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Synthetic Route to Dinuclear Platinum(II) Complexes $[\{trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-}L)]^{2+}$ (L = Aliphatic or Heterocyclic Diamine) as Potential Antitumor Agents, Exploiting the Mutual Activation of Hydroxido Ligands and Ammonium Groups

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A simple and efficient method for the synthesis of potentially antitumor-active dinuclear platinum complexes of the general formula $[\{trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-}L)]^{(n+2)+}$ (L = aliphatic or heterocyclic diamine; n = charge of L) is presented. The procedure is based on the mutual in situ activation of *trans*-$[PtCl(OH)(NH_3)_2]$ and the linker L in the form of a diammonium salt. This synthetic pathway yielded the Farrell compound $[\{trans\text{-}PtCl(NH_3)_2\}_2\{\mu\text{-}NH_2(CH_2)_6NH_2\}]Cl_2$ (BBR3005) in quantitative yield. Using the same procedure, we prepared the new pyrazolate-bridged compound $[\{trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-pz})]Cl$, determined its X-ray structure, and tested its cytotoxicity against three wild-type and one cisplatin-resistant cell lines.

Introduction

The discovery of the antitumor drug cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) has brought about a revolution in the treatment of testicular cancers. The latter were practically incurable before the advent of cisplatin; nowadays, over 90% of testicular cancers can be cured.[1] This success triggered intense efforts to find other platinum complexes that would be highly active against other types of cancer or that may be engaged in the cases where the patient develops resistance to cisplatin. Because the antitumor activity of platinum compounds is believed to derive from their ability to react with DNA and to cross-link DNA bases, the prevalent working hypothesis supposes that platinum agents forming cross-links structurally different from cisplatin−DNA cross-links would be differently recognized by cellular proteins and thus would have the potential to induce cell death via different pathways.[2−5]

One interesting class of new compounds being developed to form distinct Pt−DNA cross-links are dinuclear complexes in which two platinum centers are bridged by an aliphatic or heterocyclic diamine linker (reviewed in refs 2, 3, and 6). Some examples with two monofunctional terminal platinum centers (i.e., having one leaving group) are shown in Chart 1. Complexes such as **2**−**5** with longer, flexible linkers are likely to form with DNA long-range intrastrand and interstrand cross-links,[7−10] whereas compounds such as **6** have a rigid linker and a fixed distance between the

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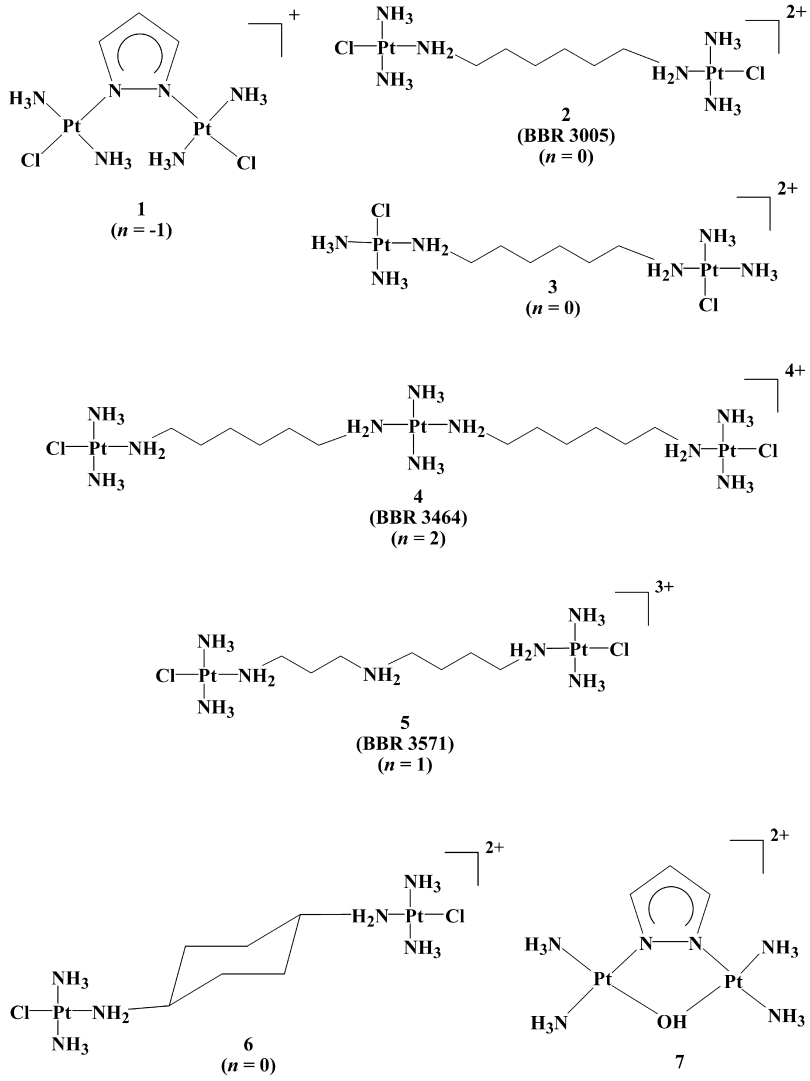
Chart 1. Dinuclear Complexes of the General Formula $[\{PtCl(NH_3)_2\}_2(\mu\text{-L})]^{(n+2)+}$ (L = Aliphatic or Heterocyclic Diamine; n = Charge of L) and the Cyclic Hydroxido-Bridged Complex **7** of Komeda et al.



platinum NH_2 ligands, thus imposing short-range (intrastrand or interstrand) cross-links.[6] Compound **7** was designed to cross-link two adjacent bases without bending the DNA helix.[11−13] Cytotoxicity tests have revealed that compounds having two $PtCl(NH_3)_2^+$ moieties bridged by a longer flexible linker, such as **2**−**5**, are the most strongly cytotoxic against leukemia L1210 cancer cell lines;[2,6] interestingly, complexes with trans geometry circumvented cisplatin resistance, whereas cis compounds did not.[14] Apparently, cross-linking of DNA bases by these trans-configurated dinuclear compounds creates structural perturbations that are processed following a different pathway than those induced by cisplatin. We report here an elegant procedure for the synthesis of such dinuclear complexes of the general formula $[\{trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-L})]^{(n+2)+}$ where L is an aliphatic or heterocyclic diamine (which may or may not contain other functional groups) and n is the charge of L. The synthesis uses a new synthon, the hydroxido complex *trans*-$[PtCl(OH)(NH_3)_2]$, which can be isolated in solid state,[15] and the diamine in the form of a protonated salt. We present two tests for this synthetic scheme. In the first, we show that the Farrell compound **2** can be prepared more easily and more efficiently than using the published procedure.[16] In the second, we used our synthetic scheme to prepare the new complex **1**. This

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new compound was characterized using X-ray crystallography and tested for cytotoxicity against four different cancer cell lines.

Experimental Section

***trans*-[PtCl(OH)(NH$_3$)$_2$]·H$_2$O.** Improved yield and purity were achieved by a modification of the published method[15] as follows. Finely ground *trans*-[PtCl$_2$(NH$_3$)$_2$] (1 mmol, 300 mg) was slowly heated in 100 mL of degassed water under argon to 65 °C (measured inside the reaction vessel) until complete dissolution (ca. 20 min), whereafter NaOH (1.5 equiv, 130 μL of a freshly prepared 35% solution) was added dropwise. The water bath was removed and stirring maintained during 20 min. The solution was quickly concentrated to 0.5−1 mL on a rotary evaporator equipped with a powerful vacuum pump (bath temperature: ≤55 °C), transferred into a microtube, and centrifuged. Upon cooling on ice of the supernatant, *trans*-[PtCl(OH)(NH$_3$)$_2$]·H$_2$O separated as pale-yellow-green crystals. The crystals were washed two times with 0.5 mL of cold water/ethanol (1:1) and dried on filter paper. Yield: 44%. The crystals were stored in a refrigerator protected from light and used within a few days.

[{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-NH$_2$(CH$_2$)$_6$NH$_2$}]Cl$_2$·0.25Acetone. Caution! *Salts with high nitrogen content are known to be explosive, so precaution is advisable and exposure of the solid diammonium salt to heat should be avoided.* 1,6-Hexanediammonium dinitrate[17] (0.31 mmol, 75 mg) was added to a suspension of *trans*-[PtCl(OH)(NH$_3$)$_2$]·H$_2$O (185 mg, 2 equiv) in 100 mL of *N*,*N*-dimethylformamide (DMF). The pale-yellow suspension was stirred at room temperature under argon, protected from light. After 2 days, a clear solution was obtained. DMF was evaporated under reduced pressure, giving a yellowish-white precipitate. ^{1}H and ^{195}Pt NMR spectra in D$_2$O indicated quantitative formation of [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-NH$_2$(CH$_2$)$_6$NH$_2$}]$^{2+}$. Recrystallization from 0.1 N HCl/acetone yielded the pure compound as a chloride salt. Anal. Calcd for C$_6$H$_{28}$Cl$_4$N$_4$Pt$_2$·0.25acetone: C, 11.09; H, 4.07; N, 11.50; Cl, 19.40. Found: C, 11.01; H, 4.13; N, 11.86; Cl, 19.22. ^{195}Pt NMR (53.7 MHz, D$_2$O, Na$_2$PtCl$_6$): δ −2440 (s).

Pyrazolium Nitrate. Caution! *Salts with high nitrogen content are known to be explosive, so precaution is advisable and exposure of the solid sample to heat should be avoided.* To pyrazole (8.74 mmol; 595 mg) in 10 mL of CHCl$_3$/ethyl acetate (1:1) was added 65% HNO$_3$ (1.6 mL, 1.5 equiv) dropwise. The mixture was stirred on ice for 30 min. The precipitate formed was washed two times with the solvent mixture and crystallized from acetone (yield 56%). Anal. Calcd for C$_3$H$_5$N$_3$O$_3$: C, 27.49; H, 3.84; N, 32.05. Found: C, 27.6; H, 3.8; N, 32.0. ^{1}H NMR (250 MHz, CD$_3$OD, TMS): δ 6.77 (t, J = 2.0 Hz, 1 H), 8.20 (d, J = 2.0 Hz, 2 H).

[{*trans*-PtCl(NH$_3$)$_2$}$_2$(μ-pz)]Cl·3H$_2$O (1). Pyrazolium nitrate (0.14 mmol, 18 mg) in 100 mL of acetone/dichloromethane (3:1) was added to powdered *trans*-[PtCl(OH)(NH$_3$)$_2$]·H$_2$O (78 mg, 1.86 equiv). The pale-yellow suspension was stirred at 70 °C, protected from light and moisture for 5 days. After cooling, the yellowish-white product was scrapped from the wall, recovered by filtration, and recrystallized from 0.1 M HCl. Crystals were filtered off and washed with 2 × 1 mL of acetone (yield 54%). Anal. Calcd for C$_3$H$_{15}$Cl$_3$N$_6$Pt$_2$·3H$_2$O: C, 5.25; H, 3.09; N, 12.25; H$_2$O, 7.88. Found: C, 5.5; H, 3.2; N, 12.0; H$_2$O (Seiko TG-DTA 320), 7.2, released between 320 and 360 K at a heating rate of 3 K min^{-1}. ^{1}H NMR (250 MHz, 0.1 M DCl, TMS): δ 6.42 (t, J = 2.0 Hz, 1 H), 7.66 (d, J = 2.0 Hz, 2 H). ^{195}Pt NMR (53.7 MHz, 0.1 M DCl, Na$_2$PtCl$_6$): δ −2267 (s).

X-ray Structure Analysis of 1. X-ray data were collected on an APEX II CCD diffractometer using the ω scan method and applying an empirical absorption correction.[18] The structure was solved by direct methods and refined by full-matrix least squares on F^2 using the *WINGX* interface.[19,20] Anisotropic displacement parameters were used for all atoms except the hydrogens (in calculated positions), treated as riding atoms. Hydrogen atoms of water molecules were not located from the difference Fourier map. See Table S1 in the Supporting Information for details. The crystallographic data were deposited as entry CCDC 648612. They can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/datarequest/cif.

Cytotoxicity Tests. A total of 96 well plates were seaded at J0 with 4500 (SKBr3), 1000 (Hela), 2800 (L1210/0), or 2800 (L1210/CDDP) cells per well in DMEM, 10% SVF, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (Gibco Invitrogen Corp., Paisley, U.K.). Cells were maintained at 37 °C in an atmosphere containing 5% CO$_2$, in a humidified incubator. At J1, potential inhibitors were added at appropriate concentrations. The complexes were dissolved in water (or predissolved in dimethyl sulfoxide (DMSO) and then diluted with water to a final concentration of DMSO of 0.2%) just before addition to the cells, and the drugs were left in contact with the cells for the full period of 48 h (J3), 72 h (J4), or 96 h (J5). After drug exposure, 10 μL of WST1 reagent was added into each well. The optical density was read at 450 nm, and the optical density at 630 nm was removed. Each point was repeated eight times. The treatment times were standard times used for cytotoxicity tests using WST1. To determine the IC$_{50}$ values, *GraphPad* software was used (GraphPad Software, Inc., San Diego, CA).

Results

In Situ Mutual Activation of *trans*-[PtCl(OH)(NH$_3$)$_2$] and a Diammonium Salt. The proposed synthesis of dinuclear platinum complexes featuring two *trans*-PtCl(NH$_3$)$_2$$^+$ centers bridged by a diamine is shown in Scheme 1. It is based on the facile isolation of the hydroxido complex *trans*-[PtCl(OH)(NH$_3$)$_2$]. The fortuitous stability of this compound[15] in the solid state offers the possibility of reacting it with $^1/_2$ equiv of a diamine linker in the form of a diammonium salt. Such salts are much more conveniently handled and quantified than the unprotonated diamines. The ammonium groups and the coordinated hydroxido ligands neutralize in situ, whereby the inert ammonium group is activated by deprotonation and the inert hydroxido ligand becomes H$_2$O, an excellent leaving group. Thus, although both isolated reactants are relatively stable, they become reactive toward each other when mixed.

Synthesis of BBR3005 (2). In the first test, we prepared the Farrell compound BBR3005[16] (**2**) in quantitative yield. The classical synthesis of this compound from transplatin and 1,6-hexanediamine suffers from the simultaneous formation of the mononuclear complex *trans*-[Pt(NH$_3$)$_2$(1,6-hexanediamine)]$^{2+}$, where the diamine acts as a trans chelator.[16] Our preparation from *trans*-[PtCl(OH)(NH$_3$)$_2$] and 1,6-

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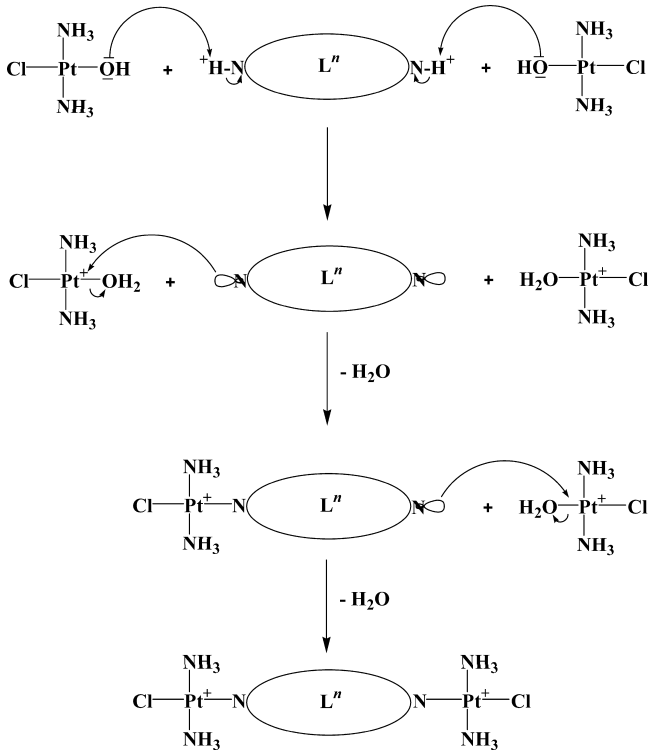
Scheme 1



hexanediammonium nitrate in DMF yields only the desired dinuclear compound, even in a diluted (6 mM) solution where the intramolecular chelation is, in principle, favored over the bimolecular reaction. This demonstrates the efficiency of our novel synthetic route and the superiority of the synthon *trans*-[$PtCl(OH)(NH_3)_2$] over *trans*-[$PtCl_2(NH_3)_2$].

Synthesis and Structure of 1. In the second test, we synthesized the novel pyrazolato (pz^-)-bridged compound [{*trans*-$PtCl(NH_3)_2$}$_2$(μ-pz)]$^+$ (**1**). **1** was considered an interesting candidate for testing as an antitumor agent because it is expected to cross-link two adjacent or nearby guanine bases of DNA, as does cisplatin or the recently reported cytotoxic pyrazolate-bridged compound [{*cis*-$Pt(NH_3)_2$}$_2$(μ-OH)(μ-pz)]$^{2+}$ (**7**)[13] but inducing a different structural distortion. **1** was prepared according to Scheme 1 from *trans*-[$PtCl(OH)(NH_3)_2$] and pyrazolium nitrate. Recrystallization from 0.1 N HCl yielded the chloride salt, [{*trans*-$PtCl(NH_3)_2$}$_2$(μ-pz)]Cl·$3H_2O$, isolated as crystals suitable for X-ray diffraction analysis. The molecular structure of the complex cation is shown in Figure 1 (see Table S2 in the Supporting Information for a list of bond lengths and bond angles). Noteworthy is the orientation of the platinum coordination planes, which are not perpendicular to the plane of the pyrazolate ring, as could have been expected for minimal steric repulsions; the dihedral angles between the platinum coordination planes and that of the pyrazolate are 54.8(2)° and 55.6(2)°, respectively. A weak "nonclassical" interaction between each platinum center and the proximal (N)−hydrogen atom (distance ~2.8 Å) may cause this distortion.[21,22] An extensive network of hydrogen bonds involving the H_2O molecules and the Cl^- ions (distances in the range 2.8−3.2 Å) completes the molecular packing.

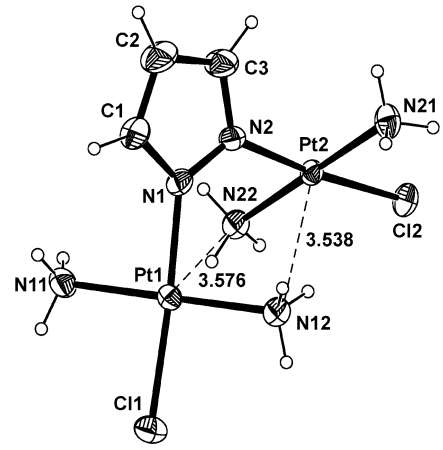


Figure 1. ORTEP view of the complex cation of **1**.

Cytotoxicity Tests of 1. The cytotoxicity of **1** (chloride salt) was tested against three common cisplatin-sensitive (SKBr3, HeLa, and L1210/0) and one cisplatin-resistant (L1210/CDDP) cancer cell lines. L1210 is a classical model frequently used in cytotoxicity tests; Hela is a cervical cancer cell line expressing the p53 tumor suppressor, and SKBr3 is a breast cancer cell line expressing the HER2 protein. Table 1 displays IC_{50} values for **1** and cisplatin. Although **1** clearly does inhibit the growth of these cancer cells, its molar activity is 1.5−3 orders of magnitude below that of cisplatin. Moreover, **1** is significantly cross-resistant with cisplatin. This is interesting in view of the fact that the related complex having a cis configuration of the NH_3 ligands, **7**, showed virtually no cross-resistance with cisplatin when tested against L1210 cancer cells.[23]

Discussion

The synthetic route reported here (Scheme 1) can be used for the convenient preparation of many dinuclear complexes [{*trans*-$PtCl(NH_3)_2$}$_2$(μ-L)]$^{(n+2)+}$ with a variety of diamine linkers L. The main advantage of this synthesis is that the two reactants, which can be conveniently stored and quantified as solids, activate each other only upon mixing. In contrast to what could be expected, water, methanol, and acetonitrile proved not to be ideal solvents for the coupling reaction; in these solvents, we observed the formation of minor products detectable by NMR, which were difficult to remove. These coordinating solvents can replace the chlorido ligand of platinum, giving rise to labile species that can react with free or monofunctionally coordinating diamine still present in solution. We therefore suspect that the observed byproducts were oligomeric species. To limit oligomerization reactions, we thus worked with less coordinating solvents (acetone/dichloromethane for **1** and DMF for BBR3005) and preferred diluted solutions. In the case of BBR3005, the diluted solutions implied, of course, the risk of favoring the formation of the mononuclear compound *trans*-[$Pt(NH_3)_2$(1,6-

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Table 1. In Vitro Toxicity of **1** (Chloride Salt and Trihydrate) and Cisplatin

		inhibitory concentration IC_{50} [μM][a]					
compound	MW	$SKBr_3$ (48 h)	$SKBr_3$ (72 h)	HeLa (96 h)	L1210/0 (96 h)	L1210/CDDP (96 h)	resistance ratio (L1210)
1	686	42 ± 22	13 ± 5	10 ± 5	5.1 ± 1.4	44 ± 25	9
cisplatin	300	1.9 ± 0.6	0.19 ± 0.06	0.005 ± 0.003	0.016 ± 0.006	3.1 ± 0.8	194

[a] The time of exposure to the drug is indicated in parentheses.

hexanediamine)]$^{2+}$, where the diamine acts as a trans chelator;[16] however, even in a 6 mM solution in platinum, this compound was not detectable by NMR. For recrystallization and for spectroscopic measurements, the products were dissolved in 0.1 M HCl rather than in pure water, in order to limit the extent of hydrolysis. For cytotoxicity tests, we first dissolved the sample in DMSO and then diluted the solution with water immediately before use.

The counterion of the diammonium salt can be selected to warrant solubility in the chosen solvent; however, it is preferable to use counterions that do not coordinate platinum. Once formed, the dinuclear product **1** was, nevertheless, resistant to 0.1 M NaCl even upon prolonged heating at 40 °C (no change of the ^{1}H NMR spectrum after 1 week at 40 °C in the dark).

Our synthetic procedure exploits the fact that *trans*-[PtCl(OH)$(NH_3)_2$]·H_2O can be obtained in pure crystalline form.[15] The cis analogue, *cis*-[PtCl(OH)$(NH_3)_2$], may be much more difficult to prepare because of the facile formation of the dimer [{*cis*-Pt(OH)$(NH_3)_2$}$_2$]$^{2+}$. However, other platinum hydroxido synthons may be obtainable. Anyway, the basic idea of reacting a hydroxido metal complex with a salt of a protonated amine ligand may deserve attention as a general method for the synthesis of metal complexes with amine ligands.

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Supporting Information Available: Tables of crystal data and structure refinement and bond lengths and angles for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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