Inorganic Chemistry
Cite This: Inorg. Chem. 2018, 57, 5004−5012
Article
pubs.acs.org/IC

Multiply Intercalator-Substituted Cu(II) Cyclen Complexes as DNA Condensers and DNA/RNA Synthesis Inhibitors

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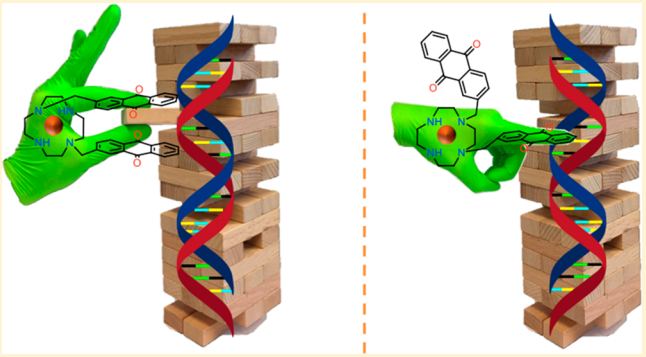
Supporting Information

ABSTRACT: Many drugs that are applied in anticancer therapy such as the anthracycline doxorubicin contain DNA-intercalating 9,10-anthraquinone (AQ) moieties. When Cu(II) cyclen complexes were functionalized with up to three (2-anthraquinonyl)methyl substituents, they efficiently inhibited DNA and RNA synthesis resulting in high cytotoxicity (selective for cancer cells) accompanied by DNA condensation/aggregation phenomena. Molecular modeling suggests an unusual bisintercalation mode with only one base pair between the two AQ moieties and the metal complex as a linker. A regioisomer, in which the AQ moieties point in directions unfavorable for such an interaction, had a much weaker biological activity. The ligands alone and corresponding Zn(II) complexes (used as redox inert control compounds) also exhibited lower activity.



INTRODUCTION

DNA condensation is as important in nature as well as in medicine. Within the 5−10 μm big nucleus of a mammalian cell, approximately 2 m of DNA are compacted into the chromatin.[1,2] Gene therapy requires compaction for the delivery of DNA into the cells to be transfected. Meanwhile, such condensation phenomena have also been observed to play a role for antitumoral drugs.[3,4]

DNA condensation and aggregation occurs in the presence of organic polyamines like spermidine, polycations under physiological conditions.[5,6] Note: (As stated by Bloomfield (*Biopolymers* **1997**, 44, 269−282) condensates of DNA represent aggregates of limited size and definite morphology, whereas aggregation and precipitation are phenomena of less order. Since it is difficult to distinguish between such effects, we use these expressions synonymously.) Among metal complexes, $[Co(NH_3)_6]^{3+}$ is a known DNA condensing agent,[6] a property that has also been reported recently for trinuclear Pt(II) complexes.[7,8] The above-mentioned systems rely on electrostatic interactions between cationic species and the negatively charged DNA backbone. In the case of metal ions like Pt(II), coordinative bonds with DNA bases are also conceivable.[9] Moreover, certain intercalating aromatic compounds carrying alkyl ammonium groups such as mitoxantrone (1,4-dihydroxy-5,8-bis-[2-(2-hydroxyethylamino)-ethylamino]-anthracene-9,10-dione), a 9,10-anthraquinone (AQ) derivative, and YOYO-1 (1,1′-(4,4,8,8-tetramethyl-4,8-diazaundecamethylene)bis[4-[(3-methylbenzo-1,3-oxazol-2-yl)-methylidene]-l,4-dihydroquinolinium]tetraiodide) can act as condensing agents. However, their mechanism of action is different from that of metal complexes, since they interact not only electrostatically but also by intercalation into DNA duplexes.[3,10,11] There are a few examples reported, mainly using Pt and Ru, where cytotoxic characteristics resulted from the combination of the respective metal ion with a DNA

Received: January 9, 2018
Published: April 23, 2018

DOI: 10.1021/acs.inorgchem.8b00027
Inorg. Chem. 2018, 57, 5004−5012

intercalating ligand moiety as, for example, in refs 12−14. DNA condensing agents based on such complexes are scarce.[15−19] The interaction of intercalating AQ cyclen and cyclam complexes with DNA has been exploited solely for the recognition of specific sequence patterns and the unwinding of DNA.[20−25]

We have observed surprising biological effects when using multiple AQ moieties as intercalators on a Cu(II) cyclen complex. Not only high cellular uptake and cytotoxicity but also very efficient condensation/aggregation of DNA and inhibition of DNA and RNA synthesis has been found, strongly depending on the degree and regiochemistry of AQ substitution. A variety of biophysical techniques was applied to evaluate the impact of metal complex/DNA interaction on the DNA conformation and enzymatic synthesis of DNA and RNA. Molecular and cell biological tools as well as molecular modeling have contributed to complete the picture of effective DNA condensing agents with a potential application as anticancer drugs.

RESULTS AND DISCUSSION

Synthesis and Characterization. Five Cu(II) cyclen complexes carrying none, one, two, and three AQ-based substituents (Figure 1, cf. S-1.1) were synthesized. All

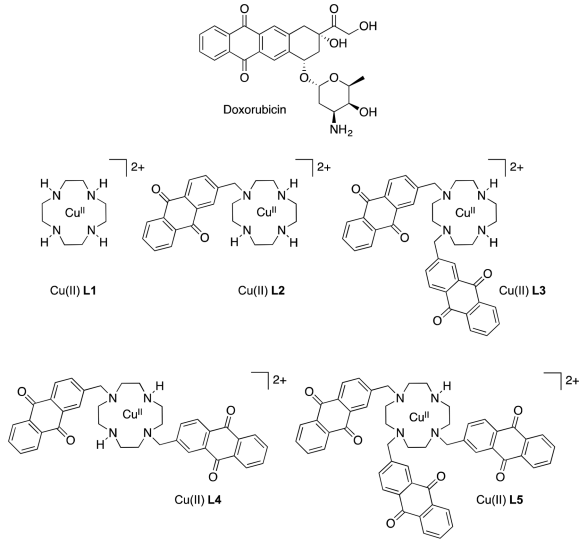


Figure 1. Structural formula of AQ-based doxorubicin and Cu(II) complexes of ligands **L1**−**L5**.

complexes were thoroughly characterized. For $[CuL2(NO_3)]NO_3$, also X-ray crystal structure analysis was performed. For the di-AQ derivative two regioisomers are conceivable, and we thus prepared the 1,4- and the 1,7-di-AQ-cyclen (**L3**, **L4**) Cu(II) complexes. Complexes comprising Zn(II) as a redox-inert metal ion were considered for comparison to exclude any artifacts due to oxidative degradation of DNA.

X-ray Crystal Structure. Although Cu(II) **L2** has already been described by Kimura and co-workers,[21] to the best of our knowledge its crystal structure is presented here for the first time (Figure 2, Table 1, cf. S-2).

Cu(II) **L2** contains $[CuL2(NO_3)]^+$ cations with square-pyramidal coordination environment and NO_3^- anions. The Cu(II) ion is elevated by 0.539 Å over the ring plane. At an angle of 115° (N1−C13−C14) the (2-anthraquinonyl)methyl group is tethered to the N1 atom. Bond lengths and angles of

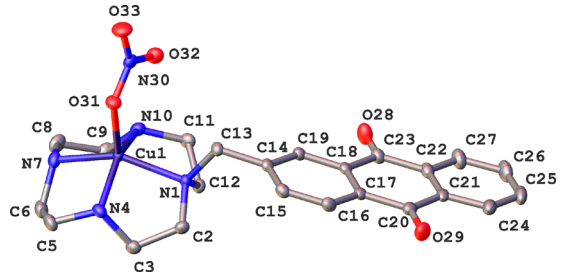


Figure 2. ORTEP[26] diagram of the molecular structure of Cu(II) **L2** showing the $[CuL2(NO_3)]^+$ cation. Thermal ellipsoids are drawn at a probability level of 50%. The counterion and H atoms are omitted for clarity.

Table 1. Selected Bond Lengths and Angles of $[CuL2(NO_3)]^+$

bond length [Å]			angle [deg]
Cu1−N1	2.075(1)	O31−Cu1−N1	104.07(5)
Cu1−N4	2.015(1)	O31−Cu1−N4	98.05(5)
Cu1−N7	2.025(1)	O31−Cu1−N7	106.35(5)
Cu1−N10	2.002(1)	O31−Cu1−N10	112.89(5)
Cu1−O31	2.164(1)	N1−Cu1−N4	85.55(5)
C2−C3	1.511(2)	N4−Cu1−N7	86.30(6)
O31−N30	1.278(2)	N7−Cu1−N10	85.08(6)
N1−C13	1.497(2)	N10−Cu1−N1	86.88(5)
C13−C14	1.513(2)	N1−Cu1−N7	149.31(6)
C14−C15	1.397(2)	N4−Cu1−N10	149.06(6)
C18−C23	1.484(2)	N1−C13−C14	114.85(13)
C23−C28	1.224(2)	C13−C14−C15	120.18(15)

the (2-anthraquinonyl)methyl group are in accordance with the published structure of 2-methylanthraquinone.[27] Furthermore, the AQ moiety is perfectly planar and shows no distortion from the mean aromatic plane compared to 2-methylanthraquinone (in average 0.024 Å deviation), whose non-hydrogen atoms differ by 0.022 Å from the mean aromatic plane.[27]

Biophysical Evaluation of Metal Complex/DNA Interaction. DNA melting experiments were performed to determine the effect of the Cu(II) complexes of ligands **L1** to **L5** on the thermal stability of calf thymus DNA (CT-DNA, Table 2). Intercalation into the double helix is known to

Table 2. Effect of the Cu(II) Complexes of Ligands L1−L5 on the Melting Temperature of CT-DNA[a]

compound	ΔT_m [°C]
Cu(II) **L1**	0.2 ± 0.3
Cu(II) **L2**	6.0 ± 2.8
Cu(II) **L3**	5.0 ± 1.5
Cu(II) **L4**	1.6 ± 0.6
Cu(II) **L5**	1.0 ± 0.1

[a] T_m = 82.4 ± 0.2 °C, cf. S-3 for conditions and diagrams including also ligands and Zn(II) complexes.

increase the melting temperature of DNA (T_m) due to increased stability.[28] The melting temperature of CT-DNA was not influenced by Cu(II) **L1**, whereas the complexes of AQ-containing ligands **L2**−**L5** increased T_m indicating thermal stabilization by intercalation. The stabilizing effect, however, decreased when going from Cu(II) **L2** carrying one AQ group (ΔT_m = 6.0 ± 2.8 °C) to Cu(II) **L5** with three substituents (ΔT_m = 1.0 ± 0.1 °C), probably due to increasing steric crowd

DOI: 10.1021/acs.inorgchem.8b00027
Inorg. Chem. 2018, 57, 5004−5012

and/or aggregation phenomena. The corresponding ligands and Zn(II) complexes showed the same tendencies on T_m, although less pronounced (cf. S-3).

The binding affinities of the Cu(II) complexes toward CT-DNA were determined employing the ethidium bromide (EB) displacement assay (Table 3). While the binding constant of Cu(II) **L1** as expected rules out an intercalative binding mode, the binding constants of the complexes of ligands **L2** to **L5** are in a range that indicates intercalative DNA binding.[29] As suggested already by the structural formula (Figure 1), Cu(II) **L4** and Cu(II) **L5** should be able to bisintercalate DNA, and such a scenario is considered also in Table 3, when the complexes displace two EB molecules at a time. Higher binding constants (e.g., at least an order of magnitude higher)[30,31] in comparison to the monointercalating complexes of **L2**–**L3** should be expected. This was not observed here in the same way it was in the melting studies due to the prompt aggregation even at low concentrations of Cu(II) **L4**–**L5** (vide infra). A quantitative comparison of K_{app} values for these complexes is thus not reasonable. Nevertheless, the general trend of binding affinity is in accordance with the one obtained by DNA melting studies. For Cu(II) **L2**, Kimura et al. have shown before that EB was more efficiently displaced from poly(dG) × poly(dC) than CT-DNA.[21] Under the conditions we used, the Cu(II) complexes of ligands **L2**–**L5** had binding constants K_{app} in the same order of magnitude toward CT-DNA and poly(dG) × poly(dC) but much lower ones for poly(dA) × poly(dT) (cf. S-4 for displacement studies of homopolymers and K_{SV} and K_{app} values for all oligonucleotides), suggesting a preference of these complexes for G-rich sequences.

Table 3. Effect of the Cu(II) Complexes of Ligands L1–L5 on K_{app} Values [M^{-1}] of CT-DNA[a]

compound	K_{app} [M^{-1}] × 10^7
Cu(II) **L1**	0.44
Cu(II) **L2**	7.26
Cu(II) **L3**	3.38
Cu(II) **L4**	0.73 (1.45)
Cu(II) **L5**	0.40 (0.80)

[a]Values for bisintercalation are considered in parentheses; cf. S-4 for conditions and diagrams.

The ability of the complexes to condense DNA was explored by total intensity light scattering.[32,33] The intensity of scattered light is low in absence or at low concentrations of a condensing agent, but it is markedly increased at a critical concentration due to the formation of condensed DNA particles; subsequently, it levels off (cf. S-5). The efficacy of a DNA condenser can be quantified by determining the EC_{50} value, which is the concentration of a condensing agent at the midpoint of DNA condensation. Cu(II) **L2** induced condensation only at concentrations higher than 30 μM (Table 4). In contrast, the remaining complexes Cu(II) **L3**–**L5** condensed DNA at concentrations ~1 μM. Spermine, a polyamine (polyammonium cations under physiological conditions) and known DNA condenser, is 4 times less efficient under the same conditions.[7]

As a fourth, complementary, biophysical technique linear dichroism (LD) spectroscopy was applied. This analysis provides a good indicator of the orientation of the ligand on the DNA. If a molecule is bound to DNA in a specific orientation with respect to the biopolymer it exhibits an LD signal. By contrast, if the molecule is unbound or bound randomly to DNA then no LD signal can be detected. The LD spectra of CT-DNA titrated with the Cu(II) complexes of **L2**–**L4** (Figure 3, cf. S-6) show a negative signal in the 300–375 nm range and negative bands in the DNA region (220–300 nm).

Table 4. EC_{50} Values of the Cu(II) Complexes of Ligands L2–L5 for CT-DNA Obtained from Total Intensity Light Scattering[a]

compound	EC_{50} [μM]
Cu(II) **L2**	>30
Cu(II) **L3**	0.85 ± 0.08
Cu(II) **L4**	0.78 ± 0.07
Cu(II) **L5**	1.16 ± 0.05
spermine	4.10 ± 0.50[7,8]

[a]EC_{50} values were determined by plotting the scattered light intensity against the concentration of condensing agents (cf. S-5). Data are means ± standard deviation (SD) of three separate measurements.

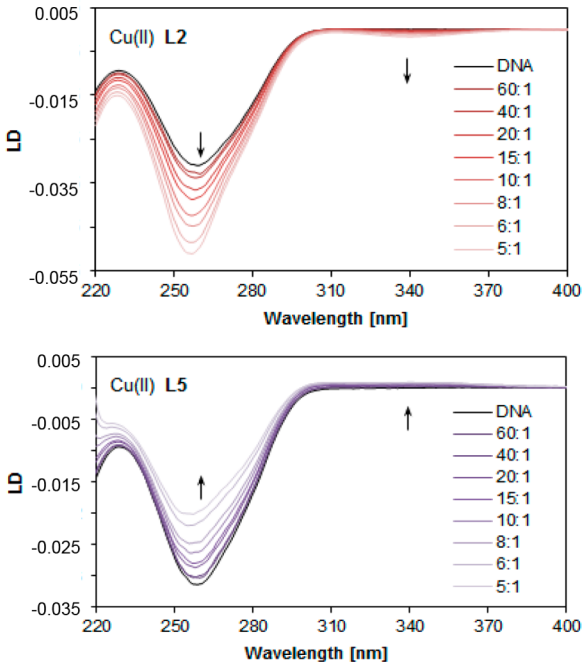


Figure 3. LD spectra of free CT-DNA and in the presence of increasing concentrations of Cu(II) **L2** (top) and Cu(II) **L5** (bottom).

As the concentration of Cu(II) complexes is increased, the intensity of LD signals increases. This is in agreement with a coplanar arrangement of the DNA base pairs and the AQ moieties, which is highly suggestive of an intercalative mode of binding.[34] Whereas the effects are distinct for Cu(II) **L2**, they are less pronounced for Cu(II) **L3** and even less for Cu(II) **L4**. This order can be correlated with the thermal stability results and binding constants (Tables 2 and 3) suggesting concordant decreasing intercalation and binding strength. The LD spectra of CT-DNA in the presence of Cu(II) **L5** (Figure 3), however, look completely different. An unexpected decrease of the LD signals is observed, which suggests that the AQ moieties are not perpendicularly arranged to the DNA helix axis anymore. This can be due to kinking, bending, or condensation/aggregation of DNA.[8,32] Since our molecular modeling studies (vide infra) indicate low perturbation of the DNA structure, bent/kinked

DOI: 10.1021/acs.inorgchem.8b00027
Inorg. Chem. 2018, 57, 5004–5012

DNA can be rather excluded, whereas condensation can be considered a conclusive explanation. Note: (We did an energy minimization into the nearest local energy minimum. Although it seems unlikely, we cannot completely exclude the possibility that there are other energy minima, which might correspond to a kinked or bent DNA structure.) The intermediate behavior of Cu(II) **L3** and Cu(II) **L4** suggests a transition from coplanar arrangement of the AQ units to condensation effects.

LD studies with poly(dG) × poly(dC) and poly(dA) × poly(dT) homopolymers showed no major differences to the behavior toward CT-DNA for Cu(II) **L3** (cf. S-6). Poly(dG) × poly(dC) exhibits an A conformation[35] with the DNA base planes being more inclined and not perfectly perpendicular to the axis like in B-form DNA. Consequently, this gives rise to the low LD signal in the absence of the complexes. Once Cu(II) **L3** is added, the signal increases significantly suggesting a straightening of the DNA duplex due to intercalation of an AQ moiety. However, Cu(II) **L4** was peculiar showing, on the one hand, first increasing and then decreasing LD signals for poly(dA) × poly(dT) (for a base/complex ratio > 15:1). On the other hand, no obvious changes were observed for poly(dG) × poly(dC). The latter observation indicates a cramping of the A-DNA conformation due to the assumed bisintercalation binding mode—even at lowest concentrations. For poly(dA) × poly(dT), however, such a cramping might occur only when a certain concentration is exceeded to allow a switching monointercalation → bisintercalation (increasing → decreasing LD signals).

Molecular Biological Evaluation of Metal Complex/DNA Interaction. A gel retardation assay provided insight in the DNA condensation ability of **L2**−**L5** and their corresponding Zn(II) and Cu(II) complexes (cf. S-7). Addition of the complexes of **L3**−**L5** (10−20 μM) led to plasmid DNA remaining in the gel loading wells. Note: (In some cases at 50 μM ligand/complex, no DNA at all could be detected, since binding of these compounds can impede DNA visualization due to disturbed intercalation of the EB gel stain (*J. Phys. Chem. B* **2014**, 118, 4832−4839).) In earlier reports similar concentrations were used but at higher complex/DNA ratios[15,36] accompanied by DNA damage.[16] No significant differences between the retardation effects of Zn(II) and Cu(II) complexes were observed. Ligands **L2**−**L4**, however, showed almost no or very little effect on DNA migration up to 50 μM concentration, and ligand **L5** alone inhibited the migration only at the highest concentration tested (50 μM). This observation is similar to reported retardation effects of macrocyclic polyamine condensers,[37] strongly suggesting the importance of the metal ion for efficient condensation. The results could be correlated with total intensity light scattering measurements (vide supra). To test representatively, if the otherwise used buffer system Tris-HCl as a potential competitive ligand for Cu(II)[38] could interfere with the biological activity of the complexes, a Good's buffer system[39] was also tested. The 3-(*N*-morpholino)-propanesulfonic acid (MOPS) buffer had no changes to the results obtained with the Tris system (cf. S-7). It should be mentioned, that the competing reaction with Tris could be more problematic in case of the Zn(II) complexes due to their lower stability. Whereas a log K = 24.8[40] is reported for Cu(II) cyclen, it is only log K = 16.2[40] for Zn(II) cyclen. The formation constants of the Tris complexes, however, differ not that much: log K_1 = 4.1 for Cu(II) vs 2.4 for Zn(II).[41] The potential release of the metal ion from the complexes in case of Zn(II), but not of Cu(II), might explain the intermediate behavior of the Zn(II) complexes between the ligands and the Cu(II) species in most of the presented experiments.

Atomic force microscopy (AFM) is a useful imaging technique for investigating DNA intercalation as well as aggregation.[7,8,42,43] The formation of DNA aggregates in the presence of Cu(II) complexes of **L3**−**L5** was observed at low micromolar concentrations (Figure 4, cf. S-8). Additionally, for

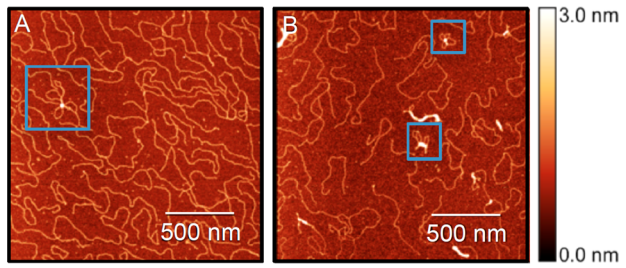


Figure 4. Representative AFM images of linearized plasmid pSP73 DNA in the presence of Cu(II) **L4** at 1.56 μM (A) and 3.13 μM (B). Untreated reference cf. S-8.

Cu(II) **L4** and Cu(II) **L5** typical loop structures and plectonemic coils (interwound DNA strands visible as thick-stranded features) were observed (blue frames in Figure 4 and S-8.4). Such DNA−DNA contacts arise from bisintercalation[42] and reduction of the negative charge of DNA when binding to positively charged molecules.[42] Increasing the complex concentration led to the formation of larger and more compact DNA aggregates and eventually to disappearance of DNA molecules from the substrate (cf., e.g., Figure S-8.4C), indicating little adhesion to the surface during rinsing after immobilization.

To examine whether the complexes Cu(II) **L1**−**L5** were able to inhibit synthesis of DNA when bound to it, we utilized the polymerase chain reaction (PCR) as an ex vivo model for the DNA replication machinery.[44] A 279 bp insert of pBR322 plasmid DNA was amplified in the presence of the complexes. Inhibition of the *Thermus aquaticus* (*Taq*) polymerase was observable through missing DNA bands on an agarose gel. In agreement with the literature,[44] the well-known DNA binder cisplatin inhibited DNA synthesis at concentrations higher than 50 μM only (cf. S-9). For the complexes Cu(II) **L2**−**L5** the extent of inhibition was dependent on the number of AQ groups coupled to the cyclen backbone. The higher the number of AQ substituents, the more efficient was the inhibition. While 3 μM of Cu(II) **L2** was needed, already 1 μM of the cis-substituted complex Cu(II) **L3**, 0.1 μM of the trans-substituted complex Cu(II) **L4** and 75 nM Cu(II) **L5** caused the same effect (Figure 5). Concordantly with the gel retardation, **L5** and Zn(II) **L5** also inhibited the PCR, although at higher concentrations (cf. S-9).

An additional DNA transcription assay showed that Cu(II) **L4** and Cu(II) **L5** also inhibited transcription of the circular form of pBR322 DNA into RNA at even milder conditions compared to the PCR assay (cf. S-10). Here the transcriptional activity was monitored by measuring the increase of fluorescence resulting from the formation of aminonaphthalenesulfonate (AmNS) by its cleavage from the terminal phosphate group of uridine triphosphate (UTP).[45] Even though an absorption effect of the AQ groups must be taken into account, the results obtained are in accordance with the PCR experiment: again Cu(II) **L4** and Cu(II) **L5** are most

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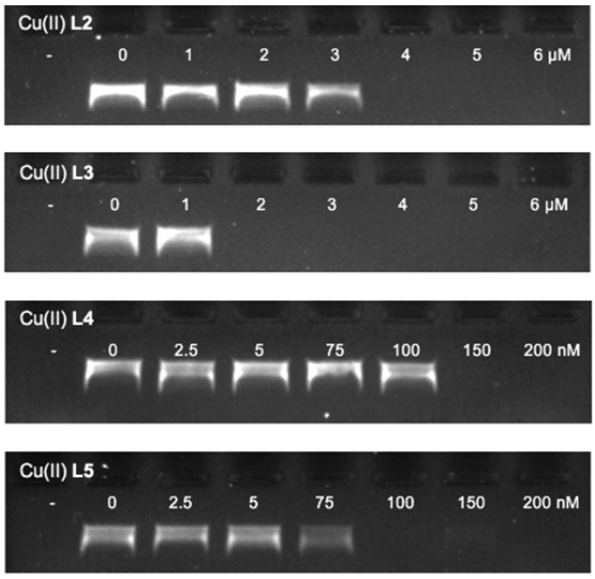

Figure 5. Inhibition of the synthesis of a 279 bp insert of linearized pBR322 plasmid DNA in the presence of Cu(II) **L2**–**L5** analyzed by agarose gel electrophoresis.

active followed by Cu(II) **L3** and Cu(II) **L2**. 50% inhibition of the transcriptional activity was achieved with only 10 μM of Cu(II) **L4**, whereas hundredfold more was needed in case of the condenser spermine under the same conditions.[45]

Although Cu(II) **L3**–**L5** are efficient condensers, Cu(II) **L4** and Cu(II) **L5** were the most potent inhibitors of DNA replication and transcription despite their relatively weak DNA binding strength. This might be due to a bisintercalative binding mode of Cu(II) **L4** and Cu(II) **L5** resulting in enhanced disturbance of the Taq polymerase and the RNA polymerase.

Evaluation of Cytotoxicity. Because of potent inhibition of DNA synthesis in vitro we studied cytotoxic properties of the Cu(II) complexes of **L1**–**L5** to assess if their activity is retained in cellulo. Adenocarcinomic human alveolar basal epithelial A549 cells were incubated with the complexes for 48 h, and their survival was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As a control normal human dermal fibroblasts (NHDF) were employed to discern if the complexes are cytotoxic specifically toward cancer cells (Table 5, cf. S-11). As expected Cu(II) **L1** only showed a slight cytotoxic effect on A549 cells (IC_{50} = 109.4 μM). The

Table 5. IC_{50} [μM] Values and 95% Confidence Intervals (CI, in Brackets) Obtained by the MTT Assay (48 h) against A549 and NHDF Cells for the Cu(II) Complexes of L1–L5, Compared with the Reference Drugs Doxorubicin and Cisplatin[a]

compound	A549	NHDF
Cu(II) **L1**	109.4 (71.3–167.7)	93.1 (79.5–109.2)
Cu(II) **L2**	96.9 (77.8–120.6)	77.6 (62.9–95.7)
Cu(II) **L3**	28.3 (20.4–39.2)	43.2 (23.5–79.3)
Cu(II) **L4**	1.3 (0.4–3.5)	13.4 (2.2–80.2)
Cu(II) **L5**	1.4 (0.6–3.5)	8.1 (3.1–21.2)
doxorubicin	2.0 (n/a)[46]	2.5 (n/a)[49]
cisplatin	13.0 (n/a)[47]	94.6 (n/a)[47]

[a]cf. S-11 for diagrams including also ligands and Zn(II) complexes.

results obtained for the AQ-substituted cyclen complexes are in accordance with the results obtained by the PCR assay: the 1,4-di-AQ complex Cu(II) **L3** is ~3 times more effective than the mono-AQ complex Cu(II) **L2** (IC_{50} = 96.9 μM vs 28.3 μM). As expected Cu(II) **L4** and Cu(II) **L5** show even higher activity (IC_{50} = 1.3 and 1.4 μM) correlating with their inhibitory activity toward DNA and RNA synthesis. Cu(II) **L4** and Cu(II) **L5** yet outperform doxorubicin and cisplatin, of which 2.0 and 13.0 μM, respectively, are needed to achieve the same effect under similar conditions.[46,47] Cu(II) **L1** and Cu(II) **L2** show lower cytotoxicity towards the studied cancer cell line A549 over normal human dermal fibroblasts. In comparison, Cu(II) **L3** shows slight selectivity for the cancer cells, whereas Cu(II) **L4** and Cu(II) **L5** show a distinct selectivity factor of 10 and 6, respectively, for the cancer over the fibroblast cells. They are thus equally (Cu(II) **L5**) or even more (Cu(II) **L4**) selective than cisplatin under similar conditions.

In contrast to other systems, for example, a Cu(II) bisterpyridine (bistpy) derivative,[16] high cytotoxicity was not accompanied by obvious damage of DNA on the agarose gel. This could be attributed to the higher stability of the presented system (log K = 24.8[40] for cyclen vs 19.1[48] for bistpy), preventing a release of "free" Cu(II), which is prone to cleave DNA.

It was noted that the dose–response relationship of Cu(II) **L4** and Cu(II) **L5** as well as Zn(II) **L5**, according to the MTT assay, was more flat than with the ligand alone or Cu(II) **L1**–**L3** with both cell types. Even at the lowest concentrations used, the calculated relative viabilities did not reach the 100% plateau, leading to less well-fitted dose–response curves (cf. S-11). As the MTT assay measures metabolic activity that is proportional to the cell number, we analyzed whether the low values resulted from an actually low cell number or from interference of the compounds with cell metabolism. With flow cytometry, intact cells were counted after incubation with the highest and lowest concentrations investigated in the MTT assay of Cu(II) **L2**, **L4**, and **L5**. This experiment revealed that the cell number was slightly increased rather than decreased at low concentrations, while at higher concentrations, the cell number was decreased (cf. S-12), similarly to the results from the MTT test. Consequently, at low concentrations Cu(II) **L4** and **L5** seem to inhibit metabolic activity rather than actually being responsible for cell death.

As in the PCR assay ligand **L5** and its corresponding Zn(II) complex also exhibited activity in the cytotoxicity assay; nevertheless, they were less effective than the Cu(II) complex (9.0 and 2.5 μM, respectively, in A549 cells, cf. S-11). The cytotoxicity of **L5** is in agreement with the literature investigating macrocyclic polyamines with intercalating moieties.[37,50]

Interestingly, metal coordination alleviates cytotoxicity in A549 cells in case of **L1**–**L3** similarly for Cu(II) and Zn(II), whereas, for **L4** and **L5**, the presence of Zn(II) and Cu(II) ions increases cytotoxicity. This indicates either a better cellular uptake for the ligands **L1**–**L3** in comparison to their metal complexes or a toxic disturbance of metal ion homeostasis by these ligands. Binding of the cyclen ligands to DNA should be easily accomplished (at pH 7.4 cyclen is doubly protonated[51]) and might also contribute to the ligand cytotoxicity. Also peculiar is the general trend that the Cu(II) complexes were more cytotoxic than the Zn(II) complexes in cancer cells, whereas it was vice versa in the fibroblast cells. It can be assumed that the different redox states of the two cell lines play

DOI: 10.1021/acs.inorgchem.8b00027
Inorg. Chem. 2018, 57, 5004–5012

a role for this observation: in the cancer cells, Cu(II) is reduced more easily to Cu(I) due to elevated levels of intracellular reductants like glutathione.[52] This leads to generation of oxidative stress and thus high cytotoxicity[53]—in contrast to the redox-inert Zn(II) complexes. The phenomenon that Zn(II) can be more toxic than Cu(II) in fibroblast cells has been described before in the literature.[54]

To gain a better insight into the importance of cellular uptake for the cytotoxicity, Cu(II) **L1** and Cu(II) **L4** as two representative compounds with low and high cytotoxicity were incubated with A549 cells, and the Cu(II) content was determined by atomic absorption spectroscopy (AAS). Whereas only 0.07 nmol Cu/mg protein was detected for Cu(II) **L1** after 24 h, it was almost 40 times as much for Cu(II) **L4** (cf. S-13). These results correlate well with the IC_{50} values and can be explained by the more hydrophobic character of Cu(II) **L4** due to the two AQ moieties, which facilitate the permeation of the complex through the lipid bilayer of cell membranes.

The use of bisintercalators as antagonists of DNA metabolism is a strategy for anticancer drugs, which was already introduced in the 1970s.[55] For instance, bifunctional anthracycline derivatives were shown to be more cytotoxic than the anthracycline alone, also in doxorubicin-resistant cell lines.[30,56] Bisintercalating AQ derivatives have shown efficacy in vivo in tumor-engrafted mice;[57] however, no studies on the molecular level like the ones performed here have been undertaken before.

Molecular Modeling. The surprising finding that the regioisomers Cu(II) **L3** and Cu(II) **L4** equally efficiently aggregate DNA but differ significantly in their DNA and RNA synthesis inhibiting and cytotoxic properties was further elucidated with molecular modeling. This method has been used before successfully to correlate structural variations of DNA-intercalating agents with their cytotoxicity.[58] The complexes were intercalated with their AQ moieties into a short G-rich DNA duplex (PDB:440D) at the minor and major groove (cf. S-14), since Cu(II) **L2** is known to selectively recognize consecutive G sequences.[21] G-rich sequences favor A-DNA conformation,[35] wherein the minor groove is wider and shallower compared to B-DNA. Such a minor groove is a preferred binding site for AQ-based antibiotics like chromomycin A3.[35]

The calculated conformational energy values did not show significant differences for Cu(II) **L3** and Cu(II) **L4** in the minor and major groove within the accuracy of the force field. For Cu(II) **L4** (and **L5**), additionally, a conformation with both AQ moieties being intercalated was found at comparable energy values as the singly intercalated complexes (Figure 6, Figure S-14.5). Whereas bisintercalation into B-DNA conformations is sterically impossible (Figure S-14.6), the bisintercalative binding mode in G-rich sequences[42] exhibiting A-DNA conformation might present the main reason for the high cytotoxicity of Cu(II) **L4** and Cu(II) **L5** as well as for efficient DNA and RNA synthesis inhibition and DNA aggregation propensity.

The found bisintercalation mode is very interesting, since it disagrees with the "neighbor exclusion principle". Whereas this principle requests intercalating molecules to have at least two base pairs between them,[59] here only a single base pair is sandwiched between the AQ moieties. The distance of these moieties in Cu(II) **L4** is only 6.5 Å (Figure S-14.7), thus geometrically adequate to span one base pair. Coplanar insertion with two base pairs between would require a larger

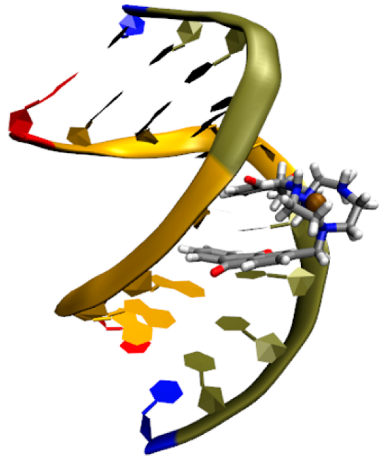

Figure 6. Cu(II) **L4** intercalated into a short DNA double helix in the minor groove showing insertion of both AQ moieties.

distance (~10 Å as, e.g., in a bisintercalating Pt-acridine compound—to our knowledge the only example besides the one presented here consisting of two intercalators linked by a metal complex[60]).

There are many examples of organic bisintercalators, natural ones like echinomycin and artificial ones like ditercalinium and diacridines with various linker lengths. Nevertheless, only very few of them (e.g., a bisintercalating peptide[61]) seem to violate the neighbor exclusion principle. Alternative conceivable binding modes like bisintercalation obeying the neighbor exclusion principle or inter/intrastrand cross-linking are improbable for Cu(II) **L4** and Cu(II) **L5**: (1) perturbed DNA structures are energetically not preferred.[62] (2) The neighbor exclusion principle seems to apply only to short sequences like pentanucleotides[63] but not to longer ones like in the present experimental and theoretical studies. (3) A cross-linking of two DNA duplexes (meaning considerable steric crowd) is possible only for longer linkers (≥8.8 Å[64,65]). (4) The cross-linking of two strands within one duplex would have resulted in higher thermal stabilization of the DNA.[65]

CONCLUSIONS

Whereas multiply AQ-substituted Cu(II) cyclen complexes exhibited modest DNA binding affinities compared to monosubstituted species, dramatic effects on the DNA morphology, efficient inhibition of DNA/RNA synthesis, and high cytotoxicity have been observed. The ligands alone and the corresponding Zn(II) complexes, which are less stable than their Cu(II) counterparts under the conditions of the experiments, showed weaker effects. This suggests the importance of the metal ion for the interaction with DNA (electrostatic and/or coordinative) next to the AQ-based intercalation. Although the interconnection of the effects could not be completely elucidated, bisintercalation was identified as the most likely reason for them. Most interesting is the behavior of Cu(II) **L3** and **L4**—regioisomeric compounds, which represent equally efficient DNA condensers, but only the latter one is an efficient inhibitor of DNA/RNA synthesis and cytotoxic agent due to bisintercalating properties. Cu(II) **L4** and Cu(II) **L5** thus fulfill the requirements for transcription inhibitors discussed as anticancer agents (modest binding affinity, bulky groups in the minor groove, polymerase inhibition).[58] By combining the endogenous metal Cu (in

DOI: 10.1021/acs.inorgchem.8b00027
Inorg. Chem. 2018, 57, 5004−5012

contrast to exogenous Pt and Ru as in some anticancer drugs/drug candidates and DNA condensers) with a structural motif of the drug doxorubicin (AQ) an application as anticancer drug with low side effects can be envisioned. Also, selective toxicity is conceivable for such bisintercalators due to discrimination of DNA sequences.[55]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.8b00027.

Experimental details for all reactions, spectroscopic characterization of all products, diagrams for biophysical, molecular, and cell biological studies (PDF)

Accession Codes

Crystallographic data for [Cu**L2**(NO_3)]NO_3. CCDC 1532772 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The Studienstiftung des deutschen Volkes is acknowledged for a predoctoral fellowship, and the Dahlem Research School is acknowledged for a postdoctoral fellowship for J.H.; O.L. and B.G.K. acknowledge support by the DFG collaborative research center CRC 765. J.M. and V.B. were supported by the Czech Science Foundation (Grant No. 17-09436S). We thank A. Hoppensack for providing us with NHDF cells, J. Heinrich and C. Schattschneider for their help with DNA melting studies, and the Kulak group for proofreading of this article.

■ REFERENCES

(1) Li, G.-W.; Xie, X. S. Central Dogma at the Single-Molecule Level in Living Cells. *Nature* **2011**, *475*, 308−315.

(2) Misteli, T. Physiological Importance of RNA and Protein Mobility in the Cell Nucleus. *Histochem. Cell Biol.* **2008**, *129*, 5−11.

(3) Kapuscinski, J.; Darzynkiewicz, Z. Relationship between the Pharmacological Activity of Antitumor Drugs Ametantrone and Mitoxantrone (Novatrone) and Their Ability to Condense Nucleic Acids. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 6302−6306.

(4) Hou, X.-M.; Zhang, X.-H.; Wei, K.-J.; Ji, C.; Dou, S.-X.; Wang, W.-C.; Li, M.; Wang, P.-Y. Cisplatin Induces Loop Structures and Condensation of Single DNA Molecules. *Nucleic Acids Res.* **2009**, *37*, 1400−1410.

(5) Gosule, L. C.; Schellman, J. A. Compact Form of DNA Induced by Spermidine. *Nature* **1976**, *259*, 333−335.

(6) Pelta, J.; Livolant, F.; Sikorav, J.-L. DNA Aggregation Induced by Polyamines and Cobalthexamine. *J. Biol. Chem.* **1996**, *271*, 5656−5662.

(7) Malina, J.; Farrell, N. P.; Brabec, V. Substitution-Inert Trinuclear Platinum Complexes Efficiently Condense/Aggregate Nucleic Acids and Inhibit Enzymatic Activity. *Angew. Chem., Int. Ed.* **2014**, *53*, 12812−12816.

(8) Malina, J.; Farrell, N. P.; Brabec, V. DNA Condensing Effects and Sequence Selectivity of DNA Binding of Antitumor Noncovalent Polynuclear Platinum Complexes. *Inorg. Chem.* **2014**, *53*, 1662−1671.

(9) Todd, R. C.; Lippard, S. J. Structure of Duplex DNA Containing the Cisplatin 1,2-$\{Pt(NH_3)_2\}^{2+}$-d(GpG) Cross-Link at 1.77Å Resolution. *J. Inorg. Biochem.* **2010**, *104*, 902−908.

(10) Kapuscinski, J.; Darzynkiewicz, Z. Condensation of Nucleic Acids by Intercalating Aromatic Cations. *Proc. Natl. Acad. Sci. U. S. A.* **1984**, *81*, 7368−7372.

(11) Kuyper, C. L.; Brewood, G. P.; Chiu, D. T. Initiating Conformation Transitions of Individual YOYO-Intercalated DNA Molecules with Optical Trapping. *Nano Lett.* **2003**, *3*, 1387−1389.

(12) Deo, K. M.; Pages, B. J.; Ang, D. L.; Gordon, C. P.; Aldrich-Wright, J. R. Transition Metal Intercalators as Anticancer Agents—Recent Advances. *Int. J. Mol. Sci.* **2016**, *17*, 1818.

(13) Lozano, H. J.; Busto, N.; Espino, G.; Carbayo, A.; Leal, J. M.; Platts, J. A.; García, B. Interstrand DNA Covalent Binding of Two Dinuclear Ru(II) Complexes. Influence of the Extra Ring of the Bridging Ligand on the DNA Interaction and Cytotoxic Activity. *Dalton Trans.* **2017**, *46*, 3611−3622.

(14) Augustus, T. M.; Anderson, J.; Hess, S. M.; Bierbach, U. Bis(acridinylthiourea)platinum(II) Complexes: Synthesis, DNA Affinity, and Biological Activity in Glioblastoma Cells. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 855−858.

(15) Sun, B.; Guan, J.-X.; Xu, L.; Yu, B.-L.; Jiang, L.; Kou, J.-F.; Wang, L.; Ding, X.-D.; Chao, H.; Ji, L.-N. DNA Condensation Induced by Ruthenium(II) Polypyridyl Complexes $[Ru(bpy)_2(PIPSH)]^{2+}$ and $[Ru(bpy)_2(PIPNH)]^{2+}$. *Inorg. Chem.* **2009**, *48*, 4637−4639.

(16) Rajalakshmi, S.; Kiran, M. S.; Nair, B. U. DNA Condensation by Copper(II) Complexes and Their Anti-Proliferative Effect on Cancerous and Normal Fibroblast Cells. *Eur. J. Med. Chem.* **2014**, *80*, 393−406.

(17) Meng, X.; Liu, L.; Zhou, C.; Wang, L.; Liu, C. Dinuclear Copper(II) Complexes of a Polybenzimidazole Ligand: Their Structures and Inductive Roles in DNA Condensation. *Inorg. Chem.* **2008**, *47*, 6572−6574.

(18) Li, C.; Tian, H.; Duan, S.; Liu, X.; Xu, P.; Qiao, R.; Zhao, Y. Controllable DNA Condensation-Release Induced by Simple Azaheterocyclic-Based Metal Complexes. *J. Phys. Chem. B* **2011**, *115*, 13350−13354.

(19) Dutta, S.; Snyder, M. J.; Rosile, D.; Binz, K. L.; Roll, E. H.; Suryadi, J.; Bierbach, U.; Guthold, M. PT-ACRAMTU, A Platinum−Acridine Anticancer Agent, Lengthens and Aggregates, but Does Not Stiffen or Soften DNA. *Cell Biochem. Biophys.* **2013**, *67*, 1103−1113.

(20) Siters, K. E.; Sander, S. A.; Devlin, J. R.; Morrow, J. R. Bifunctional Zn(II) Complexes for Recognition of Non-Canonical Thymines in DNA Bulges and G-Quadruplexes. *Dalton Trans.* **2015**, *44*, 3708−3716.

(21) Kikuta, E.; Matsubara, R.; Katsube, N.; Koike, T.; Kimura, E. Selective Recognition of Consecutive G Sequence in Double-Stranded DNA by a Zinc(II)−macrocyclic Tetraamine Complex Appended with an Anthraquinone. *J. Inorg. Biochem.* **2000**, *82*, 239−249.

(22) Ellis, L. T.; Perkins, D. F.; Turner, P.; Hambley, T. W. The Preparation and Characterisation of Cyclam/Anthraquinone Macrocyle/Intercalator Complexes and Their Interactions with DNA. *Dalton Trans.* **2003**, 2728−2736.

(23) Tucker, J. H. R.; Shionoya, M.; Koike, T.; Kimura, E. A Zinc(II)−Cyclen Complex Attached to an Anthraquinone Moiety That Acts as a Redox-Active Nucleobase Receptor in Aqueous Solution. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 2465−2469.

(24) Kimura, E.; Ikeda, T.; Shionoya, M. Macrocyclic Metal Complexes for Selective Recognition of Nucleic Acid Bases and

DOI: 10.1021/acs.inorgchem.8b00027
Inorg. Chem. 2018, 57, 5004−5012

Manipulation of Gene Expression. *Pure Appl. Chem.* **1997**, *69*, 2187−2195.
(25) Aoki, S.; Kimura, E. Zinc−Nucleic Acid Interaction. *Chem. Rev.* **2004**, *104*, 769−787.
(26) Farrugia, L. J. ORTEP-3 for Windows − a Version of ORTEP-III with a Graphical User Interface (GUI). *J. Appl. Crystallogr.* **1997**, *30*, 565.
(27) Kingsford-Adaboh, R.; Kashino, S. Disordered Structure of 2-Methylanthraquinone. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1995**, *51*, 2094−2096.
(28) Cao, Y.; He, X. Studies of Interaction Between Safranine T and Double Helix DNA by Spectral Methods. *Spectrochim. Acta, Part A* **1998**, *54*, 883−892.
(29) Sheng, X.; Lu, X.-M.; Chen, Y.-T.; Lu, G.-Y.; Zhang, J.-J.; Shao, Y.; Liu, F.; Xu, Q. Synthesis, DNA-Binding, Cleavage, and Cytotoxic Activity of New 1,7-Dioxa-4,10-Diazacyclododecane Artificial Receptors Containing Bisguanidinoethyl or Diaminoethyl Double Side Arms. *Chem. - Eur. J.* **2007**, *13*, 9703−9712.
(30) Lelle, M.; Freidel, C.; Kaloyanova, S.; Tabujew, I.; Schramm, A.; Musheev, M.; Niehrs, C.; Müllen, K.; Peneva, K. Overcoming Drug Resistance by Cell-Penetrating Peptide-Mediated Delivery of a Doxorubicin Dimer with High DNA-Binding Affinity. *Eur. J. Med. Chem.* **2017**, *130*, 336−345.
(31) Dervan, P. B.; Becker, M. M. Molecular Recognition of DNA by Small Molecules. Synthesis of Bis(methidium)spermine, a DNA Polyintercalating Molecule. *J. Am. Chem. Soc.* **1978**, *100*, 1968−1970.
(32) Hannon, M. J.; Moreno, V.; Prieto, M. J.; Moldrheim, E.; Sletten, E.; Meistermann, I.; Isaac, C. J.; Sanders, K. J.; Rodger, A. Intramolecular DNA Coiling Mediated by a Metallo-Supramolecular Cylinder. *Angew. Chem., Int. Ed.* **2001**, *40*, 880−884.
(33) Vijayanathan, V.; Thomas, T.; Shirahata, A.; Thomas, T. J. DNA Condensation by Polyamines: A Laser Light Scattering Study of Structural Effects. *Biochemistry* **2001**, *40*, 13644−13651.
(34) Rodger, A. How to Study DNA and Proteins by Linear Dichroism Spectroscopy. *Sci. Prog.* **2008**, *91*, 377−396.
(35) Gao, Y.-G.; Robinson, H.; Wang, A. H.-J. High-Resolution A-DNA Crystal Structures of d(AGGGGCCCCT). An A-DNA Model of poly(dG)·poly(dC). *Eur. J. Biochem.* **1999**, *261*, 413−420.
(36) Dong, X.; Wang, X.; He, Y.; Yu, Z.; Lin, M.; Zhang, C.; Wang, J.; Song, Y.; Zhang, Y.; Liu, Z.; Li, Y.; Guo, Z. Reversible DNA Condensation Induced by a Tetranuclear Nickel(II) Complex. *Chem. - Eur. J.* **2010**, *16*, 14181−14189.
(37) Yan, H.; Li, Z.-F.; Guo, Z.-F.; Lu, Z.-L.; Wang, F.; Wu, L.-Z. Effective and Reversible DNA Condensation Induced by Bifunctional Molecules Containing Macrocyclic Polyamines and Naphthyl Moieties. *Bioorg. Med. Chem.* **2012**, *20*, 801−808.
(38) Nagaj, J.; Stokowa-Sołtys, K.; Kurowska, E.; Frączyk, T.; Jeżowska-Bojczuk, M.; Bal, W. Revised Coordination Model and Stability Constants of Cu(II) Complexes of Tris Buffer. *Inorg. Chem.* **2013**, *52*, 13927−13933.
(39) Good, N. E.; Winget, G. D.; Winter, W.; Connolly, T. N.; Izawa, S.; Singh, R. M. M. Hydrogen Ion Buffers for Biological Research. *Biochemistry* **1966**, *5*, 467−477.
(40) Kodama, M.; Kimura, E. Equilibria of Complex Formation between Several Bivalent Metal Ions and Macrocyclic Tri- and Penta-Amines. *J. Chem. Soc., Dalton Trans.* **1978**, 1081−1084.
(41) Hanlon, D. P.; Watt, D. S.; Westhead, E. W. The Interaction of Divalent Metal Ions with Tris Buffer in Dilute Solution. *Anal. Biochem.* **1966**, *16*, 225−233.
(42) Berge, T.; Jenkins, N. S.; Hopkirk, R. B.; Waring, M. J.; Edwardson, J. M.; Henderson, R. M.; et al. Structural Perturbations in DNA Caused by Bis-Intercalation of Ditercalinium Visualised by Atomic Force Microscopy. *Nucleic Acids Res.* **2002**, *30*, 2980−2986.
(43) Malina, J.; Hannon, M. J.; Brabec, V. Iron(II) Supramolecular Helicates Condense Plasmid DNA and Inhibit Vital DNA-Related Enzymatic Activities. *Chem. - Eur. J.* **2015**, *21*, 11189−11195.
(44) Jany, T.; Moreth, A.; Gruschka, C.; Sischka, A.; Spiering, A.; Dieding, M.; Wang, Y.; Samo, S. H.; Stammler, A.; Bögge, H.; Fischer von Mollard, G.; Anselmetti, D.; Glaser, T. Rational Design of a Cytotoxic Dinuclear Cu_2 Complex That Binds by Molecular Recognition at Two Neighboring Phosphates of the DNA Backbone. *Inorg. Chem.* **2015**, *54*, 2679−2690.
(45) Luckel, F.; Kubo, K.; Tsumoto, K.; Yoshikawa, K. Enhancement and Inhibition of DNA Transcriptional Activity by Spermine: A Marked Difference between Linear and Circular Templates. *FEBS Lett.* **2005**, *579*, 5119−5122.
(46) Senwar, K. R.; Sharma, P.; Reddy, T. S.; Jeengar, M. K.; Nayak, V. L.; Naidu, V. G. M.; Kamal, A.; Shankaraiah, N. Spirooxindole-Derived Morpholine-Fused-1,2,3-Triazoles: Design, Synthesis, Cytotoxicity and Apoptosis Inducing Studies. *Eur. J. Med. Chem.* **2015**, *102*, 413−424.
(47) Jayakumar, S.; Mahendiran, D.; Viswanathan, V.; Velmurugan, D.; Kalilur Rahiman, A. Heteroscorpionate-Based Heteroleptic Copper(II) Complexes: Antioxidant, Molecular Docking and in Vitro Cytotoxicity Studies. *Appl. Organomet. Chem.* **2017**, *31*, e3809.
(48) Chaurin, V.; Constable, E. C.; Housecroft, C. E. What Is the Coordination Number of Copper(II) in Metallosupramolecular Chemistry? *New J. Chem.* **2006**, *30*, 1740−1744.
(49) Tiago, M.; Mendes de Oliveira, E.; Abdo Brohem, C.; Comune Pennacchi, P.; Duarte Paes, R.; Brandão Haga, R.; Campa, A.; Berlanga de Moraes Barros, S.; Smalley, K. S.; Stuchi Maria-Engler, S. Fibroblasts Protect Melanoma Cells from the Cytotoxic Effects of Doxorubicin. *Tissue Eng., Part A* **2014**, *20*, 2412−2421.
(50) Ding, A.-X.; Tang, Q.; Gao, Y.-G.; Shi, Y.-D.; Uzair, A.; Lu, Z.-L. [12]aneN_3 Modified Tetraphenylethene Molecules as High-Performance Sensing, Condensing, and Delivering Agents toward DNAs. *ACS Appl. Mater. Interfaces* **2016**, *8*, 14367−14378.
(51) De León-Rodríguez, L. M.; Kovacs, Z.; Esqueda-Oliva, A. C.; Miranda-Olvera, A. D. Highly Regioselective N-Trans Symmetrical Diprotection of Cyclen. *Tetrahedron Lett.* **2006**, *47*, 6937−6940.
(52) Russo, A.; DeGraff, W.; Friedman, N.; Mitchell, J. B. Selective Modulation of Glutathione Levels in Human Normal versus Tumor Cells and Subsequent Differential Response to Chemotherapy Drugs. *Cancer Res.* **1986**, *46*, 2845−2848.
(53) Stohs, S. J.; Bagchi, D. Oxidative Mechanisms in the Toxicity of Metal Ions. *Free Radical Biol. Med.* **1995**, *18*, 321−336.
(54) Monticone, R. E.; Nick, R. J.; Eichhorn, G. L. The Effect of Cellular Age on Zinc Levels in Untreated and Zinc-Treated Human Diploid Fibroblasts. *J. Inorg. Biochem.* **1987**, *30*, 291−298.
(55) Wakelin, L. P. G. Polyfunctional DNA Intercalating Agents. *Med. Res. Rev.* **1986**, *6*, 275−340.
(56) Portugal, J.; Cashman, D. J.; Trent, J. O.; Ferrer-Miralles, N.; Przewloka, T.; Fokt, I.; Priebe, W.; Chaires, J. B. A New Bisintercalating Anthracycline with Picomolar DNA Binding Affinity. *J. Med. Chem.* **2005**, *48*, 8209−8219.
(57) Murdock, K. C. Heteroalkylenebisanthraquinonens, US4278605, 1981.
(58) Wakelin, L. P. G.; Bu, X.; Eleftheriou, A.; Parmar, A.; Hayek, C.; Stewart, B. W. Bisintercalating Threading Diacridines: Relationships between DNA Binding, Cytotoxicity, and Cell Cycle Arrest. *J. Med. Chem.* **2003**, *46*, 5790−5802.
(59) Crothers, D. M. Calculation of Binding Isotherms for Heterogeneous Polymers. *Biopolymers* **1968**, *6*, 575−584.
(60) Choudhury, J. R.; Bierbach, U. Characterization of the Bisintercalative DNA Binding Mode of a Bifunctional Platinum-Acridine Agent. *Nucleic Acids Res.* **2005**, *33*, 5622−5632.
(61) Robledo-Luiggi, C.; Wilson, W. D.; Pares, E.; Vera, M.; Martinez, C. S.; Santiago, D. Partial Intercalation with DNA of Peptides Containing Two Aromatic Amino Acids. *Biopolymers* **1991**, *31*, 907−917.
(62) Fuller, W.; Waring, M. J. A Molecular Model for the Interaction of Ethidium Bromide with Deoxyribonucleic Acid. *Ber. Bunsenges. Phys. Chem.* **1964**, *68*, 805−808.
(63) Rao, S. N.; Kollman, P. A. Molecular Mechanical Simulations on Double Intercalation of 9-Amino Acridine into d(CGCGCGC)·d(GCGCGCG): Analysis of the Physical Basis for the Neighbor-Exclusion Principle. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 5735−5739.

DOI: 10.1021/acs.inorgchem.8b00027
Inorg. Chem. 2018, 57, 5004−5012

(64) Hopcroft, N. H.; Brogden, A. L.; Searcey, M.; Cardin, C. J. X-Ray Crystallographic Study of DNA Duplex Cross-Linking: Simultaneous Binding to Two $d(CGTACG)_2$ Molecules by a Bis(9-Aminoacridine-4-Carboxamide) Derivative. *Nucleic Acids Res.* **2006**, *34*, 6663−6672.
(65) Mullins, S. T.; Annan, N. K.; Cook, P. R.; Lowe, G. Bisintercalators of DNA with a Rigid Linker in an Extended Configuration. *Biochemistry* **1992**, *31*, 842−849.

DOI: 10.1021/acs.inorgchem.8b00027
Inorg. Chem. 2018, 57, 5004−5012