Guanine binding to dirhodium tetracarboxylate anticancer complexes: quantum chemical calculations unravel an elusive mechanism†‡

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The reaction mechanism leading to metalated DNA fragments in which guanine-N7,O6 spans the metal–metal bond of dirhodium antitumour complexes in a bridging fashion at equatorial sites has been unravelled by a comprehensive prediction of intermediates and transition states.

The success of the anticancer drug cisplatin has stimulated the search for new metallopharmaceuticals.[1] Metal–metal-bonded dirhodium tetracarboxylate complexes (DTs; Fig. 1, center) have been developed,[2–6] but their mode of antitumour activity is not entirely understood. DTs bind to single- as well as to double-stranded DNA and inhibit DNA replication and protein synthesis in a manner akin to cisplatin.[2,3] Whereas guanine (Gua) binds to cisplatin *via* site N7, it was controversial as to whether Gua can bind to DTs as well. Axial (ax) Gua-N7 adducts with DTs were believed to be unstable due to repulsion between Gua-O6 and the carboxylate oxygen atoms (Fig. 1, left).[2] Later studies, however, revealed that Gua bases of DNA fragments span the metal–metal bond at equatorial (eq) positions *via* sites N7/O6 (Fig. 1, right).[7,8] Despite the sound characterization of the reactants and final products, the intermediates in the reaction of guanine with DTs have not been characterized experimentally, and the reaction mechanism has remained elusive.[2,3]

The objective of this work is to unravel the mechanism of Gua binding to dirhodium(II) tetracarboxylate compounds using a combined density functional theory (DFT) and continuum dielectric model (CDM) approach.[9] This mechanism is highly relevant to the interactions of metal–metal-bonded antitumour compounds with DNA and their possible biological implications. The mechanism can be divided into two main parts: I, formation of monodentate eq Gua adducts; II, ring closure yielding the bidentate Gua adduct.

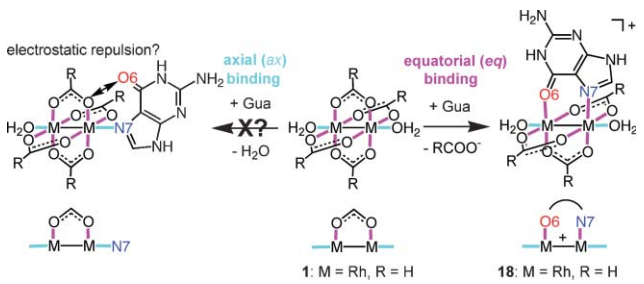
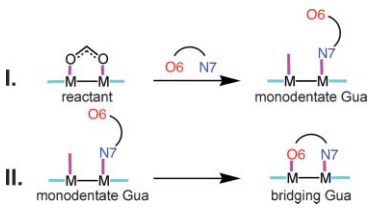


Fig. 1



The anticancer drug cisplatin traditionally serves as a reference compound in the discussion of the mode of action of non-platinum metallopharmaceuticals.[10] We compare the monodentate Gua binding (part I) with the well-known monodentate Gua binding to cisplatin (Fig. 2): (a) one of the four basal ligands at the square-planar Pt(II) center is directly exchanged, without a five-coordinate intermediate; (b) Gua-N7 is preferred to Gua-O6 binding; (c) the reaction is hydrolysis-assisted, *i.e.*, a chloride leaving group is hydrolyzed first, followed by reaction of the aqua complex with Gua.

First, we have explored the direct eq Gua attack on DT and, as a possible alternative, initial ax binding and subsequent ax to eq migration.[11] We report only the relevant intermediates and transition states and their relative free energies *G* in aqueous solution predicted at the B3LYP level; full details of all molecules **1**–**18** and all TS are provided in the ESI.‡ The calculations reveal

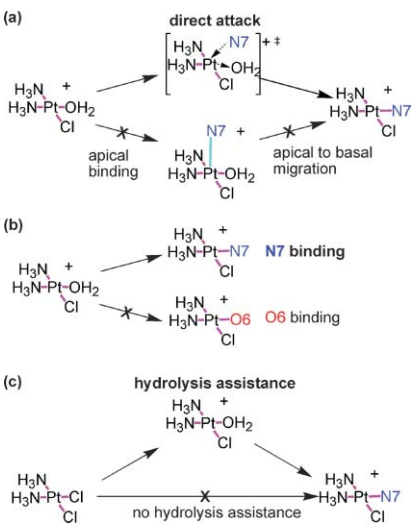


Fig. 2

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‡ Electronic supplementary information (ESI) available: Reaction profile, computational details and calculated structures and transition states. See DOI: 10.1039/b709209a
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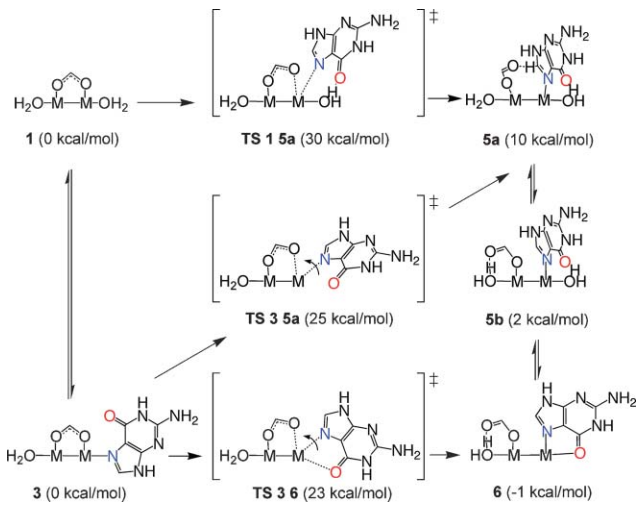
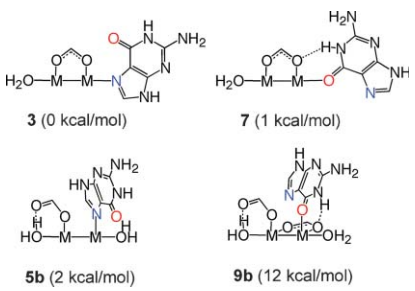
that the transition state for direct eq Gua attack, **TS 1 5a** (G = 30 kcal mol^{-1}), is much higher in free energy than the transition states for ax to eq migration, **TS 3 5a** (25 kcal mol^{-1}) and **TS 3 6** (23 kcal mol^{-1}). Contrary to conventional wisdom,[2] the ax Gua-N7 adduct **3** is *not* a highly unstable species, but is a key intermediate in the title reaction. Species **5a**, **5b** and **6** are expected to be in equilibrium involving only ax ligand exchange. In summary, ax binding and subsequent ax to eq migration is *kinetically* preferred to direct eq Gua attack.



Second, the competition of Gua-N7 *vs*. Gua-O6 binding to the dirhodium unit has been investigated. The ax Gua-O6 adduct **7** (G = 1 kcal mol^{-1}) is approximately as stable as the ax Gua-N7 adduct **3** (0 kcal mol^{-1}). However, the eq Gua-O6 adducts are ~7–10 kcal mol^{-1} less stable than their eq Gua-N7 counterparts. For example, **9b** has a relative free energy of 12 kcal mol^{-1}, which is much higher than that of **5b** (2 kcal mol^{-1}). Because the transition states leading to eq Gua-O6 and eq Gua-N7 adducts are similar in free energy (see ESI for details‡), eq Gua-N7 binding is *thermodynamically* rather than kinetically preferred to eq Gua-O6 binding.



Third, the possibility of a bridging carboxylate group to be partially hydrolyzed and become monodentate has been considered. Partial hydrolysis would yield **2a** (G = 12 kcal mol^{-1}), which would likely rearrange to the more stable isomer **2b** (G = 8 kcal mol^{-1}). In agreement with the predicted ax to eq Gua-N7 migration (*vide supra*), the calculations show partial hydrolysis of the carboxylate *via* **TS 1 2a migration** (G = 24 kcal mol^{-1}) to be kinetically preferred to the direct attack of a water molecule at the eq position *via* **TS 1 2a direct** (G = 28 kcal mol^{-1}). Because the hydrolysis barrier *via* **TS 1 2a migration** is very similar to that of cisplatin hydrolysis (G ~ 24 kcal mol^{-1}),[12] we have investigated various possible reactions of the partial hydrolysis product **2b**. Their transition states, however, are higher in free energy. Thus, the calculations suggest that the title reaction is not hydrolysis-assisted; we aim to corroborate this finding with kinetic studies.

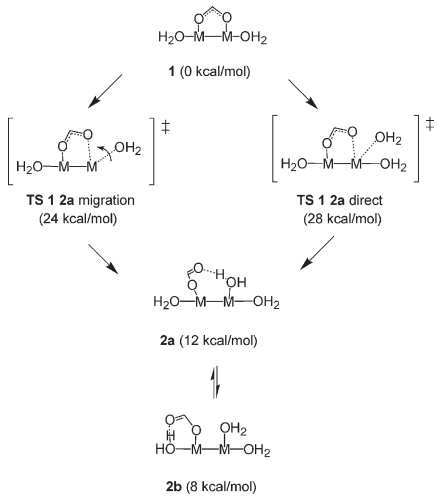


Fig. 3 summarizes the predicted characteristics of monodentate Gua binding to dirhodium tetracarboxylates, suggesting striking similarities to and differences from cisplatin chemistry (Fig. 2).

Finally, the formation of the product **18** from the monodentate eq Gua-N7 adduct **5b** has been explored. Not surprisingly, the product **18** (−5 kcal mol^{-1}) is the most stable structure (although it is much less stable than the Gua adducts of cisplatin, G = −15 kcal mol^{-1}).[9] We expected the reaction to proceed *via* a simple rotation about the Rh–Gua-N7 bond in **5b** and binding of the Gua-O6 moiety to the second metal center *via* **TS 5b 18**, yielding the bidentate Gua-N7,O6 product **18**. However, this transition state is rather high in free energy (31 kcal mol^{-1}). The calculations reveal an unexpected alternative mechanism *via* the ax–eq carboxylate chelate **11**, which readily forms from **5b** *via* loss of ax water. In the reaction of **11**, the nucleobase rotates about the

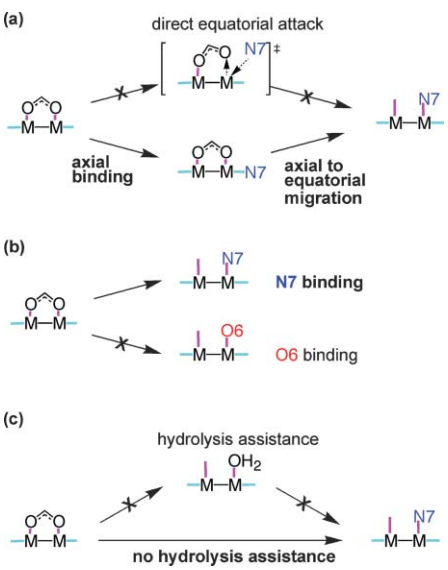
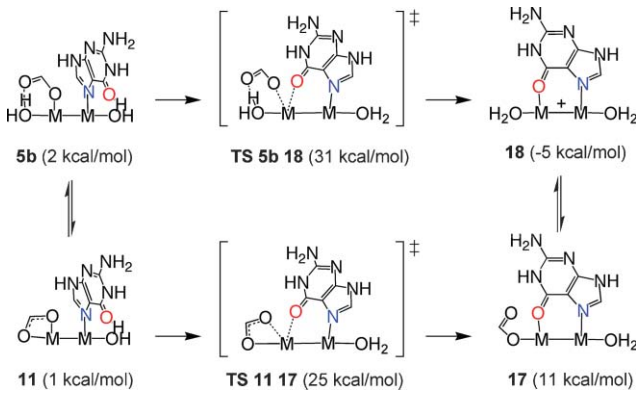
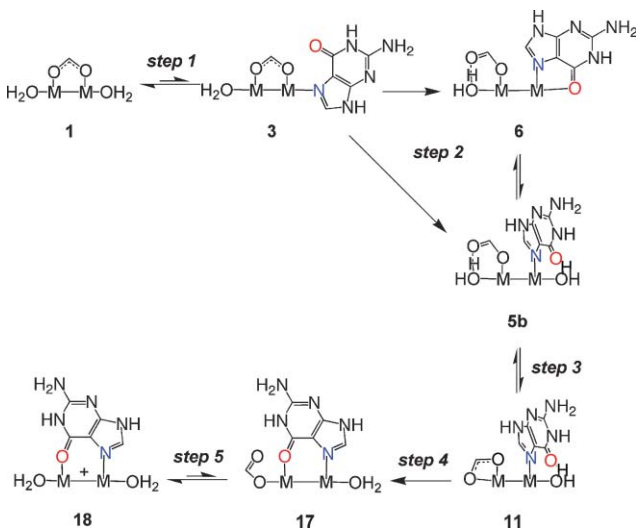


Fig. 3

Rh–Gua-N7 bond, and Gua-O6 binds *via* **TS 11 17** to the adjacent metal center. The carboxylate ring is cleaved, yielding the product precursor **17**. Species **17** readily exchanges the ax carboxylate for an ax water molecule, affording the product **18**.



These results imply the following multiple-step mechanism of the title reaction: *Step 1.* An ax water ligand in **1** is displaced by Gua-N7, yielding **3**. *Step 2.* In **3**, Gua-N7 migrates from an ax to an eq position. In the eq Gua-N7 adducts, the ax position may be occupied by a water ligand (**5b**) or by Gua-O6 (**6**). *Step 3.* An ax–eq carboxylate chelate **11** forms. *Step 4.* The Gua-N7,O6 bridge is formed, yielding **17**. *Step 5.* The ax monodentate carboxylate is substituted by a water ligand. The calculations predict activation free energies of ~23–25 kcal mol^{-1} for *steps 2 and 4*, whereas the other reaction steps involve fast ax ligand exchange.



In summary, we have unravelled the mechanism of guanine binding to dirhodium tetracarboxylates, representing an emerging class of metal–metal-bonded antitumour complexes.[2–6] Numerous experiments led to the sound characterization of the reactants and products, but the reaction mechanism was not established. Our high-level quantum chemical calculations suggest a multiple-step mechanism *via* an axial Gua-N7 adduct and an ax–eq carboxylate chelate as unexpected key intermediates. This pioneering quantum chemical investigation paves the way for future studies exploring how the differences and similarities in the Gua reactions between the metal–metal bonded complexes and cisplatin affect the activity of these antitumour agents with respect to their binding to DNA and other biomolecules.

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Notes and references

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