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Titanocene Difluorides with Improved Cytotoxic Activity

Silvia Eger,[†] Timo A. Immel,[†] James Claffey,[‡] Helge Müller-Bunz,[‡] Matthias Tacke,[‡] Ulrich Groth,[†] and Thomas Huhn*,[†]

[‡]*Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland and* [†]*Fachbereich Chemie and Konstanz Research School Chemical Biology, Universität Konstanz, Universitätsstrasse 10, Fach 720, 78457 Konstanz, Germany*

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Titanocene difluorides can be obtained by halide metathesis of the respective titanocene dichlorides with trimethyltin fluoride (Me_3SnF), giving access to a new class of cytotoxic active substances. Furthermore, an improved method for the synthesis of diaryl-substituted titanocene dichlorides is presented.

Titanocene dichloride (TDC; Cp_2TiCl_2) attracted great interest because it was the first non-platinum complex to show promising results as an antitumor agent. It reached clinical trials, but the efficacy of Cp_2TiCl_2 in phase II clinical trials in patients with metastatic renal cell carcinoma[1] or metastatic breast cancer[2] was too low to be pursued.

More recently, a large number of differently substituted titanocene derivatives have been synthesized and tested for their potential cytotoxicity.[3] By substitution of the cyclopentadienyl (cp) rings, the cytotoxicity in LLC-PK cells could be increased by a factor of 1000. The *p*-methoxybenzyl-substituted titanocene Y (**1b**) shows an IC_{50} value of 21 μM,[4] and the dimethylamino-functionalized and heteroaryl-substituted titanocene C shows an IC_{50} value of 5.5 μM,[5] compared to 2 mM for Cp_2TiCl_2.

Furthermore, the cytotoxic activity can be influenced by substitution of the two chloride ligands. The nature of these two "not-cp-ligands" affects the hydrolytic stability and thereby the bioavailability of the active substance. Ligands that show a higher hydrolytic stability than chloride but can still be hydrolyzed under physiological conditions seem to be ideal. Recently, Claffey et al. reported an IC_{50} value of 1.6 μM for oxalititanocene Y (titanocene Y with both chloride ligands substituted by the bidentate oxalate).[6] We were interested in the synthesis and cytotoxicity of fluorotitanocene derivatives because of their anticipated higher stability against solvolysis; the Ti−F bond is known to be more stable by 75 kcal/mol than the Ti−Cl bond.[7]

Within this paper, we introduce several benzyl- and diaryl-substituted titanocene difluorides that can be obtained by fluorinating the respective TDCs with trimethyltin fluoride. Preliminary cytotoxicity studies show that the fluorine analogue of titanocene Y is 4−7 times more cytotoxic than titanocene Y itself. Furthermore, we present an improved method for the synthesis of diaryl-substituted TDCs.

For comparative cytotoxicity studies between TDCs and titanocene difluorides, two different classes of titanocene derivatives have been synthesized.

Three different benzyl-substituted TDCs [*m*- (**3a**), *p*- (**3b**), and 3,5-dimethoxybenzyl (**3c**)] were synthesized according to Sweeney et al.[4] Selected fulvenes **1** are hydridolithiated by $LiB(Et)_3H$ (SuperHydride) to give the corresponding lithium cyclopentadienides **2**, which were, in turn, transmetalated with $TiCl_4$ to yield bis(benzyl)titanocene dichlorides **3** (Scheme 1).

The second class of titanocene derivatives, diaryl-substituted titanocenes **7**, was primarily synthesized according to a one-pot procedure, consisting of halogen−lithium exchange, fulvene addition, and transmetalation by $TiCl_4$ published by Pampillion et al.[8]

Neither the halogen metal exchange nor the carbolithiation of the respective fulvene **1** might proceed to completeness; i.e., the amount of lithium cyclopentadienide **6** is difficult to estimate. However, an excess of titanium tetrachloride leads to cyclopentadienyltitanium(IV) trichlorides, while an excess of ligand facilitates the reduction of titanium(IV) to titanium(III) species. These byproducts hamper the isolation of the target molecule because the respective TDCs **7** are difficult to purify. Because the solubility of lithium cyclopentadienides **6** in diethyl ether is rather limited, the halogen−lithium exchange was set up with 2 equiv of *tert*-BuLi in Et_2O instead of

*To whom correspondence should be addressed. E-mail: thomas.huhn@uni-konstanz.de. Fax: +49-7531-884424.

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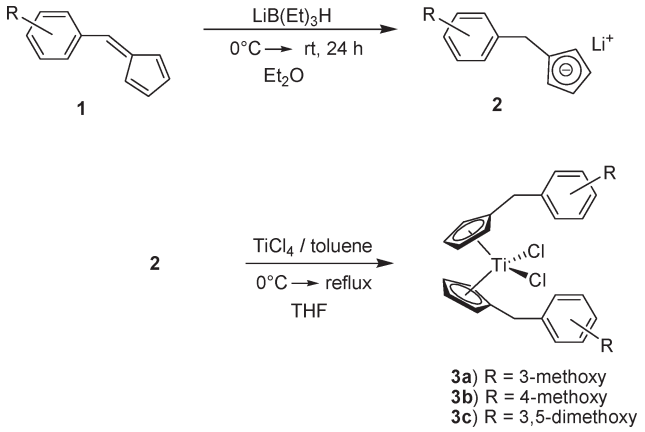
Scheme 1. Synthesis of Bis[(*m*-methoxybenzyl)cyclopentadienyl]-, Bis[(*p*-methoxybenzyl)cyclopentadienyl]-, and Bis[(3,5-dimethoxybenzyl)-cyclopentadienyl]titanium(IV) Dichlorides **3a**−**3c**



tetrahydrofuran (THF). After the addition of fulvene **1**, the precipitating lithium cyclopentadienide **6** was isolated by gravity filtration over a sintered-glass funnel. After quantification, the subsequent transmetalation was set up in a 2:1 stoichiometry with $[TiCl_4(THF)_2]$ in THF under reflux.

A vigorously stirred suspension of **5** in Et_2O reacts exhaustively with added fulvene **1** at room temperature, leading to precipitation of the colorless lithium cyclopentadienide **6** in good yield. With this improved method, the synthesis of *m*- and *p*-methoxydiaryl-substituted TDCs **7a** and **7b** proceeded in good yield and excellent purity (Scheme 2 and Table 1).

Attempts to synthesize the 3,5-dimethoxydiaryl derivative were met with failure. Apparently, the product from the halogen metal exchange in the case of 3,5-dimethoxybromobenzene rearranges to the highly stabilized 2-lithio-1,3-dimethoxybenzene.

Subsequently, the TDCs were fluorinated with trimethyltin fluoride utilizing a method reported first by Herzog et al.[9] Trimethyltin fluoride is a nonodorous, colorless, crystalline substance, and in contrast to other trimethyltin halogenides, it is insoluble in most organic solvents because of its polymeric structure.[10] It is known to be a powerful yet selective fluorinating agent especially suitable for group IV metallocene halogenides already effective at room temperature.[11] It reacts with $Cp^x{}_2MCl_2$ (M = Ti, Zr, Hf; Cp^x = differently substituted cp ligands) in toluene to $Cp^x{}_2MF_2$ in generally good yields. The byproduct, the highly toxic trimethyltin chloride, can be quantitatively recovered from the reaction mixture by vacuum sublimation. Reaction with an excess aqueous potassium fluoride solution in alcohol regenerates the fluorinating compound; Me_3SnF precipitates quantitatively from the solution as colorless crystals (Scheme 3).

Fluorination of benzyl-substituted TDCs **3** and **8** proved to be suitable with a 10% molar excess of Me_3SnF in a toluene suspension. Because the fluorinating agent is insoluble in toluene, the excess was simply filtered off. The completeness of the fluorination was traced by ^1H and ^{19}F NMR spectroscopy. Yields are essentially quantitative, while

Scheme 2. Synthesis of Bis[bis(*m*-methoxyphenyl)methylcyclopentadienyl] and Bis[bis(*p*-methoxyphenyl)methylcyclopentadienyl]titanium-(IV) Dichlorides **7a** and **7b**

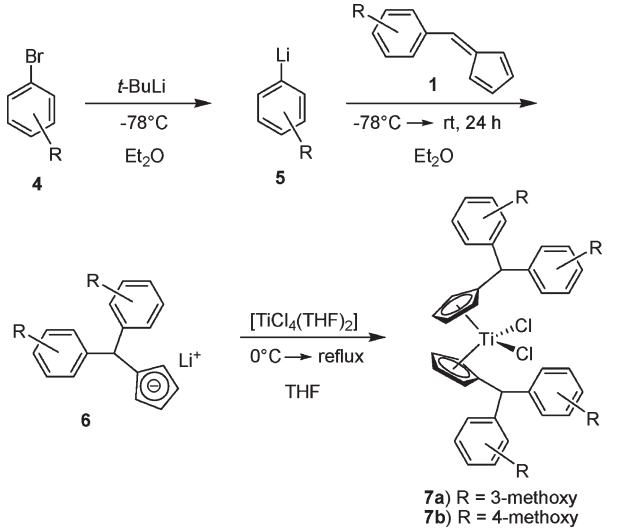
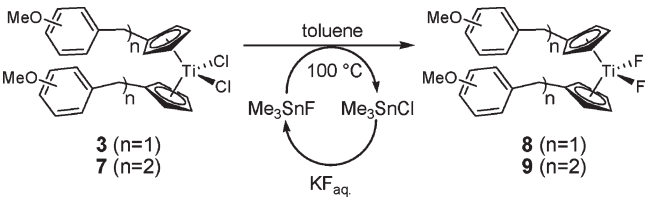


Table 1. Yields of Isolated Chloro- and Fluorotitanocenes

		3[a] (X = Cl)	**8** (X = F)			**7**[a] (X = Cl)	**9** (X = F)
a	*m*-MeO	53	82	**a**	*m*-MeO	77	66
b	*p*-MeO	74	83	**b**	*p*-MeO	76	79
c	3,5-MeO	58	60				

[a] Yields are given with respect to the isolated lithium cyclopentadienide.

Scheme 3. Use of Trimethyltin Fluoride for the Fluorination of Benzyl- and Diaryl-Substituted TDCs (**3** and **7**) and Regeneration of the Fluorinating Agent by Subsequent Recycling with an Aqueous KF Solution



the diminished yields (Table 1) result from repeated recrystallizations to remove even trace amounts of impurities that otherwise might obscure the biological assays. Following this procedure, we were able to synthesize five pairs of TDCs (**3a**, **3b**, **3c**, **7a**, and **7b**) and titanocene difluorides (**8a**, **8b**, **8c**, **9a**, and **9b**) for comparative cytotoxicity studies.

X-ray crystallographic studies established the molecular structures of **8a** and **8b** (Figures 1 and 2).[12] Neither the different substitution of the benzyl rings nor the exchange of the two chloride ligands with fluoride leads to a great difference in the molecular structures. The distance between

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(12) Crystal data for **8a**: $C_{26}H_{26}O_2F_2Ti$, M_r = 456.37 g/mol, monoclinic, space group *C*2, a = 20.841(2) Å, b = 5.6814(6) Å, c = 8.9748(9) Å, β = 91.267(2)°, V = 1062.43(19) Å^3, Z = 2, T = 100(2) K, D_{calc} = 1.427 g/cm, R_{int} = 0.0167, 5682 reflections collected, R1 (wR2) = 0.0343 (0.0857), and S = 1.044 for 2732 reflections with $I > 2\sigma(I)$. Crystal data for **8b**: $C_{26}H_{26}O_2F_2Ti$, M_r = 456.37 g/mol, monoclinic, space group *C*2/*c*, a = 26.642(5) Å, b = 5.7514(10) Å, c = 14.681(3) Å, β = 109.766(3)°, V = 2117.0(6) Å^3, Z = 4, T = 100(2) K, D_{calc} = 1.432 g/cm, R_{int} = 0.0346, 11 116 reflections collected, R1 (wR2) = 0.0954 (0.2586), and S = 1.198 for 3069 reflections with $I > 2\sigma(I)$.

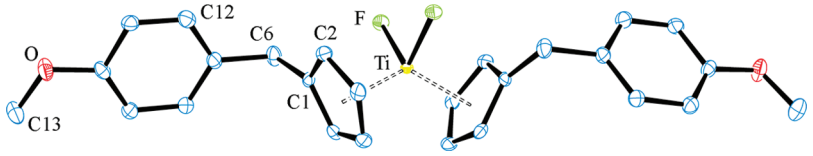

Figure 1. Molecular structure of **8b** in the crystal (thermal ellipsoids are drawn on the 50% probability level).[14] Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [deg]: Ti−F 1.866(3), Ti−centroid 2.060(1); F−Ti−F 96.61(19), centroid−Ti−F2 104.92(9), centroid−Ti−F1 105.71(9), centroid−Ti−centroid 133.20(1).

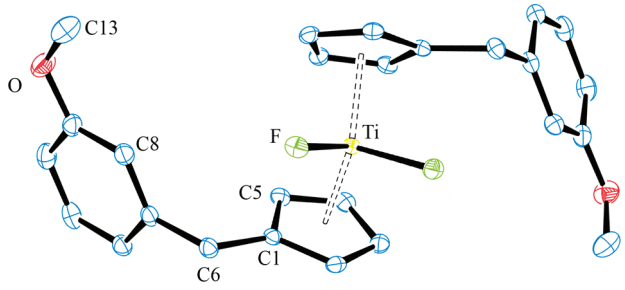

Figure 2. Molecular structure of **8a** in the crystal (thermal ellipsoids are drawn on the 50% probability level).[14] Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [deg]: Ti−F 1.8664(10), Ti−centroid 2.068(1); F−Ti−F 96.97(7), centroid−Ti−F2 105.68(3), centroid−Ti−F1 104.57(3), centroid−Ti−centroid 133.64(1).

the titanium center and the center of the cp rings is 2.068 Å for **8a** and 2.060 Å for **8b**, while the analogue bond measures 2.058 Å in **3a**[13] and 2.060 Å in **3b**. As expected, the average Ti−F bond in **8a** and **8b** is considerably shorter (1.866 Å) than the average Ti−Cl bond in **3a** and **3b** (2.364 and 2.370 Å). The F−Ti−F angle is slightly wider (96.97° for **8a** and 96.61° for **8b**) than the corresponding Cl−Ti−Cl angle in **3a** (94.13°) and **3b** (95.90°). The same tendency can be observed for the centroid−Ti−centroid angle, which is 133.64° for **8a** and 133.20° for **8b**, while the corresponding angle in the chloro analogues is 130.85° (**3a**) and 130.70° (**3b**).

All compounds were tested for their cytotoxicity on two human cancer cell lines (HeLa S3 and Hep G2). IC_{50} values were determined by Alamar Blue-based cytotoxicity assays.

The exchange of chloride with fluoride leads in most cases to enhanced IC_{50} values. In general, the effect is more pronounced for the sterically less demanding *p*-methoxybenzyl-substituted titanocenes. Hence, difluorotitanocene Y **8b** with an IC_{50} value of 13 μM showed the highest cytotoxic activity, closely followed by difluoride **9b** with an IC_{50} value of 16 μM. Both para-substituted fluorotitanocenes exhibit more than a 4-fold increased cytotoxicity compared to their chloro congeners (Table 2).

However, meta-substituted fluorotitanocene **8a** showed less improved activity (72 μM). The crystal structures of **8a** and **8b** showed no influence of the methoxy substitution pattern on the bonding length or angle. Therefore, the observed differences in cytotoxicity are not based on the electronic properties of the complexes. Fluoride **9a** even has the same cytotoxicity (35 μM) as its chloro counterpart **7a**. Here, as in the case of the 3,5-dimethoxybenzyltitanocene **3c**/**8c**, sterical reasons seemed to limit the cytotoxicity.

Table 2. Cytotoxicity Data of TDCs versus Titanocene Difluorides Estimated in Two Different Cell Lines by an Alamar Blue Assay

		IC_{50} Hela S3 [μM]		IC_{50} Hep G2 [μM]	
	compd	Cl	F	Cl	F
1	**3a**/**8a**	194 ± 36	72 ± 10	353 ± 63	220 ± 68
2	**3b**/**8b**	59 ± 15	13 ± 1	211 ± 110	30 ± 1
3	**3c**/**8c**	120 ± 4	145 ± 34	156 ± 39	139 ± 51
4	**7a**/ **9a**	35 ± 11	34 ± 2	42 ± 12	56 ± 19
5	**7b**/**9b**	84 ± 25	16 ± 1	108 ± 30	39 ± 11
6	TD-X	1340 ± 501	191 ± 151	1220 ± 744	432 ± 281
7	cisplatin[a]	7 ± 1		6 ± 1	

[a] Cisplatin was tested on all plates as an internal standard.

Recently, it has been shown that the para-substitution pattern of titanocene Y is a prerequisite for coordination onto the backbone of DNA.[15] The strongly improved cytotoxicity of the 4-methoxyaryltitanocene difluorides (entries 2 and 5), therefore, seemed to be based on a favorable molecular geometry as well as on enhanced hydrolytic stability.[16]

It should be noted that fluoride ions themselves are not cytotoxic at concentrations below 10^{-3} M.[17]

In conclusion, the halide metathesis of functionalized TDCs with Me_3SnF proceeds smoothly and yields titanocene difluorides in excellent yield. By suitable substitution of the cp rings, selected difluorides showed a cytotoxicity 3−5-fold higher than that of the respective dichlorides. Under the same conditions, the "gold standard" cisplatin showed a cytotoxicity that was only 2-fold greater than our best hit [IC_{50}(cisplatin in HeLa S3) = 7 (± 1) μM]. Studies are now underway in our laboratories to further investigate and understand the influence of the substitution pattern and hydrolytic stability on cytotoxicity.

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Supporting Information Available: Experimental details, a table of bond lengths and bond angles, synthetic schemes of all new compounds, NMR listings, and X-ray crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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