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An Antitumor Agent of *β*-Cyclodextrin–Modified Titanocene Complex: Synthesis and Characterization

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The complex of a derivative of *β*-cyclodextrin, that is mono[6-deoxy-6-(2-butenedinitrile-2,3-dimercapto sodium salt)]-*β*-cyclodextrin (6-mnt-*β*-CD), with titanocene (titanocene di[mono[6-deoxy-6-(2-butenedinitrile-2,3-dimercapto)]-*β*-cyclodextrin], $Cp_2Ti[6\text{-mnt-}\beta\text{-CD}]_2$) has been synthesized and characterized by IR spectroscopy, UV spectroscopy, elemental analysis, thermogravimetry, ^1H- and ^{13}C-NMR spectroscopy. The stoichiometry of the target molecule was determined by ^1H-NMR spectroscopy and elemental analysis.

Key words titanocene di[mono[6-deoxy-6-(2-butenedinitrile-2,3-dimercapto)]-*β*-cyclodextrin]; ^{13}C-NMR; thermogravimetry; titanocene complex; *β*-cyclodextrin

Titanocene diacido complexes ($Cp_2TiR_1R_2$, **1**) have shown antiproliferative activities against various experimental tumors, such as Ehrlich ascites tumor, sarcoma 180, B16 melanoma, colon 38 carcinoma, leukemia system L1210 and P388, and Lewis lung carcinoma as well as against xenografted human carcinomas.[1—9] The previous work of Köpf-Maier *et al.*[8] had confirmed that the diacido 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-)[2,3-dimercapto-2-butenedinitrilato(2-)-*S*,*S*′] ($Cp_2Ti[mnt]$, **2**). In this paper we described a novel *β*-cyclodextrin (*β*-CD)–modified acido ligand containing the mnt group and its titanocene complex **3**.

Results and Discussion

The pre-preparation of the acetylacetone (acac) solution with titanocene dichloride would encourage the expected reaction to produce title complex **3**. The formation of $[Cp_2Ti(acac)]^+$ has stabilized the titanocene moiety in aqueous solution so as to keep from hydrolysis and other unexpected by-products.[10]

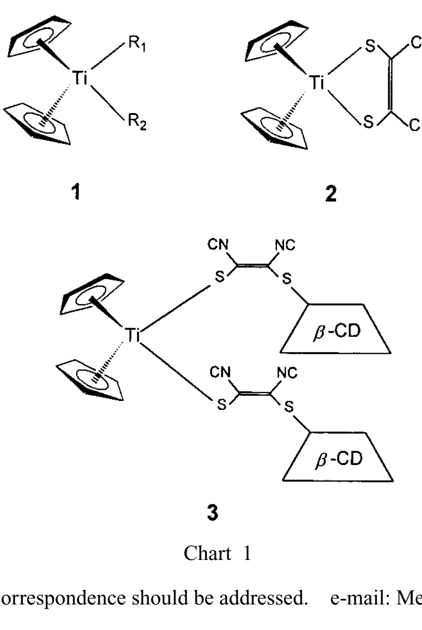

Chart 1

Analysis of ^{13}C-NMR spectrum of 6-mnt-*β*-CD suggested it contained one C=C bond at δ 118.38 and 118.79 ppm, and two nitrile groups at δ 80.94 and 81.15 ppm (Table 1). In the case of titanocene di[mono[6-deoxy-6-(2-butenedinitrile-2,3-dimercapto)]-*β*-CD] ($Cp_2Ti[6\text{-mnt-}\beta\text{-CD}]_2$, **3**), the signals of double bond and nitrile groups exhibited at δ 118.34, 118.75, 81.10, and 81.32 ppm, respectively. And the signal at δ 119.31 ppm was assigned as cyclopentene ring of the titanocene complex, which was then confirmed by the ^1H-NMR spectrum results (Table 2). The IR spectra of both the 6-mnt-*β*-CD and the titanocene complex **3** have shown the presence of nitrile groups (2189.7, 2205.4 cm^{-1}, respectively). The stoichiometry (1 : 1 molar ratio of cyclopentene ring with cyclodextrin cavity) of complex **3** that was isolated as green powder in our experiments has also been determined by ^1H-NMR spectroscopy (Table 2). Despite of the insolubility of complex **2**, complex **3** was fairly soluble and stable in water.

The obvious downfield shifts of signals of the nitrile groups observed in complex **3** in contrast to that in 6-mnt-*β*-CD (Table 1) have suggested the charge transfer from mnt group to the titanocene center, which could be interpreted as the formation of a S–Ti bonding and confirmed by the higher frequency shift of ν_{CN} (from 2189.7 to 2205.4 cm^{-1}) in IR spectra. Considering the structural hindrance in complex **3**, the cyclopentene rings couldn't be included into *β*-CD cavity (*vide infra*).

Figure 1 showed the thermogravimetric (TG) and differential thermogravimetric (DTG) curves of Cp_2TiCl_2, 6-mnt-*β*-CD, and $Cp_2Ti[6\text{-mnt-}\beta\text{-CD}]_2$. Two peaks were observed in the DTG curve of 6-mnt-*β*-CD. The first peak between 15 and 130 °C (the maximum observed at 53 °C) corresponded to the dehydration process. The TG results showed that the 6-mnt-*β*-CD contained 9 mol of water. The second peak around 292 °C was related to the fusion and degradation of 6-mnt-*β*-CD. In the case of Cp_2TiCl_2, the decomposition was clearly seen at around 322 °C. However in that of $Cp_2Ti[6\text{-mnt-}\beta\text{-CD}]_2$, the dehydration, during which 20 mol of water was lost up to 130 °C from the experimental results (*vide infra*), occurred at around 67 °C. Elemental analysis results (C, 45.17; H, 5.64; N, 2.04%, respectively) agreed well with the forma-

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Table 1. Data of ^{13}C-NMR Spectra of the Titanocene Complex[a]

Complex	C1	C2	C3	C4	C5	C6	C[b]	C[b]	C[c]	C[c]	C[d]
6-mnt-β-CD[e]	102.05	72.12	73.15	81.62	72.49	59.94	118.79	118.38	81.15	80.94	
Cp_2Ti[6-mnt-β-CD]$_2$	102.07	72.05	73.07	81.54	72.43	59.87	118.75	118.34	81.32	81.10	119.31

a) Relative to internal DMSO-d_6 (δ=39.51 ppm), C1 to C6 refer to carbon atoms of β-CD structure. *b*) Carbon atoms of the double bond in mnt. *c*) Carbon atoms of the nitrile group in mnt. *d*) Carbon atoms in the ring of cyclopentene. *e*) From refs. 11 and 12.

Table 2. Data of ^{1}H-NMR Spectra of the Titanocene Complex[a]

Complex	H-1	H-2	H-3	H-4	H-5	H-6	H[b]	$C_{p.a.}$[c]
6-mnt-β-CD[d]	4.82	3.31	3.63	3.35	3.55	3.63		
Cp_2Ti[6-mnt-β-CD]$_2$	4.82	3.32	3.63	3.42	3.55	3.63	6.47	0.79

a) H-1 to H-6 refer to protons of the β-CD structure. *b*) Protons in the cyclopentene ring of titanocene. *c*) Value of the comparison of peak areas of the cyclopentene ring and H-1. *d*) From ref. 12.

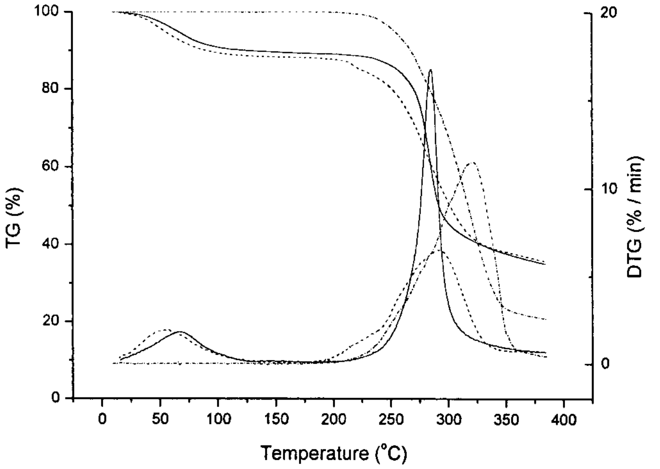

Fig. 1. TG and DTG Curves of Cp_2TiCl_2 (---------), Cp_2Ti[6-mnt-β-CD]$_2$ (———), and 6-Mnt-β-CD (··············)

tion of the dehydrated product ($C_{102}H_{148}O_{68}N_4S_4Ti$). A sharp and strong peak around 285 °C appeared clearly, which should be assigned to the decomposition of Cp_2Ti[6-mnt-β-CD]$_2$. These results have suggested complex **3** a new chemical species different from the original substances in fact. The water content of complex **3** was determined to be 20 according to TG results, namely 10 mol water per β-CD moiety that was consistent with the results of elemental analyses (see the Experimental). Accordingly, we could presume that the cyclopentene rings of complex **3** have not penetrated into the β-CD cavity so as not to release some water from it. So, complex **3** still remained the potential to include other guest molecules in the future.

In summary, the present study provided us with an ionizable derivative of β-CD (6-mnt-β-CD) and a novel molecular combination (Cp_2Ti[6-mnt-β-CD]$_2$) formed by the ionizable CD derivative with a titanocene drug material. The molecular combination **3** was soluble in water so that it might have dramatically improved the bioavailability of the original titanocene drug. On the other hand, to extend the functions of pharmaceutical additives the combination of molecular encapsulation with drug materials is effective and a valuable tool in the improvement of drug properties.[13,14] Consequently the molecular combination **3** containing pre-inclusion ligands, which could be formed by a logical complexation of the 6-mnt-β-CD ligand with other drug materials, might greatly enlarge the pharmaceutical potency of the titanocene drugs.

Experimental

General Methods 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 ^{13}C- and ^{1}H-NMR spectra were recorded on a Bruker AVANCE-300 spectrometer in dimethylsulfoxide-d_6 (DMSO-d_6) solution at 15 °C. Elemental analysis was made by a Perkin-Elmer 240C elemental analyzer. Thermogravimetric analysis curves were recorded on an American SDT-2960 thermal analyzer. Titanocene dichloride (Cp_2TiCl_2) and acetylacetone were obtained from Fluka Chemical Company. 6-Mnt-β-CD was synthesized according to the methods described in the literatures.[11,12]

Synthesis of Cp_2Ti[6-mnt-β-CD]$_2$ (3) To a solution of acetylacetone (0.25 ml) in water (20 ml) was added titanocene dichloride (0.5 g, 2.0 mmol), and the solution turning red was stirred at 60 °C for 30 min. Then to a solution of 6-mnt-β-CD (1.0 g, 0.68 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 was collected by filtration and washed with cool water to give 0.4 g the final product. ^{13}C-NMR (DMSO-d_6) δ: 59.87, 72.05, 72.43, 73.07, 81.10, 81.32, 81.54, 102.07, 118.34, 118.75, 119.31. IR (KBr) cm^{-1}: 2205.4. UV λ_{max} (DMF) nm: 388. *Anal.* Calcd for $C_{102}H_{148}O_{68}N_4S_4Ti \cdot 20H_2O$: C, 40.11; H, 6.20; N, 1.83. Found: C, 40.14; H, 6.35; N, 1.90.

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