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Dimethyl titanocene Y: A valuable precursor for libraries of cytotoxic titanocene derivatives

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ABSTRACT

Reaction of the known titanocene Y **2** with methyl lithium at −15 °C yields bis-[(p-methoxybenzyl) cyclopentadienyl]titanium(IV) dimethyl (dimethyl titanocene Y, **3**), a hitherto unknown, surprisingly robust titanium (IV) dimethyl species. Dimethyl titanocene Y was utilized in the preparation of several bis-[(p-methoxybenzyl)cyclopentadienyl]titanium(IV) dicarboxylates by the reaction with the free carboxylic acids in fair to good yields. Cytotoxicity of all new compounds has been estimated in Hela S3 cells.

1. Introduction

The development of platinum complexes such as cisplatin, carboplatin or oxaliplatin had an enormous impact on current cancer chemotherapy. Unfortunately, the spectrum of cancers treatable with those platinum agents is narrow and treatment is associated with severe dose-limiting side effects and resistance phenomena [1–3]. These unsolved problems in platinum-based anti-cancer therapy led to an increased search for novel non-platinum-containing complexes as cytostatic agents [4–10].

Titanium complexes based on the octahedral species budotitane [11–13] or on titanocene dichloride [14–18] showed encouraging antitumor activity in various cell lines and little cross resistance to cisplatin was observed. Titanocene dichloride itself was found to enrich in areas near the nuclear chromatin, inhibit DNA synthesis and induce apoptosis [19–22]. Binding studies suggested that the cellular uptake of titanocene dichloride may be mediated either by the iron transport protein transferrin or by human serum albumin [23–28].

The main disadvantages of these complexes, probably leading to the low activities observed in clinical phase II trials, are their poor solubility in aqueous media and their hydrolytic instability under physiological conditions, ultimately leading to unidentified metabolites [29,30].

Dimethylamino or methoxy derivatization of the cylopentadienyl (Cp) rings proofed to be a valuable concept to improve solubility. One of the most active titanium complexes, bis-[(p-methoxybenzyl) cyclopentadienyl]titanium(IV) dichloride (titanocene Y), was thereby found by the Tacke group in 2005 [16]. Concerning hydrolysis, it is believed that the chloride derivative hydrolyses too quickly and is therefore not an optimal candidate for therapeutic use [29]. In order to optimize its biological potential, Claffey et al. derivatized titanocene Y through anion-exchange using silver oxalate [31]. The obtained oxalititanocene Y was found to be 13-fold more cytotoxic against the porcine kidney cell line LLC-PK than titanocene Y itself and twice as active as cisplatin thus making it the most toxic titanocene derivative. Despite this success, the use of silver salts suffers from several disadvantages. The often colloidal silver chloride is hard to filter off and potential contaminations with silver species may distort the outcome of the biological assays. Moreover, for the synthesis of large libraries to systematically study the influence of the non-Cp ligands, silver salts for every compound have to be synthesized.

2. Experimental

2.1. General

All experiments requiring dry atmosphere were carried out under an atmosphere of dry nitrogen using either standard Schlenk technique or utilizing a MBraun Labmaster MB150 Glovebox. Superhydride (lithium triethylborohydride, 1 M in tetrahydrofuran [THF]) was purchased from Aldrich Chemical Company, methyllithium (2.3 M in diethyl ether) from Chemetall. All chemicals and solvents were dried and purified when necessary according to standard procedures [32]. NMR spectra were measured on a Bruker Avance DRX 600 spectrometer. Chemical shifts (δ) are referenced on the residual proton signals of the solvent and are given in parts per million

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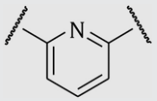
Table 1
Employed dicarboxylic acids for the derivatization of dimethyl titanocene Y and yields of the resulting dicarboxylates.

HOOC–X–COOH	–	CH_2			
	8	**9**	**10**	**11**	**12**
Product:	**13**	**14**	**15**	**16**	**17**
Yield	72%	35%	41%	45%	42%

downfield from tetramethylsilan (TMS). Structure assignments were done based on 2D-NMR (correlation spectroscopy [COSY], heteronuclear multiple bond coherence [HMBC], heteronuclear single quantum coherence [HSQC]) experiments. Signal multiplicities are assigned as multiplet (m), singlet (s), dublett (d), triplet (t) or triplet of triplets (tt). Melting points were determined with a Krüss KSP1N melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer Spectrum 100 FT ATR (attenuated total reflection) IR spectrophotometer. Elemental analyses were performed in the microanalytical laboratory of the University of Konstanz.

2.2. Chemistry

2.2.1. Bis-[(p-methoxybenzyl)cyclopentadienyl]titanium(IV) dichloride (2)

A solution of 6(4-methoxyphenyl)fulvene (**1**) (15.0 g, 81.4 mmol) in anhydrous THF (100 ml) was added to a solution of lithium triethylborohydride (77.4 ml, 77.4 mmol) in THF over 1 h and the resulting yellow solution was stirred at room temperature (RT) till turning colorless (~12 h). The solution was cooled to 0 °C and a solution of $TiCl_4$ in toluene (36.4 ml, 36.4 mmol) was added. The deep red solution was stirred for 36 h and the precipitating red solid was filtered off and washed with cold toluene (100 ml). The remaining red solid was taken up in a sufficient amount of CH_2Cl_2 and filtered again. The solvent was evaporated to give **2** as red micro crystals (12.0 g, 24.5 mmol, 63%). The characterization of compound **2** is in agreement with the parameters reported elsewhere [16].

2.2.2. Bis-[(p-methoxybenzyl)cyclopentadienyl]titanium(IV) dimethyl (3)

A solution of MeLi in Et_2O (22.4 ml, 2.3 M, 51.5 mmol, 1.05 eq.) was added to a suspension of **2** (12.0 g, 24.5 mmol) in Et_2O (250 ml) over 30 min at −15 °C. The mixture was allowed to warm to RT and stirred for 12 h. CH_2Cl_2 (150 ml) was added and the organic phase washed three times with cold water (100 ml) and finally dried over $MgSO_4$. After evaporation of the solvent and recrystallization from pentane, **3** was obtained as yellow crystalline solid (9.7 g, 21.6 mmol 88%) of m.p. 107.5–108.5 °C; ^{1}H-NMR (600.1 MHz, $CDCl_3$, s: singlet, d: doublet, t: triplet, tt: triplet of triplets): δ = 7.19 (d, J = 8.6 Hz, 4H, H_{ar}), 6.87 (d, J = 8.6 Hz, 4H, H_{ar}), 5.96 (t, J = 2.6 Hz, 4H, H_{Cp}), 5.69 (t, J = 2.6 Hz, 4H, H_{Cp}), 3.80 (s, 6H, OCH_3), 3.72 (s, 4H, CH_2), −0.14 (s, 6H, $TiCH_3$) ppm; ^{13}C-NMR (150.9 MHz, $CDCl_3$): δ = 158.0 ($CH_3O\underline{C}_{ar}$) 133.3 ($CH_2\underline{C}_{ar}$), 129.6 (HC_{ar}), 128.3 ($CH_2\underline{C}_{Cp}$), 114.5 (HC_{Cp}), 113.9 (HC_{ar}), 111.0 (HC_{Cp}), 55.3 (OCH_3), 44.5 ($TiCH_3$), 35.7 (CH_2) ppm; Elemental analysis calcd (%) for $C_{28}H_{32}O_2Ti$: C, 75.00; H, 7.19; found: C, 74.56; H, 7.29; IR (neat) ν = 3000, 1650, 1570, 1250, 820 cm^{-1}.

2.3. Ligand exchange

2.3.1. Bis-[(p-methoxybenzyl)cyclopentadienyl]titanium(IV) oxalate (13)

To oxalic acid (**8**) (213 mg, 2.37 mmol) suspended in anhydrous THF (40 ml) was added a solution of **3** (1.06 g, 2.37 mmol) in anhydrous THF (20 ml). The mixture was stirred for 12 h at RT and half of the solvent was evaporated. The evolving solid was filtered off and washed with cold THF (40 ml). Recrystallization from toluene/THF gave the oxalate **13** in 72% yield (1.70 mol, 860 mg) and m.p. 194.5–195.5 °C.

^{1}H-NMR (600.1 MHz, $CDCl_3$) δ = 7.03 (d, J = 8.7 Hz, 4H, C_{ar}), 6.82 (d, J = 8.7 Hz, 4H, C_{ar}), 6.54 (t, J = 2.6 Hz, 4H, C_{Cp}), 6.19 (t, J = 2.6 Hz, 4H, C_{Cp}), 3.77 (s, 6H, OCH_3), 3.68 (s, 4H, CH_2) ppm; ^{13}C-NMR (150.9 MHz, $CDCl_3$) δ = 162.9 (OC=OTi), 158.6 ($CH_3O\underline{C}_{ar}$), 143.6 ($CH_2\underline{C}_{Cp}$), 130.1 (HC_{Ar}), 129.5 ($CH_2\underline{C}_{ar}$), 121.6 (HC_{Cp}), 119.8 (HC_{Cp}), 114.1 (HC_{ar}), 55.3 (OCH_3), 35.1 (CH_2) ppm; Elemental analysis calcd (%) for $C_{28}H_{26}O_6Ti$: C, 66.41; H, 5.18; found: C, 66.13; H, 5.06; IR (neat) ν = 3155, 1720, 1300, 790 cm^{-1}.

2.3.2. Bis-[(p-methoxybenzyl)cyclopentadienyl]titanium(IV) malonate (14)

To malonic acid (**9**) (347 mg, 3.34 mmol) in anhydrous THF (50 ml) was added a solution of **3** (1.5 g, 3.34 mmol) in anhydrous THF (20 ml). The mixture was stirred for 12 h at RT and the solvent was evaporated. The remaining solid was recrystallized from CH_2Cl_2/toluene to give the malonate **14** as red powder (615 mg, 1.2 mmol, 35%) of m.p.: 69 °C. Although the analysis data for "C" are somewhat unsatisfactory, the spectroscopic analysis data are reasonably support the formula.

^{1}H-NMR (600.1 MHz, $CDCl_3$): δ = 7.07 (d, J = 8.4 Hz, 4H, C_{ar}), 6.83 (d, J = 8.4 Hz, 4H, C_{ar}), 6.43 (d, J = 4.6 Hz, 4H, C_{Cp}), 6.19 (s, 4H, C_{Cp}), 3.78 (s, 6H, OCH_3), 3.37 (s, 4H, $C_{ar}\underline{CH}_2$), 3.28 (s, C=OCH_2C=O) ppm; ^{13}C-NMR (150.9 MHz, $CDCl_3$): δ = 171.9 (OC=OTi), 158.5 ($CH_3O\underline{C}_{ar}$), 143.1 ($CH_2\underline{C}_{Cp}$), 130.1 ($CH_2\underline{C}_{ar}$), 129.9 (HC_{Ar}), 119.2 (HC_{Cp}), 115.0 (HC_{ar}), 55.2 (OCH_3), 37.3 ($\underline{CH}_2C_{ar}$), 34.9 (O(O=C)–$\underline{CH}_2$–(C=O)O) ppm; Elemental analysis calcd (%) for $C_{29}H_{28}O_6Ti$: C, 66.93; H, 5.42; found: C, 66.03; H, 5.18; IR (neat) ν = 2990, 1640, 1520, 1240, 815 cm^{-1}.

2.3.3. Bis-[(p-methoxybenzyl)cyclopentadienyl]titanium(IV) cyclopropane-1,1-dicarboxylate (15)

To cyclopropane-1,1-dicarboxylic acid (**10**) (247 mg, 1.9 mmol) was added a solution of **3** (848 mg, 1.9 mmol) in anhydrous THF (25 ml). The mixture was stirred for 4 h at RT and the solvent was evaporated at ambient temperature. The remaining solid was recrystallized from toluene/Et_2O to yield the cyclopropane-1,1-dicarboxylate **15** as red crystals (425 mg, 0.78 mmol, 41%) of m.p. 165.0–168 °C; ^{1}H-NMR (600.1 MHz, $CDCl_3$) δ = 7.07 (d, J = 8.6 Hz, 4H, C_{ar}), 6.82 (d, J = 8.6 Hz, 4H, C_{ar}), 6.47 (t, J = 2.6 Hz, 4H, C_{Cp}), 6.18 (t, J = 2.6 Hz, 4H, C_{Cp}), 3.78 (s, 6H, OCH_3), 3.72 (s, 4H, $C_{ar}\underline{CH}_2$), 1.76 (s, 4H, CH_2CH_2) ppm; ^{13}C-NMR (150.9 MHz, $CDCl_3$) δ = 174.7 (OC=OTi), 158.4 ($CH_3O\underline{C}_{ar}$), 142.4 ($CH_2\underline{C}_{Cp}$), 130.3 ($CH_2\underline{C}_{ar}$), 130.1 (HC_{Ar}), 119.4 (HC_{Cp}), 118.3 (HC_{Cp}), 114.0 (HC_{ar}), 55.3 (OCH_3), 34.9 ($\underline{CH}_2C_{ar}$), 23.8 (C_{quart}), 23.6 (CH_2CH_2) ppm; Elemental analysis calcd (%) for $C_{31}H_{30}O_6Ti$: C, 68.14; H, 5.53; found: C, 68.20; H, 5.36; IR (neat) ν = 3090, 1632, 1508, 1348, 1228, 831 cm^{-1}.

2.3.4. Bis-[(p-methoxybenzyl)cyclopentadienyl]titanium(IV) cyclobutane-1,1-dicarboxylate (16)

To cyclobutane-1,1-dicarboxylic acid (**11**) (107 mg, 0.74 mmol) suspended in anhydrous THF (5 ml) was added a solution of **3** (333 mg, 0.74 mmol) in anhydrous THF (5 ml). The mixture was stirred for 12 h at RT and the solvent evaporated. The remaining orange solid was recrystallized from CH_2Cl_2/pentane to give cyclobutane-1,1-dicarboxylate **16** (187 mg, 0.33 mmol, 45%) as yellowish solid of m.p. 85.5–86.5 °C; ^{1}H-NMR (600.1 MHz, $CDCl_3$): δ = 7.06 (d, J = 8.6 Hz, 4H, C_{ar}), 6.82 (d, J = 8.6 Hz, 4H, C_{ar}), 6.38 (t, J = 2.6 Hz, 4H, C_{Cp}), 6.12 (t, J = 2.6 Hz, 4H, C_{Cp}), 3.77 (s, 6H, OCH_3), 3.66 (s, 4H, $C_{ar}\underline{CH}_2$), 2.69 (t, J = 8.0 Hz, 4H, $\underline{CH}_2CH_2\underline{CH}_2$), 2.23 (tt, J = 8.0 Hz, 2H, $CH_2\underline{CH}_2CH_2$) ppm; ^{13}C-NMR (150.9 MHz, $CDCl_3$): δ = 178.7 (OC=OTi), 159.4 ($CH_3O\underline{C}_{ar}$), 143.3 ($CH_2\underline{C}_{Cp}$), 131.1 ($CH_2\underline{C}_{ar}$), 130.9 (HC_{Ar}), 119.8 (HC_{Cp}), 115.0 (HC_{ar}), 56.3 (OCH_3), 47.1 ($\underline{CH}_2C_{ar}$), 35.9