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Supramolecular Nanoencapsulation as a Tool: Solubilization of the Anticancer Drug *trans*-Dichloro(dipyridine)platinum(II) by Complexation with β-Cyclodextrin

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Abstract: A novel, water-soluble *trans*-platinum complex was synthesized by inclusion complexation with β-cyclodextrin. The complexation was confirmed by ^{1}H NMR, FT-IR, TGA, and XRD as well as by SEM and EDX. As the precursor complex is not water-soluble, it is difficult to employ it for biological applications. Here, we report that the encapsulation with cyclodextrin allowed to solubilize the complex to a solubility value of 1.6 mg/mL. Moreover, the cytotoxicity in vitro of the novel inclusion complex indicated a much higher activity after encapsulation.

Keywords: Anticancer drug; cisplatin; cyclodextrin; nanoencapsulation; platinum(II)

Introduction

Since the discovery of the antitumor properties of cisplatin (CPT), *cis*-diamminedichloroplatinum(II), by Rosenberg,[1,2] many attempts were made to prepare a large number of new platinum compounds. It was reported that complexes having cis geometry were antitumor active, with CPT showing the highest activity. In contrast, transplatin was shown to be inactive.[3] However, recently it has been found that several transplatinum derivatives exhibit enhanced cytotoxicity compared to that of *cis*- and *trans*-platinum.[4,5] One class of this kind of active trans derivatives contains planar aromatic moieties such as pyridine,[6] *N*-methylimidazole, thiazole, and quinoline.

As most of the platinum(II) complexes are insoluble in water or less soluble than CPT, different approaches have been employed to obtain water-soluble complexes of this type. The most common approach used unil recently was a structural approach, mainly to replace the chloride ligands with chelating carboxylates, oxalate, and glycolate.[7] Also, there are some reports on several anionic phosphono carboxylate complexes with a high solubility and stability in aqueous solutions.[8] However, not much work has been done to increase the solubility in water and decrease the toxicity of these compounds, which are important challenges for their biomedical applications. One promising method to eliminate these drawbacks could be the encapsulation of the drug with a macrocyclic host, e.g., cyclodextrin, which is a fascinating molecule, nontoxic, with a unique structure, able to form inclusion complexes with a variety of compounds. In the present work, we report for the first time[9] that a new technique to solubilize and enhance the cytotoxic activity of *trans*-platinum derivatives such as the *trans*-dichloro(dipyridine)platinum(II) (DDP) by nanoencapsulation with

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[†] Department of Materials Science and Engineering.

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(1) Rosenberg, B.; Van Camp, L.; Krigas, T. Inhibition of Cell Division in Escherichia coli by Electrolysis Products from a Platinum Electrode. *Nature* **1965**, *205*, 698–699.

(2) Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. Platinum Compounds: a New Class of Potent Antitumour Agents. *Nature* **1969**, *222*, 385–386.

(3) Cleare, M. J.; Hoeschele, J. D. Studies on the antitumor activity of group VIII transition metal complexes. Part I. Platinum (II) complexes. *Bioinorg. Chem.* **1973**, *2*, 187–210.

(4) Farrell, N. Current status of structure-activity relationships of platinum anticancer drugs: activation of the trans geometry. *Met. Ions Biol. Syst.* **1996**, *32*, 603–639.

(5) Van Beusichem, M.; Farrell, N. Activation of the trans geometry in platinum antitumor complexes. Synthesis, characterization, and biological activity of complexes with the planar ligands pyridine, N-methylimidazole, thiazole, and quinoline. Crystal and molecular structure of trans-dichlorobis(thiazole) platinum(II). *Inorg. Chem.* **1992**, *31*, 634–639.

(6) Farrell, N. Cytostatic trans-Platinum(II) complexes. *J. Med. Chem.* **1989**, *32*, 2240–2241.

(7) Wong, E.; Giandomenico, C. M. Current Status of Platinum-Based Antitumor Drugs. *Chem. Rev.* **1999**, *99*, 2451–2466.

(8) Hollis, L. S.; Miller, A. V.; Amundsen, A. R.; Schuring, J. E.; Stern, E. W. Cis-Diamineplatinum(II) complexes containing phosphono carboxylate ligands as antitumor agents. *J. Med. Chem.* **1990**, *33*, 105–111.

(9) Geckeler, K. E. Horvath, G. International patent application pending.

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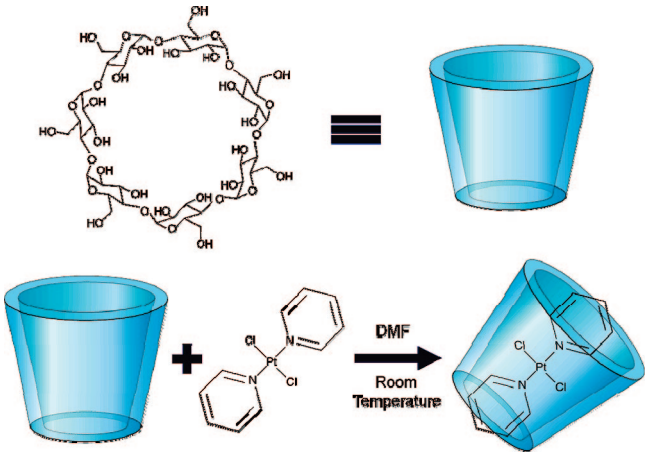
Scheme 1. Schematic Illustration of the Inclusion of trans-Dichloro(dipyridine)platinum(II) in the Cavity of β-Cyclodextrin



β-cyclodextrin (CD) leads to the synthesis, characterization, and anticancer activity of a novel class of water-soluble CD-encapsulated transplatin derivatives.

Cyclodextrins are widely used cyclic, bucket-shaped oligosaccharides consisting of six, seven, or eight glucopyranose units, namely α-, β-, γ-cyclodextrin, linked by α-1,4-glycosidic bonds to form the macrocycle.[10–12] We have chosen cyclodextrins for nanoencapsulation because they are seminatural products with very low or no toxicity, and they have the ability to enhance drug delivery through biological membranes. In comparison with other supramolecular hosts,[13] cyclodextrins are the best accessible and feature a high biocompatiblity. In addition, there are other advantages for drug delivery by the formation of inclusion complexes, e.g., in combination with different drugs, it is possible to control the release rate of drugs.[14]

We have been exploring the supramolecular chemistry with cyclodextrin[11] and recently reported water-soluble inclusion complexes of CD with different guest molecules[15–18] using the "supramolecular masking concept". Consequently, we thought to adopt this concept to solubilize the DDP antitumor complex by using CD, in turn facilitating the preparation of novel water-soluble inclusion complexes (Scheme 1) for biomedical applications. Hence, in this paper, we describe a supramolecular nanoencapsulation approach to improve the water-solubility and successively the cytotoxic activity of the platinum antitumor complex.

A schematic representation of the synthesis of the CD−DDP inclusion complex is shown in Scheme 1. In order to confirm the structure of the platinum inclusion complex, different characterization methods such as FTIR and ^{1}H NMR spectroscopy, as well as TGA, powder XRD, SEM, and EDX analyses were performed.

Results and Discussion

The DDP was prepared according to the literature,[19] and its trans isomer was confirmed by the Kurnakov test.[20] In order to authenticate the formation of the trans isomer complex, the as-prepared compound has further been characterized by far-IR spectroscopy, which was carried out in the range from 600 to 250 cm^{-1}. The vibrations corresponding to ν(Pt−N) bonds were in perfect agreement with the values found in the literature.[21,22]

It is observed that the XRD reflection peaks of the CD−DDP inclusion complex (see Supporting Information Figure S1) differ from those of the CD and the DDP complex, showing that the final product has neither the typical 2θ values of CD nor those of the DDP trans complex, suggesting the formation of an inclusion complex.[16,23] In addition, the inclusion complex shows a different pattern, especially for the 2θ values between 10° and 25°, where the peaks observed were assigned to the combination of both the DDP complex and CD. This is in accord with previously reported results of the CD complexes.[16]

The FTIR spectra of the CD−DDP inclusion compound, CD, and DDP are shown in Figures 1 and 2. FTIR spectroscopy is a very useful tool to prove the presence of both host and guest components in an inclusion complex. Thus, a sharp absorption band, noticed at 1605 and 1460 cm^{-1} due to the C=N and pyridine ring stretching vibrations, respectively, of the DDP was reduced considerably in the inclusion compound owing to the complexation. An ad-

(10) Szejtli, J. Introduction and General Overview of Cyclodextrin Chemistry. *Chem. Rev* **1998**, *98*, 1743–1754.
(11) Geckeler K. E., Ed. *Advanced Macromolecular and Supramolecular Materials and Processes*: Kluwer Academic/Plenum Publishers: New York, 2003.
(12) Geckeler K. E.; Rosenberg, E., Eds. *Functional Nanomaterials*; American Scientific Publ.: Valencia, 2006.
(13) Premkumar, T.; Geckeler, K. E. Nanosized CuO particles via a supramolecular Strategy. *Small* **2006**, *2*, 616–621.
(14) Martin del Valle, E. M. Cyclodextrins and their uses: a review. *Process Biochem.* **2004**, *39*, 1033–1046.
(15) Samal, S.; Choi, B. J.; Geckeler, K. E. The first water-soluble main-chain polyfullerene. *Chem. Commun.* **2000**, 1373–1374.
(16) Murthy, C. N.; Geckeler, K. E. The water-soluble β-cyclodextrin-[60]fullerene complex. *Chem. Commun.* **2001**, 1194–1195.
(17) Murthy, C. N.; Choi, S. J.; Geckeler, K. E. Nanoencapsulation of [60]fullerene by a novel sugar-based polymer. *J. Nanosci. Nanotechnol.* **2002**, *2*, 129–132.
(18) Nepal, D.; Samal, S.; Geckeler, K. E. The first fullerene-terminated soluble poly(azomethine) rotaxane. *Macromolecules* **2003**, *36*, 3800–3802.
(19) Thompson, L. K. Trans Platinum(II) Complexes with pyridine and substituted pyridines. *Inorg. Chim. Acta* **1980**, *38*, 117–119.
(20) Kauffman, G. B. Cis- and trans-dichloro(dipyridine)-platinum(II). *Inorg. Synth.* **1963**, *7*, 249–253.
(21) Clark, R. J. H.; Williams, C. S. The far-infrared of metal-halide complexes of pyridine and related ligands. *Inorg. Chem.* **1965**, *4*, 350–357.
(22) Kalinowska, U.; Matławska, K.; Chêcińska, L.; Domagała, M.; Kontek, R.; Osiecka, R.; Ochocki, J. Synthesis, spectroscopy and antiproliferative activity of cis- amd trans-platinum(II) complexes with diethyl(pyridin-4-ylmethyl)phosphate. X-ray crystal structure of trans-Pt(II) complex. *J. Inorg. Biochem.* **2005**, *99*, 2024–2031.
(23) Premkumar, T.; Geckeler, K. E. A green approach to fabricate CuO nanoparticles. *J. Phys. Chem. Solids* **2006**, *67*, 1451–1456.

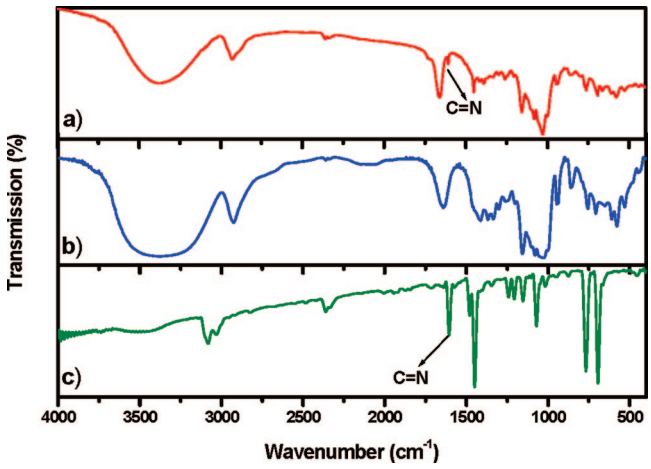


Figure 1. FT-IR spectra of (a) CD−DDP inclusion complex, (b) CD, and (c) DDP.

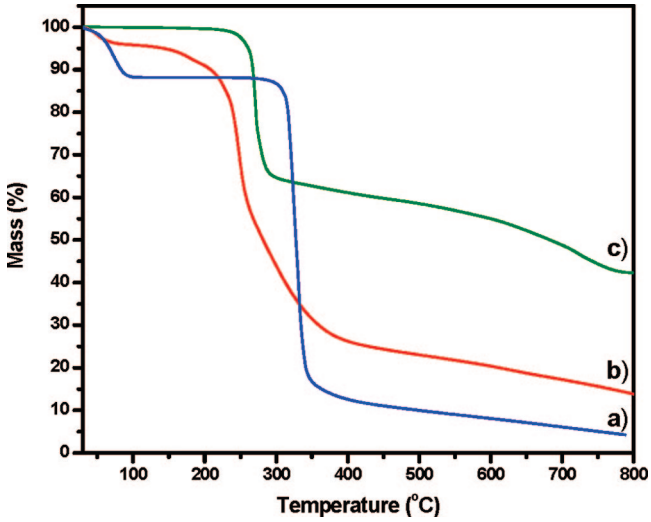


Figure 2. Thermogravimetric curves of (a) CD, (b) CD−DDP inclusion complex, and (c) DDP.

ditional key observation was the sharp decrease of the intensity and slight hypsochromic shift of the peak between 3500 and 3000 cm^{-1} in the spectrum of the inclusion compound. In agreement with earlier reports, the containment of vibrational modes in the region of 3500–3300 cm^{-1} has been correlated to the confirmation of host–guest interactions as a result of water release upon inclusion.[24] Thus, the positions and relative intensities of a few bands due to both the host and guest are affected, which corroborate the formation of an inclusion compound between CD and DDP.

In order to study the thermal stability of the inclusion complex thermogravimetric analysis (Figure 2) data were recorded in a nitrogen atmosphere. From the TG analysis, it was found that the inclusion compound showed at the beginning a small mass loss due to the loss of water molecules, followed by a larger mass loss corresponding to the decomposition of the DDP complex and CD. In most

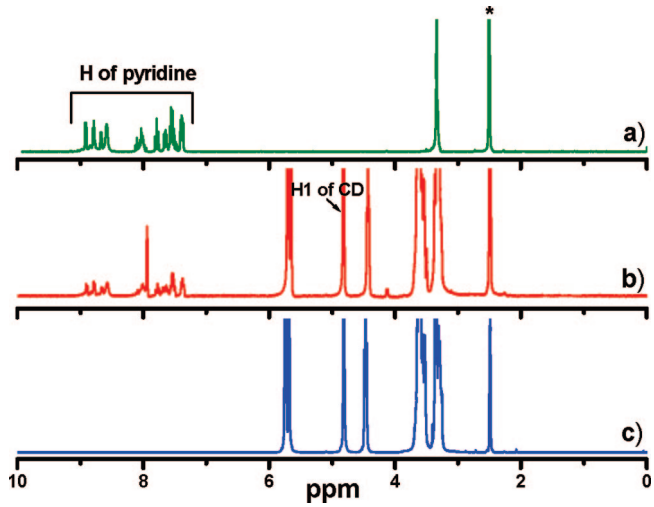


Figure 3. ^{1}H NMR spectra of (a) DDP, (b) CD−DDP inclusion complex, and (c) CD.

cases, CD can form complexes with guest molecules in a ratio of 1:1, 1:2, or 2:1. The theoretical, calculated mass content of CD in the complex for these three ratios could be 72.7, 57.2, and 84.2%, respectively. The TGA data of the inclusion compound show that the total mass loss at 400 °C is 73.6%. This value indicates that the ratio between CD and DDP complex is of 1:1 type.[16] At the same temperature, pure CD (Figure 2a) lost 87.5% of its initial mass, and the mass loss observed for the inclusion compound compared to that of the platinum complex is due to the presence of CD in the final product.

With the aim of substantiating the inclusion formation, the inclusion compound, DDP, and CD were further characterized by ^{1}H NMR spectroscopy (Figure 3). It is evident from the spectra that the peak observed in the range 7.3–9.0 ppm of DDP, assigned to the pyridine protons and also noticed in the inclusion compound as well in addition to the characteristic peaks of CD, [25] corroborates the formation of an inclusion complex. Further, we calculated the ratio between the pyridine protons and the C1−H protons of CD, which appear at 4.81 ppm, from the NMR spectra. Accordingly, the area ratio between the pyridine protons and those of CD is 8.9:7. Using this data we can estimate the number of CD rings corresponding to one molecule of DDP.[25] Thus, we have found that the structure of the inclusion complex contains only one CD ring, which means that the ratio between CD and platinum complex is of the 1:1 type, that is in good agreement with the TGA data. In addition, the pyridine protons of the DDP are shifted considerably upfield from 8.04 ppm to 7.93 ppm after the complex formation. The upfield shift (0.11 ppm) observed due to the guest protons inside the cavity of the host has been considered as an evidence for the formation of an inclusion complex.[26]

(24) Rusa, C. C.; Luca, C.; Tonelli, A. E. Polymer-Cyclodextrin Inclusion Compounds: Toward New Aspects of Their Inclusion Mechanism. *Macromolecules* **2001**, *34*, 1318–1322.

(25) Liu, Y.; Yang, Y.-W.; Chen, Y.; Zou, H-X. Polyrotaxane with Cyclodextrins as Stoppers and Its Assembly Behavior. *Macromolecules* **2005**, *38*, 5838–5840.

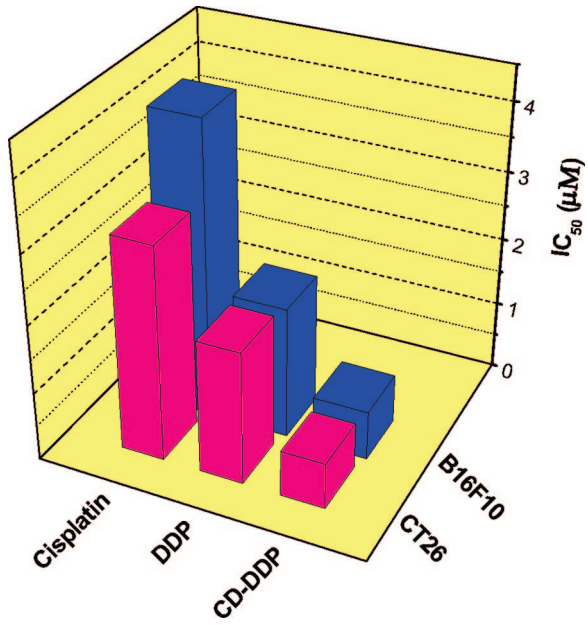


Figure 4. 3D plot showing the IC_{50} values of CPT, DDP, and CD−DDP inclusion complex in CT 26 and B16F10 cell lines.

The surface morphology of the compounds was studied by scanning electron microscopy (SEM), which also provided evidence for the formation of the inclusion complex. Initially, we observed a powdered form of CD and potassium tetrachloroplatinate(II) by SEM; however, then we also observed the powder form of DDP and the inclusion complex (see Supporting Information Figure S2). While potassium tetrachloroplatinate(II) shows an irregular structure, DDP exhibits a regular plate-rod-like structure. Further, CD shows sheetlike morphology, and the CD−DDP inclusion complex looks different from all other results. The alteration of crystals and powder can be assumed as an additional indication of the formation of new inclusion complex. Representative energy dispersive X-ray spectroscopy analyses were conducted as well in order to determine the quantitative composition of the synthesized compound. The results of EDX suggest that the platinum content in the inclusion complex is 11.4%, which is in good concurrence with the theoretical value of a 1:1 inclusion compound (calcd 12.5% for 1:1 ratio and 7.2% for 1:2 ratio of DDP and CD).

Different views of space-filling molecular models of the three-dimensional conformation of the CD−DDP inclusion complex are shown in Figure 5. The host and guest molecules were independently built up, and their geometry was optimized. The overall structure of these possible models of inclusion complexes was again subjected to energy minimization. The final energy-minimized molecular model indicates that DDP is included in the host cavity.

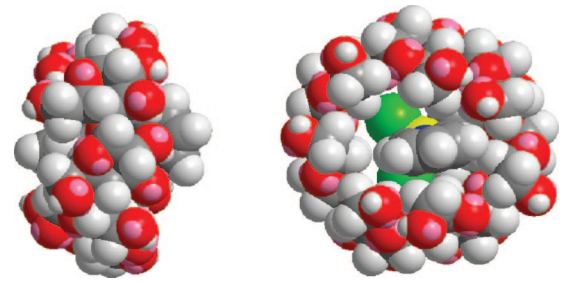


Figure 5. Space-filling energy-minimized (MM2) molecular models showing different views of DDP encapsulated in the CD cavity.

In order to investigate whether the encapsulation in CD has an effect on the cytotoxicity of DDP, a control experiment was performed with the as-prepared novel inclusion complex (CD−DDP), DDP, and CPT using two different cancer cell lines CT26 colon carcinoma and B16F10 melanoma. As shown in Figure 4, the lowest IC_{50} were obtained with as-prepared inclusion compound. The experimental data show that growth inhibition by DDP and CD-DDP against CT26 colon carcinoma cells in vitro was 1.6 and 4.6 times, respectively, more effective than the DNA-modifying drug CPT cell line. It is interesting to note that the cytotoxic activity of the as-prepared inclusion compound against these cell lines was higher than that of the DDP against different other cell lines reported.[27] Overall, the results indicate that the novel as-prepared CD−DDP is more potent than the precursor DDP and the CPT in the induction of the cytotoxicity of cancer cell lines such as CT26 colon carcinoma and B16F10 melanoma.

It is pertinent to mention here that the as-prepared supramolecular complex is well soluble in water (∼1.6 mg/mL), which is a higher solubility than the commercial chemotherapeutic drug CPT (∼1 mg/mL). Further, the literature survey (Table 1) shows that most of the platinum derivatives are insoluble in water and only soluble in organic solvents, especially problematic when used in conjunction with pharmaceutical formulations. It is noted that the only water-soluble *trans*-platinum complex reported (1 mg/mL) (Table 1) was found to be inactive, as it did not show any cytotoxic activity against human ovarian tumor cell lines (A2780/A2780cisR) and human ovarian carcinoma cell lines (CH1/CH1cisR).[22] Further studies to enhance the solubility by using derivatization reactions and other approaches are currently underway. Here we proved that the higher water-solubility and anticancer activity of hydrophobic platinum compounds can be attained by nanoencapsulation with CD. Hence, our complex and approach may be expected to have a great potential for biomedical applications.

Experimental Section

Materials. β-Cyclodextrin (CD) and potassium tetrachloroplatinate(II) were obtained from Wako Pure Chemical Ltd.

(26) Liu, Y.; Liang, P.; Chen, Y.; Zhang, Y.-M.; Zheng, J.-Y.; Yue, H. Interlocked Bis(polyrotaxane) of Cyclodextrin-Porphyrin Systems Mediated by Fullerenes. *Macromolecules* **2005**, *38*, 9095–9099.

(27) Perez, J. M.; Fuertes, M. A.; Alonso, C.; Navarro-Ranninger, C. Antitumor and Cellular Pharmacological Properties of a Novel Platinum(IV) Complex: trans-[$PtCl_2(OH)_2$(Dimethylamine) (Isopropylamine)]. *Crit. Rev. Oncol. Hematol.* **2000**, *35*, 109–113.

Table 1. Survey of Different Platinum Derivatives and Their Suitable Solvents

compd	solvent/medium	ref
trans-[$PtCl_2(OH)_2$(dimethylamine)(isopropylamine)]	DMEM[a]	28
trans-[$PtCl_2$(dimethylamine)(isopropylamine)]	DMEM	28
trans-PtL_2Cl_2 and $[PtL_3Cl]Cl$	DMF	29
L: 3-hydroxypyridine, 4-hydroxypyridine and imidazo[1,2-α]pyridine		
trans-[$PtCl_2NH_3$(3-(hydroxymethyl)pyridine)] and *trans*-[$PtCl_2NH_3$(4-(hydroxymethyl)pyridine)]	water	30
trans-platinum complexes containing phosphane groups	acetone	31
cis- and *trans*-[$PtCl_2L_2$] (L = 4-methylpyridine and NH_3)	DMF	6
trans-[$PtCl_2L_2$] (L= pyridine)	DMF	6
trans-platinum(II) complexes with diethyl (pyridin-4-ylmethyl)phosphate	DMSO[b]	22
cis-platinum(II) complexes with diethyl (pyridin-4-ylmethyl)phosphate	DMSO	22
trans- [$PtCl_2$(amine)(isopropylamine)]	PBS[c]	32
CD-DDP inclusion complex (this study)	water	9

[a] Dulbecco's modified Eagles medium. [b] Dimethyl sulfoxide. [c] Phosphate-buffered saline.

Table 2. Inhibitory Concentration (IC_{50}) (μM) Values Calculated for Cisplatin, *trans*-Dichloro(dipyridine)platinum(II) (DDP), and Inclusion Complex (CD−DDP) for the Two Different Cancer Cell Lines CT26 Colon Carcinoma and B16F10 Melanoma

	IC_{50} (μM)	
sample	CT26 colon carcinoma	B16F10 melanoma
misplatin	3.13 ± 0.42	4.31 ± 0.12
DDP	1.94 ± 0.43	1.92 ± 0.07
CD-DDP	0.67 ± 0.07	0.71 ± 0.01

β-Cyclodextrin was used after drying overnight at 80 °C under vacuum. *N*,*N*′-dimethylformamide (DMF) was purified by distillation over CaH_2 under reduced pressure. Pyridine (anhydrous, 99.8%) was obtained from Sigma-Aldrich and used without any further purification.

Measurements. Fourier-transform infrared (FTIR) spectra were taken as KBr pellets at room temperature under nitrogen using a Perkin-Elmer System 2000. ^{1}H NMR spectra were recorded at 300 MHz using a JEOL JNM-LA 300 WB instrument and DMSO-d_6 as the solvent. Thermogravimetric analyses (TGA) were recorded on a TA-2050 thermal analyzer under nitrogen at a heating rate of 10 °C/min. X-ray diffraction (XRD) measurements were carried out on a Rikagu diffractometer with a copper radiation ($\lambda = 0.15406$ nm) at 40 kV and 40 mA. Scanning electron microscopy (SEM) images and EDX were taken on a Hitachi S-4700 instrument. For the moelcular modeling studies, the software ChemOffice 2005 (Chem3D Ultra 9.0 version) was used.

Synthesis of the *trans*-Dichloro(dipyridine)-platinum(II) (DDP) Complex. The platinum complex was prepared according to a previously reported method.[19] Briefly, an aqueous solution (3 mL) of potassium tetrachloroplatinate(II) (K_2PtCl_4) (83 mg, 0.2 mmol) was mixed with an aqueous solution (1 mL) of excess pyridine (0.065 mL, 0.8 mmol) and refluxed until a clear solution was obtained. The resulting clear solution was filtered and added into a saturated aqueous solution of sodium chloride. A yellow solid was obtained after heating the aqueous solution at 90 °C for 10 h under continuous stirring. The yellow solid was isolated by filtration and thoroughly washed with distilled water and diethyl ether and dried under vacuum. The as-prepared compound is insoluble in water, but soluble in several organic solvents such as *N*,*N*-dimethylformamide, acetone, and chloroform.

To confirm the trans structure of the complex, we performed the Kurnakov test.[20] While no visible reaction was noticed upon mixing a hot aqueous solution of saturated thiourea with the as-prepared trans isomer, however, on cooling white needles of *trans*-dichlorobis(thiourea)platinum(II) were observed, which corroborate the formation of the trans complex.

(28) Perez, J. M.; Kelland, L. R.; Montero, E. I.; Boxall, F. E.; Fuertes, M. A.; Alonso, C.; Navarro-Ranninger, C. Synthesis, characterization and binding with DNA of four planaramineplatinum(II) complexes of the forms: trans-PtL_2Cl_2 and $[PtL_3Cl]Cl$, where L = 3-hydroxypyridine, 4-hydroxypyridine and imidazo(1,2-α)pyridine. *Mol. Pharmacol.* **2003**, *63*, 933–944.

(29) Chowdhury, M. A.; Huq, F.; Abdullah, A.; Beale, P.; Fisher, K. Preparation, characterization and cytotoxic activity of new compounds trans-[$PtCl_2NH_3$(3-(hydroxymethyl)-pyridine)] and trans-[$PtCl_2NH_3$(4-(hydroxymethyl)-pyridine)]. *J. Inorg. Biochem.* **2005**, *99*, 1098–1112.

(30) Ramos-Lima, F. J.; Quiroga, A. G.; Perez, J. M.; Navarro-Ranninger, C. Synthesis and Characterization of New Transplatinum Complexes Containing Phosphane Groups - Cytotoxic Studies in Cisplatin-Resistant Cells. *Polyhedron* **2003**, *22*, 3379–3381.

(31) Ramos-Lima, F. J.; Quiroga, A. G.; Perez, J. M.; Font-Bardia, M.; Solans, X.; Navarro-Ranninger, C. Preparation and characterization of novel trans-[PtCl(2)(amine)(isopropylamine)] compounds: cytotoxic activity and apoptosis induction in ras-transformed cells. *Eur. J. Inorg. Chem.* **2003**, 1591–1598.

(32) Montero, E. I.; Diaz, S.; Gonzales-Vadillo, A. M.; Perez, J. M.; Alonso, C.; Navarro-Ranninger, C. Preparation and Characterization of Novel trans-[PtCl2(amine)(isopropylamine)] Compounds: Cytotoxic Activity and Apoptosis Induction in ras-Transformed Cells. *J. Med. Chem.* **1999**, *42*, 4264–4268.

Synthesis of the water-soluble CD−DDP inclusion compound: In order to obtain the platinum inclusion compound, equimolar quantities of cyclodextrin (113.5 mg, 0.1 mmol) and DDP (44.6 mg, 0.1 mmol) in *N*,*N*′-dimethylformamide (DMF) (7 mL), in which both the cyclodextrin and platinum complex are soluble, were mixed and stirred for about 24 h at room temperature. The solid compound obtained after the evaporation of the solvents by using a rotary evaporator, was rinsed with deionized water and centrifuged to remove the unreacted materials. Finally, the aqueous supernatant was collected and the inclusion compound isolated by freeze-drying the solution and drying at 70 °C under vacuum for 24 h.

Cytotoxic Activity. We have tested the cytotoxic activity of the as-prepared inclusion complex (CD−DDP), DDP, and CPT using two different cancer cell lines CT26 colon carcinoma and B16F10 melanoma. The cytotoxicity was evaluated using the MTT[33] [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide] cell survival assay (colorimetric method), wherein a tetrazolium compound is reduced to a formazan compound by living cells. The cells were plated onto 96-well culture plates at a density of 5×10^3 cells/well. The drug treatment time was 20 h. The cell viability was evaluated by measurement of the absorbance at 570 nm. All experiments were performed six times. The IC_{50} values were defined as the concentration of drug that produces 50% of cell growth inhibition.

(33) Tsoli, M.; Kuhn, H.; Brandau, W.; Esche, H.; Schmid, G. Cellular Uptake and Toxicity of Au55 Clusters. *Small* **2005**, *1*, 841–844.

Conclusions

In conclusion, we have successfully prepared a novel cyclodextrin-encapsulated *trans*-dichloro(dipyridine)platinum(II) complex, so as to improve the water-solubility and the anticancer activity of the DDP compound. The results show that the inclusion complex is of 1:1 type. Interestingly, the growth inhibition by the as prepared CD-DDP against CT26 and B16F10 cell lines in vitro was found to be 4.6 and 6.1, respectively, times higher than the CPT. It is also worthwhile to mention here that the solubility of DDP in water (1.6 mg/mL) and the significantly higher anticancer activity of the DDP could be achieved by the complexation with CD, when compared with the commercial drug CPT.

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Supporting Information Available: SEM images and X-ray diffraction pattern of *trans*-dichloro(dipyridine)platinum(II), β-cyclodextrin, and the inclusion complex. Inhibitory concentration (IC_{50}) (μM) values calculated for cisplatin, *trans*-dichloro(dipyridine)platinum(II), and the inclusion complex for CT26 colon carcinoma and B16F10 melanoma cell lines. This material is available free of charge via the Internet at http://pubs.acs.org.

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