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Toward the Design of Novel Polynuclear Platinum Antitumor Complexes: A Polydentate Ligand System Based on Dipyridylamine and 1,3,5-Trimethylenebenzene

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A novel hexadentate ligand, *N*,*N*,*N*′,*N*′,*N*″,*N*″-hexa(2-pyridyl)-1,3,5-tris(aminomethyl)benzene (**L**), was designed and synthesized. The X-ray structure analysis reveals that the three dipyridylamine (DPA) groups of **L** are almost perpendicular to the central trimethylenebenzene, and two of them are spacially close to each other while the third one is further apart. The trinuclear Pt(II) complexes $[Pt_3LCl_6]$ (**1**) and $[Pt_3L(CBDCA)_3]$ (**2**) (where CBDCA represents cyclobutane dicarboxylic acid) were prepared and fully characterized by IR, NMR, and ESMS spectroscopy. A mononuclear complex, [PtL(CBDCA)] (**3**), was also prepared and structurally characterized, which suggests that controlled formation of mono-, di-, and trinuclear complexes with **L** is possible. Spectroscopic data showed that complexes **2** and **3** are able to bind to calf thymus DNA and their CBDCA group can be readily replaced by thiourea.

Polyamine-bridged polynuclear platinum(II) complexes have attracted considerable attention because they represent a new class of anticancer agents that possess potent and distinct biological activity from cisplatin.[1] A representative compound of the class, BBR3464, has been in clinical trials since 1997. The complex is actually composed of two monofunctional Pt moieties, and the two separated Pt−Cl bonds maintain the bifunctional binding mode on DNA.[2] Encouraged by the success of this compound, many studies have been conducted for the search for novel polynuclear complexes with desired chemical and biological properties.[3]

It has been shown recently that the introduction of bulky planar ligands such as pyridine and substituted pyridines maintained the potent cytotoxicity of the Pt(II) complexes while they significantly reduced the rate of the deactivation by thiol groups.[4] 2,2′-Dipyridylamine (DPA) is an aromatic amine that shares certain similarities to 2,2′-dipyridine; however, the central amine unit introduces more flexibility to the pyridine rings.[5] Although studies on the Pt(II)−DPA system are rare, several DPA complexes of platinum(II) and palladium(II) have demonstrated equal or higher antitumor activity than cisplatin against P388 leukemia cell lines.[6]

In this work, we designed a novel multidentate ligand **L** in which three DPA moieties are linked through a trimethylenebenzene group, a more rigid backbone compared to polyamines. Moreover, the ligand offers a certain degree of flexibility, which provides potential for both intra- and interstrand DNA cross-linking.

Ligand **L** is synthesized from the reaction of 1,3,5-tris(bromomethyl)benzene (TBB) and DPA in 1:3 molar ratio in dimethyl sulfoxide (DMSO) solution with the presence of excessive potassium hydroxide (Scheme 1 and Supporting Information). The compound is fully characterized by FT-IR, elemental analysis, ESMS, NMR and X-ray crystallography.

The ^{1}H NMR spectrum of **L** in $CDCl_3$ shows six signals at 8.22d, 7.40t, 7.09s, 6.92d, 6.81t, and 5.34s ppm which can be assigned, respectively, to the protons of the three equivalent DPA, benzyl, and methylene groups, suggesting these groups are free of rotation in solution. As expected,

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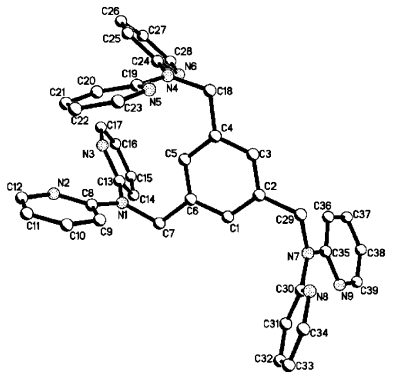
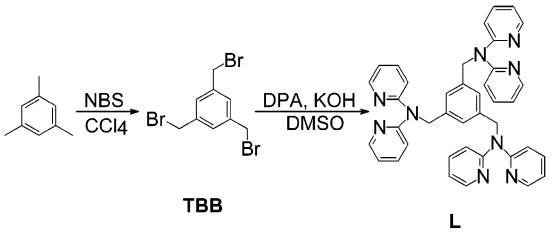


Figure 1. Molecular structure and labeling scheme of **L**.

Scheme 1. Synthetic Pathway of Ligand **L**



eight signals were observed at 156.7, 147.8, 139.2, 137.4, 123.8, 117.1, 114.7, and 51.4 ppm in the ^{13}C NMR spectrum.

Figure 1 shows an ellipsoid plot of the crystal structure of **L** with the numbering scheme. It can be noted that the three DPA groups are almost perpendicular to the central trimethylenebenzene, and two of them are spacially close to each other while the third one is further apart. The dihedral angles between the two pyridyl rings in each DPA group are 131.1°, 127.0°, and 128.2°, respectively.

The reaction of **L** with $K_2[PtCl_4]$ or $[Pt(dmso)_2CBDCA]$[7] in 1:3 molar ratio gives readily formation of $[Pt_3LCl_6]$ (**1**) or $[Pt_3L(CBDCA)_3]$ (**2**) (See Supporting Information for details) in quantitative yield. The ^{1}H NMR spectroscopy of **1** in DMSO-d_6 shows that the three DPA moieties maintain their equivalency upon coordination to platinum; the chemical shifts are 8.83d, 8.03s, 7.71t, 7.30d, 7.25t, and 5.34s ppm, respectively. The aryl proton signals are shifted to lower field compared to **L** which suggested the coordination of DPA groups to platinum. The ESMS spectrum of **1** contains three major peaks at *m*/*z* values of 1389.9, 1448.7, and 1463.7 which can be assigned to $[Pt_3LCl_5]^+$, $[Pt_3LCl_6+Na]^+$, and $[Pt_3LCl_6+K]^+$, respectively. Similarly, the ^{1}H NMR spectrum of **2** suggested the presence of three equivalent Pt(II)-bound DPA groups, and its ESMS spectrum contains one major peak with an *m*/*z* value of 1662.1, assignable to $[Pt_3L(CBDCA)_3 + Na]^+$ (Supporting Information).

The UV spectra of complexes **1** and **2** show the absorption bands at 304 and 306 nm, respectively, which can be attributed to the metal to ligand charge transfer absorption (MLCT).

In order to investigate the possibility of the stepwise coordination of **L**, the reaction of $[Pt(dmso)_2CBDCA]$ with **L** in 1:1 molar ratio in a refluxed methanol solution was conducted, which gave the formation of [PtL(CBDCA)] (**3**). In the ^{1}H NMR spectrum of **3**, two sets of signals with an integral ratio of ca. 1:2 were observed, suggesting that the coordination of Pt(II) to one of the DPA groups resulted in their inequivalency (Supporting Information).

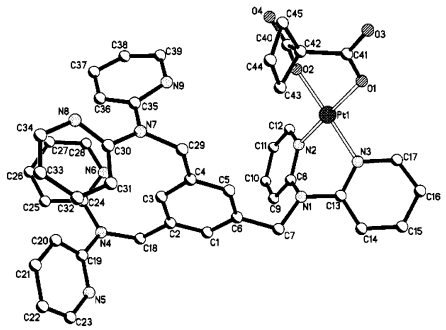


Figure 2. Molecular structure and labeling scheme of complex **3**.

The ellipsoid plot with the numbering scheme of **3** is shown in Figure 2. The Pt(II)-bound DPA group in **3** is situated above the central trimethylenebenzene plane, while the other two DPA groups remain almost the same as observed in **L**. The Pt(II) center is coordinated in a square planar geometry, and the CBDCA moiety displays a similar conformation to that described in the literature.[8,9] The two six-membered chelate rings which DPA and CBDCA formed with the Pt(II) atom adopt a boat conformation. The cyclobutane ring, which is nearly perpendicular to the Pt(II) coordination sphere, is distorted significantly from the best plane of C42−C43−C44−C45 with the dihedral angle between C42−C43−C44 and C42−C45−C44 being 16.1°.

In order to study the potential DNA binding ability of **1**, **2**, and **3**, their reactions with calf thymus (CT) DNA were followed by the UV method. Upon addition of an increasing amount of DNA (10^{-5}−10^{-4} M) to the complexes (10^{-5} M), 40% and 9% hyperchromisms were observed for **2** and **3** at 306 and 305 nm, respectively, which suggested possible interactions between complexes **2** and **3** with DNA. However, no obvious change was found for complex **1**. A similar hyperchromism was previously observed for copper complexes,[10] which was attributed to the dissociation of complex aggregates and the breakage of intermolecular hydrogen bonds when bound to DNA. A time-dependent UV experiment for the reaction of complex **3** with DNA (Figure 3) showed that, upon addition of a fixed amount of DNA (1.3×10^{-4} M) to the solution of complex **3** (6.7×10^{-5} M), a very slight hyperchromism was observed at 305 nm initially, followed by the significant decrease in intensity. After 24

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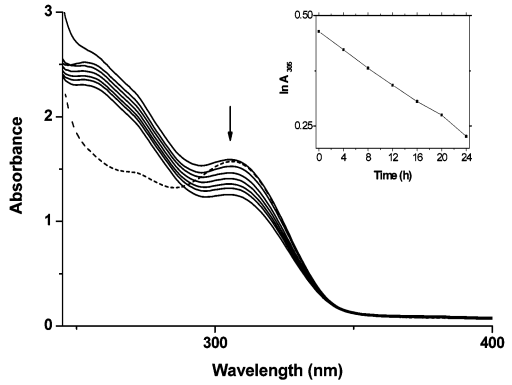

Figure 3. Time dependent absorption spectra of **3** (6.7×10^{-5} mol·L^{-1}) in the absence (- - -) and presence (—, represent 0, 4, 8, 12, 16, 20, and 24 h, respectively) of a fixed amount of CT DNA (1.3×10^{-4} mol·L^{-1}) at 37 °C. Inset shows decrease in absorbance at 305 nm (ln *A*) with time for the reaction of **3** with DNA.

h, a 21% hypochromism was observed which is much larger than that observed for potential DNA intercalators,[11] which suggests that complex **3** is able to bind to DNA strongly. The platinum(II) in complex **3** could bind covalently to the nucleobases such as guanine in DNA upon leaving the CBDCA group, and the four uncoordinated pyridine groups could form hydrogen bonding with or intercalate into DNA. As shown in the crystal data, the distances between Pt1−N4 and Pt1−N7 are 9.043 and 7.089 Å, respectively, providing the possibility of both binding modes simultaneously.

The fluorescent experiments showed that, upon titrating the solution of **2** or **3** (20 μL per scan) into the samples containing 4.9×10^{-5} M of DNA and 4.9×10^{-5} M of ethidium bromide (EB), the emission band at 600 nm of the DNA−EB system decreased in intensity with increasing the concentration of the Pt(II) complexes, which indicates that the complexes can replace EB from the DNA−EB system. Such a characteristic change is often observed in the intercalative DNA interaction.[10c] The data further suggested that intercalation may also be an alternative binding mode for the complexes.

The CBDCA bound to Pt(II) such as that in carboplatin is shown to be less susceptible to hydrolysis compared to chloride. Sulfur-containing molecules such as L-methionine have been suspected to be involved in the activation of carboplatin.[12] Transfer of Pt onto DNA via sulfur-containing peptides or proteins may also be possible.[13] Therefore, the reaction of **3** and thiourea was conducted in order to investigate the potential replacement of CBDCA. As shown by ^{1}H NMR spectroscopy (Supporting Information), the signals for free CBDCA were observed after 30 min and increased in intensity with time, suggesting that CBDCA can be readily substituted by thiourea. No ring-opened monodentate intermediate was observed under the NMR conditions used here, which indicates the fast replacement of CBDCA by the second thiourea.

In conclusion, the novel polydentate ligand designed in this work provides the possibility for the synthesis of mono- and polynuclear platinum complexes which could bind strongly to DNA and thereby be antitumor active. The rigidity of the backbone offers the possibility for designed length of DNA cross-linking, while the flexibility of the DPA groups provides the possibility for DNA intercalation. Moreover, the uncoordinated DPA moieties furnish the potential binding sites for metal ions such as Cu(II) and Ru(II), giving rise to formation of heteromultinuclear complexes that possess antitumor potential. Further work on the biological properties of this ligand and its metal compounds is currently ongoing in our group.

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Supporting Information Available: Crystallographic data of **L** and complex **3**. Experimental procedures for the preparation of **L** and **1**−**3** and their ESMS, NMR, UV−vis, and fluorescent spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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