Transition metal Kinamycin model as a DNA photocleaver for hypoxic environments: bis(9-diazo-4,5-diazafluorene)copper(II) nitrate†

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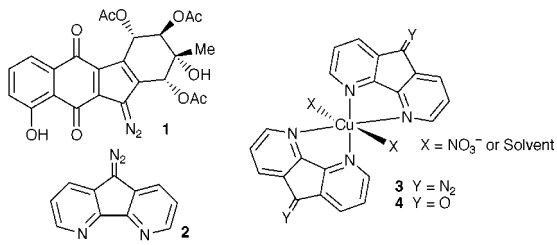
Bis(9-diazo-4,5-diazafluorene)copper(II) nitrate is an effective DNA photocleaving agent under anaerobic conditions using visible light and represents a potential model for the action of Kinamycin antitumor antibiotics.

The natural product antitumor antibiotic Kinamycin C **1**,[1,2] was recently found to contain a reactive terminal diazo group that is thought to be responsible for the DNA cleaving activity of the



molecule. Although very little is known about the details of Kinamycin's antibiotic activity, the thermal and photochemical reactivity of organic diazo compounds has been long established.[3] Recently, organic chemists have sought to mimic the function of Kinamycin C using simple synthetic diazo analogs, while monitoring biological activity through DNA cleavage assays.[4,5] Although functional, these agents are typically activated through long thermal incubations or UV photolyses, conditions that are less than ideal for medicinal uses.

In addition to their photochemical lability, the thermal decomposition of diazo groups is known to be accelerated by both oxidizing[6,7] and reducing[8] agents. Addition of Cu^{II} salts has been shown to significantly increase the thermal DNA cleaving ability of synthetic diazo compounds.[7] Notably, Kinamycin C contains a redox-active quinone unit in close proximity to the diazo group, suggesting that the antibiotic may use this functionality as an *intramolecular* redox switch to promote N_2 release. To this end, we sought to investigate the effect of binding a redox active transition metal to a diazo-containing ligand on the reactivity and DNA-photocleaving properties of the resulting complex. Ligand **2** was chosen for our initial studies because it is a simple nitrogen chelate containing an exocyclic diazo group that would direct a highly reactive carbene or radical intermediate toward potential substrates.

Compound **2** was synthesized from 4,5-diazafluorenone, using modified literature procedures.[9] The copper(II) complex **3** was synthesized by adding methanolic $Cu(NO_3)_2{\cdot}2.5H_2O$ to a solution of ligand in a 1 : 2 ratio. The full solution and solid-state characterization, as well as the X-ray crystallographic analysis, of the complex are reported elsewhere.[10]† Compound **4**, the ketonic diazo precursor complex, was prepared in an analogous manner and used as a control for DNA cleavage studies.

The effectiveness of DNA cleavage was assessed under rigorously anaerobic conditions by comparison of supercoiled (form I) DNA to the circular relaxed (form II) and linear (form III) DNA. As seen in Fig. 1(*a*), control photoreactions with DNA alone (lane 3) or 200 μM **4** (lane 4) and dark control reactions with 200 or 100 μM **3** (lane 5, 6) resulted in little or no DNA cleavage. In contrast, photoreactions of **3** using visible wavelength light resulted in significant production of linear and nicked DNA depending on the concentrations of **3** used. At a concentration of 200 μM (lane 7), no distinct bands are observable because the plasmid has been degraded into many minor fragments, resulting only in a faint streak on the gel. At concentrations of 100 (lane 8), 50 (lane 9) and 25 μM (lane 10), significant amounts of linear and nicked DNA are visible. Even at concentrations as low as 12.5 μM (lane 11), approximately half of the supercoiled plasmid has been converted to the nicked form. Discernable photocleavage by **3** is also observed at concentrations of 12.5 μM when wavelengths longer than 590 nm are used over 12 h (lane 12).‡

A comparison of efficiency between 2 equiv. of **2** and 1 equiv. of **3** was made because it is possible that both ligands of the Cu^{II} complex could react with DNA substrate. Significantly less cleavage was detected in the case of **2** alone [Fig. 1(*b*), lanes 3, 5, 7] relative to **3** (lanes 4, 6, 8) at one half the concentration, respectively. Close inspection of both gels in Fig. 1 reveals that the migration of the linear DNA product is slightly retarded relative to that of the linearized control, suggesting that the 4,5-diazafluorene moiety may be covalently bound to the DNA strand following photolysis. Evidence for alkylation of DNA by radical intermediates is well-established in the literature.[11]

In an effort to gain chemical insight into the reactivity of **3**, photolysis reactions were performed in aqueous solution in the absence and presence of calf thymus DNA and buffer. Anaerobic photolysis of **3** at $\lambda \geqslant 455$ nm in unbuffered water

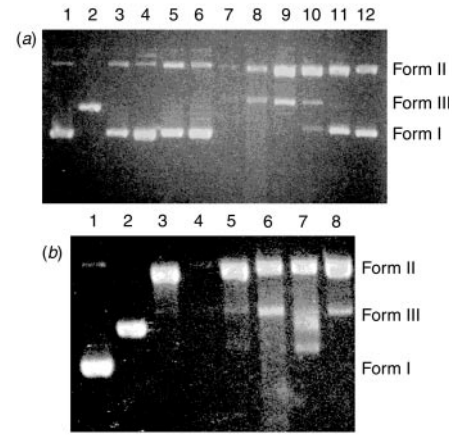


Fig. 1 Agarose gel electrophoresis of the photocleavage reaction of plasmid pUC118 (3162 bp) by **3** and related control compounds. Reactions were performed air-tight Pyrex conical vials with 50 μM bp in 10 mM Tris acetate pH 7.6 buffer at $\lambda \geqslant 455$ nm, t = 1 h, T = 20 °C unless otherwise noted. (*a*) Lane 1: pUC118 control. Lane 2: linearized pUC118 (EcoR1 digest). Lane 3: pUC118. Lane 4: 200 μM **4**. Lane 5: 200 μM **3** control. Lane 6: 100μM **3** control. Lane 7: 200 μM **3**. Lane 8: 100 μM **3**. Lane 9: 50 μM. Lane 10: 25 μM **3**. Lane 11: 12.5 μM **3**. Lane 12: 12.5 μM **3**, λ > 590 nm. (*b*) Lane 1: pUC118 control. Lane 2: linearized pUC118 (EcoR1 digest). Lane 3: 400 μM **2**. Lane 4: 200 μM **3**. Lane 5: 200 μM **2**. Lane 6: 100 μM **3**. Lane 7: 100 μM **2**. Lane 8: 50 μM **3**.

† Experimental and spectral data for this communication are available from the RSC web site, see http://www.rsc.org/suppdata/cc/1999/2405/

results in the formation of new absorption feature at λ_{max} ~ 590 nm [Fig. 2(*a*), inset]. In the presence of O_2 this transition bleaches due to reaction of Cu^I with O_2 yielding reactive oxygen species (•OH or O_2^-) which oxidize the ligand generating a new absorption feature at 415 nm. Interestingly, the absorption band at 415 nm also appears in the photolysis of **2** under anaerobic buffered conditions, ruling out assignment of this feature as a Cu^{II} ligand-to-metal charge transfer transition and suggesting that it may arise from a conjugated organic species such as a dimerization product.§ Photodimerization and thermal dimerization of carbene intermediates to form ketazine and bifluorenylidene species have been documented for **2** and the related 9-diazofluorene derivatives in the presence of bulky or unreactive solvents.[3,12] However, since the absorption feature is also observed upon anaerobic photolysis of **3** and subsequent O_2 oxidation in unbuffered aqueous solution, the growth of the feature at 415 nm likely arises from oxidation of the ligand *via* either hydroxyl radicals from Cu^I/O_2 reactivity, or carboxy radicals generated by H-atom abstraction from buffer in the absence of O_2. Both of these reactive species are capable of addition reactions with aromatic frameworks to yield hydroxylated, ketonized or carboxylated ligand products.[3,7,13,14] In fact, one of the organic products detected upon extraction with $CHCl_3$ is the 4,5-diazafluorenone (λ_{max} = 390 nm). Further oxidation of this species yields the 4,5-diazafluorene-3,9-dione which has an absorption maximum at 414 nm.[13]

Anaerobic photolysis of **3** in 10 mM Tris acetate buffer at $\lambda \geqslant 455$ nm shows clean conversion to photoproduct during the first 40 min, resulting in the formation of the absorption feature at 415 nm and a broad transition at lower energies (λ_{max} ~ 590 nm). This profile indicates partial conversion to a Cu^I species and formation of oxidized ligand product [Fig. 2(*a*)].† Photolysis of **3** in the presence of calf thymus DNA (0.33 mM in bp) under identical conditions is shown in Fig. 2(*b*). The optical spectrum indicates a significantly different product distribution relative to reaction without DNA substrate. Of note is the diminished growth of the band at 415 nm indicating that the photolysis intermediate of **3** is predominantly reacting with the CT DNA substrate rather than forming dimerization products or generating potent radical oxidants. Neither simple H-atom abstraction from the deoxyribose ring nor phosphonation of diazofluorene[15] would yield products exhibiting the strong absorption band at 415 nm. Also, the prominent growth of the Cu^I band at low energy is not evident in the reaction with DNA, demonstrating that any Cu^I formed is also reoxidized in the acetate/DNA environment.

We are currently investigating the differences in reactivity of **2** and **3** toward DNA substrates. One plausible mechanism involves the binding of the copper dication to the negatively charged DNA backbone. Although the crystal structure shows two asymmetrically bound ligands and two nitrates bound tightly to the complex, conductivity studies indicate that the nitrate anions dissociate to form a 2:1 electrolyte in aqueous solution creating a labile site that permits the DNA substrate to bind to the Cu center. The mechanism for photoactivated DNA cleavage by **3** is intriguing in that excitation at 455 nm would be expected to lead directly to decomposition and N_2 release *via* the ligand centered n $\rightarrow \pi^*$ state localized on the diazo group. However, detection of photoreduced Cu^I suggests that metal–ligand redox chemistry may also play a role in activation of the diazo unit, possibly generating a diazonium cation prior to N_2 release. This is indeed supported by precedence for electrooxidative[6,7] and bimolecular photooxidative N_2 release.[16] Electrochemical studies of **3** in DMSO show a chemically reversible Cu^{2+}/Cu^+ couple at +0.094 V *vs*. SCE, while parallel studies of **2** exhibit an irreversible oxidation at +1.3 V corresponding to N_2 release.[8] From these values a Cu^+–$L^{\cdot+}$ ligand radical state would lie in the vicinity of +1.2 V above the ground state and would be accessible *via* $\lambda \geqslant 455$ nm excitation. The ability of transition metal diazo compounds to employ an additional unimolecular photoredox pathway to release N_2 and form ligand-bound, DNA-damaging radical intermediates suggests that the quinone subunit of Kinamycin C may indeed be serving as a redox source in a parallel N_2-releasing mechanism.

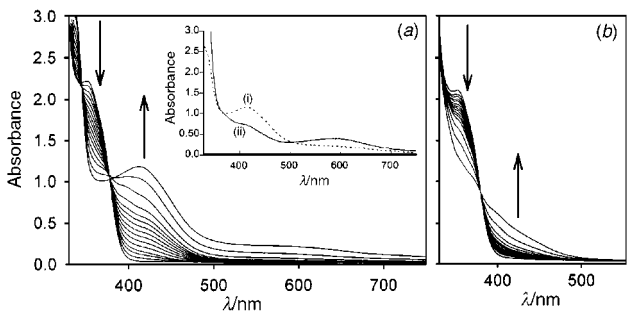


Fig. 2 Evolution of the electronic absorption spectra upon anaerobic photolyses of **3** (0.33 mM, $\lambda \geqslant 455$ nm) in the absence (*a*) and the presence (*b*) of CT DNA (0.33 mM in bp) in Tris acetate buffer (pH 7.6). Times shown are 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 24 and 40 min. The spectra obtained upon anaerobic photolysis of **3** in unbuffered aqueous solution (i) prior to and (ii) following exposure to O_2 are given in the inset.

Complexes such as **3** represent the first of a new class of potent transition metal diazo DNA photocleavers that may be activated using visible light under hypoxic conditions. The ability to employ metal–ligand photoredox chemistry *via* visible region excitation may allow compounds based on this photo-triggering unit to find potential applications in structural biology and the growing field of photomedicine.

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Notes and references

‡ Control experiments involving photolysis of the plasmid DNA alone at $\lambda \geqslant 590$ nm for 12 h yielded only slight nicking, comparable to that observed in Fig. 1(*a*), lane 3, confirming the activity of **3** at these wavelengths.

§ 9,9′-Bi(4,5-diazafluorenylidene) has been prepared recently and also shows a distinct band at 417 nm in CH_2Cl_2. Photolysis of **4**, the 4,5-diazafluorenone analog, resulted in no change in the absorption spectrum over a period of 3 h, indicating that the observed reactivity is derived from the diazo unit. Loss of the diazo group on photolysis in **3** has been unequivocally confirmed by N_2 bubble formation and disappearance of the asymmetric diazo stretch in the IR at 2080 cm^{-1} (ref. 10).

¶ Photolysis studies show that DNA cleavage does indeed occur under aerobic conditions as well.

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