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New metallocene-bridged cyclodextrin dimer: A stable derivative of the antitumor drug titanocene dichloride and its potent cytotoxity against human breast cancer (MCF-7) cells

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Abstract

A novel cyclodextrin dimer bridged with titanocene moiety, titanocene di[mono[6-deoxy-6-(2-(thio-1,2-dicyane ethylenylthio)ethyl thio-1,2-dicyane ethylenylthio)]-β-cyclodextrin] (**6**), has been synthesized and characterized by IR spectroscopy, UV spectroscopy, thermogravimetry, MALDI-TOF MS spectrum, ^{1}H and ^{13}C NMR spectroscopy. Especially, its potent cytotoxity against Human Breast Cancer (MCF-7) has also been studied.

Keywords: Titanocene di[mono[6-deoxy-6-(2-(thio-1,2-dicyane ethylenylthio)ethyl thio-1,2-dicyane ethylenylthio)]-β-cyclodextrin]; Titanocene complex; β-Cyclodextrin dimer; Cytotoxity

1. Introduction

Recently, cyclodextrin (CD) dimers, of which the binding sites show characteristic specificity and therefore give access to controllable selectivity, have been extensively investigated [1]. The variety of bridged moieties make it possible to create unique specificity and functions in addition to that of the monotonous cavity of original cyclodextrins [2]. The connecting groups, which combine recognition sites of the two cyclodextrins, have played a very important role in the embodiment of the uniqueness of cyclodextrin dimers [3]. Here we report a unique dimeric cyclodextrin (compound **6**) bridged with titanocene moiety.

It is well known that titanocene diacid complexes ($Cp_2TiR_1R_2$ (**1**) Scheme 1) have shown antitumor properties against a wide range of human and murine tumors, and therefore have received considerable attention [4,5]. The diacid ligands could be widely varied without loss of antitumor potency of titanocene complexes, such as in that of the titanium bis(η^5-2,4-cyclopentadien-1-yl-) [2,2-dimercapto-2-butenedinitrilato (2-)-S,S′] (Cp_2Ti[mnt] (**2**) Scheme 1) [5]. From this point of view, our construction of this novel molecular combination (compound **6**) will reasonably be expected to exhibit not only some structural specificity, but also the titanocene antitumor activity and the potential to conjoin other drugs via a further inclusion interaction with them.

2. Results and discussion

The synthetic strategy has been shown in Scheme 2. The ^{1}H and ^{13}C NMR of these compounds have shown satisfactory agreement with the expected structures. From the spectra, the cyclopentene signals of **6** have been evidently observed at 6.56 and 6.46 ppm in 1H NMR, and at 133.57 and 132.45 ppm in ^{13}C NMR (Table 1). The

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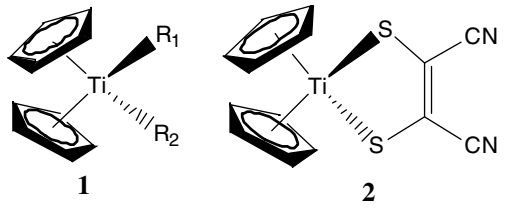
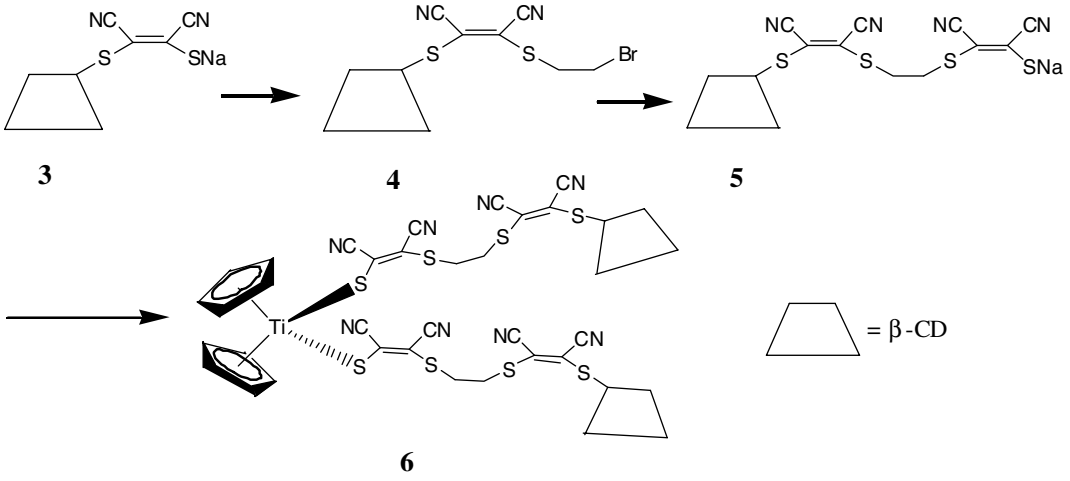


Scheme 1.

electronic spectra of compounds **4**, **5** and **6** show quite a reasonable absorption of the mnt moiety in DMF, from which the ionized and non-ionized mnt parts have been seen at 382 and 387 nm in **5** and **6**, and at 343.5 and 346.0 nm in **4** and **5**, respectively (see Section 4). Moreover in these spectra, the molar absorption coefficients are roughly proportional to the number of chromophors in the molecule. IR spectra of the three compounds also support the existence of non-ionized mnt group in **4** (2212.6 cm^{-1}), ionized mnt group in **5** (2186.4 cm^{-1}) and the formation of S–Ti bonding in **6** (2204.7 cm^{-1}), which can be confirmed by the shifts of ν_{CN}.

Fig. 1 shows the thermogravimetric (TG) and differential thermogravimetric (DTG) curves of **4**, **5**, and **6**. Two peaks are observed in the DTG curve of **4**. The first one between 25 and 100 °C (the maximum observed at 65 °C) corresponds to the dehydration process, and the TG results show 8 mol of water has been liberated in this process. The second peak around 218 °C is related to the fusion and degradation of **4**. In the case of **5**, the dehydration and decomposition processes have been seen clearly at around 55 and 300 °C respectively. And the TG results show it contains 8 mol of water, too. However in that of **6**, the dehydration, during which 21 mol of water has been lost up to 120 °C, occurs at around 55 °C. A sharp and strong peak around 264 °C appears clearly, which should be assigned to the decomposition of the structure of **6**. These results have suggested compound **6** a new chemical species different from **4** and **5** in fact. Furthermore, the water content of **6** indicates the structural hindrance of cyclopentene moiety not being inserted into β-CD cavity [6]. Therefore, compound **6** still remains the potential to include other guest molecules in the future.

The cytotoxity of **6** against the human breast cancer cells (MCF-7) was evaluated and compared with titanocene dichloride (Cp_2TiCl_2) (Fig. 2). At any concentration lower than 10^{-5} M, complex 6 has a higher cytotoxity against MCF-7. (When the concentration is about higher than 10^{-5} M, the cytotoxity of **6** cannot be tested accurately because there is some sediment of complex **6** at the bottom of the plate.) At low concentration range (10^{-6}–10^{-7} M),



Scheme 2.

Table 1
Partial ^{13}C-NMR spectroscopic data[a] for compounds **4**, **5** and **6**

Compound	C-1 (ppm)	C-2 (ppm)	C-3 (ppm)	C-4 (ppm)	C-5 (ppm)	C-6 (ppm)	C[b] (ppm)	C[c] (ppm)	C[d] (ppm)
4	102.07	72.14	73.16	81.63	72.48	60.01	56.19		
5	102.06	72.14	73.15	81.62	72.48	60.01	56.19		
6	102.19	72.25	73.27	81.77	72.58	60.13	56.33	117.40	133.57 132.45

[a] Spectra were recorded in DMSO-d_6 at 15 °C, and data were referenced to internal DMSO-d_6 (39.51 ppm); C-1 to C-6 refer to the carbon atoms of the β-CD structure.
[b] Carbon atoms of the ethyl group.
[c] Carbon atoms of the double bond in mnt.
[d] Carbon atoms of the cyclopentadienyl ring of the titanocene moiety.

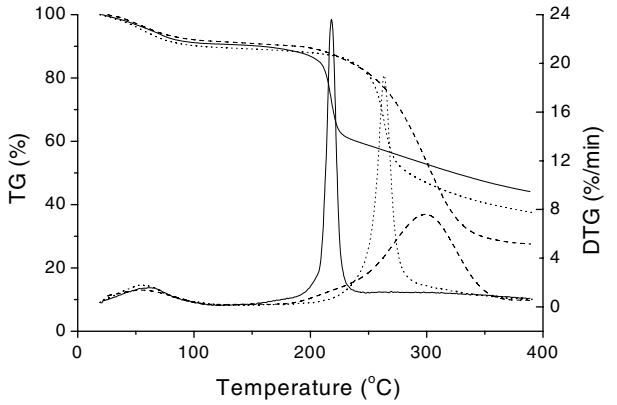


Fig. 1. TG and DTG curves of compound **4** (——), **5** (----) and **6** (……).

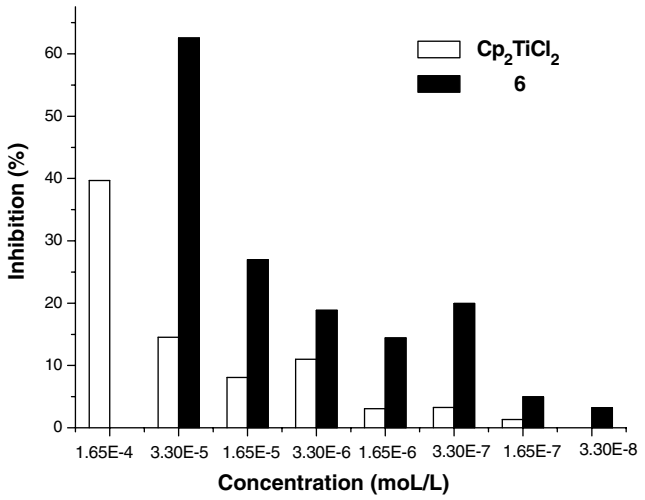


Fig. 2. Cytotoxic activity of complex **6** and Cp_2TiCl_2 against MCF-7 cell lines.

complex **6** demonstrated much higher activity than Cp_2TiCl_2. For example, at a concentration of 3.3×10^{-7} M, complex **6** still maintained an inhibition rate of 20.0% against MCF-7, while the inhibition rate of Cp_2TiCl_2 was only 3.3%. Therefore, the novel titanocene-bridged cyclodextrin dimmer **6** demonstrated higher antitumor activity than titanocene dichloride against MCF-7. Comparing with $Cp_2TiR_1R_2$, the compound **6** which contained four electron-rich groups ($[mnt]^{2-}$) and two –S–C–C–S– chains between the mnt groups was more flexible. Furthermore, the introduction of the cyclodextrins into titanocene complexes will increase the water-soluble ability, which is of great current interest [7]. The further investigation is undergoing.

3. Conclusion

In summary, the present study has provided us with a novel molecular combination **6** formed by an ionizable CD derivative with a titanocene drug material. Compound **6** is fairly soluble in water and it might dramatically improve the bioavailability of the original titanocene drug. It demonstrated much higher cytotoxity against MCF-7 than titanocene dichloride. On the other hand, to extend the functions of pharmaceutical additives, the combination of molecular encapsulation with drug material is effective and a valuable tool in the improvement of drug properties [8,9]. So, the molecular combination **6** might greatly enlarge the pharmaceutical potency of the titanocene drugs, which could be achieved by a logical inclusion complexation of the β-CD ligand with other drug material.

4. Experimental

4.1. Physical measurements and materials

A Bruker IFS66V FT-IR spectrophotometer was used, and the measurements were made by the KBr disk method. The UV spectra were recorded on a Shimadzu UV-3100 spectrometer. All ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE-300 spectrometer in DMSO-d_6 solution at 15 °C. Elemental analyses were determined by a Perkin–Elmer 240C elemental analyzer. TG analyses curves were recorded on an American SDT-2960 thermal analyzer. Ethanol, 1,2-dibromide ethane (BrC_2H_4Br) and acetyl acetone (acac) were of analytically pure grade. Titanocene dichloride (Cp_2TiCl_2) was obtained from Fluka Chemical Company. DMF was distilled under reduced pressure in N_2 atmosphere before used. 2-Butene-dinitrile-2,3-dimercapto disodium salt (Na_2mnt) and 6-mnt-β-CD (compound **3** in Scheme 1) were synthesized according to the methods described in the literatures [10,11]. MALDI-TOF-MS analyses were performed employing delayed extraction in positive ion mode on a time-of-flight mass spectrometer (Voyager- DE STR, Applied Biosystems, Framingham, MA, USA) with a 2.0-m flight tube. Desorption/ionization was obtained by using a 337-nm nitrogen laser with a 3-ns pulse width. Available accelerating potential is in the range of +20/-20 kV. The spectral shown generally represent the sums of 300 laser shots.

4.2. Synthesis of 6-[Br-mnt]-β-CD (4)

To a vigorously stirred solution of 6-mnt-β-CD (**3**) (2.0 g, 1.36 mmol) in water (30 ml), BrC_2H_4Br (1.0 g, 5.32 mmol) was added, and the mixture was stirred at room temperature for 3–4 h. After standing overnight, the precipitate formed was collected by filtration and washed with water and ethanol to give product **4** as yellow deposits (1.8 g, 90%). Anal. Calc. for $C_{48}H_{73}O_{34}BrN_2S_2 \cdot 10\ H_2O$: C, 37.28; H, 6.06; N, 1.81. Found: C, 37.38; H, 6.20; N, 1.77%. UV: λ_{max} nm (DMF): 343.5 ($\log\varepsilon$ 3.98). IR (ν, cm^{-1}): 2212.6 (w). ^1H NMR (288 K, DMSO-d_6): δ, 4.82 (s, 7H, C1–H), 3.71–3.84 (m, 4H, methylene), 3.55–3.63 (m, 28H, C3,5,6-H), 3.30–3.45 (m, 14H, C2, 4-H) ppm. ^{13}C-NMR (288 K, DMSO-d_6): δ 102.07, 81.63, 73.16,

72.48, 72.14, 60.01, 56.19 ppm. MALDI-TOF MS: 1389.7 [6-[Br-mnt]-β-CD + Na^+] (Fig. S1).

4.3. Synthesis of 6-$(mnt)_2$-β-CD (5)

The mixture of 6-[Br-mnt]-β-CD (**4**, 1.0 g, 0.732 mmol) and Na_2mnt (0.4 g, 2.15 mmol) was allowed to react in DMF with stirring at 60 °C for 2–4 h. The dark-red solution was filtrated and concentrated under reduced pressure to 3–4 ml, then poured into alcohol to precipitate crude 6-$(mnt)_2$-β-CD (**5**). The crude product was dissolved in 3–4 ml of water and then added dropwise to stirred alcohol. After standing overnight, the final product precipitated from the alcohol solution as a light yellow power (0.8 g, 80%). Anal. Calc. for $C_{52}H_{73}O_{34}N_4NaS_4 \cdot 10H_2O$: C, 38.33; H, 5.75; N, 3.44. Found: C, 38.46; H, 5.87; N, 3.28%. UV: λ_{max} nm (DMF): 382 (log ε 3.84), 346 (log ε 3.76). IR (ν, cm^{-1}): 2186.4 (w). ^{1}H NMR (288 K, DMSO-d_6): δ 4.83 (s, 7H, C1–H), 3.71–3.85 (m, 4H, methylene), 3.55–3.63 (m, 28H, C3,5,6-H), 3.31–3.45 (m, 14H, C2, 4-H) ppm. ^{13}C-NMR (288 K, DMSO-d_6): δ 102.06, 81.62, 73.15, 72.48, 72.14, 60.01, 56.19 ppm. MALDI-TOF MS: 1450.2 [6-mnt_2-β-CD + Na^+] (Fig. S2).

4.4. Synthesis of Cp_2Ti[6-$(mnt)_2$-β-CD]$_2$ (6)

To a solution of acac (0.25 ml) in water (20 ml), was added Cp_2TiCl_2 (0.5 g, 20 mmol), and the solution was stirred at 60 °C for 30 min. Then to a solution of 6-$(mnt)_2$-β-CD (**5**, 1.0 g, 0.69 mmol) in water (20 ml), was added the above-mentioned red solution (4.0 ml), and this mixture was stirred at room temperature for 1–2 h. The green deposits from the mixture were collected by filtration and washed with cool water to give the final product (0.47 g, 60%). Anal. Calc. for $C_{114}H_{156}O_{68}N_8S_8Ti \cdot 20H_2O$: C, 40.38; H, 5.83; N, 3.30. Found: C, 40.61; H, 5.47; N, 3.18%. UV: λ_{max} nm (DMF): 387 (log ε 4.13). IR (ν, cm^{-1}): 2204.7 (w). ^{1}H-NMR (288 K, DMSO-d_6) δ 6.56–6.57 (m, 5H, cyclopentene), 6.46–6.48 (m, 5H, cyclopentene), 4.82 (s, 14H, C1–H), 3.71–3.74 (m, 8H, methylene), 3.55–3.63 (m, 56H, C3,5,6-H), 3.29–3.44 (m, 28H, C2,4-H) ppm. ^{13}C-NMR (288 K, DMSO-d_6): δ 133.57, 132.45, 117.40, 102.19, 81.77, 73.27, 72.58, 72.25, 60.13, 56.33 ppm. MALDI-TOF MS: 3054.7 [Cp_2Ti[6-$(mnt)_2$-β-CD]$_2$ + Na^+] (Fig. S3).

4.5. Cytotoxicity assays

The assay is carried out in 96-well plates with an average of 0.5×10^4 cells per ml with 100 μL in each well. The cells were then treated with varying concentrations of drug for 2 days in an atmosphere of 5% CO_2/95% air. After 2 days the cells were removed from the incubator and washed with cold trichloroacetic acid (TCA) to fix the cells to the bottom of the plates. These were then washed with water to ensure the removal of all TCA. The plates were then stained with a solution of 0.02% (w\v) SRB in 1.0% (v\v) acetic acid for 30 min. The plates were washed thoroughly with acetic acid and allowed to dry overnight. A 100 μl portion of 10 mM unbuffered Tris was added to each well to resuspend the SRB dye. The plates were then read using a plate reader, which takes the mean absorbance at 530 nm of each concentration.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2006.09.052.

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