. Inorg. Chem. 49 (1999) 183;
(c) P. Köpf-Maier, Eur. J. Clin. Pharmacol. 47 (1994) 1;
(d) C.V. Christodoulou, D.R. Ferry, D.W. Fyfe, A. Young, J. Doran, T.M.T. Sheehan, A.G. Eliopoulos, K. Hale, J. Baumgart, G. Sass, D.J. Kerr, J. Clin. Oncol. 16 (1998) 2761.
[6] M. Guo, H. Sun, H.J. McArdle, L. Gambling, P.J. Sadler, Biochemistry 39 (2000) 10023.
[7] M.M. Harding, G. Mokdsi, Curr. Med. Chem. 7 (2000) 1289.
[8] (a) J.H. Toney, T.J. Marks, J. Am. Chem. Soc. 107 (1985) 947;
(b) P. Yang, M.L. Guo, Coord. Chem. Rev. 186 (1999) 189.
[9] (a) O.R. Allen, L. Croll, A.L. Gott, R.J. Knox, P.C. McGowan, Organometallics 23 (2004) 288;
(b) M.A.D. McGowan, P.C. McGowan, Inorg. Chem. Commun. 3 (2000) 337.
[10] (a) G. Mokdsi, M.M. Harding, J. Organomet. Chem. 565 (1998) 29;
(b) J.H. Murray, M.M. Harding, J. Med. Chem. 37 (1994) 1936;
(c) M.M. Harding, G. Mokdsi, J.P. Mackay, M. Prodigalidad, S.W. Lucas, Inorg. Chem. 37 (1998) 2432.
[11] (a) H. Sun, H. Li, R.A. Weir, P.J. Sadler, Angew. Chem. Int. Ed. 37 (1998) 1577;
(b) M.L. Guo, H. Sun, P.J. Sadler, J. Inorg. Biochem. 74 (1999) 150.
[12] U. Pindur, M. Haber, K. Sattler, J. Chem. Educ. 70 (1993) 263.
[13] E. Campaige, J. Ashby, S.W. Osborn, J. Heterocycl. Chem. 6 (1969) 885.
[14] R. Pech, R. Böhm, Pharmazie 39 (1984) 4, and references therein.
[15] (a) L. Brandsma, H. Verkruijsse, in: Preparative Polar Organometallic Chemistry 1, Springer, Heidelberg, 1987, p. 168;
(b) H.E. Gschwend, H.R. Rodrigues, Org. React. 26 (1979) 1.
[16] C. Kuehm-Caubère, S. Adach-Becker, S. Fort, P. Caubère, Tetrahedron 52 (1996) 9087.
[17] (a) Yu.A. Ol'dekop, V.A. Knizhnikov, Russ. J. Gen. Chem. 52 (1982) 1387;
(b) V.A. Knizhnikov, S.A. Makhnach, M.G. Novikova, Yu.A. Ol'dekop, Organomet. Chem. USSR 3 (1990) 732;
(c) G. Erker, R. Petrenz, Organometallics 11 (1992) 1647.
[18] A.N. Nesmeyanov, E.I. Fedin, O.V. Nogina, N.S. Kochetkova, V.A. Dibovitsky, P.V. Petrovsky, Tetrahedron Suppl. 8 (1966) 389.
[19] K.D. Bartle, D.W. Jones, R.S. Matthews, Tetrahedron 27 (1971) 5177.
[20] P.D. Clark, D.F. Ewing, R.M. Scrowston, Org. Magn. Reson. 8 (1976) 252.
[21] (a) R.D. Schuetz, W.L. Fredericks, J. Org. Chem. 27 (1962) 1301;
(b) P.C. Montevecchi, M.L. Navacchia, J. Org. Chem. 60 (1995) 6455.
[22] J. Giraud, C. Marzin, Org. Magn. Reson. 12 (1979) 647.
[23] L.V. Dunkerton, B.C. Barot, A. Nigam, J. Heterocycl. Chem. 24 (1987) 749.
[24] C.A. Mike, T. Nelson, J. Graham, A.W. Cordes, N.T. Allison, Organometallics 7 (1988) 2573.
[25] P.R. Stafford, T.B. Rauchfuss, A.K. Verma, S.R. Wilson, J. Organomet. Chem. 526 (1996) 203.
[26] A. Clearfield, D.K. Warner, C.H. Salderriaga-Molina, R. Ropal, J. Bernal, Can. J. Chem. 53 (1975) 1622.
[27] V. Kochman, J.C. Rucklidge, R.J. O'Brien, W. Santo, J. Chem. Soc., Chem. Commun. (1976) 1340.
[28] R.M. Schaffrin, J. Trotter, J. Chem. Soc. A (1970) 1561.
[29] S. Brink, S. Lotz, Unpublished results.
[30] C.E.J. Van Rensburg, A.J. Theron, M. Chasen, Oncol Rep. 3 (1996) 103.
[31] COLLECT, Data Collection Software, Nonius B.V., Netherlands, 1998.
[32] Z. Otwinowski, W. Minor, Processing of X-Ray Diffraction Data Collected in Oscillation Mode, in: C.W. Carter, R.M. Sweet (Eds.), Methods in Enzymology, vol. 276, Macromolecular Crystallography, Part A, Academic Press, 1997, p. 307.
[33] G.M. Sheldrick, Acta Crystallogr., Sect. A 46 (1990) 467.
[34] G.M. Sheldrick, SHELXL-97 (Release 97-2), University of Göttingen, Germany, 1997.