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Metal−Intercalator-Mediated Self-Association and One-Dimensional Aggregation in the Structure of the Excised Major DNA Adduct of a Platinum−Acridine Agent

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Platinum−acridinylthiourea conjugates are a new class of potent cytotoxic agents that associate with DNA through a combination of monofunctional metalation of nucleobase nitrogen and intercalation of the acridine chromophore of the sulfur-bound nonleaving group into the DNA base stack.[1a,b] The formation of a single purine−metal coordinative bond by these agents is in contrast to the mechanism of platinum-based chemotherapies in regular clinical use[2] or in clinical trials,[3] which are inducers of (bifunctional) purine−purine cross-links. PT-ACRAMTU, the prototype (Chart 1; ACRAMTU = 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea, HNO_3 salt), forms a unique array of DNA adducts previously unknown for platinum drugs.[1c,d] In double-stranded DNA, PT-ACRAMTU targets guanine (80%) and adenine (20%) bases. The most abundant drug-modified DNA fragment detected in enzymatic digests of native DNA treated with PT-ACRAMTU was the mononucleoside adduct, dGuo* (Chart 1; dGuo = 2′-deoxyguanosine).[1d] dGuo* forms by replacement of the chloro leaving group in PT-ACRAMTU by endocyclic nitrogen N7 of guanine, which is located in the major groove of the biopolymer. On a synthetic scale, dGuo* can also be generated by reaction of dGuo with PT-ACRAMTU or directly from the precursors of the conjugate.[1d] ^{1}H and ^{195}Pt NMR spectroscopy, reverse-phase high-performance liquid chromatography (HPLC), and electrospray (tandem) mass spectrometry have been used in a previous study to elucidate the structure of dGuo*. The data clearly demonstrated that dGuo* generated enzymatically and synthetically are chemically identical species.[1d] We have now been able to grow single crystals of this adduct suitable for X-ray analysis and report here its crystal structure. The unusual type of self-association observed in this structure provides a strong rationale for the proposed covalent−intercalative DNA binding mode of PT-ACRAMTU.

Suitable crystals of the adduct were grown by slow evaporation of sitting drops of a saturated aqueous solution (pH 6.9) of dGuo* at 4 °C. Partially deprotonated dGuo* crystallizes in the monoclinic space group C_2 as discrete $[(dGuo^*)_2\text{-}2H^+]^{4+}$ cations (**1**, Figure 1) along with 4 chloride counterions and 20 water molecules in the asymmetric unit. **1** comprises a pseudosymmetric dimeric entity, which is held together by a combination of hydrogen bonding and van der Waals forces. The structure can be best described as a mismatch base pair formed by the two platinum-modified guanines, which is sandwiched between the acridine chromophores of the ACRAMTU ligands. In this rare type of mispairing,[4] one of the guanine (G) bases exists in its N1-deprotonated form,[5] mimicking complementary cytosine. This arrangement results in three hydrogen bonds between the two coplanar nucleobases (angle between planes, 2.0°). The GG$^-$ base pair is stabilized by two acridine units stacked at distances of 3.34 and 3.28 Å, respectively, producing a high degree of chromophore overlap (Figure 2a).

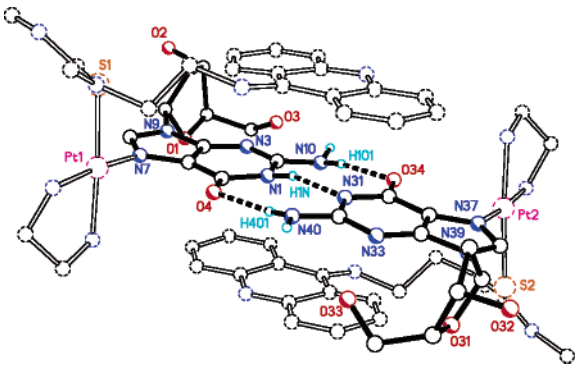
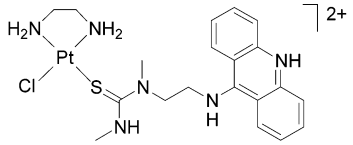
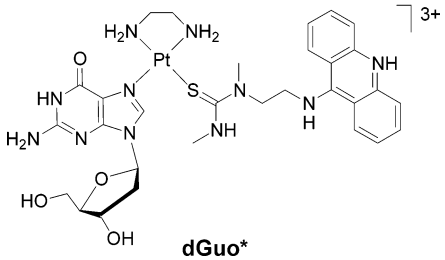


Figure 1. View of the cationic dimer **1** in the crystal with selected atoms labeled. The nucleoside moieties are highlighted using solid bonds. In both nucleosides, the guanine bases adopt a *syn* conformation about the N-glycosidic bond, and the sugar puckers are C2′-*endo*. Intranucleoside hydrogen bonds involving the 5′-hydroxo groups of the D-ribose residues and N3 of guanine stabilize this conformation (O3- - -N3 2.765(6) Å, O33- - -N33 2.75(1) Å). The [PtN$_3$S] coordination planes make large dihedral angles of 79.8° (Pt1) and 74.6° (Pt2) with the guanine bases. Hydrogen bonding within the GG$^-$ base pair is indicated by dashed lines (N1- - -N31 2.871(6) Å, N10- - -O34 2.754(6) Å, N40- - -O4 2.884(6) Å). All other hydrogens, counterions, and solvent molecules were omitted for clarity. A thermal ellipsoid drawing of **1** is shown in Figure S1 (Supporting Information).

Chart 1



PT-ACRAMTU



dGuo*

^{1}H chemical shift anomalies and nuclear Overhauser effects observed in 1-D and 2-D NMR spectra of dGuo* indicate that the dimeric form persists in solution (Supporting Information).

The asymmetric units are related by a two-fold axis of rotation, such that a one-dimensional array of stacked dimers is generated along the *c*-axis (Figure 2b). The extended stacking is mediated by $\pi-\pi$ interactions between the acridine units, which results in

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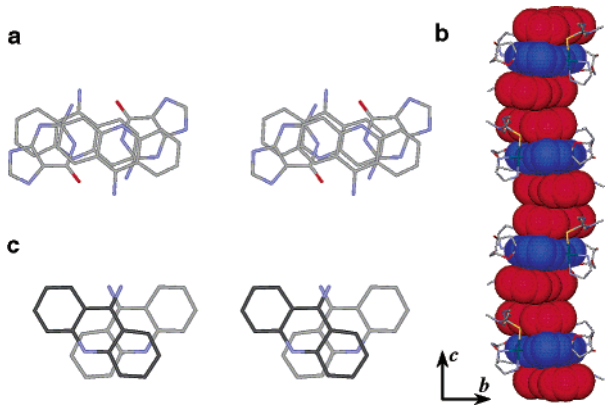

Figure 2. (a) Stereoview along the *c*-axis of the stacking interaction in the asymmetric unit. (b) View along the *a*-axis of the infinite π-stack in the crystal of [**1**]$Cl_4 \cdot 20H_2O$. A repeat of four dimers packed along the *c*-axis is shown. The GG^- base pairs (blue) and acridine chromophores (red) are highlighted as space-filling spheres. (c) Stereoview along the *c*-axis of the stacking interactions between the asymmetric units.

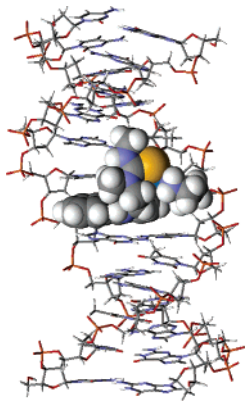

Figure 3. Energy-minimized AMBER model of a dodecamer containing the major-groove guanine-*N7* monoadduct of PT-ACRAMTU, d(5′-CC-TCT**CG***TCTCC-3′/3′-GGAGA**GC**AGAGG-5′) (the asterisk denotes the site of platination). Acridine intercalates into the central 5′-C(6)G(7)/C(18)G(19) base step. The adduct is highlighted as space-filling spheres. Calculations were based on published protocols (ref 1c).

base−base separations of 3.203 and 3.870 Å and interplanar angles of 9.0 and 11.1°. The acridine chromophores do not stack directly on top of one another but are twisted around the *c*-axis by about 60°. This positions the endocyclic nitrogen of each base above the aromatic ring system of its neighboring base, the preferred stacking mode for N-heterocyclic aromatics (Figure 2c).[6] No significant direct interactions are observed between the infinite stacks of **1**. They do, however, interact through water- and chloride-mediated hydrogen bonding.

The crystal structure of **1** shows that the flexibly linked acridine unit in dGuo* can adopt a geometry that produces nearly perfect coplanarity with the platinated guanine base. The increased $\pi-\pi$ stacking interactions with a base pair compared to a single base is probably an important organizational force in the formation ("self-recognition") of the GG^- mismatch in **1**. The apparently seamless incorporation of dGuo* in an extended one-dimensional array of coplanar bases in the structure of **1** suggests that B-type DNA should be an ideal "host" for this adduct. On the basis of the geometry observed in the crystal, intercalation of acridine into the DNA base stack perpendicular to the helical axis and parallel to the platinated base pair appears to be a highly feasible binding mode. Plasmid unwinding experiments conducted earlier[1b] demonstrated that PT-ACRAMTU untwists DNA by 21° per adduct, indicating efficient intercalation of the acridine base. While significant unwinding and extension of the DNA helix can be predicted for this combination of monofunctional platination and intercalation, the proposed dual binding mode may not cause significant bending of the duplex. This would be in contrast to the situation for cisplatin, whose major adduct and putative cytotoxic lesion, the intrastrand cross-link between adjacent purine bases, causes significant disruption of the stacking and local bending of the DNA toward the major groove.[7] A molecular mechanics/molecular dynamics (MM/MD) study of a dodecamer modified with PT-ACRAMTU, in fact, suggests that the proposed combination of platinum binding to N7 of guanine and (partial) intercalation of the acridine on the 5′ face of the platinated nucleobase untwists the DNA without bending it significantly. The model (Figure 3) suggests that the guanine adduct formed by PT-ACRAMTU is unlikely to mimic or resemble cisplatin-induced DNA damage, unlike other "pseudobifunctional" platinum complexes bearing planar nonleaving groups.[8] NMR and X-ray crystallographic studies of similar model duplexes containing dGuo* are currently underway to address these issues.

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Supporting Information Available: Details of the structure determination of [**1**]$Cl_4 \cdot 20H_2O$ (CIF); a thermal ellipsoid drawing of the asymmetric unit and 1-D ^{1}H, 2-D COSY, and 2-D NOESY NMR data for dGuo* (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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