# *cis*-Diammine(pyridine)chloroplatinum(II), a monofunctional platinum(II) antitumor agent: Uptake, structure, function, and prospects

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**We have identified unique chemical and biological properties of a cationic monofunctional platinum(II) complex, *cis*-diammine(pyridine)chloroplatinum(II), *cis*-[Pt(NH$_3$)$_2$(py)Cl]$^+$ or cDPCP, a coordination compound previously identified to have significant anticancer activity in a mouse tumor model. This compound is an excellent substrate for organic cation transporters 1 and 2, also designated SLC22A1 and SLC22A2, respectively. These transporters are abundantly expressed in human colorectal cancers, where they mediate uptake of oxaliplatin, *cis*-[Pt(DACH)(oxalate)] (DACH = *trans-R,R*-1,2-diaminocyclohexane), an FDA-approved first-line therapy for colorectal cancer. Unlike oxaliplatin, however, cDPCP binds DNA monofunctionally, as revealed by an x-ray crystal structure of *cis*-{Pt(NH$_3$)$_2$(py)}$^{2+}$ bound to the N7 atom of a single guanosine residue in a DNA dodecamer duplex. Although the quaternary structure resembles that of B-form DNA, there is a base-pair step to the 5′ side of the Pt adduct with abnormally large shift and slide values, features characteristic of cisplatin intrastrand cross-links. cDPCP effectively blocks transcription from DNA templates carrying adducts of the complex, unlike DNA lesions of other monofunctional platinum(II) compounds like {Pt(dien)}$^{2+}$. cDPCP–DNA adducts are removed by the nucleotide excision repair apparatus, albeit much less efficiently than bifunctional platinum–DNA intrastrand cross-links. These exceptional characteristics indicate that cDPCP and related complexes merit consideration as therapeutic options for treating colorectal and other cancers bearing appropriate cation transporters.**

cancer therapy | nucleotide excision repair | organic cation transporter | RNA polymerase II inhibition | x-ray crystal structure

The recent surge in clinical trials involving platinum anticancer drugs reflects the efficacy and success rate of cisplatin, the first and most potent member of the class (1). Most recently, the FDA approved oxaliplatin as a first-line therapy for the treatment of colorectal cancer (2). Since the advent of platinum-based cancer therapy, several active compounds that violate the classical structure–activity relationships for cisplatin have been identified, including platinum(IV) (1) and polynuclear platinum complexes (3), and platinum compounds with a trans stereochemistry (4). In this report, we describe the results of structural and mechanistic investigations of *cis*-diammine(pyridine)chloroplatinum(II) (cDPCP, Fig. 1). The anticancer activity of this cationic complex was established in murine tumor models almost 20 years ago (5, 6). However, perhaps because, unlike cisplatin, cDPCP forms monofunctional rather than bifunctional lesions with DNA, or because as a cation it was not expected to cross cell membranes readily, the compound was never tested in humans. Here, we describe experiments revealing (*i*) uptake of cDPCP mediated by organic cation transporters (OCT) 1 and 2, (*ii*) the x-ray crystal structure of a DNA dodecamer duplex containing a monofunctional adduct of the complex bound to a central guanosine residue, (*iii*) the ability of cDPCP–DNA adducts to inhibit transcription, and (*iv*) reduced repair of cDPCP–DNA adducts by the mammalian excinuclease relative to those of cisplatin. The latter two cellular responses (*iii* and *iv*) are crucial to the anticancer activity of cisplatin and oxaliplatin (7).

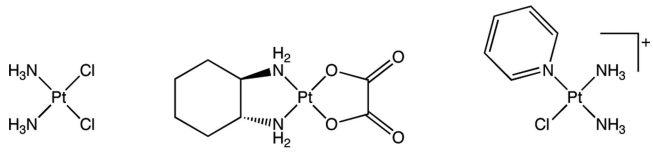


**Fig. 1.** Chemical structures of cisplatin, oxaliplatin, and cDPCP.

Cellular entry represents the first step in the mechanism of action of platinum anticancer agents. Relative differences in influx and efflux contribute to drug resistance and are a primary factor in the differential response of various tumor types to the drugs (8, 9). Copper transporters, including CTR1, facilitate cellular uptake of cisplatin and oxaliplatin, but their importance in mediating the anticancer activity of these compounds is uncertain (10). Recently, we identified OCT1 and OCT2 as critical mediators of oxaliplatin transport and toxicity in human tissue. We detected mRNA from OCT1 in 20 of 20 tumor samples from colon cancer patients and mRNA from OCT2 in 11 of 20 samples (11). There are three members of the human OCT family: hOCT1 is expressed primarily in the liver; hOCT2 in the kidney; and hOCT3 in the liver, heart, brain, and placenta (12). All are present in the intestinal/colorectal area to varying degrees (11, 13). OCTs interact with organic substrates having $M_r$ <400 Da and overall positive charge (12). Oxaliplatin, a neutral compound, is therefore transported only after loss of the oxalate group to form mono- or dicationic species. Although oxaliplatin and cisplatin form similar adducts on DNA, cisplatin is a poor substrate for OCT1 and OCT2 compared with oxaliplatin (11) and is not active against colorectal cancer. These properties suggest that OCT-mediated uptake of oxaliplatin into cancer cells is a major determinant of its activity.

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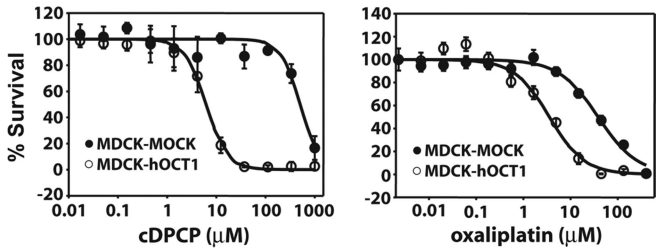
During the course of structure–function studies of Pt-based OCT substrates, we identified the monofunctional cationic complex cDPCP to have outstanding properties (see below). The antitumor properties of cDPCP were initially established in a sarcoma 180 ascites murine model study (5). The compound was subsequently shown to block DNA replication at single dG sites by replication mapping experiments, and a series of biochemical experiments established that cDPCP forms a fundamentally different adduct profile than the bifunctional cross-links of cisplatin or oxaliplatin (6). Concern over the potential lability of heterocyclic N-donor ligands, such as pyridine, in Pt(II) complexes, has led to extensive investigations of the stability of their DNA adducts. Although some complexes containing three N-donor ligands, such as *N*-methyl-2,7-diazapyrenium, do form bifunctional adducts after loss of the heterocycle (14), neither pyridine nor ammonia dissociates from cDPCP upon DNA binding (6, 14).

cDPCP is one of a few monofunctional platinum(II) compounds that is active against tumor cells; most complexes in this class, including [Pt(dien)Cl]Cl, dien = diethylenetriammine, and the trans isomer of cDPCP, have limited to no cytotoxicity (5). Exceptions include $[PtIA_2L]I$ complexes, where $A_2$ = 2,9-dimethyl-1,10-phenanthroline and L is an N-donor heterocyclic ligand. The compound in which L = 1-methylcytosine is more toxic than cisplatin against a panel of 11 cell lines (15). Of significance in the present context is the observation that the greatest differential cytotoxicity occurred for the three colon cancer lines among the 11 that were investigated.

Our primary objective in the present study was to understand more fully the molecular mechanism of action of cDPCP. The four early phases by which platinum compounds exert their anticancer activity are cellular entry, activation (typically by aquation), DNA binding, and the initial cellular responses to the DNA damage (7). We were therefore interested to determine whether cDPCP would improve upon the specificity of oxaliplatin for entry into cells expressing OCTs while sharing or exceeding the potency of cisplatin. We examined cellular accumulation of cDPCP vs. oxaliplatin because of organic cation transporters and the effect of transporter expression on the sensitivity of mammalian kidney cells to the compound. To determine the influence of cDPCP on DNA geometry, we constructed a site-specifically platinated dodecamer duplex containing a centrally located *cis*-$\{Pt(NH_3)_2(py)\}^{2+}$–dG adduct and determined its structure by x-ray crystallography. The consequences of cDPCP binding to a plasmid DNA were also studied. To investigate processing of cDPCP and cisplatin adducts by DNA damage-recognition proteins, we used an *in vitro* system to study repair of monofunctional platinum lesions by nucleotide excision repair (hereafter "excision repair") and a cell-based $\beta$-galactosidase reporter assay to study the ability of the adducts to inhibition transcription by RNA polymerase II. The results of these experiments provide compelling evidence that cDPCP and related compounds are excellent prospects for further preclinical and clinical investigations as anticancer drugs.

## Results

**Cellular Accumulation and Pt-Induced Loss of Viability in OCT+/− Cells.** Mammalian cells stably transfected with a full-length human OCT1 or OCT2 cDNA, or transfected with an empty vector as a control, were used to study the cellular accumulation and cytotoxicity of cDPCP and oxaliplatin. cDPCP is 87-fold more cytotoxic in OCT1(+) than OCT1(−) cells, whereas oxaliplatin was only 12-fold more effective. The cytotoxicity of cDPCP in OCT2(+) cells increased by a factor of 137 over OCT2(−) cells, compared with a 53-fold increase with oxaliplatin (Fig. 2). Examination of treated cells for platinum content revealed that accumulation of cDPCP is 68-fold higher in hOCT2-containing cells than in cells not expressing the transporter. In hOCT1-containing cells, a 23-fold increase in platinum accumulation was measured [supporting information (SI) Table S1]. The corresponding numbers for oxaliplatin were 23-fold for hOCT2 and 4.7-fold for hOCT1. Measurements of platinum levels on DNA after cDPCP treatment were not obtained, but DNA platination by oxaliplatin closely tracks its accumulation in cells expressing hOCT1 and hOCT2 and can be reversed by OCT1 and OCT2 inhibitors (11).




$IC_{50}$ values (μM)

| | MDCK-MOCK | MDCK-hOCT1 | Fold Change |
|---|---|---|---|
| cDPCP | 704 ± 280 | 8.1 ± 1.2 | 87 |
| Oxaliplatin | 40.5 ± 1.6 | 3.3 ± 1.0 | 12 |
| | HEK-MOCK | HEK-hOCT2 | |
| cDPCP | 206 ± 56 | 1.50 ± 0.22 | 137 |
| Oxaliplatin | 4.0 ± 1.3 | 0.075 ± 0.009 | 53 |

**Fig. 2.** Cellular response to cDPCP and oxaliplatin. (*Upper*) Cell growth inhibition assays for cDPCP (*Left*) and oxaliplatin (*Right*) in MDCK cells with and without hOCT1. (*Lower*) $IC_{50}$ values for both compounds in MDCK-OCT1 vs.-MOCK and HEK-OCT2 vs. -MOCK cells, expressed as mean ± SD from three experiments, with quadruplicate measurements obtained in each experiment.

**DNA-Binding Characteristics of cDPCP.** ***X-ray structure.*** We synthesized a DNA dodecamer duplex containing a site-specific *cis*-$\{Pt(NH_3)_2(py)\}^{2+}$–dG adduct, crystallized the compound, and collected x-ray diffraction data to 2.17-Å resolution (Table 1). The structure was solved by using phases obtained from single-wavelength anomalous diffraction data from the Pt atom. There is one molecule in the asymmetric unit and a solvent content of 56%. The structure, depicted in Fig. 3, contains linear, B-form DNA (Tables S2 and S3) with platinum coordinated to N7 of guanine in the major groove. Watson–Crick hydrogen bonding between the base pairs is maintained throughout the dodecamer duplex. The double helix is unwound by ≈8° in the vicinity of the platination site, a value in agreement with previous NMR spectroscopic results of a heptamer duplex containing the analogous *cis*-$\{Pt(NH_3)_2(4\text{-Me-py})\}^{2+}$–dG adduct (16).

The pyridine ligand of the *cis*-$\{Pt(NH_3)_2(py)\}^{2+}$ moiety is directed toward the 5′ end of the platinated strand (Figs. 3 and 4). This orientation facilitates formation of a hydrogen bond between the $NH_3$ ligand trans to pyridine and O6 of the guanosine residue (N···O distance, 2.8 Å). Analysis of the structure reveals that the platinated G-C base pair is displaced toward the major groove to accommodate the platinum adduct. The result is a base pair step to the 5′ side of the platinum adduct with abnormally large shift and slide values of 1.20 and 0.82 Å, respectively (Fig. 3 and Table S2). Marzilli *et al.* (17) first identified this structural feature as a common characteristic of 1,2-intrastrand cross-links formed by cisplatin.

***Solution structural properties.*** We used supercoiled pBR322 DNA and studied global platination with cDPCP in solution as a function of bound platinum per nucleotide ($r_b$), determined by atomic absorption spectroscopy, to assess whether the adduct unwinds the duplex in solution. This method is based on the principle that negatively supercoiled circular DNA becomes positively wound when the duplex is locally unwound, a phenomenon encountered upon the formation of intrastrand cross-

**Table 1. Data collection and refinement statistics**

| Dataset | SAD | High resolution |
|---|---|---|
| Data collection statistics* | | |
| Beamline | APS 24-ID-C† | SSRL 9–2‡ |
| Wavelength, Å | 1.072 | 0.984 |
| Space group | $C222_1$ | $C222_1$ |
| Unit cell dimensions, Å | | |
| *a* | 45.8 | 46.4 |
| *b* | 66.4 | 66.0 |
| *c* | 56.6 | 56.1 |
| Resolution range, Å | 50–2.72 | 50–2.17 |
| Obs. reflections | 14,263 | 32,977 |
| Unique reflections | 2,264 | 4,765 |
| Completeness | 94.2 (73.6) | 96.0 (76.0) |
| $I/\sigma$ | 23.2 (6.7) | 18.1 (2.7) |
| $R_{merge}$§ | 7.3 (17.6) | 10.7 (55.6) |
| Refinement statistics | | |
| $R$¶ | | 22.5 |
| $R_{free}$‖ | | 25.4 |
| rms deviation bond lengths, Å | | 0.006 |
| rms deviation bond angles, ° | | 1.435 |
| Average B-factors, Å$^2$ | | 42.4 |
| Platinum ligand | | 44.4 |
| Solvent | | 42.9 |

*Values in parentheses are for the highest-resolution shell.
†ADSC Q315 detector, 360 frames, $\Delta\Phi = 1°$, exposure time = 2 s.
‡MAR 325 detector, 180 frames, $\Delta\Phi = 1°$, exposure time = 5 s.
§$R_{merge} = \Sigma|I-\langle I\rangle|/\Sigma I$.
¶$R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
‖$R_{free}$ = $R$ obtained for a test set of reflections (5% of diffraction data).

links by cisplatin (18). Cisplatin unwinds the duplex by 13° per bound platinum atom, visibly altering the electrophoretic mobility of the supercoiled DNA on agarose gels. This result is largely a consequence of the formation of intrastrand cross-links, because monofunctional platinum complexes like $[Pt(dien)Cl]^+$, which can bind only to a single base, unwind duplex DNA by only 6° per Pt atom (19). We observe no unwinding of the superhelix by cDPCP (Fig. S1) at $r_b$ values up to 0.034. This result is consistent with the formation of monofunctional Pt–DNA adducts in solution. The amount of DNA-bound cDPCP per amount added is, within experimental error, the same as that of cisplatin, as revealed by plots of $r_b$ vs. $r_f$, the formal ratio of platinum added per nucleotide (Figs. S2 and S3).

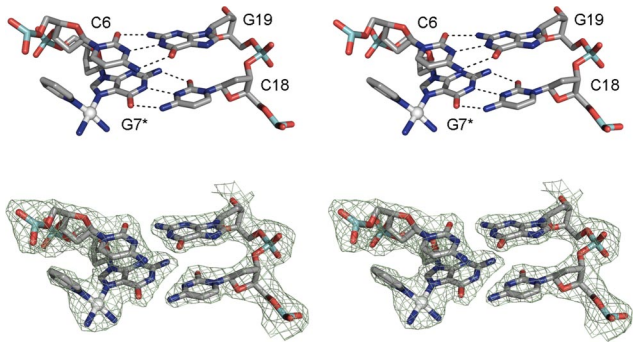



**Fig. 4.** Stereoscopic views of the cDPCP-dG adduct on duplex DNA. (*Upper*) Watson-Crick hydrogen bonding. (*Lower*) $2F_o-F_c$ electron density map contoured at $1\sigma$.

**Inhibition of Transcription by cDPCP in HeLa Cells.** Among the proteins and protein complexes that encounter cisplatin–DNA adducts is the transcription apparatus. Unlike DNA polymerases, which briefly pause at, and then bypass, cisplatin cross-links, presumably without major downstream effects (20), RNA polymerases are greatly affected by the presence of these adducts. The progression of human RNA polymerase II (Pol II) along the DNA strand is almost completely blocked by cisplatin-DNA adducts (21), and the arrest and subsequent ubiquitylation of Pol II initiate transcription-coupled repair, a subpathway of

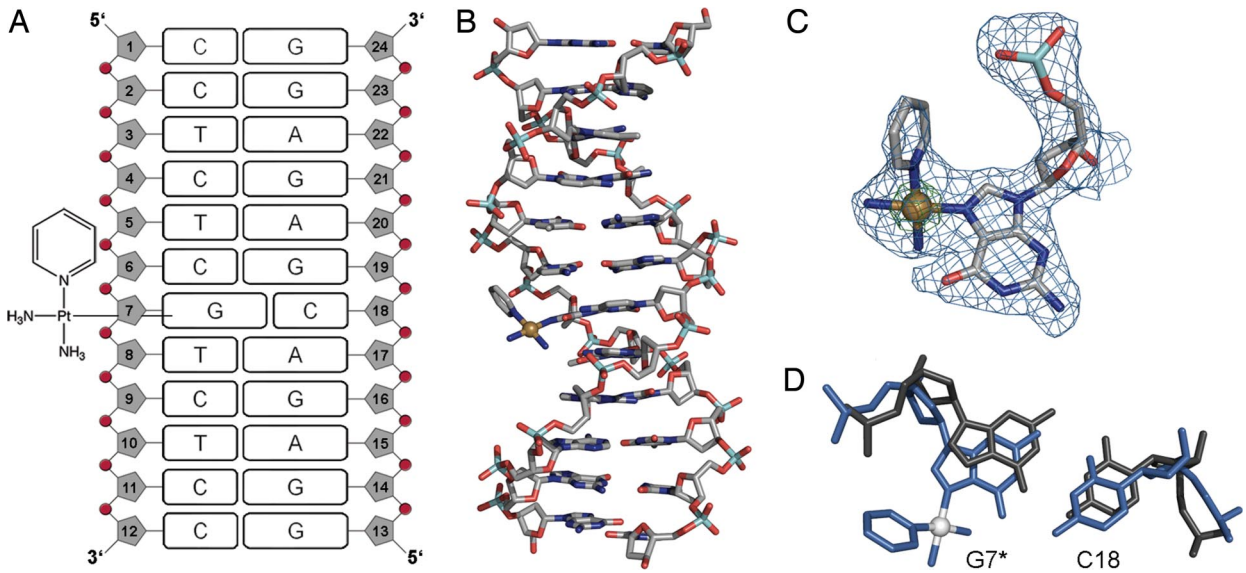



**Fig. 3.** X-ray crystal structure of cDPCP-modified DNA. (*A*) Schematic diagram showing the DNA sequence and location of the platinum adduct for the complex studied by x-ray crystallography. (*B*) Structure of the cDPCP-damaged DNA duplex, which maintains a linear B-form conformation despite binding of the Pt complex. (*C*) Close-up view of the monofunctional Pt–dG adduct, with $2F_o-F_c$ maps contoured at $1\sigma$ (blue) and $15\sigma$ (green), which show significant electron density around the platinum atom. (*D*) The platinated base pair (blue) overlaid with ideal B-form DNA (gray). Platinum-binding forces the DNA bases out into the major groove.

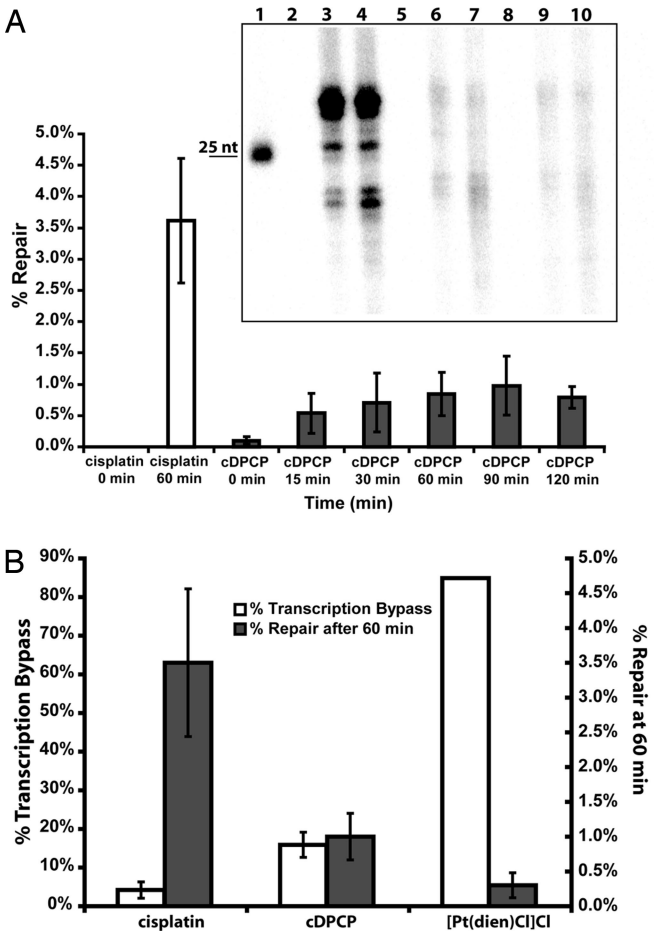



**Fig. 5.** Repair and inhibition of transcription by cDPCP-DNA adducts. (*A*) Comparison of repair of cDPCP with repair of cisplatin and [Pt(dien)Cl]Cl adducts (gel, *Inset*), and comparison of the kinetics of repair of cisplatin and cDPCP (bar plot). Gel lanes (*Inset*) are as follows: (1) ladder w/band at 25 nt, (2) cisplatin 0 min, (3) cisplatin 30 min, (4) cisplatin 60 min, (5–7) cDPCP 0/30/60 min, (8–10) [Pt(dien)Cl]Cl 0/30/60 min. The entire gel is given in Fig. S7*A*. Error bars represent the standard deviation of three separate experiments. (*B*) Comparison of successful transcription bypass and repair of various Pt-DNA adducts. Adducts of cDPCP, much like those of cisplatin, allow minimal bypass by RNA polymerase II but are inefficiently repaired, similar to adducts of [Pt(dien)Cl]Cl. Repair values report percentage excision products detected after 60 min and transcription bypass values are given at $r_b = 0.0039$ for the $\beta$-gal live-cell assay. Repair error bars are the standard deviation of five, five, and two separate experiments for cisplatin, cDPCP, and [Pt(dien)Cl]Cl, respectively. Transcription error bars are the standard deviation of samples prepared in triplicate. The entire experiment was performed twice.

nucleotide excision repair, and programmed cell death, or apoptosis (22).

Plasmids containing the *lac*Z gene downstream of an SV40 promoter were modified with cisplatin, cDPCP, or $[Pt(dien)Cl]^+$ at $r_b$ levels from 0 to 0.13 (Fig. S3) and transfected into HeLa cells. The products of $\beta$-galactosidase activity were assayed colorimetrically after 24 h by addition of *ortho*-nitrophenyl-$\beta$-galactoside (ONPG). Bypass of platinum adducts by the Pol II complex led to increased transcription of the *lacZ* gene and absorbance at 420 nm arising from ONPG cleavage by $\beta$-galactosidase. There was a clear difference between Pol II bypass of cisplatin vs. $[Pt(dien)Cl]^+$ adducts, relative to that for the unplatinated control plasmids (Fig. 5 and Fig. S4), with $[Pt(dien)Cl]^+$ requiring >5 times the platination level as cisplatin to block progression of RNA Pol II completely. In contrast, transcription inhibition by the monofunctional cDPCP adducts very nearly matched that of cisplatin and was much more effective than inhibition by $[Pt(dien)Cl]^+$. Transcription of the cisplatin-modified plasmid was effectively inhibited at an $r_b$ value of $2.5 \times 10^{-3}$. Inhibition by $[Pt(dien)Cl]^+$-modified plasmids was the same as that of the unplatinated control at $r_b = 7.8 \times 10^{-3}$, whereas transcription from the cDPCP-modified plasmid was reduced to 16% that of the control at an $r_b$ value of $3.9 \times 10^{-3}$ (Fig. 5).

**Removal of Lesions by Excision Repair.** The major product of cisplatin-induced DNA damage, the intrastrand d(GpG) cross-link (23, 24), is repaired by the excision repair pathway (25). The integrity of this pathway in human cells is a key indicator of the sensitivity of a tumor to platinum-based therapy. Human cells from disorders in which excision repair deficiency is a phenotype, such as xeroderma pigmentosum (26) and Cockayne syndrome (27), are exquisitely sensitive to cisplatin damage. A test for the presence of a key protein in the excision repair pathway, ERCC1, is in FDA Phase III trials for use as a predictive factor in tailoring chemotherapy to patients with nonsmall cell lung cancer (28). Conversely, increased efficiency of excision repair leads to rapid removal of cisplatin adducts and is associated with cisplatin resistance in human tumor cells (29, 30).

We used 156-mer DNA probes (Figs. S5 and S6) site-specifically platinated with adducts of cisplatin, cDPCP, or $[Pt(dien)Cl]^+$, and radioactively labeled with an internal $^{32}P$ modification, in combination with repair-active mammalian cell-free extracts, to analyze excision repair. The samples were resolved on denaturing polyacrylamide electrophoresis gels, and the radioactive intensity was quantified with a phosphorimager. Percentage repair was determined by comparing the intensity of unrepaired 156-mer vs. that of the primary excision products at 25–29 bp. Repair efficiency of the three different site-specifically platinated 156-mer probes in CHO nuclear extracts was quantitated as 3.5% for cisplatin-modified DNA, 1.0% for the cDPCP adduct, and 0.3% for [Pt(dien)Cl]Cl adducted DNA after 60-min reaction times (Fig. 5 and Fig. S7). The rate and amount of repair are comparable to values observed for cisplatin and oxaliplatin 1,2-intrastrand d(GpG) cross-link-containing probes (25, 31–33) and less than the 10% repair observed for cisplatin 1,3-intrastrand d(GpTpG) cross-linked probes (33). Kinetic experiments revealed that repair of the cDPCP adduct levels off after 60 min (Fig. 5 and Fig. S7), a result consistent with previous measurements (25).

## Discussion

cDPCP is an outstanding substrate for OCT1 and OCT2, as revealed by the increased accumulation in cells that express these critical transporters compared with those lacking them. The dramatic enhancements in cellular accumulation and corresponding cell sensitization indicate that cDPCP has a greater tumor-targeting potential than oxaliplatin, the colorectal cancer agent that owes its unique antitumor properties to specific uptake by OCT1 and OCT2. Compared with oxaliplatin, cDPCP is much less toxic to cells that do not express OCT1 or 2 (Fig. 1). This property suggests that, like oxaliplatin, cDPCP will be able to target colorectal or liver cancer but with a reduction in the severity of side effects for tissues that do not express OCT1 or OCT2. The presence of these transporters in certain organs, most notably kidney and liver, may require the use of cotreatments to mitigate toxic side effects. Nephrotoxicity, the dose-limiting side effect for cisplatin therapy, is less problematic in oxaliplatin treatment (34). Liver toxicity is a non-dose-limiting side effect of cisplatin and oxaliplatin (35).

Compared with cisplatin, cDPCP causes only minor distortions to double helical DNA upon binding to a guanine N7 atom in the major groove. Characteristics of the cisplatin 1,2-intrastrand d(GpG) cross-link include a roll angle of 26° between the bound guanines and a 40° bend toward the major groove (36). It is widely accepted that these structural distortions inhibit

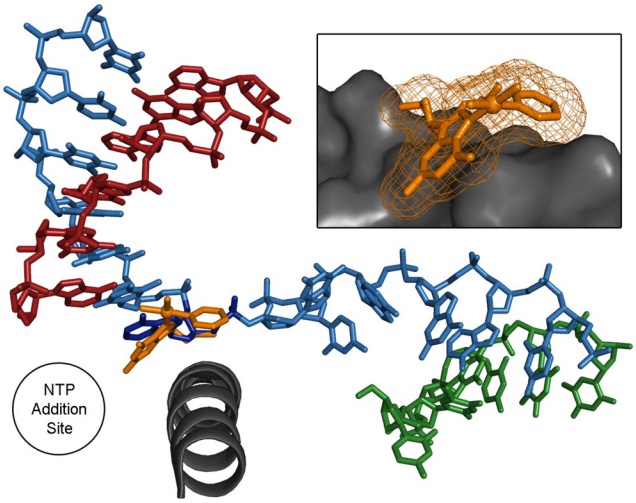



**Fig. 6.** Active site of RNA polymerase II, with a cDPCP–dG adduct (shown in orange) modeled into template DNA (blue) at the +1 site, where incoming NTPs are matched and added to synthesized RNA (red). Complementary nontemplate DNA is shown in green. This model demonstrates how cDPCP adducts may shift the template base out of its native conformation (shown in dark blue) by steric interactions between the pyridine ligand and the Pol II bridge helix, shown in gray. (*Inset*) Space-filling views of the Pt adduct and bridge helix. Pol II coordinates: Protein Data Bank (PDB) ID code 2NVQ; cDPCP-dG coordinates: this work.

transcription, leading either to nucleotide excision repair or to apoptosis. A recent x-ray crystal structure of an RNA polymerase II elongation complex containing a cisplatin 1,2-d(GpG) intrastrand cross-link in the DNA template strand suggested that the platinum adduct inhibits transcription by prohibiting translocation of the cross-link from the +2/+3 site to the +2/+1 site. This barrier stems from the inability of the covalently linked dinucleotide to twist by ≈90° for crossing the bridge helix in the +2/+1 site (37). This finding provides a structural clue for understanding transcription inhibition by cisplatin intrastrand cross-links.

By contrast, monofunctional adducts of cDPCP offer no such translocation barrier, because cDPCP binds covalently only to a single DNA base. Modeling of the *cis*-$\{Pt(NH_3)_2(py)\}^{2+}$–dG adduct into the template strand of the elongation complex suggests the absence of a significant barrier to translocation between the +2 and +1 sites (data not shown). However, when the adduct is modeled in the +1 site, where the incoming NTP is matched to the template strand, of an elongation complex solved crystallographically (38), it is clear that the pyridine ligand would sterically clash with the bridge helix, in effect twisting the base out of its native conformation (Fig. 6). This distortion could lead to NTP misincorporation, which in turn might stall the polymerase. This hypothesis explains why cDPCP lesions inhibit transcription, whereas adducts of the trans isomer and other monofunctional platinum compounds like [Pt(dien)Cl]Cl, which would not cause such steric hindrance, are much less potent inhibitors. Like cisplatin, transcription is strongly inhibited by cDPCP both in cell extracts and in live cells, but a significantly higher level of platination with $[Pt(dien)Cl]^+$ is required to block transcription to the same extent.

Although cDPCP blocks transcription nearly as efficiently as cisplatin, repair of cDPCP adducts by the mammalian excinuclease is demonstrably reduced relative to repair of cisplatin or oxaliplatin damage. Because cDPCP can largely escape repair and yet inhibit transcription very effectively, its adducts should persist longer than those of cisplatin yet produce a similar number of downstream consequences that might raise the therapeutic potential of cDPCP relative to cisplatin. The design of anticancer agents specifically as transcription inhibitors has been proposed, based on the premise that an extended delay in the restoration of transcription would induce apoptosis by p53-dependent and -independent pathways (39). If true, persistence of transcription blocks would promote cell death and enhance the potency of cDPCP. Combined with the high selectivity of cDPCP for cells expressing hOCT1 and hOCT2, which are broadly expressed in human colorectal cancer (11), these findings support the candidacy of this unique monofunctional cationic complex as an anticancer drug.

## Materials and Methods

**Materials.** Potassium tetrachloroplatinate(II) was obtained as a gift from Engelhard Corporation (now BASF). Cisplatin (40) and *cis*-$[Pt(NH_3)_2(py)Cl]Cl$ (5) were synthesized as described. Phosphoramidites and other reagents for DNA synthesis were purchased from Glen Research. Crystallization reagents were obtained from Hampton Research and Sigma. Enzymes were purchased from New England Biolabs. Plasmids pBR322 and pSV-β-galactosidase were purchased from New England Biolabs and Promega, respectively, and were amplified in 100 ml of LB cultures of *E. coli* XL1-blue cells containing ampicillin as a selecting agent and purified on Maxi-prep columns (Qiagen). [$\gamma$-$^{32}$P]ATP was obtained from Perkin–Elmer. All other chemicals and solvents were purchased from commercial suppliers.

**Cellular Accumulation and Compound Cytotoxicity: Cell Lines and Transfection.** Madin–Darby canine kidney (MDCK) cells were stably transfected with full-length human OCT1 cDNA (MDCK-hOCT1) and the empty vector (MDCK-MOCK), as established (41). Human embryonic kidney (HEK293) cells stably transfected with the full-length OCT2 cDNA (HEK-hOCT2) and with the empty vector (HEK-MOCK) were also described (11).

**Cell Culture.** The stably transfected MDCK and HEK293 cells were cultured in DMEM supplemented with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin (Invitrogen) and the respective selection antibiotics and grown at 37°C in a humidified atmosphere with 5% $CO_2$.

**Compound Cytotoxicity.** Cytotoxicities of the compounds were determined by plating cells in 96-well plates at a predetermined density. Cells were then incubated overnight, and platinum complexes were added to the culture medium. After 7 h, the medium was replaced with fresh Pt-free medium, and the incubation was continued for a total of 72 h after the initial addition. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were performed as described (42).

**Cellular Accumulation of Platinum.** These studies were performed as described (11).

**X-Ray Crystal Structure Determination of Platinated DNA Duplex.** Two deoxyoligonucleotides (5′-CCTCTCGTCTCC-3′ and its complementary strand) were synthesized and purified by standard methods (43). The site-specifically platinated duplex was prepared and purified as described (44, 45). Details of crystallization experiments, x-ray diffraction data collection, structure determination, and refinement are available in the *SI Text*.

**DNA Unwinding Assays.** Details of plasmid platination and agarose gel analysis are available in the *SI Text*.

**Transcription in Living Human Cells: Transcription Probe Preparation.** The pSV-β-galactosidase vector (Promega), containing a *lacZ* gene under the control of an SV40 promoter and enhancer, was amplified in XL1-blue, purified on a Maxi-prep column (Qiagen) and globally platinated with either cisplatin, *cis*-$[Pt(NH_3)_2(py)Cl]^+$, or $[Pt(dien)Cl]^+$ to yield $r_b$ values between 0 and 0.13. Excess platinum was removed by spin dialysis (Nanosep columns, Pall Biosciences, 3K molecular weight cutoff), and DNA and Pt concentrations were quantified by UV-vis and atomic absorption spectroscopy, respectively.

**Transcription Assay.** Experimental details are provided in *SI Text*.

**Nucleotide Excision Repair Assay.** Probes (156-mer) were prepared as described in *SI Text*. Assays were performed as described (31, 46) with 10 fmol of the platinated repair probe and 75 μg of cell-free HeLa extract. Reactions were allowed to proceed for 60 min at 30°C and were stopped by the addition of SDS

and proteinase K to final concentrations of 0.34% and 20 $\mu$g/ml, respectively. After extraction with 25:24:1 phenol:chloroform:isoamyl alcohol and careful ethanol precipitation with 10 $\mu$g of linear polyacrylamide as coprecipitant, reaction products were analyzed by 10% urea-PAGE.

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